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Xerophorus donadinii (Bon) Vizzini, Consiglio & M. Marchetti (*Callistosporiaceae*: Agaricales) from Mediterranean Region of Turkey

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Research Article

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Abstract

Xerophorus donadinii (Bon) Vizzini, Consiglio & M. Marchetti has been identified from the Mediterranean Region of Turkey based on morphological characters and molecular phylogenetic studies. A combined dataset generated from nuclear ribosomal internal transcribed spacer and nuclear ribosomal DNA large subunit gene sequences is used to assess its position within *Callistosporiaceae*. Color photographs from its natural habitat, morphological descriptions and line-drawings of microscopic characters are provided.

Keywords: Basidiomycota, nrITS, nrLSU, molecular phylogeny, Turkey

Türkiye'nin Akdeniz Bölgesi'nden *Xerophorus donadinii* (Bon) Vizzini, Consiglio & M. Marchetti (*Callistosporiaceae*: Agaricales)

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Öz

Xerophorus donadinii (Bon) Vizzini, Consiglio & M. Marchetti, morfolojik karakterlere ve moleküler filogenetik çalışmalara dayalı olarak Türkiye'nin Akdeniz Bölgesi'nden tanımlanmıştır. Nükleer ribozomal internal transcribed spacer ve nükleer ribozomal DNA büyük alt birim gen dizilerinden oluşturulan birleşik bir veri seti, bu türün *Callistosporiaceae* içindeki konumunu değerlendirmek için kullanılmıştır. Doğal ortamında çekilen renkli fotoğraflar, morfolojik açıklamalar ve mikroskobik karakterlerin çizgi çizimleri sunulmuştur.

Anahtar Kelimeler: Bazidiyomikota, nrITS, nrLSU, moleküler filogeni, Türkiye

Introduction

The genus *Xerophorus* (Bon) Vizzini, Consiglio & M. Marchetti was considered to be a subgenus within *Callistosporium* Singer by Bon [1, 2]. However, a recent study by Vizzini et al. [3] indicated that *Xerophorus* species form an independent lineage within the family *Callistosporiaceae* Vizzini,

Consiglio, M. Marchetti & P. Alvarado and should be considered as a separate genus. The species of the genus *Xerophorus* is characterized with spaced, thick lamellae, hygrophoroid basidia up to 50 µm long, whitish spore-print, smooth and amygdaliform spores up to 10 µm long, and a bluish-green reaction in basic solutions [3]. They grow mainly on soil. Only three species of this genus have been identified so far, which are *Xerophorus donadinii* (Bon) Vizzini, Consiglio & M. Marchetti and *Xerophorus olivascens* (Boud.) Vizzini, Consiglio & M. Marchetti from Europe, and *Xerophorus dominicanus* Angelini, Vizzini & Bizzi and from Dominican republic [3]. *Xerophorus donadinii* is regarded as an European macrofungi restricted to the Mediterranean regions. It is only reported from Italy, France and Hungary [3]. Additionally, only a morphological description of this species is reported from Black Sea region in Turkey [4]. No other records of this genus are determined in other parts of the world. Thus, more comprehensive studies are needed to better understand this genus and its species. In this study, we provide morphological features and genetic data of *X. donadinii* from Mediterranean region of Turkey. This study contributes significantly to the Turkish mycobiota in such a way that it not only shows that this species exists in the Mediterranean region but also provides, for the first time, genetic data from two different gene loci. This information is inevitable to discriminate the macrofungi from its most closely related species in the genus *Xerophorus*.

Material and Methods

Morphological Analysis

Macrofungi samples were collected from Isparta province of Mediterranean Region in Turkey during field works in autumn seasons. Observations of microscopic characteristics were made using the Leica DM500 light microscope (Leica Microsystems, Wetzlar, Germany) at magnifications up to 400× and 1000×. Microscopic structures were observed in dried material stained in ammoniacal Congo red. The following abbreviations are used: L^m and W^m indicate the average length and width of basidiospores, Q shows the ratios of length/width and Q^m presents the average quotient of the measured basidiospores [5]. Specimens are kept at the fungarium of Isparta University of Applied Sciences, Isparta, Turkey.

