

# Expanding the diversity of *Genea* Vittad. (Ascomycota, Pezizales) in Türkiye: Morphological and molecular insights into newly recorded species

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## Abstract

Despite their ecological significance, the diversity of *Genea* Vittad. species in Türkiye remains underexplored, highlighting the need for further research. The current study aims to expand the known distribution of *Genea* species in Türkiye by integrating morphological and molecular analyses of new collections. Fungal specimens were collected from Edirne and Kırklareli provinces between October and December 2022. Morphological characteristics were documented using light and scanning electron microscopy, and molecular phylogenetic analyses were conducted using nrITS (rDNA) sequences to confirm species identity. The studies identified the new Turkish collections as *Genea fragrans* (Wallroth) Saccardo, *Genea pseudobalsleyi* Agnello, Bratek & Cabero, *Genea pseudoverrucosa* Bratek, Konstant. & Van Vooren, and *Genea vagans* Matti., each exhibiting over 99% sequence similarity. This study provides the first records of these species in Türkiye, offering detailed descriptions of their morphological features, habitats, and phylogenetic placement.

## Özet

Ekolojik önemlerine rağmen, Türkiye'deki *Genea* Vittad. türlerinin çeşitliliği yeterince keşfedilmemiştir ve bu durum daha fazla araştırma yapılması gerektiğini vurgulamaktadır. Bu çalışma, morfolojik ve moleküler analizleri entegre ederek *Genea* türlerinin Türkiye'deki bilinen dağılımını genişletmeyi amaçlamaktadır. Mantar örnekleri Ekim ve Aralık 2022 tarihleri arasında Edirne ve Kırklareli illerinden toplanmıştır. Morfolojik özellikler ışık ve taramalı elektron mikroskobu kullanılarak belgelenirken, tür kimliğini doğrulamak için nrITS dizileri kullanılarak moleküler filogenetik analizler yapılmıştır. Çalışmalar, yeni Türk koleksiyonlarını *Genea fragrans* (Wallroth) Saccardo, *Genea pseudobalsleyi* Agnello, Bratek & Cabero, *Genea pseudoverrucosa* Bratek, Konstantinidis & Van Vooren ve *Genea vagans* Mattirollo olarak tanımladı ve her biri % 99'un üzerinde dizi benzerliği sergiledi. Bu çalışma, bu türlerin Türkiye'deki ilk kayıtlarını sunmakta ve morfolojik özellikleri, habitatları ve filogenetik yerleşimleri hakkında ayrıntılı tanımlar sunmaktadır.

**Keywords:** subterranean fungi, truffles, mycobiota, European part of Türkiye

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## Introduction

The genus *Genea* Vittad., established by Vittadini (1831), represents a group of subterranean fungi within the Ascomycota division. The name of the genus honours the distinguished zoologist Dr. Joseph Gené, with *G. verrucosa* Vittad. and *G. papillosa* Vittad. serving as the original taxa. While *Genea* is predominantly found throughout the Mediterranean region, it has not received the same scientific attention as the genus *Tuber* P. Micheli ex F.H. Wigg, which is widely recognised for its economically and gastronomically valuable truffles. It continues to dominate research priorities in mycology (Alvarado et al., 2014).

The taxonomic classification of *Genea* has undergone considerable revisions over time, reflecting advancements in understanding its phylogenetic relationships. Initially placed within the order Tuberales, the genus was reclassified under Pezizales by Trappe (1979), who retained its inclusion in the subterranean family Geneaceae due to unresolved connections with other taxa in the order. However, subsequent studies by Pfister (1984) highlighted shared structural and morphological features among *Genea* species, including similarities in excipulum architecture, pigmentation patterns, and spore ornamentation, which ultimately led to the dissolution of Geneaceae as a family and the reassignment of *Genea* to Pyrenomataceae.

Morphologically, *Genea* species are characterized by hypogeous, hollow, spherical to subspherical ascomata, which are typically black, reddish-brown, or yellowish, often adorned with warts and featuring an apical opening (Læssøe & Hansen, 2007). The ascomata may possess surface hairs and typically exhibit a tuft of basal hyphae. The gleba is divided into chambers containing a pythothecium arranged in a palisade and an epithecium formed by paraphyses at the peridial junction (Alvarado et al., 2016). Asci are hyaline, inamyloid, and contain eight uniseriate warted spores (Alvarado et al., 2014).

The genus exhibits broad ecological associations with a diverse array of host trees, including fir, larch, pine, oak, beech, birch, chestnut, hazel, hemlock, hornbeam, linden, rockrose, and Douglas fir (Zhang, 1991; Moreno-Arroyo et al., 1998; Smith, 2007; Guevara-Guerrero et al., 2012; Alvarado et al., 2014, 2016, 2021; Agnello et al., 2016; Paz et al., 2016, 2019; Ribes et al., 2016; Kaounas et al., 2016; Crous et al., 2021). Unlike specific, closely related genera, *Genea* has adapted to abandon the mechanism of active spore ejection, opting instead for dispersal facilitated by animals. The ripe ascomata emit volatile chemical signals to lure small mammals, including flying squirrels, voles, and mice. These animals consume the ascomata, and their digestive processes facilitate the effective distribution of the spores (Smith et al., 2006).

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Agnello et al., 2016; Paz et al., 2016, 2019; Ribes et al., 2016; Kaounas et al., 2016; Crous et al., 2021). Unlike specific, closely related genera, *Genea* has adapted to abandon the mechanism of active spore ejection, opting instead for dispersal facilitated by animals. The ripe ascomata emit volatile chemical signals to lure small mammals, including flying squirrels, voles, and mice. These animals consume the ascomata, and their digestive processes facilitate the effective distribution of the spores (Smith et al., 2006).

Currently, the genus includes 49 formally recognized species, described through contributions spanning nearly two centuries (Vittadini, 1831; Berkeley & Broome, 1846; Tulasne & Tulasne, 1851; Corda, 1854; Saccardo, 1889; Bresadola, 1893; Harkness, 1899; Mattiolo, 1900a, 1900b; Velenovský, 1922; Imai, 1933; Gilkey, 1939; Cribb, 1960; Trappe & Guzmán, 1971; Stewart & Heblack, 1979; Trappe, 1979; Zhang, 1991; Moreno-Arroyo et al., 1998; Smith et al., 2006; Smith, 2007; Guevara-Guerrero et al., 2012; Alvarado et al., 2014, 2016, 2021; Agnello et al., 2016; Kaounas et al., 2016; Paz et al., 2016, 2019; Crous et al., 2021). In Türkiye, five *Genea* species have been documented to date through morphological analyses carried out by various researchers. Specifically, *G. hispidula* Berk. ex Tul. & C. Tul. was reported from Trabzon, *G. klotzschii* Berk. & Broome from Samsun, *G. lobulata* (Mor.-Arr., J. Gómez & Calonge) P. Alvarado & Mor.-Arr. from Niğde, *G. sphaerica* Tul. & C. Tul. from İzmir, and *G. verrucosa* Vittad. from Muğla, with detailed descriptions provided in the studies by Uzun & Kaya (2019), Berber et al. (2019), and Türkoğlu & Castellano (2014). These findings were compiled and included in the Turkish truffles checklist by Akata et al. (2022).

