The Lichenologist **48**(3): 191–199 (2016) doi:10.1017/S0024282916000104 © British Lichen Society, 2016

Two new species of *Lichenothelia* (*Lichenotheliaceae*) from Iran

Tahereh VALADBEIGI, Matthias SCHULTZ and Wolfgang VON BRACKEL

Abstract: Two new species of *Lichenothelia*, both from Iran, are described. *Lichenothelia iranica* is characterized by a black thallus with often finely lobate, slightly effigurate, not areolate margins, eight non-amyloid spores per ascus and 1–3-septate ascospores with 1–2 longitudinal or oblique septa. *Lichenothelia ilamensis* is distinguished by a black areolate, fissured, slightly effigurate or rarely lobulate thallus. The areoles are confluent and aggregated in the centre, becoming dispersed towards the margin, and the asci contain (4–)6(–8) non-amyloid, 1-septate spores.

Key words: Ascomycota, biodiversity, Dothideomycetes, fungi, taxonomy

Accepted for publication 7 *February* 2016

Introduction

The genus *Lichenothelia* D. Hawksw. was introduced by Hawksworth (1981) for the *Microthelia aterrima* group, and included two species: *Lichenothelia metzleri* (Lahm) D. Hawksw. and *L. scopularia* (Nyl.) D. Hawksw. Henssen (1987) provided the first thorough inventory of the genus *Lichenothelia* and treated 20 species, 18 of them newly described: *L. calcarea* Henssen, *L. convexa* Henssen, *L. dendritica* Henssen, *L. echinulata* Henssen, *L. gigantea* Henssen, *L. globulifera* Henssen, *L. intermedia* Henssen, *L. intermixata* Henssen, *L. macrocarpa* Henssen, *L. minor* Henssen, *L. paradoxa* Henssen, *L. patagonica* Henssen, *L. prolifera* Henssen, *L. radiata* Henssen, *L. schindleri* Henssen, *L. solitaria* Henssen, *L. solitarioides* Henssen, and *L. tenuissima* Henssen. After this pioneering work, several more species were added: *L. antarctica* Øvstedal from quartz-mica-schist in Antarctica (Øvstedal & Lewis Smith 2001), *L. renobalesiana* D. Hawksw. & V. Atienza from limestone and on endolithic thalli of different calcicolous *Verrucariaceae* in Spain and England (Atienza & Hawksworth 2008), *L. uralensis* Zhurb. from siliceous rock in the Ural Mountains in the north-eastern part of European Russia (Zhurbenko 2008), and *L. spiralispora* ("*spiratispora*") Etayo, lichenicolous on *Acarospora* in Spain (Etayo 2010). Finally, Muggia *et al.* (2015*a*) added *L. arida* Muggia *et al.*, *L. umbrophila* Muggia *et al.* and *L. umbrophila* var. *pullata* Muggia *et al.*, all growing on siliceous rocks, from Europe and the USA. Ertz *et al.* (2013) combined *Lichenostigma rugosum* G. Thor as *Lichenothelia rugosa* (G. Thor) Ertz & Diederich due to the division of stromatic cells by septa (vs. budding in *Lichenostigma*) and the results of phylogenetic studies.

According to Hawksworth (1981), Henssen (1987), Hyde *et al.* (2013), and Muggia *et al.* (2015*a*), *Lichenothelia* is circumscribed by the following set of characters: thallus not lichenized, forming a black crust, composed of scattered stromata or of scattered to continuous areoles, pseudoparenchymatous, often with stolons bearing microthalli developing into new areoles, thus the single stromata are frequently connected by black superficial hyphae; either with perithecioid and ostiolate ascomata with interascal filaments or non-ostiolate stromata with pseudoparenchymatous locules, releasing ascospores through decay of the stroma wall

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https://doi.org/10.1017/S0024282916000104 Published online by Cambridge University Press

(some species show intermediate characters); asci bitunicate, globose to clavate, outer layers or apex of ascus K/I+ blue or not, containing (2–)4–8 ascospores; ascospores first hyaline, becoming pale, golden, dark or reddish brown, usually ornamented and halonate, ellipsoid to soleiform, 1-septate to muriform; macroconidia, if present, black, globose, multicellular, often stipitate, originating from the thallus surface; conidiomata, if present, immersed in stromata, with globose to elongated conidiogenous cells and simple, hyaline, rod-shaped conidia.

