Geliş(Recevied) :26.09.2020
Kabul(Accepted) :12.11.2020

Research Article
Doi: 10.30708.mantar.800585

A Note on *Battarrea phalloides* in Turkey

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Abstract: The current study was conducted based on a *Battarrea* sample obtained from Muğla province (Turkey). The sample was identified based on both conventional methods and ITS rDNA region-based molecular phylogeny. By taking into account the high sequence similarity between the sample (ANK Akata & Altuntaş 690) and *Battarrea phalloides* the relevant specimen was considered to be *B. Phalloides* and the morphological data also strengthen this finding. In this study, photos of macro and microscopic structures, a short description, scanning electron microscope (SEM) images of spores and elaters, and the ITS rDNA region-based molecular phylogeny of the samples were given. Also, the distribution of *B. phalloides* specimens identified thus far from Turkey was revealed for the first time in this study.

Key words: Fungal diversity, gasteroid fungi, Turkey

Türkiye'deki *Battarrea phalloides* Üzerine Bir Not

Öz: Bu çalışma, Muğla yöresinden (Türkiye) elde edilen *Battarrea* örneğine dayanılarak yapılmıştır. Örnek, hem konvansiyonel yöntemlere hem de ITS rDNA bölgesi bazlı moleküler filogeniye dayalı olarak tanımlanmıştır. Örnek (ANK Akata & Altuntaş 690) ile *Battarrea phalloides* arasındaki yüksek sekans benzerliği dikkate alınarak ilgili örnek *B. phalloides* olarak kabul edilmiş ve morfolojik veriler de bu bulguyu güçlendirmiştir. Bu çalışmada, makro ve mikroskobik yapıların fotoğrafları, kısa bir betimleme, sporların ve elaterlerin taramalı elektron mikroskobu (SEM) görüntüleri ve numunelerin ITS rDNA bölgesi bazlı moleküler filogenisi verilmiştir. Ayrıca Türkiye'den bugüne kadar tespit edilen *B. phalloides* örneklerinin dağılımı ilk kez bu çalışmada ortaya konmuştur.

Anahtar kelimeler: Mantar çeşitliliği, gasteroid mantarlar, Türkiye

Introduction

The gasteroid genus of fungi, *Battarrea* Pers. is used to be placed in the families *Battarreaceae* Corda or *Tulostomataceae* E. Fisch. (*Tulostomatales* Demoulin.). According to molecular phylogenetics, the genus is placed within the order *Agaricales* Underw. (Ivančević et al, 2016).

Battarrea phalloides (Dicks.) Pers., the type species of the genus, was firstly described in 1785 by Dickson as *Lycoperdon phalloides* Dicks , however, it was later transferred to the genus *Battarrea* by Persoon in 1801. More than sixteen species involved in the genus since 1801 but most of them are currently considered as synonyms of *B. phalloides* (Shepherd and Cooper, 2017).

B. phalloides, also known as tall stiltball, scaley-stalked puffball, mallee drumstick, desert stalked puffball or sandy stiltball, is reported from all continents except Antarctica and red-listed in nine Europen countries. The species is mainly characterized with convex to hemispherical spore sac; fibrous to scaly stipe with volva; globose, subglobose to broadly ellipsoid spores with warty ornamentation and spirally thickened elaters (Calonge, 1998; Pegler et al., 1995).

Considering the literature on Turkish mycobiota, *Battarrea phalloides* have thus far been reported from four locations in Turkey (Sesli and Denchev, 2008; Adanacıoğlu et al, 2016). In the current study, a new location was added to the distribution of Turkish *B. phalloides* along with the details of its macro and micromorphology, ITS rDNA region-based molecular phylogeny and SEM images of spores and capillitium. The aim of this study is to reveal a new locality and distribution of *B. phalloides* in Turkey.

