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The genus *Immersaria* (*Lecideaceae*) in Iran, including *I. iranica* sp. nov.

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Abstract: The species of the genus *Immersaria* in Iran are reviewed. *Immersaria iranica* is described as a new species in the *Lecideaceae*, characterized by pycnoconidia opening by stellate, winding cracks and 2′-*O*-methylsuperphyllinic acid as major secondary metabolite. The characters separating it from other taxa of the *Lecideaceae* are discussed. *Immersaria athroocarpa* and *I. cupreoatra* are new records for Iran. An identification key and a table with diagnostic characters of the species hitherto known from Iran are provided.

Key words: key, lecideoid lichens, lichenized fungi, saxicolous, taxonomy

Introduction

The genus *Immersaria* Rambold & Pietschm. was initially established as a monotypic genus for the lecideoid species *I. athroocarpa* (Ach.) Rambold & Pietschm. This taxon was previously placed in *Lecidea* [as *L. athroocarpa* (Ach.) Ach.], *Amygdalaria* [as *A. athroocarpa* (Ach.) Clauzade & Cl. Roux] and *Porpidia* [as *P. athroocarpa* (Ach.) Hertel & Rambold]. The following combination of morphological characters served to recognize the genus: a brown pigmented, areolate thallus with a distinct epinecral layer and an amyloid medulla, apothecia immersed in the thallus, with a dark, black or grey disc and a strongly reduced proper margin, during ontogeny frequently becoming separated by a fissure from the surrounding areole; *Porpidia*-type asci; and halonate, simple ascospores (Rambold 1989). Subsequently, five additional species were included, either transferred from *Lecidea* or *Bellemerea* (i.e. *I. cupreoatra* (Nyl.) Calatayud & Rambold, *I. carbonoidea* (J. W. Thomson) Esnault & Cl. Roux, and *I. usbekica* (Hertel) Barbero, Nav.-Ros. & Cl. Roux: Barbero *et al.* 1990; Calatayud & Rambold 1998) or hitherto undescribed [*Immersaria mehadiana* Calat. & Rambold and *I. olivacea* Calat. & Rambold (Calatayud & Rambold 1998)]. The inclusion of these taxa entailed only a slight extension of the original genus concept of Rambold & Pietschmann (Rambold 1989): the ascoma discs may be brown, the ascospores are occasionally uniseptate or without a halo, and the pycnoconidia, usually bacillar, can be also pyriform or clavate.

In their phylogenetic studies on selected members of the *Porpidiaceae* and *Lecideaceae*, using the LSU region of the nrDNA and the β-tubulin fragment, Buschbom & Mueller (2004) and Buschbom & Barker (2006) concluded that *Porpidia* is not monophyletic. In their phylogram *Immersaria*, represented by *I. usbekica* (not the generic type) appeared as sister to a clade with taxa currently placed in *Lecidea* and *Cecidonia*, but without support (Buschbom & Mueller 2004). In a separate analysis of LSU and β-tubulin *Immersaria usbekica* seemed more related to *Porpidia* but also without support (Buschbom & Barker 2006). For an estimate of the generic

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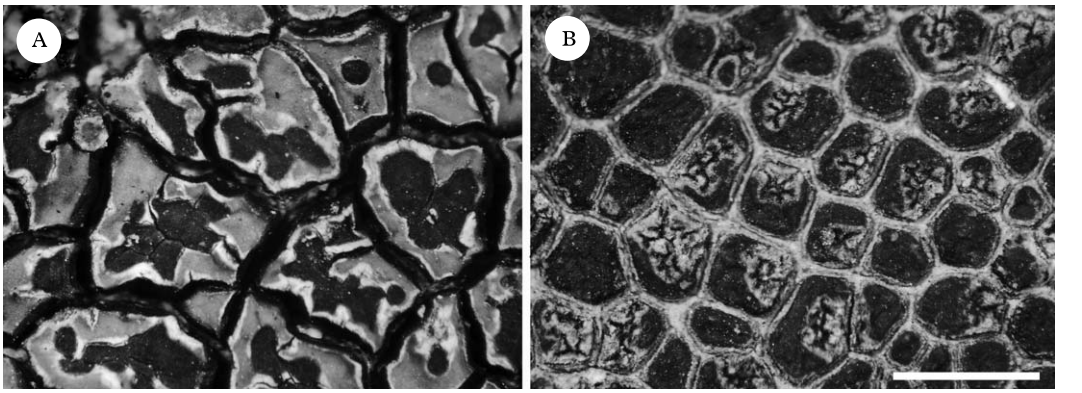

FIG. 1. *Immersaria iranica*. A, thallus areoles with ascomata (*Sipman* 55361); B, thallus areoles with stellate and winding, crack-like openings of pycnidia (*Valadbeigi* 9008). Scale = 0·5 mm.

status and precise phylogenetic position of *Immersaria* further species of the genus need to be included in phylogenetic analyses and additional markers used.

