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ARTICLE

New records of crustose lichen species for Iran and Asia

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ABSTRACT The Khorasan-Razavi Province in northeastern Iran boasts a diverse lichen flora, fostered by its climatic diversity and extensive calcareous substrates, which provide an ideal habitat for crustose saxicolous lichens. However, the distribution of these lichens in this province has remained inadequately explored. Thus, this study aims to conduct a taxonomic investigation of crustose saxicolous lichens within this area. Between 2018 and 2019, we employed survey methods to collect 436 rock substrates from 44 locations within the province. Lichen specimens were meticulously examined, considering their morphological, anatomical, and ecological attributes. Light microscopy was utilized to scrutinize morphological and anatomical features, with a specific focus on the thallus and perithecium. Our findings reveal the first-ever documentation of the species *Caloplaca atroalba* (Tuck) Zahlbr. (1930) in Asia. Additionally, we report the presence of the species *Pyrenodesmia micromontana* (Frolov, Wilk, and Vondrák) Hafellner & Türk (2016), *Arthonia mediella* Nyl. (1859), *Lecania erysibe* (Ach.) Mudd (1861), *Placopyrenium canellum* (Nyl.) Gueidan & Cl. Roux, *Verruculopsis minutum* (Hepp.) Krzewicka, and *Involucropyrenium waltheri* (Kremp.) Breuss, along with the genus *Involucropyrenium*, as new records for the Iranian flora. **Acta Biol Szeged 67(1):55-61 (2023)**

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Introduction

Iran, a vast country in southwest Asia, boasts a diverse lichen flora due to its extensive geographic range and varied climatic conditions (Seaward et al. 2004). A comprehensive update of the Iranian lichen inventory in 2008 identified a total of 590 lichen species and 55 lichenized fungal species in the country (Seaward et al. 2008). However, the complete characterization of Iran's lichen flora remains an ongoing endeavor. In this context, the Khorasan-Razavi province in northeastern Iran stands out as one of the most biotope-rich regions (Babaeian and Najafi 2010). Thanks to its diverse climatic conditions and extensive presence of calcareous substrates, this province holds the potential to serve as an ideal habitat for crustose saxicolous lichens (Moniri and Sipman 2011). Crustose saxicolous lichens are known to thrive on various types of rocks, including sandstone, lime, sandy limestone, dolomite, schist, granite, ophiolite, and volcanic rocks (Babaeian and Najafi 2010; Baradaran et al. 2020). As a result, it is anticipated that these lichens exhibit significant diversity within the Khorasan-Razavi province. However, the distribution of these lichens within this province has not received sufficient investigation to date (Asef 2010, 2007). Hence, the primary objective of this study is to conduct a taxonomic examination of the crustose saxicolous lichens in the Khorasan-Razavi province. Our fieldwork encompassed the geographical coordinates between 36° 19' 16.4892'' N and 59° 36' 57.5004'' E, covering an area that constitutes 8.8% of Iran's total landmass. Here, we provide morphological descriptions of one new species originating from Asia and four new species, along with one newly discovered genus native to Iran.

Materials and Methods

Study area

Between 2018 and 2019, we employed a survey method to collect samples from 436 rock substrates at 44 distinct locations within Khorasan-Razavi Province. This province, situated at an elevation of 970 meters above sea level in northeastern Iran, shares its borders with Turkmenistan to the north and Afghanistan to the east (Shojaee and Fallah-Ghalhari 2017). Using geological maps, sampling sites were selected in pristine and accessible locations at least one kilometer apart. Calcareous (limestone) substrates were located through geological maps, with sampling sites chosen from both the northern

and northeastern regions of Khorasan-Razavi Province, where carbonate sedimentary substrates (limestones) are more prevalent, as well as the less common southern and central regions. Moreover, due to the presence of certain species of crustose saxicolous lichens on siliceous soils, siliceous substrates were examined separately.

