www.biodicon.com BIOLOGICAL DIVERSITY AND CONSERVATION

ISSN 1308-8084 Online ISSN 1308-5301 Print

Research article/Araştırma makalesi
DOI: 10.46309/biodicon.2023.1197860
165/2 (2023) 84-97

Molecular and taxonomic studies on some *Acarospora* (Acarosporales, Ascomycota) species in Türkiye

Mithat GÜLLÜ[*1],Mehmet Gökhan HALICI [1], Fatma ÖZTÜRK KÜP[1]
ORCID: 0000-0001-7100-9609; 0000-0003-4797-1157**;** 0000-0002-4785-4017

[1] Erciyes University, Department of Biology, Faculty of Science, 38039 Kayseri, Turkey

Abstract

Acarospora is a crustose lichen genus in the family Acarosporaceae and has a wide distribution. While the lichen genus *Acarospora* has more than 200 species in the world, the number of species so far determined in Türkiye is 41. Here we report three *Acarospora* species: *A. irregularis* H. Magn., *A. rosulata* (Th. Fr.) H. Magn., *A. thamnina* (Tuck.) Herre and two lichenicolous fungal species: *Lichenostigma svandae* Vondrák & Šoun, *Stigmidium fuscatae* (Arnold) R. Sant. new to Türkiye. Detailed information on these 5 taxa is provided along with photographs. The nrITS, β tubulin and mtSSU gene regions of the new *Acarospora* records are studied and their phylogenetical positions are discussed.

Key words: Türkiye, biodiversity, lichenized fungi, ITS gene region, *Acarospora*.

---------- * ----------

Türkiye'deki bazı *Acarospora* (Acarosporales, Ascomycota) türleri üzerinde moleküler ve taksonomik çalışmalar

Özet

Acarospora, Acarosporaceae familyasında yer alan kabuksu bir liken cinsi olup, dünya üzerinde geniş bir yayılış alanına sahiptir. Acarospora cinsi içerisinde dünyada 200'den fazla tür bulunurken, Türkiye'de şu ana kadar tespit edilen tür sayısı 41'dir. Bu çalışma, Türkiye liken florası için üç likenleşmiş mantar ve iki likenikol mantar türü yeni kayıt olarak sunulmaktadır: *Acarospora irregularis* H. Magn., *Acarospora rosulata* (Th. Fr.) H. Magn., *Acarospora thamnina* (Tuck.) Herre, *Lichenostigma svandae* Vondrák & Šoun, *Stigmidium fuscatae* (Arnold) R. Sant. Bu 5 takson hakkında detaylı bilgiler fotoğraflarla birlikte verilmektedir. Yeni kayıtların nrITS, β tubulin ve mtSSU gen bölgeleri çalışılmış ve türlerin filogenetik konumu *Acarospora* cinsinde yer almış ve cinsin diğer türlerinden net olarak ayrılmıştır.

Anahtar kelimeler: Türkiye, biyoçeşitlilik, likenleşmiş mantarlar, ITS gen bölgesi, *Acarospora*.

1. Introduction

Acarospora is a cosmopolite crustose lichen genus in the family Acarosporaceae. This genus is represented by more than 200 species in the world and the number of species so far determined in Türkiye is 41. The vast majority of species belonging to the genus is characterized by having more than 100 ascospores per asci, usually the pseudo-lecanorine apothecia immersed in the thallus [1,2].

Turkey's flowering plant biodiversity is well known. The country is very rich in flowering plants, about 10765 species of flowering plants are known and the endemism rate is quite high. But compared to European countries, the

[*] *Corresponding author* / Haberleşmeden sorumlu yazar: Tel.: +903522076666; Fax.: +903522076666; E-mail: mgullu@erciyes.edu.tr
 Received: 01.11.2023; Published: 15.08.2023 BioDiCon. 1063-011122

history of lichen biodiversity studies in Türkiye is quite new. A checklist of lichenized and lichenicolous fungi was published quite recently by John and Turk (2017) and according to this checklist 1898 lichenized and lichenicolous fungal species are known from Türkiye. The estimated number of lichenized and lichenicolous fungi from Türkiye is over 2000 [3].

The floristic studies on lichenized fungi in Türkiye are mostly based on anatomical and morphological characters [4]. However, in addition to anatomical and morphological studies in lichenized fungi, studies on nuclear rDNA have started to increase in the last decades [5-8]. Among the ribosomal regions, ITS gene region has the highest probability of succesful identification. Here we present 12 ITS sequence data and related Maximum Likelihood dendrograms. This study provides three lichenized fungi and two lichenicolous fungi new records for the lichen mycobiota of Türkiye.

2. Materials and methods

2.1. Lichen Material

Lichenized and lichenicolous fungal samples were collected from different regions of the Türkiye (Table 1). The specimens examined are deposited in Erciyes University Herbarium Kayseri, Türkiye (ERCH). The "ACA" code was used for numbering the samples in the herbarium database. A dissecting binocular microscope was used to examine the morphology. Detailed examination of the anatomy of the thallus and apotechia of the samples was carried out with a compound microscope. The colour of asci, hypothecium, epithecium and medulla were recorded both before and after spot tests. Asci and ascospores were visualized in the sections taken from the apothecia and their types and sizes were recorded.

Table 1. Samples collected from different regions of Türkiye and their localities

Species & Herbarium Accession No.	**Localitiy**	**GenBank Accession No.**		
		ITS	**mtSSU**	**β-tubulin**
Acarospora irregularis ACA 0.073	Türkiye, Erzurum, Narman, alt. 2315 m, on siliceous rocks	MK996290	MN005097	MN005699
Acarospora rosulata ACA 0.011	Türkiye, Kars, Kağızman, alt. 1040 m, on siliceous rocks	MK996279	MN005094	-
Acarospora rosulata ACA 0.020	Türkiye, Kırıkkale, Keskin, alt. 1290 m, on siliceous rocks	MK996280	-	-
Acarospora rosulata ACA 0.022	Türkiye, Kırşehir, Akçakent, alt. 1475 m, on siliceous rocks	MK996281	-	-
Acarospora rosulata ACA 0.040	Türkiye, Balıkesir, Susurluk, alt. 200 m, on siliceous rocks	MK996282	-	-
Acarospora rosulata ACA 0.057	Türkiye, Erzurum, on the highway to Tortum-Erzurum, alt. 1880 m, on siliceous rocks	MK996283	-	-
Acarospora rosulata ACA 0.065	Türkiye, Kırşehir, Çiçekdağı, alt. 1085 m, on siliceous rocks	MK996284	-	-
Acarospora rosulata ACA 0.108	Türkiye, Kayseri, Erciyes Mountain, alt. 2530 m, on siliceous rocks	MK996285	MN005095	-
Acarospora rosulata ACA 0.164	Türkiye, Yozgat, Şefaatli, alt. 1010 m, on siliceous rocks	MK996286	MN005096	-
Acarospora rosulata ACA 0.171	Türkiye, Erzurum, Narman, alt. 1885 m, on siliceous rocks	MK996287	-	-
Acarospora rosulata ACA 0.309	Türkiye, Aksaray, Büyük Hasan Mountain, alt. 1960 m, on siliceous rocks	MK996288	-	-
Acarospora thamnina ACA 0.033	Türkiye, Ankara, Kızılcahamam, alt. 1380 m, on siliceous rocks	MK996289	-	-

2.2. Molecular Methods

2.2.1. DNA Isolation, PCR and Sequencing

DNA isolation and PCR processes were carried out in the Molecular Biology Laboratory of Erciyes University. The commercial kit for DNA isolation (Dneasy Plant Mini Kit-Qiagen) was performed according to the protocol provided by the manufacturer. DNAs obtained from DNA isolation were used as templates for PCR. Primers "ITS1-F (5′-CTTGGTCATTTAGAGGAAGTAA-3′)" and "ITS4 (5'- TCCTCCGCTTATTGATATGC-3')" were used in PCR to amplify the repeat of the nrITS region (nrITS1-5.8S-nrITS2, ca. 500 bp) of nuclear ribosomal DNA [9,10]. For PCR 50 µL reaction volumes were prepared using 36.8 µl dH_2O, 2 µl of genomic DNA, 0.2 µl Taq DNA polymerase, 2 µl 10 mM dNTP, 0.5 µl each primer, 4 µl $MgCl_2$ (50 mM), 4 µl of 10 x reaction buffer on a thermal cycler equipped with a heated lid. The conditions for PCR are as follows: "An initial denaturation 4 min at 95 °C; 10 cycles with 1 min at 95 °C, 1.30 min at 56 °C, and 1 min at 72 °C; and 15 cycles with 1 min at 95 °C, 1.30 min at 51 °C, and 1 min at 72 °C; a final extension step of 8 min at 72 °C was added, after which the samples were kept at 4 °C." The products obtained from PCR were run on agarose gel and DNA bands were obtained from nine lichenized fungal samples. 1.6% agarose gel was used for all samples for electrophoresis and for size estimation 1 Kb Plus DNA Ladder was used and compared with the samples. Sequencing was done at the BM Labosis laboratory.

