



## *Volvariella pilosipilea* (Volvariellaceae, Agaricales): A new record for Türkiye mycobiota

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### Abstract

**Purpose:** *Volvariella* is a cosmopolitan genus, with *V. pilosipilea* recently described from Spain following a revision of the European taxa. This study aimed to document the first record of *Volvariella pilosipilea* from Western Eurasia (Adıyaman Province, Türkiye), thereby extending its known distribution beyond the type locality.

**Method:** The identity of the collection was verified through an integrative approach combining morphological examination of macro- and microstructures with molecular phylogenetic analysis based on the nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) region. Maximum Likelihood analysis was employed to infer the phylogenetic relationships of *Volvariella*.

**Findings:** Molecular phylogenetic analysis confirmed the taxonomic identity of the Turkish specimen as *Volvariella pilosipilea*, demonstrating a strong phylogenetic affinity with the type sequence from Spain within a highly supported clade. The species forms a sister lineage to *V. nodosicystis* and *V. thailandensis*.

**Conclusion:** This record represents a significant range extension for *V. pilosipilea* from its type locality in Spain to Türkiye, suggesting a broader distribution across Mediterranean Europe and Western Eurasia.

**Keywords:** Basidiomycota, agaricoid, taxonomy, systematic, molecular phylogenetic analysis, nrDNA ITS

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## *Volvariella pilosipilea* (Volvariellaceae, Agaricales): Türkiye mikobiyotası için yeni bir kayıt

### Özet

**Amaç:** *Volvariella* kozmopolit bir cinstir ve *V. pilosipilea* Avrupa taksonlarının gözden geçirilmesinin ardından yakın zamanda İspanya'dan tanımlanmıştır. Bu çalışma, *Volvariella pilosipilea*'nın Batı Avrasya'dan (Adıyaman İli, Türkiye) ilk kaydını belgelendirmeyi ve böylece türün bilinen yayılışını tip lokalitesinin ötesine genişletmeyi amaçlamaktadır.

**Metod:** Koleksiyonun kimliği, makro ve mikro yapıların morfolojik incelemesi ile nükleer ribozomal DNA (rDNA) internal transcribed spacer (ITS) bölgesine dayalı moleküler filogenetik analizinin birleştirildiği bütüncül bir yaklaşımla doğrulanmıştır. *Volvariella*'nın filogenetik ilişkilerini çıkarmak için Maksimum Olabilirlik analizi kullanılmıştır.

**Bulgular:** Moleküler filogenetik analiz, Türkiye örneğinin taksonomik kimliğini *Volvariella pilosipilea* olarak doğrulamış olup, İspanya'daki tip dizi ile yüksek destekli bir klad içinde güçlü bir filogenetik yakınlık göstermiştir. Tür, *V. nodosicystis* ve *V. thailandensis* ile bir kardeş soy oluşturmuştur.

**Sonuç:** Bu kayıt, *V. pilosipilea*'nın bilinen dağılımını İspanya'daki tip lokalitesinden Türkiye'ye genişleterek, Akdeniz Avrupası ve Batı Avrasya'da daha geniş bir yayılışa sahip olabileceğine işaret etmektedir.

**Anahtar kelimeler:** Bazidiyomikota, agarikoid, taksonomi, sistematik, moleküler filogenetik analiz, nrDNA ITS

### 1. Introduction

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The genus *Volvariella* Spegazzini is widely distributed across tropical, subtropical, and temperate regions of both hemispheres [1-6]. Species of the genus are saprotrophic, terrestrial, wood-occupying habitats, or living on decaying agarics [3, 4, 6]. Globally, more than 67 species have been described, of which 30 are reported from Europe [1-11].

Historically, *Volvariella* was circumscribed within the classification of the family Pluteaceae Kotl. & Pouzar [1, 2]. *Volvariella* is morphologically delineated from the genera *Volvopluteus* Vizzini, Contu & Justo and *Pluteus* Fr. by a suite of diagnostic characters, including the presence or absence of a volva, basidiospore size, and the structure of the pileipellis [1-6].

