Kastamonu Uni., Orman Fakültesi Dergisi, 2025, 25(1): 1-8 Research Article
Kastamonu Univ., Journal of Forestry Faculty
Doi:10.17475/kastorman.1660474

Statesia pompholyx: A Newly Reported Species for Turkish *Hysterangiales*

Ilgaz AKATA[1*], Gülce EDİŞ[2], Eda KUMRU[2], Emre KESKİN[3], Ergin ŞAHİN[4]

[1]Ankara University, Faculty of Science, Department of Biology, Ankara, TÜRKİYE
[2]Ankara University, Graduate School of Natural and Applied Sciences, Ankara, TÜRKİYE
[3]Ankara University, Faculty of Agriculture, Department of Fisheries and Aquaculture, Ankara, TÜRKİYE
[4]Dokuz Eylül University, Faculty of Science, Department of Biology, Izmir, TÜRKİYE
*Corresponding Author: fungus@hotmail.com.tr

Received Date: 13.05.2024 Accepted Date: 09.09.2024

Abstract

Aim of study: This research seeks to deepen our understanding of Turkish mycobiota by incorporating a newly identified species from *Hysterangiales*.

Area of study: The area is approximately 4 kilometres from the centre of Demirköy in Kırklareli and is predominantly covered with beech trees.

Material and method: The fungal samples were rigorously examined through morphological evaluation and DNA sequencing of the nrITS rDNA region and *TEF1a* gene, incorporating microscopic and macroscopic features for phylogenetic analysis.

Main results: Following fieldwork and laboratory research, the species *Statesia pompholyx* belonging to the order *Hysterangiales* was identified and recorded for the first time in Türkiye.

Research highlights: This study presents a newly reported species of hypogeous fungi within the mycobiota of Türkiye and identifies *S. pompholyx* as the second officially documented species within the Turkish *Statesia* genus.

Keywords: Hypogeous Fungi, Mycobiota, New Record, Türkiye

Statesia pompholyx: Türkiye *Hysterangiales*'leri İçin Yeni Rapor Edilen Bir Tür

Öz

Çalışmanın amacı: Bu araştırmanın temel amacı, *Hysterangiales* takımına ait yeni tanımlanmış bir tür ekleyerek Türkiye mikobiyotası hakkındaki bilgileri zenginleştirmektir.

Çalışma alanı: Kırklareli'nin Demirköy ilçe merkezine yaklaşık 4 kilometre uzaklıktaki alan ağırlıklı olarak kayın ağaçlarıyla kaplıdır.

Materyal ve yöntem: Örnekler, hem morfolojik değerlendirme hem de nrITS rDNA bölgesi ve *TEF1a* geninin DNA dizilimi dahil olmak üzere ayrıntılı analize tabi tutulmuştur. Bu süreç, filogenetik analiz yapmak için hem mikroskobik hem de makroskobik özelliklerin incelenmesini içermektedir.

Temel sonuçlar: Arazi çalışmaları ve laboratuvar araştırmalarının ardından, *Hysterangiales* takımına ait Statesia pompholyx türü tespit edilmiş ve Türkiye'de ilk kez kaydedilmiştir.

Araştırma vurguları: Bu çalışma, Türkiye mikobiyotası içinde yeni rapor edilen bir hipogean mantar türünü sunmakta ve *S. pompholyx*'i Türkiye *Statesia* cinsi içinde resmi olarak belgelenmiş ikinci tür olarak tanımlamaktadır.

