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Phytopathogenic hyphomycetes determined in Yedigöller National Park, Bolu Province

Bolu ili, Yedigöller Milli Parkı'ndan tespit edilen fitopatojenik hifomisetler

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Abstract
Sixteen cercosporoid and ramularioid species belonging to nine genera are reported from Yedigöller National Park, Bolu Province, Türkiye. The species were identified based on conventional methods. Of these, *Cercospora datiscicola*, *Cercospora mercurialis*, *Filiella pastinacae*, *Pseudocercospora sambucigena*, *Ramularia linaria,* and *Ramularia sphaeroidea* are new records for Türkiye mycobiota. The description of the new recorded species are presented and discussed with their macro and micromorphological photographs.

Özet
Bolu ili Yedigöller Milli Parkı'ndan 9 cinse ait 16 cercosporoid ve ramularioid tür kaydedilmiştir. Tür teşhisleri geleneksel yöntemlere dayanarak yapılmıştır. Tespit edilen türlerden *Cercospora datiscicola*, *Cercospora mercurialis, Filiella pastinacae, Pseudocercospora sambucigena, Ramularia linaria* ve *Ramularia sphaeroidea* Türkiye mikobiyotası için yeni kayıttır. Yeni kaydedilen türlerin deskripsiyonları, makro ve mikromorfolojik fotoğrafları ile birlikte verilmiş ve tartışılmıştır.

INTRODUCTION

Genera of phytopathogenic hyphomyceteous fungi with relationship to *Mycosphaerellaceae*, such as *Cercospora* Fresen., *Cercosporella* Sacc., *Passalora* Fr., *Ramularia* Unger etc., show high morphological diversity in contrast to their teleomorphs (Kirschner 2009) and cause typical leaf spot, necrosis and chlorosis in a broad host range, including many cultivars. Some species may develop almost without symptoms (e.g., *Cercosporella hypoestis* Hansf.), attack stems, or cause foot-rot (e.g., *Ramulispora herpotrichoides* (Fron) v. Arx). A few species are hyperparasitic (e.g. *Ramularia coleosporii* Sacc.). They also have the potential to be used as biocontrol agents of weeds (Braun et al. 2016).

Yedigöller National Park, which has seven lakes and numerous streams, is situated in the Bolu Province in Türkiye. According to Emberger's climate classification, the region is of the type having a semi-arid, upper, cold winters Mediterranean climate of daily and seasonal photoperiodism. This indicates that summer precipitation is little in this region; vegetation is under the effect of the summer drought, and the precipitation regime is Eastern Mediterranean precipitation regime Type 1.

The research area mainly consists of mixed forest vegetation. At lower elevations, it is covered by *Fagus orientalis* Lipsky, *Carpinus orientalis* Mill. subsp. *orientalis*, some *Quercus* L. species, *Cornus mas* L., *Corylus colurna* L., *Sorbus aucuparia* L., *Sambucus nigra* L., *Acer campestre* L., *Acer platanoides* L., *Alnus glutinosa* (L.) Gaertn. Regions at upper elevations are covered with

Gymnospermae, such as *Abies nordmanniana* (Steven) Spach, *Pinus nigra* J.F.Arnold, *Pinus sylvestris* L., and *Taxus baccata* L. Under parts of forest-covered shrubs such as *Rhododendron ponticum* L., *Daphne pontica* L., and *Juniperus oxycedrus* L. The research area has some marshy habitats; the aquatic plants are growing in the lake (like *Lemna* L. sp., and *Potamageton* L. sp.), and marsh plants are growing in near the lake (like *Carex* L. ssp., *Lythrum* L. sp., and *Typha* L. sp.).

The purpose of the current study is to determine the phytopathogenic hyphomycetes of Yedigöller National Park and make a contribution to mycobiota of Türkiye.

MATERIALS AND METHODS

Specimens of fungi were collected from Yedigöller National Park in Bolu Province of Türkiye between 2018 and 2021. The Flora of Türkiye and the East Aegean Islands was used to identify the host plants (Davis 1965–85). A Leica DM E light microscope was used to examine and measure the preparations prepared from the host tissue. A Leica EZ4D stereo microscope was used for close-up photos of infected host surfaces. Taxa were identified using relevant literature (Vassilevskiy and Karakulin 1950, Esfandiari 1951, Chupp 1954, Vimba 1970, Şvartsman et al. 1975, Deighton 1976, Ellis and Ellis 1987, Braun 1995, 1998, Braun and Melnik 1997, Braun and Crous 2005, Bakalova and Borisova 2010, Pirnia et al. 2010, Bensch et al. 2012, Crous et al. 2013, Świderska-Burek 2015, Heydari et al. 2017).