The present study documents and characterizes these four newly recorded *Genea* species in Türkiye, thereby contributing to a deeper understanding of the genus diversity and distribution across the country.

## Materials and Methods

### Field Study

The *Genea* specimens were systematically collected from various forest habitats, including pine-dominated woodlands in the Uzunköprü district of Edirne and oak forests in the Demirköy and Pınarhisar districts of Kırklareli, employing trained *Lagotto Romagnolo* truffle dogs as part of the collection process (Figure 1). Extensive field investigations were conducted to document the macroscopic and ecological characteristics of the collected specimens, facilitating a detailed assessment of their morphological variability and environmental preferences. High-resolution imaging was performed using a Nikon D810 camera fitted with an AF-S NIKKOR 105 mm f/1.4E ED lens, ensuring the capture of intricate structural details essential for taxonomic analysis. Simultaneously, a comprehensive dataset was compiled, systematically recording vital metadata, including collection dates, precise geographical coordinates, habitat descriptions, and unique specimen identifiers.

## Morphological Study

The samples were examined using light microscopy (LM) and scanning electron microscopy (SEM). For LM (Euromex Oxion), visualization was achieved with distilled water, 5% KOH, and Congo red. The dimensions of ascospores were assessed by measuring at least 30 randomly selected spores outside the asci, while the length-to-width ratio, noted as Q, was calculated independently of ornamentation. Spore sizes were reported without ornamentation, with the ornamentation width defined with the base of the warts. For SEM, sections of the gleba were affixed to stubs using double-sided tape and coated with gold particles to enhance conductivity. Imaging was conducted using a ZEISS EVO 40 SEM at an acceleration voltage of 20 kV. The specimens were then prepared for long-term preservation and stored in the Fungarium of the Faculty of Science at Ankara University (ANK).

## Molecular Study

### Determination of Internal Transcribed Spacer (ITS) rDNA Sequences

The nuclear ribosomal ITS (nrITS) rDNA region was amplified from genomic DNA isolated from *Genea* specimens using the

polymerase chain reaction (PCR) method. This amplification strictly adhered to established and well-validated methodologies outlined in previous studies (Akata et al., 2023a, 2023b, 2024a, 2024b). These standardized protocols ensured the accuracy and reproducibility of the molecular data generated, thus providing a robust foundation for subsequent analyses.

### Molecular Phylogenetic Analysis

The phylogenetic relationships among the fungal samples were meticulously analyzed with the MEGA-X software (Kumar et al., 2018), using nucleotide sequences obtained directly from the specimens. The reference sequences were selected from GenBank using a rigorous screening process that designates closely related taxa as the ingroup and more distantly related sequences as the outgroup, using NCBI BLAST searches. Multiple sequence alignment was performed with the MUSCLE algorithm (Edgar, 2004) to optimize accuracy and consistency. Following these alignments, K2+G+I was determined as the most suitable nucleotide substitution model to guide the construction of phylogenetic trees. The Neighbour-Joining method was employed to infer evolutionary relationships, and the robustness of the branching patterns was assessed with 1,000 bootstrap replicates. This analytical approach aligned with established methodologies outlined in previous research (Akata et al., 2024a, 2024b, 2024c, 2024d), ensuring methodological rigour and comparability.

## Results

This study assessed the macroscopic and microscopic characteristics of the samples and evaluated their ribosomal DNA (rDNA) sequences using ITS sequencing.

### Taxonomy

Phylum **ASCOMYCOTA** (Berk) Caval.-Sm.

Ordo **Pezizales** J. Schöt.

Family **Pezizaceae** Dumort.

Genus *Genea* **Vittad.**

*Genea fragrans* (Wallr.) Sacc. (1889), (Figures 2, 6a, and 6b).

**Material examined:** Türkiye-Kırklareli, Pınarhisar, under oak, 350 m, 41°41' N, 27°42' E, 4 Oct. 2022, ANK Akata TT 098; *ibid.*, 417 m, 41°41' N, 27°38' E, 18 Nov. 2022, ANK Akata TT 207.

### Macroscopic and Microscopic Features

Ascomata 7–8 mm across, hypogeous, hollow, subglobose and lobed, with a peridium surface displaying a brownish-black hue and covered by tiny, blunt, inconspicuous dark warts; a small tuft of hyphae occupies the base. Peridium up to 150 µm thick, with an outer surface brownish-black and exhibiting small, blunt, inconspicuous dark warts, composed of a single layer of pseudoparenchymatous tissue, comprising hyaline to light brownish angular to subglobose cells measuring 15–35 µm in width. Internal chamber consists of unique, winding chambers leading to a gleba with a labyrinth-like configuration, and the chamber walls lined with a black epithecium, up to 70 µm, formed by brownish to dark brown angular cells



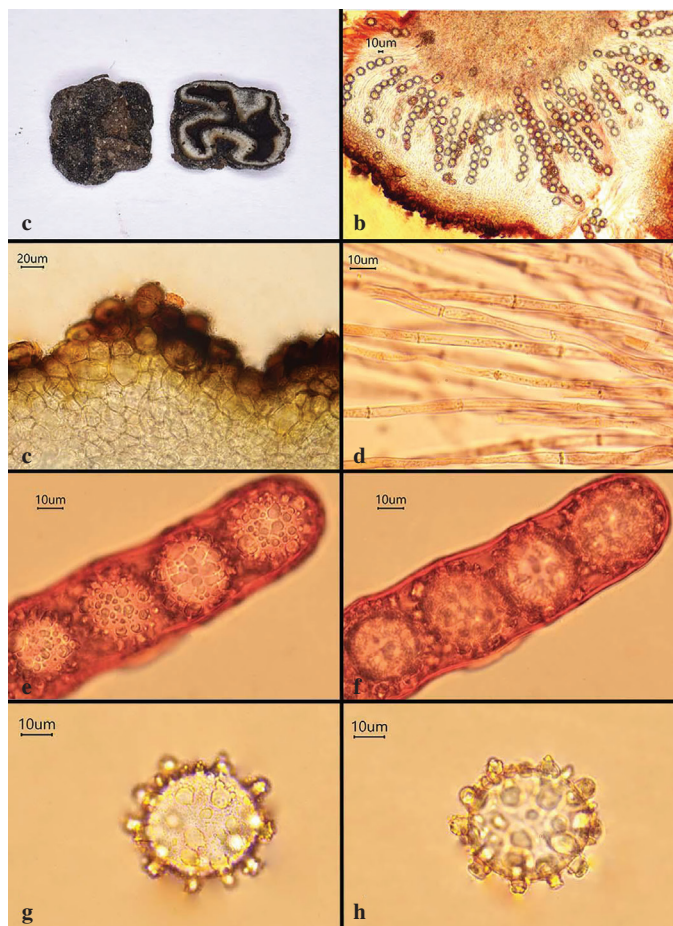
**Figure 1.** (a–d) Collection areas; (e) truffle dogs (*Lagotto Romagnolo*).

measuring 20–45 µm wide. Asci 240–300 × 25–30 µm, 8-spored, cylindrical, with a short peduncle. Paraphyses cylindrical, thread-like, 3–6 µm broad, septate, with some swollen cells. Ascospores (29)31–41(43) × (27)28–36(38) µm, (mean: 38 × 34), [(n = 30), Q = (1.02)1.06–1.22(1.31), Qav = 1.14], subglobose to broadly ellipsoid, hyaline to pale yellow, ornamented with truncated warts, measuring 1.5–4 × 2–4.5 µm, mixed with micro-warts.

