



Two new species and one new record of *Kretzschmaria* (Ascomycota, Xylariales) from Iran

Pourmoghaddam MJ^{1,2}, Khodaparast SA^{1*}, Krisai-Greilhuber I², Voglmayr H² and Stadler M³

¹Department of Plant Protection, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran

²Division of Systematic and Evolutionary Botany, Department of Botany and Biodiversity Research, University of Vienna, Rennweg 14, 1030 Wien, Austria

³Helmholtz-Zentrum für Infektionsforschung GmbH, Dept. Microbial Drugs, Inhoffenstrasse 7, 38124, Braunschweig, Germany

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Abstract

Kretzschmaria hedjaroudei and *Kretzschmaria iranica* are described as new species based on collections from dead wood in northern Iran and on evidence from morphology and molecular phylogenetic data. *Kretzschmaria hedjaroudei* is phylogenetically close to *Kretzschmaria deusta*, from which it differs in its stromatal morphology, ascospore size and the size of the apical apparatus. *Kretzschmaria iranica* is similar to *Kretzschmaria pavimentosa*, but distinguishable by smaller ascospore size. Phylogenetic analyses of a combined matrix of the internal transcribed spacer (ITS) region of the nuclear rDNA and of α -actin (ACT1) gene sequences strongly support their status as two distinct new species within the genus *Kretzschmaria*. Moreover, *Kretzschmaria zonata*, a species previously only known from tropical countries, is encountered for the first time in Iran.

Key words – 2 new species – Biodiversity – Biosystematics – Phylogeny – Xylariaceae

Introduction

The Xylariaceae have traditionally comprised the stromatic genera of the Xylariales, but recently, the family was re-organised and now comprises mainly genera with a geniculosporium-like anamorph (Daranagama et al. 2018) and this was followed in the latest classification of Wijayawardene et al. (2018). Wendt et al. (2018) resurrected and emended the *Hypoxylaceae* for *Hypoxylon* and most other genera featuring a nodulisporium-like anamorph and transferred *Biscogniauxia*, *Camillea* and related genera to the Graphostromataceae. The Xylariaceae *sensu stricto* is, with regard to the number of genera and species, still the largest family in the Xylariales and even contains several genera that form very small stromata or are only recognised to belong to it from studies of the anamorphs and molecular phylogenies (Daranagama et al. 2018). As recently summarised by Helaly et al. (2018), these fungi have a very interesting lifestyle, as they are able to live as saprotrophs on wood or dung, but also constitute one of the predominant taxonomic groups among the endophytes of seed plants, while only a few species have been recognised as plant pathogens or associates of insects.

The genus *Kretzschmaria* Fries was described in 1849 with *K. clavus* as the type species.

Martin (1970) emphasized the close relationship between *Xylaria* and *Kretzschmaria*, segregating them by the vertical orientation of perithecia and umbonate or aristate apices on the fertile parts. The last monographic treatment of the genus, in which the taxonomy of *Kretzschmaria* has been linked to the holomorphic concept of the Xylariaceae, goes back to Rogers & Ju (1998). Strikingly, the genus has received little attention since then, and many species are still only known from their teleomorphs and have never been cultured and studied by molecular phylogenetic methods.

Stromata of *Kretzschmaria* species are superficial, peltate, discoid, restricted pulvinate, to effused pulvinate, usually gregarious, discrete, or fused into crusts, stipitate or sessile. Mature stromata lack KOH extractable pigments and have a carbonaceous outer layer encrusting a rather soft white to blackish inner layer. The asci are cylindrical, stipitate, evanescent or persistent, with an urn-shaped, amyloid apical apparatus. Ascospores are medium, dark, or blackish brown, ellipsoid to fusoid with a straight or sigmoid germ slit ranging over the entire spore length to much less than spore-length on the less convex side and have a perispore indehiscent in 10% KOH (Rogers & Ju 1998, Mugambi et al. 2009).

The genus is informally divided into kretzschmarioid and ustulinoid taxa. Kretzschmarioid taxa are characterised by stipitate or sessile stromata, with the fertile parts and/or stipes often fused, usually with entire margins. Individual fertile parts rarely exceed 1 cm diam. and the anamorphs may be coremioid or noncoremioid. Ustulinoid taxa have more or less sessile stromata, to which frequently rhizoid-like processes or narrow connectives are attached. Their stromata usually have crenate margins, with fertile parts usually exceeding 1 cm diam., and the anamorphs (where known) are noncoremioid.

Species of this genus are identified from all over the world, and they are widespread in temperate and tropical areas (Rogers & Ju 1998). Currently, about 23 species are accepted (Rogers & Ju 1998, Hladki & Romero 2001, Rogers & Ju 2004, Mugambi et al. 2009, Pereira et al. 2009, Yun et al. 2016), most of which are causing white rot and root rot (Rogers & Ju 1998). Analysis of β -tubulin, α -actin and RPB2 sequence data confirmed that *Kretzschmaria* is phylogenetically closely related to *Xylaria* (Hsieh et al. 2010). In Iran, *K. deusta* is the only species of the genus reported so far (Saber 2002).

During a survey of Xylariaceae in Guilan and Mazandaran provinces in Iran, two *Kretzschmaria* taxa were found that could not be identified to species. The combination of morphological and molecular data did not result in any matches with known taxa, hence they are described here as new species. Furthermore, an additional *Kretzschmaria* species was recorded as new for Iran.

