CZECH MYCOLOGY 69(2): 113–123, JULY 26, 2017 (ONLINE VERSION, ISSN 1805-1421)

A noteworthy record of endophytic *Quambalaria cyanescens* from *Punica granatum* in Iran

MOHAMMAD EBRAHIM VAHEDI-DARMIYAN[1], MEHDI JAHANI[1]*,
MOHAMMAD REZA MIRZAEE[2], BITA ASGARI[3]

[1] Department of Plant Protection, College of Agriculture, University of Birjand, Birjand, Iran
[2] Plant Protection Research Department, South Khorasan Agricultural and Natural Resources Research and Education Center, AREEO, Birjand, Iran
[3] Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
*corresponding author; mjahani@birjand.ac.ir

Vahedi-Darmiyan M.E., Jahani M., Mirzaee M.R., Asgari B.: A noteworthy record of endophytic *Quambalaria cyanescens* from *Punica granatum* in Iran. – Czech Mycol. 69(2): 113–123.

During an investigation into endophytic fungi associated with pomegranate in the South Khorasan Province of Iran, 2015–2016, five isolates were recovered with the morphological and molecular characteristics of *Quambalaria cyanescens*. The present study is the first fully documented report of *Q. cyanescens* from Iran, providing insight into its geographic distribution and host range. Our study is also the first report of occurrence of *Q. cyanescens* as an endophyte in a member of the *Lythraceae* family.

Key words: endophyte, flower, pomegranate, *Quambalariaceae*.

Article history: received 16 February 2017, revised 10 May 2017, accepted 17 June 2017, published online 26 July 2017

Vahedi-Darmiyan M.E., Jahani M., Mirzaee M.R., Asgari B.: Pozoruhodný nález endofytické *Quambalaria cyanescens* z *Punica granatum* v Íránu. – Czech Mycol. 69(2): 113–123.

Během výzkumu endofytických hub v pletivech marhaníku granátového v íránské provincii Jižní Chorásán v letech 2015–2016 bylo nalezeno pět izolátů s morfologickými i molekulárními znaky *Quambalaria cyanescens*. Předložená studie představuje první plně doložený záznam o výskytu tohoto druhu v Íránu, doplněný o informace o jeho rozšíření ve světě a hostitelském spektru. Přináší též první záznam o výskytu *Q. cyanescens* jako endofyta v rostlině z čeledi kyprejovitých.

INTRODUCTION

The pomegranate (*Punica granatum* L.), a fruit-bearing deciduous tree belonging to the *Lythraceae* family, is an important and one of the oldest fruit crops of the tropical and subtropical regions of the world. Pomegranates are naturally

adapted to environments with cool winters and hot summers; however, they can also be grown in the humid tropics or subtropics (Stover & Mercure 2007, Bazargani-Gilani et al. 2014, Farag & Emam 2016). Iran is the second largest producer and largest exporter of pomegranate in the world.

Recently, the incidence of a physiological disorder called aril browning or aril paleness, thought to occur due to an oxidative stress, has been threatening the popularity of pomegranate fruit (Mirdehghan & Vatanparast 2013).

Plants provide numerous and diverse niches for endophytic organisms which produce a high diversity of substances with potential medical, agricultural, and industrial applications (Costa et al. 2012, Paul & Lee 2014). Fungal endophytes are broad-spectrum microorganisms colonising plants without causing any visible symptom of infection (Muvea et al. 2014). Information on endophytic fungi of pomegranate and their impacts on the biology of the host is quite limited. To date, *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, *Penicillium glabrum* (Wehmer) Westling, *Colletotrichum* spp., *Phomopsis* sp., *Nigrospora* sp. and *Phyllosticta* sp. have been reported as endophytes of pomegranate (Shankar Naik et al. 2008, Huang & Wang 2011, Hammerschmidt et al. 2012, Mussi-Dias et al. 2012).

During an investigation into endophytic fungi associated with pomegranates in Ferdows County, South Khorasan Province, Iran in 2015–2016, five isolates belonging to *Quambalaria* were obtained specifically on flowers and studied.