### Ecology and Distribution

*Genea fragrans* is typically found from September to December, mainly beneath deciduous trees like oak, beech, hornbeam, and common hazel, and less frequently associated with chestnut, poplar, willow, blackthorn, and European hop-hornbeam (Venturella et al., 2006; Riva, 2009; Zotti et al., 2010; Kajevska et al., 2013; Landi et al., 2015; Alvarado et al., 2016). Its known distribution covers Greece, Germany, Hungary, France, Italy, Macedonia, Serbia, Spain, and Morocco (Venturella et al., 2006; Diamandis & Perlerou, 2008; Saitta et al., 2008; Bratek et al., 2013; Kajevska et al., 2013; Alvarado et al., 2016, 2021; Savić et al., 2018).

*Genea pseudobalsleyi* Agnello, Bratek & J. Cabero (2014), (Figures 3, 6c, and 6d).



**Figure 2.** *Genea fragrans*. (a) ascomata; (b) microscopic cross-section of ascomata; (c) peridium; (d) septa of paraphyses; (e, f) spores contained within the ascus; (g, h) single ascospore.

**Material examined:** Türkiye- Kırklareli, Pınarhisar, under oak, 480 m, 41°44' N, 27°34' E, 5 Oct. 2022, ANK Akata TT 127; ibid., 312 m, 41°39' N, 27°36' E, 5 Dec. 2022, ANK Akata TT 281; Edirne, Uzunköprü, under pine, 106 m, 41°12' N, 26°44' E, 7 Dec. 2022, ANK Akata TT 350. Alvarado et al. (2014) offered a comprehensive overview of the type specimens from the original collection.

### Macroscopic and Microscopic Features

Ascomata 7–9 mm across, hypogeous, hollow, subglobose to moderately lobed, with a uniformly warted peridium surface adorned with small polygonal black warts; a small tuft of hyphae occupies the base. Peridium consisting of a single layer of pseudoparenchymatic tissue up to 160 µm thick, composed of hyaline, subglobose cells measuring 15–45 µm in diam., transitioning towards the outer layer into angular cells with thicker, darker walls. Internal chamber displaying variability, ranging from a single chamber to multiple sinuous chambers formed by wall projections, producing a brain-like appearance; chamber walls lined with a blackish or brown-black epithecium up to 60 µm thick, comprising pseudoparenchymatous tissue with angular cells featuring thick, melanized walls measuring 15–40 µm in width. Asci 180–240 × 35–40 µm, eight-spored, somewhat cylindrical with a short peduncle. Paraphyses cylindrical, septate, 4–9 µm diam., with some cells appearing swollen. Ascospores (24)25–29 (31) × 23–26 µm, (mean: 28 × 24), [(n = 30), Q = 1.1–1.26, Qav = 1.18], spherical to broadly ellipsoid, pale yellow, containing prominent, unevenly positioned oil droplets; surface ornamented with truncated warts, occasionally featuring small digitations at the top, measuring 2–3.5 × 2–2.5 µm.

### Ecology and Distribution

*Genea pseudobalsleyi* is primarily observed between June and December and is mainly associated with oak trees, particularly *Quercus ilex* L. and *Q. pyrenaica* Willd. Its geographical distribution encompasses France, Hungary, Italy, and Spain (Alvarado et al., 2014; Perez, 2024).

*Genea pseudoverrucosa* Bratek, Konstant. & Van Vooren (2014), (Figures 4, 6e, and 6f). Material examined: Türkiye-Kırklareli, Pınarhisar, under oak, 14 m, 41°44' N, 27°34' E, 5 Oct. 2022, ANK Akata TT 124. Alvarado et al. (2014) presented a thorough account of the type specimens from the original collection.

### Macroscopic and Microscopic Features

Ascomata 10–12 mm across, hypogeous, hollow, spherical to extensively lobed, peridium surface black and adorned with tiny, rounded warts, with an inconspicuous basal tuft of dark hyphae. Peridium consisting of a single layer of pseudoparenchymatic tissue up to 200 µm thick, made up of light brownish angular cells measuring 18–35 µm in width. Internal chamber slightly partitioned yet primarily intact, coated with a dark epithecium resembling the peridium, epithecium structure pseudoparenchymatous made of isodiametric cells up to 160 µm. Asci 200–250 × 30–35 µm, 8-spored, somewhat cylindrical. Paraphyses 4–8 µm broad, cylindrical, septate, with some swollen cells. Ascospores (26)–28–

33 (–34) × (23–) 24–28(–29) µm, (mean: 31 × 26), [(n = 30), Q = (1.05)1.1–1.32 (1.4), Qav = 1.2], subglobose to broadly ellipsoid, hyaline, ornamented with conical-truncated to irregular warts, measuring 2–4 × 1.5–3 µm.

### Ecology and Distribution

*Genea pseudoverrucosa* is typically found from July to January in temperate deciduous forests associated with oak, hornbeam, linden, and common hazel. It also thrives in Mediterranean habitats featuring oaks (*Quercus ilex* L. and *Q. coccifera* L.). Its distribution spans Greece, Hungary, France, Romania, and Morocco (Alvarado et al., 2014).

*Genea vagans* Mattir. (1900), (Figures 5, 6g, and 6h). Material examined: Türkiye-Kırklareli, Demirköy, under oak, 14 m, 41°51' N, 27°56' E, 8 Oct. 2022, ANK Akata TT 163.

