



## New records of anamorphic fungi from North of Iran

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**Abstract:** Fungi associated with leaf spot symptoms of plants in forest areas of Golestan province, Iran, were collected and examined based on morphological characteristics of conidiophores, conidia and conidiogenous loci. Ten taxa were identified. Among the taxa, *Passalora* cf. *bacilligera* and *Pseudocladosporium hachijoens* are new records for the mycobiota of Iran. *Cercospora* on *Eruca* sp. was morphologically different from other *Cercospora* hitherto been reported on Brassicaceae and compared with *Cercospora* species on this plant family. *Cercospora pantoleuca* was newly reported from Golestan province (North of Iran). Furthermore, *Plantago major* and *Sambucus nigra* were found to be new hosts for *Cercospora pantoleuca* and *Ramularia sambucina* in Iran, respectively.

**Key words:** mycobiota, leaf spot, morphology, taxonomy

## INTRODUCTION

Some species of the genera *Cercospora* Fresen., *Passalora* Fr., *Pseudocladosporium* U. Braun and *Ramularia* Unger treated in this paper. The genus *Cercospora* is distinguished from other similar genera by having acicular, hyaline and septate conidia with conspicuous hila produced on pigmented, unbranched, septate and smooth conidiophores with conspicuously thickened and darkened conidiogenous loci (scars). The genus *Passalora* is characterized by having

pigmented conidiophores and pigmented ellipsoid-fusiform to obclavate-subcylindrical conidia (Crous & Braun 2003). *Ramularia* species are identified by having colorless structures, solitary to fasciculate conidiophores forming small to sporodochial fascicles and solitary to catenate conidia with high morphological variation and sexual morphs are belonging to the genus *Mycosphaerella* s. str. (Videira et al. 2016). In the genus *Pseudocladosporium*, conidiogenous cells forming as lateral protuberant denticles on creeping hyphae. Conidia are subcylindric, fusiform to ellipsoid-ovoid forming in simple or often branched acropetal chains (Braun 1998). In recent years, increasing attempts to examine cercosporoid and ramularioid fungi (Mycosphaerellaceae) in Iran have been made (Pirnia et al. 2010, 2012 a, b, c, d; Hessami et al. 2011, 2012; Khodaparast et al. 2012; Bicharanlou et al. 2013 a, b, c; Pirnia, 2014; Behrooz et al. 2015 a, b, 2017 a, b). Recently, Pirnia & Braun (2017) published a new species, *Ramularia ranuncolicola*, and new records of the genus for the mycobiota of Iran. In this paper, two new records for mycobiota of Iran and two records of new host plants for previously reported species are listed.

## MATERIALS AND METHODS

Specimens with leaf spot symptoms from different locations in North of Iran (Golestan province) were collected during spring-autumn 2014-15. Host plants were identified by local botanists of Golestan Research Center for Agricultural and Natural Resources, Gorgan, Iran. In infected leaves, stromata, conidiophores and conidia were picked up directly by a needle from leaves and microscopic slides were prepared in 25% lactic acid. A combination of morphological features including shape, size and septation of conidia, conidiophores, conidiogenous loci and hila were examined using standard light microscopy (Olympus CH40) and used for identification of species (Braun 1998, Crous & Braun 2003). Fifty conidia and conidiophores per specimen were randomly selected and measured. Drawings were made using a drawing tube attached to the microscope.

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## RESULTS AND DISCUSSION

The species are listed in alphabetical order. Two records, viz, *Passalora bacilligera* and *Pseudocladopodium hachijoens* found to be new for the mycobiota of Iran. *Plantago major* and *Sambucus nigra* proved to be new hosts for *Cercospora pantoleuca* and *Ramularia sambucina* in Iran. All specimens are deposited in the Fungal Reference Collection of the Ministry of Jihad-e Agriculture (IRAN) at the Iranian Research Institute of Plant Protection.

### *Cercospora mercurialis* Pass., Mycoth. Univ., No. 783 (1877)

Leaf spots circular to irregular, pale-brown with a brown line border, center yellowish grey, 2–8 mm in diameter. Stromata present, small, substomatal, brown, 20–35  $\mu\text{m}$  wide. Caespituli amphigenous, mostly hypophyllous, punctiform. Conidiophores in small

fascicles, 3–8 stalks, arising from stromata, through stomata, pale-brown to brown, erect, geniculate to sinuous, rarely dichotomously branched in the upper part, smooth, thin, irregular in width, narrower towards the tip, 0–1-septate, (12–)15–67  $\times$  2.5–5  $\mu\text{m}$ . Conidiogenous cells integrated, terminal. Conidial scars conspicuous, thickened and darkened. Conidia formed singly, hyaline, obclavate to subcylindrical, straight to slightly curved, smooth, thin, indistinctly multiseptate, base subtruncate to obconically truncate, tip mostly obtuse, 47–100  $\times$  2.5–5  $\mu\text{m}$ . Hila thickened and darkened (Fig. 1).

*Specimen examined.* IRAN, Golestan Province, Gorgan, (Nahar-Khoran), On *Mercurialis annua* L. (Euphorbiaceae), 22 May 2015, N. Heydari, IRAN 16805F.