Materials & Methods

Morphological characterisation

Samples were collected from Guilan and Mazandaran provinces (N Iran) during 2016. Parts of corticated branches and trunks of infested trees containing xylariaceous stromata were transferred to the laboratory. Single ascospores cultures were obtained from ascostromata based on the method described by Rogers & Ju 1998. The fungus was described from cultures grown at 20 °C on 2% Difco oatmeal agar (OA). Microscopy of ascospores and asci was done in distilled water, and Melzer's reagent was used for staining of the apical ascus apparatus. At least 30, 10 and 5 measurements were determined for ascospores, asci and ascus apical apparatus, respectively. Macrophotographs were obtained with a Keyence VHX-6000 microscope. Light microscopy with Nomarski differential interference contrast (DIC) was done using a Zeiss Axio Imager.A1 compound microscope equipped with a Zeiss AxioCam 506 colour digital camera. Usually, more than one photo was taken and selected photos of each structure were mounted in a single photo plate using Photoshop (version CS5). Living cultures have been deposited in the Fungal Herbarium of the Iranian Research Institute of Plant Protection (IRAN) and STMA (HZI culture collection, Helmholtz Centre for Infection Research, Braunschweig, Germany). Also, dried vouchers have

been deposited in the fungarium of the Department of Plant Protection, Faculty of Agricultural Science, University of Guilan, Guilan, Iran (GUM).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted directly from perithecia or mycelia produced in culture using Chelex 5% (Walsh et al. 1991, Hirata & Takamatsu 1996). DNA preparations were stored at -20 °C until used for PCR. The DNA amplification was obtained by polymerase chain reaction (PCR). A region spanning ITS1, 5.8S and ITS2 of rDNA was amplified as described by Khodaparast et al. (2012) using the primers ITS5 and ITS4 (White et al. 1990). Part of the α -actin gene (ACT1) region was amplified with primers ACT-512F and ACT-783R (Carbone & Kohn 1999). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington, UK) and the PCR primers. Sequencing was performed on an automated DNA sequencer (ABI 3730xl Genetic Analyzer, Applied Biosystems). Sequences derived from this study were deposited at the NCBI GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov>); for GenBank accession numbers see Table 1.

Sequence alignment and phylogenetic analyses

The raw trace files were inspected and edited with MEGA v. 7 software (Kumar et al. 2016). The sequences were compared with sequences from GenBank using BLAST searches; sequences with high similarity were added to the matrices. Sequences of an accession of *Hypoxylon fragiforme* (JN979419, AY951831) and of *H. howeanum* (JQ009323, AY951839) from Hypoxylaceae were included as outgroups to root the trees. The details on the sequences included in the phylogenetic analyses are given in Table 1. A basic alignment of the obtained sequences in this study together with the sequence data from GenBank and the outgroup sequences was first done using MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/index.html>) (Katoh et al. 2002); and when considered necessary, manual adjustments were made in MEGA v. 7 (Kumar et al. 2016). Maximum likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI 1.5 (Silvestro & Michalak 2012), using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates. The matrix was partitioned for the different gene regions. Bootstrap support (BS) below 70 % was considered low, between 70–85 % medium and above 85 % high. The matrix and the resulting tree have been deposited at Treebase under submission number S23376 (<http://purl.org/phylo/treebase/phyloids/study/TB2:S23376?x-access-code=f5b91e2c76a8997905731af44909f2d5&format=html>)

Results

Sequencing and molecular phylogeny

The final alignment comprised sequences of 27 accessions of *Kretzschmaria* spp. and *Xylaria* spp. from GenBank and from the Iranian specimens and two *Hypoxylon* species as outgroups. Amplification of the ITS and ACT1 loci yielded fragments of approximately 700 and 300 bp, respectively. The final matrix contained 741 bp from ITS and 234 bp from ACT1. Of the 975 characters included in the combined analyses, 368 were parsimony informative (110 from ACT1, 258 from ITS). The best ML tree (-lnL = 7791.715), revealed by RAxML, is shown as a phylogram in Fig. 1. In the phylogenetic analyses, the genus *Xylaria* was revealed as paraphyletic; however, without significant support of the tree backbone. The genus *Kretzschmaria* formed a monophylum but without significant support as well. Within *Kretzschmaria*, *K. guyanensis* and *K. megalospora* formed a highly supported (97 % BS) subclade in an unsupported sister group relationship to a highly supported (89 % BS) core *Kretzschmaria* subclade. The latter contained all taxa of our study from Iran. Moreover, five new sequences generated from Iranian material were clearly distinct from already published sequences belonging to the genus *Kretzschmaria*, of which four were

morphologically revealed to belong to two undescribed species, while two others matched with *K. zonata* and *K. deusta*, respectively (Fig. 1).

Table 1 List of used strains for molecular phylogeny. Sequences in bold were generated during the present study