Identification of lichen specimens

To identify lichen specimens, we relied on relevant literature and identification keys, focusing on the morphology and anatomy of the lichens. In this context, a stereomicroscope (Carl Zeiss C. Stemi, Germany) was used to examine the morphology of both the vegetative and reproductive components of the lichens. Furthermore, hand sections of the thallus and perithecium were mounted in water for anatomical analysis, carried out under a light microscope (Carl Zeiss Axioskop 40, Germany). For this purpose, ten samples of each lichen species were collected, and ten measurements were recorded for each specimen following the standard calibration slide protocol based on Vondrak et al. (2013). The lichen specimens were further identified using the standard chemical spot test, which involved placing a drop of 10% KOH (K), NaClO (C), KC, N, and KOH/I solutions on the thallus and apothecia of lichen specimens. Additionally, hydrochloric acid was used to distinguish between calcareous and siliceous rocks (Orange et al. 2001)

Results and Discussion

During floristic surveys, we discovered *Caloplaca atroalba* (Tuck) Zahlbr. (1930) for the first time in Asia. Furthermore, we recorded five species of crustose lichens that were previously unreported in Iran. All specimens are kept in the Research Institute of Forest and Rangelands (TARI), Tehran, Iran (number of herbarium codes: 12174 for *Caloplaca atroalba,* 12175 for *Pyrenodesmia micromontana,* 12176 for *Arthonia mediella,* 12177 for *Lecania erysibe,* 12180 for *Placopyrenium canellum*, and 12179 for *Involucropyrenium waltheri*).

Caloplaca atroalba **(Tuck) Zahlbr. (1930)**

Description

Thallus crustose, areolate, gray to olive-gray, no prothallus. Apothecia 0.5-0.8 mm in diameter, lecanorine, disc black-brown, slightly convex, smooth, and without pruinose, thalline exciple, hymenium hyaline, 82.5-91.4 µm in length, paraphyses with 2-3 distinct swollen apical cells, simple; subhymenium hyaline; asci 8-spored; ascospores hyaline, 14.2-15.2×7.1-8.2 µm, space between locules 1.5-2.5 µm (Fig. 1). No secondary metabolite is known.

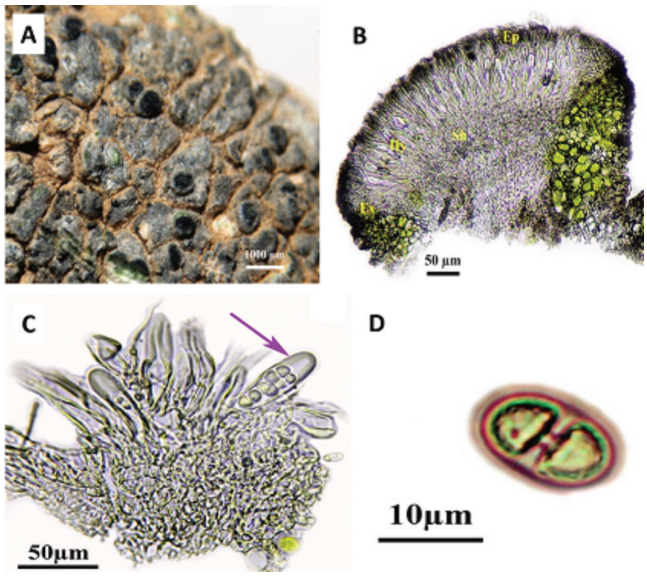

Figure 1. Morphological and anatomical features of *Caloplaca atroalba* (Tuck) Zahlbr. (A) areolate thallus and lecanorine apothecia; (B) apothecia cross-section, EP (epithecium), Hy (hymenium), Sh (subhymenium), Ex (exciple); (C) the tip of the arrow indicates a teloschistes-type ascus; (D) Polarilocular and bicellular spore.

Spot test

Epihymenium K+ purple; apothecia margin K+ bright purple.

Geographical distribution and Substrate

Caloplaca atroalba can be found in North America, in particular Southern California, Utah, Ohio, and Arizona in the United States, as well as Alberta, Canada. Furthermore, this species has been reported in Sweden. The substrate is calcareous and non-calcareous rocks, as well as sandstone (Wetmore 1994; Kondratyuk et al. 2013). In our study, *C. atroalba* specimens were collected in Bakharz in April 2019, Latitude: 35° 11' 39" N, Longitude: 60° 20' 57" E, 2120 m, calcareous substrate, semi-dry-cold climate.