Notes— The species is characterized by having moderately short and occasionally branched conidiophores. The first report of the species in Iran published by Pirnia et al. (2010).

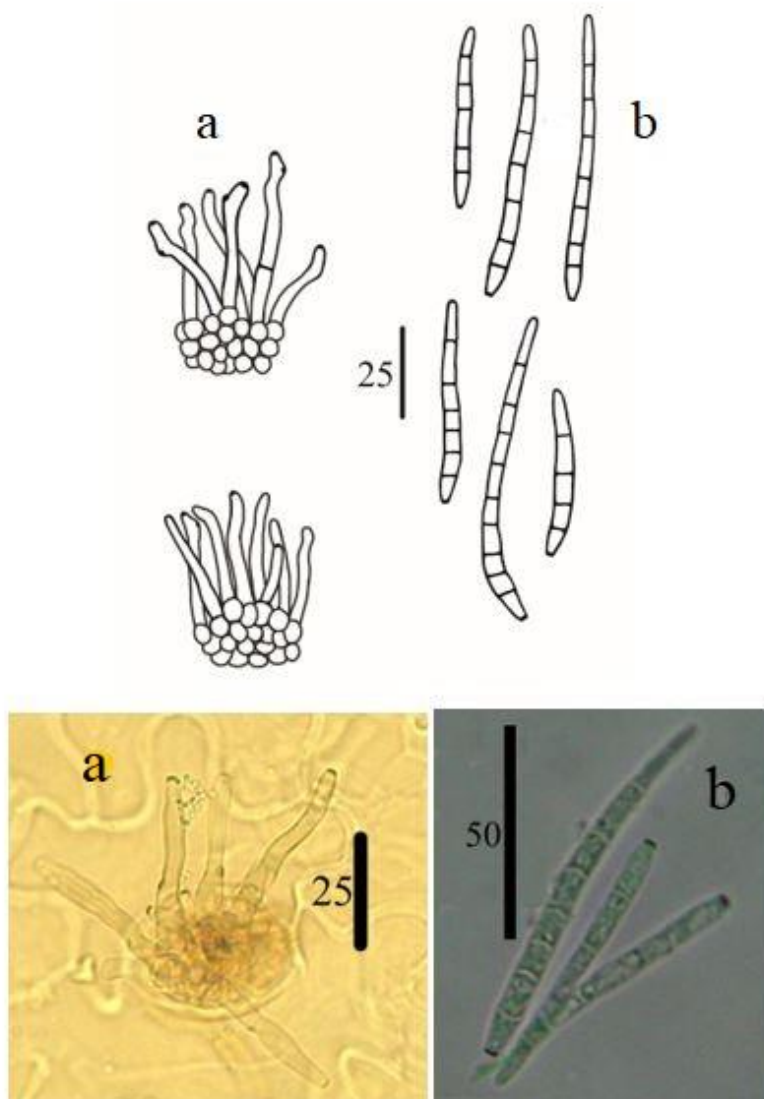


Fig. 1. *Cercospora mercurialis* on *Mercurialis annua* a. Stromata and conidiophores b. Conidia (Drawing: M. Pirnia).

***Cercospora pantoleuca* Sacc., *Michelia* 1: 268 (1878)**

Leaf spots circular, numerous, brown to blackish brown with gray center, 1–3 mm in diameter. Caespituli amphigenous, mostly epiphyllous, punctiform; stromata small to fairly prominent, brown, 20–25  $\mu\text{m}$  wide. Conidiophores fasciculate, 8–15 stalks, arising through stomata, pale olivaceous-brown, paler towards the tip, aseptate or with few inconspicuous septa, straight, subcylindrical to geniculate-sinuous, not branched, smooth, thin, 15–50  $\times$  2.5–5  $\mu\text{m}$ . Conidial scars conspicuous, thickened and darkened, terminal and lateral, 1.5–2  $\mu\text{m}$  wide. Conidia formed singly, hyaline, narrowly obclavate, straight to slightly curved, smooth, thin, 3–7-septate, base truncate, tip obtuse to subacute, 40–90  $\times$  2.5–4 (–5)  $\mu\text{m}$ . Hila thickened and darkened (Fig. 2).

*Specimen examined.* IRAN, Golestan Province, Gorgan, (Qarnabad), On *Plantago major* L. (Plantaginaceae), 13 July 2014, N. Heydari, IRAN 16806F.

*Notes*— Characters of specimen examined closely resembles Braun's (1995) description of this species, but slightly differs in having faintly pigmented conidiophores and is previously reported by Pirnia et al. (2012a) from North Khorasan Province (North East

