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Molecular phylogeny and morphology of four *Ramularia* species from Iran along with a checklist of ramularia-like taxa

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Abstract

The genus *Ramularia* includes important plant pathogens with worldwide distribution, commonly associated with leaf spot diseases on a broad range of plant hosts. Although these fungi are common in Iran, most of the species found to date have been identified on the basis of morphological characteristics, and DNA data are available for limited number of them. During our investigation of fungi associated with leaf spot diseases in north and northwest of Iran, *Ramularia* isolates were recovered from leaves with leaf spots on different herbaceous and woody plants in the *Asteraceae*, *Apiaceae*, and *Vitaceae* families. Based on sequence data of five genomic loci (ITS, *actA*, *tef1*, *rpb2* and *gapdh*), host, cultural and morphological data; four species including *R. cynarae* on *Cirsium arvense*, *R. heraclei* on *Heracleum* sp., *R. hydrangeae-macrophyllae* on *Vitis vinifera*, and *R. inaequalis* on *Taraxacum campylodes*, were identified. *Ramularia hydrangeae-macrophyllae* represents a new record for the mycobiota of Iran as well as Asia, and *V. vinifera* is a new host for this species in the world. Moreover, *C. arvense* and *T. campylodes* are new hosts for *R. cynarae* and *R. inaequalis* in Iran, respectively. Additionally, a comprehensive literature-based checklist for 50 ramularia-like species known to occur on different plant species in Iran was provided. The complete annotated list covers 41 *Ramularia* species, two *Cercosporella*, two *Neoovularia*, two *Neoramularia*, one *Microcyclosporella*, one *Neopseudocercosporella*, and one *Ramulariopsis*.

Keywords: Hyphomycetes, leaf spotting fungi, *Mycosphaerella*, phylogeny, systematic

فیلوژنی مولکولی و ریخت‌شناسی چهار گونه رامولاریا از ایران به همراه فهرستی از آرایه‌های شبه‌رامولاریا

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مونس بخشی: استادیار پژوهش، بخش تحقیقات رستنی‌ها، مؤسسه تحقیقات گیاه‌پزشکی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران (mounesbakhshi@gmail.com)

خلاصه

جنس *Ramularia* بیمارگرهای گیاهی مهمی را شامل می‌شود که در سراسر جهان پراکنده‌اند و اغلب با علایم لکه‌برگی در دامنه گسترده‌ای از میزبان‌های گیاهی همراه هستند. با وجود پراکنش گسترده این قارچ‌ها در ایران، تاکنون بیشتر گونه‌ها براساس صفات ریخت‌شناختی شناسایی شده‌اند و توالی DNA برای تعداد بسیار اندکی از آن‌ها وجود دارد. طی مطالعه عوامل ایجاد لکه برگی روی گیاهان مختلف در نواحی شمال و شمال‌غرب کشور، جدایه‌هایی از جنس رامولاریا از برگ‌های دارای علایم لکه‌برگی میزبان‌های چوبی و علفی در تیره‌های *Asteraceae*، *Apiaceae* و *Vitaceae* به دست آمد. براساس ترکیب داده‌های توالی پنج ناحیه ژنی (ITS، *actA*، *tef1*، *rpb2* و *gapdh*)، اطلاعات میزبان و صفات ریخت‌شناختی، چهار گونه شامل: *R. cynarae* روی کنگر صحرایی (*Cirsium arvense*)، *R. heraclei* روی گیاه *Heracleum* sp.، *R. hydrangeae-macrophyllae* روی انگور (*Vitis vinifera*) و *R. inaequalis* روی گیاه گل قاصد (*Taraxacum campylodes*) شناسایی شدند. این نخستین گزارش از وجود آرایه *R. hydrangeae-macrophyllae* برای میکوبیوتای ایران و همچنین قاره آسیا بوده و انگور به عنوان میزبان جدیدی برای این گونه در دنیا گزارش می‌شود. همچنین، کنگر صحرایی و گل قاصد به ترتیب به عنوان میزبان‌های جدیدی در ایران برای دو گونه *R. cynarae* و *R. inaequalis* هستند. علاوه‌براین، در این پژوهش، فهرست کاملی از ۵۰ گونه رامولاریا و جنس‌های مشابه که روی میزبان‌های مختلف در ایران گزارش شده‌اند، ارایه گردیده است. این فهرست دربردارنده ۴۱ گونه از جنس رامولاریا، دو گونه از جنس *Cercosporella*، دو گونه از جنس *Neoovularia*، دو گونه *Neoramularia*، یک گونه *Microcyclosporella*، یک گونه *Neopseudocercosporella* و یک گونه *Ramulariopsis* می‌باشد.

واژه‌های کلیدی: سیستماتیک، فیلوژنی، قارچ‌های عامل لکه‌برگی، میکوسفرلا، هیفومیست

Introduction

Ramularia Unger is a species-rich genus, that belongs to the family *Mycosphaerellaceae* (*Ascomycota*, *Dothideomycetes*, *Capnodiales*) and was originally described by Unger (1833) based on *Ramularia pusilla* Unger (Videira *et al.* 2015b, Bakhshi & Arzanlou 2017). The genus was monographed by Braun (1995, 1998) who considered *Ramularia* as a genus of hyphomycetous fungi characterized by having colorless structures (conidiophores and conidia) with distinct, thickened, darkened and refractive conidiogenous loci and conidial hila. A combination of morphological features including the color (hyaline or pigmented) and the structure of conidiophores (simple or branched), the structure of conidiogenous loci and conidial hila (conspicuous or inconspicuous, by being thickened and darkened or not) have been used in the taxonomy of *Ramularia* and allied genera (Braun1995, 1998). Genera with hyaline structures and conspicuous, thickened and darkened conidial loci include *Cercosporella* Sacc., *Hawksworthiana* U. Braun, *Neoovularia* U. Braun, *Phacellium* Bonord., *Pseudodidymaria* U. Braun, *Ramularia* and *Ramulariopsis* Speg., while genera with inconspicuous conidial loci include *Monodidymaria* U. Braun, *Neoramularia* U. Braun, and *Pseudocercosporella* Deighton (Videira *et al.* 2016).

The genus *Ramularia* includes many plant pathogenic species with worldwide distribution, causing generally leaf spots on various host plants (Verkley *et al.* 2004, Videira *et al.* 2015a, b, 2016, Bakhshi & Arzanlou 2017). Some important phytopathogenic species include *R. beticola* Fautrey & Lambotte on sugar beet (Wieczorek *et al.* 2014), *R. collo-cygni* B. Sutton & J.M. Waller on barley (Walters *et al.* 2008) and *R. grevilleana* (Oudem.) Jørst. on strawberry (Carisse *et al.* 2000) that cause severe economic losses to these crops. For most of the *Ramularia* species, sexual morph is unknown, but those identified, were linked to the sexual genus *Mycosphaerella* Johanson (*Mycosphaerellaceae*) (Braun 1998, Park & Shin 2016, Videira *et al.* 2016). Since January 2013, following the new rules for naming of pleomorphic fungi outlined in the International Code of Nomenclature for Algae, Fungi and Plants (ICN) (Hawksworth 2011, Norvell 2011, Wingfield *et al.* 2012), the older asexual name *Ramularia* (1833) has been proposed as preferred name instead of the younger sexual name *Mycosphaerella* (1884) (Kirk *et al.* 2013, Wijayawardene *et al.* 2014, Rossman *et al.* 2015).

Historically, a combination of morphological traits such as the shape, size and septation of conidia and the type of conidiogenous loci and conidial hila along with host taxonomy have been used to identify species of *Ramularia* and allied genera (Braun 1995, 1998). However, considering that, the morphological characters by which to describe and identify *Ramularia* and ramularia-like species are rather reduced; reliable identification of these species based on morphological characters alone is difficult (Videira *et al.* 2015a, b, 2016, Bakhshi & Arzanlou 2017). In recent years, the use of DNA phylogenetic markers, also known as DNA barcoding is becoming an increasingly prevalent tool for taxonomy of the different groups of fungi (Bakhshi *et al.* 2014, 2015, 2018, Crous *et al.* 2009a, 2013, Groenewald *et al.* 2013, Verkley *et al.* 2013, Quaedvlieg *et al.* 2013, Videira *et al.* 2015a, b, 2016). To date, the most inclusive molecular study in order to improve the delimitation of *Ramularia* from allied genera and the circumscription of species within this genus, is that of Videira *et al.* (2016) who compared 420 isolates belonging to *Ramularia* and allied genera based on multilocus DNA sequences, morphological and cultural data. The robust multi-gene phylogeny inferred showed that the genus *Ramularia* proved to be polyphyletic, and not monophyletic as previously thought, and the species non-congeneric with the type *R. pusilla* were assigned to the new genera *Xenoramularia* Videira, H.D. Shin & Crous, *Epicoleosporium* Videira & Crous, and *Teratoramularia* Videira, H.D. Shin & Crous (Videira *et al.* 2016). Therefore these data and other molecular studies on the genus *Ramularia* (Videira *et al.* 2015a, b, Bakhshi &

Arzanlou 2017) show that the identifications in *Ramularia* and ramularia-like fungi will have to rely on DNA sequence data to support morphological conclusions.