The genus *Quambalaria* J.A. Simpson (*Quambalariaceae*, *Microstromatales*) comprises six species, including *Q. cyanescens* (de Hoog & G.A. de Vries) Z.W. de Beer, Begerow & R. Bauer (de Beer et al. 2006), *Q. coyrecup* T. Paap (Paap et al. 2008), *Q. eucalypti* (M.J. Wingf., Crous & W.J. Swart) J.A. Simpson (Wingfield et al. 1993, Simpson 2000), *Q. pitereka* (J. Walker & Bertus) J.A. Simpson (Simpson 2000), *Q. pusilla* (U. Braun & Crous) J.A. Simpson (Simpson 2000) and *Q. simpsonii* Cheew. & Crous (Cheewangkoon et al. 2009).

Members of this genus are mainly differentiated by pigment production, conidial and conidiogenous cell dimensions and the production of secondary conidia (Paap et al 2008).

All isolates from Ferdows County were assigned to *Q. cyanescens* based on morphological and molecular data (a combined sequence dataset of the ITS region and partial LSU rDNA).

This species was previously reported from Iran (Ershad et al. 1998, as *Sporothrix cyanescens*; Kolařík et al. 2006, as *Fugomyces cyanescens*), but it has remained poorly documented, with identification restricted to non-detailed morphological features of isolates associated with gall-making insects.

The present study is the first fully documented report of *Q. cyanescens* from a region of Iran with a semi-arid ecosystem, which provides insight into the geographic distribution and host range of *Q. cyanescens*.

MATERIAL AND METHODS

Isolation of endophytic fungi. A total of 216 flower segments were collected from pomegranate trees in hotspot areas of aril paleness disorder and from healthy, disease-free pomegranate trees in non-hotspot areas (representing 131 and 85 segments, respectively) for isolation of endophytic fungi.

Fungal strains were isolated from tissue segments of *Punica granatum* cv. Shishe-cap, native to Ferdows County, with a predominantly semi-arid climate. The segments were thoroughly washed under tap water and the surface sterilised by immersion in 75% ethanol for 1 min, 1% sodium hypochlorite for 3 minutes and 95% ethanol for 30 s, then rinsed three times in sterile distilled water, and finally dried on sterilised paper. The surface-sterilised flower segments were placed on potato-dextrose agar (PDA, Merck, Germany) supplemented with streptomycin sulphate (50 mg/l) and incubated at 25 °C in the dark.

The relative frequency of colonisation was calculated according to Kumar & Hyde (2004).

Morphological observation. Colony morphology and microscopic characteristics were determined on 1/2 PDA (Paap et al. 2008) at 25 °C in the dark. Mycelium, conidiophores and conidia were examined from slide cultures prepared according to Riddell (1950) and mounted in water and Lacto-Cotton Blue. Photographs were taken using an Olympus DP25 digital camera installed on an Olympus BX51 light microscope. Subcultures of the strains included in this study are preserved at the Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran.

DNA extraction and amplification. Total genomic DNA was extracted from single-conidial isolates grown on 2% malt extract agar (MEA) for 7 d, using the Chelex 100 chelating resin method, modified by Walsh et al. (1991) and Hirata & Takamatsu (1996). The ITS region (ITS1–5.8S–ITS2) and the D1–D2 portion of the LSU ribosomal DNA of the representative strain were amplified with primer pairs ITS4/ITS5 and NL1/NL4, respectively (White et al. 1990, Boekhout et al. 1995).

PCR amplification was carried out using a MJ Mini thermal cycler (Bio-Rad, Hercules, USA). The PCR reaction (25 µl) contained 1 ng genomic DNA, 0.2 mM dNTPs, 1.6 mM $MgCl_2$, 1× PCR buffer, 1.25 U of Taq DNA polymerase, and 1 µl of each primer (10 pmol) (SinaClon Co., Karaj, Iran). Both amplification reactions

were performed with the following cycling parameters: 3 min at 94 °C, followed by 30 cycles at 1 min at 94 °C, 45 s at 54 °C and 2 min at 72 °C, with a final extension for 7 min at 72 °C. The PCR products were purified using a PCR purification kit (Bioneer, Daejeon, Korea). Sequencing was performed in an Applied Biosystems Automated 3730 xl DNA analyzer (Applied Biosystems, Foster City, USA).

Phylogenetic analysis. To clarify the relationships of these isolates within the genus *Quambalaria*, we conducted phylogenetic analyses using sequences of the ITS region (610 bp) and LSU rDNA (560 bp) individually (not shown) and combined (Fig. 1). The sequences recovered in this study were aligned against the ex-type culture (CBS 357.73) or authentic strains of *Quambalaria* species from GenBank. Already published sequences of *Quambalaria* species together with our own sequence of *Q. cyanescens* were included in the phylogenetic analyses (Tab. 1).