Molecular Sequencing, Phylogenetic and Genetic Analyses

DNA isolation, polymerase chain reaction (PCR) amplifications and sequencing methods were carried out according to Kaygusuz et al. [5]. The primer pairs ITS1F/ITS4 [6, 7] and LR0R/LR5 [8] were used for amplifying nrITS and nrLSU gene regions, respectively. DNA sequencing was performed with the same primers used in PCR amplifications at the Source Bioscience company (Berlin, Germany). Chromatograms were visually examined and manually edited using BioEdit 7.0.5 [9]. *Cleistocybe vernalis* Ammirati, A.D. Parker & Matheny [ADP 050506 (WTU)] was used as the outgroup taxa. All newly generated nrITS and nrLSU sequences were submitted to the GenBank database. The phylogenetic trees were constructed using the Maximum Likelihood (ML) and Bayesian Inference (BI)

analyses. The newly generated nrITS and nrLSU sequences were selected from *Callistosporiaceae* dataset following the sampling of Vizzini et al. [3]. The ML analysis of the concatenated dataset was constructed in RAxML v8.2.10 [10] under the GTRGAMMA substitution model with 1000 bootstrap replicates. The BI analysis of the concatenated data matrix was performed in MrBayes 3.2.2 [11] by Markov chain Monte Carlo (MCMC) method. Six simultaneous Markov chains were run each for 1.000.000 generations, trees were sampled every 100th generation. A color-coded pairwise identity matrix of sequences belonging to *Xerophorus* was inferred using Sequence Demarcation Tool Version 1.2 (SDTv1.2) software [12].

Results

Molecular Analysis

The phylogenetic position of the three collections of *Xerophorus* was carried out by the phylogenetic analysis of the combined 14 nrITS sequences (3 newly generated, 11 downloaded from GenBank) and 14 nrLSU sequences (3 newly generated, 11 downloaded from GenBank). The genus *Xerophorus* formed a well-supported monophyletic group (MLB = 98%, Fig. 1). The phylogenetic analyses of combined nrITS/nrLSU sequences shows that all newly generated sequences of *X. donadinii* from Turkey are clustered together with three Italian collections in a highly supported clade (MLB = 99%, Fig. 1).

Xerophorus donadinii is sister to *X. olivascens* with high bootstrap support (MLB = 100%). *X. dominicanus* formed a basal clade in the phylogenetic analyses and is distantly related with the other two *Xerophorus* species. The pairwise nucleotide sequence of nrITS regions of *X. donadinii* from Turkey showed 89.9-99.9% identity with three collections of *X. donadinii* from Italy (CA 20181110.02, 18223 AMB, 18222 AMB) (Fig. 2). Turkish collection of *X. donadinii* showed 73.8% identity with *X. olivascens* (1237/05 EMB) from Italy (Fig. 2)