Anahtar Kelimeler: Hipogean Mantarlar, Mikobiyota, Yeni Kayıt, Türkiye

Introduction

The order *Hysterangiales* K. Hosaka & Castellano, classified within the *Basidiomycota* division, was formally established by Hosaka et al. (2006). Following comprehensive phylogenetic analyses, this taxonomic classification was introduced to group the family *Hysterangiaceae* E. Fisch and its phylogenetically related species (Davoodian et al., 2021). Predominantly, species within the order are obligate ectomycorrhizal, forming symbiotic relationships with a wide range of vascular plants, except for some species in the family *Phallogastraceae* Castellano, T. Lebel, Davoodian & K. Hosaka (Hosaka et al., 2006; Davoodian et al., 2021). These associations include interactions with plants from families

Citation (Atıf): Akata, I., Edis, G., Kumru, E., Keskin, E., & Sahin, E. (2025). *Statesia pompholyx*: A Newly Reported Species for Turkish *Hysterangiales*. *Kastamonu University Journal of Forestry Faculty*, 25 (1), 1-8.

such as *Pinaceae* Spreng. ex Rudolphi, *Myrtaceae* Juss., *Fagaceae* Dumort., and *Nothofagaceae* Kuprian. (Castellano, 1999; Castellano et al., 2000; Hosaka et al., 2008). The order is characterized by their underground existence and reliance on other organisms to distribute their spores (Hosaka et al., 2008). Its members exhibit distinctive morphological features, such as the powdery spore aggregations in the family *Mesophelliaceae* (G. Cunn.) Jülich, the gelatinous or robust gleba with a distinct columella in the families *Gallaceaceae* Locq. ex P.M. Kirk and *Hysterangiaceae* families (Davoodian et al., 2021). While many species in the *Mesophelliaceae* and *Hysterangiaceae* families have spores within utricles, those in the *Gallaceaceae* and *Phallogastraceae* families typically do not (Hosaka et al., 2006).

Davoodian et al. (2021) discovered 26 possible new genera and improved the categorization within the *Hysterangiales* order by forming two new suborders, reassigning two species, and confirming the classification of 11 established taxa. The research emphasized the intricate taxonomy of the *Hysterangium* Vittad., which has been widely classified based on distinct molecular and morphological traits and, as part of the ongoing work to refine the taxonomy of *Hysterangium* and its related genera, also proposed a new genera called *Statesia*, a Castellano, T. Lebel, Davoodian & K. Hosaka as a tribute to Jack States, a mycologist known for his passion for truffles. This new genus was defined by four distinct species, including two newly identified species, *S. cazaresii* Castellano, T. Lebel, Davoodian & K. Hosaka, and *S. zelleri* Castellano, T. Lebel, Davoodian & K. Hosaka. Additionally, two species previously classified under the genus *Hysterangium*, *H. calcareum* R. Hesse and *H. pompholyx* Tul. & C. Tul. were reclassified into the *Statesia* genus as *S. calcarea* (R. Hesse) Castellano, T. Lebel, Davoodian & K. Hosaka and *S. pompholyx* (Tul. & C. Tul.) Castellano, T. Lebel, Davoodian & K. Hosaka, respectively (Lebel et al., 2022).

The establishment of *Statesia* as a separate genus is supported by both phylogenetic and morphological analyses. Species of the genus are characterised by its underground, secluded basidiomata, which are usually roundish and feature surfaces that are either smooth or finely hairy, starting white and often shifting to shades of brown or red. Peridium may consist of one or two layers and displays a variety of textures, either woven-like, straight, or composed of angular cells. Its colour ranges from transparent to brownish. Gleba is divided into chambers that may be empty, partially, or filled, exhibiting hues from red to brown or olive. Rhizomorphs are commonly abundant, sprawling across the surface. The central columella resembles a branching tree. Typically, each basidium produces two spores, which range in shape from ellipsoid to spindle-like and have surfaces that are generally smooth or verrucose, lacking spines, and enclosed within a uniformly inflated utricle that may be distinctly visible or somewhat vague (Lebel et al., 2022).

The checklist on Turkish truffles conducted by Akata et al. (2022) documented nine species under the order *Hysterangiales* in the country. Yet, to date, there has been no scientific evidence to verify the presence of *Statesia pompholyx* (Tul. & C. Tul.) Castellano, T. Lebel, Davoodian & K. Hosaka within the borders of Türkiye.