Species	GenBank Acc No ITS	GenBank Acc No ACT	Specimen or strain ID	Origin	Reference
<i>Kretzschmaria clavus</i>	EF026126	EF025596	YMJ 114, CBS 122872	French Guiana	Hsieh et al. (2010)
<i>K. deusta</i>	KT281901	KU684090	CBS 826.72, CBS 163.93	USA, Thailand	U'Ren et al. (2016), Senanayake et al. (2015)
<i>K. deusta</i>	MH084755	MH056202	IRAN 3060c, GUM1547	Iran	This study
<i>K. guyanensis</i>	GQ408901	GU300079	89062903 (HAST)	Taiwan	Hsieh et al. (2010)
<i>K. hedjaroudei</i> sp. nov.	MH084757	MH056204	IRAN 3061c, STMA 18005, GUM1549	Iran	This study
<i>K. iranica</i> sp. nov.	MH084758	MH056205	GUM1550	Iran	This study
<i>K. iranica</i> sp. nov.	MH084759	MH056206	GUM1551	Iran	This study
<i>K. iranica</i> sp. nov.	MH084760	MH056207	GUM1552	Iran	This study
<i>K. lucidula</i>	EF026125	EF025595	YMJ 112	French Guiana	Hsieh et al. (2010)
<i>K. megalospora</i>	EF026124	EF025594	YMJ 229	Malaysia	Hsieh et al. (2010)
<i>K. neocaledonica</i>	GU300078	GQ398236	94031003 (HAST)	Taiwan	Hsieh et al. (2010)
<i>K. pavimentosa</i>	GU300077	GQ398235	109 (JDR)	Taiwan	Hsieh et al. (2010)
<i>K. sandvicensis</i>	GU300078	GQ398234	113 (JDR)	USA, Hawaiian Islands	Hsieh et al. (2010)
<i>K. zonata</i>	MH084756	MH056203	GUM1548	Iran	This study
<i>Xylaria arbuscula</i>	KY610394	GQ421286	89041211 (HAST), CBS126415	Germany, Taiwan	Hsieh et al. (2010), Wendt et al. (2018)
<i>X. areolata</i>	GU300080	GQ408902	89041211 (HAST), 543 (HAST, JF)	French West Indies	Hsieh et al. (2010)
<i>X. bambusicola</i>	EF026123	AY951873	YMJ 205	Taiwan	Hsieh et al. (2010)
<i>X. coccophora</i>	GU300093	GQ421289	786 (HAST, JF)	French Guiana	Hsieh et al. (2010)
<i>X. cranioides</i>	GU300075	GQ398233	226 (HAST)	Taiwan	Hsieh et al. (2010)
<i>X. fissilis</i>	GU300073	GQ398231	367 (HAST, JF)	French West Indies	Hsieh et al. (2010)
<i>X. hypoxylon</i>	KY204024	GQ427195	95082001 (HAST), CBS 122620	Taiwan, Sweden	Hsieh et al. (2010), Sir et al. (2016)
<i>X. multiplex</i>	GU300098	GQ427198	580 (HAST, JF)	French West Indies	Hsieh et al. (2010)
<i>X. muscula</i>	GU300087	GQ408909	520 (HAST, JF)	French West Indies	Hsieh et al. (2010)
<i>X. oligotoma</i>	GU300092	GQ421288	784 (HAST, JF)	French Guiana	Hsieh et al. (2010)
<i>X. polymorpha</i>	GU322460	GQ452364	1012 (JDR)	USA	Hsieh et al. (2010)

Table 1 Continued.

Species	GenBank Acc No ITS	GenBank Acc No ACT	Specimen or strain ID	Origin	Reference
<i>X. tuberooides</i>	GU300074	GQ398232	475 (HAST, JF)	French West Indies	Hsieh et al. (2010)
<i>X. venustula</i>	GU300091	GQ421287	88113002 (HAST)	Taiwan	Hsieh et al. (2010)
<i>Hypoxylon fragiforme</i>	AY951831	JN979419	YMJ 387	France	Hsieh et al. (2005)
<i>H. howeanum</i>	JQ009323	AY951839	YMJ 388	France	Hsieh et al. (2005)

