

First report of *Rutstroemia elatina* (Ascomycota) from Turkey

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ABSTRACT

The purpose of this research was to identify *Rutstroemia* samples from Bolu province (Turkey) on June 22, 2019. The samples were identified based on both conventional and molecular methods (ITS region of the rDNA). By considering the high sequence similarity of the collected samples (Akata 7020) with *Rutstroemia elatina* (Alb. & Schwein.) Rehm, the relevant specimen was considered to be *R. elatina* and the morphological data supported this finding. This species was firstly reported from Turkey. The results of the molecular analysis and a short description of the newly reported species along with its colored images associated with macroscopic and microscopic structures were conferred.

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ÖZET

Bu araştırmanın amacı 22 Haziran 2019'da Bolu (Türkiye)' den toplanan *Rutstroemia* örneklerini tanımlamaktır. Numunelerin tanımlanması hem geleneksel hem de moleküler yöntemlere (rDNA'nın ITS bölgesi) dayanarak yapılmıştır. Toplanan örneklerin (Akata 7020) *Rutstroemia elatina* (Alb. & Schwein.) Rehm ile olan yüksek sekans benzerliği dikkate alındığında, ilgili örnek *R. elatina* olarak kabul edilmiş ve morfolojik veriler bu bulguyu desteklemiştir. Bu tür ilk defa Türkiye'den rapor edilmiştir. Moleküler analizin sonuçları, makroskobik ve mikroskobik yapılarla ilişkin renkli fotoğraflarıyla ile birlikte yeni rapor edilen türün kısa bir betimlemesi yapılmıştır.

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Anahtar Kelimeler

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Yeni kayıt

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INTRODUCTION

Rutstroemia is the largest genus of the family *Rutstroemiaceae* within the order *Helotiales* (*Ascomycota*). The genus comprises roughly 75 widely distributed species particularly in temperate regions and its members are mainly characterized by brownish to black, cup to funnel or goblet shaped apothecia with short stipe, eight-spored, uni to biseriata, amyloid and cylindrical asci, branched to unbranched, cylindrical to filiform paraphyses sometimes thickenings toward to tips, ellipsoid to allantoid, hyaline, smooth, mostly uni to bisepate, more rarely multiseptate spores (Hansen and Knudsen, 2000; Kirk et al., 2008).

R. elatina is an uncommon species growing on the fallen branches, twigs or needles of *Abies alba* (silver fir). Although it is considered to be specific to silver fir, it has also been reported on *Picea abies* (Palmer et al., 1994). According to the literature (Işık and Türkekül, 2018; Sesli and Denchev, 2008; Öztürk et al., 2010), *R. conformata* (P. Karst.) Nannf. and *R. firma* (Pers.) P.

Karst. have hitherto been registered from Turkey but there was no report of *R. elatina* (Alb. & Schwein.) Rehm for Turkish *Rutstroemia*. The purpose of this study was to make a contribution to the larger *Ascomycota* of Turkey.

MATERIALS and METHODS

Rutstroemia specimens were collected from Bolu province during the field study in 2019. Macroscopic characteristics of the fresh specimens were noted and their photographs were taken at the growth sites. Microscopic features such as asci, paraphyses and spores were examined with Leica DM 1000" bright field light microscope and some chemicals (Congo red, Melzer's reagent, 5% potassium hydroxide, 10% ammonium hydroxide etc.) were utilized for this purpose. We benefited from the relevant literature (Breitenbach and Kränzlin, 1984; Hansen and Knudsen, 2000) for identification. The identified voucher samples were deposited to the Herbarium of

Ankara University (ANK).

Molecular study

DNA Isolation

The genomic DNA was isolated from the sporophores of the specimen, according to the modified CTAB method (Aras et al., 2013). NanoDrop One Microvolume UV-Vis Spectrophotometer (ThermoFisher) was used to calculate the concentration and purity of the extracted DNA.

PCR Amplification and Sequencing

The ITS1, 5.8s rRNA gene and ITS2 regions were amplified with PCR by using the universal ITS1 and ITS4 oligonucleotides (White et al., 1990). PCR was conducted in a reaction volume of 25µl. The final concentrations of the PCR ingredients were adjusted as follows: 1× Taq DNA polymerase buffer, 2 units of Taq DNA polymerase (Fermentas), 0,4 mM dNTPs, 3 mM MgCl₂, and 15 pmol of both ITS 1 and ITS4 primers. PCR was carried out in a Thermal Cycler (Applied Biosystems MiniAmp Plus) with the following thermal cycling protocol: first denaturation step of 95°C for 4 min, persued by 35 cycles of 95°C for 30s, 56°C for 15s, and 72 °C for 40s, and a last elongation step of 7 min at 72°C. The PCR amplicons were electrophoretically analyzed in 1.2% agarose gel containing ethidium bromide, and the amplicon sizes were defined by the aid of a DNA size marker (GeneRuler 100 bp Plus DNA Ladder, ThermoFisher). The sequences of the amplicons were determined with

Sanger dideoxy chain termination method at the laboratory of MacroGen Europe in Amsterdam, The Netherlands using the same oligonucleotide primers.