Material and metod

Morphological study: The materials used in this study originates from both a research trip and fungarium samples kept in Biology Department of Muğla Sıtkı Koçman University. In the field, necessary macroscopic features of the specimens were noted; in the laboratory, microscopic structures were scrutinized using both simple light microscope and scanning electron microscope (SEM). Averagely 30 measurements were done using Euromex Oxion Trinocular microscope, 100X magnification rates were utilized for each structure and the compiled data were statisitically analyzed. For SEM studies, pieces of spore mass reside inside the gleba were fixed on stubs using double-sided sticky tape, coated with gold particles, and examined using an EVO 40XVP (LEO Ltd., Cambridge, UK) scanning electron microscope with an accelerating voltage of 20 kV. Identification of the samples was performed in accordance with the relevant literature (Pegler et al., 1995; Hansen and Knudsen, 1997; Calonge, 1998). The exsiccatae were deposited in the Ankara University Herbarium (ANK).

Determination of the ITS rDNA sequences: The genomic DNA was isolated from ANK Akata & Altuntaş 690 using the CTAB method as described before (Rogers and Bendich, 1994). After the spectrophotometric verification of the quality and quantity of the extracted genomic DNA, it was used as a template in polymerase chain reaction for the amplification of the Internal Transcribed Spacer (ITS) rDNA regions. The ITS rDNA regions were amplified by PCR using the universal ITS1 forward and ITS4 reverse oligonucleotides as described before (Stielow et al., 2015). After confirming the presence of amplification product as single, distinct band on agarose gel, the amplicon was cleaned-up with PCR purification kit (QIAquick PCR Purification kit, QIAGEN) and its sequence was determined by Sanger sequencing method. The sequencing PCR was executed with the same ITS1 and ITS4 primers using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™) and the fragment analyses were conducted using ABI Prism 3130 Genetic Analyzer. Both the agarose gel electrophoresis and the Sanger sequencing were conducted as described elsewhere (Chen et al., 2014).

Molecular phylogeny study: For the phylogenetic analysis, the raw sequence data were assembled using Sequencher version 5.4.6 sequence assembly software (Gene Codes Corporation) and later BLASTn search was performed with the assembled sequence for determining the best hits. Based on this BLAST search, the in-group and the out-group members were selected for the phylogenetic tree construction. The assembled sequence was aligned with the nucleotide sequences of the predetermined in-group and out-group members obtained from the NCBI GenBank database using the ClustalW algorithm of MEGAX software (Kumar et al., 2018). The phylogenetic tree that reveals the evolutionary relationship of ANK Akata & Altuntaş 690 was constructed using the Maximum Likelihood method and GTR nucleotide substitution model with invariant + gamma distribution (Nei and Kumar, 2000). The phylogenies of the specimens were predicted using the bootstrap method with applying 1000 bootstrap replicates (Felsenstein, 1985).

Results

Agaricaceae Chevall.

Battarrea phalloides (Dicks.) Pers. (1801), (Figure1-3).

Syn.: *Lycoperdon phalloides* Dicks. (1785), *Dendromyces stevenii* Libosch. (1814), *Phallus campanulatus* Berk. (1842), *Ithyphallus campanulatus* (Berk.) Sacc. (1888), *Sphaericeps lignipes* Welw. & Curr. (1868), *Sphaerocybis lignipes* (Welw. & Curr.) Clem. (1909), *Battarrea stevenii* (Libosch.) Fr. (1829), *B. gaudichaudii* Mont. (1834), *B. guicciardiniana* Ces. (1875), *B. muelleri* Kalchbr. (1880), *B. tepperiana* F. Ludw. (1889), *B. guachiparum* Speg. (1898), *B. patagonica* Speg. (1898), *B. laciniata* Underw. ex V.S. White (1901), *B. levispora* Massee (1901), *B. franciscana* Copel. (1904), *B. phalloides* var. *stevenii* (Libosch.) Cleland & Cheel (1916), *B. katzlerae* Ulbr. (1936), *B. phalloides* f. *stevenii* (Libosch.) Calonge (2004).