Hitherto, most of the ramularia-like taxa reported from Iran, have been identified based on morphological characteristics and host range (Ershad 2009, Pirnia *et al.* 2012, Bicharanlou *et al.* 2014, Heidari *et al.* 2015, Behrooz *et al.* 2015, 2017, Heydari *et al.* 2017, Pirnia & Braun 2018) and DNA data are available for a limited number of these (Videira *et al.* 2016, Bakhshi & Arzanlou 2017), rendering their identifications unproven in the light of recent molecular revisions of this genus (Videira *et al.* 2015a, b, 2016). In this regard, the aim of this study was to characterize *Ramularia* species obtained from the infected leaves of several plant species collected from the northwest of Iran; based on morphology, cultural characteristics and phylogenetic analyses of the DNA sequence data. In addition, we have also assembled a checklist of ramularia-like taxa recorded to date from Iran, with the hope of what is reported here, may encourage other mycologists to study the diversity of this group of fungi in Iran.

Materials and Methods

- List of the species

The list of ramularia-like fungi was compiled using reports available in the literature (Ershad 2009, Pirnia *et al.* 2012, Bicharanlou *et al.* 2014, Heidari *et al.* 2015, Behrooz *et al.* 2015, 2017, Bakhshi & Arzanlou 2017, Heydari *et al.* 2017, Pirnia & Braun 2018, Farr & Rossman 2018). The list includes ramularia-like taxa together with their host species and families from which they have been collected. Synonyms were identified and related to currently accepted names with the help of recent papers and Mycobank (http:// http://www.mycobank.org). The checklist is organized alphabetically by genus and species name (Table 1).

- Sample collection, isolation and morphological characterization

Symptomatic leaves were collected in the field from East and West Azarbaijan provinces (Iran). Leaves were examined in the laboratory directly under a Nikon SMZ 1500 stereo-microscope. Isolates were obtained in pure culture by direct transfer of conidia from a single leaf spot onto plates containing 2% malt extract agar (MEA; Fluka, Hamburg, Germany) with a sterile fine pointed needle using a previously described procedure (Bakhshi *et al.* 2011). Representative cultures were deposited in the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS), Utrecht, The Netherlands. Dried specimens were preserved in the Fungarium of the Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN).

- DNA extraction, PCR amplification and sequencing

Fungal mycelia of isolates grown on MEA plates for 15 days at 25 °C in the dark, were harvested with a sterile scalpel and the genomic DNA was extracted according to the protocol developed by Möller *et al*. (1992). Five partial nuclear genes were initially targeted for PCR amplification and sequencing, namely, internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) of the nrDNA operon, actin (*actA*), translation elongation factor 1-α (*tef1*), RNA polymerase II second largest subunit (*rpb2*) and glyceraldehyde-3-phosphate dehydrogenase (*gapdh*). The PCR amplifications were performed in a total volume of 12.5 µL on a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The primers, protocols and conditions for standard amplification and subsequent sequencing of the loci were according to Bakhshi *et al*. (2015) for ITS, *tef1* and *actA* loci, Videira *et al*. (2015a) for *rpb2* and Bakhshi & Arzanlou (2017) for *gapdh*.

The resulting fragments were sequenced in both directions using the PCR primers and the ABI Prism BigDye® Terminator Cycle Sequencing Reaction Kit Ver. 3.1 (Applied Biosystems™, Foster City, CA, USA), following the protocol of the manufacturer. DNA sequencing amplicons were purified through Sephadex® G-50 Superfine columns (Sigma Aldrich, St. Louis, MO) in MultiScreen HV plates (Millipore, Billerica, MA) as outlined by the manufacturer. Purified sequence reactions were run on an Applied Biosystems™ 3730xl DNA Analyzer (Life Technologies Europe BV, Applied Biosystems™, Bleiswijk, The Netherlands).

- Sequence alignment and phylogenetic analysis

DNA sequence data were analyzed in MEGA (Molecular Evolutionary Genetics Analysis) Ver. 6 software (Tamura *et al.* 2013) and consensus sequences were manually generated from the forward and reverse sequences. The consensus regions of ITS, *actA*, *tef1*, *rpb2* and *gapdh* were compared with sequences available in National Center for Biotechnology Information (NCBI) GenBank nucleotide database using a megaBLAST search. The obtained sequences from GenBank together with the newly generated sequences during this study, were aligned with MAFFT Ver. 7 online interface using default settings (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013) for each gene and whenever necessary, manually improved in MEGA Ver. 6. The alignments were concatenated with Mesquite Ver. 2.75 (Maddison & Maddison 2011).

MrModeltest Ver. 2.3 (Nylander 2004) was used to determine the appropriate gene models for each gene partition. A Bayesian phylogenetic reconstruction was performed with MrBayes Ver. 3.2.6 (Ronquist *et al.* 2012) based on the results of MrModeltest. The heating parameter was set at 0.15 and burn-in was set to 25% and trees were saved each 1000 generations. The Markov Chain Monte Carlo (MCMC) analysis of four chains was started in parallel from a random tree topology and lasted until the average standard deviation of split frequencies reached a value of 0.01 (stopval = 0.01). The resulting phylogenetic tree was printed with Geneious Ver. 8.1.8 (Kearse *et al.* 2012). All new sequences generated in this study, were deposited in NCBIs GenBank nucleotide database (www.ncbi.nlm.nih.gov), and the accession numbers of the sequences used for the phylogenetic analyses are detailed in table 2.

- Taxonomy

Microscopic structures were studied on synthetic nutrient-poor agar (SNA) (Crous *et al.* 2009b) after incubation at 21 °C for 7–15 days. Slides were prepared using the inclined coverslip method (Nugenta *et al.* 2006) in clear lactic acid as mounting medium and also transparent adhesive tape (Bensch *et al.* 2012), with at least 30 measurements per structure, with extreme values given in parentheses. Observations of the microscopic structures were performed at ×1000 magnification using a Nikon Eclipse 80i light microscope with differential interference contrast illumination. The terminology of morphological structures followed those used for the description of *Ramularia* species by Crous *et al.* (2011). Images were recorded with a Nikon digital sight DS-f1 high-definition color camera mounted on the above-mentioned light microscope. For culture characterization, the isolates were inoculated on SNA, MEA and Oatmeal Agar (OA; Crous *et al.* 2009b), and incubated in the dark at 25 °C. After 14 days, the colony diameter was measured and the colony color was rated according to the mycological color charts of Rayner (1970). The layout of acquired images and photographic preparations was carried out in Adobe Photoshop CS5.