Materials and Methods

Material

The study is based on specimens collected between 2003 and 2008. Most were collected in NW Iran (East Azerbaijan), but single specimens were from Gilan, Kohgiluyeh o Boyer Ahmad, Mazandaran and Zanjan.

Morphological and chemical analyses

Morphology was studied under a stereomicroscope. Anatomy of the thallus and apothecia was studied on hand-cut sections mounted in water using a conventional high power light microscope. Amyloid reactions were tested using Lugol's solution with and without pre-application of KOH solution. TLC was performed according to Orange *et al*. (2001) using Merck silica gel 60 F254 pre-coated glass TLC plates and charring with 10% sulphuric acid for the visualization of the spots. The presence of 2′-*O*-methylsuperphyllinic acid was demonstrated by co-chromatography of a reference sample of *Porpidia rugosa* (Taylor) Coppins & Fryday (Arnold, *Lich. Exs*. 808, B).

The Species

Immersaria iranica Valadbeigi, Sipman & Rambold sp. nov.

Thallus crustaceus, areolatus, fuscus ad rufofuscus, saxicola, prothallo nigro, areolis marginalibus radiantibus. Ascomata immersa, disco fusconigro, epruinoso, hymenio *c*. 160 µm alto, ascosporis subglobosis *c*. (5−)7·5−8–10(−14)×(5−)7·5−8–10(−14) µm. Conidiomata pycnidia, frequentia, apertura rimiformi, sinuosa ad stellata, conidiis clavatis vel raro bacilliformibus, *c*. 4×2 µm. Acidum 2′-*O*-methylsuperphyllinicum continens.

Typus: Iran, Mazandaran, Haraz road, 20 km to Aamol, 36°17′42″N, 52°21′40″E, on calcareous rock, 1475 m, 7 April 2006, *T. Valadbeigi* 9008 (TARI—holotypus; B, hb. Valadbeigi—isotypi).

(Fig. 1A & B)

Thallus crustose, areolate, up to several cm diam.; surface brown to slightly reddish brown, dull, smooth. Areoles flat to slightly convex, sometimes slightly undulate, occasionally with irregular grooves delimiting crystal-like warts, *c*. 0·5–1 mm diam., frequently with rounded, whitish rims, with blackish sides and usually sharp, sometimes rounded edges; marginal areoles larger and slightly radiating, *c*. 1 mm wide and 1·5 mm long. *Hypothallus* black to bluish black or whitish, frequently visible between the areoles. In section, thallus *c*. 0·3–0·5 mm thick; epinecral layer *c*. 15–25 µm high, I+ violet; (pheno-)cortical layer 30–37 µm thick, I+ violet with uppermost cells pale brown, hyaline below; medulla white, without crystals, I+ violet; *photobiont* chlorococcoid, cells globose, 5–13(−17) × 7·5–13 µm; medullary hyphae 2·5–3 µm thick.

Ascomata immersed, mostly one per areole, 0·3–0·5 mm diam., round or lobed

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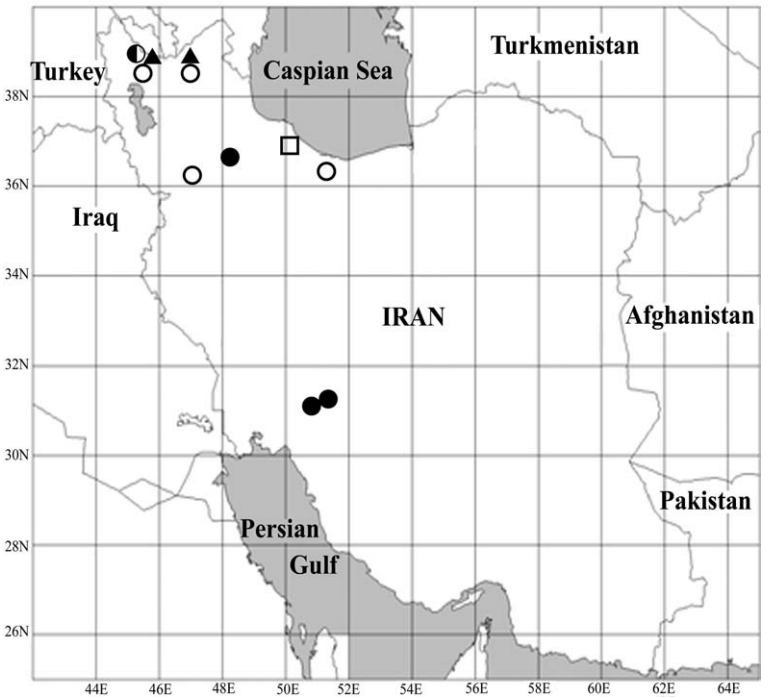

FIG. 2. Map showing the distribution of *Immersaria athroocarpa* (□), *Immersaria cupreoatra* (▲), *Immersaria iranica* (○), and *Immersaria usbekica* (●) in Iran.