Molecular phylogenetic studies have demonstrated the polyphyletic nature of *Volvariella*. A significant number of members of its taxa fall outside the Pluteineae (*Pluteoid* clade), instead exhibiting phylogenetic affinities with hygrophoroid fungi such as *Camarophyllus* (Fr.) P. Kumm. and *Cantharocybe* H.E. Bigelow & A.H. Sm. [3]. Consequently, contemporary phylogenetic treatments have reclassified *Volvariella* within the newly erected family Volvariellaceae Vizzini, Consiglio & P. Alvarado, which is situated within the suborder Pluteineae Aime, Dentinger & Gaya [11].

In recent years, numerous agaricoid fungi have been documented from Türkiye [12-18]. A total of thirteen *Volvariella* species have been recorded in Türkiye [4, 6, 19, 20]. Among these, four of which were recently described as new to science: *Volvariella izmirensis* Kaygusuz & Knudsen [6], *V. neoparvula* F. Caballero, Justo, G. Ferisin, Kaygusuz, Ševčíková, Dovana, Consiglio & L.A. Parra [6], *V. ranulicystis* Kaygusuz, J.L. Siquier, G. Ferisin, F. Caballero & Justo [6], and *V. turcica* Kaygusuz & Knudsen [4].

This study presents the first morphological and molecular confirmation of *Volvariella pilosipilea* Fern. Caball., Justo, Pérez-del-Amo & C. Roqué from Türkiye, representing the species' first recorded occurrence outside its type locality worldwide.

## 2. Materials and Methods

### 2.1. Morphological observation

*Volvariella* was collected in Adıyaman Province, Türkiye. Field documentation of the specimens included in situ macro-morphological characterization, georeferencing, and habitat description, accompanied by photographic documentation. Specimens were subsequently transported to the laboratory and subjected to lyophilisation utilizing an electric dryer maintained at approximately 30°C. For micro morphological examination, dried tissue samples were rehydrated in a 3% potassium hydroxide (KOH) solution and mounted in Congo Red for observation under optical microscopy. A minimum of thirty basidiospores were measured per specimen. The following morphometric parameters were employed:  $L^m$  (mean basidiospore length),  $W^m$  (mean basidiospore width),  $Q$  (length-to-width ratio for an individual basidiospore), and  $Q^m$  (mean quotient for all measured basidiospores).

Following comprehensive morphological and phylogenetic characterization, the specimens were deposited at the Department of Biology, Karamanoğlu Mehmetbey University.

### 2.2. Molecular procedures

For genomic analysis, basidiomata were lyophilized and subjected to DNA isolation using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, Irvine, California) according to the manufacturer's instructions. The quality and integrity of the isolated genomic DNA (gDNA) were verified via agarose gel electrophoresis. The internal transcribed spacer (ITS) region was subsequently amplified from the extracted gDNA by polymerase chain reaction (PCR) with the primer pair ITS1F and ITS4 [21, 22]. Amplification products were visualized on a 1% agarose gel to confirm successful PCR prior to sequencing. Raw sequence data were manually inspected and edited using BioEdit version 7.0.5 [23].

To determine phylogenetic affiliations, the newly generated sequence was compared with the GenBank nucleotide database using the MegaBLAST algorithm. Multiple sequence alignment was executed with MAFFT v.7 under the FFT-NS-I strategy [24], followed by manual inspection and curation in BioEdit. Phylogenetic reconstruction was performed under the Maximum Likelihood (ML) framework, following established methodologies [25]. The resulting phylogenetic tree was visualized using FigTree v.1.4.2 and subsequently edited for graphical enhancement in Adobe Illustrator CS6.

## 3. Results

### 3.1. Phylogenetic analysis

The nrDNA ITS dataset consists of 54 sequences representing *Volvariella* species, one of which was newly obtained in the present study. The Maximum Likelihood (ML) method was used to reconstruct the phylogenetic tree based on a final aligned dataset of 1,069 sites, which incorporated gap regions.

Molecular analysis confirms the identity of the Turkish specimen as *Volvariella pilosipilea* (PX279498), showing it clusters in a highly supported clade (MLB = 100% bootstrap) with the type sequence from Spain (PP660709). Phylogenetically, this species is sister to *Volvariella nodosicystis* Fern. Caball., Justo & A. Valverde (PP660710) and *V. thailandensis* Suwannar., Kumla & Lumyong (OM417504), with the Turkish and Spanish samples forming a distinct, monophyletic lineage (Fig. 1). This finding extends the known distribution range of *V. pilosipilea* from its type locality in Spain to Türkiye, indicating that the species may have a wider biogeographic range across Europe and Western Eurasia.