For scanning electron microscopy (SEM), infected leaves bearing conidiophores and/or conidia were mounted on stubs with double-sided tapes. They were coated with gold in Polaron SC 502 Sputter Coater and examined with Jeol JSM 6060 SEM at 5–10 kV in the Gazi University, Faculty of Science.

RESULTS AND DISCUSSION

During our study in Yedigöller National Park on determining the microfungi on vascular plants, we collected several phytopathogenic hyphomycetes species that cause foliar spots. Some of the microfungus species determined in this study, such as *Cercospora datiscicola* Esfand*, Cercospora mercurialis* Pass.*, Filiella pastinacae* (P. Karst.) Videira & Crous, *Pseudocercospora sambucigena* U. Braun, Crous & K. Schub., *Ramularia linaria* Baudyš & Picb. and *Ramularia sphaeroidea* Sacc., were the first records for Türkiye. The list of microfungi with their descriptions (for new records only), host plants, localities, habitats, collection dates, and voucher numbers were presented below according to the systematics given in Index Fungorum (Kirk 2023).

Dothideomycetes

Mycosphaerellales

Mycosphaerellaceae

***Cercospora datiscicola* Esfand Sydowia 5(3-6):368 (1951).**

Leaf spots amphigenous, single or confluent, subcircular, irregular, sometimes delimited by veinlets, 2–6 mm diameter, up to 15 mm long when confluent, creamy to pale grayish, spots margin dark brown. Caespituli on both sides of leaves, punctiform, brown. Conidiophores fasciculate, sparsely to almost densely dispersed, arising from brown stromata, simple, straight, and cylindric, geniculate-sinuous, unicellular or sparsely septate, guttulate, 38–119 × 3.6–4.7 µm, brown below, paler towards the apex; conidial scars conspicuous, darkened and thickened. Conidia solitary, subcylindric to obclavate, straight to curved, gradually attenuated at apex, apex obtuse, base obconically truncate, 41–86.4 × 3–4.7 µm, 2–6 septate, not constricted, hyaline; hila darkened, thickened (Figure 1).

Material Examined: Türkiye, Bolu: Yedigöller National Park, on living leaves of *Datisca cannabina* L. (*Datiscaceae*), 40°56′52″N, 31°45′02″E, 716 m asl, 27.06.2018, Doğan 1328; Bolu: Yedigöller National Park, Mengen road, 40°56′30″K, 31°44′40″D, 811 m asl, 03.08.2019, GD 2041.

This fungus was reported in Armenia (Braun and Melnik 1997, Crous and Braun 2003), and Iran (Esfandiari 1951, Chupp 1953). Although the specimen of *Cercospora datiscicola* collected from Yedigöller area is morphologically similar to descriptions made by Braun and Melnik (1997), Esfandiari (1951), and Chupp (1953), it differed slightly, especially in terms of conidia size and number of septa: 30–100 × 4–5 µm and (2)3–7(9) septate (Braun and Melnik 1997); 32–85 × 4–5 µm and 2–7 rarely 8–9 septate (Esfandiari 1951); 30–90 × 4–5 µm and 2–9

Figure 1. *Cercospora datiscicola*: A. the appearance of infected leaf, B. conidiophores and conidia on leaf (SEM), C. conidiophores, D. conidia. Scale bars: 1 mm (A), 50 µm (B), 10 µm (C, D)

septate (Chupp 1953). The conidia in the specimen from Türkiye were narrower and 2–6 septate. Esfandiari (1951) and Chupp (1954) described hypophyllous caespituli and continuous conidiophores, while Braun and Melnik (1997) cite continuous or sparsely septate conidiophores. The specimen collected in Türkiye had amphigenous caespituli and continuous or sparsely septate conidiophores.