2.2.2. Sequence alignment and phylogenetic analysis

Sequence analyzes were performed by the BM Labosis Laboratory. Sequence results were compared on the NCBI website using the BLAST program. diting of the sequences was carried out with the Bioedit program [11]. Alignment of the sequences was done with the "Clustal W" option in the Bioedit program. Data selection was made from GenBANK, considering both the molecular results obtained and the species with which the samples were morphologically related. The selection of lichen samples from genbank was chosen by taking into consideration the molecular results as well as morphological relationships the with the studied samples. Maximum Likelihood phylogenetic trees were constructed on the "MEGA 6 (Molecular Evolutionary Genetics Analysis)" program using the Kimura 2-parameter model [12]. Gaps in the data were removed by pairwise deletion and the reliability of the obtained phylogenetic tree was tested with 1000 bootstrap replications. The gaps in data were deleted by pairwise deletion and 1000 bootstrap replications tests was used for controlling the reliability of the inferred tree. The species associated with the in-groups were selected as out-groups for the phylogenetic trees.

3. Results

3.1. Acarospora irregularis H. Magn.

Specimens examined: Türkiye, Erzurum, Narman, on the highway to Pasinler-Oltu, on siliceous rocks, 40º 09' 578" N, 41º 53' 810" E, alt. 2315 m, 29 July 2013 [ACA 0.073].

Description: Thallus dispersed or contiguous, convex to flat areaoles or squamules, edges crenulate or entire, covering rocks up to 10 mm in diam. Lateral and upper surfaces brown to reddish brown, epruinose (Figure 1). Lower surface brown to reddish brown. Algal layer thick, up to 150-200 mm thick, lower and upper surfaces uneven, forming algal palisades, interrupted by hyphal bundles, ca. 30–50 mm wide. Algal cells ca. 10 mm in diam. Apothecia to 1 mm in diam., usually 1, sometimes 2 or 3 per areole or squamules. Disc reddish brown, epruinose. Hymenium hyaline (70–)100–120(–150) µm high, epihymenium reddish brown and 15 µm high. Asci clavate, ca. 70–80 x 15–20 µm. Ascospores hyaline, simple, ca. 4–6 x 2.0– 3.0 µm broadly ellipsoid to ellipsoid [13].

Chemistry: all spot tests negative.

Ecology: This species can be found on non-calcareous rocks in low and high altitudes. The sample we studied was collected from the siliceous rock from approximately 2300 m.

Distribution: Europe (Slovakia, Russia, Italy, Hungary, Greece, Czech Republic) [13].

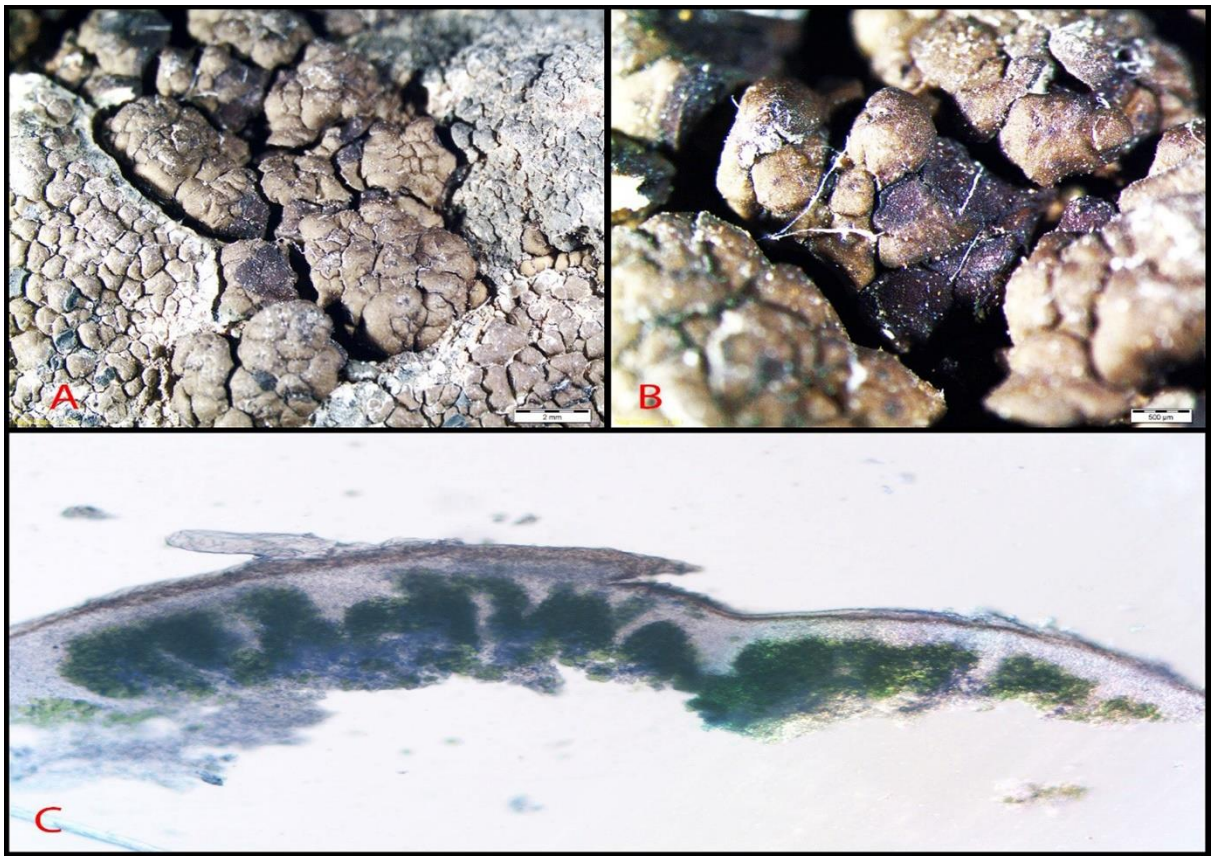

Figure 1. *Acarospora irregularis* A and B. Thallus with apothecia. C. Algal palisades with wide hyphal bundles.

