Cryptogamie, Mycologie, 2017, 38 (1): 13-25

Kirschsteiniothelia arasbaranica sp. nov., and an emendation of the Kirschsteiniotheliaceae

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Abstract – A new species of *Kirschsteiniothelia* from dead branches of sessile oak, *K. arasbaranica*, is described from Arasbaran forests of Iran, using morphological and molecular data. *Kirschsteiniothelia arasbaranica*, lacking an anamorph, is characterized by possessing eight-spored, cylindrical-clavate, bitunicate, fissitunicate asci, 120-180 × 30-40 µm, with an apical ocular chamber 5-8 µm wide, and ascospores narrowly to broadly ellipsoidal, 1-septate, verrucose to finely spinulose, (30-)34-42(-44) × (12-)13-16(-18) µm, with a distinct mucilaginous sheath. Phylogenetic analysis of a combined sequence dataset of the ITS, LSU and SSU rDNA also supports the recognition of this fungus as a new species. We hereby propose an emendation of the family Kirschsteiniotheliaceae. A dichotomous key for identification of 20 sufficiently documented *Kirschsteiniothelia* species is also provided.

***Dendryphiopsis* / Dothideomycetes / identification key / phylogeny / ribosomal DNA / taxonomy**

INTRODUCTION

Kirschsteiniothelia D. Hawksw. was introduced by Hawksworth (1985), and typified by *K. aethiops* (Sacc.) D. Hawksw. based on *Sphaeria aethiops* Berk. & M.A. Curtis. Most species assigned to this genus are widespread in tropical regions and commonly occur on dead wood. This ascomycetous genus is characterized by superficial, erumpent, globose to subglobose, dark brown to black, membranaceous, scattered or loosely aggregated ascomata with or without central papilla; a thick pseudoparenchymatous peridium consisting of thick-walled cells arranged in palisadic configuration at base angles (*textura angularis*); hamathecium comprising numerous, filiform, hyaline, pseudoparaphyses; bitunicate, fissitunicate, cylindrical-clavate, 8-spored asci with a long pedicel and an ocular apical chamber; biseriately arranged ascospores, ellipsoidal, slightly curved, smooth-walled, olive brown to dark brown, 1-2-septate with median or submedian septum, without a distinct gelatinous sheath (Hawksworth, 1985; Boonmee *et al.,* 2012; Hyde *et al.,* 2013).

The anamorph of *Kirschsteiniothelia* has been listed as *Dendryphiopsis* S. Hughes, a connection proven by morphological and molecular data (Hawksworth, 1985; Schoch *et al.,* 2006; Hernandez *et al.,* 2008; Boonmee *et al.,* 2012; Hyde

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doi/10.7872/crym/v38.iss1.2017.13

et al., 2013; Wijayawardene *et al.,* 2014). *Dendryphiopsis* anamorph of *Kirschsteiniothelia* has macronematous, mononematous, erect, branched or unbranched, septate, brown to dark brown, smooth-walled conidiophores; monoblastic, terminal, delimited conidiogenous cells, constricted at the septa; apically produced conidia, broadly ellipsoid-obovoid, 1-2(-3)-septate, light brown, red brown to dark brown, smooth-walled (Hawksworth, 1985; Hyde *et al.,* 2013; Su *et al.,* 2016).

Originally *Kirschsteiniothelia* was included within the Pleosporaceae (Hawksworth, 1985; Barr, 1987); however, based on host, morphology and anamorph, Barr (1993) argued that *Kirschsteiniothelia* belongs to the Pleomassariaceae. Based on molecular data, Schoch *et al.* (2006) showed that the type species, *K. aethiops*, was not phylogenetically close to the Pleosporaceae, and further suggested that this genus should be transferred to a separate family. In further phylogenetic analysis of the Dothideomycetes, *K. elaterascus* Shearer grouped within Morosphaeriaceae, while *K. maritime* (Linder) D. Hawksw. clustered with *Mytilinidion* spp. as a sister group in the Mytilinidiaceae clade (Schoch *et al.,* 2009; Suetrong *et al.,* 2009). The family Kirschsteiniotheliaceae was then proposed by Boonmee *et al.* (2012) to accommodate taxa grouping with *K. aethiops*. The higher order taxonomy of the genus *Kirchsteiniothelia* has not been clarified due to insufficient data (Boonmee *et al.,* 2012; Hyde *et al.,* 2013).