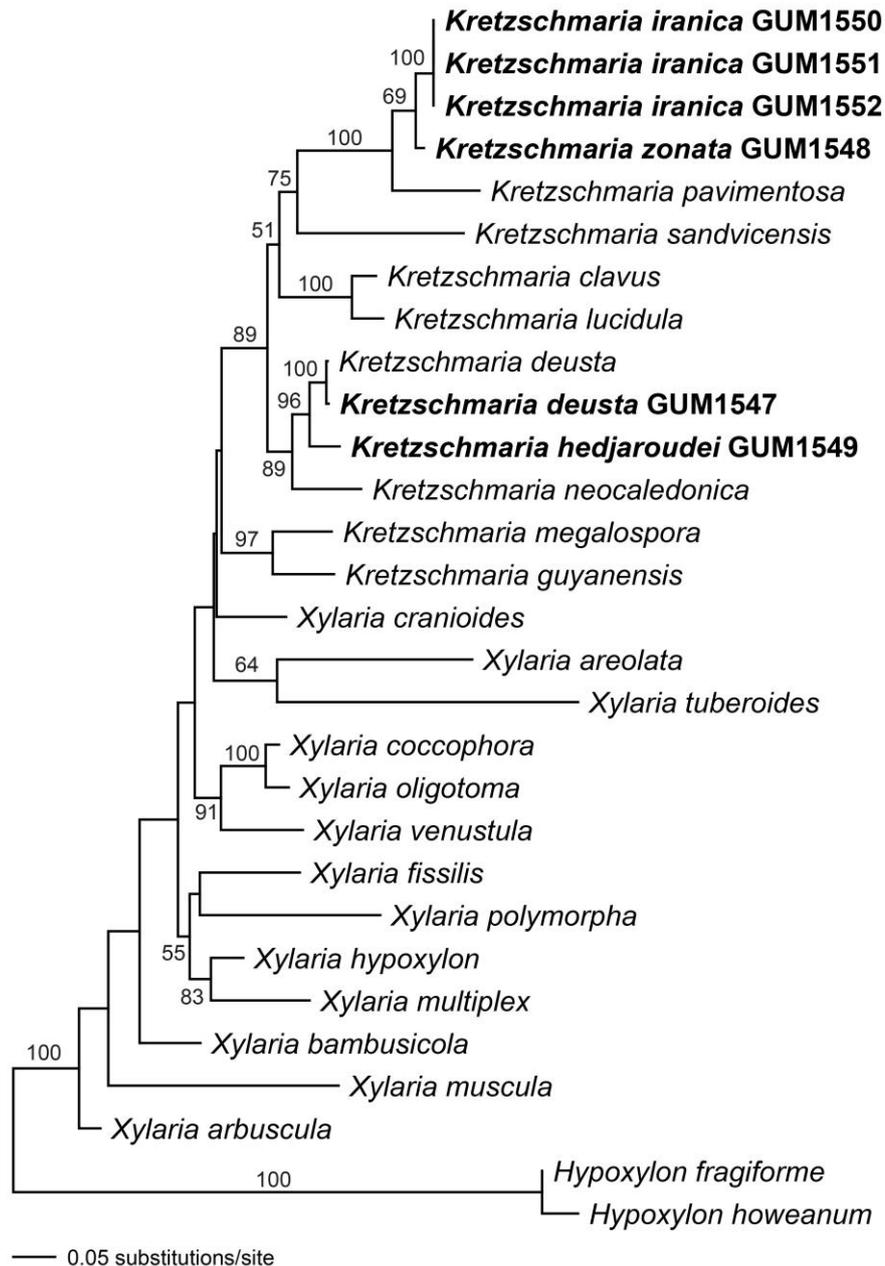


Figure 1 – Phylogram of the best ML tree ($-\ln L = 7791.715$) revealed by RAxML from an analysis of the combined ITS-ACT1 matrix of *Kretzschmaria* and *Xylaria* species, showing the phylogenetic position of the Iranian *Kretzschmaria* isolates (in bold, with herbarium numbers following the taxon names). ML bootstrap support above 50% is given above or below the branches. The tree was rooted to with two *Hypoxylon* species.

Taxonomic part

The combination of morphological and molecular data led to the conclusion that *Kretzschmaria hedjaroudei* and *K. iranica* represent undescribed species of the Xylariaceae, and are therefore described as new. In addition, *K. zonata* is a new record for Iran.

Kretzschmaria hedjaroudei Pourmoghaddam & Khodap, sp. nov.

Fig. 2

Mycobank: MB 827993; Facesoffungi number: FoF 04958

Holotype – Iran, Mazandaran Province, Tonekabon County, Dohezar forest, on wood or bark of a dead branch, N 36°47'21.02", E 50° 52'34.07", 1 m elev., 28 Oct 2016; M.J. Pourmoghaddam (GUM1549, consisting of a dried specimen. Ex-type cultures IRAN 3061C & STMA 18005. GenBank Acc. No: ITS – MH084757, ACT1 – MH056204).

Etymology – In honor of the Iranian mycologist Ghorban Ali Hedjaroude, a pioneer in the exploration of Iranian fungi.

Teleomorph – Stromata superficial, pulvinate, discrete, sessile, up to 2 cm long × 1–1.4 cm wide × 2.28–2.54 mm thick, attachment to substrate with strong connective, steep margins; surface black to dark brown, with inconspicuous perithecial mounds and cracks, carbonaceous immediately beneath surface; tissue between and beneath perithecia black to dark brown. Perithecia obovoid to cylindrical, 0.75–1.15 mm wide × 1.15–1.8 mm high, ostioles coarsely papillate. Asci with amyloid, urn-shaped apical apparatus, 6–8 µm high × 3–5 µm wide, stipe up to 200 µm long, spore-bearing part 90–140 × 12–14 µm. Ascospores smooth, unicellular, brown to dark brown, fusoid to ellipsoid, inequilateral, with narrowly or broadly rounded ends, 24–30 × 5–7 µm, with straight germ slit much less than spore-length on flattened side; perispore indehiscent in 10% KOH.

Cultures and anamorph – Cultures on 2% OA reaching 90 mm diam. in 4 weeks, first whitish, felty and zonate, then becoming gray from center outwards with concentric zones. Asexual morph not produced in culture.

Host – on wood or bark of a dead branch

Known distribution – Iran

Notes – *Kretzschmaria hedjaroudei* is closely related to *K. deusta*; however, it can be easily distinguished by its stromatal shape, smaller diameter and steep margins of stromata, coarsely papillate ostioles, smaller ascospores (24–30 × 5–7 vs. 27–35 × 7–9 µm) and larger apical apparatus (6–8 × 3–5 vs. 4–6 × 3–4 µm) (Rogers & Ju 1998). It is also similar to *K. parvistroma* according to the shape of stromata, which according to Mugambi et al. (2009) differs by much larger ascospores (34–)37–38(–39) × (10–)12–13 µm.

Kretzschmaria iranica Pourmoghaddam & Khodap, sp. nov.

Fig. 3

Mycobank: MB 827994; Facesoffungi number: FoF 04959

Holotype – Iran, Guilan Province, Fouman County, Ghalerodkhan forest, on fallen wood of *Alnus subcordata*, N 37°04'05.02", E 49°14'40.08", 480 m elev., 10 Nov 2016, M.J. Pourmoghaddam GUM1551, consisting of a dried specimen. GenBank Acc. No: ITS – MH084759, ACT1 – MH056206).

Etymology – Named after the country from where it was collected, Iran

Teleomorph – Stromata superficial, pulvinate to effused-pulvinate, densely aggregated, up to 8.5 cm long × 2–4 cm wide × 1.8–3.45 mm thick, with broad attachment to substrate and narrow connective, margins steep; surface brown to dark brown, with inconspicuous perithecial mounds, often with reticulate cracks, carbonaceous immediately beneath surface; tissue between and beneath perithecia brown to dark brown. Perithecia obovoid to lanceolate, 0.6–0.9 mm wide × 0.75–1.15 mm high, ostioles finely papillate. Asci with amyloid, urn-shaped apical apparatus, 7–10 µm high × 4.5–7 µm wide, stipe up to 150 µm long, spore-bearing part 80–130 × 10–15 µm. Ascospores smooth, unicellular, dark to blackish brown, fusoid to ellipsoid, inequilateral, with narrowly or broadly rounded ends, 29–40 × 8–12 (–13) µm, with straight germ-slit slightly less than spore-length on flattened side; perispore indehiscent in 10% KOH.