***Cercospora mercurialis* Pass., in Thümen, Mycoth. Univ., cent. 8: no. 783 (1877).**

Leaf spots single or confluent, irregular, subcircular, 1–10 mm diameter, up to 22 mm long when confluent, whitish to yellowish, spots margin brown. Caespituli hypophyllous, punctiform, brown. Conidiophores fasciculate, sparsely to almost densely dispersed, arising from brown stromata, geniculate to sinuous, erect, not branched, narrower towards the tip, unicellular or rarely one septate, guttulate, 32.7–56.5 × 4.5–6.1 µm, brown below, paler towards the apex; conidial scars conspicuous, darkened, thickened. Conidia solitary, cylindrical, straight or slightly curved, apex obtuse, base subtruncate, 5–15 septate, not constricted, guttulate, 37.4–124.7 × 3.1–5.1 µm, hyaline; hila darkened, thickened (Figure 2).

Material Examined: Türkiye, Bolu: Yedigöller National Park, on living leaves of *Mercurialis* L. sp. (*Euphorbiaceae*), 40°55′40″N, 31°44′59″E, 1180 m asl, 02.08.2019, Doğan 1982; Bolu: Yedigöller National Park, around the Kapankaya Observation Terrace, 40°55′39″N, 31°45′06″E, 1200 m asl, 25.06.2021, Doğan 2409.

It was reported from Austria, Bulgaria, Germany, England, Greece, Iran, Italy, Portugal, Poland, Romania, Scotland, Russia, Spain, Ukraine, and the United Kingdom (Farr and Rossman 2020). The Turkish specimen *Cercospora mercurialis* morphologically fits to literature (Chupp 1954, Pirnia et al. 2010, Świderska-Burek 2015, Heydari et al. 2017), but the conidiophores of Turkish members are not branched (Chupp 1954, Świderska-Burek 2015), while those reported by Pirnia et al. (2010) and Heydari et al. (2017) dichotomously branched in the upper part.

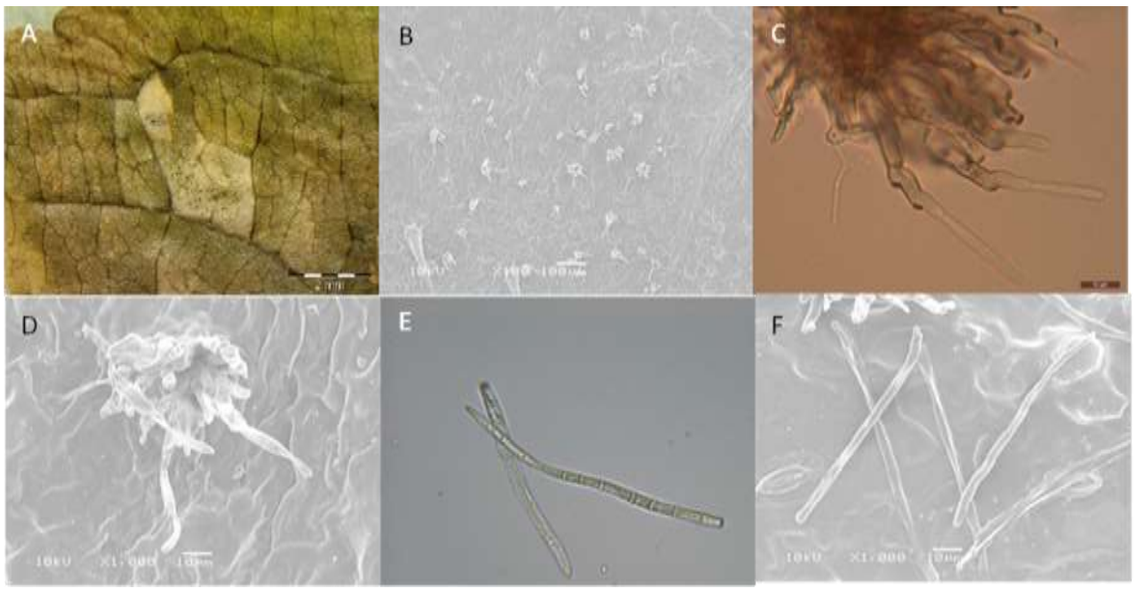

Figure 2. *Cercospora mercurialis*: A. the appearance of infected leaf, B. conidiophores and conidia on leaf (SEM), C. conidiophores and conidia, D. conidiophores and conidia (SEM), E. conidia, F. conidia (SEM). Scale bars: 2 mm (A), 100 µm (B), 10 µm (C-F)