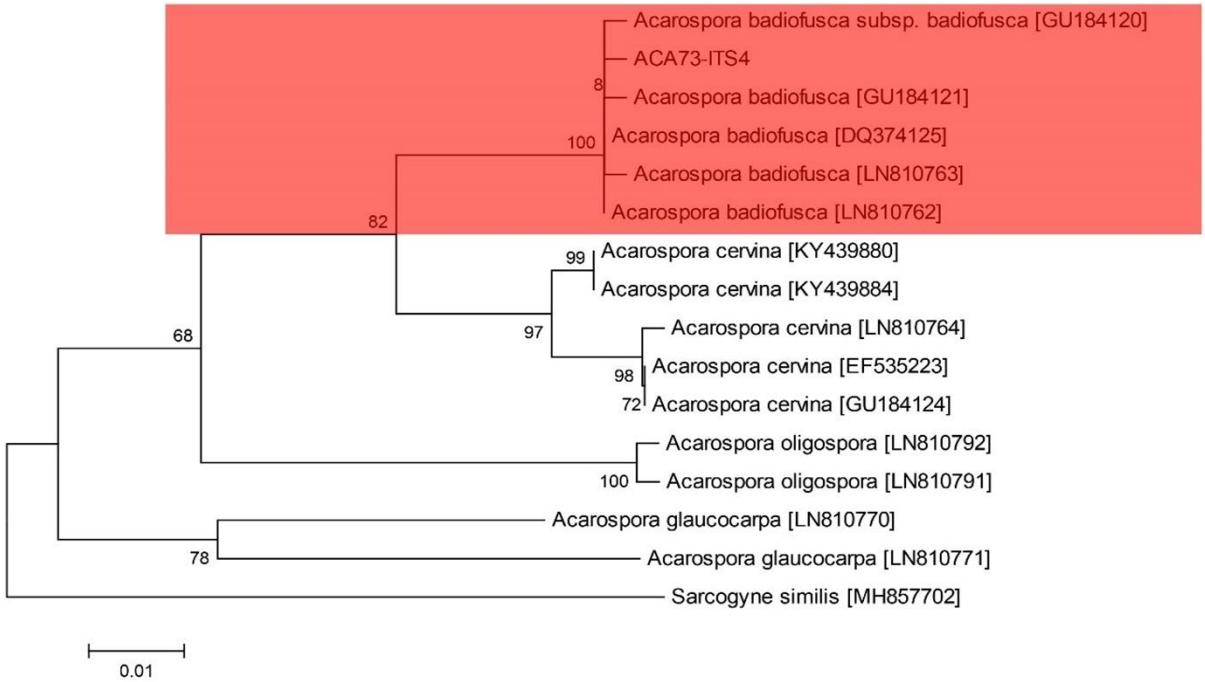

Figure 2. Maximum likelihood analysis inferred from ITS gene region sequences of *Acarospora irregularis* and related species.

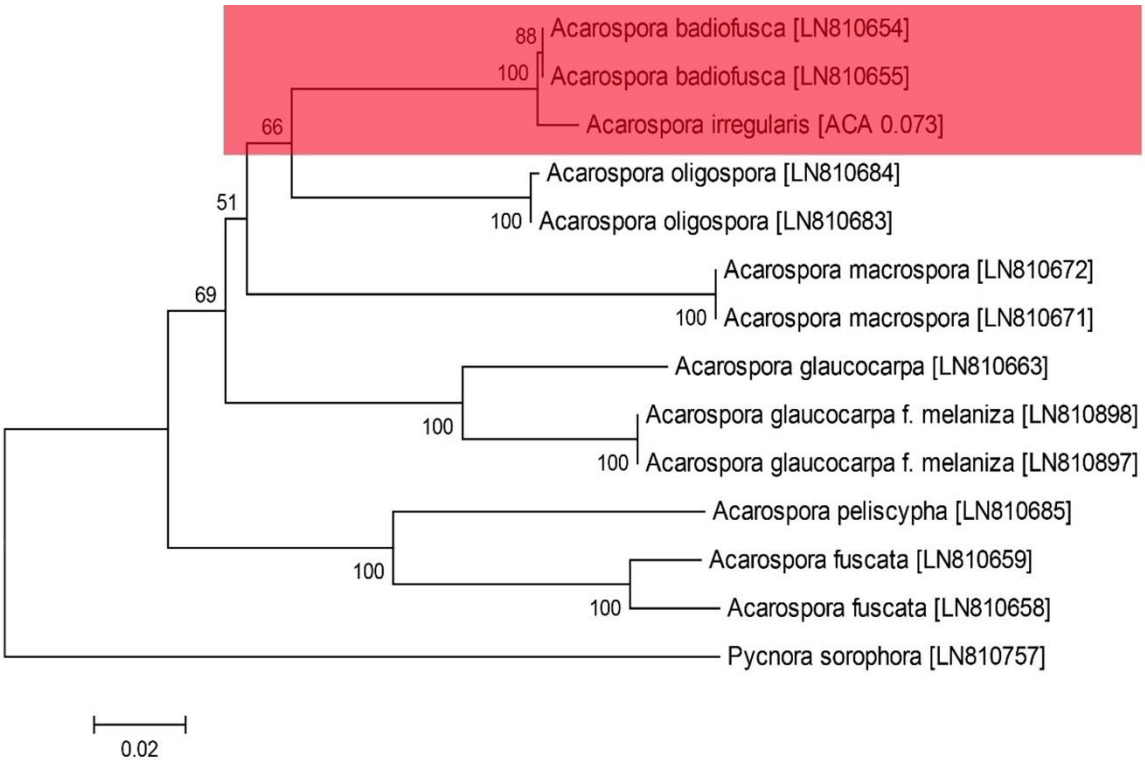

Figure 3. Maximum likelihood analysis inferred from β tubulin gene region sequences of *Acarospora irregularis* and related species.

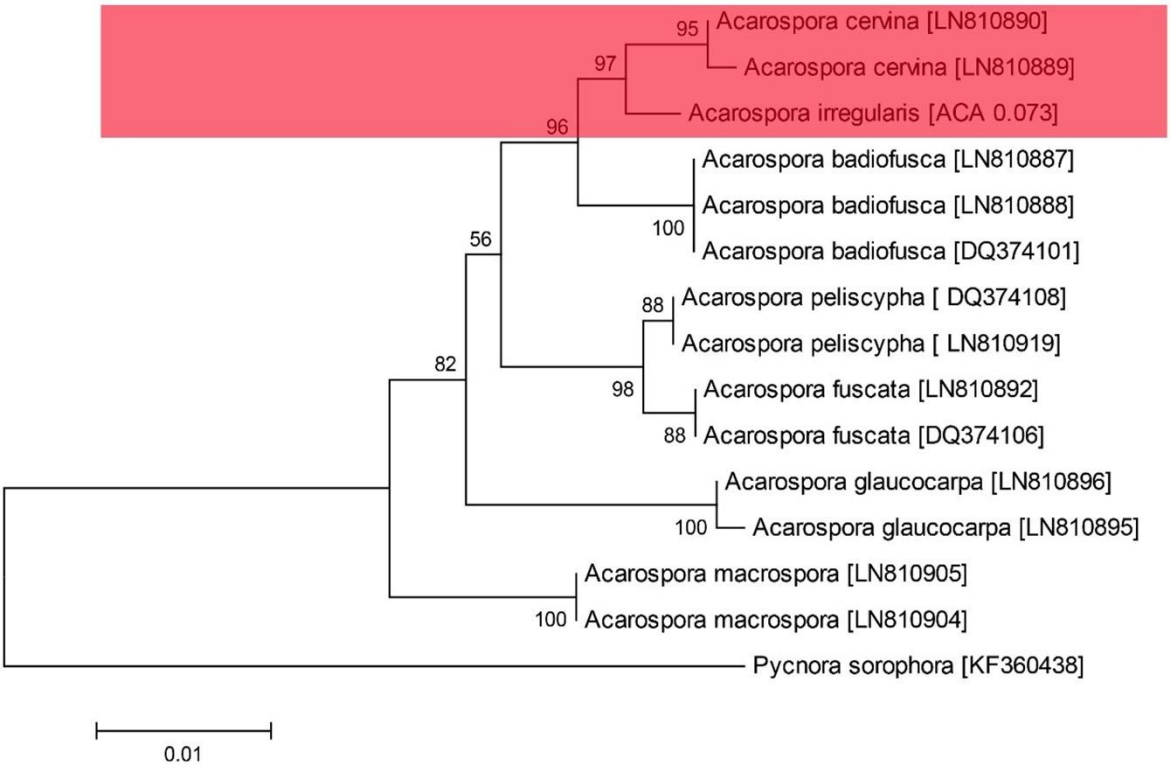

Figure 4. Maximum likelihood analysis inferred from mtSSU gene region sequences of *Acarospora irregularis* and related species.