Host – on fallen wood of *Alnus subcordata*

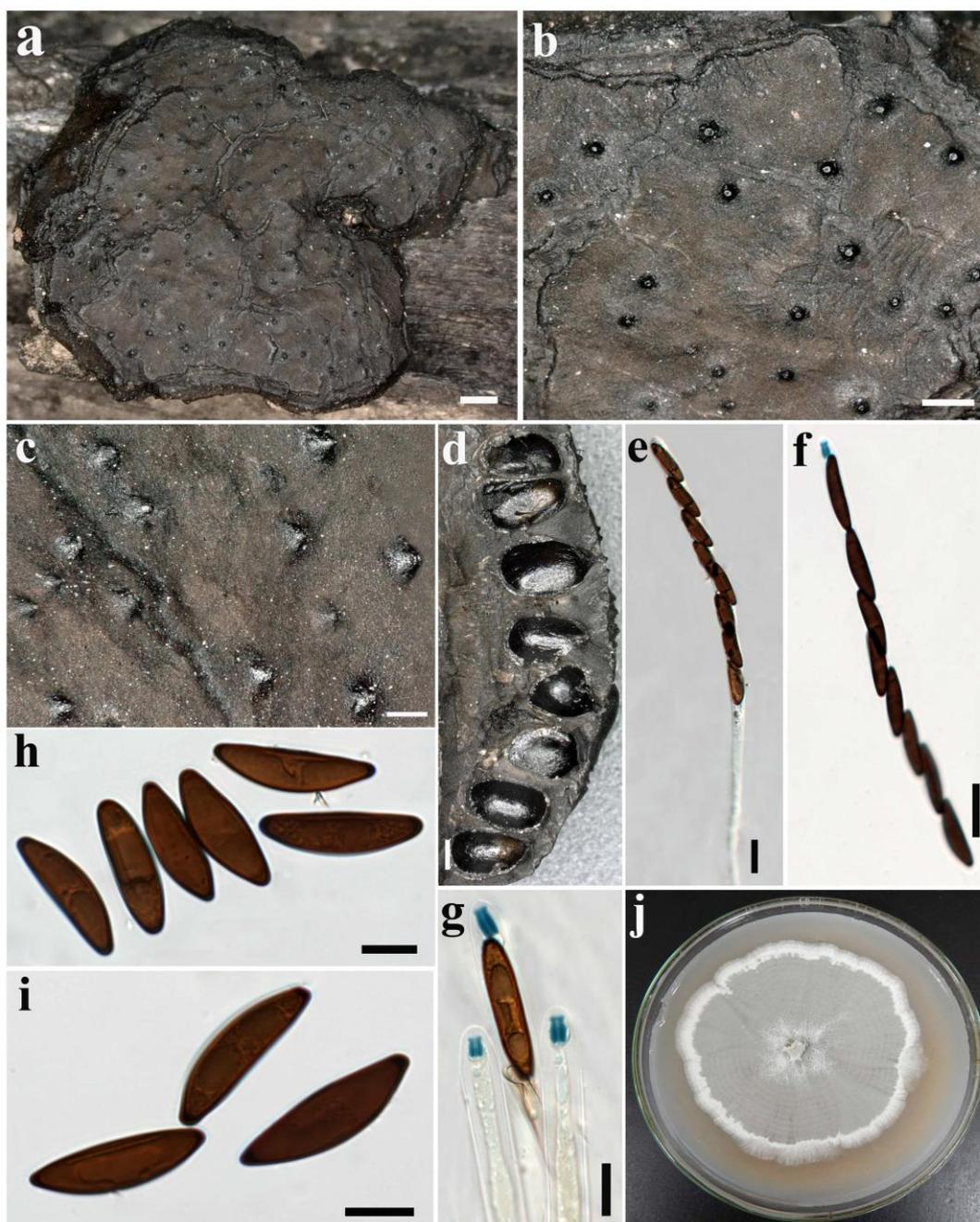


Figure 2 – *Kretzschmaria hedjaroudei* (Holotype). a Stromata habit. b Close-up view of stromata surface. c Close-up view of ostiolar discs. d Stroma in vertical section showing perithecia. e Asci. f Asci, showing apical apparatus in Melzer's reagent. g Apical apparatus in Melzer's reagent. h–i Ascospores in water, showing germ slits. j Culture on OA. Scale bars: a = 1 mm, b = 0.4 mm, c = 0.2 mm, d = 0.5 mm, e–f = 20 μ m, g–i = 10 μ m.

Known distribution – Iran

Additional material examined – Iran, Guilan Province, Fouman County, Ghalerodkhan forest, on dead trunk of *Quercus* sp., N 37°04'10.26", E 49°14'40.18", 551 m elev., 10 Nov 2016, leg. M.J. Pourmoghaddam (GUM1552, consisting of a dried specimen. GenBank Acc. No: ITS – MH084760, ACT1 – MH056207). Iran, Mazandaran Province, Tonekabon County, Dohezar forest, on dead trunk of *Pterocarya fraxinifolia*, N 36°47'21.02", E 50° 52'34.07", 1 m elev., 28 Oct 2016, leg. M.J. Pourmoghaddam (GUM1550, consisting of a dried specimen. GenBank Acc. No: ITS – MH084758, ACT1 – MH056205).

Notes – *Kretzschmaria iranica* has morphological similarities to *K. pavimentosa*, but differs by a larger apical apparatus (7–10 × 4.5–7 vs. 6–9 × 4.5–6 μm), smaller ascospores (29–40 × 8–12 (–13) vs. 35–54 (–56) × 7.5–11 μm) and in the length of germ-slit, which is similar to *K. deusta*.