The sequences were aligned with MAFFT (Katoh et al. 2005) using the Q-INS-i algorithm. Maximum likelihood (ML) analysis was carried out with RAxML, as implemented on a RAxML webserver (http://phylobench.vital-it.ch/raxml-bb/) with the gamma model of rate heterogeneity (Stamatakis et al. 2008). Five runs with 100 bootstrap replicates using the "Maximum likelihood search" and "Estimate proportion of invariable sites" were performed. Minimum evolution (ME) analysis was generated using MEGA 6.0 (Tamura et al. 2013), with a Tamura-Nei substitution model and 1000 bootstrap replications. The Bayesian inference algorithm was performed using MrBayes (Ronquist et al. 2012) at the Trease webserver [Mishra et al. on-line (unpubl. data, with kind permission of Marco Thines, Goethe University, Frankfurt am Main, Germany), Bogner et al. 2016]. The GTR+G nucleotide substitution model was selected with jModelTest 2 (Darriba et al. 2012) under the Akaike information criterion (AIC) for the dataset.

Rhodotorula hinnulea (R.G. Shivas & Rodr. Mir.) Rodr. Mir. & Weijman, *R. bacarum* (Buhagiar) Rodr. Mir. & Weijman, *Microstroma album* (Desm.) Sacc., *M. juglandis* (Bérenger) Sacc. and *Sympodiomycopsis paphiopedili* Sugiy., Tokuoka & Komag. were chosen as outgroup taxa (Fig. 1). The combined dataset consisted of 1425 characters, of which 232 were parsimony informative (data not shown). The sequences generated in this study were deposited at GenBank (Tab. 1).

RESULTS AND DISCUSSION

Phylogeny and description

NCBI BLAST search revealed that the ITS region and D1/D2 domains of the LSU rDNA in our examined strain of *Quambalaria cyanescens* (IRAN 2465C) had 100% similarity to the ex-type culture of *Q. cyanescens* (CBS 357.73, DQ317622).

Tab. 1. Species and isolates included in this study, their sources and GenBank accession numbers. Sequences with numbers in bold were generated in this study, others are from GenBank. (T) = ex-type strain.
Abbreviations: CBS – Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; CMW – Culture Collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; IRAN – Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran; R.B. – Herbarium R. Bauer, Tübingen, Germany; WAC – Department of Agriculture, Western Australia Plant Pathogen Collection, Perth, Australia.

Taxon	Strain	Source	Country	GenBank accession no.		References
				ITS	LSU	
Microstroma album	R.B. 2072[a]	*Quercus robur*	Germany	DQ317624	AF352052	Paap et al. 2008
Microstroma juglandis	R.B. 2042[a]	*Juglans regia*	Germany	DQ317634	DQ317617	Paap et al. 2008
Quambalaria cyanescens (T)	CBS 357.73	Skin of man	Netherlands	DQ317622	DQ317615	de Beer et al. 2006
Quambalaria cyanescens	WAC 129555	*Corymbia calophylla*	Australia	DQ823421	DQ823441	Paap et al. 2008
Quambalaria cyanescens	WAC 12952	*Corymbia calophylla*	Australia	DQ823419	DQ823440	Paap et al. 2008
Quambalaria cyanescens	IRAN 2465C	*Punica granatum*	Iran	**KX377510**	**KX377511**	This study
Quambalaria cyanescens	WAC 12953	*Corymbia ficifolia*	Australia	DQ823422	DQ823443	Paap et al. 2008
Quambalaria cyanescens	CBS 127353	*Betula pendula*	Russia	HG799003		Antropova et al. 2014
Quambalaria cyanescens	CBS 127352	*Betula pendula*	Russia	HG799002		Antropova et al. 2014
Quambalaria simpsonii	CBS 124773	*Eucalyptus* sp.	Thailand	GQ303291	GQ303322	Antropova et al. 2014
Quambalaria simpsonii	CBS 124772	*Eucalyptus tintinnans*	Australia	GQ303290	GQ303321	Antropova et al. 2014
Quambalaria eucalypti (T)	CMW 1101	*Eucalyptus grandis*	South Africa	DQ317625	DQ317618	Paap et al. 2008
Quambalaria eucalypti	CMW 11678	*Eucalyptus grandis*	South Africa	DQ317626	DQ317619	de Beer et al. 2006
Quambalaria coyrecup (T)	WAC 12947	*Corymbia calophylla*	Australia	DQ823431	DQ823444	Paap et al. 2008
Quambalaria coyrecup	WAC 12949	*Corymbia calophylla*	Australia	DQ823432	DQ823445	Paap et al. 2008
Quambalaria pitereka	CMW 6707	*Corymbia maculata*	Australia	DQ317627	DQ317620	de Beer et al. 2006
Quambalaria pitereka	CBS 118828	*Corymbia citriodora*	Australia	DQ317628	DQ317621	de Beer et al. 2006
Rhodotorula hinnulea	CBS 8079	*Banksia collina*	Australia	AB038130	AF190003	de Beer et al. 2006
Rhodotorula bacarum	CBS 6526	*Ribes nigrum*	UK	DQ317629	AF352055	de Beer et al. 2006
Sympodiomycopsis paphiopedili	CBS 7429	*Paphiopedilum primulinum*	Japan	DQ317631	AF190005	de Beer et al. 2006