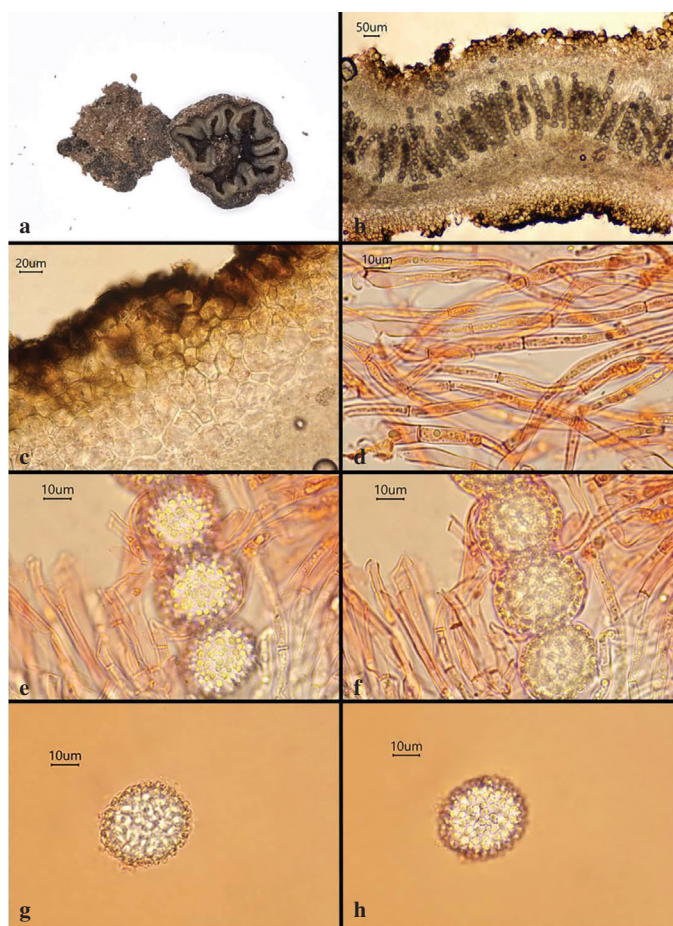
### Macroscopic and Microscopic Features

Ascomata 10–15 mm across, hypogeous, hollow, subglobose to moderately lobed, with an apical pore and a tuft of brownish hairs at the base, peridium black, finely verrucose, surface adorned with small polygonal black warts. Peridium consisting of a single layer of pseudoparenchymatic tissue up to 300 µm thick, composed

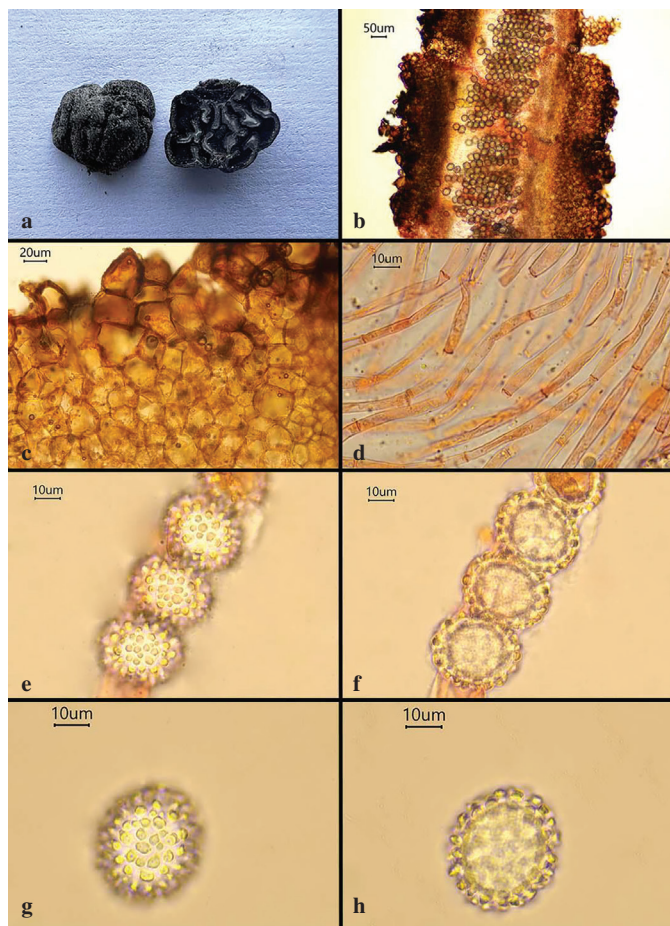
of hyaline, angular cells measuring 25–50 µm in width. Internal chamber usually solitary, occasionally divided, coated with a dark epithecium, up to 150 µm thick, comprising pseudoparenchymatous tissue with angular cells measuring 20–40 µm wide. Asci 220–250 × 30–40 µm, 8-spored, somewhat cylindrical with a long peduncle. Paraphyses cylindrical, septate, 5–10 µm diam., with some swollen cells. Ascospores (29)31–38(39) × (25)27–30(31) µm, (mean: 35 × 29), [(n = 30), Q = (1.1)1.15–1.22(1.3), Qav = 1.18], broadly ellipsoid to ellipsoid, hyaline, ornamented with conical or conical-truncated warts, measuring 4–6 × 3–5 µm, sometimes showing single digitations at the top.

### Ecology and Distribution

*Genea vagans* is typically observed in autumn, particularly beneath trees such as fir and beech, with a tentative association with chestnuts, as indicated in early studies (Mattiolo, 1900b). Recent research has documented its presence under oak, beech, alder, common hazel, and hornbeam (Alvarado et al., 2016, 2021; Savić et al., 2018). Geographically, the species is distributed across Italy, France, Spain, Russia, and Serbia, as evidenced by studies spanning over a century (Mattiolo, 1900a; Bucholtz, 1901; Ceruti, 1960; Vidal, 1997; Alvarado et al., 2014, 2016, 2018, 2021; Kaunas et al., 2016; Ribes et al., 2016; Savić et al., 2018).



**Figure 3.** *Genea pseudobalsleyi*. (a) ascomata; (b) microscopic cross-section of ascomata; (c) peridium; (d) septa of paraphyses; (e, f) spores contained within the ascus; (g, h) single ascospore.



**Figure 4.** *Genea pseudoverrucosa*. (a) ascomata; (b) microscopic cross-section of ascomata; (c) peridium; (d) septa of paraphyses; (e, f) spores contained within the ascus; (g, h) single ascospore.

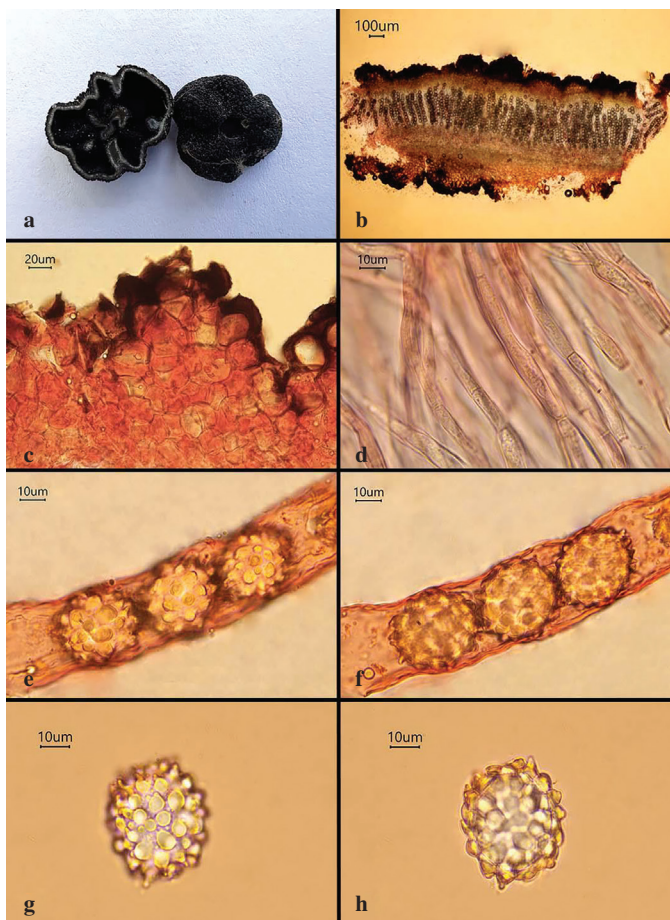
### Evolutionary History of the *Genea* Specimens