Most of the described species of this globally distributed genus are either epi- or endolithic, either endocapylic or episubstratic on lichens or are saprophytic. Commonly they live in close contact with colonies of different algae or cyanobacteria (Henssen 1987). Hawksworth (1981) suggests that this mixture of algae and cyanobacteria is a source of carbohydrate for *Lichenothelia*.

The genus belongs to the polyphyletic ecological group of rock-inhabiting fungi (RIF) or black meristematic fungi (in a strict sense RIF are invariably asexual). These organisms are specialists in extremotolerance, living in different stressful environments such as deserts, exposed rocks, or even saltpans or acidic sites (Selbmann *et al.* 2015). On exposed rocks these fungi have to face extreme conditions such as exposure to drought, strong winds, substantial changes in temperature (including repeated freeze-thaw cycles), high evaporation (also causing osmotic stress), and strong solar radiation delivering both visible and ultraviolet light. Their stress-resistance is thought to result from the high melanin content and thick cell walls. Melanin is believed to protect the RIF from extreme cold or heat, extreme pH or osmotic conditions, toxicity of metals, and ultraviolet or even ionizing radiation (Dadachova *et al.* 2007; Selbmann *et al.* 2013*a*, *b*, 2015). Another adverse condition of exposed rocks, especially in deserts, is the low availability of energy-rich carbon sources. RIF are thought to gain these from neighbouring algae and cyanobacteria. This putative mode of nutrition is coupled with extremely slow metabolism and growth rates (Selbmann *et al.* 2015).

Recently, several attempts have been made to clarify the phylogeny of *Lichenothelia* species. Whereas Henssen & Jahns (1974) and Hawksworth (1981) placed the *Lichenotheliaceae* in the order *Dothideales*, Hyde *et al.* (2013) erected the new order *Lichenotheliales* K. Knudsen *et al.* within Dothideomyceta. Ertz *et al.* (2013) realized that *Lichenostigma* s. str. does not belong to the *Lichenotheliaceae* but to the new order *Lichenostigmatales* within the Arthoniomycetes, whereas species of *Lichenostigma* with vegetative cells separating by septation (vs. budding in the *Lichenostigmatales*), which represent most species of the subgenus *Lichenogramma*, belong to the *Lichenotheliaceae*. However, a recombination was made only for *Lichenostigma rugosum* to *Lichenothelia rugosa*.

Ertz *et al.* (2013) and Muggia *et al.* (2013, 2015*a*, *b*) provided cladograms of the phylogenetic relationships within Dothideomyceta including *Lichenothelia* species and showed that most of the species or samples cluster within the *Lichenotheliales*. In Muggia *et al.* (2013) single specimens were placed outside of this group, into *Teratosphaeriaceae* or unnamed groups, in one type of analysis and in another analysis within the *Lichenotheliales*. *Lichenothelia tenuissima* clustered twice outside the *Lichenotheliales*, once near the *Myriangiales* and once in a group with Saxomyces. The authors stated that these placements might be due to the amplification of contaminating fungi but this was not mentioned in Muggia *et al.* (2015*b*). Nevertheless, it appears that the risk of contamination in the analysis was high, consequently the placement of single samples of *Lichenothelia* outside the *Lichenotheliales* group is not sufficient to doubt the monophyly of *Lichenothelia* as described above. We therefore place our two new species, in *Lichenothelia* since they fit the features of the genus perfectly.

Materials and Methods

This study is based on specimens collected by the first author in 2004 and 2009. Morphological and anatomical observations were made using standard microscopic

https://doi.org/10.1017/S0024282916000104 Published online by Cambridge University Press

techniques. Microscopic measurements were made on hand-cut sections mounted in water with an accuracy of up to 0·5 µm. Measurements of ascospores are recorded as (minimum–) $\bar{x}$ – 1SD–$\bar{x}$ + 1SD (–maximum) followed by the number of measurements.