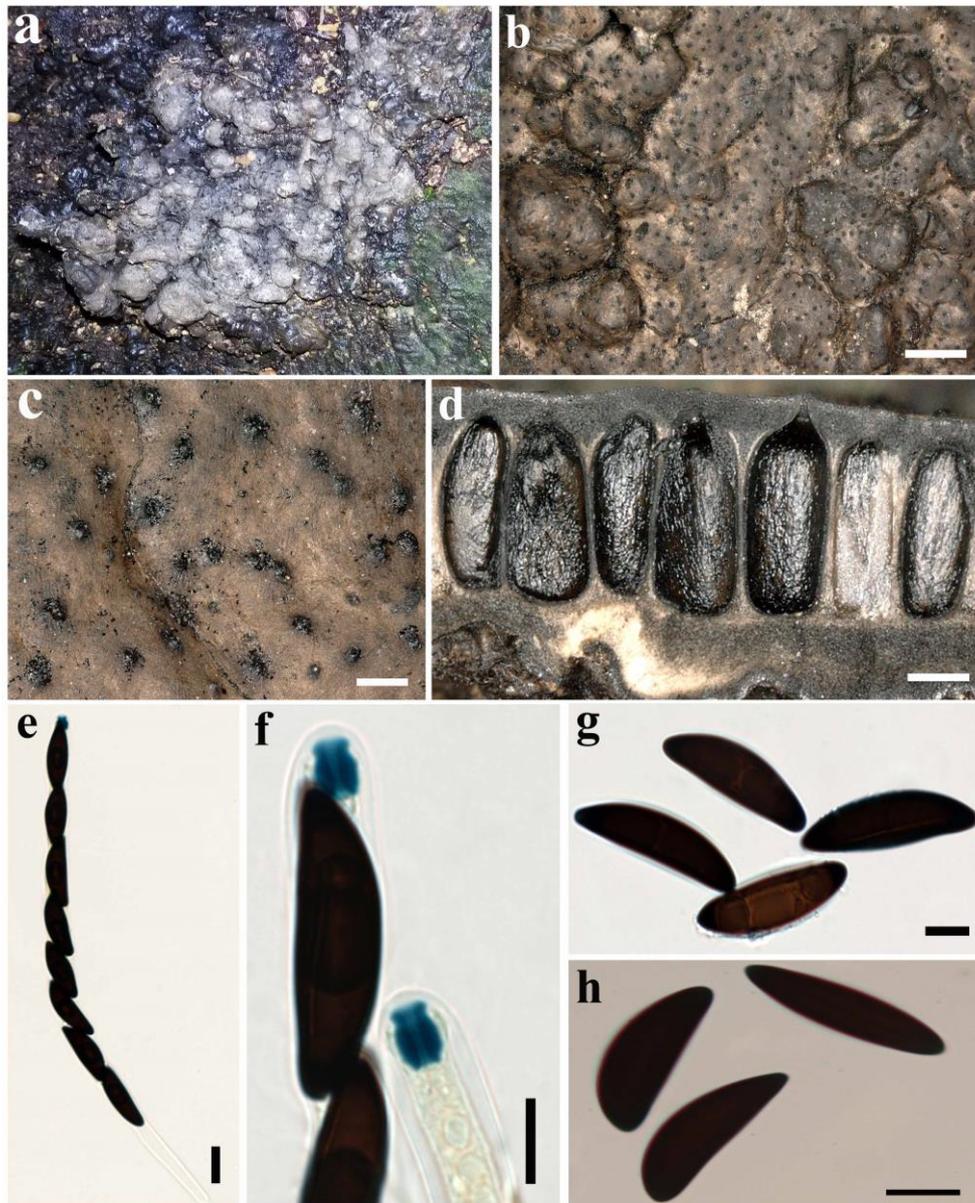


Figure 3 – *Kretzschmaria iranica* (Holotype). a Stromatal habit. b Close-up view of stromata surface. c Close-up view of ostiolar discs. d Stroma in vertical section showing perithecia. e Asci, showing apical apparatus in Melzer’s reagent. f Apical apparatus in Melzer’s reagent. g Ascospores in water, showing germ slits. h Ascospores in 10% KOH, showing indehiscent perispore. Scale bars: b = 2 mm, c = 0.3 mm, d = 0.4 mm, e = 20 μm, f–h = 10 μm.

Kretzschmaria zonata (Lév.) P. Martin, J. S. African Bot. 42: 75 (1976).

Fig. 4

Teleomorph – Stromata superficial, pulvinate to effused-pulvinate, densely aggregated, up to 8.5 cm long × 2–3.5 cm wide × 1.8–2.54 mm thick, with broad attachment to substrate and narrow connective, sloped margins; surface brown to dark brown, with inconspicuous perithecial mounds, often with reticulate cracks, carbonaceous immediately beneath surface; tissue between and beneath perithecia brown to dark brown. Perithecia with very variable shapes, 0.6–0.75 mm diam × 0.5–0.9 mm high, ostioles papillate. Asci with amyloid, urn-shaped apical apparatus, 7–9 μm high × 5–7 μm wide, stipe up to 150 μm long, spore-bearing part 80–130 × 11–13 μm. Ascospore smooth,

unicellular, dark to blackish brown, fusoid to ellipsoid, inequilateral, $30\text{--}35(40) \times 8\text{--}11 \mu\text{m}$, with straight germ-slit slightly shorter than spore-length on flattened side; perispore indehiscent in 10% KOH.

Material examined – Iran, Guilan Province, Talesh, Gisom forest, on fallen wood of *Parrotia persica*, N $37^{\circ}37'30.95''$, E $48^{\circ}58'15.11''$, 471 m elev., 6 Oct 2016, leg. M.J. Pourmoghaddam (GUM1548, consisting of a dried specimen. GenBank Acc. No: ITS – MH084756, ACT1 – MH056203).

Notes – *Kretzschmaria zonata* is similar to *K. deusta* in having the same stromatal morphology and an indehiscent perispore in 10% KOH. However, it differs by darker and wider ascospores with a slightly longer germ slit (Rogers & Ju 1998). The characters of the Iranian specimen are in accordance with *K. zonata* as defined by Rogers & Ju (1998), except that the ascospores were slightly longer ($30\text{--}35(40) \times 8\text{--}11$ vs. $21\text{--}34 \times 8.5\text{--}12 \mu\text{m}$).