Phylogenetic analysis (Fig. 1) revealed that the examined Iranian strain was clustered in a clade accommodating strains of *Q. cyanescens* occurring on a broad range of hosts, and grouped with ex-type cultures of *Q. cyanescens* with varying support of 90% bootstrap support in ME, 65% in ML, and 0.99 in Bayesian analysis.

Although *Quambalaria* sp. has recently been reported on almond in Iran (Baradaran Baghery et al. 2015), this is the first documented record of *Q. cyanescens* from Iran to our knowledge.

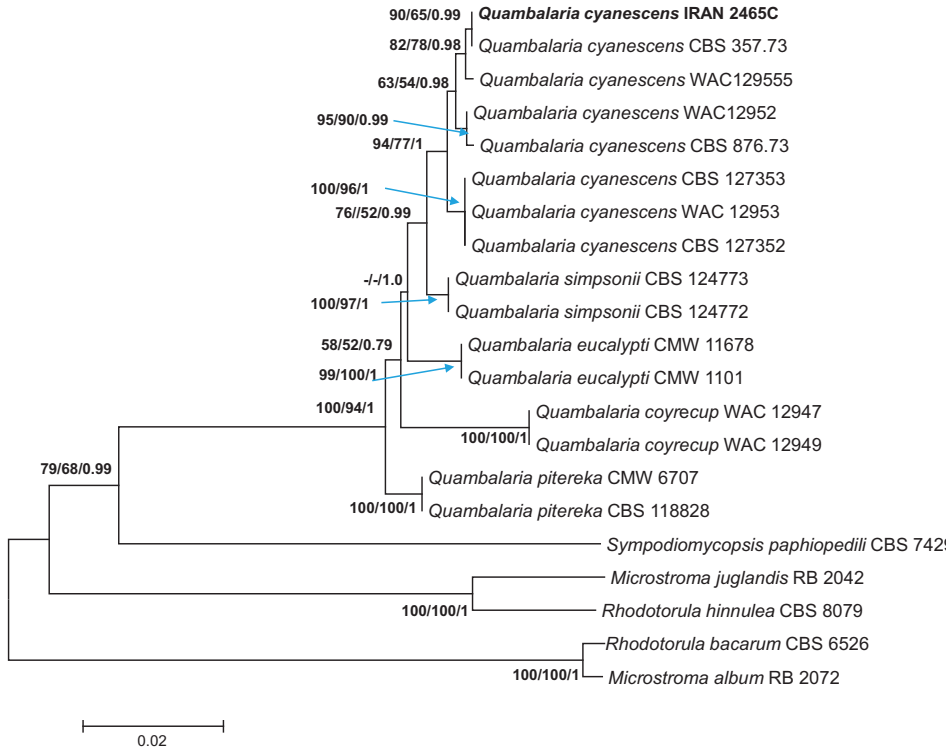

Fig. 1. Phylogenetic tree resulting from analysis of concatenated ITS and LSU constructed using Minimum evolution, Maximum likelihood and Bayesian approaches. Values at the nodes present the percentage of bootstrap support.