***Chuppomyces handelii* (Bubák) U. Braun, C. Nakash., Videira & Crous, in Videira, Groenewald, Nakashima, Braun, Barreto, De Wit & Crous, Stud. Mycol. 87:370 (2017).**

Material Examined: Türkiye, Bolu: Yedigöller National Park, near the Deringöl, on living leaves of *Rhododendron ponticum* L. (*Ericaceae*), 40°56′47″N, 31°44′51″E, 869 m asl, 25.06.2021, Doğan 2417.

***Filiella pastinacae* (P. Karst.) Videira & Crous, in Videira, Groenewald, Braun, Shin & Crous, Stud. Mycol. 83: 88 (2016).**

Leaf spots amphigenous, angular, subcircular or irregular, scattered or confluent, 1–4 mm diameter, up to 12 mm long when confluent, ochraceous to greyish brown centre, surrounded by a dark brown border. Caespituli amphigenous, punctiform, greyish white. Conidiophores in dense fascicles, arising from stromata, simple, subcylindrical, straight to flexuous, unicellular, 5–26 × 1.2–2.5 µm, hyaline to pale yellowish, smooth. Conidia solitary, acicular, filiform, subcylindrical, straight or arched, apex subacute, base truncate, 0–6 septate, 35–85 × 1.8–2.5 µm, hyaline, smooth, thin-walled; hila not darkened and unthickened (Figure 3).

Material Examined: Türkiye, Bolu: Yedigöller National Park road, on living leaves of *Astrantia maxima* Pall. (*Apiaceae*), 40°55′42″N, 31°42′38″E, 869 m asl, 12.07.2019, Doğan 1918.

***Filiella* was introduced to accommodate *Pseudocercosporella pastinacae* (P. Karst.) U. Braun, since it is not congeneric with *Pseudocercosporella* s. str. based on *Pseudocercosporella bakeri* (Syd. & P. Syd.) Deighton (Videira et al. 2016).**

Filiella pastinacae was originally described on *Pastinaca sativa* L. from Finland, but it can infect various Apiaceous hosts worldwide. The Turkish specimen *Filiella pastinacae* morphologically fits to literature (Braun 1995, Bakalova and Borisova 2010, Videira et al. 2016), but conidia are slightly narrower and shorter.

***Paracercosporidium microsorum* (Sacc.) U. Braun, C. Nakash., Videira & Crous, in Videira, Groenewald, Nakashima, Braun, Barreto, de Wit & Crous, Stud. Mycol. 87: 319 (2017).**

Material Examined: Türkiye, Bolu: Yedigöller National Park, near the main entrance, on living leaves of *Tilia* L. sp. (*Malvaceae*), 40°92′92″N, 31°74′44″E, 1166 m asl, 28.06.2018, Doğan 1372.