In an investigation of Diatrypaceae in Arasbaran forests of Iran, 2013-2016, we found two specimens of *Kirschsteiniothelia* on dead branches of sessile oak (*Quercus petraea* (Matt.) Liebl.). Based on morphological and molecular data, combined sequence dataset of ITS, LSU, SSU rDNA, a new species of *Kirschsteiniothelia* is described. The familial concept of Kirschsteiniotheliaceae is emended in asci and ascospores characteristics, and anamorph state. We present an identification key to well-documented *Kirschsteiniothelia* species. This key is clearly not a substitute for a necessary critical revision of all described species of this highly diversified genus.

MATERIALS AND METHODS

Morphological observation: Two specimens of *Kirschsteiniothelia* were collected from Arasbaran forest of Iran (East Azerbaiejan province). Vertical and horizontal sections were cut through the ascomata by hand with the aid of an Olympus SZH stereo microscope. Microscopic preparations were made in distilled water, with 30 measurements per structure. Photographs were taken using an Olympus (DP25) digital camera installed on a BX51 Olympus light microscope. Single-ascospore cultures were obtained by serial dilutions and transferring a single germinating ascospore to a new Petri dish containing potato-dextrose agar (PDA, Merck, Germany). Colony morphology, color (Rayner, 1970) and growth rate were determined on PDA at 25°C. Voucher specimens are preserved at the Fungus Reference Collection (IRAN…F) of Herbarium Ministerii Iranici Agriculturae “IRAN”, Iranian Research Institute of Plant Protection (Tehran). Living cultures are deposited at the Iranian Fungal Culture Collection (IRAN…C) of the “IRAN” herbarium.

DNA extraction and amplification: Fresh fungal mycelium (500 mg) was scraped from the margin of a PDA plate incubated at 25°C for 7-10 days and transferred into a 1.5 mL centrifuge tube and was ground using liquid nitrogen. DNA extraction was performed according to Liu *et al.* (2000). The ITS region (ITS1-5.8S-

ITS2) was amplified using primers ITS1 [5′-tcc gta ggt gaa cct gcg g] and ITS4 [5′-tcc tcc gct tat tga tat gc] (White *et al.,* 1990). Amplification of D1-D3 of the 28S rDNA (LSU) was achieved using primers LROR [5′-gta ccc gct gaa ctt aag c] and LR7 [5′-tac tac cac caa gat ct] (Rehner & Samuels, 1994; Vilgalys & Hester, 1990). The primers NS1 [5′-gta gtc ata tgc ttg tct c] and NS4 [5′-ctt ccg tca att cct tta ag] (White *et al.,* 1990) were used to amplify the 18S rDNA (SSU). The PCR reaction (25 µl) contained 2.5 µL 10× high yield PCR buffer (Jena Bioscience, Germany) containing 1 µL MgCl2 (25 mM), 0.5 µL dNTPs (10 mM), 1 µL of each primer (10 pmol/µL, Takapouzist Inc.), 1.5 unit Taq polymerase (Jena Bioscience, Germany) and 1 µL genomic DNA (30 ng/µL). PCR amplification of all the three regions was carried out according to Mehrabi *et al.* (2016). The PCR products were purified using a multiscreen filter plate (Millipore Corp., Bedford, MA, USA) in Macrogen Company, South Korea. The purified DNA samples were then submitted for sequencing to a capillary sequencing machine (ABI Prism 3730XL, Applied Biosystem, Foster City, CA) of the same company.

Phylogenetic analysis: New sequences generated in this study were checked with FinchTV v. 1.4.0 (Geospiza Inc.). The alignments were obtained using ClustalX version 2.0 (Thompson *et al.,* 1997). Sequences of the ITS, LSU and SSU were analyzed individually and in combination in PAUP* v4.0b10 (Swofford, 2003). The evolutionary history was inferred with maximum parsimony (MP) method. Characters were equally weighted, and gaps were treated as missing data. Trees were inferred with the heuristic search option with TBR branch swapping and 1000 random sequence additions. Branches of zero length were collapsed and all parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (Hillis & Bull 1993). Trees were drawn with FigTree v. 1.4.0 (Rambaut, 2012). The partition homogeneity test (PHT) was applied from PAUP v4.0b10 (Swofford, 2003). The sequences generated in this study were deposited in GenBank (Table 1).