Macroscopic and microscopic features: Basidioma initially developing underground is ovoid,

covered by a two-layered peridium. **Mature basidioma** consisting of a long stipe with an apical spore sac. **Spore sac** 30-90 mm across, convex to hemispherical covered by a smooth, whitish, grayish endoperidium exposing a sticky, rust-brown spore mass at times. **Gleba** cinnamon to rust-brown, powdery at maturity. **Stipe** 120-350 × 5-15 mm, cylindrical and hollow. **Stipe surface** longitudinally striate, fibrous to scaly in circles, grayish to pale brown, often covered by rust-brown spore mass. **Volva** at the base of stipe, sac shaped, whitish covered by rust-brown spore mass, often disappears. **Basidia** not seen. **Basidiospores** 4-6 µm diam, globose to broadly elliptical, yellowish to yellow-brown and warted with short and smooth pedicel, densely verruculose, sometimes coalescing to form anastomosing ridges. **Elaters** 4-7 µm broad, thin-walled, cylindrical to narrowly clavate, consist of spiral threads, pale yellow to honey-colored. **Pseudocapillitium** 4-5 µm broad, mostly thin-walled, smooth, hyaline to pale yellow or honey-colored and septate.

Ecology: Widely distributed but rare, summer to late autumn, solitary to scattered, in warm temperate, Mediterranean to tropical climate, frequently distributed in several kind of xerophytic vegetation, arid and semiarid regions and dry savannas steppes, coastal dunes, and woodlands; on dry, usually sandy, more rarely chalky or calcareous soils (Calonge, 1998; Howladar et al., 2013; Ivančević et al., 2016; Shepherd and Cooper, 2017; Abdel-Azeem and Nafady, 2019).

Distribution: Africa (Tunisia, Algeria, Libya, Egypt, Morocco, Equatorial Guinea, São Tomé and Príncipe, Cape Verde, Congo, Somalia, Namibia, Mauretania, Ethiopia, Angola, Kenya, Burundi Mozambique and South Africa), Asia (Azerbaidjan, Georgia, Armenia, Israel, Iraq, Pakistan, Iran, Saudi Arabia, China, India, Yemen and Mongolia), Europe (Greece, Bulgaria, Romania, Ukraine, Macedonia, Hungary, Serbia, Crotia, Cyprus, Slovakia, Austria, Poland, Spain, Czech Republic, Germany, Belgium, England, France, Russia, Italy, Malta, Switzerland and Turkey), North America (USA, Canada, Puerto Rico, Jamaica and Mexico), South America (Peru, Argentina, Chile, Ecuador, Brazil and Uruguay), Australia (Commonwealth of Australia and New Zealand) (Pegler et al. 1995, Watling et al. 1995, Calonge 1998, Nieves-Rivera 1998, Jacobson et al. 1999, Denchev and Assyov, 2010, Kreisel 2001, Esqueda et al. 2002, Gates and Ratkowsky, 2004, Yılmaz Ersel and Solak 2004, Sobestiansky, 2005; Hong and Li, 2006; Allı et al. 2007, Madrid 2007, Lacheva, 2012, Seyidova and Hüseyin, 2012, Howladar et al. 2013, Martín et al. 2013, Yousaf et al. 2013, Ivančević et al. 2016, Karadelev and Rusevska 2016, Shepherd and Cooper, 2017, Abdel-Azeem and Nafady 2019).

Material examined: TURKEY—Muğla: Bodrum, Turgutreis, in meadow, sea level, 37° 01' N, 27°15' E, 12.12.2019, ANK Akata & Altuntaş 690.

Molecular phylogenetic characterisation: The ITS rDNA sequence of ANK Akata & Altuntaş 690 determined by conventional PCR and subsequent sequencing was submitted to NCBI GenBank with the accession number MT823465. By considering the BLASTn results of the ITS sequence of ANK Akata & Altuntaş 690, the ITS sequences of the genera *Mycenastrum*, *Tulostoma*, *Bovista*, and *Lycoperdon*, some of the well-supported genera of the gasteroid fungi, were choosen as ingroup members and the ITS sequence of *Pluteus squarrosus* Iqbal Hosen & T.H. Li was selected as the outgroup member for revealing the evolutionary relationship of ANK Akata & Altuntaş 690. As a result of the phylogenetic analysis, five distinct clades appeared along with an outgroup (Figure 4). While the clade 5 contained *Battarrea* species and the specimen Ank Akata & Altuntaş 690, the Clades 1, 2, 3, and 4 included species from the genera *Bovista*, *Lycoperdon*, *Mycenastrum*, and *Tulostoma* respectively. On the other hand, *Pluteus squarrosus* was divaricated separately from the rest of the fungal taxa and constituted an outgroup as predicted. The BLASTn analysis conducted with the ITS sequence of Ank Akata & Altuntaş 690 revealed evidence for more than 99.80 % similarities with *B. phalloides* species. The phylogenetic analysis conducted with the ITS sequence of the specimen, further verified the significant evolutionary relationship of the specimen with *B. phalloides* with a bootstrap value of 100%.