Note

C. atroalba is similar to *C. variabilis*, but the latter is distinguished from *C. atroalba* by having a pruinose disc, an apothecial border, and compact granules that are insoluble in KOH. Furthermore, some *C. atroalba* specimens found on sandstone have a thin and irregular thallus similar to that of *C. oblongula*, but the latter species has a lecidecine apothecial margin (Nash 2002; Wilk 2011). *Caloplaca atroalba* (Tuck) Zahlbr. (1930) is reported here for the first time from Asia.

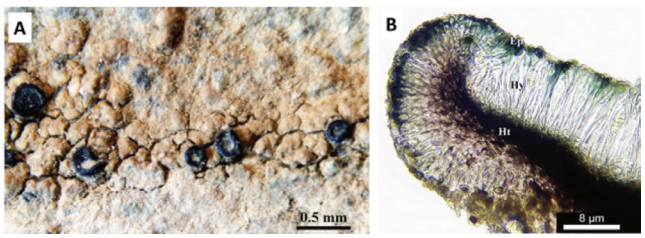

Figure 2. Demonstration of the morphological and anatomical features of *Arthonia mediella* Nyl. (1859); (A) representation of crustose thallus and apothecia; (B) a cross-section of apothecia, EP (epithecium), Hy (hymenium), Ht (hypothecium).

Arthonia mediella **Nyl. (1859)**

Description

Thallus crustose, pale, light brownish yellow, cracked-areolate. Apothecia single, circular, 0.14-0.31 mm in diameter, disc black to dark brown, shiny to matt; hypothecium brown to reddish-brown, 34.2-59 µm tall; hymenium colorless to yellow, 45.7-86 µm tall; epihymenium dark; paraphyses agglutinated and rarely branched, swollen near apex, 3.4-4.6 µm in wide; asci 8-spored, ascospores 3-5 septate, spindle-shaped, 11.4-19.9×2.8-4.2 µm, yellow, not integrated, and disc black to brown (Fig. 2). A secondary metabolite is unknown.

Spot test

Hymenium I+ red to orange.

Geographical distribution and Substrate

Arthonia mediella grows in far and cold regions of the Alps as well as in the northern lands. Besides, this species has been documented in Russia (Tarasova et al. 2016), Turkey (Öztürk and Güvenç 2010), North America, and Alaska to a limited extent (Candan and Halici 2009). Limestone and siliceous rocks serve as the growth substrate for *A. mediella*. It also grows on deadwood and woody plants (Candan and Halici 2009; Frisch et al. 2014). In April 2019, we collected *A. mediella* specimens from Zoshk, Latitude: 36° 16' 04" N, Longitude: 59° 09´ 14" E, 2433 m, calcareous substrate, cold semi-dry climate.

Note

The *A. mediella* resembles *A. muscigena* and grows alongside it on occasion, but it differs in having upright, capitate, apices to paraphyses, and 3-septate ascospores. Furthermore, *A. mediella* does not have *Trentepohila* as a photobiont, and its photobiont is *Chloroccoid*. A saxicolous morph of this species is the type of *A.myriocarpella* (Coppins 2002). According to the second revised checklist of lichenized, lichenicolous, and allied fungi for Iran, 13 species of *Arthonia* have been documented from Iran (Seaward et al. 2008). The species *A. mediella*, which is reported in this paper for the first time from Iran, will be added to that group.

