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New records of five *Staurothele* species (Verrucariaceae, Ascomycota) from Iran

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ABSTRACT This study aimed to the taxonomic investigation of the genus *Staurothele* (Ascomycota, Verrucariaceae) in northeast Iran. Lichen samples were collected from the Khorasan Razavi province and their morphological, anatomical, and ecological features were studied. Five species (*Staurothele lecideoides*, *Staurothele frustulenta*, *Staurothele drummondii*, *Staurothele rufa*, and *Staurothele polygonia*) were documented for the first time from Iran.

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Introduction

Iran has a diverse lichen flora due to its vastness and climatic diversity (Valadbeigi and Sipman 2010; Sohrabi and Sipman 2020). According to the 2008 update of the Iranian lichen checklist, Iran has 590 lichen species and 55 lichenized fungal species (Seaward et al. 2008). However, identifying Iran's lichen flora is still a long way off. In this regard, the large calcareous substrates and climatic diversity of Khorasan Razavi province in north-eastern Iran have created an ideal environment for the growth of saxicolous crustose lichens such as the Verrucariaceae (Ascomycota) species (Moniri and Sipman 2011; Abkouh et al. 2020; Baradaran et al. 2020). The Verrucariaceae family consists of about 50 genera and 750 species of lichenized fungi. However, only three Verrucariaceae genera have been identified in Khorasan Razavi province since 2000, including Dermatocarpon, Endocarpon, and Placidium (Breuss and Moniri 2017; Abkouh et al. 2020). Within the Verrucariaceae family, the genus *Staurothele* Norman (1853) contains 70 species that are distinguished by hymenial algae and (1) 2 or (4) 8 muriform ascospores per ascus (Morse and Douglas 2019; Heiðmarsson et al. 2017). In this genus, hymenial algae differ in size and shape from algae in the thallus's photosynthetic layer. Moreover, recent molecular studies show that many species of *Staurothele*

are polyphyletic (Harada 1992; Thomson 1991; Gueidan et al. 2014). According to the second revised checklist of lichenized, lichenicolous, and allied fungi for Iran, only five species of *Staurothele* have been documented from Iran (Seaward et al. 2008), furthermore, no research has been conducted on the distribution of the genus *Staurothele* in Khorasan Razavi province to date. This study aimed to the taxonomic investigation of the genus *Staurothele* (Ascomycota, Verrucariaceae) in Khorasan Razavi province: morphological and anatomical descriptions of five new records of *Staurothele* are presented from this region.

Material and Methods

Study area

This study is based on specimens collected by the first author between 2018 and 2019 using the survey method from 436 rock substrates in 44 localities in Khorasan Razavi Province. The province of Khorasan Razavi is in northeast Iran, bordering Turkmenistan to the north and Afghanistan to the east (Gholamhosseinian et al. 2021). It is located at an altitude of 970 meters above sea level, between 36° 17' 16.4892" N and 59° 36' 57.5004" E. The location of calcareous substrates and limestone rocks was determined using geological maps, and sampling sites were chosen from pristine and accessible areas separated by at

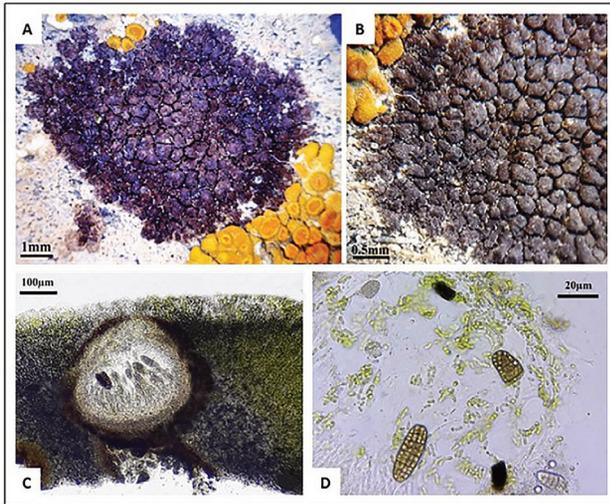


Figure 1. *Staurothele drummondii*. (A-B) Representation of crustose thallus. The thallus's central areoles are thick and have immersed perithecium. The areolas at the margins are smaller and lobed, (C) *S. drummondii* thallus and perithecium photomicrograph. A dark layer of thallus or an involucrellum usually extends to the lower level of the exciple, (D) Muriform spores with hyemial algae.

least one kilometre. Carbonate sedimentary substrates are more common in the northern and north-eastern regions of Khorasan Razavi province than in the southern and central regions (Abkough et al. 2020). Moreover, siliceous substrates were studied independently because some Verrucariaceae species grow on siliceous substrates.