This study seeks to contribute to the knowledge of Türkiye's fungal biodiversity by documenting a novel record from the genus *Statesia* under the order *Hysterangiales*.

Material and Methods

This study adopted an integrative research strategy that combined classical taxonomic methods with molecular techniques to investigate and classify fungal specimens from the Demirköy region in Kırklareli, Türkiye. The analysis included carefully evaluating morphological characteristics, sequence alignment, and phylogenetic assessment, particularly on the nrITS rDNA and *TEF1a* gene regions.

Morphological Studies

On October 8, 2022, *Statesia* specimens were collected using truffle-hunting dogs, and their macroscopic and ecological features were documented in situ. Detailed microscopic examinations were conducted, followed by statistical analysis. For SEM imaging, gleba sections were mounted on stubs with adhesive tape and gold-coated for

conductivity. The identified specimens were preserved in the Fungarium of Ankara University's Faculty of Science.

Determination of the ITS rDNA and TEF1a Sequences

Genomic DNA was extracted from the sample ANK AKATA TT 159 using the CTAB method described by Rogers & Bendich (1994), with purity and concentration assessed via a Thermo Fisher Scientific Nanodrop Lite. The DNA was used as a template for PCR amplification of the ITS rDNA region and the *TEF1a* gene, employing ITS1/ITS4 primers (Martin & Rygiewicz, 2005) and EF1-983F/EF1-1567R primers (Rehner & Buckley 2005), respectively. Amplified fragments were verified on agarose gel, purified with the GeneAll Expin Gel and CleanUp Kit, and sequenced using the BigDye™ Direct Cycle Sequencing Kit. Sequencing was performed on an ABI Prism 3130 Genetic Analyzer following Chen et al. (2014).

Molecular Phylogeny

Molecular phylogenetic analysis of sample ANK AKATA TT 159 was conducted using Sanger sequencing data from ITS1/ITS4 and EF1-983F/EF1-1567R primers, compiled with DNAMAN Version 10. Species for comparison were identified via BLASTn against the NCBI GenBank database, and sequences were aligned using ClustalW in MEGAX (Kumar et al., 2018). Phylogenetic trees were constructed employing the Maximum Likelihood method with K2+G and K2+G+I substitution models (Kimura, 1980) and validated through 1000 bootstrap replicates (Felsenstein, 1985).

Results

The description outlines the collection date, precise location, habitat characteristics, geographical coordinates, and specimen identifiers. It also incorporates an analysis of the specimens' macro- and micromorphological features, supported by Scanning Electron Microscope (SEM) images of the spores. The taxonomy of the newly identified species was established following the guidelines set out by Lebel et al. (2022).

Systematic overview

Basidiomycota Whittaker ex R.T. Moore

Agaricomycetes Doweld

Phallomycetidae K. Hosaka, Castellano & Spatafora

Hysterangiales K. Hosaka & Castellano

Hysterangiaceae E. Fisch

Statesia Castellano T.Lebel, Davoodian & K.Hosaka

Statesia pompholyx (Tul. & C.Tul.) Castellano, T.Lebel, Davoodian & K.Hosaka (2022) (Figure 1).

The comprehensive description was compiled by Lebel et al. (2022).