Involucropyrenium waltheri (kremp.) Breuss. Synonyms: Catapyrenium waltheri

Description

Prothallus black, extends between and around the squama. Thallus squamulose-crustose, (70-)100-200(300) µm thick, less than 1 mm wide, brown to grey, matt, smooth to convex, squama margins entire to slightly dentate-lobate, lower surface dark with blackish rhizohyphae. Upper cortex paraplectenchyma, 10-25 µm thickness, necrotic layer up to 25 µm or thin and invisible. The algal layer is distributed in the thallus. Medulla thin, sometimes invisible, and dark brown, the lower cortex may be detectable. Perithecia globose, usually integrated, black, formed in hypothallus and interareolar space, involucrellum twisting completely around the exciple. Asci 8-spored, biseriate, clavate. Ascospore hyaline, ellipsoid, (15-)17-21(-23) × (7.5-)10-8(-11) µm (Fig. 3).

Spot tests

Negative.

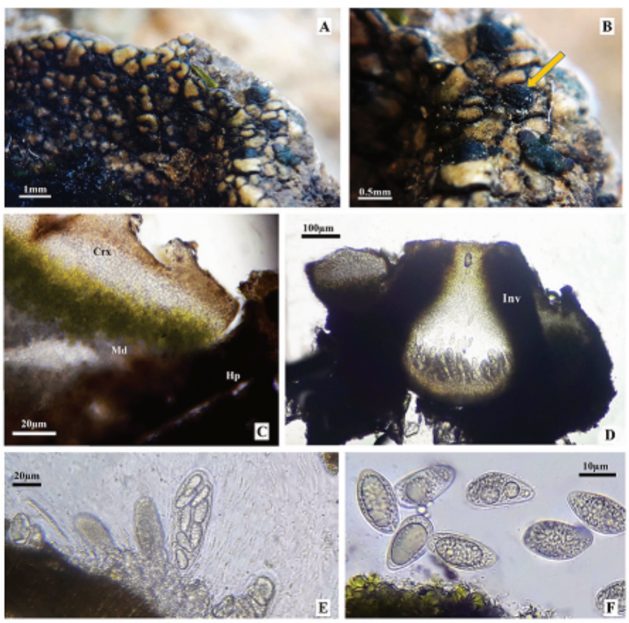

Figure 3. Demonstration of the morphological and anatomical features of *Involucropyrenium waltheri*. (A-B) Dark prothallus extends between the squama, globose perithecia observed interareolar spaces; (C) the cortex (Crx) is cinereum, the medulla (Md) is small in diameter, and a broad hypothallus (Hp) extends along and below the squama; (D) this species is distinguished by an involucrellum that completely encloses the perithecium; (E) ascus with biseriate spores; (F) ascospores.

Geographical distribution and Substrate:

Involucropyrenium waltheri is distributed globally in Europe and North America. This species grows on the calcareous substrate, siliceous rock, sandstone, and, dolomite (Prieto, Aragon, and Martinez 2010). In March and June 2018, we collected *I. waltheri* specimens from Kuh-e Sefid Valley (Khaf county), Latitude: 35° 02' 14.3" N, Longitude: 60° 24' 48.2" E, 1150 m above sea level, limestone, arid-desert-cold climate. We report the genus *Involucropyrenium* as a new record for the flora of Iran.

Lecania erysibe (Ach.) Mudd (1861)

Thallus crustose, scurfy, thin, cracked-areolate, pale gray to dirty green, areoles separated or fused, when wet pale green to slightly dark, most margins with blastidia. Apothecia up to 0.4 mm in diameter, sporadic, biatorine, discs brownish orange to dark brown, convex; thalline margin with granule-like blastidia, blastidia up to 65 µm in wide; thalline exciple, proper margin; epithecium pale brown to yellow-brown or green-yellow; hymenium 57-76.9 µm tall, 3.2-4.6 µm in wide, colorless; asci 8-spored; ascospores hyaline, 1-septate, oval, 11.4-15.5×4.6-5.7 µm (Fig. 4).

Geographical distribution and Substrate:

Lecania erysibe reported from North and south America, Australia, Britannia, Russia, Belarus, and Baltic countries (Gagarina et al. 2020; Smith 2009). It grows on acidic siliceous rocks and limestone. It can also be found on dead wood, tree trunks, branches, and leaves (van den Boom and Moniri 2018). In this study, we collected the species from three localities: 1- Dehbar, Latitude: 36° 14' 34" N, Longitude: 59° 17' 07" E, 1880 m, siliceous substrate, semi-dry ultra-cold climate. 2- Kalate-Ahan, Latitude: 36° 15' 48" N, Longitude: 59° 20' 08", 1500 m, siliceous substrate, semi-dry ultra-cold climate. 3- Kang, Latitude: 36° 16' 50" N, Longitude: 59° 12' 45" E, 2043 m, siliceous substrate, semi-dry ultra-cold climate.