RESULTS

Phylogenetic analyses: To clarify the relationships of the newly described species within the genus we conducted phylogenetic analyses using sequences of ITS (465-497 bp), LSU (1024-1094 bp) and SSU (970-1040 bp) individually (not shown) and combined (Fig. 1). The sequences generated in this study were aligned against all sequences of well-documented *Kirschsteiniothelia* species (Kirschsteiniotheliaceae) and a few members of Acrospermaceae, Aliquandostipitaceae, Dyfrolomycetaceae, Manglicolaceae, Strigulaceae and Tubeufiaceae, mostly from Li *et al.* 2016 and Hyde *et al.* 2013 (Table 1).

A partition homogeneity test in PAUP 4.0b10 (Swofford, 2003) did not show any significant divergence (P = 0.01), indicating that the individual datasets were congruent and produced trees with similar topology. Therefore the three datasets were combined in a single analysis, with *Pleospora herbarum* (Pers.) Rabenh. as outgroup (Fig. 1). The combined dataset of ITS, LSU and SSU rDNA contained 2622 positions, of which 1138 were constant, 639 variable, parsimony uninformative and 845 parsimony informative. Parsimony analysis resulted in 15 most parsimonious trees of 3368 steps with a CI of 0.643, RI of 0.694 and RC of 0.447.

Table 1. Isolates used in the phylogenetic analysis

Taxon	*Strain*	*Source*	*GenBank accession numbers*		
			ITS	*LSU*	*SSU*
Acrospermum adeanum	M133	Bryophyte, Finland	EU940180	EU940104	EU940031
Acrospermum compressum	M151	Bryophyte, Finland	EU940161	EU940084	EU940012
Acrospermum gramineum	M152	Bryophyte, Finland	EU940162	EU940085	EU940013
Aliquandostipite crystallinus (T)	R 076-1	Submerged wood, USA	–	EF175651	EF175630
Aliquandostipite khaoyaiensis (T)	CBS 118232	Decaying branch, Thailand	–	GU301796	–
Dendryphiopsis atra	DAOM 231155	?, ?	–	DQ678046	DQ677996
Dyfrolomyces rhizophorae	JK5456A	Mangrove wood, Havaii	–	GU479799	GU479766
Dyfrolomyces tiomanensis (T)	MFLUCC13-0440	Decaying mangrove wood, Malaysia	–	KC692156	KC692155
Flavobathelium epiphyllum	MPN67	Lichen, Panama	–	GU327717	JN887382
Helicomyces paludosa (T)	CBS 120503	Rotten wood, USA	–	GU301877	GU296203
Helicomyces roseus	CBS 283.51	Submerged bark, Switzerland	AY916464	AY856881	AY856928
Jahnula aquatica	R68-1	Submerged wood, USA	JN942354	EF175655	EF175632
Jahnula bipileata (T)	F49-1	Submerged wood, USA	JN942353	EF175657	EF175635
Jahnula sangamonensis (T)	A402-1B	Submerged wood, USA	JN942349	EF175661	EF175639
Jahnula seychellensis	SS 2113.2	Submerged wood, Thailand	–	EF175664	EF175643
Kirschsteiniothelia aethiops	CBS 109.53	?, ?	–	AY016361	AY016344
Kirschsteiniothelia aethiops	MFLUCC 15-0424	Submerged wood, China	KU500571	KU500578	KU500585
Kirschsteiniothelia arasbaranica (T)	IRAN 2508C	Dead branch of *Quercus petraea*, Iran	<u>KX621983</u>	<u>KX621984</u>	<u>KX621985</u>
Kirschsteiniothelia arasbaranica	IRAN 2509C	Dead branch of *Quercus petraea*, Iran	<u>KX621986</u>	<u>KX621987</u>	<u>KX621988</u>
Kirschsteiniothelia emarceis (T)	MFLUCC 10–0106	Decaying wood of unidentified tree, Thailand	HQ441570	HQ441571	HQ441572
Kirschsteiniothelia lignicola (T)	MFLUCC 10-0036	Decaying wood of unidentified tree, Thailand	HQ441567	HQ441568	HQ441569
Kirschsteiniothelia submerse (T)	MFLUCC 15-0427	Submerged wood, China	KU500570	KU500577	KU500584
Kirschsteiniothelia tectonae (T)	MFLUCC 15-1883	*Tectona grandis*, Thailand	KU144916	KU764707	–
Kirschsteiniothelia thujina	JF13210	*Abies alba*, France	KM982716	KM982718	KM982717
Manglicola guatemalensis	BCC20157	*Nypa fruticans*, Thailand	JN819282	FJ743450	FJ743444
Manglicola guatemalensis	BCC20156	*Nypa fruticans*, Thailand	JN819283	FJ743448	FJ743442
Phyllobathelium anomalum	MPN 242	Lichen, Panama	–	GU327722	JN887386
Pleospora herbarum (T)	CBS 191.86	*Medicago sativa*, India	NR111243	GU238160	GU238232
Tubeufia helicomyces	CBS 271.52	Submerged leaf of *Fraxinus excelsior*, the Netherlands	AY916461	AY856887	AY856933
Tubeufia javanica (T)	MFLUCC 12-0545	Dead culm of *Bambusa vulgaris*, Thailand	KJ880034	KJ880036	KJ880035