Table 1. Ramularia-like species known from Iran

Taxon	Host	Family	Reference
Cercosporella primulae Allesch.	*Primula veris* subsp. *macrocalyx* (Bunge) Lüdi [syn.: *Primula macrocalyx* Bunge]	*Primulaceae*	Pirnia *et al.* 2012
C. virgaureae (Thüm.) Allesch.	*Erigeron bonariensis* L. [syn.: *Conyza bonariensis* (L.) Cronquist]	*Asteraceae*	Pirnia *et al.* 2012
Microcyclosporella mali J. Frank, Schroers & Crous	*Malus pumila* Mill.	*Rosaceae*	Heidari *et al.* 2015
M. mali	*Pyrus communis* L.	*Rosaceae*	Heidari *et al.* 2015
Neoovularia nomuriana (Sacc.) U. Braun	*Astragalus odoratus* Lam.	*Fabaceae*	Ershad 2009
N. ovata (Fuckel) U. Braun	*Salvia hypoleuca* Benth.	*Lamiaceae*	Ershad 2009
N. ovata	*S. nemorosa* L.	*Lamiaceae*	Ershad 2009
N. ovata	*Salvia* sp.	*Lamiaceae*	Ershad 2009
Neopseudocercosporella capsellae (Ellis & Everh.) Videira & Crous = *Pseudocercosporella capsellae* (Ellis & Everh.) Deighton	*Capsella bursa-pastoris* (L.) Medik.	*Brassicaceae*	Behrooz *et al.* 2015
N. capsellae	*Sinapis arvensis* L.	*Brassicaceae*	Behrooz *et al.* 2015
Neoramularia esfandiarii (Petr.) U. Braun	*Scrophularia* sp.	*Scrophulariaceae*	Ershad 2009
N. rubi (Bubák) U. Braun	*Rubus caesius* L.	*Rosaceae*	Ershad 2009
Ramularia alpina (C. Massal.) Nannf.	*Alchemilla* sp.	*Rosaceae*	Ershad 2009
R. anchusae C. Massal.	*Anchusa azurea* Mill. [syn.: *Anchusa italica* Retz.]	*Boraginaceae*	Ershad 2009, Pirnia *et al.* 2012
R. anchusae	*A. arvensis* subsp. *orientalis* (L.) Nordh. [syn.: *Anchusa ovata* Lehm.]	*Boraginaceae*	Ershad 2009, Pirnia *et al.* 2012
R. anchusae	*A. azurea*	*Boraginaceae*	Behrooz *et al.* 2017
R. armoraciae Fuckel	*Barbarea plantaginea* DC.	*Brassicaceae*	Behrooz *et al.* 2017
R. beccabungae Fautr.	*Veronica anagallis-aquatica* L.	*Scrophulariaceae*	Behrooz *et al.* 2015
R. beccabungae	*V. beccabunga* L.	*Scrophulariaceae*	Ershad 2009
R. bornmuelleriana (Magnus) U. Braun	*Onobrychis sintenisii* Bornm.	*Fabaceae*	Ershad 2009
R. brunnea Peck	*Tussilago farfara* L.	*Asteraceae*	Pirnia *et al.* 2012
R. carletonii (Ellis & Kellerm.) U. Braun	*Lactuca tuberosa* Jacq.	*Asteraceae*	Pirnia & Braun 2018
R. cupulariae Pass.	*Inula* sp.	*Asteraceae*	Behrooz *et al.* 2017
R. cynarae Sacc.	*Carthamus oxyacantha* M.Bieb.	*Asteraceae*	Ershad 2009, Pirnia *et al.* 2012
R. cynarae	*C. tinctorius* L.	*Asteraceae*	Ershad 2009, Pirnia *et al.* 2012, Behrooz *et al.* 2017
R. epilobiana (Sacc. & Fautrey) B. Sutton & Piroz.	*Epilobium hirsutum* L.	*Onagraceae*	Behrooz *et al.* 2017

Table 1. (contd)

R. geranii Fuckel var. *geranii*	*Geranium pyrenaicum* Burm.f.	*Geraniaceae*	Ershad 2009, Pirnia *et al.* 2012
R. glennii Videira & Crous	*Acalypha australis* L.	*Euphorbiaceae*	Bakhshi & Arzanlou 2017
R. glennii	*Ficus carica* L.	*Moraceae*	Bakhshi & Arzanlou 2017
R. glennii	*Platanus* sp.	*Platanaceae*	Bakhshi & Arzanlou 2017
R. grevilleana (L.R. Tul. & C. Tul.) Jørst., var. *grevilleana* U. Braun	*Fragaria ananassa* (Duchesne ex Weston) Duchesne ex Rozier	*Rosaceae*	Pirnia *et al.* 2012
R. grevilleana var. *grevilleana*	*Fragaria* sp.	*Rosaceae*	Pirnia *et al.* 2012
R. grevilleana var. *grevilleana*	*Potentilla reptans* L.	*Rosaceae*	Pirnia *et al.* 2012, Behrooz *et al.* 2017, Heydari *et al.* 2017
R. heraclei (Oud.) Sacc.	*Heracleum persicum* Desf. ex Fisch., C.A.Mey. & Avé-Lall.	*Apiaceae*	Ershad 2009, Pirnia *et al.* 2012
R. heraclei	*Heracleum* sp.	*Apiaceae*	Ershad 2009, Pirnia *et al.* 2012
R. inaequalis (Preuss) U. Braun	*Calendula persica* C.A.Mey.	*Asteraceae*	Pirnia *et al.* 2012
R. iranica Petr.	*Acantholimon* sp.	*Plumbaginaceae*	Ershad 2009
R. lamii Fuckel	*Leonurus cardiaca* L.	*Lamiaceae*	Ershad 2009
R. lamii	*Mentha arvensis* L.	*Lamiaceae*	Ershad 2009
R. lamii	*M. longifolia* L.	*Lamiaceae*	Behrooz *et al.* 2017
R. lamii Fuckel var. *lamii* U. Braun	*M. arvensis* L.	*Lamiaceae*	Pirnia *et al.* 2012
R. lamii var. *lamii*	*M. piperita* L.	*Lamiaceae*	Pirnia *et al.* 2012
R. lamii var. *lamii*	*Mentha* sp.	*Lamiaceae*	Pirnia *et al.* 2012
R. macrospora Fres.	*Campanula rapunculus* L.	*Campanulaceae*	Ershad 2009
R. macularis (J. Schröt.) Sacc. & P. Syd.	*Chenopodium album* L.	*Chenopodiaceae*	Pirnia *et al.* 2012
R. mali Videira & Crous	*Prunus cerasus* L.	*Rosaceae*	Bakhshi & Arzanlou 2017
R. mali	*Vitis vinifera* L.	*Vitaceae*	Bakhshi & Arzanlou 2017
R. marrubii C. Massal.	*Sideritis montana* L.	*Lamiaceae*	Behrooz *et al.* 2015
R. marrubii	*Sideritis* sp.	*Lamiaceae*	Ershad 2009
R. nagornyi Karak.	*Centaurea solstitialis* L.	*Asteraceae*	Pirnia & Braun 2018
R. picridis Fautrey & Roum.	*Picris strigosa* M.Bieb.	*Asteraceae*	Pirnia & Braun 2018
R. pratensis Sacc.	*Rumex crispus* L.	*Polygonaceae*	Pirnia *et al.* 2012, Behrooz *et al.* 2015
R. pratensis	*Rumex* sp.	*Polygonaceae*	Pirnia *et al.* 2012, Behrooz *et al.* 2015

Table 1. (contd)

R. primulae Thum.	*Primula vulgaris* Huds. [Syn.: *P. aqualis* (L.) Hill]	*Primulaceae*	Aghapour *et al.* 2010
R. ranunculicola Pirnia & U. Braun	*Ranunculus muricatus* L.	*Ranunculaceae*	Pirnia & Braun 2018
R. rhabdospora (Berk. & Broome) Nannf.	*Plantago lanceolata* L.	*Plantaginaceae*	Ershad 2009, Pirnia *et al.* 2012, Behrooz *et al.* 2015
R. rubella (Bonord) Nannf.	*Rumex acetosa* L.	*Polygonaceae*	Bicharanlou *et al.* 2014
R. rubella	*R. conglomeratus* Murray	*Polygonaceae*	Pirnia *et al.* 2012, Behrooz *et al.* 2015
R. rubella	*R. crispus*	*Polygonaceae*	Ershad 2009
R. rubella	*Rumex* sp.	*Polygonaceae*	Ershad 2009
R. rufomaculans Peck	*Polygonum* sp.	*Polygonaceae*	Ershad 2009
R. rumicis Kalchbr. & Cooke	*Rumex crispus*	*Polygonaceae*	Ershad 2009, Pirnia *et al.* 2012
R. rumicis	*Rumex* sp.	*Polygonaceae*	Ershad 2009, Pirnia *et al.* 2012, Behrooz *et al.* 2015, Heydari *et al.* 2017
R. rumicis-scutati Allesch.	*R. scutatus* L.	*Polygonaceae*	Ershad 2009
R. sambucina Sacc.	*Sambucus ebulus* L.	*Caprifoliaceae*	Ershad 2009, Pirnia *et al.* 2012
R. sambucina	*S. nigra* L.	*Caprifoliaceae*	Heydari *et al.* 2017
R. simplex Pass.	*Ranunculus acris* L.	*Ranunculaceae*	Behrooz *et al.* 2017
R. simplex	*R. oxyspermus* Willd.	*Ranunculaceae*	Ershad 2009, Pirnia *et al.* 2012
R. simplex	*R. sahandicus* Murr.?	*Ranunculaceae*	Pirnia *et al.* 2012
R. taleshina M. Bakhshi & Arzanlou	*Alnus subcordata* C.A.Mey.	*Betulaceae*	Bakhshi & Arzanlou 2017
R. uredinicola Khodap. & Braun	*Melampsora* sp. on *Salix babylonica* L.	*Melampsoraceae*	Ershad 2009, Pirnia *et al.* 2012
R. urticae Ces.	*Urtica dioica* L.	*Urticaceae*	Ershad 2009, Pirnia *et al.* 2012
R. urticae	*U. urens* L.	*Urticaceae*	Ershad 2009, Pirnia *et al.* 2012
R. valeriana (Speg.) Sacc.	*Valeriana sisymbriifolia* Vahl.	*Valerianaceae*	Ershad 2009
R. variabilis Fuckel	*Verbascum blattaria* L.	*Scrophulariaceae*	Bicharanlou *et al.* 2014, Behrooz *et al.* 2015
R. variabilis	*V. sinuatum* L.	*Scrophulariaceae*	Behrooz *et al.* 2015
R. veronicae Fuckel	*Veronica anagallis-aquatica* L.	*Scrophulariaceae*	Behrooz *et al.* 2015
R. winteri Thüm.	*Ononis spinosa* L.	*Fabaceae*	Behrooz *et al.* 2015