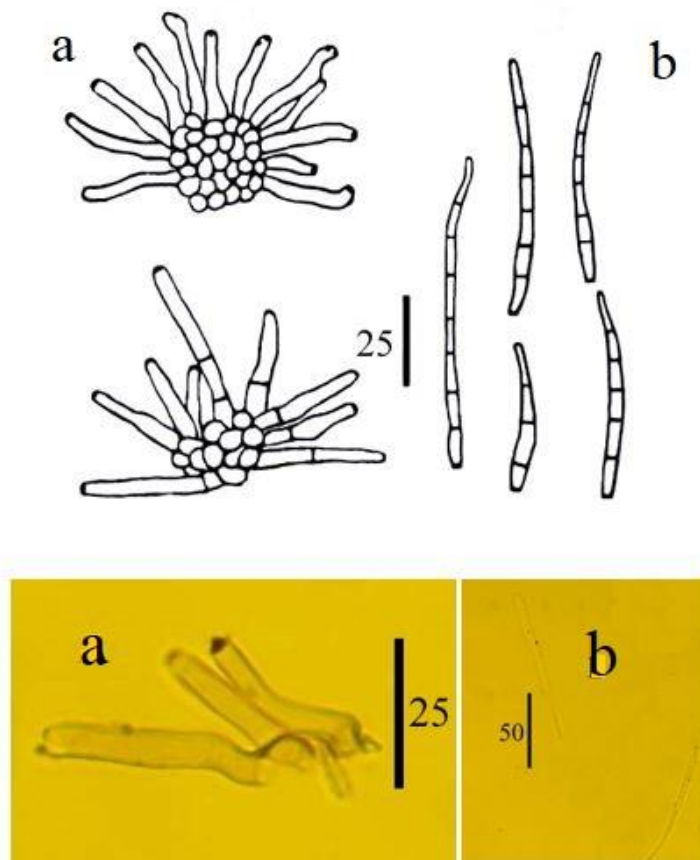
of Iran). This is the first report of the species from Golestan Province (North of Iran) and *Plantago major* is a new host for *C. pantoleuca* in Iran.

***Cercospora* sp.**

Leaf spots circular, center grayish white, 3–10 mm in diameter. Caespituli amphigenous. Stromata small, composed of a few pale brown cells, 12–25  $\mu\text{m}$  wide. Conidiophores in not dense fascicles, 4–6 stalks, pale olivaceous brown erect, not branched, 0–1-septate, geniculate to sinuous in the upper part, 15–50  $\times$  3–5  $\mu\text{m}$ . Conidiogenous loci conspicuous, mostly terminal. Conidia solitary, hyaline, obclavate to cylindrical, straight to slightly curved, smooth, thin, indistinctly multiseptate, base truncate, tip obtuse, 40–130 (–150)  $\times$  3–5  $\mu\text{m}$ . Hila thickened and darkened (Fig. 3).

*Specimen examined.* IRAN, Golestan Province, Gorgan, Tuskestan forest, On *Eruca* sp. (Brassicaceae), 30 June 2015, N. Heydari, IRAN 16804F.

*Notes*— Numerous morphologically distinguishable *Cercospora* species have been described on the basis of collections on various host genera belonging to the Brassicaceae, excluding the genus *Eruca* (Table 1).



**Fig. 2.** *Cercospora pantoleuca* on *Plantago major* a. Stromata and conidiophores b. Conidia (Drawing: M. Pirnia).

*Cercospora* sp. on *Eruca* is morphologically differ from other *Cercospora* species on Brassicaceae by having not dense fascicles, a few conidiogenous loci (scars) on conidiophores which are mostly terminal, short conidiophores (more than 50  $\mu\text{m}$  was not observed) and obclavate to cylindrical conidia (acicular conidia was not observed). According to our investigation, there is not any report of *Cercospora* species on *Eruca* in the world.

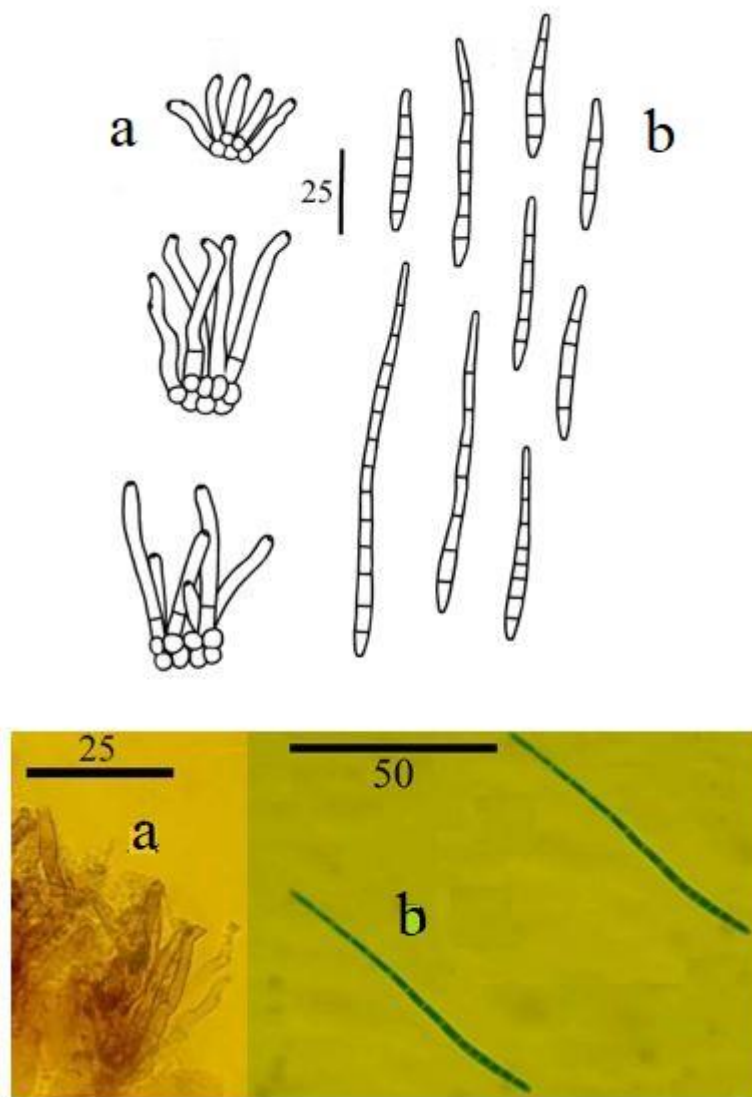
***Cercospora violae* Sacc., Nuovo Giornale Botanico Italiano 8: 187 (1876)**