Microscopy

The morphology of vegetative and reproductive structures of lichens was studied using a stereomicroscope (Carl ZEISS-C Stemi, Germany). Furthermore, hand sections from the thallus and perithecium were prepared with razor blades and evaluated in water under a light microscope (Carl ZEISS-Axioskope 40, Germany) for anatomical evaluation. Ten samples of each lichen species were collected for this purpose, and ten measurements were made for each specimen using a standard calibrated slide, as described by Vondrak and colleagues (Vondrak et al. 2013). In addition, thallus and perithecium sections were stained with 10% potassium hydroxide (KOH) and Lugol solution in spot tests. The hydrochloric acid (HCL 10%) test was also used to distinguish between calcareous and siliceous substrates (Karunaratne et al. 2005)

Results and Discussion

By survey sampling from 44 discrete localities in Khorasan Razavi province, we collected 436 rock substrate samples.

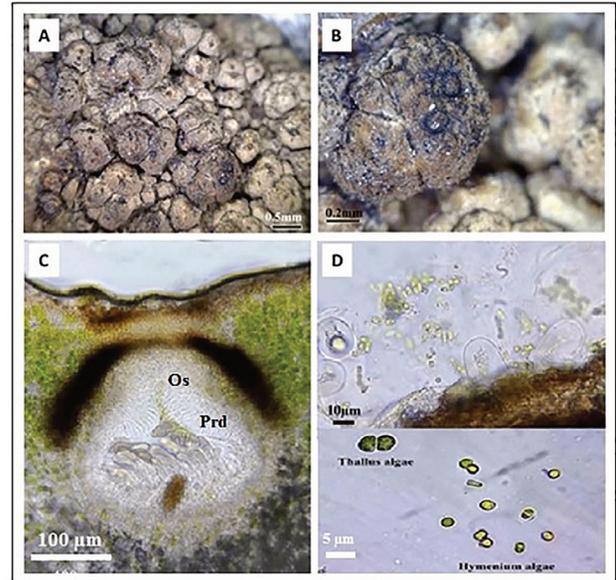


Figure 2. *Staurothele frustulenta*. (A-B) Two different brown and convex thallus morphs with immersed perithecia; (C) Micrograph of thallus and perithecium, a thallus layer is visible on the perithecium, the involucrellum (Inv) is restricted to the apex, and the spores and hyemial algae are ejected from the perithecium via the ostiole (Os) and the periphysoids (Prd); (D) Comparison of Hymenial Algae and thallus Algae. At the ostiole's exit, hyemial algae and a portion of the ascus apex can be seen. Hymenial algae in this species are almost globular.

Our study resulted in new records of five *Staurothele* species from Iran, including *Staurothele drummondii* (Tuck.) Tuck, *Staurothele frustulenta* Vain, *Staurothele lecideoides* B. de Lesd, *Staurothele polygonia* B. de Lesd, and *Staurothele rufa* (A. Massal.) Zschacke. All specimens are kept in the third author's (Mahroo Haji Moniri) collection.

Staurothele drummondii (Tuck.) Tuck (Fig. 1). Synonyms: *Verrucaria fuscocupera* (Nyl.) Nyl; *Staurothele fuscocupera* (Nyl.) Zsch. *Verrucaria fiddusilis* (Nyl.) Zahlbr; *Endocarpon wilmsoides* Zahlbr, *Staurothele septentrionalis* Lynge.

