A PRELIMINARY STUDY OF RHIZOCARPON MACROSPORUM IN RAZAVI KHORASAN PROVINCE (NE IRAN)
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The present paper is a part of a more extensive investigation of Rhizocarpon based on collections made since 2007 in Razavi Khorasan province in northeastern Iran. Here, we provide details of the morphology, anatomy and lichen substances R. macrosporum. A distribution map is also presented.

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Introduction
Understanding the diversity and taxonomy of Iran's lichens has never been more important than it is today, and this is undoubtedly the most exciting subject to be studied in the cryptogamic flora of the country. The early history of lichenological investigation in the northeast of Iran was documented by Szatala (1940, 1957). Checklists of lichens for Iran by Seaward et al. (2004, 2008) provided an up-to-date perspective on lichen biodiversity in several provinces.

The cosmopolitan genus Rhizocarpon includes ca. 200 species (Feuerer 1978) occurring on siliceous and calcareous rocks. Rhizocarpon species were traditionally divided into two groups differing in thallus color. The yellow-green species, containing rhizocarpic acid, were assigned to subgenus Rhizocarpon, and the non-yellow-green (white, gray, orange and brown) species to subgenus Phaeothallus (Thomson 1967). It is now known that these groups are polyphyletic (Ihlen & Ekman 2002). The yellow-green Rhizocarpon species have been monographed by Runemark (1956) for European and occur mainly in arctic-alpine areas (Magnusson 1927; Lynghe 1928, 1932, 1935).

Rhizocarpon was first recorded in Iran by Müller (1892). By 2008, four species had been reported for Razavi Khorasan province (Seaward et al. 2008), but the list has been recently revised (Moniri et al. 2009). According to the last reference, there are 10 species of the genus in Iran, including six species and one subspecies in Razavi Khorasan. One of these new records is R. macrosporum Räsänen, a yellow-green species very similar to R. geographicum (L.) DC. but recognizable by its larger ascospores.

Material and Methods
Razavi Khorasan province, with an area of c. 127,432 km², is located in the northeast of Iran. Thirty mountainous localities in the province were investigated; these range from ca. 900 to 2300 m. Three thalli of R. macrosporum were found on Binaloud Mt., Zoshk, 27 km west of Mashad, at 1750 m elevation (Fig. 1). The samples were examined with...
light microscopy, standard chemical spot-test reagents and TLC (Orange et al., 2001). TLC was done on one sample by M. Kukwa (Gdansk, Poland), and another by S. R. Clayden (New Brunswick Museum, Canada). The original material is deposited in the lichen collection of M. Haji Moniri, with duplicates at NBM and the herbarium of M. Kukwa. The most important references for identification were Runemark (1956) and Fletcher et al. (2009).

Results and Discussion

Rhizocarpon macrosporum Räsänen

Description. Thallus to 5 cm in diam., of closely spaced areoles separated by thin lines of prothallus; black prothallus distinct at the thallus margin (Figs. 2a-b; 6a). Areoles 0.5-1.4 mm in diam., light yellow to yellow, plane to slightly convex, surface pruinose, matt (Figs. 3a-b), with limited secondary fissuring of areoles; secondary areoles angular, cortex ca. 25-30 µm thick, medulla white, 70-90 µm thick, I + violet; distinct hypothallus 80-150 µm thick (Fig. 4); sorediate absent. Apothecia 0.3-0.7 mm in diam., black, thick, angular to roundish, usually plane or slightly convex, more or less pruinose, not surrounded by crescent-shaped areoles, with 50-80 µm thick proper excipulum. Subhymenium usually 30-200 µm, brown. Hymenium 150-220 µm tall, colourless to light brown. Epiphyllum 30-100 µm thick, reddish brown, K+ more reddish; without black granules. Spores 8/ascus, muriform, long ellipsoid, dark olivaceous to brown, 32-60 x 15-22 µm (Figs. 5, 6b-d).

Lichen substances. Rhizocarpic acid and an bourgeanic acid, identified by TLC. Rhizocarpon macrosporum is part of the “Geographicum group” of species (Runemark 1956a). These have multiseptate, mostly muriform, ascospores, an ephymenium lacking dark granules, and a medulla reacting positively with Lugol’s iodine (I+ blue). Within this group, R. macrosporum is distinguished mainly by its large ascospores, each with an average of about 30 cells visible in optical section (Feuerer 1978). Rhizocarpon sphaerosporum Räsänen reportedly differs in lacking reddish-brown, K+ more reddish, pigmentation in the ephymenium, in its more rounded areolae, and in having a more diverse medullary chemistry (Runemark 1956a). However, Timdal and Holtan-Hartwig (1988) found that these two species could not be consistently separated, and they synonymized R. sphaerosporum with R. macrosporum. Runemark (1956a) inferred that specimens of R. macrosporum reacting negatively with paraphenylenediamine (PD) lacked any characteristic secondary metabolites other than the cortical pigment rhizocarpic acid. However, with refined TLC methods, many PD– specimens of R. macrosporum proved to contain the aliphatic depside bourgeanic acid as the main medullary compound (Clayden, 2004). The Iranian collections reported here belong to this bourgeanic acid chemotype. If the synonymy of R. sphaerosporum and R. macrosporum is accepted, then R. macrosporum includes three additional chemotypes: with psoromic acid, stictic acid, or barbatic acid (Runemark, 1956a; Timdal and Holtan-Hartwig, 1988). Rhizocarpon macrosporum has a broad, but discontinuous, range in temperate areas of North America and western Eurasia. It is also known from Morocco in North Africa (Runemark 1956b). It occurs as far north as southern Scandinavia, but is apparently absent from areas with oceanic climates, including the British Isles. Records from Greenland and Arctic North America (Thomson 1997) are in need of critical
Fig. 2. (a). Secondary areoles and (b) thallus in *Rhizocarpon macrosporum* (Kamyabi, 2009).

Fig 3. (a) Primary areole on the prothallus and (b) Thick prothallus in the margin of *Rhizocarpon macrosporum* (Kamyabi, 2009).

Fig 4. Longitudinal section of an areole of *Rhizocarpon macrosporum*, Co: cortex, Gl: algal layer, Me: medulla with lemon yellow crystal of (probably) rhizocarpic acid (Kamyabi, 2009).
Fig. 5. (a): Longitudinal section of apothecium, Ep: epihymenium, Ex: exciple, Hy: hymenium, Sh: subhymenium and (b) Muriform ascospores in *Rhizocarpon macrosporum* (Kamyabi, 2009).

Fig. 6. Schematic pictures of thallus and reproductive organ in *Rhizocarpon macrosporum*: (a). Thallus, (b). Longitudinal section of apothecium, (c). Ascus containing 8 muriforme ascospores and (d). Different shapes of ascospores (Kamyabi, 2009).
reevaluation. In Eurasia, the easternmost documented occurrences of *R. macrosporum* are in Afghanistan (Steiner & Mayrhofer 1987, sub *R. sphaerosporum*) and Tajikistan (Kudratov & Mayrhofer 2002). The collections from Iran reported here fill in a gap in the known range between these two countries to the east, and Turkey and Georgia to the west. All collections from this general region were made in montane areas, with elevations above 1600 m. *Collection examined.* Binaloud Mt., Zoshk, 27 km west of Mashad, UTM 40S, 696399 4022685, 1750 m elev., 6. Sept. 2007, Saleh Kamyabi 2425b (NBM, hb. Haji Moniri, hb. Kukwa).

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