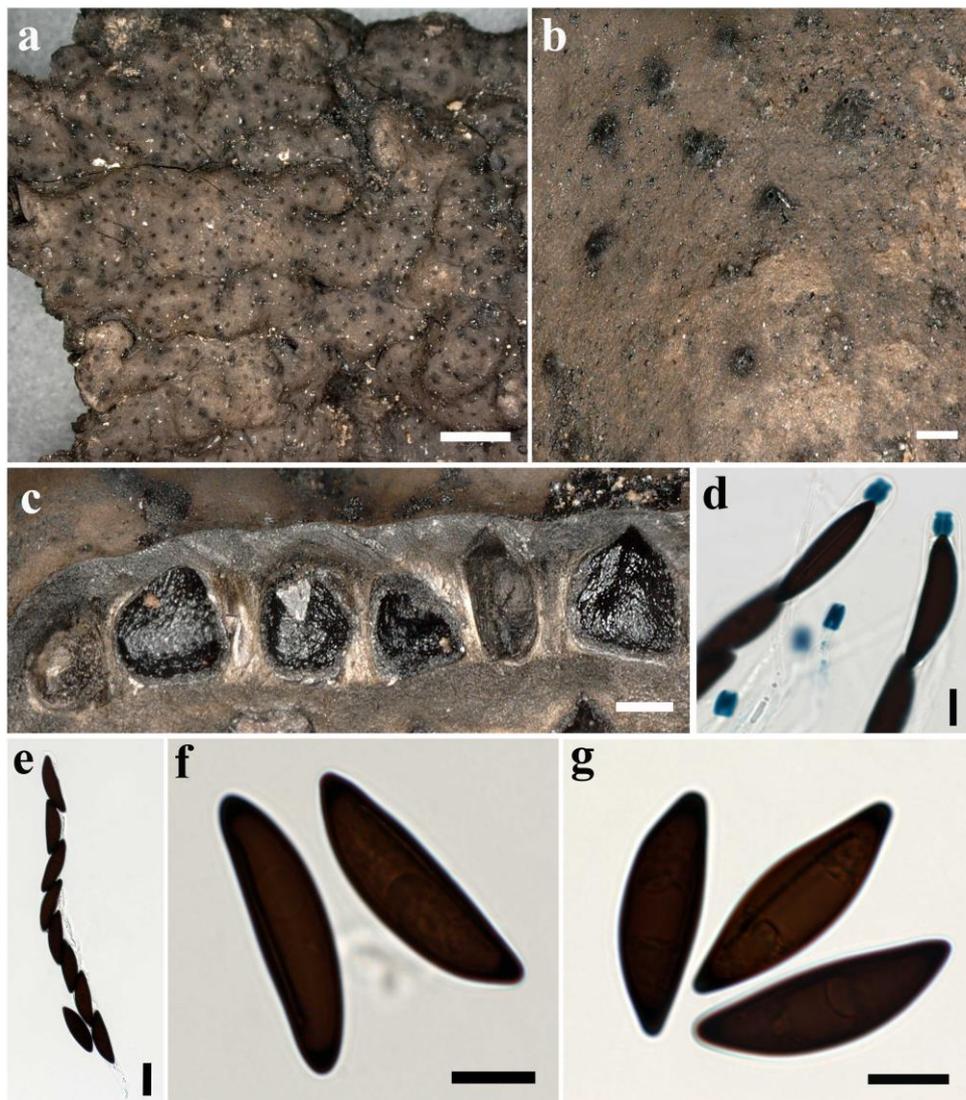


Figure 4 – *Kretzschmaria zonata*. a Close-up view of stromatal surface. b Close-up view of ostiolar discs. c Stroma in vertical section showing perithecia. d Apical apparatus in Melzer's reagent. e Asci. f–g Ascospores in water, showing germ slits. Scale bars: a = 2 mm, b = 0.2 mm, c = 0.4 mm, d, f, g = 10 μm , e = 20 μm .

Kretzschmaria deusta (Hoffm.: Fr.) P. Martin, J. S. African Bot. 36: 80 (1970).

Fig. 5

Teleomorph – Stromata superficial, pulvinate to effused-pulvinate, densely aggregated, up to

7.5 cm long × 2–5.2 cm wide × 2.8–4.5 mm thick, with broad attachment to substrate and narrow connective, sloped margins; surface black to blackish brown, with inconspicuous perithecial mounds, often with reticulate cracks, carbonaceous immediately beneath surface; tissue between and beneath perithecia brown to dark brown. Perithecia spherical to obovoid, 1–1.8 mm wide × 1–2 mm high, ostioles papillate. Asci with amyloid, urn-shaped apical apparatus, 4–6 μm high × 2.5–4 μm wide, stipe up to 250 μm long, spore-bearing part 100–150 × 10–14 μm. Ascospores smooth, unicellular, brown to dark brown, fusoid-inequilateral, 25–34 × 5.5–8 (–9) μm, with straight germ-slit much less than spore-length on flattened side; perispore indehiscent in 10% KOH.

Cultures and anamorph – Cultures on 2% OA reaching 90 mm diam. in 5 weeks, first whitish, felty and zonate, then becoming grey from centre outwards with concentric zones. Asexual morph not produced in culture.