3.2. Acarospora rosulata (Th. Fr.) H. Magn.

Specimens examined: Türkiye, Kars, Kağızman, Kuloğlu Village, on siliceous rocks, 40º 06' 342" N, 42º 59' 208" E, alt. 1290 m, 31 July 2013 [ACA 0.011]; Kırıkkale, Keskin, southwest of Keskin, Cabatobası Village, 39º 38' 11,5" N, 33º 15' 16" E, alt. 1040 m, 19 July 2012 [ACA 0.020]; Kırşehir, Akçakent, northwest of Halaçlı Köyü, on siliceous rocks, 39º 36' 01" N, 34º 12' 55" E, alt. 1475 m, 19 July 2012 [ACA 0.022]; Balıkesir, Susurluk, southeast of Karaköy, on siliceous rocks, 39º 52' 46" N, 28º 10' 52" E, alt. 200 m, 24 May 2012 [ACA 0.040]; Erzurum, on the highway to Tortum-Erzurum, on siliceous rocks, steppe vegetation, 40º 07' 267" N, 41º 25' 085" E, alt. 1880 m, 28 July 2013 [ACA 0.057]; Kırşehir, Çiçekdağı, 4 km southwest of Çiçekdağı, North of Alimpınar Village, 39º 35' 21" N, 34º 24' 14" E, alt. 1085 m, 19 July 2012 [ACA 0.065]; Kayseri, Erciyes Mountain, on siliceous rocks, 38º 33' 59.64" N, 35º 26' 02.32" E, alt. 2530 m, 20 August 2015 [ACA 0.108]; Yozgat, Şefaatli, on the highway to Karanlıkdere valley, on siliceous rocks, steppe vegetation, 39º 33' 569" N, 34º 41' 962" E, alt. 1010 m, 20 March 2015 [ACA 0.164]; Erzurum, Narman, on the highway to Pasinler-Oltu, southwest of Savatlı Village, on siliceous rocks, 40º 03' 700" N, 41º 49' 717" E, alt. 1885 m, 29 July 2013 [ACA 0.171]; Aksaray, Büyük Hasan Mountain, Near Karbeyaz Hotel, North of Basanbucağı, on siliceous rocks, steppe vegetation, 38º 09' 624" N, 34º 09' 917" E, alt. 1960 m, 05 June 2013 [ACA 0.309].

Description: Thallus areolate, dispersed to contiguous. Areoles angular to round, up to 1 mm diam, broadly attached wide. Surface dark brown to pale yellow brown, epruinose (Figure 5). Lateral and upper cortices paraplectenchymatous to subparaplectenchymatous, 30–50 µm thick, cells 3–7 × 2·5–6 µm, globose to elongate. Lower surface usually white or occasionaly brown. Photobiont green, chlorococcoid, algal cells up to 10 µm diam., 40– 100 µm thick as a continuous stratum. Medulla prosoplectenchymatous, hyaline. Apothecia one or many per areole, immersed and punctiform, 0.3–0.6 mm diam. Disc dark brown, epruinose, rough. Hymenium 70–120 µm tall, epihymenium reddish brown to dark brown. Paraphyses lax in water, 2-4 µm diam., septate, oil droplets sometimes present. Asci clavate, 80–100 × 22–26 µm. Ascospores 100–200 per asci, hyaline, simple, mostly 4–5 × 1.5–3 µm.
Chemistry: cortex KC+ pinkish red, C+ pinkish red.
Ecology: On siliceous and granite rocks, rarely on calcareous or limestone rocks. The samples we studied were collected from the siliceous rock at altitudes between 200-2500 m.
Distribution: Asia, western North America and Europe.

When examined morphologically, *Acarospora rosulata* (Th. Fr.) H. Magn. can often be confused with *A. bullata* (Th. Fr.) H. Mag. This species has morphological differences *A. bullata* - *Polysporinopsis rugulosa* (Körb.) Vězda. *Acarospora rosulata* differs from *A. bullata* in lacking large determinate thalli and distinctly rugulose apothecial discs [14]. In the Maximum Likelihood Dendrogram from ITS gene region sequences (Figure 6); *Acarospora rosulata* showed a close branch with *Acarospora bullata*. When we examined the dendrogram of the mtSSU gene region (Figure 7), *Acarospora rosulata* showed a good distinction.

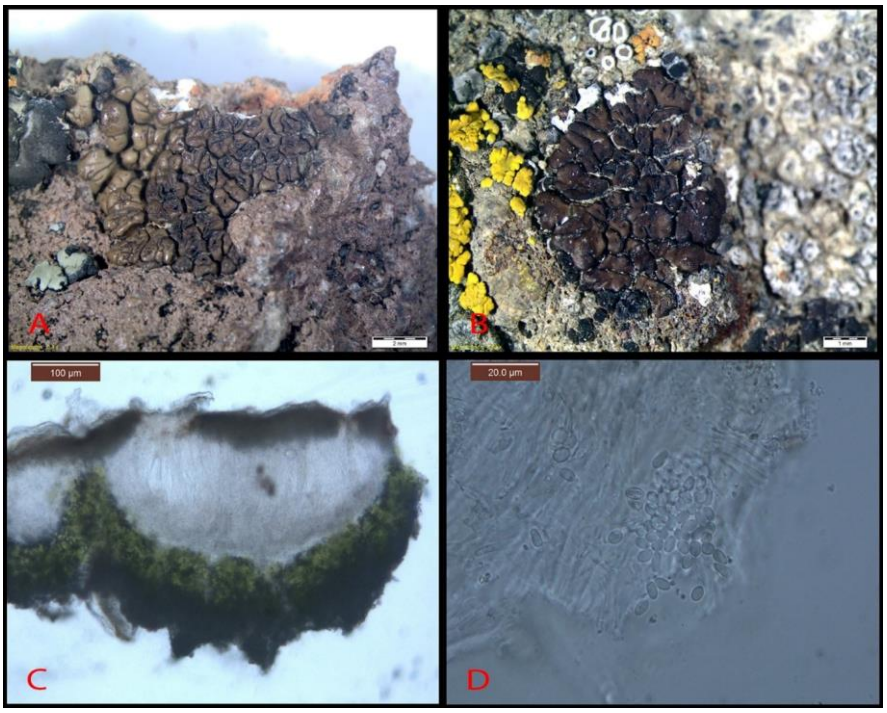

Figure 5. Morphological and anatomical images of *Acarospora rosulata* A and B. Thallus and Apothecia C. Hymenium D. Ascospores.