Table 1. (contd)

Ramularia sp.	*Foeniculum vulgare* Mill.	*Apiaceae*	Ershad 2009
Ramularia sp.	*Potentila* sp.	*Rosaceae*	Ershad 2009
Ramularia sp.	*Rumex* sp.	*Polygonaceae*	Ershad 2009
Ramulariopsis gossypii (Speg.) U. Braun	*Gossypium hirsutum* L.	*Malvaceae*	Pirnia *et al.* 2012
R. gossypii	*Gossypium* sp.	*Malvaceae*	Ershad 2009, Pirnia *et al.* 2012

Results and Discussion

- Checklist of the known species of ramularia-like taxa from Iran

The present check list contained 41 species of *Ramularia*, two species of *Cercosporella*, two species of *Neoovularia*, two species of *Neoramularia*, one species of *Microcyclosporella* J. Frank, Schroers & Crous, one species of *Neopseudocercosporella* Videira & Crous and one species of *Ramulariopsis* (Table 1). The highest numbers of ramularia-like taxa were recorded on the representatives of the *Asteraceae* (eight species), *Polygonaceae* (five species), *Rosaceae* (five species), *Lamiaceae* (four species) and *Scrophulariaceae* (four species) families.

The preliminary checklist of ramularia-like taxa gathered here, is useful for a wide range of activities. It provides both an overview on the known diversity of ramularia-like taxa in Iran and a basis for ongoing and future taxonomic studies on this group of fungi in the country. However, of the 50 ramularia-like taxa reported from Iran, very few records are supported by cultures and DNA data (Bakhshi & Arzanlou 2017), rendering their identifications unproven in the light of recent molecular revisions of these fungi (Videira *et al.* 2015a, b, 2016). In addition, conservation of microbial isolates which constitute part of a country's Heritage, in culture collections which play a significant role in conserving biological diversity, is much crucial (Mahilum-Tapy 2009). Therefore, a more exhaustive re-sampling of these important plant pathogens from diverse host plants in various geographical regions of the country is urgent to preserve these fungi in a culture collection, sequence them and resolve the taxonomic rank of them in the light of recent molecular revisions of this genus (Videira *et al.* 2015a, b, 2016).

- Phylogenetic analysis

The final concatenated alignment consisted of 2476 characters (including the alignment gaps), representing 69 taxa of *Ramularia* spp. (including 60 taxa from NCBI, and nine taxa from this study), and *Zymoseptoria halophila* (Speg.) M. Razavi, Quaedvl. & Crous (isolate CBS 128854) as an outgroup (gene boundaries of ITS: 1–524, *actA*: 525–747, *tef1*: 748–1202, *rpb2*: 1203–1871 and *gapdh*: 1872–2476). The five characters artificially introduced as spacers between partitions were excluded from the phylogenetic analysis (Fig. 1).

Based on the results of MrModelTest the Bayesian analysis performed with the GTR+I+G substitution model, with inverse gamma rates and with dirichlet base frequencies for *actA*, *rpb2* and *gapdh*. The *tef1* partition was analyzed with a HKY+I+G substitution model with inverse gamma rates and with dirichlet base frequencies while the ITS partition was analyzed with the SYM+I+G substitution model with fixed frequencies. The alignment contained a total of 1061 unique site patterns (101, 133, 233, 300 and 294 for ITS, *actA*, *tef1*, *rpb2* and *gapdh*, respectively). The Bayesian analysis lasted 1110000 generations and saved a total of 2222 trees. After discarding the first 25% of sampled trees for burn-in, the consensus trees and posterior probabilities (PP) were calculated from the remaining 1668 trees and the final tree is depicted in figure 1.

Table 2. List of fungal isolates included in phylogenetic analyses. Culture accession numbers of the isolates for the present study and new generated sequences are in bold

Taxon	Isolate	ITS	*actA*	*tef1*	*rpb2*	*gapdh*
Ramularia beticola	CBS 141109	KX287338	KX287619	KX287897	KX288176	KX288497
R. cynarae	CBS 114729	KX287398	KX287681	KX287959	KX288238	KX288558
	CPC 25897	KX287397	KX287680	KX287958	KX288237	KX288557
	CPC 25896	KX287396	KX287679	KX287957	KX288236	KX288556
	CBS 128779	HQ728118	KX287676	KX287954	KX288233	KX288553
	CBS 114728	KX287395	KX287678	KX287956	KX288235	KX288555
	CBS 135969	**MK290603**	**MK290612**	**MK290621**	**MK290628**	**MK290637**
	P 1238	**MK290604**	**MK290613**	**MK290622**	**MK290629**	**MK290638**
	P 1244	**MK290605**	**MK290614**	–	**MK290630**	**MK290639**
	P 1245	**MK290606**	**MK290615**	–	**MK290631**	**MK290640**
	CBS 128912	HQ728117	KX287677	KX287955	KX288234	KX288554
	CPC 18427	KX287394	KX287675	KX287953	KX288232	KX288552
R. geranii	CBS 159.24	KX287413	KX287696	KX287974	KX288253	KX288572
R. glennii	CBS 129441	KJ504769	KJ504433	KJ504684	KJ504552	KJ504640
R. heraclei	CPC 11505	KX287423	KX287706	KX287984	KX288263	KX288582
	CPC 11506	KX287424	KX287707	KX287985	KX288264	KX288583
	CPC 11507	KX287425	KX287708	KX287986	KX288265	KX288584
	CBS 108987	KX287421	KX287704	KX287982	KX288261	KX288580
	CBS 108988	KX287422	KX287705	KX287983	KX288262	KX288581
	CBS 135974	**MK290607**	**MK290616**	**MK290623**	**MK290632**	**MK290641**
	CBS 108969	KX287419	KX287702	KX287980	KX288259	KX288578
	CBS 108972	KX287420	KX287703	KX287981	KX288260	KX288579
	CBS 113976	KX287426	KX287709	KX287987	KX288266	KX288585
	CBS 194.25	KX287427	KX287710	KX287988	KX288267	KX288586
R. hydrangeae-macrophyllae	CPC 25902	KX287439	KX287722	KX288000	KX288279	KX288598
	CBS 341.49	KX287444	KX287727	KX288005	KX288284	KX288603
	CPC 25905	KX287436	KX287719	KX287997	KX288276	KX288595
	CPC 25906	KX287440	KX287723	KX288001	KX288280	KX288599
	CPC 20406	KX287446	KX287729	KX288007	KX288286	KX288605
	CPC 19854	KX287441	KX287724	KX288002	KX288281	KX288600
	CBS 114117	KX287452	KX287735	KX288013	KX288292	KX288611
	CBS 118408	KX287456	KX287739	KX288017	KX288296	KX288615
	CBS 122273	KX287433	KX287716	KX287994	KX288273	KX288592
	CPC 20484	KX287447	KX287730	KX288008	KX288287	KX288606
	CBS 122272	KX287438	KX287721	KX287999	KX288278	KX288597

Table 2. (contd)