Discussions

B. phalloides is a terricolous and distinctive saprobic species which can be easily recognized by its unique appearance such as umbrella-shaped basidiome, up to 400 mm long fibrous to scaly stipe covered by brown spore mass at maturity. The species appears at summer to late autumn, especially growing on dry, sandy soils of arid and semiarid regions from sea level up to over 2.500 m high and distributed in sixty-five countries within the five continents. Despite its wide distribution, *B. phalloides* is a rare species included in the Red List of Armenia, Bulgaria, Czech Republic, England, Macedonia, Poland, Romania, Russia and Slovakia (Denchev and Assyov, 2010; Rimóczi et al. 2011; Fraiture and Otto, 2013; Karadelev and Rusevska, 2016; Ivančević et al., 2016; Smith et al., 2016; Shepherd and Cooper, 2017).

Regarding the identification of fungal taxa which exhibit enormous genetic diversity, the morphological data may not always be conclusive for the accurate identification of fungal species. Therefore, the sequence data from the preserved genomic DNA regions such as ITS, nrSSU and nrLSU are taken into consideration as a hallmark in

molecular taxonomic studies for over decades (Raja et al., 2017). Apart from this, ITS is one of the most useful and widely used DNA barcoding marker and therefore confer crucial information for molecular phylogenetic studies. Hence, in this study, we benefited from the ITS region for the molecular identification of Ank Akata & Altuntaş 690. The phylogenetic analysis carried out with the ITS region pointed out as much as 100% genetic similarity between the *B. phalloides* and the specimen (GenBank ID: MT823465.1) (Figure 4).

With the current study, *B. phalloides* was reported from Muğla province for the first time and it was the fifth record from Turkey. The distribution of Turkish *Battarrea phalloides* was given in Figure 5 and Table 1.

Acknowledgement

Mustafa Sevindik is thanked for his valuable help on arrangement of the figures.

Figure 1. Basidiomata of *Battarrea phalloides*.