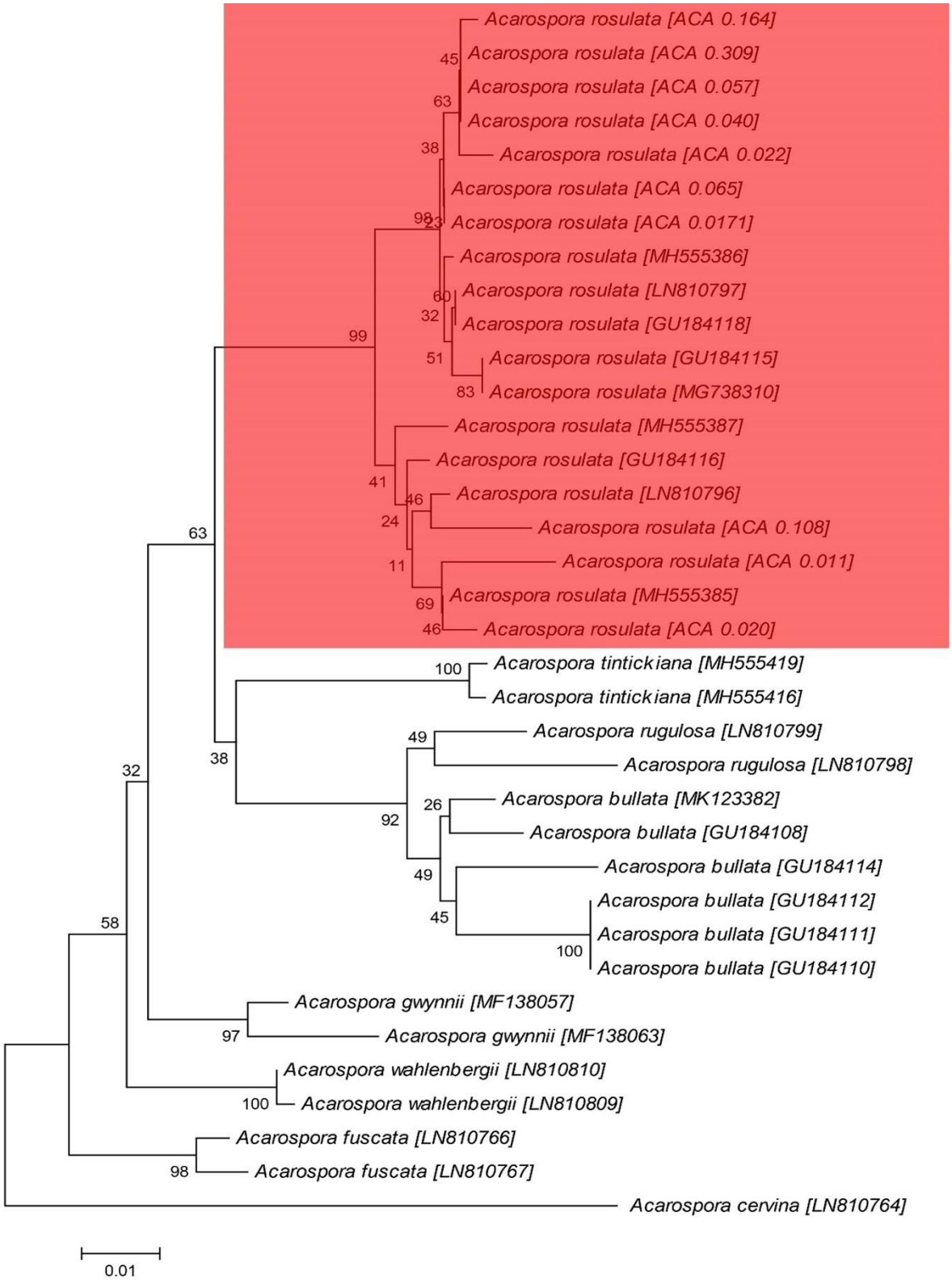

Figure 6. Maximum likelihood analysis inferred from ITS gene region sequences of *Acarospora rosulata* and related species.

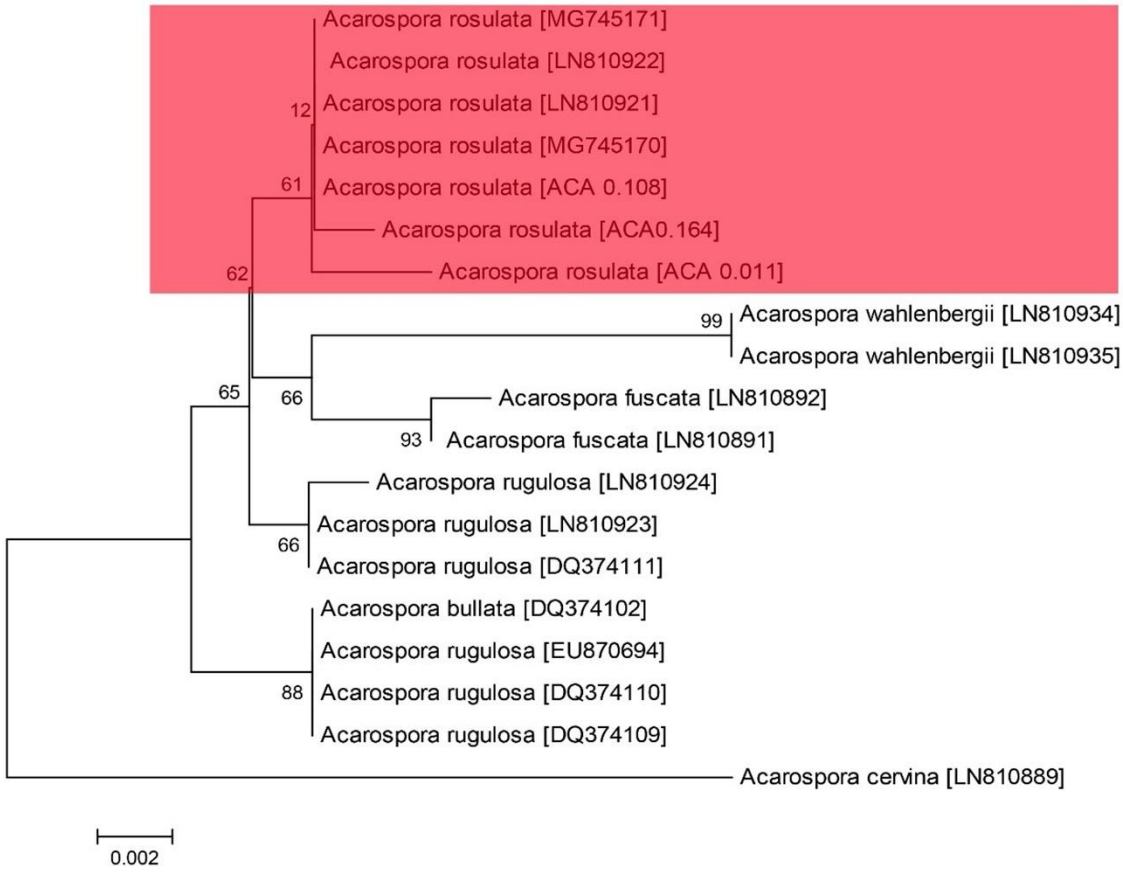

Figure 7. Maximum likelihood analysis inferred from mtSSU gene region sequences of *Acarospora rosulata* and related species.

3.3. Acarospora thamnina (Tuck.) Herre

Specimen examined: Türkiye, Ankara, Kızılcahamam, northwest of Dereiçi Village, on sliceous rocks, 40º 36' 44" N, 32º 31' 39" E, alt. 1380 m, 21 July 2012 [ACA 0.033].

Description: Tallus squamulose, on or among other lichens. Squamulose, irregular, sometimes lobed, thick, 0.3-1.5 mm in diam. The upper surface is in various shades of Brown (Figure 8). Medulla dirty brown or white. The lower surface carbonized or not, black. Apothecia, one or more per squamulose, round, 0.5 mm. The disc is very rough and usually black. Hymenium, golden yellow, 60-80 µm in diam. Asci 50-70 x 10-20 µm, with about 100 and more spores. Ascospores, hyaline, simple, often narrow ellipsoid, 5-7 x 2-3 µm.

Chemistry: strongly C + red and KC + red in cortex.

Ecology: In acidic rocks, it is generally found from the coast towards the inner parts. The sample we studied was collected from the siliceous rock from approximately 1400 m.

Distribution: Russia, Sweden, North America.

When examined morphologically, *Acarospora thamnina* (Tuck.) Herre can often be confused with *A. fuscata* (Ach.) Arnold. There are morphological differences between *Acarospora thamnina* and *A. fuscata*. Although *A. thamnina* sometimes grows on the same rock with a thallus in many shades of brown from yellowish to reddish, it has a strong C+ cortical reaction and thallus of *A. thamnina* is always shiny. In the Maximum Likelihood Dendrogram from ITS gene region sequences (Figure 9); the closest branching to *A. thamnina* is *A. insignis*. *Acarospora thamnina* is separated from *A. insignis* H. Magn. by its typical shininess, and its higher hymenium [15].