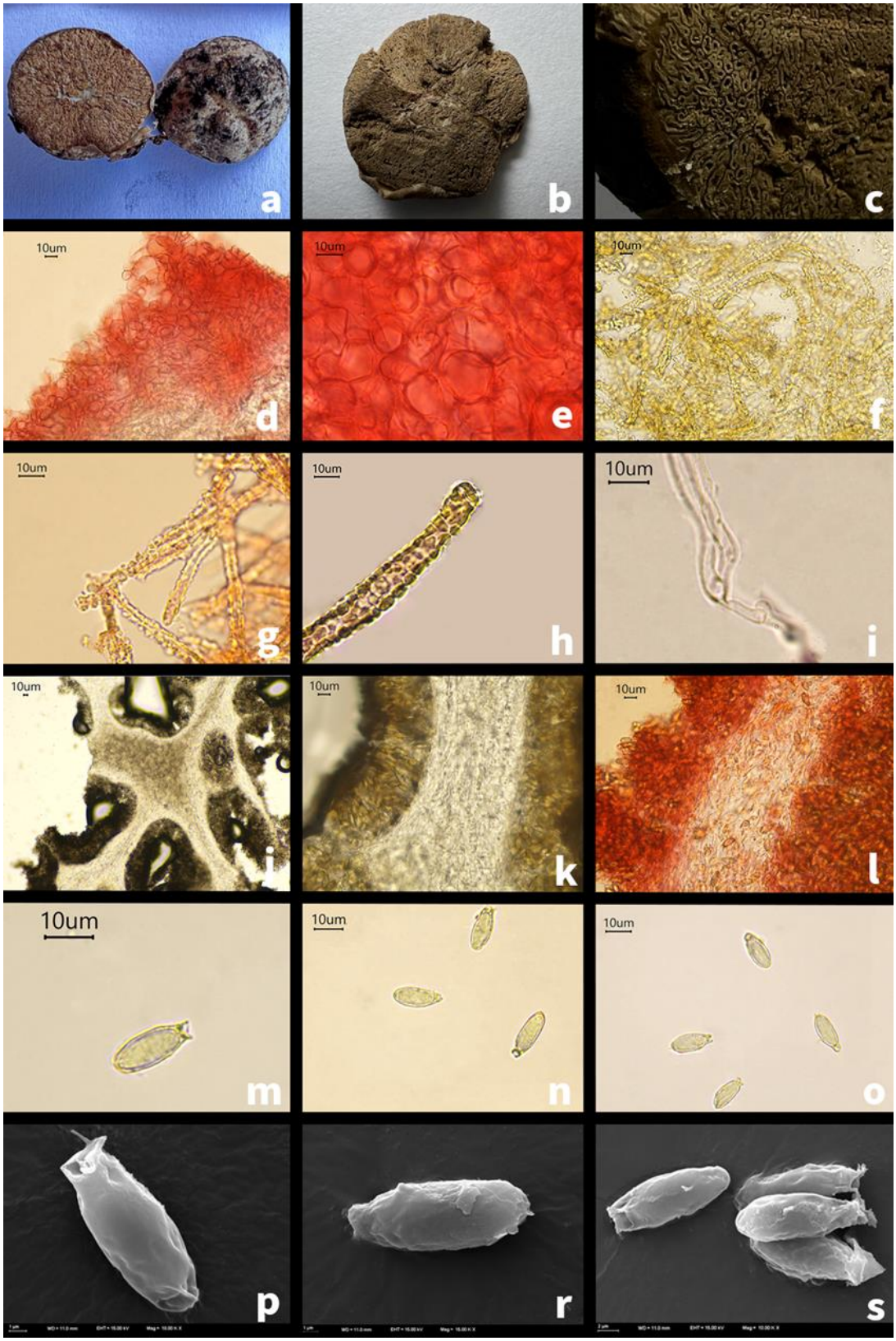

Figure 1. *Statesia pompholyx*: a. Basidomata, b, c. gleba, d. peridium (in Congo red), e. polyhedral cells of peridium (in Congo red), f. hyphae of peridium (in 5% KOH), g, h. hyphae of peridium (in Congo red), i. hyphae of peridium with clamp connection (in 5% KOH), j. cross-section of the gleba (in 5% KOH), k. hymenophoral trama (in 5% KOH), l. hymenophoral trama (in Congo red), m-o. spores (LM, in 5% KOH), p-s. spores (SEM)