Sequences with underlined numbers are generated in this study, others are from GenBank. (T) = ex-type strain. **CBS,** Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **IRAN…C,** Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran; **DAOM,** National Mycological Herbarium, Department of Agriculture, Ottawa, Ontario, Canada; **BCC,** BIOTEC Culture Collection, Bangkok, Thailand; **MFLUCC,** Mae Fah Luang University Culture Collection, Thailand.

Analysis of the combined dataset provided higher support than the individual datasets and resolved the relationships among *Kirschsteiniothelia* species. This analysis strongly supports the position of *K. arasbaranica* as a new species within the family Kirschsteiniotheliaceae, concordant with morphological traits.

Taxonomy

Kirschsteiniotheliaceae Boonmee & K.D. Hyde, *Mycologia* 104: 705. 2012. **emend.**

Asci 4-8-spored with a short or long stalk. Ascospores 2- to 4-seriate, smooth-walled to verrucose or finely spinulose, with or without a mucilaginous sheath, 1-(-2)-septate with median or submedian septum slightly or deeply constricted. *Dendryphiopsis* anamorph present or absent.

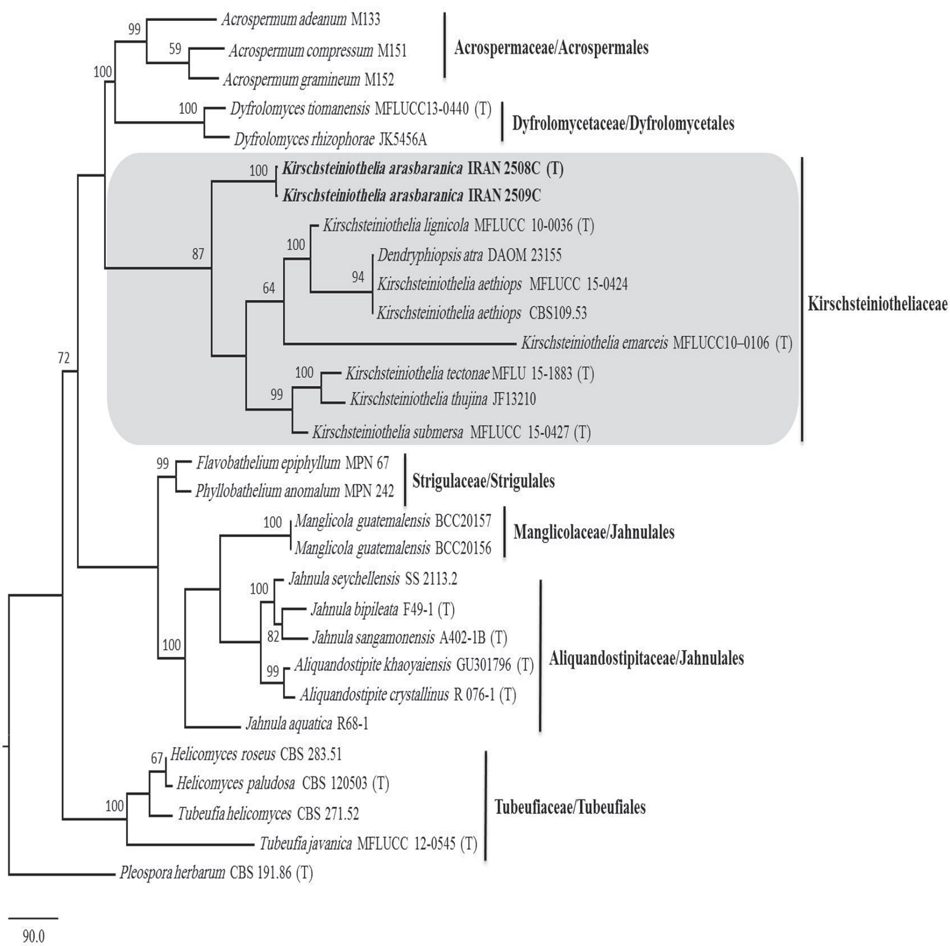

Fig. 1. Phylogram generated from maximum parsimony analysis based on combined dataset of ITS/ LSU/SSU rDNA. Bootstrap values greater than 50% (1000 replicates) are indicated above branches. *Pleospora herbarum* is outgroup. (T) = ex-type strain.