A list of the main distinguishing characters of *Lichenothelia* species (Tables 1 and 2) was compiled from our own observations, the original descriptions and other literature (Hawksworth 1981; Thor 1985; Henssen 1987; Øvstedal & Lewis Smith 2001; Atienza & Hawksworth 2008; Zhurbenko 2008; Etayo 2010; Knudsen & Kocourková 2010; Kocourková & Knudsen 2011; Muggia *et al.* 2015*a*). As noted by Muggia *et al.* (2015*a*), several species described by Henssen are almost impossible to determine due to the scarce information provided. Tables 1 and 2 are therefore no more than a guide to identification and it is hoped will be helpful in some cases.

The Species

Lichenothelia iranica Valadbeigi, M. Schultz & Brackel sp. nov.

MycoBank No.: MB 801475

Thallus saxicola, niger, lobulatus, ascomata frequentia, rotunda vel subglobosa, ad *c.* 0·2–0·3 mm lata. Ascosporae 1–3-septatae vel submuriformes, (20·0–)25·0–27·5(–30·0) × (6·25–)9·75–14·50(–15·50) µm. Conidia *c.* 12·5–14·5 µm longa, macroconidia *c.* 15 µm longa.

Typus: Iran, Ilam, Tonele reno, 33°42'N, 46°24'E, on calcareous rock, alt. 2038 m, 12 July 2009, *T. Valadbeigi* 101015 (HBG—holotypus; TARI, hb. Valadbeigi—isotypi).

(Fig. 1A–E)

Thallus saxicolous, black, 2–6 mm diam., rounded, cracked, margin often finely lobate (marginal areoles slightly effigurate); lobes 0·1–0·4 mm, without stolons.

Ascomata unilocular stromata, frequent, crowded, rounded, shiny black, ostiolate, *c.* 0·1–0·3 mm wide; *disc* flat, sometimes umbonate. Ascomatal wall pseudoparenchymatous, up to 45 µm wide, of 3–4 rows of suborbicular cells, outside dark brown with a granular pigment, inside pale brown to hyaline. Centre with branching interascal filaments, 2·0–3·5 µm wide, some slightly swollen apically (up to 5 µm) and here pale brownish or with a brownish cap. Section I−, K/I− in all parts, partly dextrinoid. *Asci* ellipsoid to pyriform, *c.* 66 × 32 µm, 8-spored. *Ascospores* hyaline at first, soon brown to dark brown, smooth but seemingly ornamented due to the granular pigmentation, 1–3-septate, sometimes with 1–2 longitudinal or oblique septa, (20·0–)25·0–27·5(–30·0) × (6·25–)9·75–14·50(–15·50) µm (n = 20).

Pycnidia resembling apothecia, smaller (*c.* 150 µm diam.) and less flattened, with a thick pseudoparenchymatous wall (up to 45 µm wide) of ±isodiametric cells, 6–13 µm wide; *conidiophores* short, branched, *c.* 5–7 × 2–3 µm, lining the inner wall of the cavity, conidiogenous cells *c.* 10 × 2–3 µm; *conidia* bacilliform, hyaline, non-septate, *c.* 1·5–2·0 × 6–8 µm. External conidia (macroconidia) brown, *c.* 15 um.

Chemistry. Not investigated.

Etymology. The species epithet refers to its occurrence in Iran.

Habitat and distribution. So far recorded on limestone from a mountainous region in the west of Iran (south-western Asia).

Remarks. *Lichenothelia iranica* should be compared with other species on limestone and with those growing on unspecified 'rock'. *Lichenothelia intermixta*, *L. minor*, and *L. prolifera* have much smaller ascospores (Table 2). *Lichenothelia calcarea* differs in the variable number of ascospores per ascus ((4–)6(–8)), whereas in *L. iranica* there are always 8 (Table 2). Moreover, *L. calcarea* has warty ascospores (smooth in *L. iranica*) and the net-forming lobes are much longer. *Lichenothelia gigantea* also differs in the variable number of spores per ascus (2–8) and the rosette-shaped thallus with long radiating lobes (Table 1). *Lichenothelia renobalesiana* differs in the thallus lacking radiating lobes and the almost consistently 1-septate ascospores. As we cannot exclude the possibility that *L. metzleri* and *L. schindleri* might also occur on calcareous rocks, we must compare the new species with these. The former differs in the I+ reaction of the asci and the golden to reddish brown colour of the ascospores, and the latter in the warty ornamentation of the ascospores.