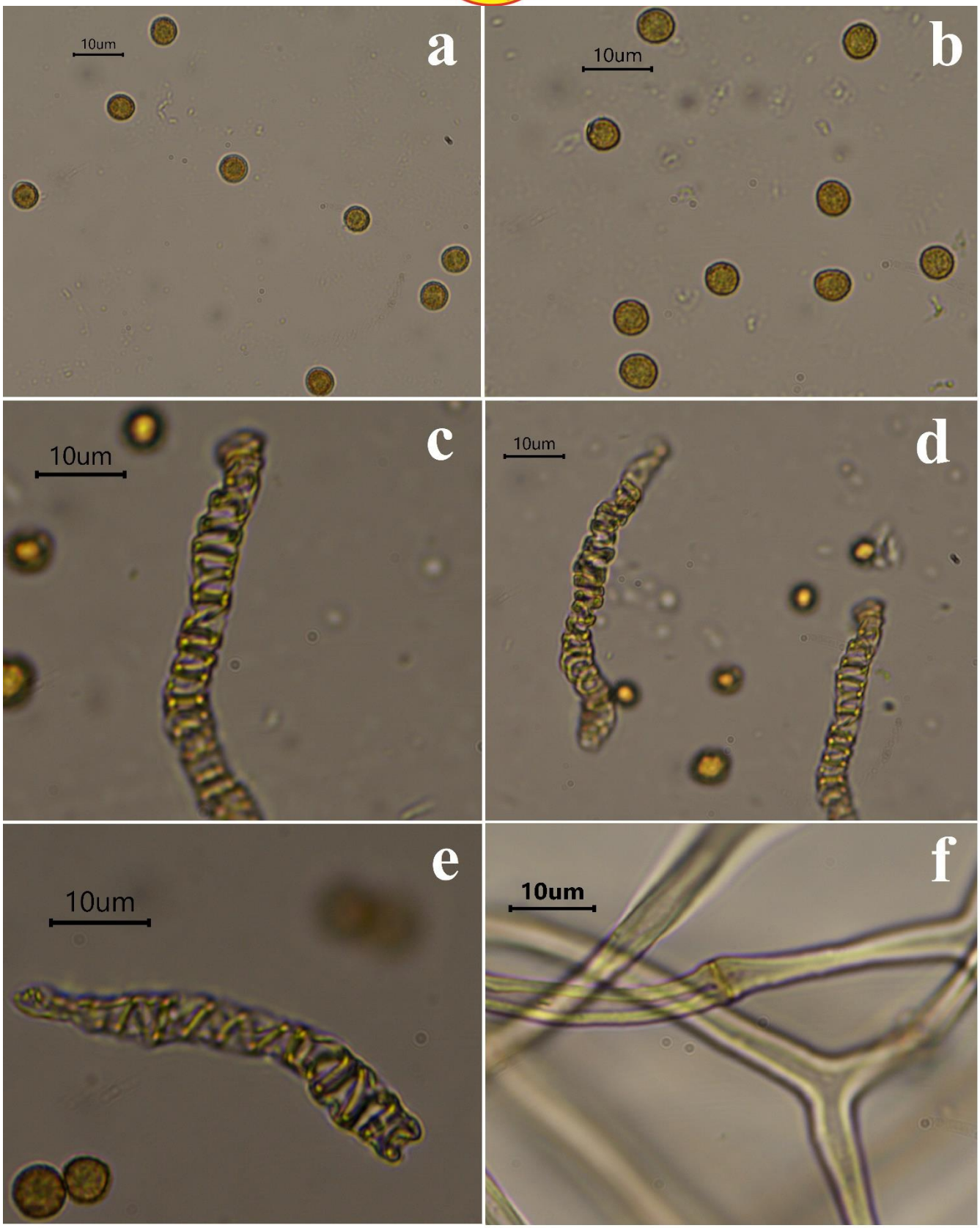

Figure 2. *Battarrea phalloides*: **a-b.** spores, **c-e.** elaters, **e.** pseudocapillitial threads.