Sequence Analysis

The ITS gene sequences of some relevant fungal species were obtained from GenBank and used for the phylogenetic analysis of the 'Akata 7020. While the ITS sequences of the genera *Rutstroemia* and *Lanzia*, two of the most well-known genera of the *Rutstroemiaceae* family, were used as ingroup sequences, the ITS sequences of *Gyromitra esculenta* and *Morchella angusticeps* were used as the outgroup sequences. The sequences were assembled by using Geneious Prime 2019.1.3 software (Biomatters Ltd) and used for the sequence identity analysis with Basic Local Alignment Search Tool (BLAST). The DNA sequences were aligned using the CLUSTALW and molecular phylogenetic analyses were conducted by using the neighbor joining method based on the Kimura 2-parameter substitution model via MEGAX software with using 1000 bootstrap replicates (Felsenstein, 1985; Kumar et al., 2018).

RESULTS

Ascomycota

Helotiales

Rutstroemiaceae

Rutstroemia elatina (Alb. & Schwein.) Rehm (Figure 1,2).



Figure 1. *Rutstroemia elatina*: a-d. apothecia
Şekil 1. *Rutstroemia elatina*: a-d. apotezyumlar



Figure 2. *Rutstroemia elatina*: a-d. asci, e,f. a portion of paraphyses, g-m. spores.

Şekil 2. *Rutstroemia elatina*: a-d. askuslar, e,f. parafizlerin bir bölümü, g-m. sporlar.

Macroscopic and microscopic features:

Apothecia: 3-5 mm broad, dark brown to black, goblet to cup shaped. **Hymenium** smooth. **Outer surface** slightly fibrous. **Stipe** up to 3 mm long, the same color. **Flesh** black and gelatinous. **Odor and taste** not distinctive. **Asci** 145–160 × 11–14 µm, uniseriate, cylindric, eight-spored, hyaline and amyloid. **Paraphyses** up to 5 µm broad, hyaline, filiform, with slight clavate thickening at the apex. **Spores** 16–18 × 5–6 µm, hyaline, ellipsoid to allantoid, guttulate, smooth, sometimes with a septum in the centre.

Ecology: April to June, on fallen branch or needles of *Abies alba* Mill. (silver fir) and *Picea abies* (L.) H.Karst. (European spruce) (Breitenbach and Kränzlin, 1984; Palmer et al., 1994).

Distribution: Bosnia and Herzegovina, Denmark, France, Germany, Poland, Romania, Russia, Switzerland and the Czech Republic (Breitenbach and Kränzlin, 1984; Palmer et al., 1994).

Specimen examined: Turkey—Bolu: Yedigöller road, 2 km distance to entrance of Ayıkayası Nature Park, on branch of *Abies nordmanniana* subsp. *equi-trojani* (Asch. & Sint. ex Boiss.) Coode & Cullen, 40° 53' 42" N–39° 40' 05" E, 1628 m, 22.06.2019, Akata 7020.

Using phylogenetic analysis, two distinct clades were revealed along with an outgroup. While the clade 1 contained fungi species from the genus *Lanzia*, the clade 2 included species from the genus *Rutstroemia* together with 'Akata 7020'. On the other hand,

Gyromitra esculenta and *Morchella angusticeps* were branched far from the other fungi species and constituted an outgroup as expected. The BLAST analysis performed with the ITS sequence of Akata 7020 provided evidence for 100% similarities of this new records with *Rutstroemia. elatina*. The phylogenetic tree constructed based on the ITS sequences further supported the close identity relationship of the new record with *R. elatina* with a bootstrap value of 100. The ITS sequence of the isolate ANK Akata 7020 was deposited to Genbank under the accession number MN263048.

DISCUSSION

R. elatina is a sabrobe species and its most common host species is considered to be *Abies alba*. Moreover, its samples have also been collected on *Picea abies* (Palmer et al., 1994). *R. elatina* is characterized by dark brown to black, stipitate, goblet to cup shaped apothecia up to 5mm in width, smooth hymenium, uniseriate, amyloid and cylindric asci containing eight-spores, septate, unbranched and filiform paraphyses slightly thickening at the tips, smooth, hyaline, ellipsoid to allantoid spores sometimes with a single central septum (Hansen and Knudsen, 2000).

R. elatina may be confused with *Rutstroemia bulgarioides* (P. Karst.) P. Karst. because of their similar color and macroscopic appearance. Though both species have dark olive brown to black, goblet to

cup shaped and stipitate apothecia, the latter is separated from the former by its shorter asci (up to 100 µm long) and spores (up to 9 µm long). The most important characteristics of *R. bulgarioides* are to grow on damp spruce (*Picea* Link) cone lying on the ground between february and may, especially after the snow melts (Breitenbach and Kränzlin, 1984).