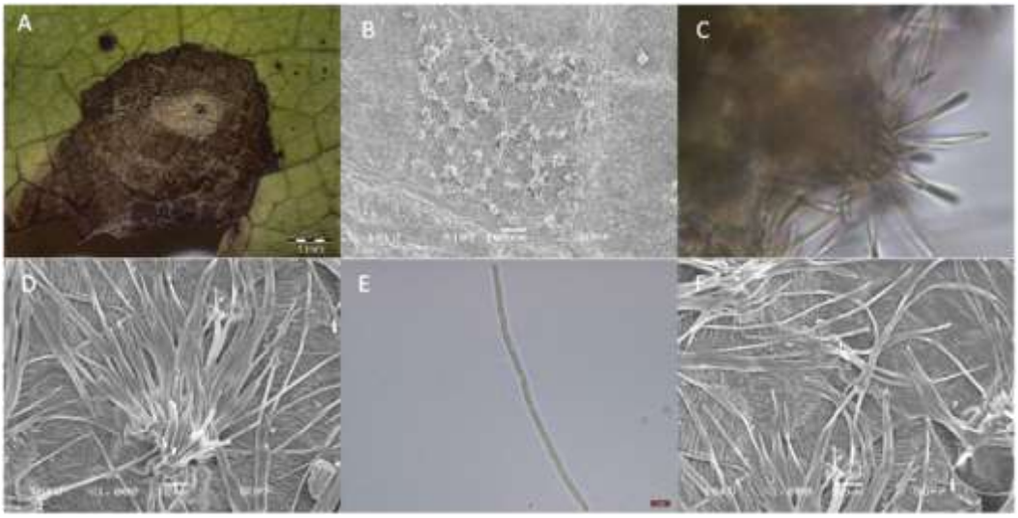

Figure 3. *Filiella pastinacae*: A. the appearance of infected leaf, B. conidiophores and conidia on leaf (SEM), C. longitudinal section of infected leaf, D. conidiophores and conidia, E. conidia (SEM), F. conidia (SEM). Scale bars: 1 mm (A), 100 µm (B), 5 µm (C, E), 10 µm (D, F).

***Passalora bacilligera* (Mont. & Fr.) Mont. & Fr., Syll. gen. sp. crypt. (Paris): 305 (1856).**

Material Examined: Türkiye, Bolu: Yedigöller National Park, the stream's edge, on living leaves of *Alnus glutinosa* (L.) Gaertn. subsp. *glutinosa* (*Betulaceae*), 40°51′35″N, 31°39′46″E, 1200 m asl, 12.08.2018, Doğan 1617.

***Pruniphilomyces circumscissus* (Sacc.) Crous & Bulgakov, in Crous, Wingfield, Schumacher, Akulov, Bulgakov, Carnegie, Jurjević, Decock, Denman, Lombard, Lawrence, Stack, Gordon, Bostock, Burgess, Summerell, Taylor, Edwards, Hou, Cai, Rossman, Wöhner, Allen, Castlebury, Visagie & Groenewald, Fungal Systematics and Evolution 6: 215 (2020).**

Material Examined: Türkiye, Bolu: Yedigöller National Park, near the main entrance, on living leaves of *Cerasus* L. sp. (*Rosaceae*), 40°94′76″N, 31°75′04″E, 716 m asl, 27.06.2018, Doğan 1326.

***Pseudocercospora sambucigena* U. Braun, Crous & K. Schub., Mycotaxon 92: 400 (2005).**

Leaf spots angular, sometimes irregular, often deformed, 25-45 mm diameter, beige to brown centre, surrounded by a yellow border. Caespituli both sides of leaves, mostly hypophyllous, punctiform, brown. Conidiophores in fascicles, arising from stromata, simple, erect, subcylindrical, straight to flexuous, slightly geniculate to sinuous, unbranched, pluriseptate, constricted at the septa, (21.7) 36.5–56.6 × 3.8–5.4 µm, brown, paler towards the apex. Conidia solitary, obclavate, subcylindrical, apex obtuse, base obconically truncate, 0–5 septate, not constricted, guttulate, 43–111 × 4.2–5.2 µm, pale brown; hila not darkened, unthickened (Figure 4).

Material Examined: Türkiye, Bolu: Yedigöller National Park, near the Nazlıgöl, in the forest, on living leaves of *Sambucus ebulus* L. (*Adoxaceae*), 40°56′38″N, 31°44′28″E, 899 m asl, 18.10.2020, Doğan 2332.

It was reported from Germany (Lotz-Winter et al. 2011), Italy, Holland, South Korea, and the USA (Crous et al. 2013). The specimen was collected from the Yedigöller area morphologically fit to described by a previous study (Braun and Crous 2005, Crous et al. 2013), but differed in having slightly narrower and fewer septa.