Leaf spots circular, center white with dark brown boarder, 2–6 mm in diameter. Caespituli amphigenous, mostly epiphyllous. Stromata 20–35  $\mu\text{m}$  wide.

Conidiophores fasciculate, 10–15 stalks, pale brown and paller towards the tip, not branched, 0–2-septate, geniculate sinuous, 35–65  $\times$  3–5  $\mu\text{m}$ . Conidiogenous loci conspicuous, terminal and lateral. Conidia solitary, hyaline, obclavate to cylindrical, straight to slightly curved, smooth, thin, indistinctly multiseptate, base truncate, tip obtuse to subacute, 45–130  $\times$  2.5–4  $\mu\text{m}$ . Hila thickened and darkened (Fig. 4).

*Specimens examined.* IRAN, Golestan Province, Golestan National Park, On *Viola odorata* L. (Violaceae), 12 June 2015, N. Heydari, IRAN 16807F.

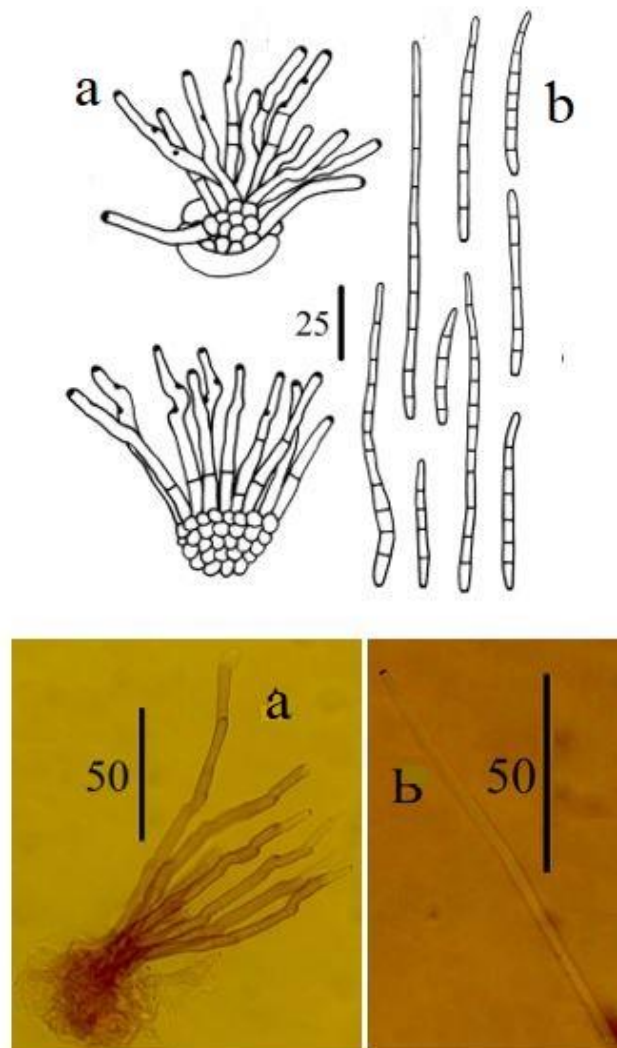
Notes— The last previous report of the species on *V. odorata* in Iran, date back to 58 years ago from Mazandaran Province (Khabiri 1958). This is the first report of the species on *V. odorata* from Golestan province.



**Fig. 3.** *Cercospora* sp. on *Eruca* sp. **a.** Stromata and conidiophores **b.** Conidia (Drawing: M. Pirnia).

**Table 1.** Conidiophore and conidium size of *Cercospora* species have hitherto been reported from host plant genera belonging to the plant family, Brassicaceae.

Species	Host plant	Conidiophore size ( $\mu\text{m}$ )	Conidium size ( $\mu\text{m}$ )
<i>Cercospora armoraciae</i>	<i>Armoracia rusticana</i>	15-50(-140)×4-6.5	25-150×3-5(-6.5)
<i>Cercospora barbarea</i>	<i>Barbarea vulgaris</i>	20-50(-80) × 4-6	50-110 × 3.5-5
<i>Cercospora bertoreae</i>	<i>Berteroa incana</i>	50-125 × 4-5.5	30-75 × 3-5
<i>Cercospora bizzozeriana</i>	<i>Lepidium latifolium</i>	10-100 × 4-7	30-60(-160)×3-7
<i>Cercospora brassicicola</i>	<i>Brassica chinensis</i>	25-500 × 3.5-7	25-200 × 2-5
<i>Cercospora cardaminae</i>	<i>Cardamina pratensis</i>	50-70 × 3-5	50-105 × 4
<i>Cercospora cheiranthi</i>	<i>Cheiranthus cheirus</i>	10-35(-100)×4-6.5	20-100 × 3-5
<i>Cercospora cruciferarum</i>	<i>Raphanus sativus</i>	15-60(-120)×4-5.5	40-150 × 2-4.5
<i>Cercospora erysimi</i>	<i>Erysimum cheiranthoides</i>	10-75 × 4-6	20-145 × 3-6
<i>Cercospora nasturtii</i>	<i>Nasturtium officinale</i>	20-100(-150)×4-6.5	20-85(-125)×3-5(-6)
<i>Cercospora stanleyae</i>	<i>Stanleya pinnatifida</i>	10-45 × 4.5-6	40-125 × 3-5.5