Among the species on siliceous rocks with similar ascospore dimensions, *L. echinulata*,

https://doi.org/10.1017/S0024282916000104 Published online by Cambridge University Press

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TABLE 1. *Descriptions of the main distinguishing characters of* Lichenothelia *species.*

Species	Substratum	Colour	Morphology	Areole size (mm)	Lobe size (mm)	Ascoma size (mm)	Iodine
L. antarctica	quartz-mica-schist	dark brown to black	areolate, clusters of short isidial outgrowths	0·1	–	0·5, semiglobose, rough to cerebriform	− (pale pinkish)
L. arida	siliceous rock	black	areolate-lobate		0·13 × 0·03–0·11	0·18–0·45, stalked	−
L. calcarea	limestone	black	lobate, rosette-shaped	–	0·4–0·75 × 0·02–0·05	0·15–0·37, stalked	−
L. convexa	siliceous rock, lichens	black	dispersed to congregated	0·1–0·4	–	0·1–0·2, pulvinate	+
L. dendritica	granite	deep brown	areolate-lobate, effigurate	0·08	0·25–0·26 × 0·03–0·05	0·2, flat	−
L. echinulata	quartz	black	areolate-dispersed	0·08–0·2(–0·3)	–	0·2, pulvinate	+
L. gigantea	limestone	black	lobate, rosette-shaped	–	2·0 × 0·1(–0·2)	0·15	−
L. globulifera	granite	deep brown	areolate, dispersed-single	0·13–0·14 × 0·065–0·07	–	0·14, flat	+
L. ilamensis	limestone	black	areolate, fissured, slightly effigurate or rarely lobulate	0·12–0·28	0·01	0·12–0·28, flat, stipitate	−
L. intermedia	granite	black	areolate-lobate, effigurate-rosette-shaped	0·075–0·15	0·2–0·6 (–0·8) × 0·03–0·08	0·2	−
L. intermixta	limestone	black	areolate, dispersed	0·04–0·06, immersed	–	0·08	?
L. iranica	limestone	black	margin often finely lobate, slightly effigurate, not areolate	–	0·1–0·4	0·1–0·3, flat-pulvinate	−
L. macrocarpa	schist	black	areolate, dispersed	0·15–0·25	–	0·3	−
L. metzleri	serpentine, basic rocks	black	areolate to granular	0·10–0·15	–	0·1–0·15(–0·3)	?
L. minor	rock cliff	black	areolate, dispersed-single	0·06(–0·08)	–	0·1, flat	+
L. paradoxa	quartz	black	areolate-warty, dispersed	0·15–0·30	–	0·16, pseudoangiocarp	?
L. patagonica	granite	black	areolate-effigurate	0·08–0·16	0·14–0·32	0·2, pulvinate	+
L. prolifera	rock	black	areolate-lobate, effigurate-rosette-shaped	?	3 × 1·4	0·18, pseudoangiocarp	+
L. radiata	quartz	brown-black	radiate-lobate	–	1·4 × 0·08–0·12	0·15, flat	+
L. renobalesiana	limestone, *Verrucariaceae*	black	non areolate, non lobate	–	–	(0·05–)0·15–0·20 (–0·25)	+
L. rugosa	*Diploschistes* spp.	black	aggregated, irregular	–	–	0·05–0·20	−
L. schindleri	rock	black	areolate-lobate, effigurate	0·12–0·27	0·15–0·80	0·4	−
L. scopularia	siliceous rock	black	areolate or ± continuous	0·07–0·12	–	0·125–0·17, pulvinate	−
L. solitaria	quartz	black	areolate-lobate, dispersed	0·3	1·5	0·3, flat	+
L. solitarioides	quartz	black	areolate, single to confluent	0·3	–	0·2, flat- pulvinate	+
L. spiralispora	*Acarospora*	black	dispersed	–	–	0·06–0·16	+
L. tenuissima	sandstone	black	areolate, single to confluent	0·08–0·16	–	0·15, pulvinate	+
L. umbrophila	gneiss, granite	black	aggregated	–	–	0·2–0·5	+
L. umbrophila var. *pullata*	conglomerate, granite	black	areolate	0·05–0·10	–	0·08–0·15	+
L. uralensis	rock, mostly quartz crystals	brown-black	rimose-areolate, single to confluent	0·10–0·25	–	0·075–0·2(–0·4) × (0·1–)0·2–0·3	+

– = either no or not applicable except the Iodine test where it = no reaction; ? = data not available. New species in bold.

