Albatrellopsis flettii, A New Genus for Turkish Albatrellaceae

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ABSTRACT
Fungal samples were collected from Kazdağları National Park (Balıkesir/Turkey) on October 25, 2014, and they were identified by implementing both traditional methods and ITS rDNA-based molecular phylogenetic analysis. By taking into account the high sequence similarity between the collected samples (ANK DNZ 023) and Albatrellopsis flettii (Morse ex Pouzar) Audet, the collected specimen was regarded as A. flettii, which was also verified by the morphological data. As a result, A. flettii was reported for the first time from Turkey at the genus and species level. A short description of the newly reported species was provided together with its macro-photography, illustrations of spores and hyphae, and spore images taken by a scanning electron microscope (SEM).

Albatrellopsis flettii, Türkiye Albatrellaceae’leri İçin Yeni Bir Cins

ÖZET
Mantar örnekleri 25 Ekim 2014 tarihinde Kazdağları Milli Park'ından (Balıkesir / Türkiye) toplandı ve hem geleneksel yöntemler hem de ITS rDNA tabanlı moleküler analiz uygulanarak tanımlandı. Toplanan örnekler (ANK DNZ 023) ve Albatrellopsis flettii (Morse ex Pouzar) Audet arasındakı yüksek sekans benzerliği dikkate alınarak toplanan örnek A. flettii olarak kabul edildi ve morfolojik veriler de bu bulguyu pekiştirdi. Sonuç olarak, A. flettii Türkiye'den ilk kez cins ve tür seviyesinde rapor edilmiştir. Yeni rapor edilen türlerin kısa açıklanması, makro fotoğrafları, spor ve hifsel yaplarının çizimleri ve tarama elektron mikroskobu (SEM) ile alınan spor görüntüleri ile birlikte verilmiştir.

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MATERIAL and METHOD

Albatrellopsis specimens were collected from Kazdağ National Park (Balıkesir-Turkey) on October 25, 2014. Macroscopic and ecological characteristics of the specimens were recorded at the collection site. In the fungarium, microscopic structures were scrutinized using both simple light microscope and scanning electron microscope (SEM). For light microscopy, approximately 50 measurements were taken under a light microscope (Euromex Oxion Trinocular microscope). 100X magnification rates were utilized for each microscopic structure and the compiled data were statistically processed. We also benefited from some chemicals including Melzer's reagent, 5% KOH, and congo red. For SEM analysis, pieces of hymenium were buried in a carbon paste, coated with gold particles, and visualized with an EVO 40XVP (LEO Ltd., Cambridge, UK) scanning electron microscope by using an accelerating voltage of 15 kV. Herbarium materials were prepared from the identified samples (ANK DNZ 023) and deposited into Ankara University Herbarium (ANK).

Determination of the ITS rDNA Sequences

For the genomic DNA extraction from ANK DNZ 023, the CTAB method was employed as previously described (Rogers and Bendich, 1994). The extracted genomic DNA was spectrophotometrically (Nanodrop Lite Theromo Scientific) analyzed for the quality and quantity measurements and then it was utilized in a polymerase chain reaction as the template in order to amplify the Internal Transcribed Spacer (ITS) rDNA regions. PCR amplification of the ITS rDNA regions was implemented by using the ITS1 forward and ITS4 reverse universal oligonucleotides as previously represented (Stielow et al, 2015). The presence of amplicons was electrophoretically confirmed as single and clear bands on an agarose gel and later they were purified with Expin Gel, PCR, and CleanUp SV Kit (GeneAll) and sequenced with Sanger dideoxy sequencing method. Same ITS1 and ITS4 oligonucleotides were employed for the sequencing PCR conducted using the BigDye™ Direct Cycle Sequencing Kit (Thermo Fisher Scientific) and the fragment analyses were carried out using ABI Prism 3130 Genetic Analyzer. Agarose gel electrophoresis and the Sanger sequencing were performed as described elsewhere (Chen et al., 2014).

Molecular Phylogeny Study

For the molecular phylogeny, the sanger reads obtained from ITS1 and ITS4 primers were assembled using DNAMAN Version 10 sequence assembly software (Lynnon Corporation) and BLASTn search was implemented with the assembled sequence for the similarity index analysis. Based on this BLAST search, the in-group and the out-group members were retrieved from NCBI GenBank for the phylogenetic analysis. The assembled sequence and the nucleotide sequences of the retrieved in-group and out-group members were aligned using the ClustalW algorithm of MEGAX software (Kumar et al., 2018). The phylogenetic tree demonstrating the evolutionary history of ANK DNZ 023 was inferred using the Maximum Likelihood method and K2 nucleotide substitution model with gamma distribution (Kimura, 1980). The bootstrap method was utilized for the accuracy estimation using 1000 bootstrap replicates (Felsenstein, 1985).

RESULTS

Albatrellaceae Nuss

Albatrellopsis Teixeira

A. flettii (Morse ex Pouzar) Audet (2010), (Figure 1).

Syn.: Albatrellus flettii Morse ex Pouzar (1972), Polyplius flettii (Morse ex Pouzar) Teixeira (1992), Polyporus flettii Morse, Mycologia (1941).