Kirschsteiniothelia arasbaranica Mehrabi, R. Hemmati & Asgari, sp. nov. Fig. 2

MycoBank: MB 817764

Etymology. The epithet refers to Arasbaran forests in Iran where it was found.

Saprobic on dead branch. **Sexual morph**: Ascomata 189-380 µm high × 350-480 µm diam, superficial with the base remaining slightly immersed in the substrate, subglobose to globose, scattered or loosely clustered in small groups, black, inside white, with apex papilla or plane. Peridium 50-80 µm thick, comprising several layers of cells of *textura angularis*; inner layer cells hyaline, outer layer cells pale brown to dark. Hamathecium comprising numerous filiform pseudoparaphyses 2-2.7 mm wide, hyaline, branched, embedded in a gelatinous matrix. Asci 120-180 × 30-40 µm, cylindrical-clavate, 8-spored, occasionally 4- or 6-spored, bitunicate, fissitunicate, with a short stalk up to 10 µm long, apically rounded, with an apical ocular chamber 5-8 µm wide. Ascospores arranged 2- to 4-seriate in the lower 2/3 and 1-seriate in the upper 1/3 of asci, (30-)34-42(-44) × (12-)13-16(-18) µm ($\bar{x}$ = 36.6 × 15.2, n=40), brown to dark brown at maturity, narrowly to broadly ellipsoidal with rounded apex, thick-walled, verrucose to finely spinulose, covered with a mucilaginous sheath, 1-septate, septum deeply constricted and submedian, the upper cell distinctly larger than the lower cell, each cell containing a distinct large guttule. **Asexual morph**: Undetermined.

Culture characteristics: Ascospores germinating on PDA within 24-48 h. Colonies on PDA dense, reaching 5 mm diam in 7 d at 25°C and 20-23 mm diam. within a month, first grayish blue (45''''b), surface covered with a white mat of aerial hyphae, becoming dark brick (7''k) with white margin at 3 mo, composed of brown to dark brown, septate, smooth or verruculose hyphae up to 6 µm wide and hyaline to pale brown hyphal elements at the periphery, with abundant anastomosis, up to 4 µm wide; reverse at first dark bluish green (39m) with hyaline margin, then becoming black at 3 mo. No conidiogenous structures observed.

Specimens examined: Iran, East Azerbaijan province, Arasbaran forests, on dead branch of *Quercus petraea*, 11 July 2015, M. Mehrabi (IRAN 16767 F, holotype), ex-type culture, IRAN 2508C; *Ibid.* (IRAN 16768 F, isotype), ex-isotype culture, IRAN 2509C.

Known geographical distribution: Northwestern Iran.

Notes: Kirschsteiniothelia arasbaranica is close to *K. dolioloides* (Rehm) You Z. Wang, Aptroot & K.D. Hyde and to *K. thujina* in ascospore size and septation, and in lacking an anamorph (Wang *et al.,* 2004; Hawksworth, 1985). However, it can be distinguished from these by having wider asci and ellipsoidal ascospores with rounded apex, covered by a distinct mucilaginous sheath.

DISCUSSION

The fungus described and illustrated here conforms well to the genus *Kirschsteiniothelia* by the morphology of its ascomata and its pseudoparaphysate hamathecium associated with bitunicate, obclavate asci and large, brown, unequally two-celled ascospores. This was further supported by our phylogenetic analysis. Among described species of *Kirschsteiniothelia*, the status of six species, viz. *K. aethiops* (Boonmee *et al.,* 2012; Wijayawardene *et al.,* 2014; Hyde *et al.,* 2013),