**Fig. 4.** *Cercospora violae* on *Viola odorata* a. Stromata and conidiophores b. Conidia (Drawing: M. Pirnia).

***Passalora* cf. *bacilligera* (Mont. & Fr.) Mont. & Fr. in Mont., *Sylloge generum specierumque cryptogamarum* 112: 10 (1967)**

Leaf spots circular, center brown with dark red boarder, 2–5 mm in diameter. Caespituli amphigenous, mostly epiphyllous. Stromata present, 30–40  $\mu\text{m}$  wide.

Conidiophores fasciculate, 10–15 stalks, pale brown, 0–2-septate, geniculate sinuous, 30–60 × 2.5–4  $\mu\text{m}$ . Conidiogenous loci conspicuous, terminal and lateral. Conidia solitary, olivaceous brown, obclavate to cylindrical, smooth, thin, 1–3-septate, base truncate to obconically truncate, tip rounded to obtuse, 20–60 × 3–6  $\mu\text{m}$ . Hila thickened and darkened (Fig. 5).

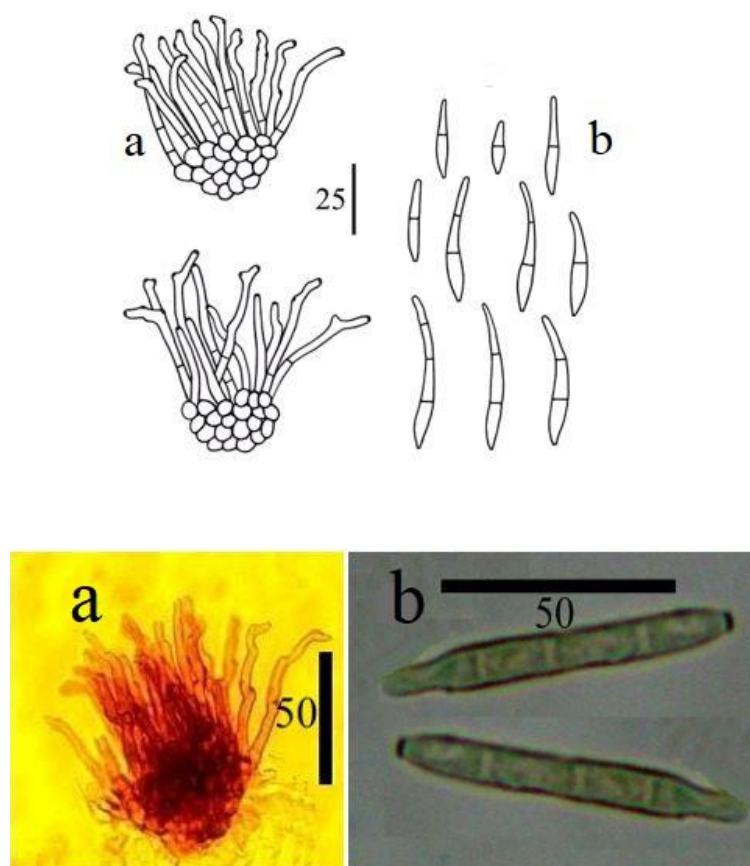


Fig. 5. *Passalora* cf. *bacilligera* on *Alnus* sp. a. Stromata and conidiophores b. Conidia (Drawing: M. Pirnia).

*Specimen examined.* IRAN, Golestan Province, Gorgan (Alangdareh), On *Alnus* sp. (Betulaceae), 23 Jul. 2014, *N. Heydari*, IRAN 16808F.

Notes— Characters of conidiophores and septation of conidia are identical to *P. bacilligera*, but conidia are narrower in our specimen. Pirnia et al. (2012c) and Bicharanlou et al. (2013b) have already been reported some *Passalora* species from North of Iran. This is the first report of *Passalora* cf. *bacilligera* for the mycobiota of Iran.

***Pseudocladosporium hachijoens* (Matsush.) U. Braun. A monograph of *Cercospora*, *Ramularia* and allied genera (phytopathogenic hyphomycetes). 2: 393 (1998)**

Fungus is saprobic, Conidiophores pale brown, very small, reduced to conidiogenous cells, forming as lateral protuberant denticles on creeping hyphae,  $7\text{--}10 \times 3\text{--}5 \mu\text{m}$ . Conidiogenous loci unthickened, Conidia pale brown, subcylindric, fusiform to ellipsoid-ovoid, forming in simple or often branched acropetal chains, 0–1-septate, ends truncate,  $11\text{--}34 \times 2.5\text{--}5 \mu\text{m}$ . Hila slightly darkened (Fig. 6).

