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NEW RECORD OF RICCIA PSEUDO-FROSTII (RICCIACEAE) FOR THE BRYOFLORA OF IRAN

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Morphological and anatomical studies indicate interspecific differences with the existing samples which demonstrate a new record in Iran. *Riccia pseudo-frostii* was seen on the western parts of Mazandaran in the North of Iran. This species was distributed often on the wet soils understory of gardens containing high calcium and magnesium which formed community with *Marchantia polymorpha* and *Funaria* sp. Thallus is averagely dichotomously two or three times divided and has apical notch with rosette and regular discoidal shape. The young thallus base is tinged purple and is monoecious with less antheridium frequency in comparison with archegonium and is hairless. In many of samples there are not scales on the ventral surface. Tetragonal spores with average diameter 70 µm are regularly reticulated surface and the length of wings reach to 4 µm. The average diameter of pores, lumens and odontos were determined 5 µm, 5–7 µm, and 2 µm, respectively. There are two types of smooth and tuberculate rhizoids in the species.

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Key words: Bryoflora; tetragonal spores, tuberculate rhizoid; north of Iran

گزارش جدید Riccia pseudo- frostii، تیره Ricciaceae برای فلور خزه گیان ایران

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بررسی های ریخت شناختی و تشریحی حاکی از تفاوت های بین گونه ای با نمونه های موجود و اثبات رکورد جدید در ایران است. گونه Riccia pseudo- frostii در بخش غربی استان مازندران در شمال ایران مشاهده شد. این گونه بر روی خاک های مرطوب باغ‌ها با کلسیم و منیزیوم زیاد دیده شد و با گونه هایFunaria و Marchantia تشکیل جوامعی را داده اند. تال به طور متوسط دو تا سه بار دیکوتومی شده، در انتها شکاف دار با شکل کلی رزت و دیسکوئیدی منظم است. قاعده تال جوان، ارغوانی کم رنگ، تک پایه و با فراوانی کمتر آنتریدی نسبت به آرکگن و فاقد مو است. سطح شکمی اکثرا فاقد فلس است. اسپورها چهار وجهی با میانگین قطر ۷۰µm با آراستار مشبک منظم هستند و بال به طول ۴µm می‌رسد. میانگین قطر منفذها ۵µm، حفره ها ۵–۷µm و دندانه ها ۲µm تعیین شد. ریزویید بر دو نوع صاف و زگیل دار است.

INTRODUCTION

Akhani & Kürschner (2004) published an annotated and updated checklist of the Iranian bryoflora, but this species was not reported in the list. Bryophytes belong to a highly specialized group of plants, neglected by most botanists due to their small size. They can survive under a wide range of environmental conditions, and often form a striking part of the vegetation in forests, wetlands, terrestrial and on rocks habitats (Kürschner 2004). *Riccia* plays an important role in the stabilisation of soils, as a component of the fragile, biological soil crusts of arid and semi-arid zones (Beckmann 2007). Bryophytes can be pioneering colonizers on highly stressed substrates in modern hot-spring analogues (e.g. New Zealand Burns 1997).

Pande & Udar in 1958 have given key to identification of *Riccia huebeneriana*: Thallus with loosely arranged assimilatory zone and wider air space. Subgenus *Riccella.* Plants monoecious. Talli narrow repeatedly branched. Spore small, up to 60 µ.

Systematic position of *Riccia* (Kour 2006, Bhandary 2011):

Division: Bryophyta

Subdivision: Hepaticophytina (Hepaticae)
Class: Marchantiopsida
Subclass: Marchantiidae
Order: Ricciales
Family: Ricciaceae
Genus: *Riccia*
Species: *pseudo- frostii*
Study area: *Riccia pseudo-frostii* in this report was accidentally found in Tonekabon in west of Mazandaran (N Iran), with longitude of 51°, 36´ and latitude of 36°, 43´ and 2 m a.s,

MATERIALS AND METHODS

Covering surface and density of *Riccia pseudo-frostii* were determined. *Riccia pseudo-frostii* samples were morphologically and anatomically studied. In the first method, the samples were fixed in 10% formalin for 48 hours, and then anatomical studies were carried out and imaged by inverted optical microscope. In another method, the samples were fixed in Hoyer's Medium (Anderson 1954). Studies were performed by the optical microscope and stereo microscope equipped with imaging analysis software and then the photography was done. Electron micrographs of spores were prepared by SEM. To determine texture, elements and other properties of the soil, the soil samples were taken at a depth of 5 cm and analyzed**.**

Determining the suitable size for quadrates

For determining the suitable size for quadrates, methodology of Cain & Castro (1959) was applied. The quadrates area was doubled each time in a sense that no new species can be added or no considerable change in species increase is made. Therefore, the dimensions were started from $1\times1 cm^2$. Ultimately, the most suitable quadrate were selected 8×8 cm^2 with the area of 64 cm^2. Increase in quadrates surface was helical in this method. The produced quadrate was called "minimal area".