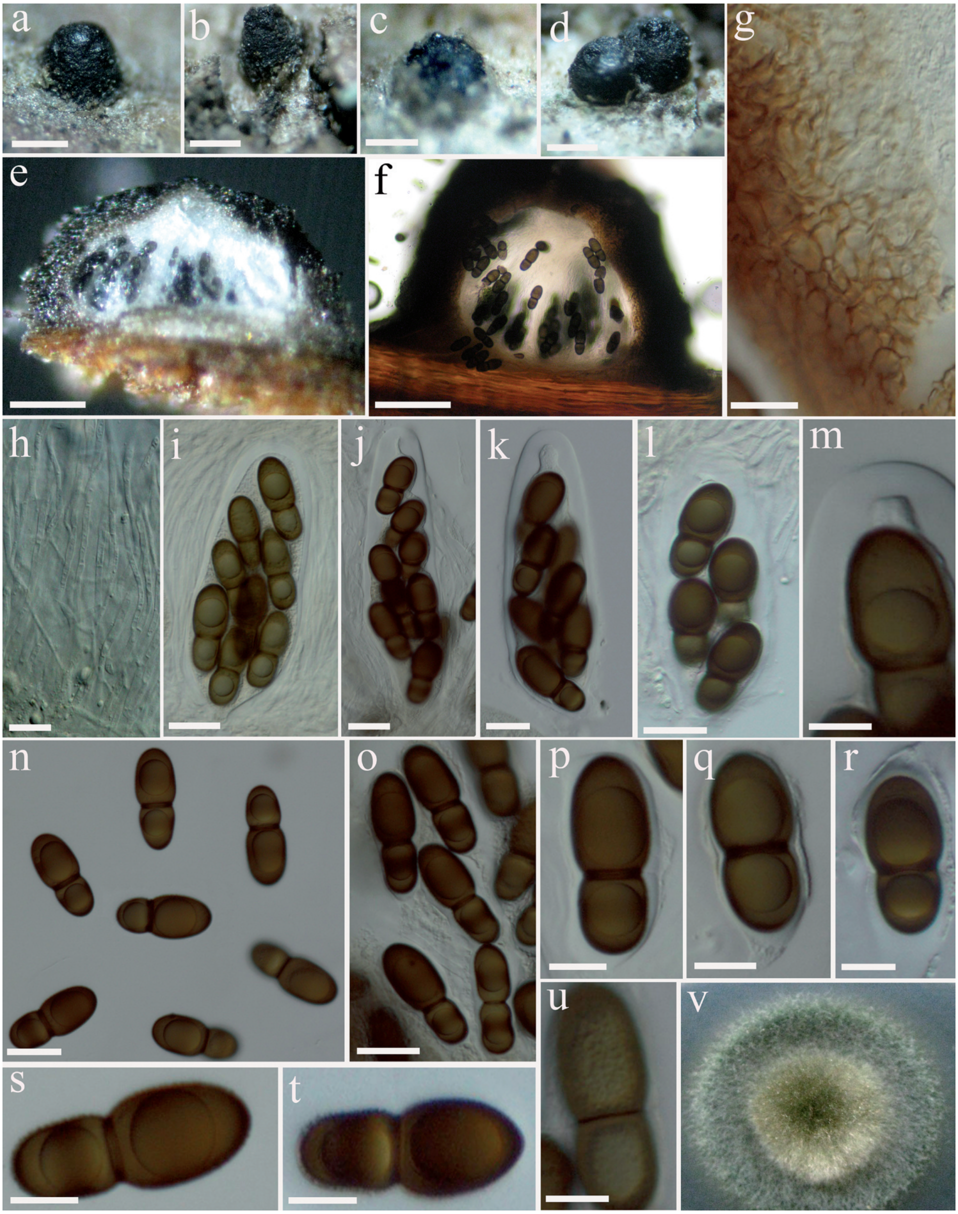

Fig. 2. *Kirschsteiniothelia arasbaranica* (holotype). **a-d.** Ascomata on dead branch of *Quercus petraea*, **e, f.** Section through ascomata, **g.** Section through peridium, comprising several layers of cells of *textura angularis*, **h.** Pseudoparaphyses, **i-l.** Asci, **m.** Apical inner layer of an ascus with small ocular chamber **n-u.** Ascospore, **v.** Culture on PDA incubated for two weeks at 25°C. Bars: **a-d** = 200 µm; **e, f** = 100 µm; **g, i-l, n, o** = 20 µm; **h, m, p-u** = 10 µm.

K. lignicola S. Boonmee & K.D. Hyde (Boonmee *et al.,* 2012), *K. emarceis* S. Boonmee & K.D Hyde (Boonmee *et al.,* 2012), *K. submersa* H.Y. Su & K.D. Hyde (Su *et al.,* 2016), *K. tectonae* Doilom, D.J. Bhat & K.D. Hyde (Liu *et al.,* 2016) and *K. thujina* (Peck) D. Hawksw. (Zhang & Fournier, 2015) has been resolved by molecular data. All these species were also included in our phylogenetic analysis of the combined ITS, LSU and SSU rDNA sequences (Fig. 1).