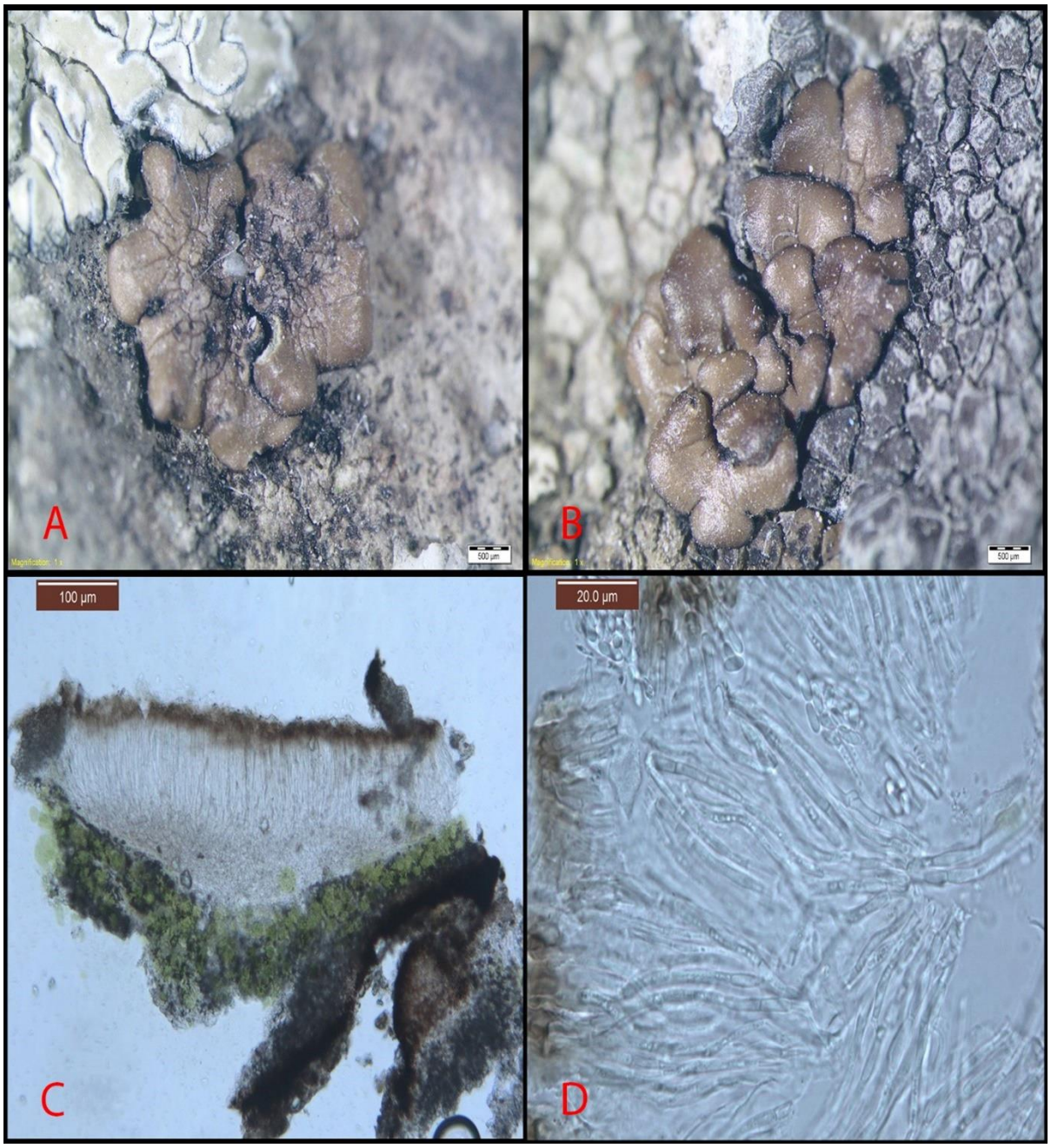

Figure 8. Morphological and anatomical images of *Acarospora thamnina* A and B. Thallus and Apothecia C. Hymenium D. Ascospores.

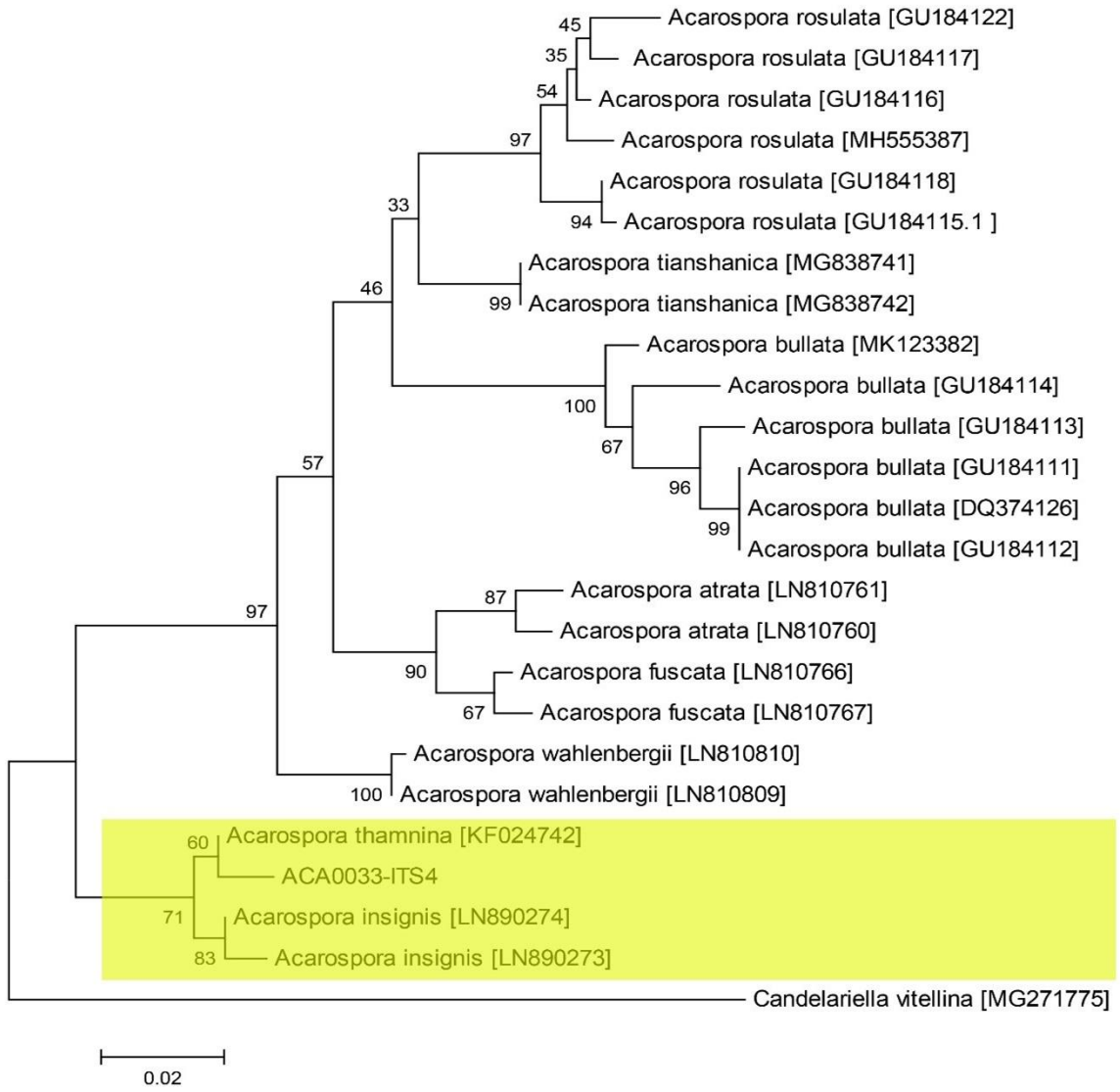

Figure 9. Maximum likelihood analysis inferred from ITS region sequences of *Acarospora thamnina* and related species.

3.4. Lichenostigma svandae Vondrák & Šoun

Specimen examined: Türkiye, Aydın, Çine, on sliceous rocks, 37º 38' 31.4" N, 28º 06' 07.4" E, alt. 520 m, 21 July 2016.

Description: Lichenicolous, on the thallus of *Acarospora cervina* A. Massal. It has irregular and superficial vegetative hyphae forming dark web-like patches over the apothecial discs or/and on the thallus (Figure 10). Ascomata black, shiny, scattered, cushion-like, rounded, 30–70 µm tall, 80–150 µm wide, Asci 6–8-spored, subglobose to broadly clavate. Ascospores hyaline at first, when old becoming greyish and then brownish, 1 septate. 11.5–12.5–13.5 × 6.2–7.1–8.0 µm [16].

Chemistry: Ascomatal tissue I-.

Ecology: This species grows on the apothecia and thalli of *Acarospora cervina* which grows on sun-exposed limestone rocks [16]. The sample we studied is grows on the areoles and apothecia of *Acarospora cervina* on calcareous rock.

Distribution: This species is only reported from Czech Republic and Ukraine (Crimean Peninsula) [16].

During our studies of the biodiversity of lichenicolous fungi in Turkey, we collected a brown, pruinose *Acarospora cervina* calcerous rocks from Turkey infected by a *Lichenostigma* species. The specimen was compared with three other *Lichenostigma* species (*Lichenostigma anatolica, L. gracile and L. subradians*) also found growing on *Acarospora spp.* [16,17]. We concluded that it is new to Turkey and describe it here. *Lichenostigma anatolica* does not have vegetative hyphae or poorly developed. However, vegetative hyphae in *L. svandae, L. gracile and L. subradians* are superficial and form black structures. Whereas *Lichenostigma svandae*, *L. gracile* and *L. subradians* has a negative reaction with ascomata tissue I, *L. anatolica* gives blue colour with ascomata tissue I. *Lichenostigma svandae* is found on *Acarospora cervina* species, while other *Lichenostigma* species are distributed on different *Acarospora* species [18].