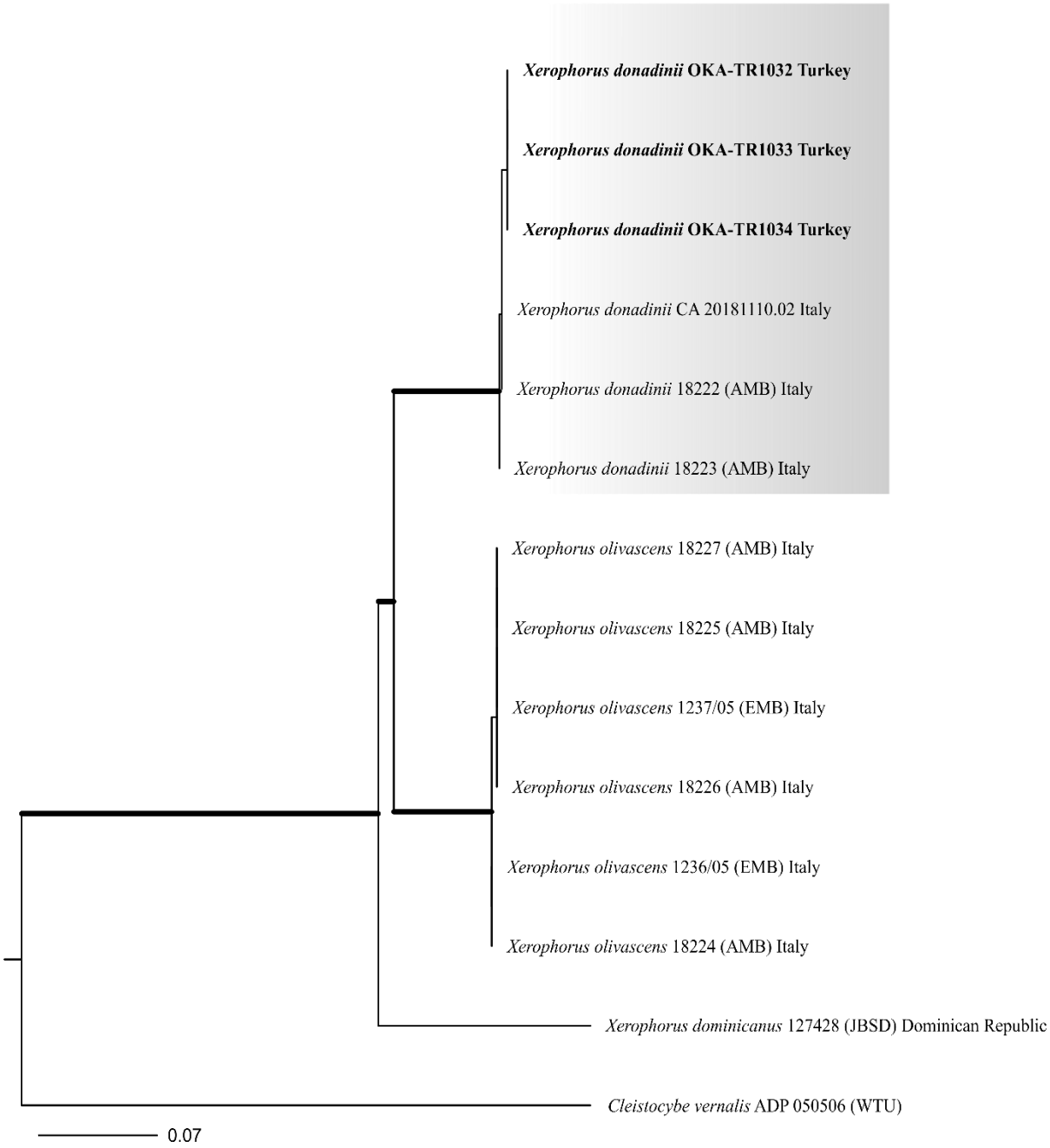

Figure 1. *Phylogenetic tree inferred from Maximum-likelihood analysis based on combined dataset (nrITS and nrLSU) of Xerophorus species. Bold branches represent Maximum-likelihood bootstrap (MLB) ≥ 90% and Bayesian posterior probabilities (BPP) ≥ 0.95. The newly generated sequences are indicated in bold. Bar indicates 0.07 anticipated changes per site per branch. Cleistocybe vernalis [ADP 050506 (WTU)] is the outgroup species.*