RESULTS

Quadrate establishment in the region

Surface of species %= number of species samples × mean surface of each species sample × surface of quadrate

Density of species %= number of species samples × number of samples of all species

Therefore, *Riccia pseudo-frostii* with the mean coverage of 42.66% and density of 60% along with *Funaria* sp. and *Marchantia polymorpha* formed communities (fig. 1. B).

Morphological and anatomical studies

Riccia pseudo-frostii often grows in *Citru*s gardens. Every thallus is 2–5 mm tall. The plant form is regular discoidal rosette. Each rosette has 0.5–2cm length, which consist of twice to three times from dichotomously branches (fig. 1. A). Base of the young thallus in rosette center was tinged purple. These parts will turn to yellow after maturity and will finally become sponged (fig. 1. C). In *Riccia pseudo-frostii*, the midrib of thallus (groove) is not complete on the dorsal surface. The groove is averagely formed at the apical one-third of thallus and is ended to an apical notch. Rhizoid is against the groove on the ventral surface.

In these observations, *Riccia pseudo-frostii* was determined monoecious, homothallic and bisexual with less antheridium frequency than archegonium. Thallus margin is swollen and epidermal cells are transparent without chloroplast (fig. 2). Epidermal cells are tetragonal, pentagonal, and hexagonal and have scattered pores reaching to the air chamber. The air pores are seen more transparent as bright spots under the stereomicroscope. The pores are slightly stretched and the surrounding cells are smaller than other cells (fig. 3).

There is a photosynthetic layer on gametophyte dorsal surface. The cells of this layer contain a mass of discoidal chloroplasts. The prominent capsules that are actually stalkless sporophytes are seen under epidermal cells. They are formed one, two, or more in a thallus and are extended from the groove in the middle of thallus (midrib) to the base. Spores are only released by death of thallus that is accompanied by dryness of the environment. In the dry sites, thallus misses its main water and seems flat and narrow and older parts have a perforated surface and becomes spongy near the center of a rosette. Spores remain in calyptra bag, are protected among gametophyte, and are released by death of sponged parts of thallus (fig. 1. C and 2).

There were smooth and tuberculate rhizoids (with folded wall), usually branchless and sometimes branched on the gametophyte ventral surface. Tuberculate rhizoids were more frequent.

Spores are black, winged, tetrahedral or pyramidal and sometimes triangular-spherical and tetraspore. The average diameter of spores is 70 µm and their wing diameter reaches to 4 µm. The mean diameter of pores, lumens, and odontos were determined 5 µm, 5-7 µm, and 2 µm, respectively. They have regular reticulations in exospore (fig. 4 A-E).

Fig. 1. A, *Riccia pseudo-frostii* as dichotomique and discoidal rosette (×40); B, association, (1) *Riccia pseudo-frostii*, (2) *Funaria sp.*, (3) *Marchantia polymorpha* (×20). C: decapsulated old basal parts of thallus (red arrow) (×60).

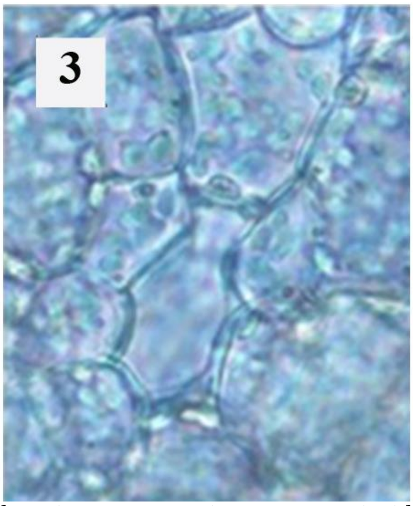

Fig. 2. Optical micrograph with stereomicroscope. Two complete, intact sporangium are seen inside calyptra bag under epidermis. Capsules are prominent underneath the older parts of thalli (× 200).