Macroscopic and Microscopic Features

Basidomata hypogeous, 15–20 mm in diam., ranging from globose to slightly irregular, initially, white or light yellowish-brown, but upon maturation, reddish-brown or deep brown. **Gleba** initially white but darkens to shades of light brown, pale reddish brown, brown, olive, or greenish brown upon drying, with locules elongated and not filled. **Columella** thin, gelatinous, and dendroid in structure. **Rhizomorphs** abundant and firmly attached to the entire surface of the basidiomata, displaying the same color as peridium. **Peridium** up to 230 µm thick, solitary layer, dark reddish-brown hue, and composed of polyhedral cells, 18–63 µm, hyphae 4–13 µm broad, numerous crystal particles dispersed throughout, with some hyphae tips along the periphery featuring oxalate crystals, clamp connections are frequently observed. **Trama** 50–90 µm thick, consists of hyaline hyphae tightly interwoven up to 4 µm broad, embedded in a gelatinous matrix, clamp connections not observed. **Basidia** 32–36 × 7–8 µm, hyaline, roughly cylindrical and two-spored. **Spores** (16–) 16.2–18.9 (–19) × (6–) 6.5–-7.1 (–7.3) µm, Q = 2.3–2.7 (–2.8), Qav = 2.5 µm, narrowly elliptical to broadly fusiform, thick-walled, smooth, and appearing yellowish brown in distilled water and pale brown in KOH. The utricle noticeable and exhibiting a rough or wrinkled texture, with a thickness of 1 µm, frequently, spores found to adhere together in pairs, extending from the middle of the spore down to its base.

Ecology: March to November, hypogeous, likely forming mycorrhizal associations with broadleaved trees such as beech, common hazel, hawthorn, hornbeam, and oak (Lebel et al., 2022).

Material examined: Turkey-Kırklareli, Demirköy, in beech forest, 41°48′ N, 27°48′ E, 212 m, 08.10.2022, AKATA TT 159.

Evolutionary History of ANK AKATA TT 159

Specimen ANK AKATA TT 159 was analysed for its evolutionary relationships using nrITS rDNA and *TEF1a* gene sequences submitted to GenBank under accession numbers OR223352 and PP622673, respectively. Comparative sequences from other *Hysterangium* species and outgroup representatives, including *Peziza montirivicola* (ITS rDNA) and *Amanita vidua* (*TEF1a*), were incorporated into the analysis. Phylogenetic trees constructed from these markers grouped ANK AKATA TT 159 with *Statesia pompholyx* isolates (voucher 17032 and strain Gross495), showing high bootstrap support and confirming its close relationship to *S. pompholyx*. The analyses achieved log-likelihood values of -5536.25 (ITS rDNA) and -3655.54 (*TEF1a*), with BLAST results indicating over 99% similarity to the referenced *S. pompholyx* isolates.

Discussion

S. pompholyx demonstrates morphological and ecological characteristics that align closely with other species within the genus *Statesia*. However, distinct differences are evident when comparing its structural features to those of its congeners, specifically in the peridium and spore morphology. In the genus *Statesia*, variations in peridium structure are notable. Within the genus, species like *S. calcarea* and *S. cazaresii* are distinguished by their notably two-layered peridium, a structural feature that uniquely defines them. In contrast, *S. pompholyx* and *S. zelleri* are identified by possessing a peridium with a single, uniform layer. The peridium of *S. pompholyx* is particularly unique; it is composed of polyhedral cells, which might suggest specialized functions or adaptations. This cellular arrangement contrasts markedly with the peridium of *S. zelleri*, which is not polyhedral but consists of densely packed hyphae, each measuring 5-8 µm in width. Moreover, the spores of *S. pompholyx* are notably more extended and slender than those of *S. zelleri* (Lebel et al., 2022).