The evolutionary lineages of the specimens ANK Akata TT 098, ANK Akata TT 124, ANK Akata TT 127, ANK Akata TT 163, ANK Akata TT 207, ANK Akata TT 281, and ANK Akata TT 350 were examined based on their nrITS rDNA sequences, which were obtained using standard molecular techniques and stored in the NCBI GenBank under the accession numbers provided in Table 1. To infer their evolutionary relationships, nrITS rDNA sequences from various members of *Genea* were selected for comparison, using a sequence of *Scutellinia verruculosa* M. Zeng, Q. Zhao & K.D. Hyde as outgroup. Our molecular phylogenetic analysis identified eight distinct clades (Figure 7). While Clade 1 comprised different isolates of *G. vagans* and the specimen ANK Akata TT 163, Clade 2 included an isolate of *G. pseudobalsleyi*, which clustered with the specimens ANK Akata TT 127, ANK Akata TT 281, and ANK Akata TT 350. In contrast, Clade 3 contained various isolates of *G. fragrans*, alongside the specimens ANK Akata TT 098 and ANK Akata TT 207. Lastly, the specimen ANK Akata TT 124 grouped with different isolates of *G. pseudoverrucosa* in a well-supported Clade 4. The remaining clades (Clades 5–8) incorporated other

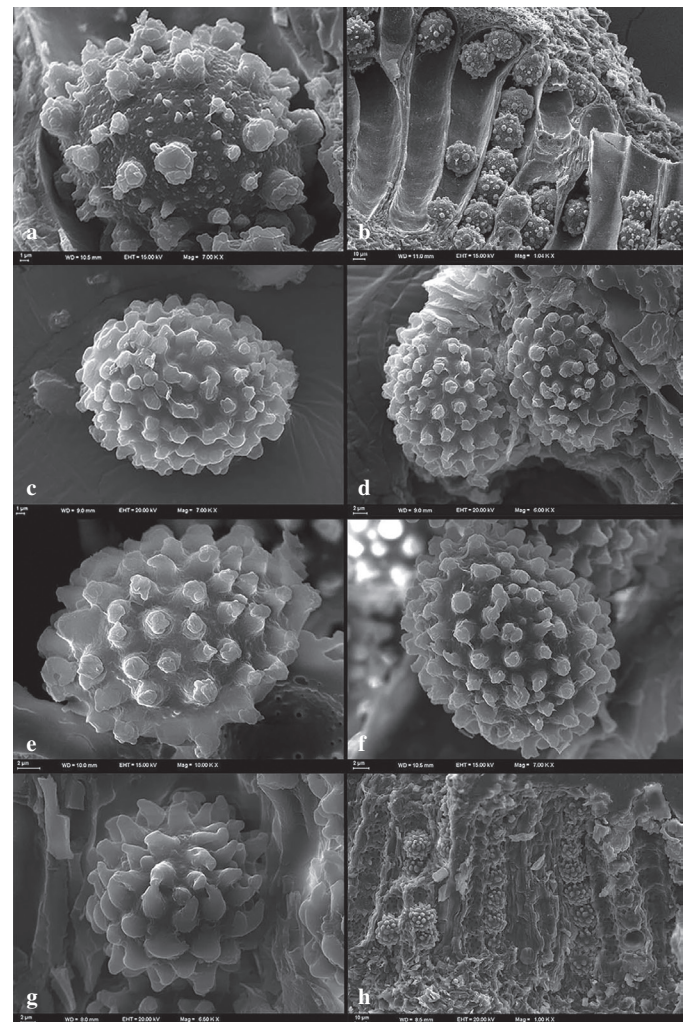
*Genea* species. Phylogenetic analyses confirmed the close relationship between our specimens and their associated *Genea* species, with high bootstrap branch support rates, thus validating their identity.

### Discussion

*Genea verrucosa*, frequently compared with *G. fragrans*, is a darkly pigmented species characterized by significantly smaller ascospores and unique spore ornamentation (Bertolini, 2014). The ascospores of *G. verrucosa* measure  $21\text{--}26 \times 19\text{--}22 \mu\text{m}$  and exhibit conical warts approximately measuring  $2 \times 1.5\text{--}4.5 \mu\text{m}$ , which are smaller and less prominent than the larger and more distinct ornamentation observed in *G. fragrans* (Türkoğlu & Castellano, 2014; Alvarado et al., 2016). Despite similarities in macroscopic appearance, the ascospores and asci of *G. verrucosa* are generally smaller, with asci not exceeding  $250 \times 35 \mu\text{m}$ . Over time, the conical spore warts of *G. verrucosa* may develop into aculeate forms but remain less prominent than the ornamentation



**Figure 5.** *Genea vagans*. (a) ascomata; (b) microscopic cross-section of ascomata; (c) peridium; (d) septa of paraphyses; (e, f) spores contained within the ascus; (g, h) single ascospore.



**Figure 6.** Scanning electron microscope micrographs. (a, b) *Genea fragrans*; (c, d) *Genea pseudobalsleyi*; (e, f) *Genea pseudoverrucosa*; (g, h) *Genea vagans*.