Description. Colonies reaching 1.5 mm diam. in 4 days at 21 °C on 1/2 PDA, and 16 mm diam. in 4 days at 25 °C on PDA. Colonies on 1/2 PDA farinose or velvety, often compact, white; reverse cream coloured at first, then turning to purple. Hyphae hyaline, smooth-walled, 2.5 µm wide, often with numerous yeast-like, hyaline, ovoid, ellipsoidal or cylindrical cells, 2–3 × 2 µm. Conidiogenous cells usually arising terminally, occasionally laterally, undifferentiated, cylindrical, 5–40 × 2 µm, apically with a cluster of small denticles often repeatedly proliferating and forming similar clusters. Primary conidia hyaline, one-celled, smooth-walled or finely verrucose, oblong, ellipsoidal or subcylindrical, with rounded apex and attenuated base, 4–7 × 2–4 µm, often producing one or several obovoid or narrowly cylindrical secondary conidia, 1.5 × 2.5 µm, either by budding or from short conidiogenous cells (Fig. 2). Teleomorph absent.

The diagnostic features of isolates assigned to *Q. cyanescens* in this study were largely consistent with the original description provided by de Hoog & de

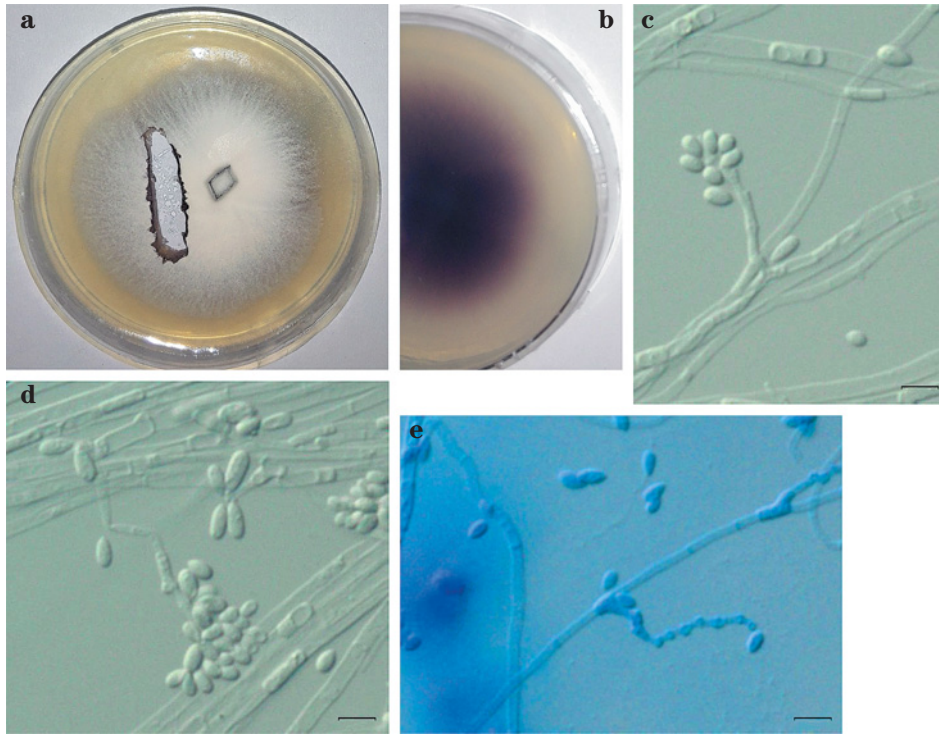

Fig. 2. *Quambalaria cyanescens* (IRAN 2465C). **a, b** – colony on 1/2 PDA; **c** – hyphae, conidiogenous cells and conidia; **d, e** – primary and secondary conidia. Scale bar = 5 µm. Photos B. Asgari.

Vries (1973). However, secondary conidia reaching up to 25 µm long were not produced by Iranian specimens.

Specimens examined

Iran. South Khorasan Province, Ferdows, on fresh flowers of *Punica granatum*, 30 June 2015, leg. & det. M.E. Vahedi-Darmiyan (IRAN 2465C). – Ibid., 1 July 2015, leg. & det. M.E. Vahedi-Darmiyan (IRAN 2593C, IRAN 2594C).

Ecology and future prospects

Quambalaria cyanescens has been isolated from a broad range of ecological niches, including air, plant materials, soil and insects from several countries in North Africa, North America, South Asia, Australia, Europe and the Middle East (de Hoog & de Vries 1973, Kolařík et al. 2006, Abdel-Sater et al. 2016) and in association with diverse plants, including *Corymbia* spp. and *Eucalyptus* spp. in Australia

(Paap et al. 2008; Farr & Rossman on-line) and *Betula pendula* in Russia (Antropova et al. 2014). This fungal species, as a potential opportunistic pathogen in immunocompromised or debilitated patients, has also been isolated from blood, human skin and hospital-acquired infections in individuals with pneumonia, peritoneal inflammation and invasive pulmonary infection (Jackson et al. 1990, Tambini et al. 1996, Fan et al. 2014, Kuan et al. 2015).