(Fig. 1A), sometimes surrounded by a white rim, with crypto-lecanorine margin, occasionally separated from the areole by a fissure. Disc flat to slightly convex, black-brown, epruinose. *Excipulum* thin to absent, with an epinecral layer *c*. 12·5–15 μm high, laterally *c*. 10 μm thick. *Hypothecium* hyaline; photobiont cells present below the hymenium. *Hymenium* hyaline, *c*. 160 μm high. *Paraphyses* apically branched, occasionally anastomosing, *c*. 5 μm thick. *Epihymenium* brown, *c*. 12–25 μm high. *Asci* of *Porpidia*-type, clavate, *c*. 70–125(−130) × 30 μm. *Ascospores* 8 per ascus, (sub)globose, aseptate, halonate, non-amyloid, *c*. (5−)7·5−8–10(−14) × (5−)7·5−8–10(−14) μm.

Conidiomata (*pycnidia*) frequent on thalli bearing only few apothecia, opening by ± stellate, winding cracks up to 0·5 mm long (Fig. 1B); *conidia* clavate to rarely bacilliform, simple, hyaline, *c*. 4 × 2 μm.

Chemistry. 2′-*O*-methylsuperphyllinic acid and traces of an unidentified substance, by TLC.

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

Distribution and habitat. *Immersaria iranica* occurs mainly in the north-west of Iran but extends to Mazandaran. We found it in five localities and thus it is probably the most common species of the genus in Iran. It grows on smooth faces of rock outcrops in sloping *Astragalus* steppe, on horizontal and inclined sides of more or less calcareous silicate rock, and it seems to prefer rather humid sites. The species was found once associated with *I. cupreoatra*.

Remarks. The new species fits perfectly into the current morphological concept of

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TABLE 1. *The major diagnostic characters and distribution data for* Immersaria *species in Iran*

Characters	*I. athroocarpa*	*I. cupreoatra*	*I. iranica*	*I. usbekica*
Thallus [colour]	pale brown	brown	brown to reddish brown	brown
Subhymenial algae [presence]	–	±	±	–
Medulla [iodine reaction]	+ violet	+ violet or −	+ violet	+ violet
Apothecial margin	crypto-lecideine	crypto-lecanorine	crypto-lecanorine	crypto-lecanorine/ lecideine
Pigmentation				
Hypothecium	colourless to brown	colourless	colourless	colourless
Epihymenium	olivaceous brown	brown	brown	olivaceous grey
Ascospore size [µm]	14–17 × 8–11	5–10 × 5–9	5–14 × 5–14	13–22 × 7–14
Pycnoconidia	bacilliform	bacilliform	clavate to rarely bacilliform	bacilliform
Pycnidia opening	simple	simple	± stellate, winding cracks	simple
Chemistry	confluentic acid	gyrophoric acid	2′-*O*-methylsuper-phyllinic acid	confluentic and/or gyrophoric acid
Substratum	calcareous	siliceous	calcareous or siliceous	calcareous
Distribution in Iran	Gilan	East Azerbaijan	East Azerbaijan, Mazandaran, Zanjan	Kohgiluyeh o Boyer Ahmad, East Azerbaijan, Zanjan
Elevation in Iran	700 m	1700–1800 m	1450–2100 m	1700–2800 m

Immersaria. *Immersaria iranica* has a brown thallus with distinct epinecral layer, I+ violet medullary layer of the thallus, ascomata completely immersed in the thallus areoles, a thin to indistinct proper margin and *Porpidia-type* asci with non-amyloid ascospores. Strikingly, the conidia are clavate and pyriform. Such conidia are known within the genus *Immersaria* from *I. olivacea* Calat. & Rambold from Spain (Calatayud & Rambold 1998), but not from any other species currently included in the genera *Porpida* or *Lecidea*. Within the genus, *I. iranica* clearly differs from all hitherto known species by its pycnidia opening by stellate, winding cracks and by the presence of 2′-*O*-methylsuperphyllinic acid (Table 1), both characters hitherto being unique in the genus.