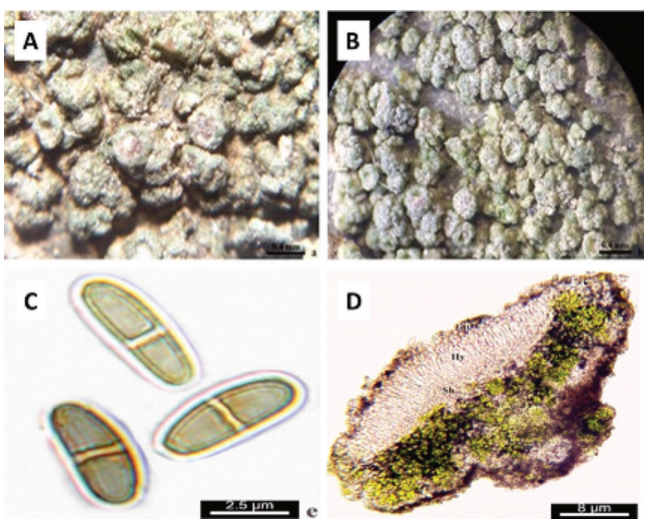

Figure 4. Demonstration of the morphological and anatomical features of *Lecania erysibe* (Ach.) Mudd (1861). (A) Cracked-areolate thallus and sporadic apothecia; (B) thallus and apothecia; (C) one-septate and oval spores; (D) cross-section of apothecia, EP (epihymenium), Hy (hymenium), Sh (subhymenium), Ex (exciple).

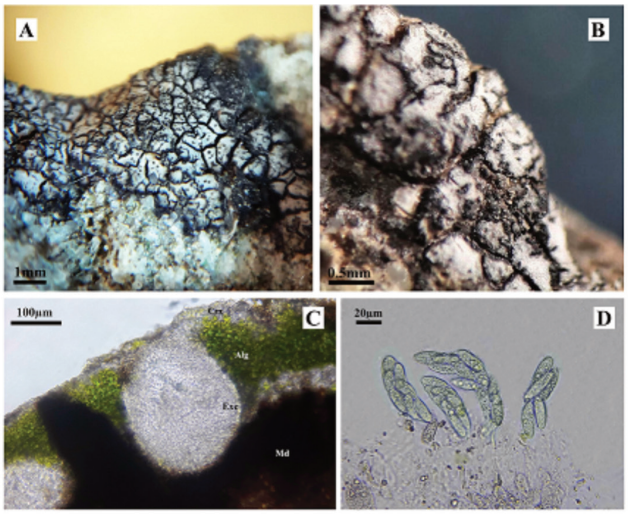

Figure 5. *Placopyrenium canellum*. (A) The thallus is crustose, with a distinct black prothallus at the thallus and areole margins. Deep cracks divide the areolas into smaller units; (B) perithecium pores appear as dark spots on the areola's surface, and ornaments appear as dark lines on the areolas; (C) thallus and perithecium micrographs. The pseudocortex (Crx) is composed of a thin layer of paraplectenchyma cells. The algal layer is sometimes subdivided by brown pigmented medulla (Md). The lower surface of the pigmented medulla occasionally reaches the surface and forms dark lines on the thallus. The medulla gives rise to the perithecium (immature peritoneum on the left), and the ostiole is located at or slightly above the surface of the cortex in mature perithecium, the exciple is colorless and sometimes dark in the apex; (D) ascus with hyaline, simple, and spindle-shaped ascospores.