Quambalaria cyanescens has further been reported as a symbiont of plants. Recently, few studies have reported broad-spectrum antimicrobial activity for *Q. cyanescens*, including antibacterial and antifungal activities (Kuan et al. 2015, Stodůlková et al. 2015).

There are many examples of endophytic occurrence of fungi previously known as saprophytes (Hosoya et al. 2014). Endophytic fungi can confer stress tolerance to host species in different environmental stresses (Bezerra et al. 2013). However, little is known about occurrence of fungal endophytes in semi-arid ecosystems (Porras-Alfaro et al. 2011).

We found that the colonisation rate of *Q. cyanescens* isolates was 5.88% in all flowers of healthy pomegranates, which showed the highest colonising frequency among endophytic fungi from this habitat. However, 11 segments had been collected from mountainous regions, which have a different climate than the hotspot areas of aril paleness disorder. Excluding these, the colonisation rate is 6.76% for non-hotspot areas of disorder with similar topology to hotspot areas.

Interestingly, this fungus could not be isolated from the other parts of the host. It was found specifically on flowers of pomegranate located in non-hotspot areas of aril paleness disorder. The isolation of *Q. cyanescens* as an endophyte from healthy pomegranate plants only suggests that this endophytic fungus may help host plants to better resist the disease or can confer stress tolerance to host species under environmental stress.

The finding that disease-free pomegranate trees are hosted by *Q. cyanescens* opens the way to investigate the epidemiological occurrence of aril paleness disorder compared to healthy trees. Based on this data, endophytic *Q. cyanescens* can be considered a source for epidemiological modelling and prediction of this physiological plant disorder.

ACKNOWLEDGEMENTS

This research was partially supported by the Agricultural Research, Education and Extension Organization (AREEO), Iran. We thank two anonymous reviewers for their thoughtful comments on earlier drafts of the manuscript.

The third author would also like to show his gratitude to Prof. Heshmat Rahimian and Dr. Mojtaba Mohammadi for allowing him to develop as a researcher and Prof. Marco Thines, Goethe-Universität Frankfurt am Main, who has been a source of inspiration through his researches.

REFERENCES

ABDEL-SATER M.A., MOUBASHER A.H., SOLIMAN Z.S.M. (2016): Diversity of filamentous and yeast fungi in soil of citrus and grapevine plantations in the Assiut region, Egypt. – Czech Mycology 68: 183–214.

ANTROPOVA A.B., BILANENKO E.N., MOKEEVA V.L., CHEKUNOVA L.N., KACHALKIN A.V., SHTAER O.V., KAMZOLKINA O.V. (2014): Report of *Quambalaria cyanescens* in association with the birch (*Betula pendula*). – Mikrobiologiya 83: 690–698.

BARADARAN BAGHERY M., ARZANLOU M., BABAI-AHARI A. (2015): Identification of the fungal agents associated with almond trunk diseases in East Azerbaijan Province. – Journal of Applied Researches in Plant Protection 4: 13–27.

BAZARGANI-GILANI B., TAJIK H., ALIAKBARLU J. (2014): Physicochemical and antioxidative characteristics of Iranian pomegranate (*Punica granatum* L. cv. Rabbab-e-Neyriz) juice and comparison of its antioxidative activity with *Zataria multiflora* Boiss essential oil. – Veterinary Research Forum 5: 313–318.

BEZERRA J.D.P., SANTOS M.G.S., BARBOSA R.N., SVEDESE V.M. (2013): Fungal endophytes from cactus *Cereus jamacaru* in Brazilian tropical dry forest: a first study. – Symbiosis 60: 53–63.

BOEKHOUT T., FELL J., O'DONNELL K. (1995): Molecular systematics of some yeast-like anamorphs belonging to the *Ustilaginales* and *Tilletiales*. – Studies in Mycology 38: 175–183.

BOGNER C.W., KARIUKI G.M., ELASHRY A., SICHTERMANN G., BUCH A.K., MISHRA B., THINES M., GRUNDLER F.M.W., SCHOUTEN A. (2016): Fungal root endophytes of tomato from Kenya and their nematode biocontrol potential. – Mycological Progress 15: 30.