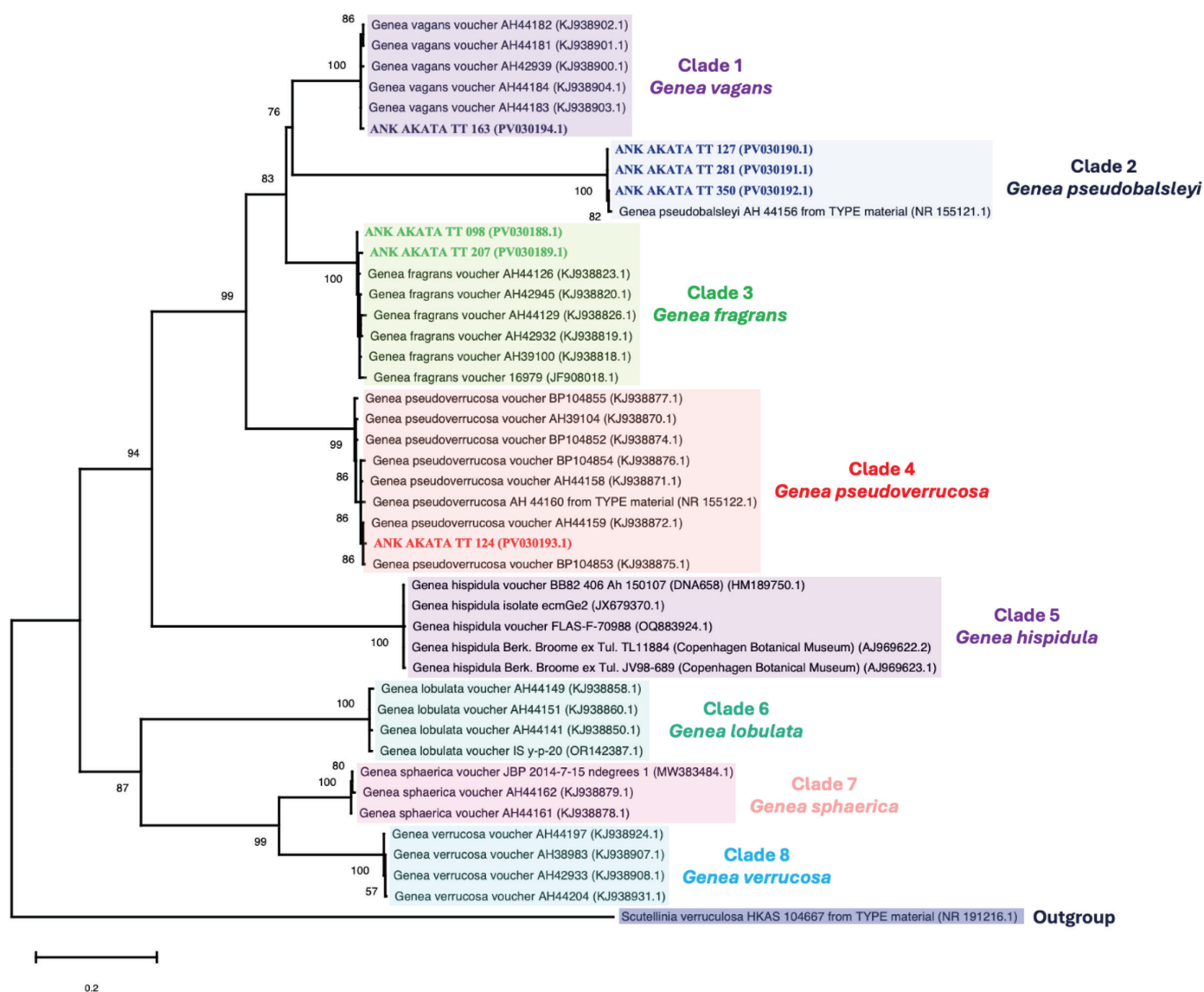
of *G. fragrans*. The ecological preferences of *G. verrucosa* further distinguish it, because it primarily associates with oak trees and is widely distributed across Europe, particularly in the Mediterranean region (Hollós, 1911; Diamandis & Perlerou, 2008; Alvarado et al., 2014; Bertolini, 2014).

Other species within the genus also display significant morphological and ecological distinctions: *G. brunneocarpa* G. Moreno, Cabero & Kaounas shares some similarities with *G. fragrans* but differs in ascospore size, measuring  $28\text{--}31.5 \times 19.5\text{--}24\text{ }\mu\text{m}$ , and by its ornamentation, which consists of truncate-conical warts that are occasionally nearly cubic, reaching  $3.8\text{--}5.5\text{ }\mu\text{m}$  high. The brownish peridium and simpler internal chamber structure of *G. brunneocarpa* further set it apart. Unlike *G. fragrans*, this species is chiefly associated with holm oak and Aleppo pine,

thriving on calcareous soils and fruiting between February and April. Its distribution is limited to Mediterranean regions, with records from Greece and Spain (Alvarado et al., 2014).

Similarly, *G. compressa* Merényi, Cabero & G. Moreno is a closely related species that can be distinguished by its smaller ascospores, measuring  $23.5\text{--}30.5 \times 18\text{--}25.5\text{ }\mu\text{m}$ , and ornamentation consisting of conical warts with digitate apices, up to  $2.3\text{--}5.3\text{ }\mu\text{m}$  high. It is commonly associated with oak species and found in mixed habitats alongside common hornbeam or Atlas cedar, with a distribution spanning Hungary, Morocco, and Spain (Kaounas et al., 2016).

Another closely related species, *G. fageticola* Konstantin., Cabero & Faust. García shares overlapping spore dimensions with *G. fragrans*,  $28\text{--}36.5 \times 18\text{--}26\text{ }\mu\text{m}$ , but it is distinguishable by its



**Figure 7.** Phylogram of ML of a set of 44 *Genea* sequences based on the nrITS rDNA, rooted with *Scutellinia verruculosa* (outgroup). Nodes with  $\geq 50\%$  ML bootstrap support are annotated with their support values. Terminals that represent sequences obtained during this study are in bold. Each sequence is accompanied by its respective GenBank accession number, and a scale bar in the lower left corner indicates a genetic distance of 0.2. ML = maximum likelihood; nrITS = nuclear ribosomal internal transcribed spacer.