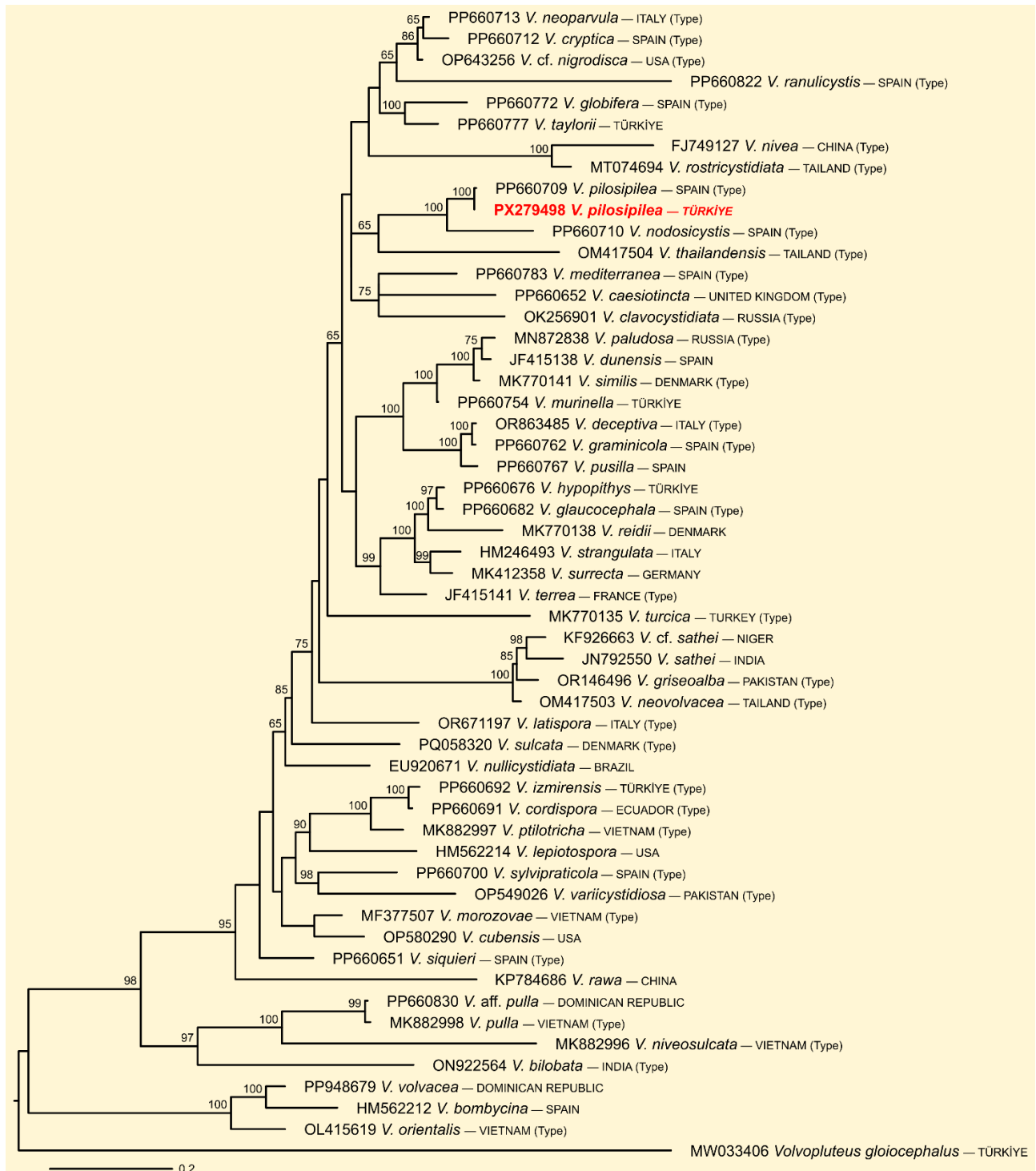


Figure 1. Phylogenetic relationships within *Volvariella* based on a Maximum Likelihood (ML) analysis of nrDNA ITS sequence data. The tree is rooted with *Volvopluteus gloiocephalus* (DC.) Vizzini, Contu & Justo. Branch support is indicated by MLB values (≥ 65%). A specimen of Türkiye origin is emphasized in bold red.