As the morphological data is not sufficient per se for the accurate identification of fungal species, the use of sequence data from the conserved DNA regions such as ITS is utilized as a useful tool in taxonomic studies for the last three decades (Baldwin, 1992; Raja et al., 2017). Additionally, ITS is one of the most common DNA barcoding markers and therefore serves as an

important source of information for the researchers to make comparisons of data. Hence, we used ITS region for the molecular identification of the Akata 7020. The BLAST and phylogenetic analyses carried out based on the ITS regions revealed 100% genetic similarity between the *Rutstroemia elatina* and the new record (GenBank ID: MN263048) (Figure 3).

In this present study, *R. elatina* was firstly recorded from Turkey and species numbers of Turkish *Rutstroemia* increased to total of three. Additionally, after *Abies alba* and *Picea abies*, *Abies nordmanniana* subsp. *equi-trojani* was also reported as a new host species for *R. elatina*.

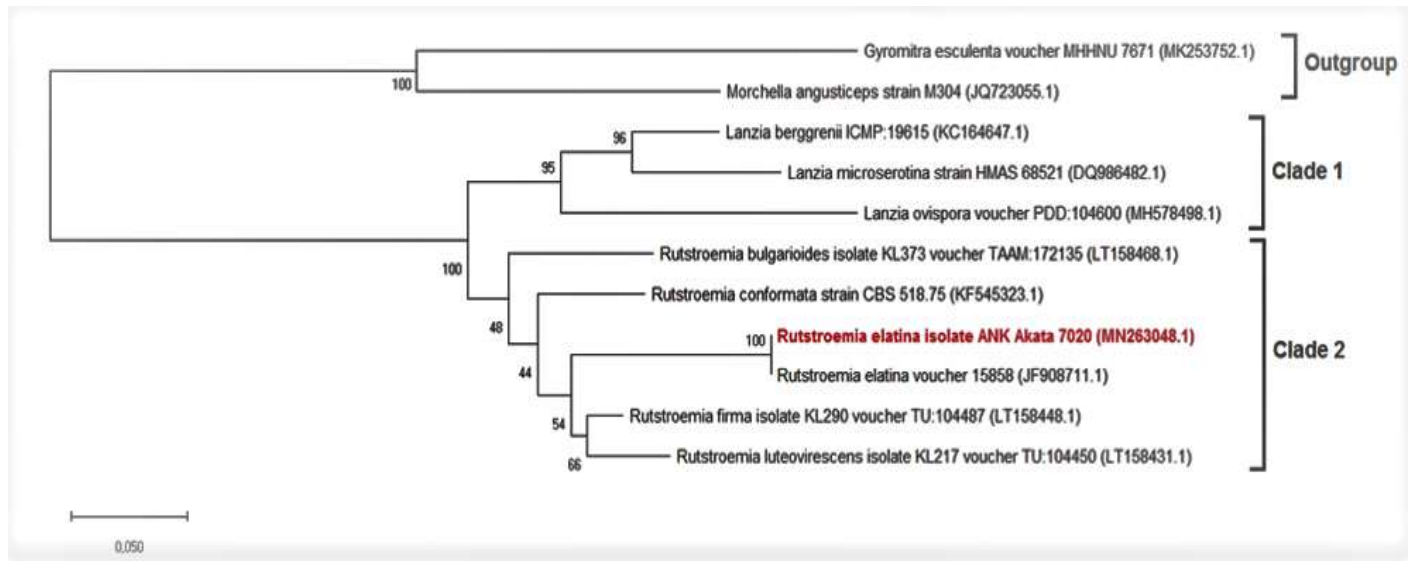


Figure 3. The neighbor joining tree demonstrating the phylogenetic relationships of 11 fungi inferred from the nuclear ITS region. Bootstrap values from 1000 bootstrap replicates were given next to the branches. All sequences were obtained from GenBank except for Akata 7020. *Gyromitra esculenta* and *Morchella angusticeps* were used as the outgroup samples. Accession numbers are given in parentheses. The scale bar shown at the lower left indicates a genetic distance of 0.05.

Şekil 3. 11 mantar türünün çekirdek ITS bölgelerinden çıkarılan filogenetik ilişkilerini gösteren komşu katılımı ağacı. 1000 önyükleme tekrarlarına ait önyükleme değerleri dalların yanında verilmiştir. Akata 7020 hariç geri kalan tüm diziler GenBank'tan alınmıştır. *Gyromitra esculenta* ve *Morchella angusticeps* dış grup örnekleri olarak kullanılmıştır. Dizilerin erişim numaraları parantez içerisinde verilmiştir. Sol altta gösterilen ölçek çubuğu 0.05 genetik uzaklığı belirtmektedir.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

The contribution of the authors is equal.

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