	CPC 19030	KX287451	KX287734	KX288012	KX288291	KX288610
	CBS 122625	KX287437	KX287720	KX287998	KX288277	KX288596
	CPC 25907	KX287445	KX287728	KX288006	KX288285	KX288604
	CPC 25908	KX287434	KX287717	KX287995	KX288274	KX288593
	CBS 118410	KX287435	KX287718	KX287996	KX288275	KX288594
	CPC 19026	KX287442	KX287725	KX288003	KX288282	KX288601
	CPC 19027	KX287443	KX287726	KX288004	KX288283	KX288602
	CPC 25901	KX287448	KX287731	KX288009	KX288288	KX288607
	CPC 25904	KX287453	KX287736	KX288014	KX288293	KX288612
	CBS 113614	KX287454	KX287737	KX288015	KX288294	KX288613
	CBS 159.82	KX287450	KX287733	KX288011	KX288290	KX288609
	CPC 25903	KX287455	KX287738	KX288016	KX288295	KX288614
	CBS 766.84	KX287449	KX287732	KX288010	KX288289	KX288608
	CBS 135970	**MK290608**	**MK290617**	**MK290624**	**MK290633**	**MK290642**
R. inaequalis	CPC 25742	KP894228	KP894336	KP894446	KP894556	KP894667
	CBS 135972	**MK290609**	**MK290618**	**MK290625**	**MK290634**	**MK290643**
	CBS 141111	KP894227	KP894335	KP894445	KP894555	KP894666
	CBS 250.96	KP894224	KP894332	KP894442	KP894552	KP894663
	P 1182	**MK290610**	**MK290619**	**MK290626**	**MK290635**	**MK290644**
	P 1226	**MK290611**	**MK290620**	**MK290627**	**MK290636**	**MK290645**
	CPC 15752	KP894225	KP894333	KP894443	KP894553	KP894664
	CPC 15753	KP894226	KP894334	KP894444	KP894554	KP894665
	CPC 15815	KX287457	KX287740	KX288018	KX288297	KX288616
R. lamii var. *lamii*	CBS 108970	KX287462	KX287744	KX288022	KX288301	KX288620
R. mali	CBS 129581	KJ504778	KJ504442	KJ504693	KJ504561	KJ504649
R. pratensis var. *pratensis*	CPC 19448	KX287489	KX287771	KX288049	KX288329	KX288648
R. rumicis	CBS 114300	KJ504787	KJ504451	KJ504702	KJ504570	KJ504658
R. taleshina	CCTU 1097; IRAN 2763C	KY967380	KY967386	KY967392	–	KY967397
R. uredinicola	CBS 141120	KX287521	KX287806	KX288084	KX288365	KX288684
R. urticae	CBS 105.26	KP894276	KP894384	KP894494	KP894604	KP894715
R. variabilis	CBS 141121	KP894280	KP894388	KP894498	KP894608	KP894719
R. veronicicola	CBS 113981	KX287538	KX287822	KX288100	KX288380	KX288700
Zymoseptoria halophila	CBS 128854	KF251645	KF253946	KF253592	–	KX348110

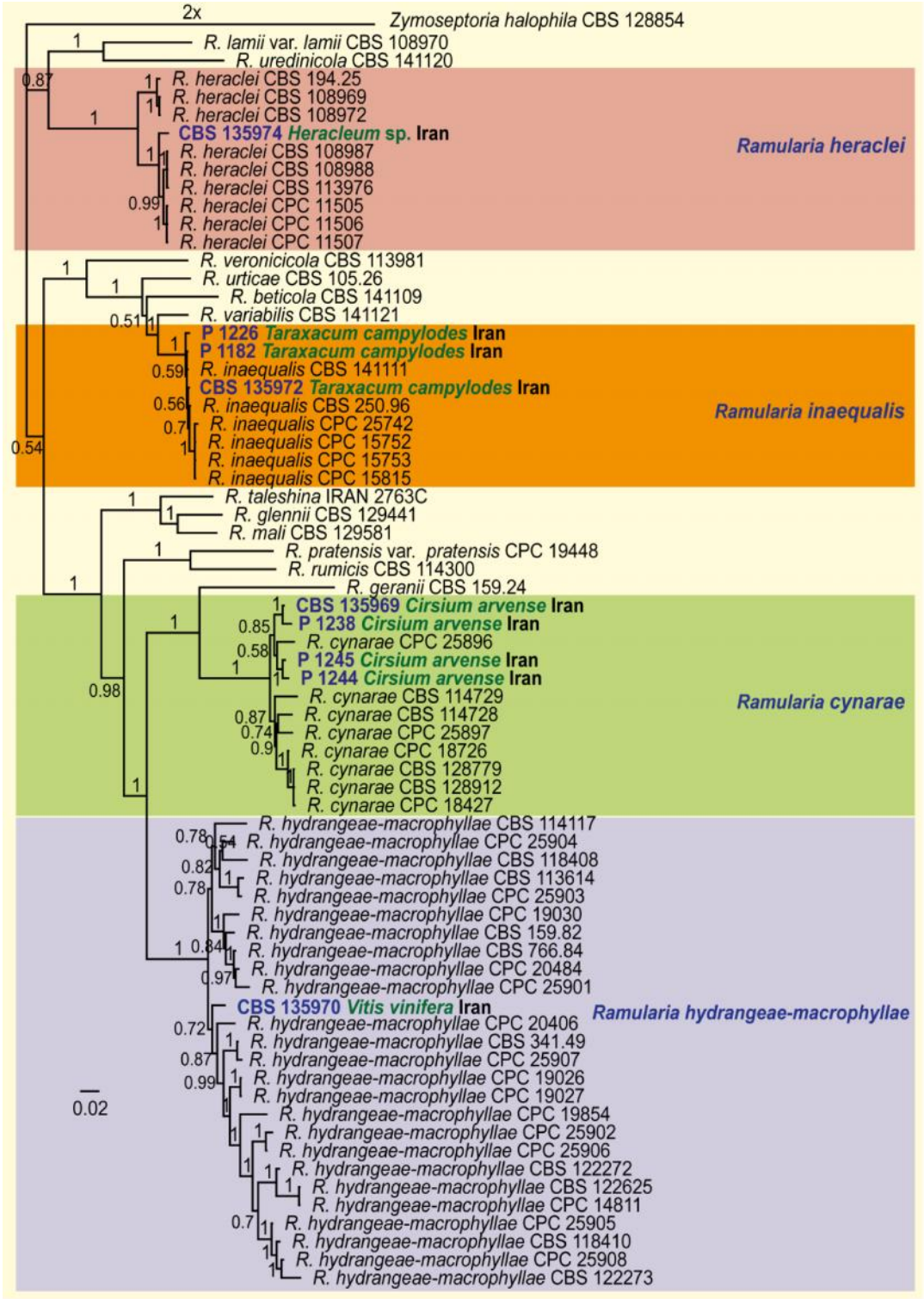

Fig. 1. Consensus phylogram (50% majority rule) of 1668 trees resulting from a Bayesian analysis of the combined five-gene (ITS, *actA*, *tef1*, *rpb2* and *gapdh*) sequence alignment using MrBayes Ver. 3.2.6. The scale bar indicates 0.02 expected changes per site. The tree was rooted to *Zymoseptoria halophila* (CBS 128854).

- Taxonomy

In this research, the Consolidated Species Concept (Quaedvlieg *et al.* 2014), a polyphasic approach combining the concordance of multiple gene genealogies with morphological and ecological information, was employed to distinguish *Ramularia* species from Iran. Based on the phylogenetic analyses, the *Ramularia* isolates recovered from four host species and three host families including *Asteraceae*, *Apiaceae*, and *Vitaceae*, were grouped in four species clades.

The following species of the genus *Ramularia* have been identified in the present investigation:

1. *Ramularia cynarae* Sacc., Michelia 1 (5): 536 (1879). (Fig. 2)

Morphology on SNA: Mycelium consisting of septate, branched, smooth, hyaline, 1–2 µm diam hyphae. Conidiophores hyaline, thin-walled smooth, erect, septate, cylindrical-oblong, straight to geniculate-sinuous, unbranched, (7–)12–17(–26) × 1–2.5 µm, or reduced to conidiogenous cells. Conidiogenous cells thin-walled, smooth, hyaline, integrated in the mycelium or terminal on the conidiophore, (6–)10–12(–15) × 1–2.5 µm, sympodially proliferating with 1–3 apical loci almost flat or protuberant, cylindrical; scars thickened, darkened, refractive, 0.5–1.5 µm diam. Conidia in general hyaline, thin-walled, smooth to slightly verruculose, catenate, with hila thickened, darkened and refractive. Ramoconidia cylindrical-oblong, 0–2-septate, (67–)14–20(–28) × 2–3 µm, with 2 apical hila. Intercalary conidia aseptate, fusoid, ellipsoid, (5–)6–8(–14) × 2–3 µm, in branched chains of up to 9. Terminal conidia, hyaline, smooth to slightly verruculose, aseptate, subcylindrical to obovoid, (3–)5–8 × 1.5–2.5 µm; hila thickened, darkened, refractive, 0.5–1 µm diam.

Culture characteristics: On MEA surface dirty white, with a green-gray tinge, folded, radially striated with undulate and concave margin, reverse iron-grey, reaching 25 mm after 2 wk at 25 °C. On OA surface flat, smooth, with sparse aerial mycelium, white, with olivaceous green entire margins, colony reverse olivaceous grey, reaching 24 mm after 2 wk at 25 °C. On SNA surface flat, smooth, dirty rosy white, with fluffy aerial mycelium, entire edge, reverse white-grey, reaching 20 mm after 2 wk at 25 °C.

Specimens examined: Iran: East Azerbaijan province, Marand, on *Cirsium arvense* (L.) Scop. (*Asteraceae*), Oct. 2012, M. Bakhshi (IRAN 17135F; living culture CBS 135969); East Azerbaijan province, Kaleibar, on *Cirsium arvense*, Nov. 2012, M. Arzanlou (P 1238; P 1244; P 1245).