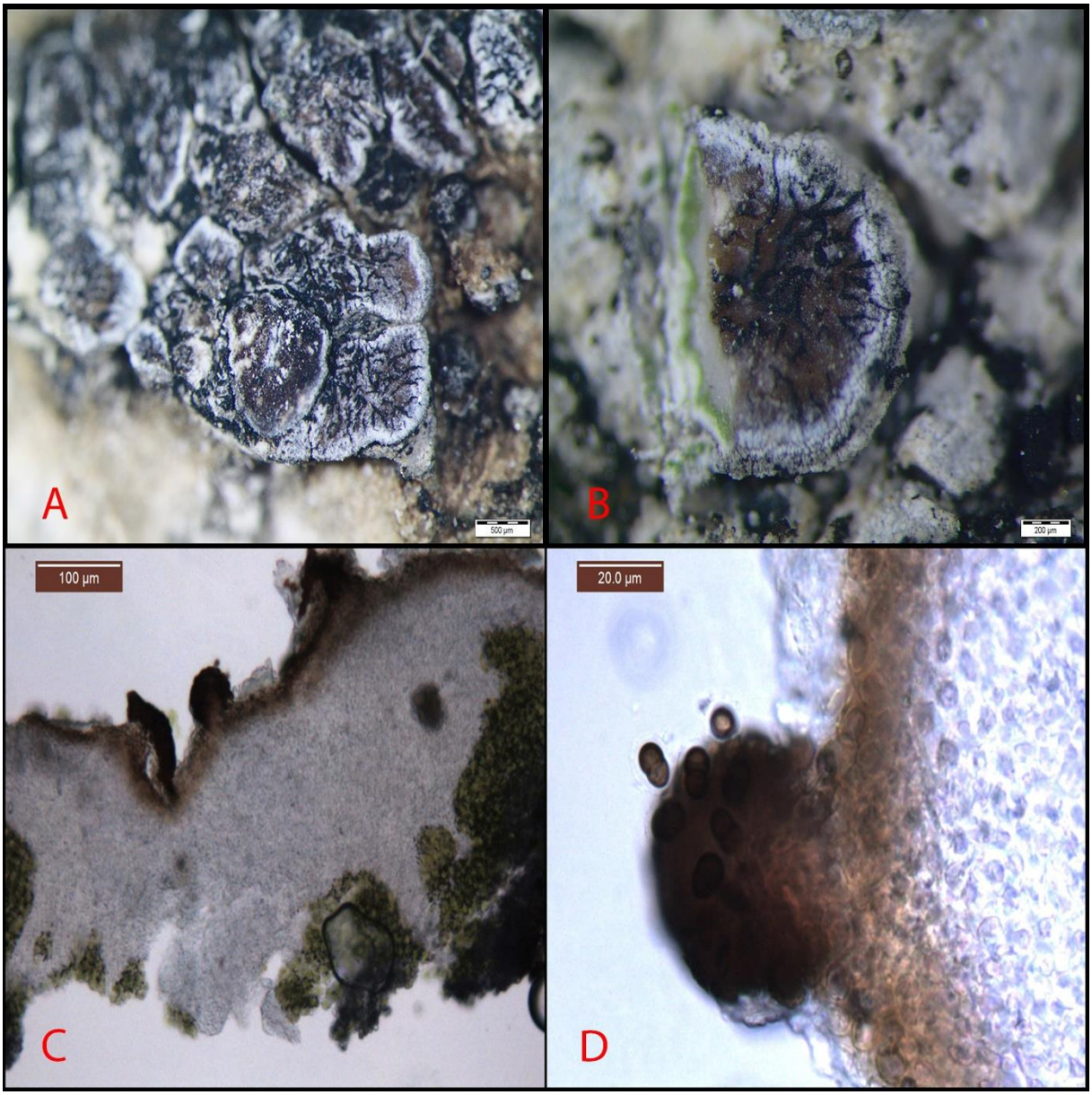

Figure 10. *Lichenostigma svandae*. A and B. Strongly infected thallus and apothecia of *Acarospora cervina* C. cross-section of an ascoma D. brown ascospores.

3.5. *Stigmidium fuscatae* (Arnold) R. Sant.

Specimen examined: Türkiye, Aydın, Çine, on sliceous rocks, 37º 38' 31.4" N, 28º 06' 07.4" E, alt. 520 m, 21 July 2016.

Description: Lichenicolous, on the thallus of *Acarospora fuscata* (Ach.) Arnold (Figure 11). Pseudothecia, black, globose, 50-100 µm wide and 50-100 µm high. Upper part of the wall dark brown. Asci, 8-spored, 30-40 x 10-15 µm, I-. Ascospores, hyaline, 1-septate, elipsoid, 10-13 x 4-5 µm. Pycnidia not seen [19].

Chemistry: Ascomatal tissue I-.

Ecology: *Stigmidium fuscatae* grows on apothecia and thalli *Acarospora fuscata* which grows on siliceous rocks. The sample we studied is grows on the areoles and apothecia of *Acarospora fuscata* on siliceous rock.

Distribution: *Stigmidium fuscatae* is known from Europe, Africa (South Africa) and North [19].

During our studies of the biodiversity of lichenicolous fungi in Turkey, we collected a brown, *Acarospora fuscata* siliceous rocks from Turkey infected by a *Stigmidium* species. The specimen was compared with two other *Stigmidium* species (*Stigmidium epixanthum, S. rouxianum*) also found growing on *Acarospora spp.*. We concluded that it is new to Turkey and describe it here. We compared *Stigmidium* species found on *Acarospora* in Table 2.

Table 2. Comparison of *Stigmidium* species growing on *Acarospora*.

Species	Ascomata size (µm)	Asci size (µm)	Ascospores (µm)	Host lichen	References
S.epixanthum	100–140 × 120–180	40–60 × 12–15	11–17× 5–7	yellow *Acarospora*	[19,20]
S.rouxianum	110–170 × 140–180	40–75× 13–18	(13.5–) 16–17.5–19 (–21) × 5.5–6.5	*A.cervina* *A.obpallens*	[21]
S. fuscatae	50–100× 50–100	30–40 × 10–15	(8–) 10–12× 4–5 (–5.5)	*A.fuscata*	Present paper