By its Iranian distribution the new species agrees with most *Immersaria* species which were all described from, and are restricted to the circum-Mediterranean and south-west Asian regions (Barbero *et al*. 1990; Calatayud & Rambold 1998). The only exceptions are *I. athroocarpa*, which has a cosmopolitan distribution, and *I. carbonoidea*, which is known only from Alaska.

Specimens examined. **Iran:** *East Azerbaijan*: Shabestar district, W along road Tabriz-Marand, 7 km N of Sufiyan, 38°18′31″N, 45°57′69″E, 1450 m, 2007, *H. Sipman, U. Søchting & M. R. Asef* 55335 (B 60 0175446, IRAN); Jolfa district, 1 km S of Daran village, E of Hadi Shahr, 38°48′42″N, 45°49′09″E, 1700 m, 2007, *H. Sipman, U. Søchting & M. R. Asef* 55361 p.p. (B 60 0175471, IRAN); Arasbaran protected area, top mountain to Mazgar, 38°40′ N, 47°00′ E, 1800 m, 2008, *T. Valadbeigi* 9127 (hb. Valadbeigi). *Mazandaran:* Karaj-chalous road, Zangoole bridge, *c*. 2 km on the way from bridge, 36°21′ N, 51°45′ E, 2000 m, 2003, *T. Valadbeigi* 9046 (TARI, hb. Valadbeigi). *Zanjan*: Zanjan to Dandy, 5 km to Broon Gheshlagh village, near to Ghezel ozan river, 36°36′ N, 48°07′ E, 2096 m, 2006, *T. Valadbeigi* 9107 (TARI, hb. Valadbeigi).

Immersaria athroocarpa (Ach.) Rambold & Pietschm.

in Rambold, *Biblioth. Lichenol.* **34:** 240 (1989).

Lichen athroocarpus Ach., *Lich. suec. prodr.* (Linköping): 77 (1798)—*Lecidea athroocarpa* (Ach.) Ach., *Method. Lich.*: 41 (1803)—*Porpidia athroocarpa* (Ach.) Hertel & Rambold, in Hertel, *Lecideaceae Exs.*, Fasc. VIII, nos 141–160: 8 (1985)—*Amygdalaria athroocarpa* (Ach.) Clauzade & Cl. Roux, *Bull. Soc. bot. Centre-Ouest*, Nouv. sér., num. spec. **7:** 157 (1985); type: Sweden, [no date, locality or collector] (H-Ach—lectotype – Hertel 1977).

For a description see Fletcher *et al.* (2009).

Distribution. Cosmopolitan. In Iran it is known from a single collection only from Gilan where it occurred at low altitude on a calciferous outcrop in the Hyrcanian forest zone.

Specimen examined. **Iran:** *Gilan*: on the road to Javaherdasht, 37°00′ N, 50°18′ E, 700 m, 2003, *T. Valadbeig* 9022 (TARI, hb. Valadbeigi).

I. cupreoatra (Nyl.) Calatayud & Rambold

Lichenologist **30:** 232 (1998).

Lecanora cupreoatra Nyl., *Not. Sällsk. Fauna et Fl. Fenn. Förh.* **8** (*Lichenes Lapponiae orientalis*): 181 (1866)—*Aspicilia cupreoatra* (Nyl.) Arn., *Flora* **53:** 470 (1870).

For a description see Clauzade & Roux (1985).

Distribution. Mediterranean (Barbero *et al.* 1990). In Iran known so far from two sites only in the north-west.

Specimens examined. **Iran:** *East Azerbaijan*: Jolfa district, 1 km S of Daran village, E of Hadi Shahr, 38°48′42″N, 45°49′09″E, 1700 m, 2007, *H. Sipman, U. Søchting & M. R. Asef* 55361 [B 60 0175471, IRAN]; Arasbaran protected area, top mountain to Mazgar, 38°40′ N, 47°00′ E, 1800 m, 2008, *T. Valadbeigi* 9988 (hb. Valadbeigi).

I. usbekica (Hertel) M. Barbero, Nav.-Ros. & Cl. Roux

Bull. Soc. Linn. de Provence **41:** 140 (1990).

Lecidea usbekica Hertel, *Khumbu Himal* **6**(3)**:** 288 (1977)—for further synonyms see Barbero *et al.* (1990).

For a description see Esnault & Roux (1987, sub *Amygdalaria tellensis*).