CHEEWANGKOON R., GROENEWALD J.Z., SUMMERELL B.A., HYDE K.D., TO-ANUN C., CROUS P.W. (2009): *Myrtaceae*, a cache of fungal biodiversity. – Persoonia 23: 55–85.

COSTA I.P.M.W., MAIA L.C., CAVALCANTI M.A. (2012): Diversity of leaf endophytic fungi in mangrove plants of northeast Brazil. – Brazilian Journal of Microbiology 43: 1165–1173.

DARRIBA D., TABOADA G.L., DOALLO R., POSADA D. (2012): jModelTest 2: more models, new heuristics and parallel computing. – Nature Methods 9: 772.

DE BEER Z.W., BEGEROW D., BAUER R., PEGG G.S., CROUS P.W., WINGFIELD M.J. (2006): Phylogeny of the *Quambalariaceae* fam. nov., including important *Eucalyptus* pathogens in South Africa and Australia. – Studies in Mycology 55: 289–298.

DE HOOG G.S., DE VRIES G.A. (1973): Two new species of *Sporothrix* and their relation to *Blastobotrys nivea*. – Antonie Van Leeuwenhoek 39: 515–520.

ERSHAD D.J., ASGARI M., AKBARIAN J. (1998): *Sporothrix cyanesens*, a fungus new to Iran, isolated from gall making insects. – In: Proceedings of the 13th Iranian Plant Protection Congress, August 1998, Karaj Junior College of Agriculture, Karaj, Iran.

FAN X., XIAO M., KONG F., KUDINHA T., WANG H., XU Y.-C. (2014): A rare fungal species, *Quambalaria cyanescens*, isolated from a patient after augmentation mammoplasty – environmental contaminant or pathogen? – PLoS ONE 9: e106949.

FARAG R.S., EMAM S.S. (2016): Insecticidal activities of pomegranate peels and leave crude juices. – Bulletin of Environment, Pharmacology and Life Sciences 5: 68–75.

FARR D.F., ROSSMAN A.Y. (on-line): Fungal Databases, U.S. National Fungus Collections, ARS, USDA. – https://nt.ars-grin.gov/fungaldatabases/. [accessed 29 April 2017]

HAMMERSCHMIDT L., WRAY V., LIN W., KAMILOVA E., PROKSCH P., ALY A.H. (2012): New styrylpyrones from the fungal endophyte *Penicillium glabrum* isolated from *Punica granatum*. – Phytochemistry Letters 5: 600–603.

HIRATA T., TAKAMATSU S. (1996): Nucleotide sequence diversity of rDNA Internal Transcribed Spacer extracted from conidia and cleistothecia of several powdery mildew fungi. – Mycoscience 37: 265–270.

HOSOYA T., ZHAO Y.J., DEGAWA Y. (2014): *Poculum pseudosydowianum*, sp. nov. (*Rutstroemiaceae*, Ascomycota) from Japan and its endophytic occurrence. – Phytotaxa 175: 216–224.

HUANG C.-L., WANG Y.-Z. (2011): New records of endophytic fungi associated with the *Araucariaceae* in Taiwan. – Collection and Research 24: 87–95.

JACKSON L., KLOTZ S.A., NORMAND R.E. (1990): A pseudoepidemic of *Sporothrix cyanescens* pneumonia occurring during renovation of a bronchoscopy suite. – Journal of Medical and Veterinary Mycology 28: 455–459.

KATOH K., KUMA K.-I., TOH H., MIYATA T. (2005): MAFFT version 5: Improvement in accuracy of multiple sequence alignment. – Nucleic Acids Research 33: 511–518.

KOLAŘÍK M., SLAVÍKOVÁ E., PAŽOUTOVÁ S. (2006): The taxonomic and ecological characterisation of the clinically important heterobasidiomycete *Fugomyces cyanescens* and its association with bark beetles. – Czech Mycology 58: 81–98.

KUAN C.S., YEW S.M., TOH Y.F., CHAN C.L., LIM S.K., LEE K.W., NA S.L., HOH C.-C., YEE W.-Y., NG K.P. (2015): Identification and characterization of a rare fungus, *Quambalaria cyanescens*, isolated from the peritoneal fluid of a patient after nocturnal intermittent peritoneal dialysis. – PLoS ONE 10: e0145932.

KUMAR D.S.S., HYDE K.D. (2004): Biodiversity and tissue-recurrence of endophytic fungi in *Tripterygium wilfordii*. – Fungal Diversity 17: 69–90.