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TABLE 2. *Further descriptions of the distinguishing characters of* Lichenothelia *species and their distribution.*

Species	Spores / ascus	Spore septation	Spore colour/structure (mature spores)	Spore size (µm)	Stolons	Macroconidia (µm)	Distribution
L. antarctica	8	muriform	hyaline	42 × 18	–	–	South Orkney Is.
L. arida	4–8	1–3-septate(–submuriform)	light/dark reddish brown	(17–)20–25·5(–28) × (7–)10–13 (–13·5)	–	–	USA
L. calcarea	(4–)6(–8)	1-septate(–submuriform)	brown, warty	(18–) 20–24(–27) × 9–14(–17)	–	?	USA
L. convexa	(3–)8	1–4-septate(–submuriform)	brown, ornamented	(10–)10·5–11·5(–12) × (5–)6–6·5	+	globose, 10–15(–22)	USA, Europe
L. dendritica	8?	1–2-septate	brown, warty	15–17 × 6·5–9	–	–	Seychelles
L. echinulata	2–8	1–3-septate	brown, warty, spiky	19–23 × 10–11	–	–	Australia
L. gigantea	2–8	sub–eumuriform	brown	21–22 × 13–15, 30–32 × 13–15·5	–	–	Australia
L. globulifera	6–8	1-septate	brown, warty	13–14 × 7–8·5	–	–	Seychelles
L. ilamensis	(4–)6(–8)	1-septate	medium to dark brown, smooth	(22–)23·8–26·8(–27·5) × (11–)11·7–14·9(–17)	+	–	Iran
L. intermedia	4–8	submuriform	brown, warty	20–24(–30) × 9–11(–13)	+	globose-ellipsoid, stipitate, 7–14(–17)	South Africa, Transvaal
L. intermixta	4–8	submuriform	brown	15·5–17 × 7–9	+	–	USA
L. iranica	8	1–3-septate (–submuriform)	medium to dark brown, smooth	(20–)25–27·5(–30) × (6·25–)10–14·5 (–15·5)	–	globose, 15	Iran
L. macrocarpa	4–8	1-septate	brown, warty	19–24 × 8–15	+	globose, 7–11	Italy
L. metzleri	8	1-septate (–submuriform)	golden to reddish brown	21–24·5 × 9–11	–	–	USA, Austria
L. minor	8	1–3-septate	brown	10–11 × 6–7·5	–	–	Australia, Mexico
L. paradoxa	8	submuriform	brown	10·5–13(–14) × 5–6·5(–8·5)	+	7·5–8·5	Slovakia
L. patagonica	8	1-septate–muriform	brown, warty	13–16 × 7·5–9	+	ellipsoid, 10–12	Argentina
L. prolifera	4	1–3-septate–submuriform	brown, warty	18–22 × 8–10·5	+	globose, 8–10	Australia
L. radiata	4–8	1-septate	hyaline, warty	15–18 × 7·5–9·5	–	–	Australia
L. renobalesiana	8	1-septate	hyaline, pale brown, reddish brown, smooth	25·5–30 × 11·5–15	–	–	Europe
L. rugosa	8	1(–4)-septate	brown to brown black, rough	10–13 × 5–7	–	–	cosmopolitan
L. schindleri	?	1–3-septate–submuriform	brown, warty	(17–)22–28·5 × 9–12	–	–	Morocco
L. scopularia	8	1–3-septate–submuriform	golden to dark brown	(13–)14–18(–21) × (5·5–)7–9(–11)	?	?	USA, Europe, Israel
L. solitaria	4–8	3-septate	brown, warty	15·5–16·5(–19·5) × (7·5–)9·5(–11·5)	–	–	Australia
L. solitarioides	8	1-septate–submuriform	brown, warty	12·5–15·5 × 6·0–7·5	+	+	Australia
L. spiralispora	(6–)8	1-septate	black, ornamented	14–19 × 6–8	–	–	Spain
L. tenuissima	8	1-septate	hyaline to brown, warty	11·0–14·5 × 6·5–7·5	+	globose to ellipsoid, 9·5–10·5	USA
L. umbrophila	8	1–3-septate (–muriform)	brown, verruculose	(18·5–)21·0–25·5(–27·0) × (9·5–)11·0–14·0(–14·5)	–	–	USA
L. umbrophila var. *pullata*	4–8	1–3-septate (–submuriform)		(11–)12·5–15·5(–16·0) × (7–)7·0–8·5 (–9·0)	–	–	Czech Republic
L. uralensis	8	1(–2)-septate	hyaline to dark brown	(13–)16–17·5(–21) × (7–)8–11(–15)	–	–	Russia

– = either no or not applicable except the Iodine test where it = no reaction; ? = data not available. New species in bold.