Description: Prothallus blackish, formed around the thallus. Thallus crustose, surface brown to blackish brown, pruinose or matte, central areoles thicker and bigger than the peripheral ones, marginal areoles lobes can be scattered or stretched-radiate, cortex thin, paraplectenchyma, contains pigmented cells, algal layer diffused in the thallus, medulla thin or thick. Perithecia are found fully within the central thick areoles, 0.4-0.6 mm thick, exciple hyaline, involucrellum dark, prominent at apex, twisting completely around the exciple, sometimes a dark thalloid layer formed between the exciple and the involucrellum. Asci 2-spored. Ascospore brown, muriform, ellipsoid or narrowly ellipsoid, 20-50 (-58) x 11-21 μ m. Spot test: negative.

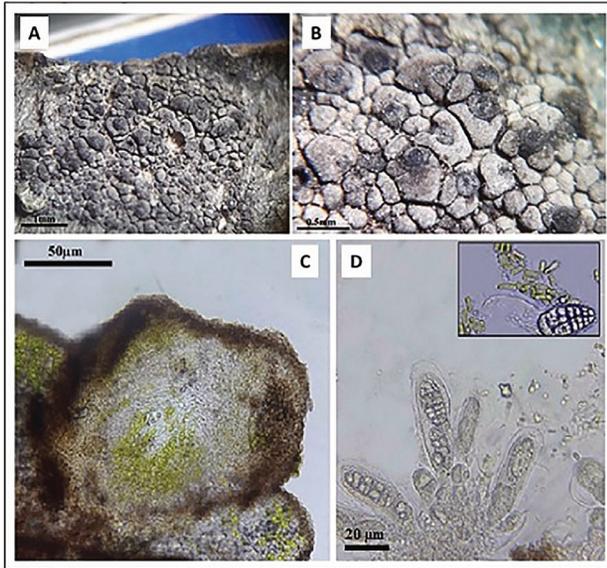


Figure 3. *Staurothele lecideoides*. (A-B) The surface of the grey thallus is dotted with prominent ridges of perithecia; (C) Thallus micrograph with perithecium. Perithecium has no involucrellum, and the exciple is pigmented; (D) Each ascus contains one or two colorless muriform ascospores; the hymenium algae with a spore is shown in the box above.

Geographical distribution and substrate: *Staurothele drummondii* tends siliceous and occasionally calcareous substrates near cold-water rivers. It has been found in Eurasia, Greenland, and North American highlands. The morphology of *S. drummondii* is influenced by environmental and substrate changes. In flat, fine-grained beds and very humid environments, the marginal lobes are elongated, whereas, in rough and uneven beds or low-humidity environments, they are short and indistinct (Morse and Douglas 2019). In March 2018, we collected *S. drummondii* specimens from Azghad, Latitude: 36°11'49.3" N, Longitude: 59°19'47.3" E, 1845 m above sea level, limestone substrate, semi-arid ultra-cold climate. The species *S. Drummond*, reported for the first time from Iran in this paper, will be added to the list of *Staurothele* species that have been documented from Iran.

Note: According to Thomson (1991), the distinguishing short and indistinct marginal areoles are more difficult to notice when growing on rough substrates, so *S. drummondii* resembles *S. areolata* species (Thomson 1991). In *S. areolata*, the fruiting and sterile areoles are nearly the same size and abruptly terminate at the margin, in contrast to the presence of smaller, more rotund marginal areoles in *S. drummondii*.

Staurothele frustulenta Vain. (1921) (Fig. 2). Synonyms: *Endocarpon elegans* (Wallr.) Lönnr.; *Endocarpon fuscillum* var. *cataleptum* Ach.; *Staurothele catalepta* (Ach.) Zschazke;

Staurothele catalepta var. *cinerea* B.de Lesdain; *Staurothele elegans* (Wallr.) Zwackh; *Stigmatomma cataleptum* (Ach.) Korb.; *Verrucaria catalepta* (Ach.) Spreng. *Verrucaria elegans* Wallr.

Description: Prothallus absent. Thallus crustose, brown, areolate, cracked into small convex areoles, sterile areoles smaller than fertile areoles. Cortex thin, paraplectenchyma, without a clear border with the algal layer. Perithecia black, with involucrellum, involucrellum apical or rarely extended to the base-level; exciple colourless. Hymenial algae globose-cuboid to oblong, 3.5-5.7 x 3-4.5 μm . Asci 2-spored. Ascospore brown, muriform, oblong to ellipsoid, (29-) 46-39-33.5(-48) \times 13-) 14.5-21-18 μm . Spot test: Negative.