### 3.2. Taxonomy

*Volvariella pilosipilea* F. Caballero, Justo, Pérez-del-Amo & C. Roqué, in Caballero, Justo, Parra, Angelini, Consiglio, Dovana, Ferisin, Kaygusuz, Knudsen, Llimona, Muñoz, Daniëls, Pérez-de-Gregorio, Ševčíková, Valverde & Vizzini, Phytotaxa 680(1): 71 (2025) (Figs. 2, 3)



Figure 2. Fresh basidiomata of *Volvariella pilosipilea*. Scale bars = 5 mm

**Macroscopic description:** *Pileus* 5–10 mm diam., hemispherical, then hemispherical-convex, later convex to plano-convex, usually with broad, low umbo; surface (sub)lanose-fibrillose with radiating fibrils that aggregate into small scales, particularly towards the margin, not hygrophanous, not translucently striate, white to beige, mostly with cream

tinge towards the margin when old; margin undulate, slightly inflexed lamellae. *Lamellae* free, moderately crowded to distant, ventricose, white, then pinkish brown or salmon when old, with concolorous, entire edge. *Stipe* 7–12 × 0.5–1.5 mm, cylindrical, surface smooth, white to white-off. *Volva* small, up to 5 mm high, fragile, saccate, with 2-3 lobed, distinctly brown. *Context* fragile, white. *Smell* and *taste* indistinct.

**Microscopic description:** *Basidiospores* (5.5–)5.7–7.5(–8.0) × (4.4–)4.5–5.7(–6.0) μm,  $L^m \times W^m = 6.5 \times 5.1$  μm,  $Q = (1.1–)1.2(–1.5)$ ,  $Q^m = 1.2$ , mostly ellipsoid to globose, smooth, hyaline, thick-walled. *Basidia* 20–35 × 7.5–11 μm, tetrasporic, clavate, hyaline, thin-walled. *Lamellar* margin sterile. *Pleurocystidia* scattered, 35–75 × 10–25 μm, usually lageniform, hyaline, thin-walled. *Cheilocystidia* numerous to scattered, 40–85 × 10–30 μm, lageniform, utriform or fusiform, hyaline, thin-walled. *Pileipellis* a cutis composed of cylindrical hyphae, up to 35 μm in diam., smooth, hyaline, thin-walled. *Clamp connections* not observed.