*Specimen examined.* IRAN, Golestan Province, Golestan National Park, On *Diospyros lotus* L. (Ebenaceae), 22 May 2015, *N. Heydari*, IRAN 16809F.

Notes— The fungus is new for the mycobiota of Iran at both generic and species level. The genus *Pseudocladosporium* differs from *Cladosporium*. The latter genus is distinguished by having branched, geniculate macronematous conidiophores, sympodial conidiogenous cells with dark convex scars and amero to phragmoconidia.

***Ramularia grevilleana* (Tul. & C. Tul.) Jørst., Meld. Stat. plantenpatol. Inst. 1: 17 (1954)**

Leaf spots irregular, center white with red brown boarder, 1–3 mm in diameter. Caespituli amphigenous, mostly epiphyllous. Stromata present, conidiophores fasciculate, hyaline, 0–septate, geniculate sinuous,  $10\text{--}50 \times 2\text{--}4 \mu\text{m}$ . Conidiogenous loci conspicuous, terminal and lateral. Conidia catenate, hyaline, cylindrical, ellipsoid-ovoid smooth to rough, 0–3–septate, base truncate to obconically truncate, tip rounded to obtuse,  $20\text{--}45 \times 2.5\text{--}4 \mu\text{m}$ . Hila slightly thickened and darkened (Fig. 7).

*Specimen examined.* IRAN, Golestan Province, Golestan National Park, On *Potentilla reptans* L. (Rosaceae), 25 June 2015, *N. Heydari*, IRAN 16812F.

Notes— The species has wide host range on hosts of various genera of the Rosaceae. Pirnia et al. (2012d) reported the species on *Fragaria ananassa* Duchesne from Golestan province.

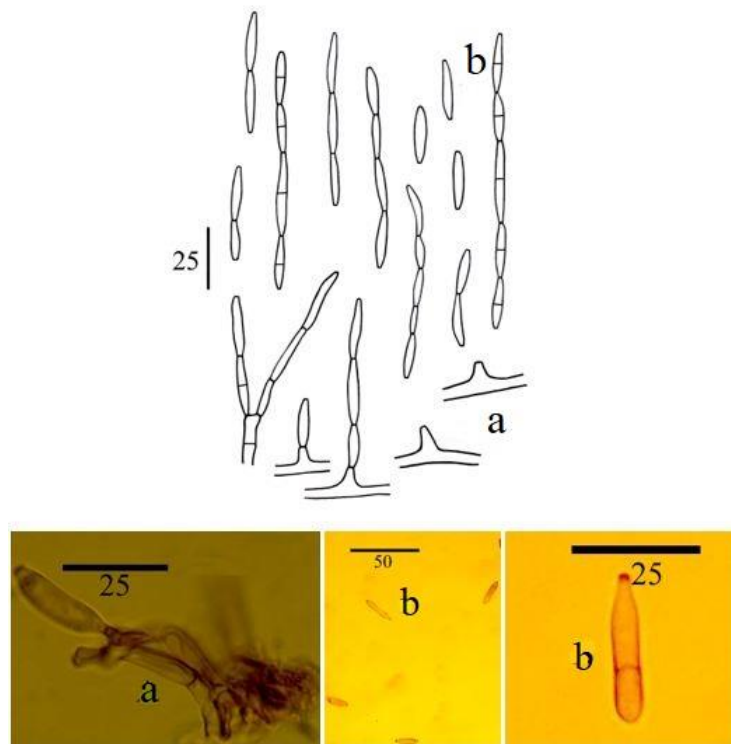


Fig. 6. *Pseudocladosporium hachijoens* on *Diospyros lotus* a. Conidiophores b. Conidia (Drawing: M. Pirnia).

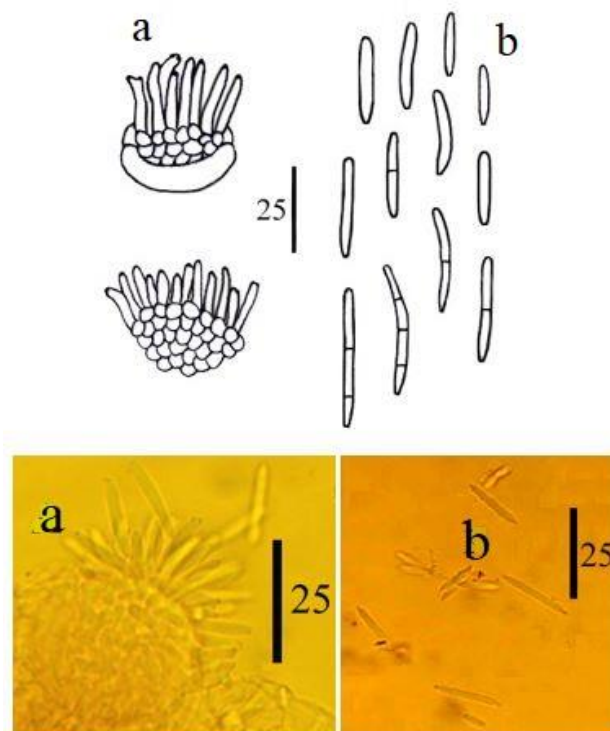


Fig. 7. *Ramularia grevilleana* on *Potentilla reptans* a. Stromata and conidiophores b. Conidia (Drawing: M. Pirnia).