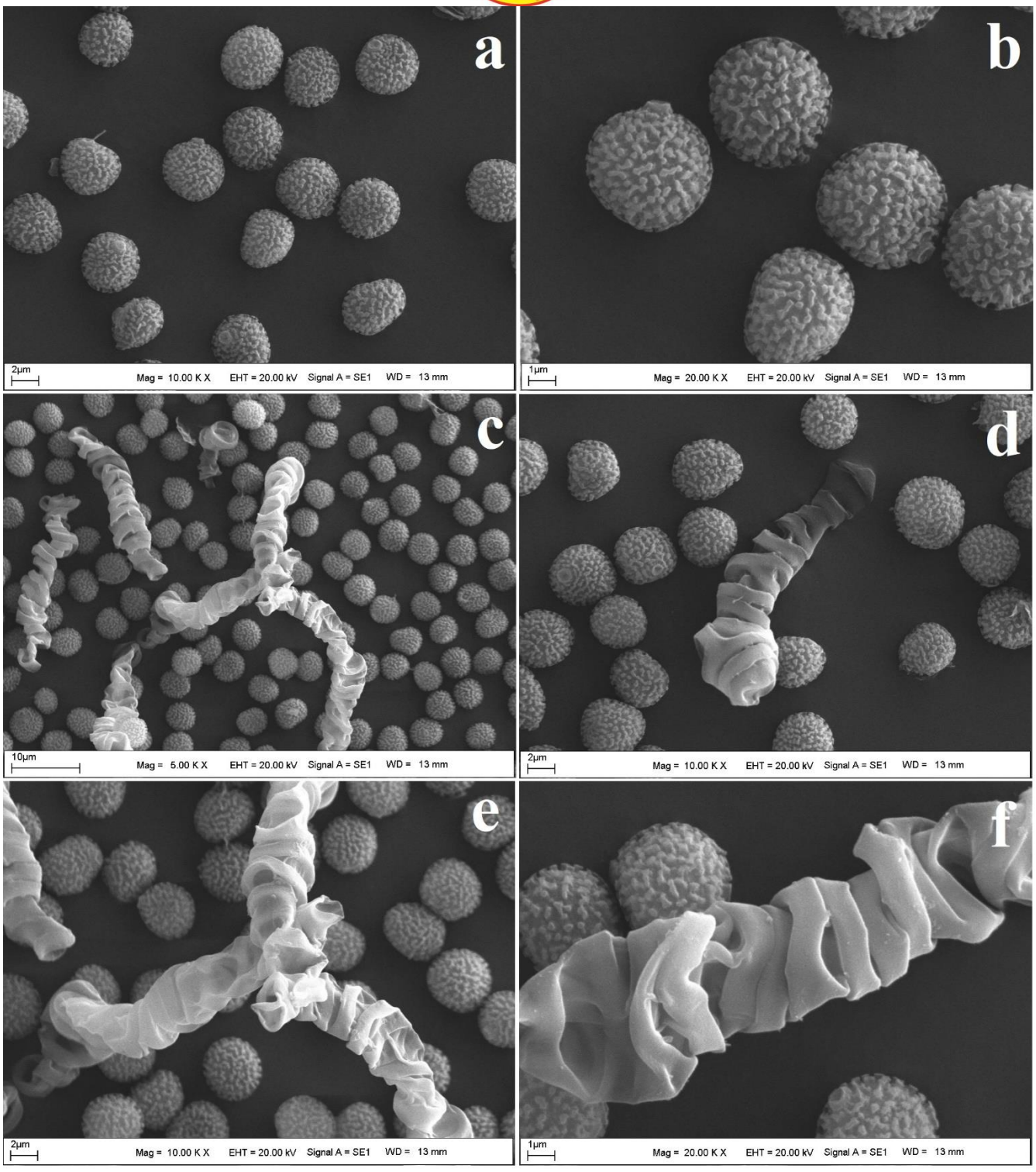

Figure 3. *Battarrea phalloides*: as viewed by a scanning electron microscope (SEM): **a-b.** spores, **c-f.** spores and elaters.