Note

The minutely blastidiate, non-pruinose thallus distinguishes *L. erysibe* from other Lecania species, which can form large sterile colonies. Sparsely blastidiate specimens may occur, in which the ascospores and hymenium resemble *L. inundata*, which has a thicker thallus, or *L. rabenhorstii* which is dark grey-brown with pruinose apothecia. According to the second updated lichen checklist for Iran, eleven species of Lecania have been documented (Seaward et al. 2008), and the species *Lecania erysibe* (Ach.) Mudd (1861), reported for the first time from Iran in this study, is an addition.

Table 1. Morphological comparison of *Placopyrenium canellum*, *Placopyrenium ariyanense*, *Placopyrenium bucekii*, and *Placopyrenium iranicum*.

	P. canellum	***P. ariyanense***	***P. bucekii***	***P. iranicum***
Prothallus	Black	Absent	Absent	Absent
Thallus	Areolate	Aareolate	Squamolose-Areolate	Areolate
Areoles diameter (mm)	0.1-0.8 (1.1)	0.5-1 (1.5)	0.4 -1.2 (2.5)	0.5- 1 (3)
Ascospores (µm)	20-23 (-28) ×6-7 (-10)	17-21(-22.5) × 10 -12(-12.5)	13.5- 17 (17.5) × (4) 5-7 (7.5)	16 -21 × 6 -7(- 8)

***Placopyrenium canellum* (Nyl.) Gueidan & Cl. Roux**

Description

Prothallus blackish, formed around the thallus or absent. Thallus crustose, well developed, 0.2-0.6 mm thick, areolate, areoles angular, partially, or completely separated by deep cracks, pale or dark grey to brownish, pruinose, 0.12-0.8 (1.1) in diameter. The upper surface is subdivided by dark lines, areoles margin thin, dark, and broken by cracks into a separate area. Upper cortex false, paraplectenchyma. The algal layer is continuous or subdivided by brown-pigmented parts of the medulla. Medulla's upper surface is colorless and thin, medulla's lower surface is densely pigmented and thick, sometimes occupying half the areole thickness, the pigmented area sometimes reaches the surface and forms dark lines on the surface of the areola. Lower cortex absence. Perithecia 1-3 (Baradaran et al. 2020) in each mature areole, visible as small dots on the areoles, involucrellum absent, exciple 150-280 µm wide, colorless or pale brownish. Asci 8-spored. Ascospore, simple, hyaline, ellipsoid, (18-) 20-23 (-28) ± 6-7 (-10) µm, Pycnidia not observed (Fig. 5).

Spot test

Negative.

Geographical distribution and Substrate:

Young thallus grows on *Aspicilia calcarea* as their Dedicated host before surviving on sunny limestone substrates. It has been found in the Mediterranean basins, as well as Poland, England, France, Hungary, Sweden, and Wales (Navarro-Rosinés et al. 2007; Krzewicka 2009). In July 2019, we collected *P. canellumon* specimens from Durbadam (Quchan), Latitude: 37° 28' 48.4" N, Longitude: 58° 27' 37.3" E, 1530 m above sea level, limestone substrate, semi-arid ultra-cold climate. According to the second revised checklist of lichenized, lichenicolous, and allied fungi for Iran, only one species of *Placopyrenium* has been documented from Iran (Seaward et al. 2008). The species *P. canellum*, which is reported in this paper for the first time from Iran, will be added to that group.

Note

Placopyrenium canellum differs from *P. fuscellum* and *V. polysticta* by larger ascospores and the presence of perispore. In addition, young thalli of *P. canellum* are parasitic on *Aspicilia* sp. Three other *Placopyrenium* species have been formerly reported from Iran; *P. ariyanense* (Breuss and Moniri 2017), *P. bucekii* (Seaward et al. 2008), and *P. iranicum* (Breuss 2009). The differential morphological characters of these taxa are listed in Table 1.

Pyrenodesmia micromontana (Frolov, Wilk & Vondrák) (2016). Synonymus: Caloplaca micromontana Frolov, Wilk & Vondrák – in Frolov et al. (2016).