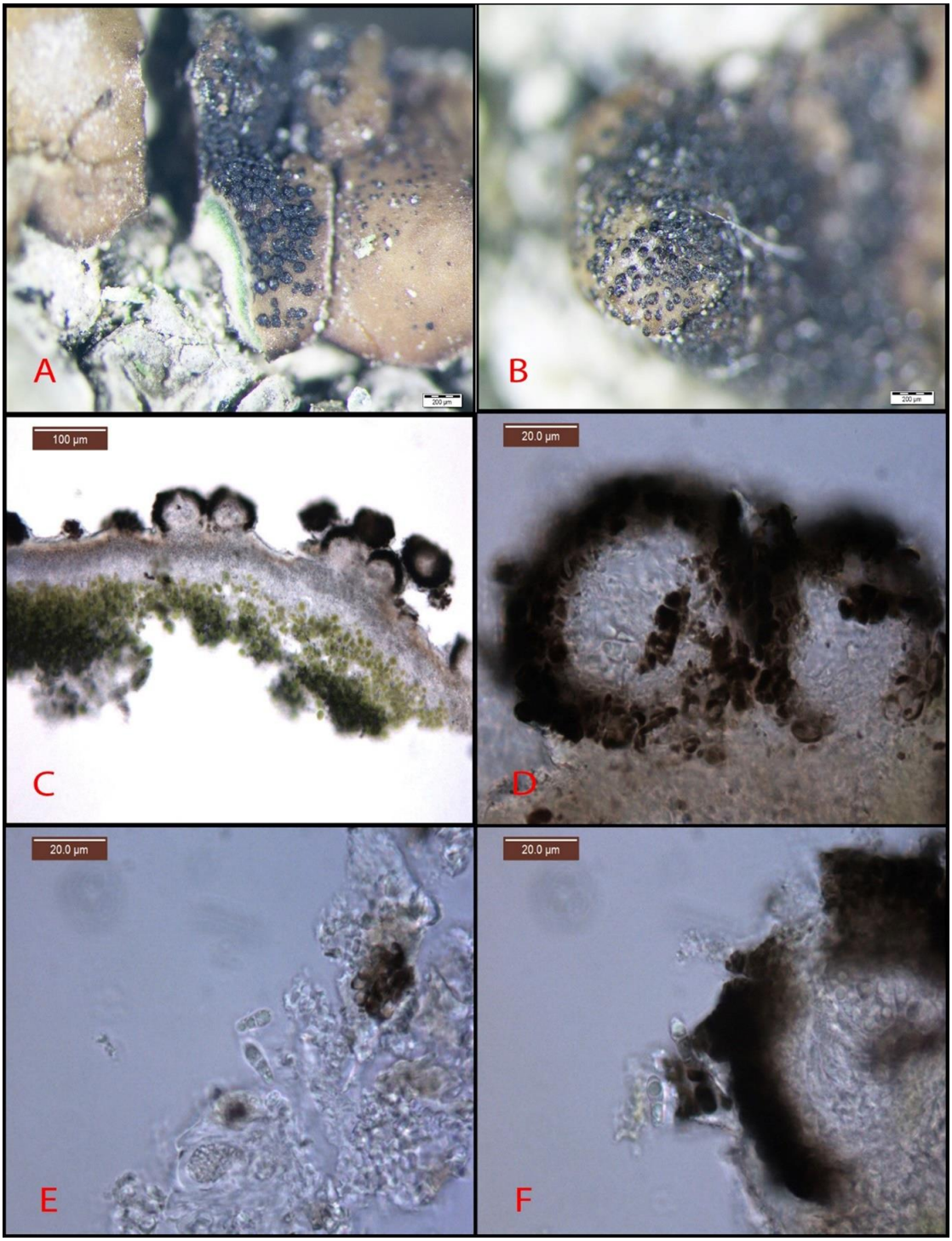

Figure 11. A and B. infected thallus of *Acarospora fuscata*. C and D. section of ascoma. E and F. Ascospores.

4. Conclusions and discussion

The circumscription of lichen-forming fungal species has traditionally been guided by morphological, chemical and ecological features. However, because lichens generally display few taxonomically useful characters, of which many are widely variable, the homology of character states within and among groups is difficult to assess. Therefore, molecular data have gained importance in lichen systematics and now have a significant impact on the classification and taxonomy of lichenized ascomycetes. In most cases, our phylogenetic analyses support the traditional species delimitation based on morphological and chemical traits.

Türkiye is a country rich in lichen biodiversity. It has a widespread distribution in our country in species belonging to the genus *Acarospora*. The *A. rosulata* (Th. Fr.) H. Magn. species described in this article is actually a lichen species found in almost every region of our country. However, this species has never been determined in previous studies. In this study, we concluded that some *A. bullata* Anzi species, which are herbarium material, are actually *A. rosulata* species as a result of molecular studies. *A. bullata* is a species confused with *A. rosulata*. *A. rosulata* differs from *A. bullata* in that it lacks a prominent thallus with fan-shaped lobes and a clearly rugulous apothecia disc [14]. When examined molecularly, these species showed good discrimination on ITS and mtSSU phylogenetic trees (Figure 6 and Figure 7).

In this study, molecular analyzes of ITS, mtSSU and β-tubulin gene regions were performed for 3 lichenized fungi (*Acarospora irregularis*, *A. rosulata* and *A. thamnina*), which are generally distributed on siliceous rocks in Türkiye. In addition, 2 lichenicolous fungi (*Lichenostigma svandae* and *Stigmidium fuscatae*) on brown *Acarospora* were studied morphologically and anatomically. Sometimes morphological characters used in lichen classification lead to misidentifications. Because of this, it is also important to make molecular studies in lichen taxonomy. ITS, mtSSU and β-tubulin sequences provided for this study are now in GenBank and can be used in the future phylogenetic studies of lichens.

Acknowledgements

This study was supported by the Fund of Erciyes University Scientific research Project (Project no: FDK-2015-5927). Thanks to Dr. Kerry Knudsen for the identification of the lichen specimens.

References

[1] Temina, M. & Nevo, E. (2009). Lichens of Israel: diversity, ecology, and distribution. *BioRisk*, 3, 127-136. DOI: 10.3897/biorisk.3.25

[2] John, V., Türk, A. (2017). Türkiye likenleri listesi. Nezahat Gökyiğit Botanik Bahçesi Yayını.

[3] Halıcı, M.G., Hawksworth, D. & Aksoy, A. (2007). Contributions to the lichenized and lichenicolous fungal biota of Turkey. *Mycotaxon*, 102.

[4] Türk, A., Halıcı, M. G., Candan, M., Yavuz, Y. (2015). The lichenized fungus genus Peltigera in Turkey. *Biological Diversity and Conservation* 8(2), 146-156.

[5] Aras, S. & Duman, D.C. (2006). Isolation of DNA for sequence analysis from herbarium material of some lichen specimens. *Turkish Journal of Botany*, 30(6), 449-453.

[6] Halıcı, M.G., Vondrák, J., Demirel, R., Ceylan, A. & Candan, M. (2014). Teloschistaceae (lichenized ascomycetes) in Turkey II.-Some poorly known taxa. Supported by molecular data. *Nova Hedwigia*, 98. DOI: 10.1127/0029-5035/2013/0163

[7] Barak, M.Ü., Halıcı, M.G. & Güllü, M. (2016). Identification of some lichenized fungi species of Erciyes Mountain Kayseri/Turkey by using ITS rDNA marker. *Biyolojik Çeşitlilik ve Koruma*, 9(2), 84-95.

[8] Vondrak, J., Halıcı, M. G., Güllü, M. & Demirel, R. (2016). Taxonomy of the genus *Athallia* and its diversity in Turkey. *Turkish Journal of Botany*, 40(3), 319-328. DOI: 10.3906/bot-1502-12

[9] White, T., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications*, 18(1), 315-322.

[10] Gardes, M. & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes- application to the identification of mycorrhizae and rusts. *Molecular ecology*, 2(2), 113-118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

[11] Hall, TA. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. *Acids Symposium Series*, 41: 95-98.

[12] Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular biology and evolution*, 30(12), 2725-2729. doi: 10.1093/molbev/mst197

[13] Knudsen, K., Kocourková, J. & Nordin, A. (2014). Conspicuous similarity hides diversity in the *Acarospora badiofusca* group (Acarosporaceae). *The Bryologist*, 117(4), 319-328. DOI: 10.1639/0007-2745-117.4.319

[14] Brinker, S. R. & Knudsen, K. (2019). The first confirmed report of *Acarospora bullata* from North America. *Opuscula Philolichenum*, 18, 11-16.

[15] Westberg, M., Timdal, E., Asplund, J., Bendiksby, M., Haugan, R., Jonsson, F., … Millanes, A. (2015). New records of lichenized and lichenicolous fungi in Scandinavia. *MycoKeys*, 11, 33-61. DOI: 10.3897/mycokeys.11.6670

[16] Vondrak, J. & Jaroslav, Š.O.U.N. (2007). *Lichenostigma svandae*, a new lichenicolous fungus on *Acarospora cervina*. *Lichenologist*, 39(3), 211-216. DOI: 10.1017/S0024282907006731

[17] Calatayud, V., Navarro-Rosinés, P. & Hafellner, J. (2002). A synopsis of *Lichenostigma* subgen. Lichenogramma (Arthoniales), with a key to the species. *Mycological Research*, 106(10), 1230-1242. https://doi.org/10.1017/S095375620200655X

[18] Halıcı, M. G., Aksoy, A. (2009). Lichenised and Lichenicolous Fungi of Aladağlar National Park (Niğde, Kayseri and Adana Provinces) in Turkey. *Turkish Journal of Botany*. 33/3. 169-189. DOI: 10.3906/bot-0810-14

[19] Triebel, D and Cáceres, M. (2004). *Stigmidium*. – In: Nash, T. H. III, Ryan, B. D., Diederich, P., Gries, C. & Bungartz, F. (eds). Lichen Flora of the Greater Sonoran Desert Region 2: 703–707. – Tempe, Arizona: Lichens Unlimited, Arizona State University.

[20] Hafellner, J., D. Triebel, B.D. Ryan and T.H. Nash III. (2002). On lichenicolous fungi from North America. II. *Mycotaxon* 84: 293–329.

[21] Kocourková, J. & Knudsen, K. (2018). *Stigmidium rouxianum* (Mycosphaerellaceae, Dothideomycetes), reported new for North America and California. *Opuscula Philolichenum*, 17, 293-298.