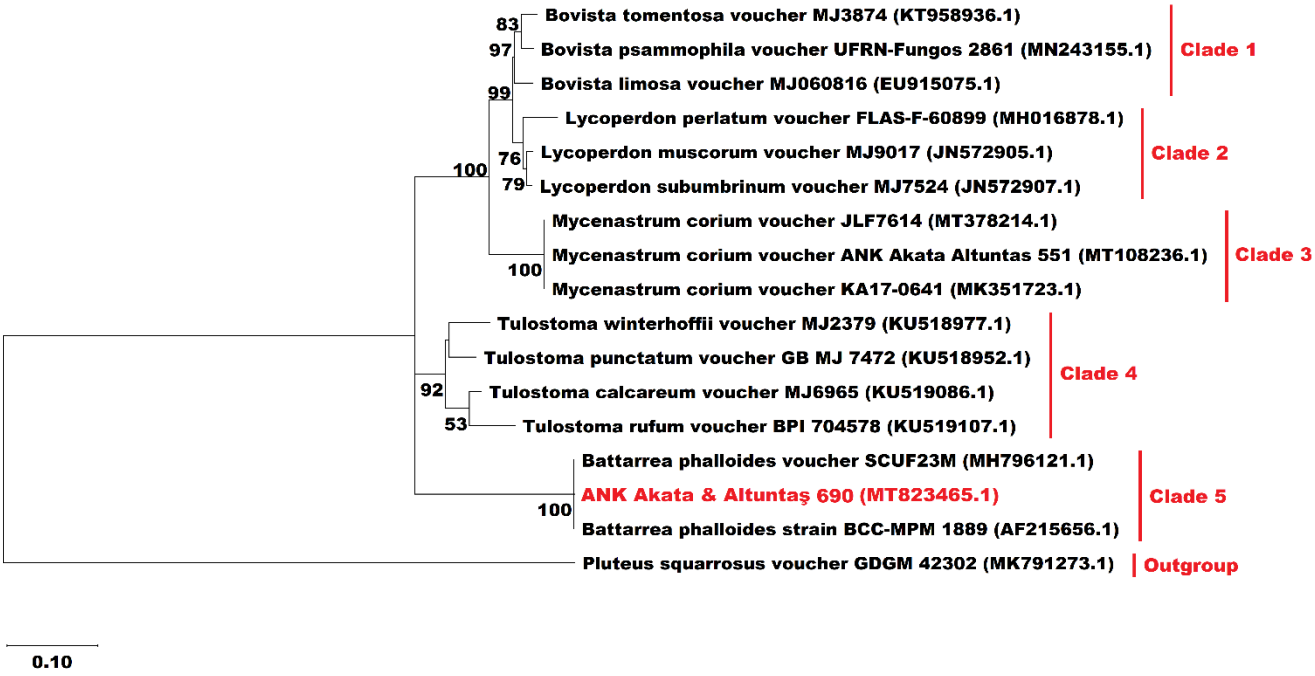

Figure 4. The Maximum Likelihood phylogenetic tree showing the evolutionary relationships of 17 fungal taxa deduced from their ITS region. Percentage bootstrap values (>50%) were stated next to the branches. All the reference sequences utilized in the phylogenetic analysis were retreived from GenBank and their accession numbers were indicated in parantheses. *Pluteus squarrosus* was used as the outgroup member. The scale bar (lower left) exhibits a genetic distance of 0.1.

Figure 5. Distribution of *Battarrea phalloides* in Turkey.

Table 1. Studies on *Battarrea phalloides* in Turkey.

STUDIES	LOCATIONS
Watling et al., 1995	Yalova
Ersel and Solak, 2004	İzmir
Allı et al. 2007	Aydın
Adanacıoğlu et al., 2016	İzmir
Current study	**Muğla**

References

Abdel-Azeem, A. M. and Nafady, N. A. (2019). New records on the genus Tomophagus and Battarrea for mycobiota of Egypt. *Current Research in Environmental and Applied Mycology*, 9(1), 77-84.

Adanacıoğlu, N., Yıldız, Ü., Oğur, E., Aykas, L., Tan, A. ve Taylan, T. (2016). Türkiye Makromantar Genetik Kaynakları I. Ege Bölgesi. *Anadolu Ege Tarımsal Araştırma Enstitüsü Dergisi*, 26(1), 46-61.

Allı, H., Işıloğlu, M. and Solak, M. H. (2007). Macrofungi of Aydın Province, Turkey. *Mycotaxon*, 99, 163-165.

Calonge, F. D. (1998). *Gasteromycetes, I. Lycoperdales, Nidulariales, Phallales, Sclerodermatales, Tulostomatales*. Volume 3., Real Jardin Botanico, Stuttgart.

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

Ersel, F. Y. and Solak, M. H. (2004). Contributions to the macrofungi of İzmir province. *Turkish Journal of Botany*, 28(5), 487-490.

Esqueda, M., Herrera, T., Perez-Silva, E., Aparicio, A. and Moreno, G. (2002). Distribution of *Battarrea phalloides* in Mexico. *Mycotaxon*, 82, 207-214.

Denchev, C. M. and Assyov, B. (2010). Checklist of the larger basidiomycetes in Bulgaria. *Mycotaxon*, 111(1), 279-282.

Fraiture, A. and Otto, P. (2013). Distribution, ecology and status of 51 macromycetes in Europe: Results of the ECCF Mapping Programme. Scripta Botanica Belgica, 53.

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

Gates, G. and Ratkowsky, D. A. (2004). Some interesting fungal records for Tasmania. *The Tasmanian Naturalist*, 126, 2-5.

Hansen, L. and Knudsen, H. (1997). *Nordic macromycetes. Vol. 3, Heterobasidioid, Aphyllophoroid and Gastromycetoid Basidiomycetes*. Nordsvamp, Copenhagen.

Hong, L. and Li, F. (2006). The genus Battarrea (Tulostomatales, Basidiomycota) in China. *Acta Botanica Yunnanica*, 28(1), 19-21.

Howladar, S., Mahmoud Y.A.G. and Meriseel, A. (2013). *Battarrea phalloides* – new for Saudi Arabia. *Österreichische Zeitschrift für Pilzkunde*, 22, 1-6.