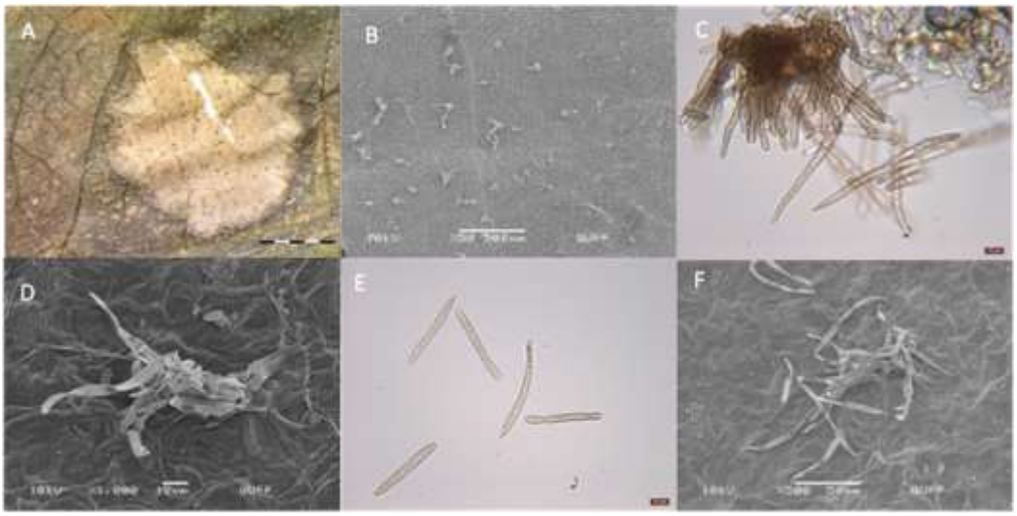

Figure 4. *Pseudocercospora sambucigena*: A. the appearance of infected leaf, B. conidiophores and conidia on leaf (SEM), C. conidiophores and conidia, D. conidiophores and conidia (SEM), E. conidia, F. conidiophores and conidia (SEM). Scale bars: 2 mm (A), 500 µm (B), 10 µm (C-E), 50 µm (F)

Ramularia hellebori **Fuckel, Jb. nassau. Ver. Naturk. 23-24: 361 (1870) [1869-70]**

*Material Examine*d: Türkiye, Bolu: Yedigöller National Park, in the forest, on living leaves of *Helleborus orientalis* Lam. (*Ranunculaceae*), 40°55′38″N, 31°45′06″E, 1200 m asl, 21.07.2018, Doğan 1402; Bolu: Yedigöller National Park, near the İncegöl, under the forest, 40°55′42″N, 31°42′47″E, 1454 m asl, 11.08.2018, Doğan 1588; Bolu: Yedigöller National Park, under the forest, 40°55′38″N, 31°45′06″E, 1196 m asl, 02.08.2019, Doğan 1993.

Ramularia linariae **Baudyš et Picb., Prace Morav. Prir. Spol. 1 (5): 304 (1924).**

Leaf spots usually marginal or terminal, amphigenous, brown to fuscous-chesnut, dried areas surrounded by a dark brown border. Caespituli amphigenous, punctiform, whitish. Conidiophores usually numerous, dense fascicles, emerging through stomata, simple, subcylindric to flexuous, geniculate-sinuous, continuous or sparsely septate, 70–94 × 2.4–2.8 µm, hyaline, smooth. Conidia formed singly, cylindric, ellipsoid to ovoid, rounded at apex, rounded or attenuated at the base, 0–1 septate, non-constricted or slightly constricted at the septum, 11–28 × 3.6–6.4 µm, hyaline, smooth to verruculose; hilum darkened and thickened (Figure 5).

Material Examined: Türkiye, Bolu: Yedigöller National Park road, on living leaves of *Linaria* Mill. sp. (*Plantaginaceae*), 40°56′51″N, 31°45′01″E, 716 m asl, 27.06.2018, Doğan 1333.

Ramularia linariae was reported from the Czech Republic, Bulgaria, Germany, Estonia, Denmark, Great Britain, Hungary, Poland, Russia, Italy, Romania, Sweden, Switzerland, Ukraine, and Turkmenistan (Farr and Rossman 2020). The specimens were collected from Yedigöller area of *Ramularia linariae* morphologically fits to described by a previous study (Braun 1998), but conidia are slightly narrower and shorter.

Ramularia parietariae **Pass., in Rabenhorst, Fungi europ. exsicc.: no. 2066. (1876).**

Material Examined: Türkiye, Bolu: Yedigöller National Park, Gülen Kayalar location, in the forest, on living leaves of *Parietaria officinalis* L. (*Urticaceae*), 40°56′14″N, 31°44′38″E, 891 m asl, 13.07.2019, Doğan 1946.