The genetic diversity of fungal species far surpasses their morphological diversity. Consequently, genetic data is frequently combined with traditional methods that predominantly rely on morphological characteristics for more accurate identification of fungal species. For this purpose, various valuable genetic markers have been employed in molecular systematics over the years. These include rRNA gene regions such as nrITS, nrSSU, and nrLSU, as well as protein-coding genes like translation

elongation factor 1a (*TEF1a*) and tubulin (TUB2), which have proven instrumental in these studies (Raja et al., 2017). The ITS rDNA region and *TEF1a* gene are extensively employed in molecular taxonomy studies throughout the fungal kingdom, acting as essential tools for generating valuable insights into fungal classification and relationships (White et al., 1990; Akata et al., 2023; 2024a; 2024b). Moreover, advancements in high-throughput sequencing technologies and bioinformatics resources facilitate comprehensive genome comparisons and phylogenomic analyses across fungal taxa, potentially replacing molecular phylogenetic analyses reliant on a limited number of marker genes shortly (Marian et al., 2024). Our investigation utilised nuclear ITS rDNA and *TEF1a* gene sequences to characterise ANK AKATA TT 159. The molecular analysis, based on nrITS rDNA and *TEF1a* gene sequences, revealed a similarity of over 99% between reference sequences of *S. pompholyx* and the specimen (GenBank ID: OR223352 and PP622673) (Figure 2 and 3).

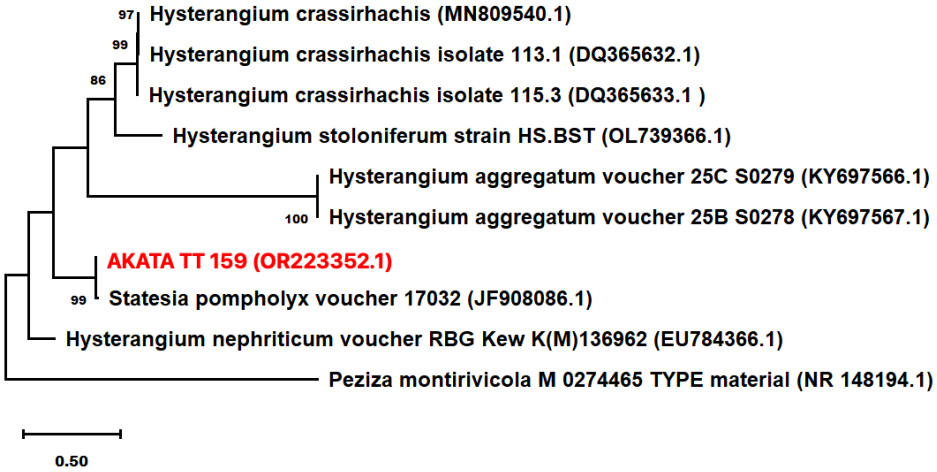

Figure 2. A phylogenetic tree, depicting the evolutionary relationships among 10 fungal specimens, is generated using the nrITS rDNA region and the maximum likelihood (ML) method. Bootstrap values are assigned to each branch to indicate confidence levels. All sequences utilized in tree construction were retrieved from the NCBI GenBank, except for ANK AKATA TT 159. Moreover, *Peziza montirivicola* was integrated into the phylogenetic tree as the outgroup representative. GenBank accession numbers are furnished for each sequence, and the scale bar located in the lower left corner represents a genetic distance of 0.50