Geographical distribution and substrate: In Europe and Asia, *Staurothele frustulenta* can be found on limestone or basic siliceous rocks in open habitats, often below the subalpine belt (Orange 2013). In March and April 2018, we collected *S. frustulenta* specimens from three localities: 1- Azghad, Latitude: 36°11'49.3" N, Longitude: 59°19'47.3" E, 1845 m above sea level, limestone substrate, semi-arid ultra-cold climate. 2- Akhlamad, Latitude: 36°35'57.6" N, Longitude: 58°55'43.5" E, 1860 m above sea level, limestone substrate, semi-arid-cold climate. 3- Akhlamad, Latitude: 36°35'41.2" N, Longitude: 58°54'48.8" E, 1740 m above sea level, limestone substrate, semi-arid-cold climate. The species *S. frustulenta*, reported for the first time from Iran in this paper, will be added to the list of *Staurothele* species that have been documented from Iran.

Note: *Staurothele frustulenta* is distinguished from *Staurothele areolata* by having smaller hymenial algae (Orange 2013).

Staurothele lecideoides B. de Lesd. (1932) (Fig. 3).

Description: Prothallus absent. Thallus crustose, grey, areolate, up 300 μm thick. Areoles small, angular, slightly flat. Perithecia single in areoles, pruinose, tiny, up to 200 μm wide, apex flat. Upper cortex paraplectenchyma, thin, 10-30 μm . Involucrellum absent; exciple black; Hymenium I+ blue; Hymenial algae 7-5 (-8) \times (2.8-) 4-3 (-4.7) μm . Asci 1 or 2-spored. Ascospore colorless, muriform, ellipsoid to oblong, (24-)38-29(-43) \times 20.5-18-12(-23) μm . Spot tests: negative.

Geographical distribution and substrate: *Staurothele lecideoides* has been reported from North America. Its substrate is siliceous rocks substrates (Thomson 1991). In July 2018, we collected *S. lecideoides* specimens from localities: 1- Durbadam (Quchan), Latitude: 37°29'39.7" N, Longitude: 58°26'07.1" E, 1890 m above sea level, limestone substrate, semi-arid ultra-cold climate. 2- 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. The species *S. lecideoides*, reported for the first time from Iran in this paper, will be added to the list of

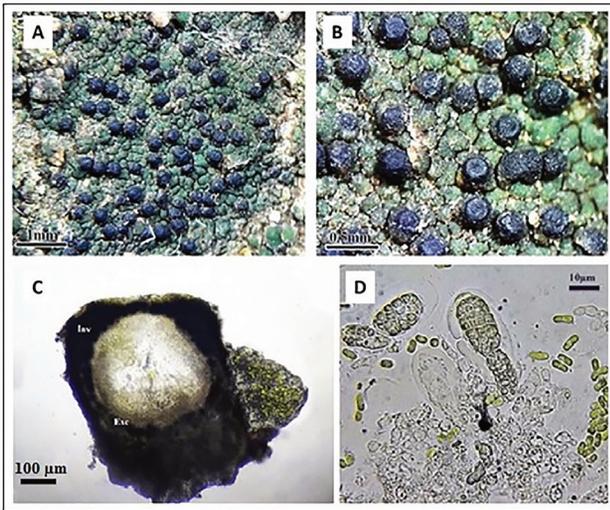


Figure 4. *Staurothele polygonia*. (A-B) The wet thallus is olive-green to dark green (dry thallus is dark brown). The involucrellums protrude from the thallus surface as distinct ridges with a perfectly smooth surface; (C) Perithecium micrograph with a dark exciple surrounded by a thick involucrellum; (D) Each ascus has two colorless muriform spores with globular to rectangular hyemial algae with slightly rounded edges.

Staurothele species that have been documented from Iran.

Staurothele polygonia B. de Lesd (Fig. 4).