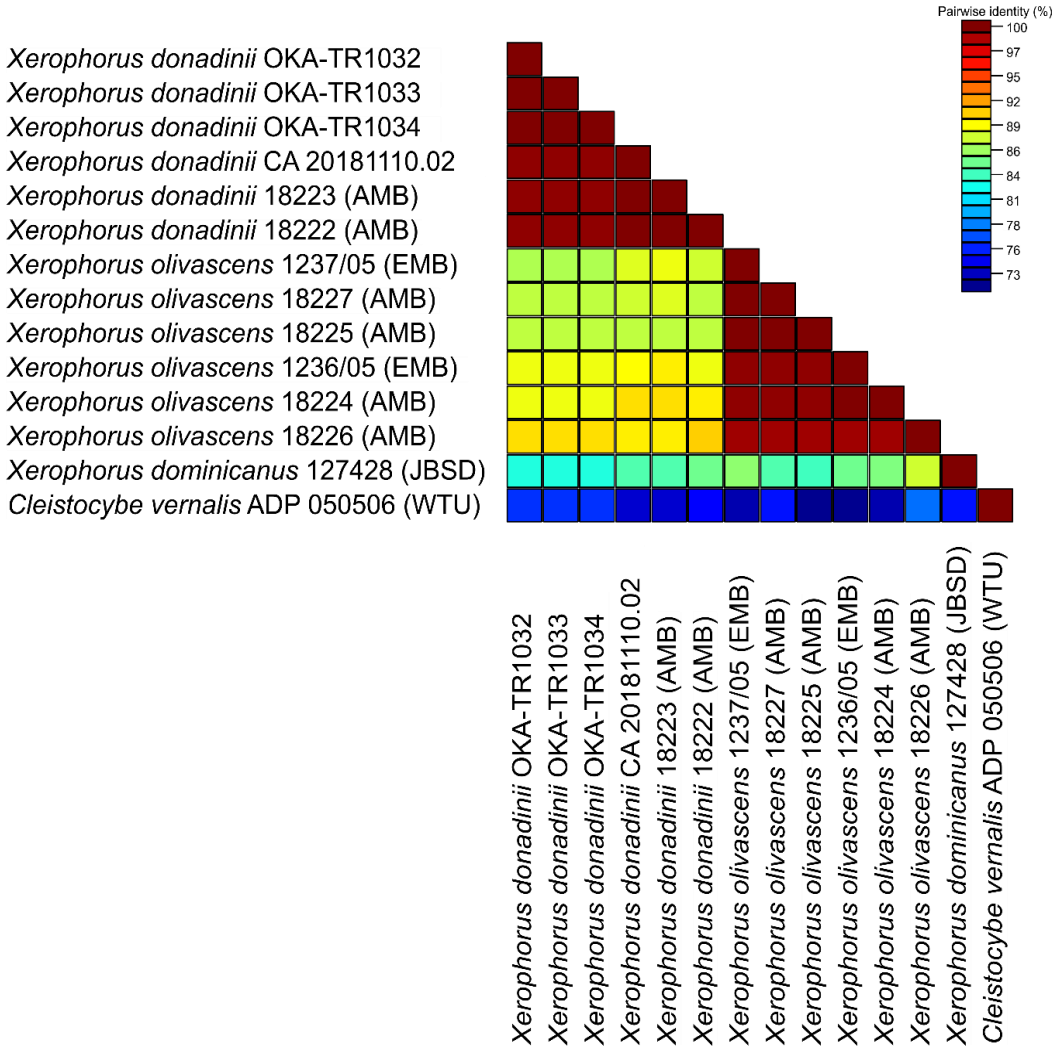

Figure 2. *The two-dimensional color-coded matrix showing pairwise identity scores between sequences belonging to Xerophorus.*

Taxonomy

Callistosporiaceae Vizzini, Consiglio, M. Marchetti & P. Alvarado.

Xerophorus donadinii (Bon) Vizzini, Consiglio & M. Marchetti, in Vizzini, Consiglio, Marchetti & Alvarado, Fungal Diversity 101: 241 (2020).