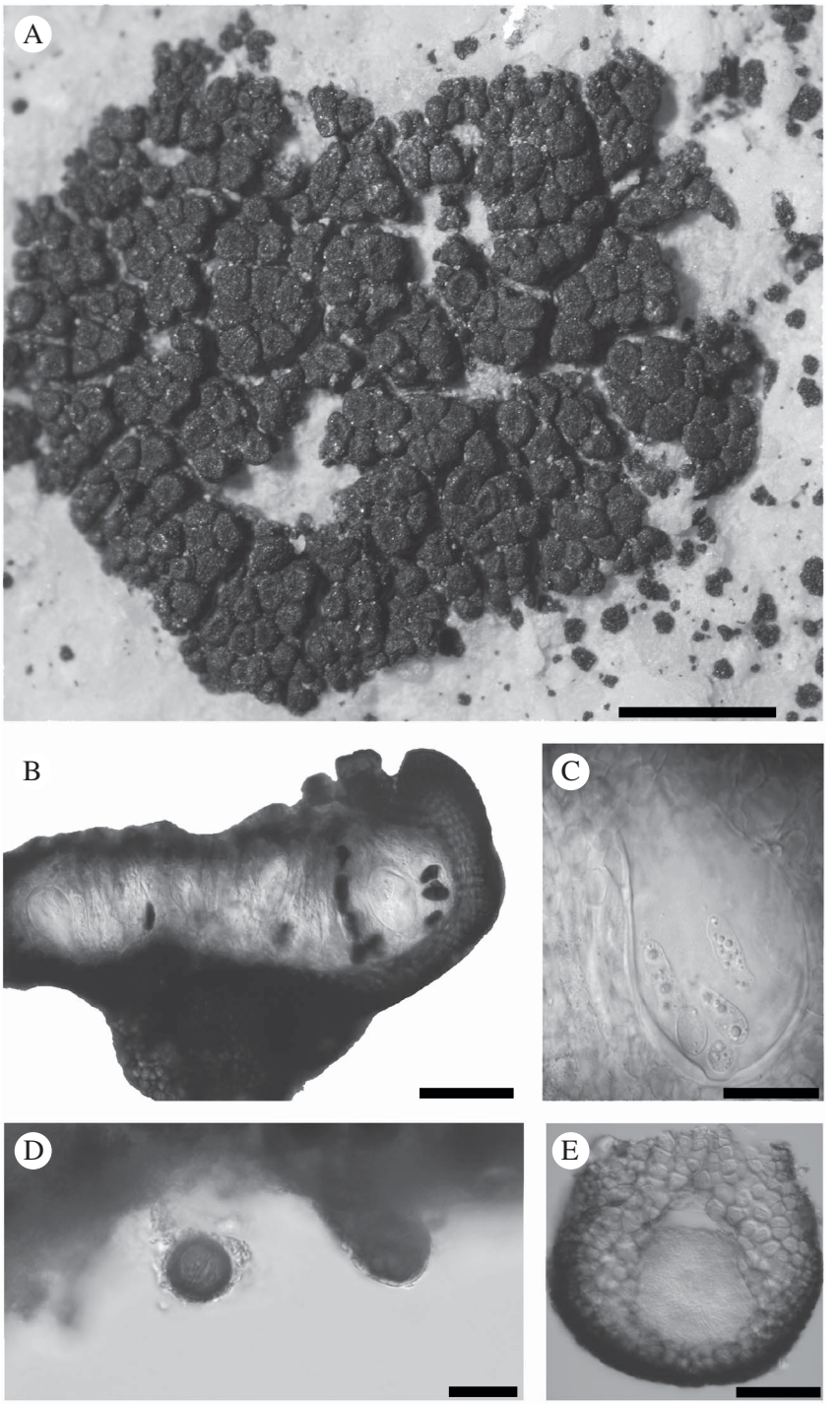

FIG. 1. *Lichenothelia iranica*, holotype. A, thallus; B, section through an ascoma; C, ascus; D, external conidium (macroconidium); E, section through a pycnidium. Scales: A = 0·9 mm; B = 60 µm; C = 20 µm; D = 15 µm; E = 50 µm.