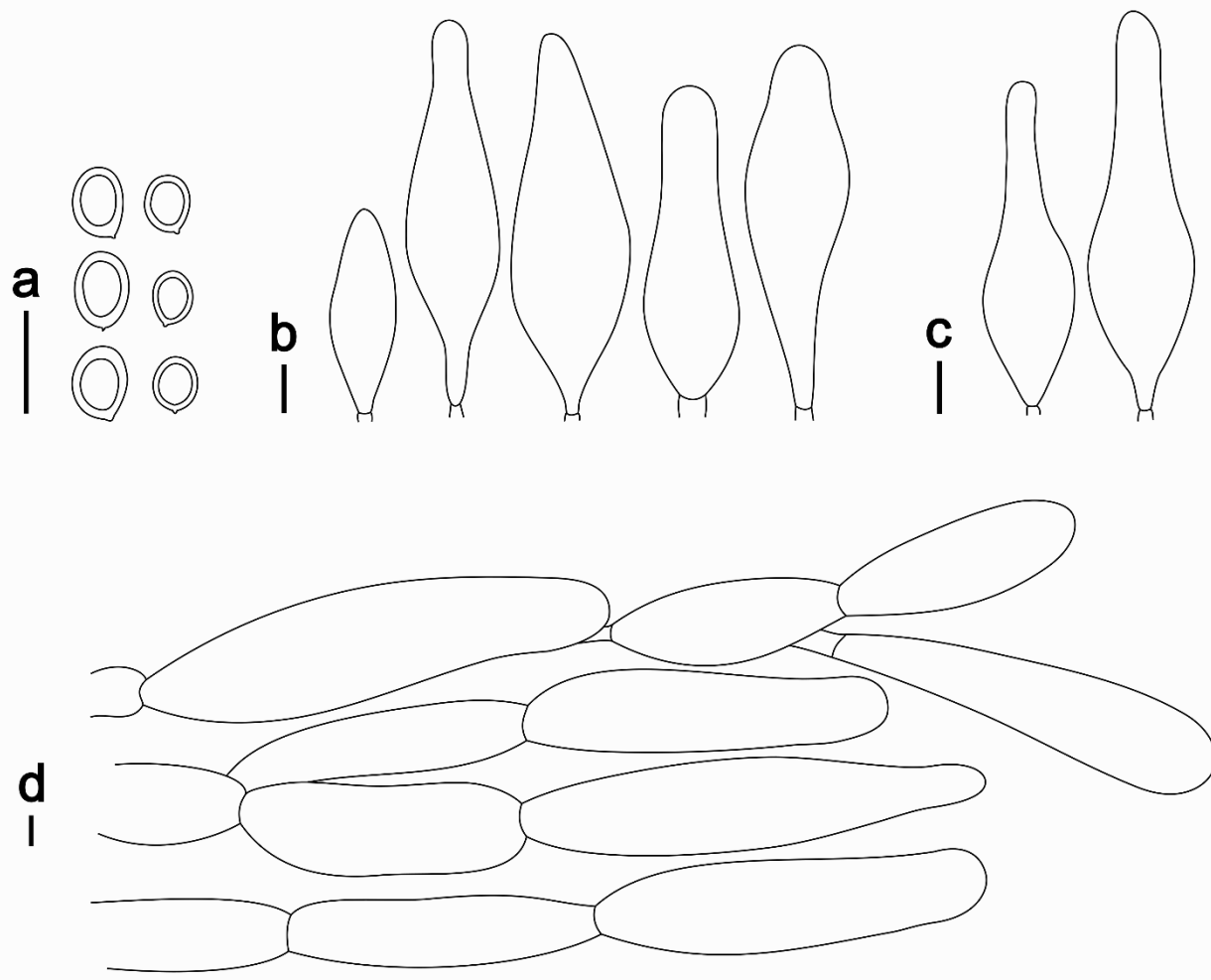


Figure 3. Microscopic features of *Volvariella pilosipilea*. (a) Basidiospores. (b) Cheilocystidia. (c) Pleurocystidia. (d) Pileipellis. Scale bars: 10 μm

**Ecology:** Occurring gregariously or in small groups, terrestrial, in mossy grassland.

**Specimen examined:** Türkiye, Adıyaman Province, Samsat district, in Dol village, on mossy, damp ground among grass, elev. 1290 m, 10.05.2009, leg. A. Kaya (K.6360), GenBank [nrDNA ITS]: PX279498.

#### 4. Conclusions and Discussions

*Volvariella pilosipilea* is morphologically similar to *V. hypopithys* (Fr.) Shaffer and *V. pusilla* (Pers.) Singer, both originally described from Europe. However, *Volvariella hypopithys* differs from *V. pilosipilea* by its larger basidiomata (up to 35 mm diam.), a white pileus with yellow to yellow-ochre tones at the center, and longer cheilocystidia (up to 124 μm) [6]. *Volvariella pusilla* can be distinguished by its larger basidiomata (up to 50 mm diam.) and broader pileipellis elements, reaching up to 50 μm in width [6].

Phylogenetically, *Volvariella pilosipilea* is closely related to *V. nodosicystis* and *V. thailandensis*. Morphologically, *Volvariella nodosicystis*, originally described from Spain, differs from *V. pilosipilea* by its pale grey pileus, pure white bilobed volva, slightly smaller basidiospores (5.4–7.1 × 4.0–5.2 μm), and predominantly utriform or clavate cheilocystidia [6]. *Volvariella thailandensis* is separated from *V. pilosipilea* by its larger basidiomata (up to 50

mm diam.), greyish-brown pileus, greyish-brown to dark-brown stipe, slightly smaller basidiospores ( $5.5\text{--}7.0 \times 4.0\text{--}6.0$   $\mu\text{m}$ ), and narrowly to broadly clavate cheilocystidia [25].

**Conflicts of interest:** No conflict of interest.

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**Ethical statement:** This study does not require ethical approval.

**Author contributions:** Abdullah Kaya collected the fungal specimen. Both authors contributed equally to the conception and design of the study, material preparation, data analysis, and manuscript drafting. All authors read and approved the final manuscript.

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