The new species described here, *K. arasbaranica*, grouped within the familial clade of Kirschsteiniotheliaceae that clustered together with Acrospermaceae (Acrospermales) and Dyfrolomycetaceae (Dyfrolomycetales) and formed a sister group with Aliquandostipitaceae and Manglicolaceae (Jahnulales) and Strigulaceae (Strigulales). This is in agreement with previous studies by Hyde *et al.* (2013) and Su *et al.* (2016). All these families, belonging to Dothideomycetes, have persistent pseudoparaphyses and bitunicate (fissitunicate) asci with a distinct ocular chamber in common. However, members of Kirschsteiniotheliaceae are distinguished by possessing mostly ellipsoidal, septate, colored ascospores, and a *Dendryphiopsis* anamorph.

Ascospores of the genus *Kirschsteiniothelia* were defined by Hawksworth (1985) as being 'without a distinct mucilaginous sheath'. In contrast, Chen & Hsieh (2004) documented the presence of ascospores with mucilaginous sheath in *K. aethiops*. Then, Chen *et al.,* (2006) described *K. reticulata* Chi Y. Chen, C.L. Wang & J.W. Huang and *K. smilacis* Chi Y. Chen, C.L. Wang & J.W. Huang, both characterized by ascospores with mucilaginous sheath. Based on a few available molecular data of *Kirschsteiniothelia* species, Boonmee *et al.* (2012) defined the family Kirschsteiniotheliaceae as having smooth-walled ascospores, without a mucilaginous sheath. Here clustering of *K. arasbaranica* within the familial clade of Kirschsteiniotheliaceae suggests that the familial circumscription proposed by Boonmee *et al.,* (2012) needs to be emended in ascospore characteristics.

Kirschsteiniothelia aethiops has been linked with the asexual genus *Dendryphiopsis* (Hughes, 1953; Hawksworth, 1985; Boonmee *et al.,* 2012; Hyde *et al.,* 2013; Wijayawardene *et al.,* 2014). Based on phylogenetic analysis, Boonmee *et al.* (2012) showed that *K. aethiops* grouped with *D. atra* (Corda) S. Hughes, the type species of *Dendryphiopsis*, *K. lignicola* and *K. emarceis*, all having a *Dendryphiopsis* anamorph. Therefore, they introduced the family Kirschsteiniotheliaceae to accommodate the genus *Kirschsteiniothelia* and its anamorph, *Dendryphiopsis*. However, in the present study, *K. arasbaranica* and *K. thujina*, both lacking anamorph, grouped within the Kirschsteiniotheliaceae.

Both isolates assigned to *K. arasbaranica* (IRAN 2508C and IRAN 2509C) were clustered together with high bootstrap support (100%) in a clade that formed a sister group with the other clade accommodating the remaining species of *Kirschsteiniothelia*. The phylogenetic distance between *K. arasbaranica* and the remaining species of *Kirschsteiniothelia* is also reflected by its unique morphological traits of ascospores that are verrucose to finely spinulose with a distinct mucilaginous sheath, and lacking a *Dendryphiopsis* anamorph. All species included in the clade Kirschsteiniotheliaceae herein can be accurately differentiated by details of the ascomata, asci, ascospores, and the anamorph.

Our phylogenetic analysis showed that *K. emarceis* formed a sister relationship with *K. aethiops*, *K. lignicola* and *D. atra* with a long branch as was previously shown by Boonmee *et al.* (2012). *Kirschsteiniothelia emarceis* differs from all other *Kirschsteiniothelia* species in having sparse ascomatal hairs. While individual phylogenetic analyses of ITS and SSU rDNA sequences (not shown), demonstrated inclusion of *K. emarceis* (MFLUCC10-0106) within the Kirschsteiniotheliaceae, analysis of LSU sequences (not shown) supported its exclusion from this family. The closest BLAST (Altschul *et al.,* 1990) hits for the LSU sequence of *K. emarceis* were members of the Pleosporales such as *Decaisnella formosa* Abdel-Wahab & E.B.G. Jones, GQ925846 (identities 786/834, 94%) and *Sporormiella pulchella* (E.C. Hansen) S.I. Ahmed & Cain, GQ203747 (identities 784/847, 93%). We, therefore, suggest the resequencing of the LSU gene of *K. emarceis*.