Basionym: [*Callistosporium olivascens* var. *donadinii* Bon, Docums Mycol. 20 (79): 57 (1990) ≡ *Callistosporium donadinii* (Bon) Contu, Micol. Ital. 22(1): 55 (1993)].

Pileus (Fig. 3a-d) 10–25 mm diam, convex to broadly convex, then applanate, without umbo or with broad low umbo, surface glabrous and dry, not hygrophanous, yellow-orange to vinous-purple, margin not straight, sinuous. ***Lamellae*** moderately crowded, thin, slightly decurrent to emarginated, pale yellow or yellow brown. ***Stipe*** (Fig. 3a-d) 25–40 × 2–7 mm, cylindrical with tapering at base, sometimes curved, stuffed to hollow, surface fibrillose, pale yellow-brown, covered with abundant white rhizomorphs at the base. ***Smell*** and ***taste*** not distinctive.

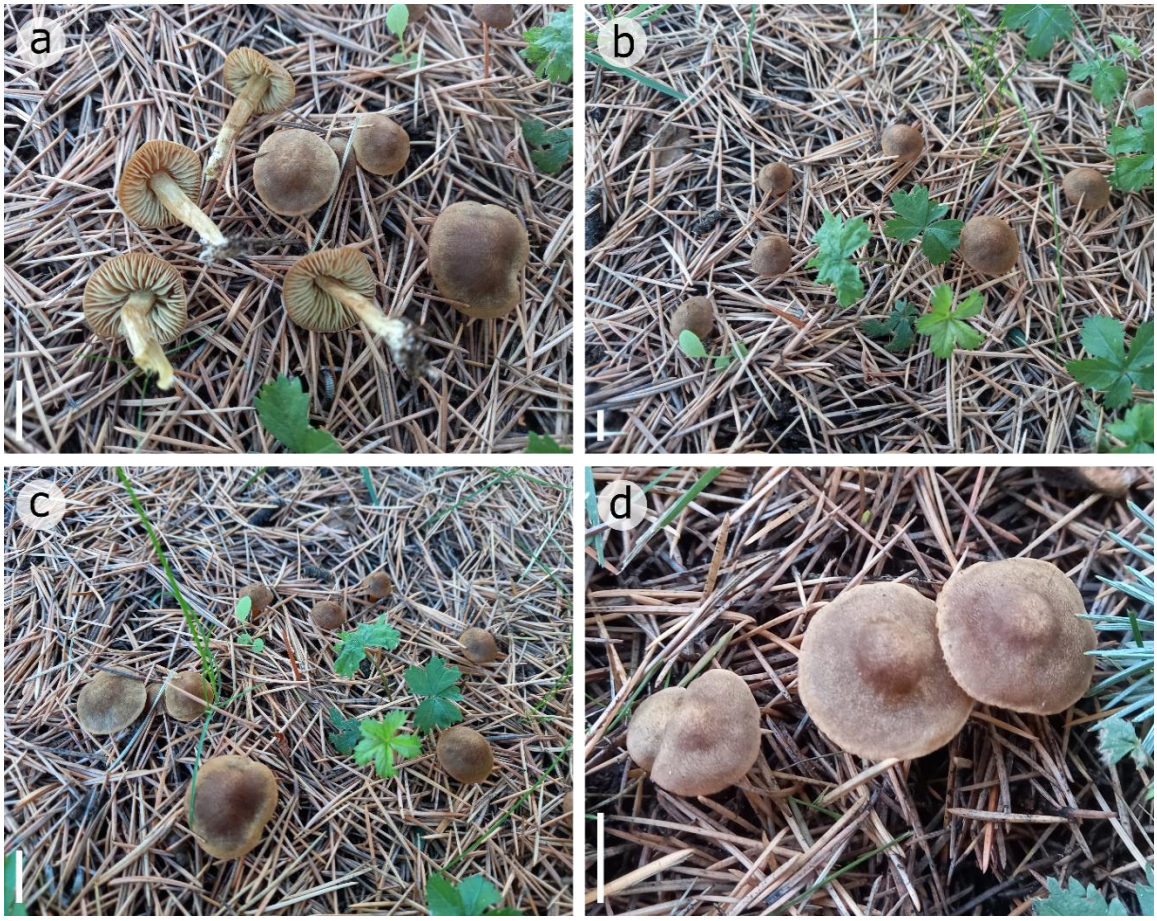

Figure 3. *X. donadinii: a-d basidiomes growing in its natural habitat. Scale bars = 10 mm.*

Basidiospores (Fig. 4a) (7.0–)7.5–8.7(–9.0) × (4.6–)4.9–5.3(–5.5) µm, $L^m \times W^m$ = 8.3 × 5.1 µm, Q = 1.5–1.7, Q^m = 1.6, amygdaliform to ovoid ellipsoid, uni- or two pale greenish guttulate, hyaline, thin-walled. ***Basidia*** (Fig. 4b) (25.0–)27.5–38.5(–48.0) × (7.0–)8.5–10.0(–11.0) µm, clavate, 4-spored, hyaline and thin-walled. ***Pleurocystidia*** absent. ***Cheilocystidia*** (Fig. 4c) (29.0–)32.5–42.0(–45.5) × (7.0–)8.5–9.0(–11.0) µm, narrowly cylindraceous to slightly flexuose