***Ramularia rumicis* Kalchbr. & Cooke, Grevillea 8: 23 (1880)**

Leaf spots circular to subcircular, sometimes irregular, gray to pale brown, 2–10 mm in diameter.

Caespituli amphigenous, mostly hypophyllous, arising from stromata, through stomata, Conidiophores fasciculate, hyaline, 0–1- septate, geniculate sinuous, (20–) 30–60 (–100) × 3–6 µm. Conidiogenous loci conspicuous, terminal and lateral. Conidia solitary

catenate, hyaline, subcylindric, 0–3-septate, ends obtuse, rounded or somewhat attenuated  $15\text{--}40 \times 3\text{--}8$   $\mu\text{m}$ . Hila thickened and darkened (Fig. 8).

*Specimen examined.* IRAN, Golestan Province, Gorgan (Qarnabad), On *Rumex* sp. (Polygonaceae), 13 Nov. 2015, *N. Heydari*, IRAN 16810F.

Notes— The species was previously reported from Guilan, and Kordestan provinces (Ershad 2009, Pirnia et al. 2012d). This is the first report of the species from Golestan province (North of Iran).

***Ramularia sambucina* Sacc., Fungi. ital. del., Tab. 989. 1881, Michelia 2: 551 (1882)**

Leaf spots subcircular to irregular, center white with dark brown border, 1.5–5 mm in diameter. Caespituli amphigenous, arising from stomata, through stomata, Conidiophores fasciculate, hyaline, 0–1-septate, geniculate sinuous,  $10\text{--}38 \times 3\text{--}5$   $\mu\text{m}$ . Conidiogenous loci conspicuous, terminal and lateral. Conidia catenate, rarely in branched chains, hyaline, cylindrical to fusoid, ellipsoid to ovoid, 0–1-septate, ends truncate to rounded  $16\text{--}40 \times 5\text{--}8$   $\mu\text{m}$ . Hila slightly thickened and darkened (Fig. 9).

*Specimen examined.* IRAN, Golestan Province, Golestan National Park, On *Sambucus nigra* L.

(Adoxaceae), 22 May 2015, *N. Heydari*, IRAN 16811F.

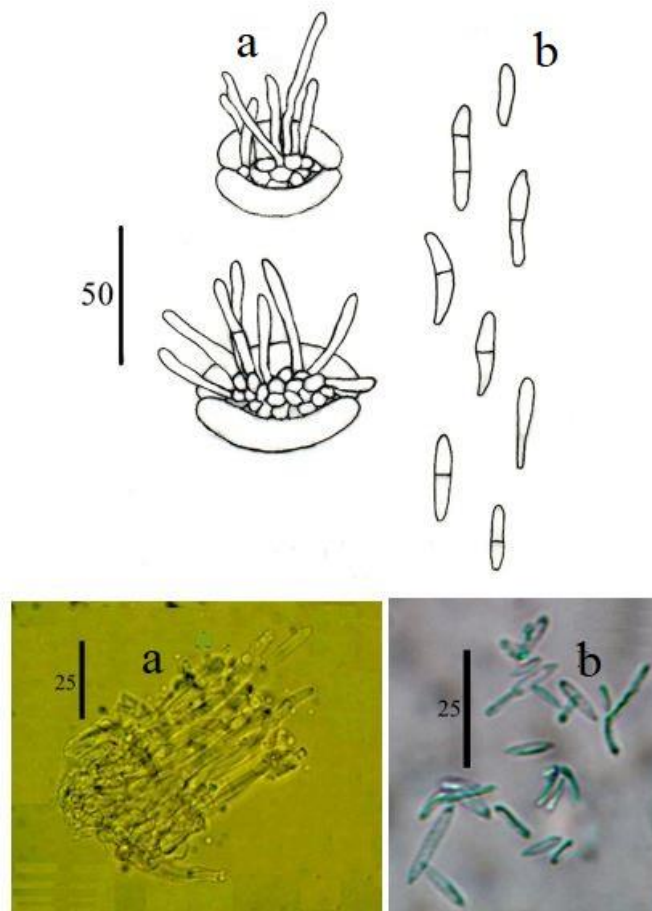
Notes— *Ramularia sambucina* was reported 75 years ago on *Sambucus ebulus* L. in Iran (Petrak and Esfandiari 1941). The morphology of the specimen examined agrees perfectly with the description of *R. sambucina* in Braun (1998). *Sambucus nigra* is a new host species for *R. sambucina* in Iran.

***Sirosporium celtidis* (Biv.) M.B. Ellis, Mycol. Pap. 87: 4 (1963)**

Fungal structures are hypophyllous, reddish brown to dark brown, velvety. Conidiophores small, not branched, 0–2 geniculate,  $12\text{--}20 \times 7\text{--}9$   $\mu\text{m}$ . Conidiogenous loci (scars) thickened, terminal. Conidia dark reddish brown, cylindrical to obclavate, often curved, thick walled, smooth, 14–30 transversely septate and occasionally with 1–4 longitudinal or oblique septa, broadly rounded at both ends,  $55\text{--}110 \times 5\text{--}8$   $\mu\text{m}$  (Fig. 10).