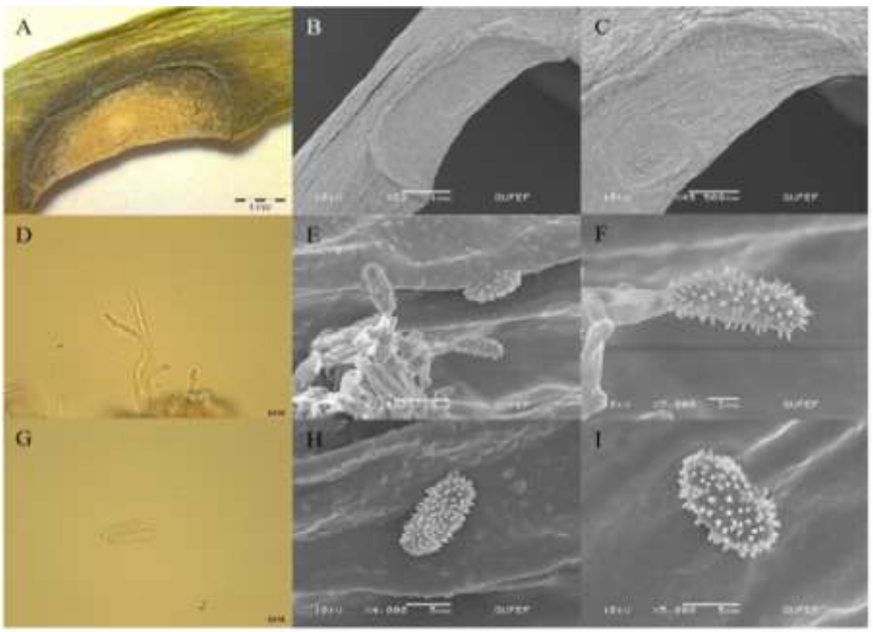

Figure 5. *Ramularia linariae*: A. the appearance of infected leaf, B, C. the appearance of infected leaf (SEM), D. conidiophores, E. conidiophores and conidia (SEM), F. conidiophores and conidium (SEM), G. conidia, H, I. conidium (SEM). Scale bars: 1 mm (A, B), 500 µm (C), 10 µm (D, E, G), 2 µm (F), 5 µm (H, I).

***Ramularia rhabdospora* (Berk. & Broome) Nannf., in Lundell & Nannfeldt, Fungi Exsiccati Suecici 39-40: 32 (1950).**

Material Examined: Türkiye, Bolu: Yedigöller National Park, around the Kapankaya Observation Terrace, roadside, on living leaves of *Plantago* L. (*Plantaginaceae*) 40°55′40″N, 31°44′12″E, 1290 m asl, 21.09.2019, Doğan 2179.

***Ramularia sambucina* Sacc., Michelia 2(no. 8): 551 (1882).**

Material Examined: Türkiye, Bolu: Yedigöller National Park, on living leaves of *Sambucus ebulus* L. (*Adoxaceae*), 40°55′08″N, 31°41′38″E, 1355 m asl, 08.09.2018, Doğan 1667.

***Ramularia sphaeroidea* Sacc., Michelia 1(no. 2): 130 (1878).**

Leaf spots usually marginal or terminal, amphigenous, irregular, sometimes bounded by the veins, scattered or confluent, 1–5 × 1–2 mm, brown. Caespituli on both sides of leaves, mostly hypophyllous, punctiform to effuse, white. Conidiophores in fascicles, erect, geniculate-sinuous, flexuous, continuous or sparsely septate, simple, rarely branched, 40–108 × 4–7.3 µm, hyaline, smooth, sometimes rough; conidial scars conspicuous, slightly darkened, thickened. Conidia formed singly, subglobose to ovoid, obovoid, apex rounded, base rounded or occasionally slightly narrowed and subtruncate, smooth to faintly rough, aseptate, 5–13.5 × 3.5–12.3 µm, hyaline; basal hilum thickened, slightly darkened (Figure 6).

Material Examined: Türkiye, Bolu: Yedigöller National Park, around the Kapankaya Observation Terrace, roadside, on living leaves of *Vicia* L. sp. (*Fabaceae*), 40°55′40″N, 31°44′12″E, 1290 m asl, 21.09.2019, Doğan 2181.