or clavate, with sometimes subcapitate apex, hyaline and thin-walled. ***Pileipellis*** (Fig. 4d) a cutis made up of radially arranged, cylindrical or clavate elements, with sometimes subcapitate apex, 3.0–12.0 µm wide hyphae, hyaline and thin-walled. ***Stipitipellis*** a cutis, consisting of narrowly cylindrical to cylindrical or clavate, 3.0–15.0 µm wide hyphae, smooth, hyaline and thin-walled. ***Caulocystidia*** (Fig. 4e) 35.0–60.0 × 5.5–13.0 µm, irregularly cylindrical, hyaline and thin-walled. ***Clamp*** connections absent in all studied tissues.

Ecology: Gregarious or sometimes in small groups, present at elev. 1000 m, in grasslands under of *Abies cilicica* subsp *cilicica*, growing naturally in Taurus Mountains.

Collections examined: TURKEY, Isparta Province, Atabey district, around Islamkoy village, under *Abies cilicica* subsp *cilicica*, at 37°55′35″N, 30°39′52.8″E, alt. 1010 m, 07 September 2019, O. Kaygusuz, OKA-TR1032; GenBank: OK442662 for nrITS, OK442659 for nrLSU; *ibid.*, under *A. cilicica* subsp *cilicica*, at 37°55′35″N, 30°39′52.5″E, alt. 1000 m, 07 September 2019, O. Kaygusuz,

OKA-TR1033; GenBank: OK442663 for nrITS, OK442660 for nrLSU; *ibid.*, under *A. cilicica* subsp *cilicica*, at 37°55′35″N, 30°39′52.2″E, alt. 1011 m, 11 September 2020, O. Kaygusuz, OKA-TR1034; GenBank: OK442664 for nrITS, OK442661 for nrLSU.