KEY TO THE SPECIES OF *KIRSCHSTEINIOTHELIA*

1. Ascomata undetermined, *Dendryphiopsis* anamorph always present..................2
1. Ascomata determined, *Dendryphiopsis* anamorph present or absent..................3
    2. Conidia on natural substrate 4-6-septate, 37.5-51.5 × 8.5-9.5 µm ....*K. submersa*
    2. Conidia on natural substrate multi-septate (9-25 or more septa), (85-)135-150(-212) × (15-)16-17(-19) µm..*K. tectonae*
3. Ascomata non-ostiolate; Asci 85-95 × 20-24 µm; ascospores 22-26 × 8-11 µm. ....*K. acerina*
3. Ascomata ostiolate 4
    4. Ascospores mostly with mucilaginous sheath 5
    4. Ascospores always without mucilaginous sheath..8
5. Ascospores (30-)34-42(-44) × (12-)13-16(-18) µm, verrucose to finely spinulose; mucilaginous sheath always present...*K. arasbaranica*
5. Ascospores mostly narrower than 13 µm 6
    6. Ascospores with reticulate ornamentation, 17-23 × 7-10 µm; mucilaginous sheath always present...*K. reticulata*
    6. Ascospore without reticulate ornamentation...7
7. Ascomata papillate; ascospores (21-)25-33(-38) × (7.5-)8.5-12.0(-14.0) µm; mucilaginous sheath present or absent; *Dendryphiopsis* anamorph present ....*K. aethiops*
7. Ascomata non-papillate; ascospore 20-24 × 6-8 µm; mucilaginous sheath always present; anamorph unknown...*K. smilacis*
    8. Ascospores with striate ornamentation, (14-)15-18(-19) × 5.0-6.5 µm; on *Juniperus communis* ....*K. striatispora*
    8. Ascospore smooth or verrucose 9
9. Ascospores mostly shorter than 20 µm...10
9. Ascospores mostly longer than 20 µm...13
    10. Ascomata subcuticular; asci 70-90 × 9-16; ascospores 14-16 × 5-6 µm; on leaves of *Freycinetia arnotti* ....*K. atkinsonii*
    10. Ascomata superficial or immersed; asci mostly narrower than 11 µm; on bark of various hosts 11
11. Ascospores (11-)13-17(-20) × 3-4(-5) µm; on *Protea cynaroides* ....*K. proteae*
11. Ascospores mostly wider than 4 µm...12
    12. Asci 84-112 × 9-10 µm; ascospores 1-seriate, 14-18 × 5-6 µm..... *K. populi*
    12. Asci 90 × 10 µm; ascospores 2-seriate, (14-)15-17.5 × 5-6.5(-7) µm ....*K. recessa*

13. Ascospores 20-22.5 × 7-8.5 µm; asci 50-80 × 15-25 µm .... *K. xera*
13. Ascospores mostly wider than 8 µm 14
    14. Asci 150-200 × 21-23 µm; ascospores smooth or verrucose, 31-41 × 12-16 µm .... *K. dolioloides*
    14. Asci mostly shorter than 150 µm 15
15. Ascomata covered with a few sparse hair-like setae; asci 88-140 × 18-24 µm; ascospores (23.5-)25-28 × 8-9(-10) µm .... *K. emarceis*
15. Ascomata glabrous 16
    16. Ascospores 1-2-septate, 27-30(-33) × 10-12(-13) µm; *Dendryphiopsis* anamorph present .... *K. lignicola*
    16. Ascospores always 1-septate; anamorph unknown 17
17. Ascospores (29-)36-50(-55) × (12-)15-17(-19) µm .... *K. thujina*
17. Ascospores smaller 18
    18. Ascomata semi-immersed, ascospores 22 × 10 µm; on *Tilia americana* .... *K. phileura*
    18. Ascomata superficial, erumpent 19
19. Ascospores 22-26 × 8-11 µm; on *Aesculus hippocastanum* .... *K. umbrinoidea*
19. Ascospores 25-28 × 7.5-10 µm; on *Tsuga Canadensis* .... *K. abietina*

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Acknowledgments. The Research Institute of Modern Biological Techniques (University of Zanjan) is acknowledged for providing the laboratory equipments and facilities. The authors thank Dr. A. Aptroot)ABL Herbarium, The Netherlands(for being so kind to send us some relevant literature and also Dr. R. Asghari for his assistance in phylogenetic analysis.

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