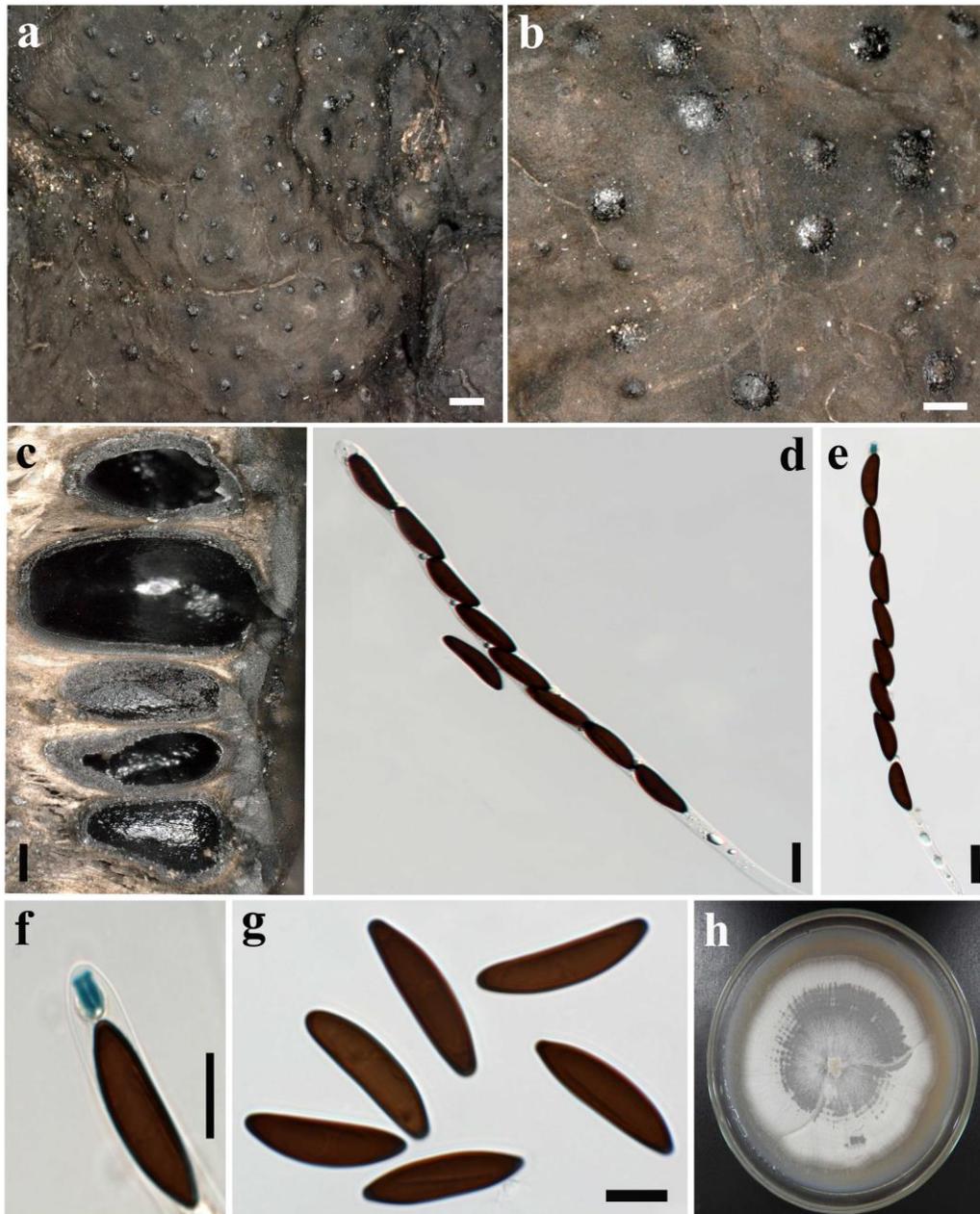


Figure 5 – *Kretzschmaria deusta*. a Close-up view of stromatal surface. b Close-up view of ostiolar discs. c Stroma in vertical section showing perithecia. d Asci. e Asci, showing apical apparatus in Melzer's reagent. f Apical apparatus in Melzer's reagent. g Ascospores in water, showing germ slits. h Culture on OA. Scale bars: a = 1 mm, b = 0.4 mm, c = 0.4 mm, d–e = 20 μm, f–g = 10 μm.

Material examined – Iran, Guilan Province, Siahkal, Deilaman forest, on dead trunk of *Fagus orientalis*, N 36°53'11.27", E 49°54'31.67", 1432 m elev., 3 Oct 2016, M.J. Pourmoghaddam (GUM1547, consisting of a dried specimen. Ex-type cultures IRAN 6030C. GenBank Acc. No: ITS – MH084755, ACT1 – MH056202).

Notes – *Kretzschmaria deusta* belongs to the ustulinoid taxa and can be distinguished from other ustulinoid *Kretzschmaria* species by smaller ascospores, except for *K. zonata* (Rogers & Ju 1998). Rogers & Ju (1998) believed that *K. deusta* is a fungus of the Northern temperate regions. Up to now, there are indeed no reliable reports of this species from tropical (1998), except for slight differences in ascospore size ($25\text{--}34 \times 5.5\text{--}8$ (–9) vs. $27\text{--}35 \times 7\text{--}9$ μm).

Discussion

From the results of this study, we introduce two new species of *Kretzschmaria*, based on morphological and molecular phylogenetic data. In addition to morphological differences, *K. hedjaroudei* is phylogenetically distinct from its closest relatives, *K. deusta*. The second new species, *K. iranica*, differs from *K. pavimentosa* in morphology and is also phylogenetically distinct. According to morphological data, *K. zonata* is similar to *K. deusta* but in the combined phylogenetic tree it is closely related to *K. pavimentosa* and *K. iranica*. Unfortunately, no sequence of the type or any other specimen of *K. zonata* was available for comparison to the Iranian specimens. Hsieh et al. (2010) conducted the first phylogenetic study of this genus based on a combined matrix of β -tubulin, α -actin and RPB2 genes and emphasized that molecular data did not correspond to morphological data. That way, the two subgroups of *Kretzschmaria* (kretzschmarioid and ustulinoid) did not group together. A recent phylogenetic study by Yun et al. (2016) using partial DNA sequences of multiple genes (β -tubulin, ITS and RPB2) revealed similar results to those of Hsieh et al. (2010), which is also the case in our phylogenetic analyses. These results show that this genus needs additional comprehensive studies with polyphasic approaches before a stable taxonomy of the group can be achieved.

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