*Specimen examined.* IRAN, Golestan Province, Aliabad (Zarringol), On *Celtis australis* L. (Cannabaceae), 29 June 2014, *N. Heydari*, IRAN 16813F.

Notes— The species was previously reported from Iran (Ershad 2009; Pirnia et al. 2012a).



**Fig. 8.** *Ramularia rumicis* on *Rumex* sp. **a.** Stromata and conidiophores **b.** Conidia (Drawing: M. Pirnia).



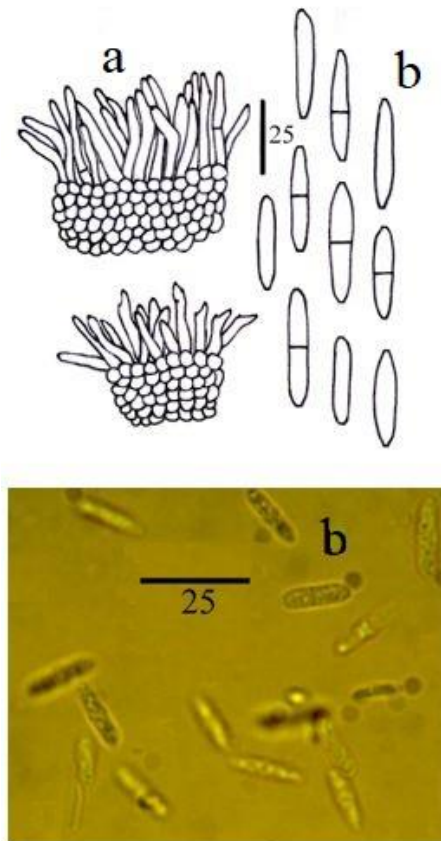


Fig. 9. *Ramularia sambucina* on *Sambucus nigra* A. Stromata and conidiophores B. Conidia (Drawing: M. Pirnia).

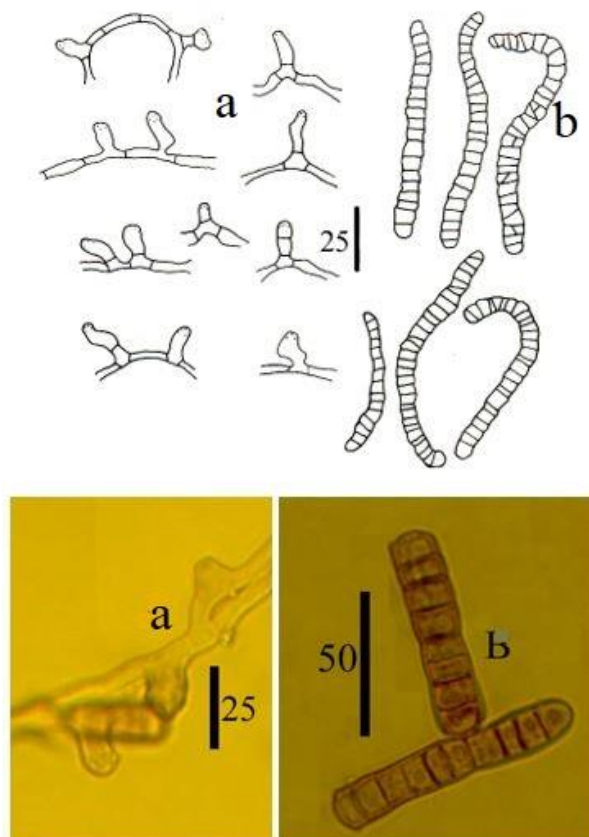


Fig. 10. *Sirosporium celtidis* on *Celtis australis* a. Conidiophores b. Conidia (Drawing: M. Pirnia).

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## گزارش های جدید از قارچ های آنامورفیک از شمال ایران

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**چکیده:** در این مقاله قارچ های مرتبط با علایم لکه برگی از گیاهان مناطق جنگلی در استان گلستان جمع آوری و بر اساس خصوصیات ریخت شناسی مربوط به کنیدیوفور، کنیدیوم و سلول مولد کنیدیوم مورد بررسی قرار گرفتند. ده آرایه شناسایی شدند. از بین آنها *Passalora bacilligera* و *Pseudocladosporium hachijoens* به عنوان گزارش های جدید برای مایکوبیوتای ایران معرفی می شوند. *Cercospora* روی *Eruca* sp. از لحاظ خصوصیات ریخت شناسی با سایر سرکوسپوراهای گزارش شده روی خانواده Brassicaceae متفاوت بود و با گونه های گزارش شده روی این خانواده مقایسه شد. *Cercospora pantoleuca* برای اولین بار از استان گلستان (شمال ایران) گزارش می شود. علاوه بر این، *Plantago major* و *Sambucus nigra* به عنوان میزبان های جدید، به ترتیب برای *C. pantoleuca* و *Ramularia sambucina* در ایران معرفی می شوند.

**واژگان کلیدی:** میکوبیوتا، لکه برگی، ریخت شناسی، تاکسونومی