Description: Thallus crustose, areolate. Areoles tiny, flat, angular, 0.1-0.2 mm wide, surface almost rough, dark-brown or olive-green to dark-green when moist, marginal areoles showing a little slight tendency to form radial lobes. Hypothallus blackish, formed around the thallus. Cortex paraplectenchyma, thin. Algal cells diffuse (unsorted). Medulla dark. Perithecia immersed in the areola, 0.6-1.0 mm wide, a broadened black flat involucrellar tip visible, a black thick involucrellar wrapping around the dark exciple, broadened flat involucrellar tip visible, ostiole not visible, hyemial gelatine I-, hyemial algae globose to oblong. Asci 1 or 2-spored. Ascospore colorless, muriform, $39-23 \times 18-10 \mu\text{m}$. Spot tests: negative.

Geographical distribution and substrate: *Staurothele polygonia* has been reported from North America, United States. It grows on granitic rocks and siliceous substrates (Thomson 1991). In April 2018, we collected *S. polygonia* specimens from Kalateh-Ahan (Torghabeh), Latitude: $36^{\circ}15'34.5''$ N, Longitude: $59^{\circ}20'00.4''$ E, 1520 m above sea level, siliceous substrate adjacent to the seasonal river, semi-arid ultra-cold climate. The species *S. polygonia*, reported for the first time from Iran in this paper, will be added to the list of *Staurothele* species that have been documented from Iran.

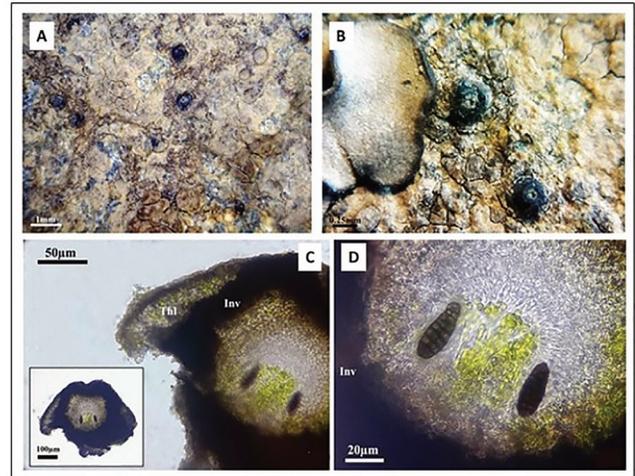


Figure 5. *Staurothele rufa*. (A-B) Thallus cream, inconspicuous with shallow cracks. Perithecia appear as black protrusions; (C) A perithecium micrograph revealed a well-developed involucrellum (Inv) that extends to the lower level, as well as a thin thallus (Thl); (D) Muriform spores and spherical-shaped hyemial algae are visible.

***Staurothele rufa* (A. Massal.) Zschacke** (Fig. 5). Synonyms: *Polyblastia rufa* A. Massal., *Polyblastia rufa* f. *orbicularis* Anzi, *Polyblastia rufa* f. *rufa* A. Massal., *Sporodictyon rufum* (A. Massal.) Trevis., *Verrucaria rufa* (A. Massal.) Garov.

Description: Thallus epilithic, cream, thin ($40 \mu\text{m}$), nearly invisible, smooth or rimose-cracked. Perithecia dark, protruding semi globose, $360-480 \mu\text{m}$ in diameter, well-developed around the dark exciple. Asci 1 or 2-spored. Ascospore brownish, ellipsoid to oblong, $(34.5-40.5-38.2-36(42-) \times (11.5-18-15.1-12(20-)) \mu\text{m}$. Hyemial algae equal or length about one and a half times the width, stretched ellipsoid, $2.5-3.7 \times 2.5$. Spot tests: negative.

Geographical distribution and substrate: *Staurothele rufa* has been reported from North America and Europe. It grows on limestone and calcareous substrates (Nimis and Nascimbene 2021). In April 2018, we collected *S. rufa* specimens from Akhlamad (Chenaran), Latitude: $36^{\circ}35'5''$ N, Longitude: $58^{\circ}55'10.3''$ E, 1920 m above sea level, calcareous substrate, semi-arid-cold climate. The species *S. rufa*, reported for the first time from Iran in this paper, will be added to the list of *Staurothele* species that have been documented from Iran.

Note: *Staurothele arctica* is distinguished from *S. rufa* by stretched algae, darker thallus, and distribution in northern latitudes.

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