Macroscopic and microscopic features

Pileus 120-160 mm broad, convex and inrolled at first, then enlarged and becoming plane, centrally depressed finally. Margin lobed or wavy and paler. Surface blue or blue-green at first, later dingy ochraceous, rusty stains in age, salmon to brick red when dry. Pores 1-4 per mm, circular to angular. Pore surface white at first, becoming apricot or salmon when mature, brick red when dry. Flesh white, thick, and firm. Odor and taste not distinctive. Stipe 50-100 mm long, centric or eccentric, circular or irregular, smooth, solid, firm, whitish when young, salmon to brick red when mature or dry. Spore print white. Hyphal system up to 10 μm broad, clamped, strongly inflated in the flesh of the cap and stem. Cystidia not seen. Basidia 15-18 × 5-6 μm, four spored, clavate with basal clamp. Basidiospores 3.5-4 × 2.5-3 μm, ellipsoid to subglobose, smooth, hyaline, and weakly amyloid.

Distribution: North America and East Asia (Zheng and Liu, 2008).

Material examined: TURKEY—Balikesir: Kazdağ National Park, under pine, 1470 m, 39° 42′ N - 26° 53′ E, 25.10.2014, ANK DNZ 023.

Molecular Phylogeny of ANK DNZ 023

The ITS rDNA sequence of ANK DNZ 023 obtained from Sanger sequencing was deposited into NCBI GenBank with the accession number of MT253103.1. In a phylogenetic analysis of ANK DNZ 023, considering the BLAST search results of the specimen’s nuclear ITS rDNA sequence, the genera Albatrellopsis, Albatrellus, Leucogaster, and
Jahnoporus, some of the well-defined genera of the family Albatrellaceae, were selected for ingroup sequences and the nuclear ITS rDNA sequences of Russula delica and Lactarius salmonicolor were selected for the outgroup sequences.

Figure 1. Albatrellopsis flettii: a–c fruit bodies, d. spores viewed under a light microscope (LM), e. spores viewed by a scanning electron microscope (SEM).

As a result of the phylogenetic analysis, four apparent clades were come out along with an outgroup (Figure 2). While the clade 1 included different isolates of Albatrellopsis flettii and the specimen ANK DNZ 023, the Clades 2, 3, and 4 contained species from the genera Albatrellus, Leucogaster, and Jahnoporus respectively. On the other side, Russula delica and Lactarius salmonicolor fell into a distinct branch separate from the ingroup species and formed an outgroup as predicted. The BLAST analysis implemented with the nuclear ITS rDNA sequence of ANK DNZ 023 revealed identity rates as high as 99.3% between the specimen and different isolates of A. flettii. The phylogenetic analyses performed herein, further strengthen the close identity relationship of this specimen with A. flettii with a high bootstrap value.

DISCUSSION

The distinct characteristics of A. flettii are bluish-green pileus developing dingy ochraceous, rusty stains in age, apricot or salmon-colored pores, clamped hyphae, small and weakly amyloid basidiospores. The species may be confused with Albatrellus confluens in terms of its morphology and ecology. Like the former species, the latter has up to 20 cm broad pileus, small and amyloid basidiospores and grow under conifers but the former can easily be separated from the latter by including blue tint on its pileus (Zeng et al., 2004; Zheng and Liu, 2008; Audet, 2010).

For the precise identification of fungal species, conventional methods that relied on morphological data may not be always sufficient. For this reason, conserved regions of genomic DNA including nrITS, nrSSU, and nrLSU as well as sequences of protein-coding genes are utilized for molecular taxonomic studies for the last few decades (Raja et al., 2017). Besides, ITS is the most generally used DNA barcoding marker for fungi and thus confers precious information for molecular phylogenetic studies. Therefore, we
employed nuclear ITS rDNA sequences for the molecular identification of ANK DNZ 023. Nuclear ITS rDNA-based molecular phylogeny exhibited almost 100% identity between A. flettii and the specimen (GenBank ID: MT253103.1) (Figure 2).

Figure 2. The Maximum Likelihood tree revealing the phylogenetic relationships of 18 fungi estimated from the nuclear ITS rDNA region. Percentage bootstrap values (≥50) were indicated for each branch. All of the sequences included in the phylogenetic analysis were retrieved from GenBank except for ANK DNZ 023. *Russula delica* and *Lactarius salmonicolor* were included as the outgroup samples in the phylogenetic analysis. GenBank accession numbers are also given. The scale bar (lower left) represents a genetic distance of 0.11.

Şekil 2. Çekirdek ITS rDNA bölgelerine göre tahmin edilen, 18 mantarın filogenetik ilişkilerini ortaya koyan en olası agaç. Her dal için, yüzde önyükleme değerleri (≥50) belirtilmiştir. ANK DNZ 023 hariç filogenetik analize dahil edilen tüm diziler GenBank'tan alınmıştır. *Russula delica* ve *Lactarius salmonicolor* dış grup örnekleri olarak filogenetik analize dahil edilmiştir.

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Statement of Conflict of Interest
Authors have declared no conflict of interest.

Contribution of the Authors as Summary
Authors declares the contribution of the authors is equal.

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