**Table 1.** GenBank accession numbers and collection sites of *Genea* specimens examined.

| Species                   | Specimen voucher/isolate/strain                               | nrITS GenBank Accession Number | Geographical origin                                 | Reference              |
|---------------------------|---------------------------------------------------------------|--------------------------------|-----------------------------------------------------|------------------------|
| <i>G. fragrans</i>        | ANK Akata TT 098                                              | PV030188                       | Türkiye, Kırklareli, Pınarhisar                     | Current study          |
|                           | ANK Akata TT 207                                              | PV030189                       | Türkiye, Kırklareli, Pınarhisar                     | Current study          |
|                           | AH44129                                                       | KJ938826                       | Spain, Zamora, Peleagonzalo                         | Alvarado et al., 2016  |
|                           | AH42932                                                       | KJ938819                       | Spain, Madrid, Torrelaguna                          | Alvarado et al., 2016  |
|                           | AH42945                                                       | KJ938820                       | Morocco, Chefchaouen                                | Alvarado et al., 2016  |
|                           | AH39100                                                       | KJ938818                       | Morocco, Chefchaouen                                | Alvarado et al., 2016  |
|                           | AH44126                                                       | KJ938823                       | Greece, Pigi Kilkis                                 | Alvarado et al., 2016  |
|                           | 16979                                                         | JF908018                       | Italy                                               | Osmundson et al., 2013 |
| <i>G. pseudobalsleyi</i>  | ANK Akata TT 127                                              | PV030190                       | Türkiye, Kırklareli, Pınarhisar                     | Current study          |
|                           | ANK Akata TT 281                                              | PV030191                       | Türkiye, Kırklareli, Pınarhisar                     | Current study          |
|                           | ANK Akata TT 350                                              | PV030192                       | Türkiye, Edirne, Uzunköprü                          | Current study          |
|                           | AH44156                                                       | NR_155121                      | Italy, Brindisi, Bosco Compare                      | Alvarado et al., 2016  |
| <i>G. vagans</i>          | ANK Akata TT 163                                              | PV030194                       | Türkiye, Kırklareli, Demirköy                       | Current study          |
|                           | AH44182                                                       | KJ938902                       | Spain, Zamora, Quintana de Sanabria                 | Alvarado et al., 2016  |
|                           | AH44183                                                       | KJ938903                       | Spain, Zamora, Quintana de Sanabria                 | Alvarado et al., 2016  |
|                           | AH44184                                                       | KJ938904                       | Spain, Girona, Campelles                            | Alvarado et al., 2016  |
|                           | AH44181                                                       | KJ938901                       | Spain, Asturias, Pola de Somiedo                    | Alvarado et al., 2016  |
|                           | AH42939                                                       | KJ938900                       | Spain, Guadalajara, El Ordial                       | Alvarado et al., 2016  |
| <i>G. pseudoverrucosa</i> | ANK Akata TT 124                                              | PV030193                       | Türkiye, Kırklareli, Pınarhisar                     | Current study          |
|                           | AH44158                                                       | KJ938871                       | -                                                   | Alvarado et al., 2016  |
|                           | AH44159                                                       | KJ938872                       | -                                                   | Alvarado et al., 2016  |
|                           | AH44160                                                       | NR_155122                      | -                                                   | Alvarado et al., 2016  |
|                           | AH39104                                                       | KJ938870                       | -                                                   | Alvarado et al., 2016  |
|                           | BP104852                                                      | KJ938874                       | Hungary, Heves county, Bükk mountains, Szilvásvárad | Alvarado et al., 2016  |
|                           | BP104853                                                      | KJ938875                       | Hungary, Pest county, Budapest                      | Alvarado et al., 2016  |
|                           | BP104854                                                      | KJ938876                       | Hungary, Győr-Moson-Sopron, Fertőrákos              | Alvarado et al., 2016  |
| <i>G. verrucosa</i>       | BP104855                                                      | KJ938877                       | Romania, Harghita, Cristuru Secuiesc                | Alvarado et al., 2016  |
|                           | AH42933                                                       | KJ938908                       | Spain, Guadalajara, Algora                          | Alvarado et al., 2016  |
|                           | AH44204                                                       | KJ938931                       | Spain, La Rioja, Torrecilla en Cameros              | Alvarado et al., 2016  |
|                           | AH38983                                                       | KJ938907                       | Spain, Guadalajara, Valdearenas                     | Alvarado et al., 2016  |
|                           | AH44197                                                       | KJ938924                       | Spain, Zamora, Peleagonzalo, Monte San Miguel       | Alvarado et al., 2016  |
| <i>G. hispidula</i>       | FLAS-F-70988                                                  | OQ883924                       | USA: VT, Rutland Co.                                | Unpublished            |
|                           | Berk. & Broome ex Tul. TL11884 (Copenhagen Botanical Museum)  | AJ969622                       | Denmark: Broby Vesterskov, South Zealand            | Tedersoo et al., 2006  |
|                           | Berk. & Broome ex Tul. JV98-689 (Copenhagen Botanical Museum) | AJ969623                       | United Kingdom: Shropshire, Dudmaston Estate        | Tedersoo et al., 2006  |
|                           | BB82_406_Ah_150107 (DNA658)                                   | HM189750                       | -                                                   | Unpublished            |
|                           | ecmGe2                                                        | JX679370                       | Czech Republic                                      | Unpublished            |

Table 1. Continued.

| Species                        | Specimen voucher/isolate/strain | nrITS GenBank Accession Number | Geographical origin                         | Reference             |
|--------------------------------|---------------------------------|--------------------------------|---------------------------------------------|-----------------------|
| <i>G. lobulata</i>             | AH44141                         | KJ938850                       | Cyprus                                      | Alvarado et al., 2016 |
|                                | AH44149                         | KJ938858                       | Greece, Attica, Parnitha                    | Alvarado et al., 2016 |
|                                | AH44151                         | KJ938860                       | Greece, Attica, Parnitha                    | Alvarado et al., 2016 |
|                                | IS_y-p-20                       | OR142387                       | Israel                                      | Unpublished           |
| <i>G. sphaerica</i>            | AH44161                         | KJ938878                       | Germany, Grockstädt                         | Alvarado et al., 2016 |
|                                | JBP 2014-7-15-1                 | MW383484                       | France: Sexey aux Forges Meurthe-et-Moselle | Unpublished           |
|                                | AH44162                         | KJ938879                       | Germany, Leinefelde                         | Alvarado et al., 2016 |
| <i>Scutellinia verruculosa</i> | HKAS:104667                     | NR_191216                      | China, Sichuan                              | Zeng et al., 2022     |

ANK = Ankara University; nrITS = nuclear ribosomal internal transcribed spacer.

spherical ascomata, the absence of labyrinthine hymenium folds, and the presence of small polygonal warts on its peridium instead of minute papillae. Additionally, *G. fageticola* has a brownish epithecium and a basal tuft of reddish or brownish hyphae, both absent in *G. fragrans*. This species, reported from Greece and Spain, associates with European beech and occasionally with sessile oak, emerging during late summer and winter (Alvarado et al., 2014; Kaounas et al., 2016).

*Genea pseudobalsleyi* exhibits notable morphological similarities with *G. balsleyi* M.E. Sm., primarily due to its black peridium surface, ascospore dimensions ( $24\text{--}30 \times 20\text{--}25 \mu\text{m}$ ), and ornamentation patterns (Alvarado et al., 2014). Nevertheless, it is distinguished by unique structural features, such as prominent projections on the peridium wall that create intricate, labyrinthine, chambered cavities absent in *G. balsleyi* (Smith, 2007). Geographic distribution further differentiates these species, as *G. balsleyi* is restricted to North America and typically associated with oak, while *G. pseudobalsleyi* is exclusively recorded in Europe (Smith, 2007; Alvarado et al., 2014). Additionally, *G. balsleyi* is characterized by a bilayered peridium structure, a feature not found in *G. pseudobalsleyi* (Alvarado et al., 2014).

In contrast, the rare American species *G. macrosiphon* Gilkey, while showing macroscopic resemblance to *G. pseudobalsleyi*, is distinct due to its significantly larger ascospore dimensions ( $36\text{--}40 \times 24\text{--}28 \mu\text{m}$ ) and highly inflated pseudoparenchymatous peridium cells (Gilkey, 1939; Alvarado et al., 2014).

Distinctive morphological features of *G. pseudobalsleyi* set it apart from closely related species. Compared to *G. verrucosa*, *G. pseudobalsleyi* is characterized by densely packed and truncated ascospore ornamentation, in stark contrast to the thinner, sharper, and more scattered ornamentation observed in *G. verrucosa* (Alvarado et al., 2014; Bertolini, 2014; Türkoğlu & Castellano, 2014). Furthermore, although *G. compressa* shares macroscopic similarities and identical ascospore dimensions,  $23.5\text{--}30.5 \times 18\text{--}25.5 \mu\text{m}$ , with *G. pseudobalsleyi*, it is readily distinguishable by its

unique conical spore ornamentation. *Genea compressa* primarily grows in autumn under various host plants, including oak, and is distributed across regions such as Spain and Hungary (Alvarado et al., 2014).