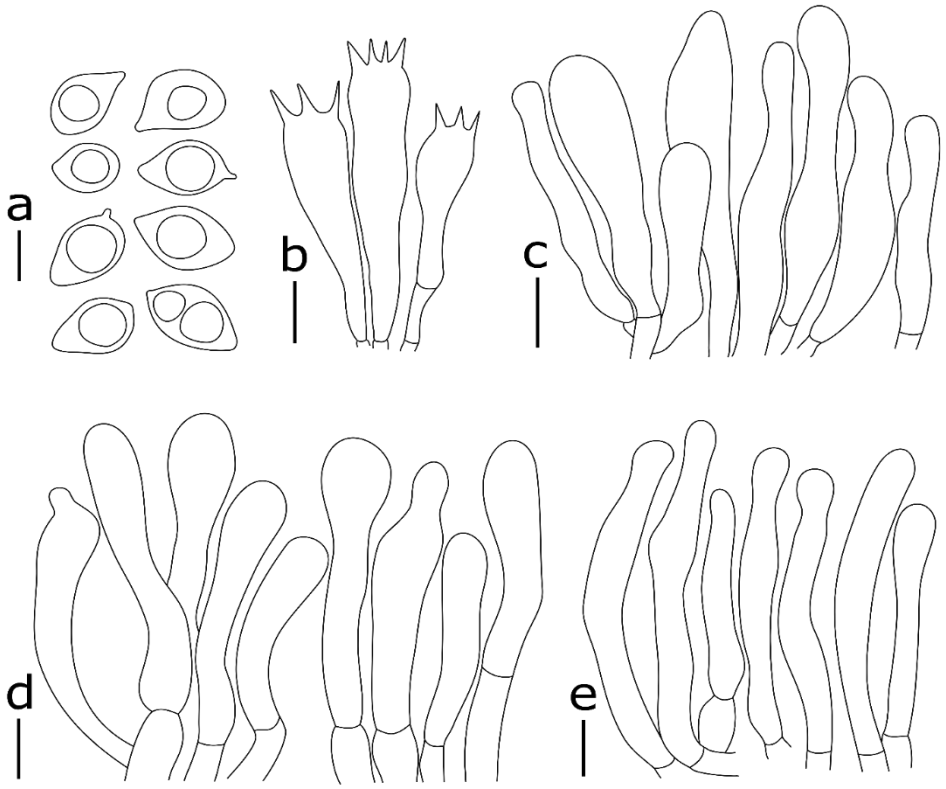

Figure 4. *Microcharacters of X. donadinii: a- basidiospores, b- basidia, c- cheilocystidia, d- pileipellis elements, e- caulocystidia. Scale bars: a = 5 µm, b-e = 10 µm.*

Discussions

Xerophorus is a genus in the family *Callistosporiaceae* containing the species *X. donadinii* and *X. olivascens* from Europe, and *X. dominicanus* from Dominican Republic [3]. Phylogenetically, *X. donadinii* and *X. olivascens* are two closely related species [3]. Morphologically, *X. donadinii* differs from *X. olivascens* because of its smaller basidiocarps (up to 30 mm), vinaceous to dark red-brown pileus, whitish-yellow to lemon yellow lamellae, and slightly smaller basidiospores (7.0–9.0 × 4.8–5.5 µm) [3]. Also, *X. donadinii* prefers habitats with *Quercus suber, Pinus, Cupressus, Acer* and *Prunus* forests, while *X. olivascens* grow under cedars in parks and gardens [3, 4]. *X. dominicanus* is a more distantly related species to both *X. donadinii* and *X. olivascens*. It is only reported from the Dominican Republic so far. It has small basidiomes with cyclamen pink tinges and bears clamp connections in the basidia and pileipellis elements [3].

Conclusion

There is not much study in the genus *Xerophorus*, which is represented by only three species worldwide. It is necessary to study this genus by describing its species and separate from species of other closely

related genera. In this respect, this study provides both morphological and molecular description of *X. donadinii* species from the Mediterranean region of Turkey. This species is believed to be restricted to Mediterranean coastal regions, but has also been reported from the Black Sea region of Turkey. When describing a macrofungi, the use of genetic data is inevitable and provides important data for comparison with its counterparts in other parts of the world. We believe morphological and molecular data of *X. donadinii* in this study significantly contributes to our knowledge on distribution of this rare macrofungi species and helps understand its ecological preferences in Turkey and worldwide.

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Ethics Committee Approval and Permissions -

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Authors Contribution The authors have contributed sufficiently in the planning, execution, and analysis of this study.

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