Notes: *Ramularia cynarae* has a wide host range within the *Asteraceae* (Videira *et al.* 2016, Farr & Rossman 2018). So far, this species has been reported from Iran on *Carthamus oxyacantha* and *Carthamus tinctorius* (*Asteraceae*) (Ershad 2009, Pirnia *et al.* 2012). In this investigation, *R. cynarae* was found for the first time on *Cirsium arvense* in Iran based on multi-gene phylogeny and morphological data.

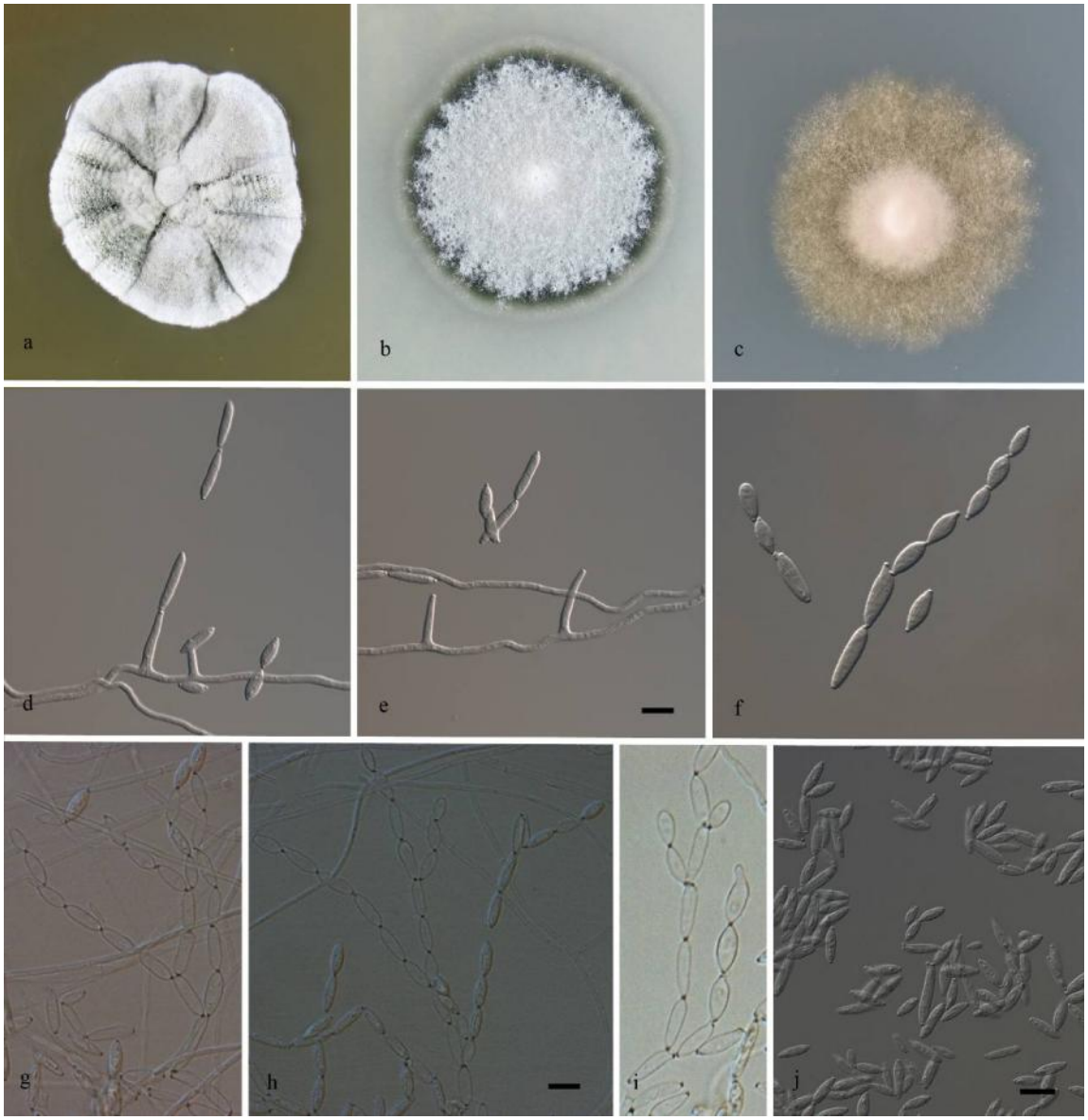

Fig. 2. *Ramularia cynarae* (CBS 135969): a. Culture on MEA, b. Culture on OA, c. Culture on SNA, d-j. Hypha, conidiophores and conidia (Bars = 10 µm).

2. *Ramularia heraclei* (Oudem.) Sacc., Fungi ital. del., Tab. 1008 (1881). (Fig. 3)

Morphology on SNA: Mycelium consisting of septate, branched, smooth, hyaline, 1–3 µm diam hyphae. Conidiophores hyaline, thin-walled smooth, erect, septate, cylindrical-oblong, straight to geniculate-sinuous, unbranched, (15–)20–35(–50) × 1–4 µm. Conidiogenous cells thin-walled, smooth, hyaline, terminal and lateral, (10–)13–20(–25) × 1–4 µm, sympodially proliferating with 1–3 apical loci almost flat or protuberant, cylindrical; scars thickened, darkened, refractive, 0.5–1.5 µm diam. Conidia in general hyaline, thin-walled, smooth to finely verruculose. Ramoconidia subcylindrical to clavate, 0–3-septate, (10–)12–18(–28) × (2.5–)4–5 µm. Intercalary conidia 0–2-septate, subcylindrical to ovoid, (8–)12–15(–20) × 2.5–5 µm, in branched chains of up to 10. Terminal conidia, hyaline, smooth to finely verruculose, 0–1-septate, obovoid, clavate, (5–)8–12 × 2.5–5 µm; hila thickened, darkened, refractive, 0.5–1 µm diam.

Culture characteristics: On MEA surface raised, folded, with sparse aerial mycelium, white, with undulate and

concave margin, reverse white-grey, reaching 16 mm after 2 wk at 25 °C. On OA surface flat, smooth, fluffy aerial mycelium, white, reaching 16 mm after 2 wk at 25 °C. On SNA surface flat, smooth, ochraceous white, flat aerial mycelium, entire edge, reverse white-grey, reaching 17 mm after 2 wk at 25 °C.

Specimen examined: Iran: East Azerbaijan province, Kaleibar, on *Heracleum* sp. (*Apiaceae*), Nov. 2012, M. Arzanlou (IRAN 17136 F; living culture CBS 135974).

Notes: *Ramularia heraclei* has been reported previously from Iran on *Heracleum persicum* and *Heracleum* sp. (Ershad 2009, Pirnia *et al.* 2012) based on morphological data on the herbarium material. Here, it has been studied and reported by molecular data of the living culture for the first time in Iran.