Additional distinctions are evident in other closely related species. For instance, *G. fageticola* displays notably larger ascospores, measuring  $28\text{--}36.5 \times 18\text{--}26 \mu\text{m}$ , and unique ornamentation characterized by truncated or rounded warts measuring  $2.1\text{--}6.5 \times 2\text{--}6 \mu\text{m}$  (Alvarado et al., 2014). In contrast, *G. cephalonica* Kaounas, Agnello & P. Alvarado, which is exclusively associated with Greek fir (*Abies cephalonica* Loudon) in Greece, features slightly smaller ascospores,  $24\text{--}35 \times 15.5\text{--}26 \mu\text{m}$ , but it is distinguished by its distinct ornamentation, with warts reaching  $3\text{--}6 \mu\text{m}$  in height (Kaounas et al., 2016).

The macroscopic features of *G. pseudoverrucosa* resemble *G. fragrans* but are often misidentified as *G. verrucosa* due to similarities in ascospore dimensions. Its spore ornamentation, characterized by truncate warts, is similar to that of *G. fragrans*; however, the warts in *G. pseudoverrucosa* are lower, denser, and more uniform. In contrast, *G. fragrans* has more prominent and scattered warts, often interspersed with smaller ones. *G. pseudoverrucosa* also shares similarities with *G. brunneocarpa*, yet the latter displays more irregularly scattered warts, necessitating ecological and macroscopic observations to separate them. Similarly, *G. pseudobalsleyi* features a black, warted peridium and comparable ascospores but it is distinguished by its thinner warts,  $1.5\text{--}2 \mu\text{m}$  wide. The North American species *G. balsleyi* exhibits a bilayered peridium, a hollow inner cavity, and conical spore warts (Gilkey, 1939; Alvarado et al., 2014).

The original Italian collections of *G. vagans*, first described by Mattirollo (1900a), were subsequently detailed and illustrated by Ceruti (1960), who characterized the species as producing small, rounded ascomata with a black verrucose peridium. The ascospores were ellipsoidal, measuring  $35\text{--}38 \times 27\text{--}28 \mu\text{m}$ , and adorned with prominent conical warts that frequently coalesce at their bases.

Lawrynowicz (1988) reported nearly identical dimensions, with ascospores measuring  $31\text{--}38 \times 27\text{--}28 \mu\text{m}$ . However, Vidal (1997) and Montecchi and Sarasini (2000) documented significantly smaller ascospore sizes, ranging from  $18\text{--}22 \mu\text{m}$ , highlighting variability in reported measurements. A later study by Ribes et al. (2016) aligned more closely with Ceruti (1960) and Lawrynowicz (1988), recording ascospore dimensions of  $31.2\text{--}38.4 \times 27.1\text{--}30.2 \mu\text{m}$ . Alvarado et al. (2016) further corroborated these findings, reporting ascospore sizes of  $32\text{--}41 \times 27\text{--}37 \mu\text{m}$  based on authentic material from Mattiolo and additional specimens. In our study, ascospore dimensions of  $31\text{--}38 \times 27\text{--}30 \mu\text{m}$  were observed, consistent with the findings of Lawrynowicz (1988) and Ribes et al. (2016), reinforcing the morphological variability within this species.

*Genea vagans* has often been misidentified due to its resemblance to other species, such as *G. anthracina* Heblack & Stewart, an American species. While both species share similar macroscopic features, including small, black fruiting bodies with a single basal cavity, *G. anthracina* is distinguishable by its smaller ascospores, which measure  $22\text{--}26 \times 18\text{--}20 \mu\text{m}$  and are ornamented with subglobose, irregular, and occasionally truncated warts (Stewart & Heblack, 1979). Confusion also arises with *G. pinicola* V. Kaounas, J. Cabero & F. García, a species that transitions from an initial yellowish hue to a yellowish-brown (Alvarado et al., 2014). Although typically associated with pine, collections of *G. pinicola* have also been documented under oak. This species has ascospores measuring  $26\text{--}29 \times 17\text{--}20 \mu\text{m}$ , with ornamentation made of small conical warts,  $1\text{--}3 \mu\text{m}$  in height (Alvarado et al., 2014; Ribes et al., 2016). Externally, *G. vagans* also resembles *G. sphaerica* Tul. & C. Tul., sharing similarities in shape and colour (Vacek, 1951; Hawker, 1954). However, detailed microscopic analysis reveals key differences between the two species: *G. sphaerica* is characterized by broadly elliptical to sub-spherical ascospores, measuring  $20\text{--}25 \times 18\text{--}20 \mu\text{m}$ , densely ornamented with fine, truncated, or brush-like warts  $0.5\text{--}1.5 \mu\text{m}$  in height (Alvarado et al., 2014, 2016). Additionally, *G. sphaerica* possesses a basal cavity with numerous folds, a feature absent in *G. vagans* (Ribes et al., 2016).

The genetic diversity among fungal species significantly surpasses their morphological appearance, necessitating the integration of genetic data with traditional morphological methods for species identification. Genetic markers, such as rRNA gene regions (nrITS, nuclear ribosomal small subunit, and nuclear ribosomal large subunit) and protein-coding gene sequences, have been employed in molecular systematics for decades (Raja et al., 2017). Among these, the ITS region is extensively used in fungal molecular taxonomy, providing valuable insights (White et al., 1990). Additionally, advancements in high-throughput sequencing and bioinformatics now enable whole-genome comparisons and phylogenomic analyses, which may soon surpass traditional molecular phylogenetic studies based on a limited number of marker genes (Marian et al., 2024).

In this study, nuclear ITS rDNA sequences were used to identify the fungal specimens ANK Akata TT 098, ANK Akata TT 124, ANK Akata TT 127, ANK Akata TT 163, ANK Akata TT 207,

ANK Akata TT 281, and ANK Akata TT 350. This analysis demonstrated a genetic similarity of over 99% between the specimens and their related isolates of *Genea* (Table 1, Figure 6).

This study reports the first occurrence of *G. fragrans*, *G. pseudobalsleyi*, *G. pseudoverrucosa*, and *G. vagans* in Türkiye. The research achieved precise taxonomic identification using a combined approach of detailed morphological analyses and molecular phylogenetic methods focused on nrITS sequences. As a result, the number of documented *Genea* species known in Türkiye has increased to nine.

#### Ethics

**Ethics Committee Approval:** Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

**Data Sharing Statement:** All data are available within the study.

#### Footnotes

**Authorship Contributions:** Conceptualization: E.K., G.D., and I.A.; Design/methodology: E.K., G.D., and I.A.; Execution/investigation: E.K., G.D., and I.A.; Resources/materials: E.K. and I.A.; Data acquisition: E.K., G.D., Em.K., and I.A.; Data analysis/interpretation: E.Ş., Em.K., and I.A.; Writing – original draft: E.Ş. and I.A.; Writing – review & editing/critical revision: I.A.

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