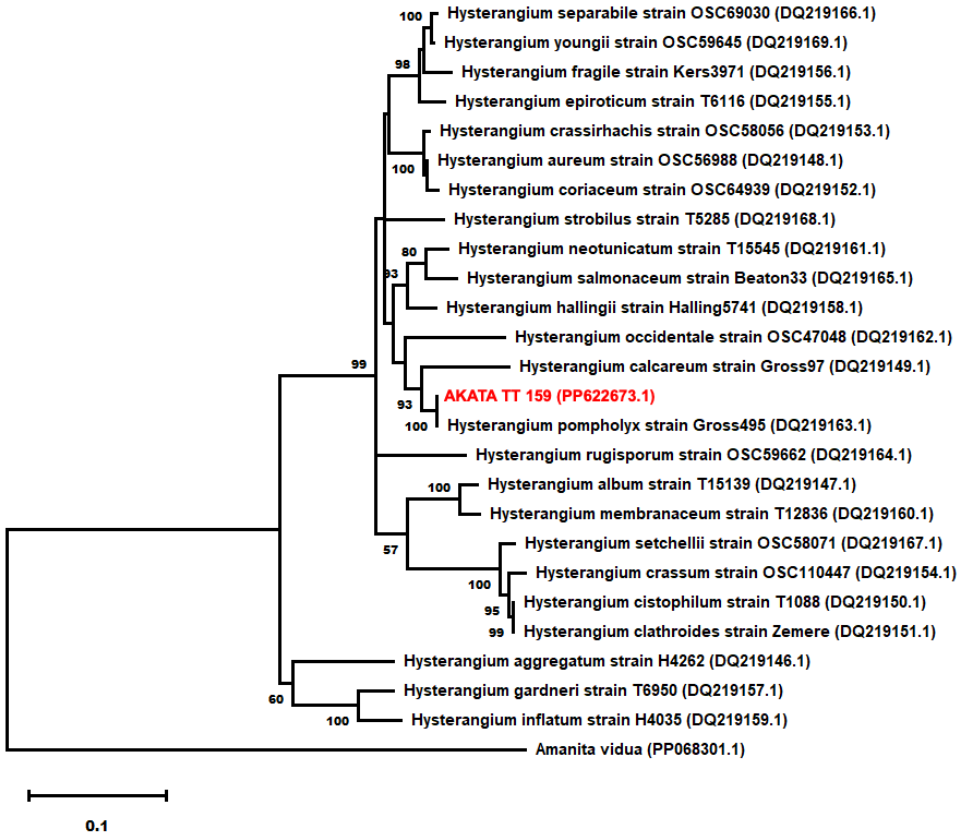

Figure 3. A phylogenetic tree, portraying the evolutionary relationships among 26 fungal specimens, is depicted using the partial sequence of the *TEF1a* gene and the maximum likelihood (ML) method. Bootstrap values are assigned to each branch to indicate confidence levels. All sequences employed in constructing the tree were sourced from the NCBI GenBank, except for ANK AKATA TT 159. Furthermore, *Amanita vidua* was integrated into the phylogenetic tree as the outgroup representative. GenBank accession numbers are furnished for each sequence, and the scale bar located in the lower left corner represents a genetic distance of 0.1

Conclusion

The current study has reported *S. pompholyx* within Türkiye, marking a significant enhancement to the diversity of the *Statesia* genus within this region. Before this revelation, the occurrence of a species known as *Hysterangium calcareum* in Türkiye was highlighted in a report by Elliott et al. (2016). A pivotal taxonomic overhaul undertaken by Lebel et al. (2022) led to the reclassification of *H. calcareum* into the genus *Statesia*, which resulted in its renaming to *S. calcarea* (R. Hesse) Castellano, T. Lebel, Davoodian & K. Hosaka. Consequently, *S. pompholyx* is now the second officially recorded member of Turkish *Statesia*.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: I.A., G.E., Ed.K.; Investigation: G.E, Ed.K., I.A..; Material and Methodology: I.A., G.E., Ed.K..; Supervision: I.A., E.Ş., Em.K.,; Visualization: G.E., Ed. K..; Writing-Original Draft: I.A.; Writing-review & Editing: I.A., Em.K., E.Ş. All authors have seen and accepted the publication of the version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

Turkish Scientific and Technological Research Council (TÜBİTAK) supported the study with project number 121Z924.

References

Akata, I., Şen, İ., Sevindik, M. & Kabaktepe, Ş. (2022). Truffle Checklist of Turkey II with A New Record. *Turkish Journal of Agriculture-Food Science and Technology*, 10(10), 1913-1920.