Description

Thallus crustose, epilithic, gray; Apothecia zeorine or rarely biatorine, wet, mature apothecia without stalk

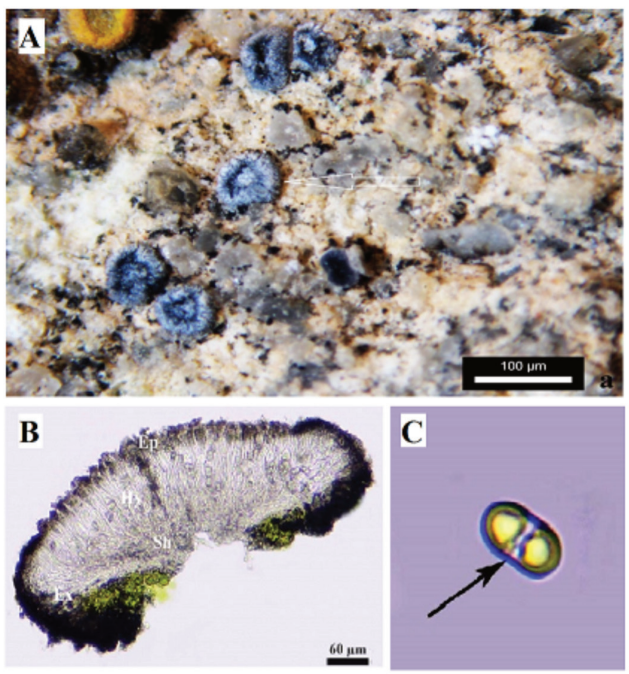

Figure 6. Morphological and anatomical features of *Pyrenodesmia micromontana* (Frolov, Wilk & Vondrák) (2016). (A) Crustose thallus confined to around the apothecia, concave apothecia; (B) longitudinal section of apothecium, EP (epithecium), Hy (hymenium), Sh (subhymenium), Ex (exciple); (C) hyaline polarilocular ascospores with a thick cytoplasmic channel between two locules.

and dense, 0.1-0.3 mm in diameter, concave to rarely flat, immersed in adnate, disc black with pruinose, unknown prothallus; hymenium hyaline, sometimes with hyaline crystals, 108.3-119.7 µm tall, epihymenium pale, an algal layer at the bottom; exciple 108.3-119.7 µm in wide, thalline exciple up to 15 µm thick with few hyaline crystals; paraphyses slightly branched; asci 8-spored, 52.4-68.8±15-22.6 µm; ascospores hyaline, 1-septate, polarilocular, 14.2-17.1±5.7-8.5 µm, septa up to 2.6 µm in wide, with a thick cytoplasmic channel between two locules (Fig. 6).

Spot test

Apothecia UV-, K-, and C-; true exciples faintly K+ violet; hymenium I+.

Geographical distribution and Substrate

In Europe and Asia, *Pyrenodesmia micromontana* can be found on limestone outcrops, stones, and pebbles, as well as lime-rich schist and sandstone. Also, it was recorded from mountains at various altitudes. For example, *P. micromontana* is found in the Alps (Austria) at altitudes ranging from 1700 to 2100 meters, whereas it grows in Poland and Slovakia at altitudes ranging from 550 to 1500 meters. Its Pakistani locality is at 4100 meters, while Russian records range from 150–400 meters in the Ural Mountains and 2200 meters in the Sayan Mountains (Frolov et al. 2016). We collected *P. micromontana* in April 2019 from Bakharz, Latitude: 35° 11' 39.3" N, Longitude: 60° 20' 56.8" E, 2120 m, calcareous substrate, semi-dry-cold climate.

Note

P. micromontana is similar to *Caloplaca albopruinosa,* although the latter is distinct from *P. micromontana* by having an ascospore septum wider, thalline exciple indistinct, thallus distinctly endolithic, grey or white (Frolov et al. 2016). Furthermore, *C. albopruinosa* was also found in 2007 on limestone rocks at an altitude of 1290 meters in Iran's West Azerbaijan province (Seaward et al. 2008), and the species *P. micromontana* (Frolov, Wilk & Vondrák) (2016) which is reported in this study for the first time from Iran, is an addition.

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