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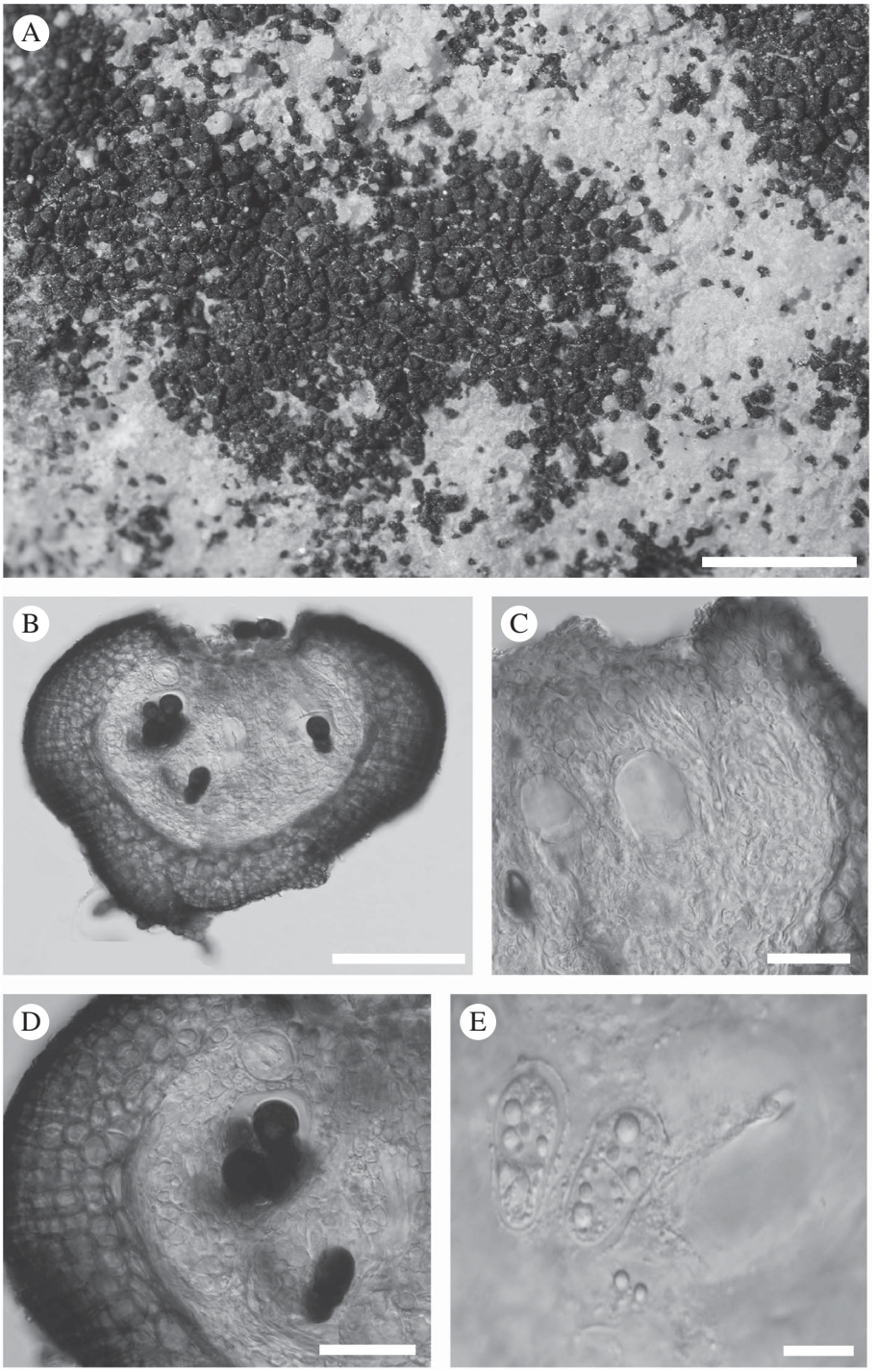

FIG. 2. *Lichenothelia ilamensis*, holotype. A, thallus; B, section through an ascoma; C, asci; D, asci with dark brown ascospores; E, hyaline ascospores with perispores. Scales: A = 4 mm; B & C = 30 µm; D = 35 µm; E = 10 µm.