It was originally described on *Lotus uliginosus* Schkuhr but later is able to infect *Fabaceae* members in the world (Videira et al. 2016). The Turkish specimen agrees with other species descriptions concerning the morphology of caespituli, conidiophores, and conidia, the only observable difference being in the size of conidia and conidiophores. Braun (1998) described conidia measuring 7–15 (18) × (5) 6–12.5 µm and conidiophores measuring (15) 30–100 (150) × (2.5) 3–6 (7) µm; and Ellis and Ellis (1987) described conidia measuring 8–12 µm diameter and conidiophores measuring 40–120 × 3 µm.

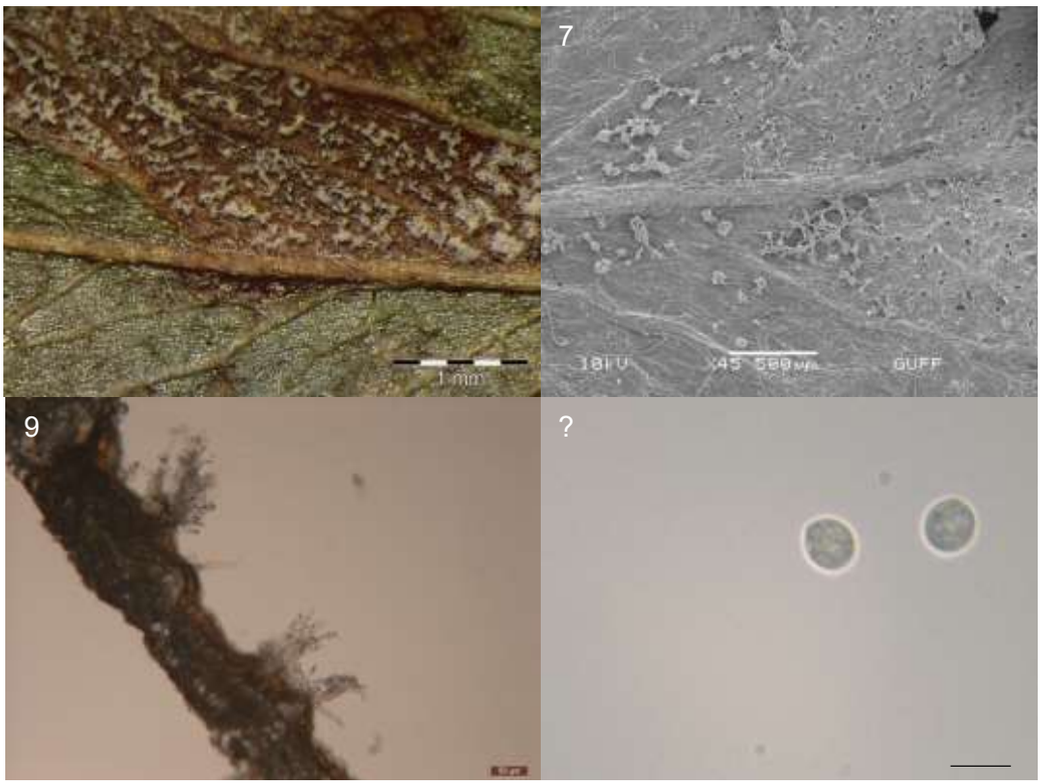

Figure 6. *Ramularia sphaeroidea*: A. the appearance of infected leaf, B. the appearance of infected leaf (SEM), C. longitudinal section of infected leaf, D. conidia. Scale bars: 1 mm (A), 500 µm (B), 50 µm (C), 10 µm (D).

***Ramularia uredinearum* Hulea, J. Pl. Prot. Japan 22(4): 210 (1939).**

Material Examined: Türkiye, Bolu: Yedigöller National Park, near the Dilek Çeşmesi, on living leaves of *Lonicera* L. sp. (*Caprifoliaceae*), 40°56′22″N, 31°44′47″E, 823 m asl, 21.09.2019, Doğan 2198.

***Zasmidium lythri* (Westend.) U. Braun & H.D. Shin, in Schubert, Braun, Groenewald & Crous, Stud. Mycol. 72(1): 320 (2012)**

Material Examined: Türkiye, Bolu: Yedigöller National Park, near the main entrance, roadside, on living leaves of *Lythrum salicaria* L. (*Lythraceae*), 40°56′51″N, 31°44′25″E, 716 m asl, 27.06.2018, Doğan 1977.

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