MIRDEHGHAN S.H., VATANPARAST G. (2013): Avoiding the paleness of pomegranate arils by preharvest application of salicylic acid and potassium sulfate. – Acta Hort. (ISHS) 1012: 815–819.

MISHRA B., PLOCH S., WEILAND C., THINES M. (on-line) [unpublished manuscript]: TrEase – a webserver to infer phylogenetic trees with ease. – http://www.thines-lab.senckenberg.de/trease. [accessed August 2016]

MUSSI-DIAS V., ARAÚJO A.C.O., SILVEIRA S.F., ROCABADO J.M.A., ARAÚJO K.L. (2012): Endophytic fungi associated with medicinal plants. – Revista Brasileira de Plantas Medicinais 14: 261–266.

MUVEA A.M., MEYHOFER R., SUBRAMANIAN S., POEHLING H.-M., EKESI S., MANIANIA N.K. (2014): Colonization of onions by endophytic fungi and their impacts on the biology of *Thrips tabaci*. – PLoS ONE 9: e108242.

PAAP T., BURGESS T.I., MCCOMB J.A., SHEARER B.L., HARDY G.E.S.J. (2008): *Quambalaria* species, including *Q. coyrecup* sp. nov., implicated in canker and shoot blight diseases causing decline of *Corymbia* species in the southwest of Western Australia. – Mycological Research 112: 57–69.

PAUL N.C., LEE H.B. (2014): First record of endophytic *Paraconiothyrium brasiliense* isolated from Chinese maple leaves in Korea. – The Korean Journal of Mycology 42: 349–352.

PORRAS-ALFARO A., HERRERA J., NATVIG D.O., LIPINSKI K., SINSABAUGH R.L. (2011): Diversity and distribution patterns of soil fungal communities in a semiarid grassland. – Mycologia 103: 10–21.

RIDDELL R.W. (1950): Permanent stained mycological preparations obtained by slide culture. – Mycologia 42: 265–270.

RONQUIST F., TESLENKO M., VAN DER MARK P., AYRES D.L., DARLING A., HOHNA S., LARGET B., LIU L., SUCHARD M.A., HUELSENBECK J.P. (2012): MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. – Systematic Biology 61: 539–542.

SHANKAR NAIK B., SHASHIKALA J., KRISHNAMURTHY Y.L. (2008): Diversity of fungal endophytes in shrubby medicinal plants of Malnad region, Western Ghats, Southern India. – Fungal Ecology 1: 89–93.

SIMPSON J.A. (2000): *Quambalaria*, a new genus of eucalypt pathogens. – Australasian Mycologist 19: 57–62.

STAMATAKIS A., HOOVER P., ROUGEMONT J. (2008): A rapid bootstrap algorithm for the RAxML web servers. – Systematic Biology 57: 758–771.

STODŮLKOVÁ E., CÍSAŘOVÁ I., KOLAŘÍK M., CHUDÍČKOVÁ M., NOVÁK P., MAN P., KUZMA M., PAVLŮ B., ČERNÝ J., FLIEGER M. (2015): Biologically active metabolites produced by the basidiomycete *Quambalaria cyanescens*. – PLoS ONE 10: e0118913.

STOVER E.W., MERCURE E.W. (2007): The pomegranate: a new look at the fruit of paradise. – HortScience 42: 1088–1092.

TAMBINI R., FARINA C., FIOCCHI R., DUPONT B., GUÉHO E., DELVECCHIO G., MAMPRIN F., GAVAZZENI G. (1996): Possible pathogenic role for *Sporothrix cyanescens* isolated from a lung lesion in a heart transplant patient. – Medical Mycology 34: 195–198.

TAMURA K., STECHER G., PETERSON D., FILIPSKI A., KUMAR S. (2013): MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. – Molecular Biology and Evolution 30: 2725–2729.

WALSH P.S., METZGER D.A., HIGUCHI R. (1991): Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. – Bio Techniques 10: 506–513.

WHITE T.J., BRUNS T., LEE S., TAYLOR J. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. – In: Innis M.A., Gelfand D.H., Sninsky J.J., White T.J., eds., PCR Protocols: a guide to methods and applications, pp. 315–322. Academic Press, New York.

WINGFIELD M.J., CROUS P.W., SWART W.J. (1993): *Sporothrix eucalypti* (sp. nov.), a shoot and leaf pathogen of *Eucalyptus* in South Africa. – Mycopathologia 123: 159–164.