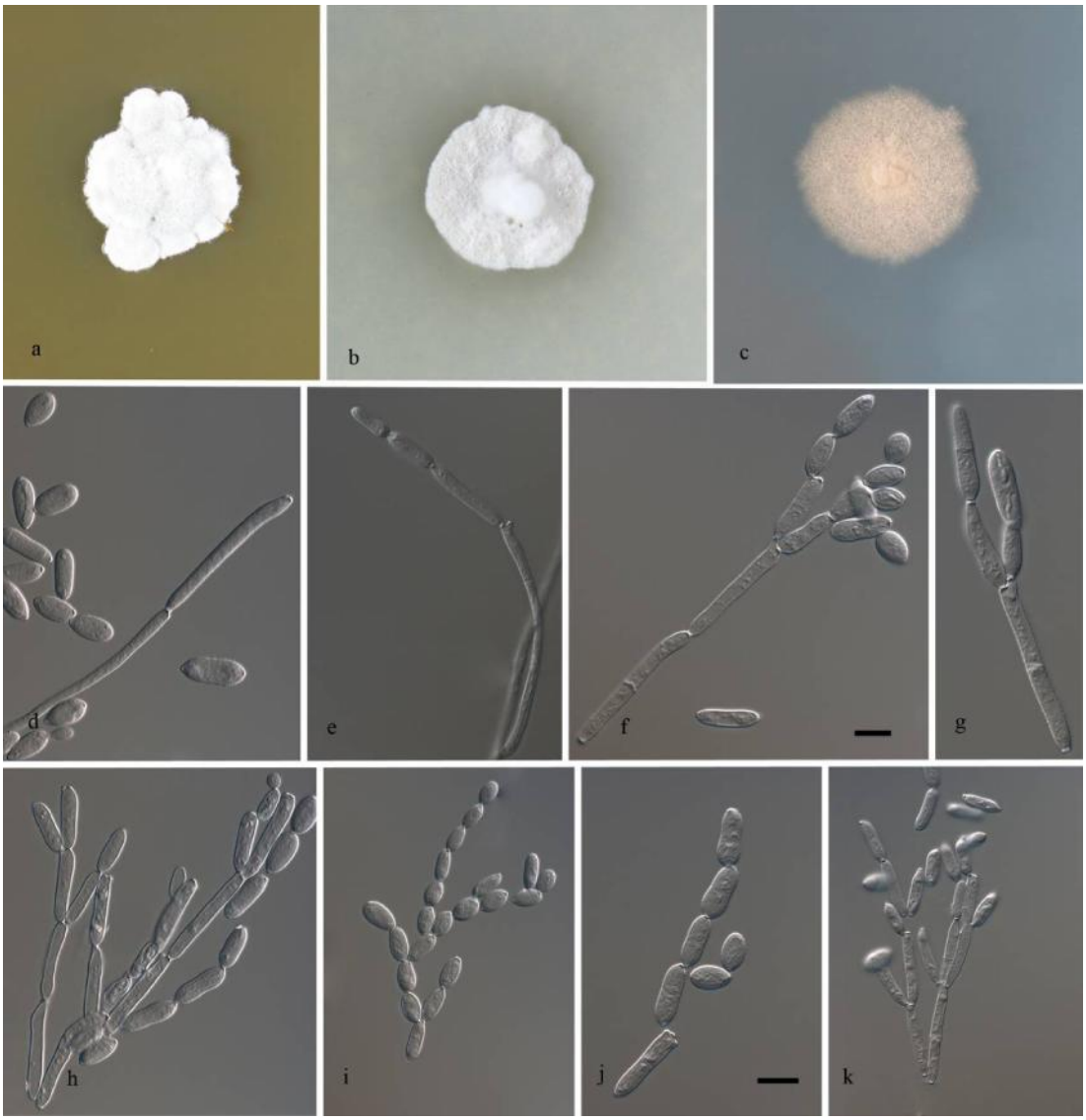

Fig. 3. *Ramularia heraclei* (CBS 135974): a. Culture on MEA, b. Culture on OA, c. Culture on SNA, d-K. Hypha, conidiophores and conidia (Bars = 10 µm).

3. *Ramularia hydrangeae-macrophyllae* U. Braun & C.F. Hill, Australasian Mycologist 27 (2): 53 (2008). (Fig. 4)

Morphology on SNA: Mycelium consisting of septate, branched, smooth, hyaline, 1–2 µm diam hyphae. Conidiophores hyaline, thin-walled smooth, erect, septate, cylindrical-oblong, straight to geniculate-sinuous, unbranched, (8–)12–19(–30) × 1–2.5 µm, or reduced to conidiogenous cells. Conidiogenous cells thin-walled, smooth, hyaline, terminal and lateral, (6–)12–16(–25) × 1–2.5 µm, sympodially proliferating with 1–5 apical loci almost flat or protuberant, cylindrical; scars thickened, darkened, refractive, 0.5–1.5 µm diam. Conidia in general hyaline, thin-walled, smooth to slightly verruculose, catenate, with hila thickened, darkened and refractive. Ramoconidia cylindrical-oblong, 0–2-septate, (6–)8–15(–22) × (2–)2.5–3 µm, with 2–3 apical hila. Intercalary conidia 0–1-septate, fusoid, ellipsoid, (5–)6–8(–13) × 1.5–2.5 µm, in branched chains of up to 15. Terminal conidia, hyaline, smooth to slightly verruculose, aseptate, subcylindrical to obovoid, (4–)8–11 × 1.5–2.5 µm; hila thickened, darkened, refractive, 0.5–1 µm diam.

Culture characteristics: On MEA surface folded, with sparse fluffy aerial mycelium, dirty white but with pale greenish grey tones, with undulate and concave margin, reverse iron-grey, reaching 19 mm after 2 wk at 25 °C. On OA surface flat, rosy-buff, while fluffy aerial mycelium covers the centre, entire margin, reaching 23 mm after 2 wk at 25 °C. On SNA surface flat, smooth, ochraceous white, flat aerial mycelium, undulate margin, reverse white-grey, reaching 14 mm after 2 wk at 25 °C.

Specimen examined: Iran: East Azerbaijan province, Kaleibar, on *Vitis vinifera* (*Vitaceae*), Nov. 2012, M. Arzanlou (IRAN 17137F; living culture CBS 135970).

Notes: Recently based on the combination of morphological and multi-gene phylogenetic analysis, it has been demonstrated that, *R. hydrangeae-macrophyllae* is a plurivorous species with multiple family-associations including *Apiaceae*, *Asteraceae*, *Cyperaceae*, *Fabaceae*, *Hydrangeaceae*, *Iridaceae*, *Juncaceae*, *Lauraceae*, *Myrtaceae*, *Platanaceae*, *Poaceae*, *Ranunculaceae*, *Rosaceae*, *Sapindaceae*, and *Typhaceae* (Videira *et al*. 2016).

In the present research, *Ramularia hydrangeae-macrophyllae* has been found for the first time in Asia. Additionally, this is the first report of the species on *Vitis vinifera* in the world, thus a further family, *Vitaceae* was added to the host range of this species. In addition, Bakhshi & Arzanlou (2017) reported *R. mali* on *Vitis vinifera*. Although, both the *R. hydrangeae-macrophyllae* and *R. mali* reported on *Vitis vinifera* from Iran (collected from Kaleibar region in East Azerbaijan province), but they are phylogenetically quite distinct from each other (Fig. 1).

These data, therefore, emphasizes the importance of using sequence data for identification of *Ramularia* species.

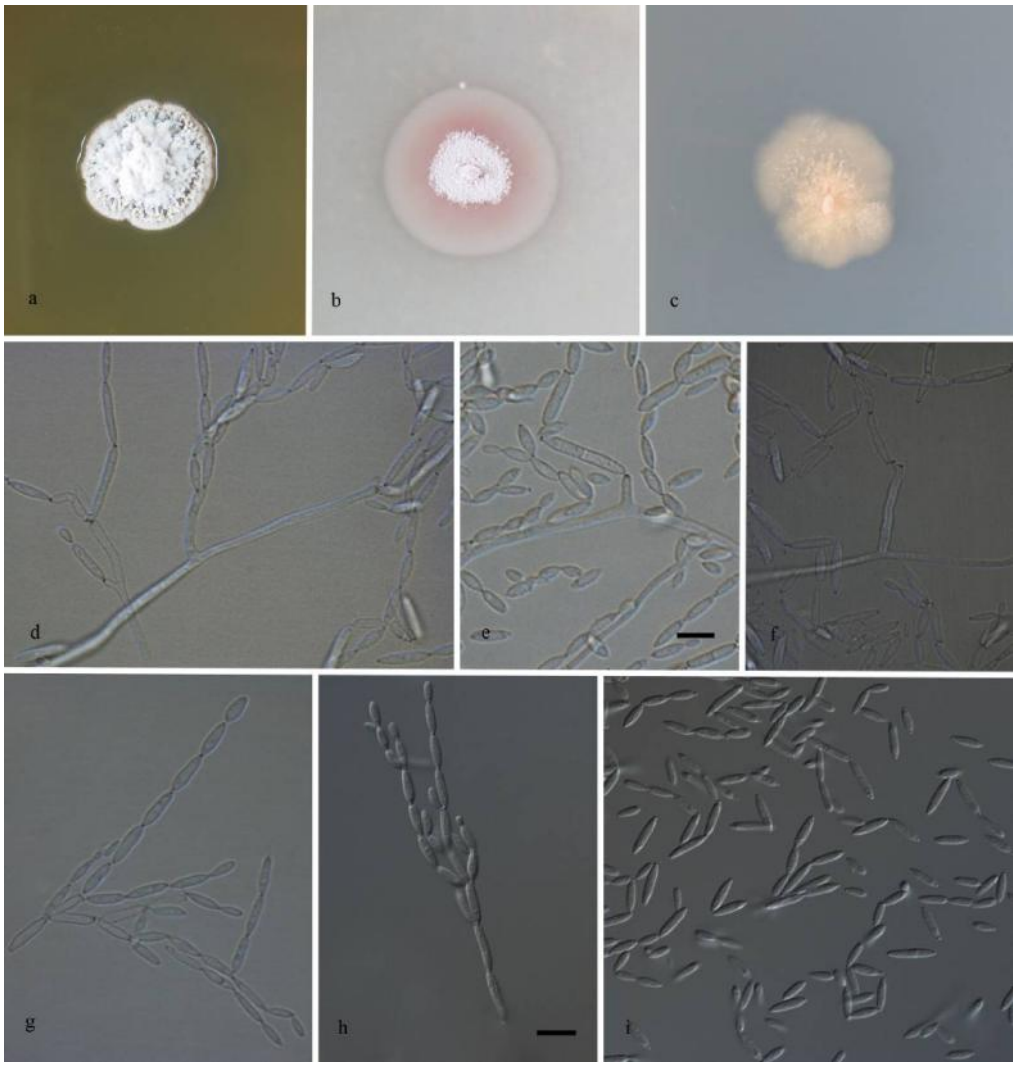

Fig. 4. *Ramularia hydrangeae-macrophyllae* (CBS 135970): a. Culture on MEA, b. Culture on OA, c. Culture on SNA, d–i. Hypha, conidiophores and conidia (Bars = 10 µm).