Distribution. Known from the western Mediterranean (Spain, Algeria) to Central Asia (Barbero *et al.* 1990). Hertel (2001) reported it for the first time from Iran. Our specimens are from the north-west and west of Iran (East Azerbaijan, Kohgiluyeh o Boyer Ahmad and Zanjan), while Hertel (2001) reported it from Markazi province. The species probably has the widest ecological amplitude of any of the four Iranian species and may grow under the driest conditions. It was found in *Quercus* woodland (20 km from Semirom to Yassuj at 1750 m alt.) and in hilly steppe at higher elevations of 2470 m in Kohgiluyeh o Boyer Ahmad and 2800 m in Zanjan.

Specimens examined. **Iran:** *Kohgiluyeh o Boyer Ahmad*: *c.* 20 km from Semirom to Yassuj, 31°17′ N, 51°26′ E, 2470 m, 2003, *A. A. Maassoumi & S. R. Safavi* 2657 [B 60 0133417, TARI]; *c.* 71 km from Semirom to Yassuj, 31°09′ N, 51°14′ E, 1750 m, 2003, *A. A. Maassoumi & S. R. Safavi* 2670 (B 60 0133421, TARI). *East Azerbaijan*: Jolfa district, 1 km S of Daran village, E of Hadi Shahr, 38°48′42″N, 45°49′09″E, 1700 m, 2007, *H. Sipman, U. Søchting & M. R. Asef* 55363 (B 60 0175473, IRAN), 55368 (B 60 0175477, IRAN). *Zanjan*: Zanjan to Dandy, 5 km to Broon Gheshlagh village, near to Ghezel Ozan River, 36°36′ N, 48°07′ E, 2800 m, 2006, *T. Valadbeigi* 4067 (TARI, hb. Valadbeigi).

Key to the species of *Immersaria* in Iran

1 Apothecial disc black, more or less white-pruinose; epihymenium olivaceous grey or olivaceous brown; apothecial margin cryptolecanorine to lecideine; subhymenial algae absent; confluentic and/or gyrophoric acid present 2
Apothecial disc dark brown to black, epruinose; epihymenium brown; apothecial margin cryptolecanorine; subhymenial algae usually present; gyrophoric acid or 2′-*O*- methyl superphyllinic acid present 3

2(1) Thallus surface brown to yellowish brown, C− (confluentic acid); apothecial margin cryptolecideine, separated from thallus; hypothecium brown in lower part; ascospores 14–17 × 8–11 μm . . . . . **I. athroocarpa**
Thallus surface olivaceous brown, C+ rose (gyrophoric and confluentic acids); apothecial margin cryptolecanorine, adnate to thallus; hypothecium colourless; ascospores 13–22 × 7–14 μm . . . . . **I. usbekica**

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3(1) Thallus brown, C+ rose (gyrophoric acid); conidiomata opening by simple, elongate pores; conidia bacilli form. . . . . **I. cupreoatra**
Thallus brown to slightly reddish brown, C− (2′-O-methylsuperphyllinic acid); conidiomata opening by ± stellate, winding cracks; conidia mostly clavate to rarely bacilli form. . . . . **I. iranica**

The major diagnostic characters and distribution of *Immersia* species in Iran is provided in Table 1.

Discussion

The detailed new information about the genus *Immersaria* in Iran presents an opportunity to re-evaluate the distribution patterns of the genus. The presence of four out of the seven known species of *Immersaria* in Iran implies a south-west Asian focus of distribution for the genus. The next diverse countries with three species each are Spain, with *I. athroocarpa* (Llimona & Hladun 2001), *I. olivacea* Calatayud & Rambold (Calatayud & Rambold 1998) and *I. usbekica* (Barbero *et al.* 1990), Italy with *I. athroocarpa*, *I. cupreoatra* and *I. usbekica* (ITALIC, http://dbiodbs.univ.trieste.it/global/chkpwforkeys) and Romania with *I. athroocarpa*, *I. cupreoatra* and *I. mehadiana* (Ciurchea in http://www.bgbm.org/BGBM/STAFF/Wiss/Sipman/Zschackia/Rumania/index.htm; Calatayud & Rambold 1998).

Within the genus two species groups are recognizable. One with black or white-pruinose ascocarp discs, with a wide or global distribution, including the species *I. athroocarpa*, *I. carbonoidea*, *I. olivacea* and *I. usbekica*; and a second group with dark brown, epruinose ascocarp discs, extending from SE Europe to Central Asia, including the species *I. cupreoatra*, *I. iranica* and *I. mehadiana*. This last group includes species with a more complex chemistry, such as *I. mehadiana* (Calatayud & Rambold 1998) and *L. cupreoatra* (unpublished data from Greece, in B). Further investigation of their chemistries and genotypic traits may lead to the recognition of additional species.

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