Akata, I., Kumru, E., Ediş, G., Özbey, B. G. & Sahin, E. (2023). Three New Records For Turkish Agaricales Inhabiting Ankara University Beşevler 10th Year Campus Area. *Kastamonu University Journal of Forestry Faculty*, 23(3), 250-263.

Akata, I., Kumru, E., Şahin, E., Acar, İ. & Kaya, E. (2024a). *Amanita vidua*: A new record for Turkish *Amanita* Section *Phalloideae* based on morphological and molecular data. *Trakya University Journal of Natural Science*, 25(1), 97-110.

Akata, I., Kumru, E., Ediş, G., Acar, İ. & Sahin, E. (2024b). Two Newly Reported Agaricales Species from Türkiye with Morphological and Molecular Data. *Kastamonu University Journal of Forestry Faculty*, 24(3), 260-280.

Castellano, M. A. (1999). Hysterangium. In: Cairney JWG, Chambers SM, Cairney SW (eds), *Ectomycorrhizal Fungi: key genera in profile.* Springer-Verlag, New York, 311-323.

Castellano, M. A., Verbeken, A., Walleyn, R. & Thoen, D. (2000). Some new or interesting sequestrate Basidiomycota from African woodlands. *Karstenia*, 40, 11-21.

Chen, L., Cai, Y., Zhou, G., Shi, X., Su, J. & et al. (2014). Rapid Sanger sequencing of the 16S rRNA gene for identification of some common pathogens. *PloS one*, 9(2), e88886.

Davoodian, N., Lebel, T., Castellano, M. A. & Hosaka, K. (2021). Hysterangiales revisited: expanded phylogeny reveals new genera and two new suborders. *Fungal Systematics and Evolution*, 8, 65-80.

Elliott, T. F., Türkoğlu, A., Trappe, J. M. & Güngör, M.Y. (2016). Turkish truffles 2: eight new records from Anatolia. *Mycotaxon*, 131, 439-453.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*. 39(4), 783-791.

Hosaka, K., Bates, S. T., Beever, R. E., Castellano, M. A., Colgan, III. & et al. (2006). Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass Phallomycetidae and two new orders. *Mycologia* 98, 949-959.

Hosaka, K., Castellano, M. A. & Spatafora, J. W. (2008). Biogeography of Hysterangiales (Phallomycetidae, Basidiomycota). *Mycological Research* 112, 448-462.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular evolution*, 16, 111-120.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution*, 35(6), 1547-1549.

Lebel, T., Davoodian, N., Bloomfield, M., Syme, K., May, T. & et al. (2022). A mixed bag of sequestrate fungi from five different families: Boletaceae, Russulaceae, Psathyrellaceae, Strophariaceae, and Hysterangiaceae. *Swainsona*, 36, 33-65.

Marian, I. M., Valdes, I. D., Hayes, R. D., LaButti, K., Duffy, K. & et al. (2024). High phenotypic and genotypic plasticity among strains of the mushroom-forming fungus Schizophyllum commune. *bioRxiv*, 2024-02.

Martin, K. J. & Rygiewicz, P. T. (2005). Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. *BMC microbiology*, 5, 1-11.

Raja, H. A., Miller, A. N., Pearce, C. J. & Oberlies, N. H. (2017). Fungal identification using molecular tools: a primer for the natural products research community. *Journal of Natural Products*, 80(3), 756-770.

Rehner, S. A. & Buckley, E. (2005). A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia*, 97, 84-98.

Rogers, S. O. & Bendich, A. J. (1994). Extraction of total cellular DNA from plants, algae and fungi. *Plant molecular biology manual*, 183-190.

White, T. J., Bruns, TD, Lee, S. & Taylor, J.W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, 315-322. In: Innis, M.A. & Gelfand, D.H. (eds). *PCR Protocols: A Guide To Methods And Applications.* Academic Press, London, 482.