4. *Ramularia inaequalis* (Preuss) U. Braun, Monogr. *Cercosporella*, *Ramularia* Allied Genera (Phytopath. Hyphom.) 2: 68 (1998). (Fig. 5)

Morphology on SNA: Mycelium consisting of septate, branched, smooth, hyaline, 1–2 µm diam hyphae. Conidiophores hyaline, thin-walled smooth, erect, septate, cylindrical-oblong, straight to sinuous, unbranched, (20–)30–55(–75) × 1–2 µm, or reduced to conidiogenous cells. Conidiogenous cells thin-walled, smooth, cylindrical-oblong, hyaline, terminal on conidiophores or intermediate in the mycelium, (8–)17–20(–30) × (1–)2(–2.5) µm, sympodial proliferation with one apical, flattened or protuberant, thickened, darkened and refractive locus. Conidia hyaline, thin-walled, smooth. Ramoconidia cylindrical-oblong, 0–1(–2)-septate, (15–)20–27(–35) × 1.5–3 µm, with 1–2(–3) apical loci. Intercalary conidia 0–1-septate, cylindrical-oblong, fusoid or clavate, 10–17(–27) × 1.5–2(–2.5) µm, in branched chains of up to 7. Terminal conidia aseptate, cylindrical-oblong to obovoid, (9–)11(–18) × (1–)2–2.5 µm; hila thickened, darkened, refractive, 0.5–1 µm diam.

Culture characteristics: On MEA surface raised, with sparse aerial mycelium, white, with entire margin, reverse white-grey, reaching 18 mm after 2 wk at 25 °C. On OA surface flat, smooth, with sparse aerial mycelium, white, with olivaceous grey entire margins, colony

reverse olivaceous grey, reaching 18 mm after 2 wk at 25 °C. On SNA surface flat, smooth, dirty rosy white, with fluffy aerial mycelium, entire edge, reverse white-grey, reaching 15 mm after 2 wk at 25 °C.

Specimens examined: Iran: East Azerbaijan province, Marand, on *Taraxacum campylodes* G.E.Haglund. (*Asteraceae*), Oct. 2012, M. Bakhshi (P 1226); East Azerbaijan province, Mianeh, on *Taraxacum campylodes*, Nov. 2012, Z. Abdollahi (IRAN 17138F; living culture CBS 135972); West Azarbaijan province, Khoy, Firouragh, on *Taraxacum campylodes*, Sept. 2012, M. Arzanlou (P 1182).

Notes: *Ramularia inaequalis* has been reported previously from Iran on *Calendula persica* (*Asteraceae*) (Pirnia *et al.* 2012). In this investigation, this species was found for the first time on *Taraxacum campylodes* in Iran based on multi-gene phylogeny and morphological data.

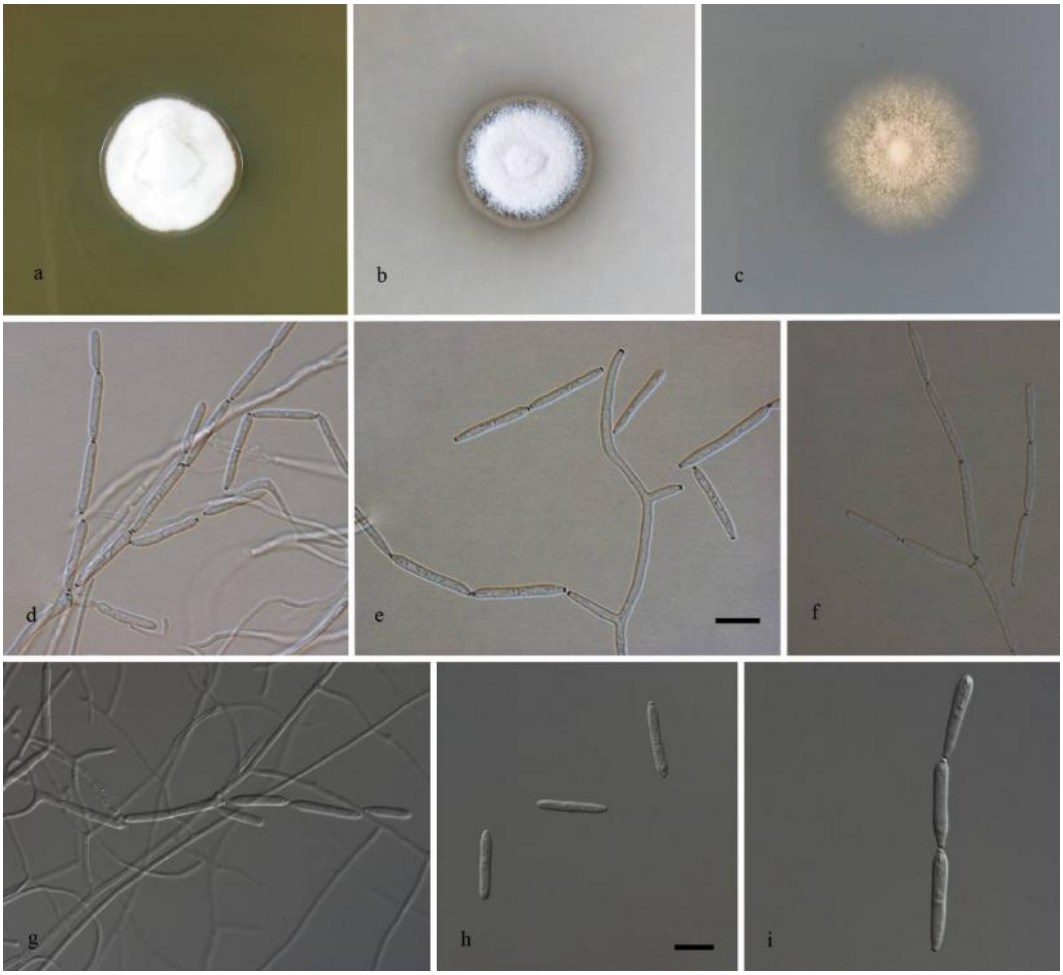

Fig. 5. *Ramularia inaequalis* (CBS 135972): a. Culture on MEA, b. Culture on OA, c. Culture on SNA, d–i. Hypha, conidiophores and conidia. (Bars = 10 µm).

Host family index for ramularia-like taxa from Iran

The taxa reported from Iran are listed below according to the host family:

Apiaceae
Ramularia heraclei
Ramularia sp.
Asteraceae
Cercosporella virgaureae
Ramularia brunnea
R. carletonii
R. cupulariae
R. cynarae
R. inaequalis
R. nagornyi
R. picridis
Betulaceae
Ramularia taleshina
Boraginaceae
Ramularia anchusae
Brassicaceae
Neopseudocercosporella capsellae
Ramularia armoraciae
Campanulaceae
Ramularia macrospora
Caprifoliaceae
Ramularia sambucina
Chenopodiaceae
Ramularia macularis
Euphorbiaceae
Ramularia glennii
Fabaceae
Neoovularia nomuriana
Ramularia bornmuelleriana
R. winteri
Geraniaceae
Ramularia geranii
Lamiaceae
Neoovularia ovate
Ramularia lamii
R. lamii var. *lamii*
R. marrubii
Malvaceae
Ramulariopsis gossypii
Moraceae
Ramularia glennii
Onagraceae
Ramularia epilobiana
Plantaginaceae
Ramularia rhabdospora
Platanaceae
Ramularia glennii
Plumbaginaceae
Ramularia iranica
Polygonaceae
Ramularia pratensis
R. rubella
R. rufomaculans
R. rumicis
R. rumicis-scutati
Ramularia sp.
Primulaceae
Cercosporella primulae
Ramularia primulae
Ranunculaceae
Ramularia ranunculicola
R. simplex
Rosaceae
Microcyclosporella mali
Neoramularia rubi
Ramularia alpina
R. grevilleana
R. mali
Ramularia sp.
Scrophulariaceae
Neoramularia esfandiarii
Ramularia beccabungae
R. variabilis
R. veronicae
Urticaceae
Ramularia urticae
Valerianaceae
Ramularia valeriana
Vitaceae
Ramularia hydrangeae-macrophyllae
R. mali

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