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L. intermedia, *L. macrocarpa*, and *L. umbrophila* differ in the ornamented ascospores, while *L. arida* differs in the reddish brown colour of the ascospores, the varying number of spores per ascus and the areolate-lobate thallus. All other species on siliceous rocks have either much smaller or much larger ascospores.

Additional specimen examined. **Iran:** *Ilam*: Tonele reno, 33°42'N, 46°24'E, *c.* 2500 m, on calcareous rock, 2004, *T. Valadbeigi* 101000 (hb. Valadbeigi).

Lichenothelia ilamensis Valadbeigi, M. Schultz & Brackel sp. nov.

MycoBank No.: MB 801476

Thallus saxicola, crustaceus, areolatus, nigricans, ascosporae 1-septatae, (22·0–)23·8–26·8(–27·5) × (11·0–)11·7–14·9(–17·0) µm.

Typus: Iran, Ilam, Tonele reno, 33°42'N, 46°24'E, alt. 3038 m, on calcareous rock, 12 July 2009, *T. Valadbeigi* 101022 (HBG —holotypus; TARI, hb. Valadbeigi—isotypi).

(Fig. 2A–E)

Thallus saxicolous, black, areolate, dispersed at margins, confluent and aggregated in the centre, up to 20 mm diam., not or slightly effigurate or rarely lobulate, with stolons, fissured; areoles *c.* 0·12–0·28 mm wide.

Ascomata unilocular stromata, frequent, rounded, shiny black, ostiolate, 0·12–0·28 mm, stipitate; *disc* flat, sometimes concave. Ascomatal wall pseudoparenchymatous, up to 70 µm wide, of 6–10 rows of suborbicular cells, outside dark brown with a granular pigment, inside pale brown to hyaline. Centre with branching interascal filaments, 1·0–2·5 µm wide, not easy to see except in KOH where they swell up to 4 µm. Section I−, K/I− in all parts, partly dextrinoid. *Asci* ellipsoid to pyriform 40–65 × 25–35 µm, (4–)6(–8)-spored. *Ascospores* hyaline at first, soon dark brown, smooth but seemingly ornamented owing to the granular pigmentation, 2-celled, (22·0–)23·8–26·8(–27·5) × (11·0–)11·7–14·9 (–17·0) µm, l/b =(1·6–)1·7–2·1(–2·3) ($n = 20$), hyaline spores with perispore.

Pycnidia not observed.

Chemistry. Not investigated.

Etymology. The species epithet refers to the occurrence in Ilam, western Iran.

Habitat and distribution. Known on limestone in mountainous regions, from one locality only in Western Iran.

Remarks. Lichenothelia ilamensis differs from *L. iranica* in the consistently 1-septate ascospores and in the varying number of spores per ascus. It also has to be compared with the other calcicolous species of the genus and with others growing on unspecified 'rock'. Of these, *L. intermixta*, *L. minor*, and *L. prolifera* have smaller ascospores; *L. metzleri* and *L. schindleri* have narrower ascospores. *Lichenothelia calcarea* and *L. gigantea* differ in the 1-septate to submuriform ascospores, whereas the ascospores in *L. ilamensis* are always 1-septate. *Lichenothelia renobalesiana* differs in the consistently 8-spored asci (Table 2).

Most of the other species of the genus have at least some pluriseptate or submuriform spores. Among the species with 1-septate ascospores, *L. spiralispora*, *L. tenuissima*, *L. radiata* and *L. globulifera* have smaller ascospores, and *L. macrocarpa* has warty ascospores.

The authors are most grateful to Kerry Knudsen (Riverside, California), Lucia Muggia (Graz, Austria), Laura Selbmann (Viterbo, Italy), and Mikhail Zhurbenko (St. Petersburg, Russia) for sending us their papers. Harrie Sipman (Berlin, Germany) supported our work greatly with his hospitality and extensive discussions. The study was financially supported by a grant from Ilam University, awarded to the first author.

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