Ivančević, B., Mešić, A., Tkalčec, Z., Kušan, I. and Horjan, I. (2016). Studies on Croatian Basidiomycota 3: the first record of Battarrea phalloides (Agaricales) with a worldwide taxonomic review of Battarrea species. *Nova Hedwigia*, 197-209.

Jacobson, K. M., Jacobson, P. J. and Miller Jr, O. K. (1999). The autecology of *Battarrea stevenii* in ephemeral rivers of southwestern Africa. *Mycological Research*, 103(1), 9-17.

Karadelev, M. and Rusevska, K. (2016). Distribution maps of critical endangered species from Macedonian Red List of Fungi. *Hyla: Herpetological bulletin*, 2016(1), 14-18.

Kreisel, H. (2001). Checklist of the gasteral and secotioid Basidiomycetes of Europe, Africa, and the Middle East. *Österreichische Zeitschrift für Pilzkunde*, 10, 213-313.

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

Lacheva, M. (2012). New data of some rare larger fungi of Agaricaceae (Agaricales) in Bulgaria. *Sci Technol*, 2(6), 24-29.

Madrid, H. (2007). Battarrea stevenii (Liboschitz) Fr. en Paposo II Región de Chile. *Boletín Micológico*, 22.

Martín, M. P., Rusevska, K., Dueñas, M. and Karadelev, M. (2013). Battarrea phalloides in Macedonia: genetic variability, distribution and ecology. *Acta Mycologica*, 48(1), 113–122.

Nei, M. and Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford university press.

Nieves-Rivera, A. M., Lodge, D. J. and Miller, O. K. (1998). *Contributions to the study of Gasteromycetes of Puerto Rico*. McIlvainea. Vol. 13, no. 2 (1998).: p. 50-58: ill., map.

Pegler, D. N., & Laessøe, T. and Spooner, B. (1995). *British Puffballs, Earthstars and Stinkhorns: an account of the British gasteroid fungi*. Royal Botanic Gardens, Kew.

Raja, H. A., Miller, A. N., Pearce, C. J. and 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.

Rimóczi, I., Jeppson, M. and Benedek, L. (2011). *Characteristic and rare species of Gasteromycetes in Eupannonicum*. Fungi non delineati 56–57. Alassio, Italy: Edizioni Candusso.

Rogers, S. O., and Bendich, A. J. (1994). Extraction of total cellular DNA from plants, algae and fungi. *In Plant Molecular Biology Manual*. (pp. 183-190). Springer, Dordrecht.

Sesli, E. and Denchev, C. M. (2008). Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. *Mycotaxon*, 106(2008), 65.

Seyidova, H. and Hüseyin, E. (2012). Macrofungi of Nakhchivan (Azerbaijan) Autonomous Republic. *Turkish Journal of Botany*, 36(6), 761-768.

Shepherd, L. D. and Cooper, J. A. (2018). First record of the fungus *Battarrea phalloides* (Agariacaceae) in New Zealand. *New Zealand Journal of Botany*, 56(1), 109-114.

Smith, J. H., Suz, L. M. and Ainsworth, M. A. (2016). *Red List of Fungi for Great Britain: Bankeraceae, Cantharellaceae, Geastraceae, Hericiaceae and selected genera of Agaricaceae (Battarrea, Bovista, Lycoperdon & Tulostoma) and Fomitopsidaceae (Piptoporus)*. http://fungi. myspecies. info/sites/fungi. myspecies. info/files/Smith% 20et% 20al, 20(282015), 29.

Sobestiansky, G. (2005). Contribution to a macromycete survey of the States of Rio Grande do Sul and Santa Catarina in Brazil. *Brazilian Archives of Biology and technology*, 48(3), 437-457.

Stielow, J. B., Levesque, C. A., Seifert, K. A., Meyer, W., Iriny, L., Smits, D. and Lomascolo, A. (2015). One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, 35, 242.

Watling, R., Gucin, F. and Isiloglu, M. (1995). *Battarraea phalloides*--its history, biology and extension to its distribution. *Nova Hedwigia*, 60(1), 13-18.

Yousaf, N., Khalid, A. N. and Niazi, A. R. (2013). Taxonomy of gasteroid fungi from some arid regions of Punjab, Pakistan. *Journal of Biodiversity and Environmental Sciences*, 3(12), 253-263.