Fig. 3. Optical micrograph with invert microscope. Photosynthetic layer cells are on gamethophytic dorsal surface accompanied with discoidal chloroplasts. A pore is seen in the center. The surrounding cells are arranged differently from other cells (×400).

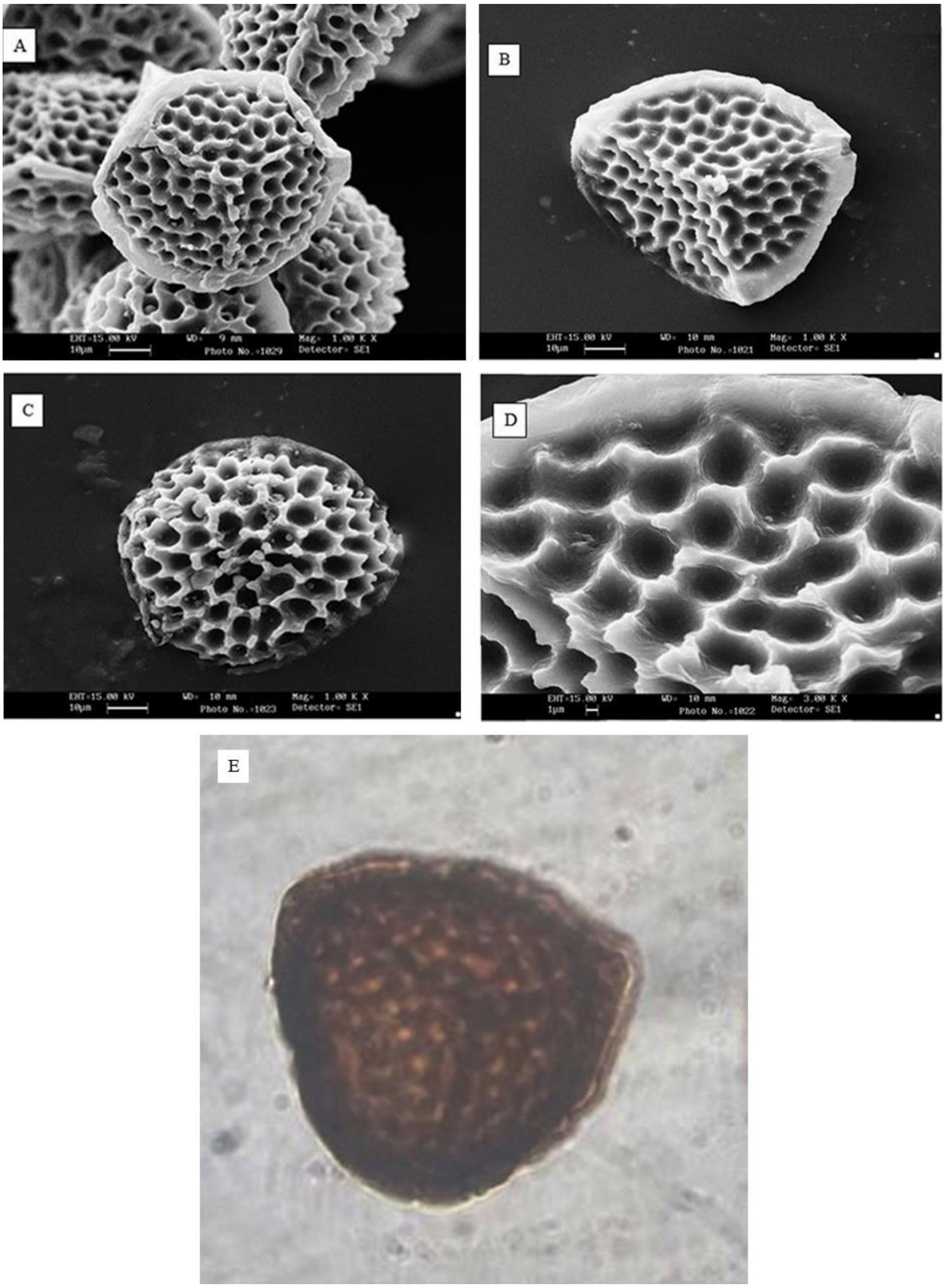

Fig. 4. A-D: SEM electron micrographs of *Riccia pseudo- frostii* spore. A-B: Proximal surface, trilete; Exospores ornamentation, reticulate and regular; C, distal surface, convex; D, Lumen; E, light micrograph. proximal surface of *Riccia pseudo-frostii* spore; tetrahedral, triangular-spherical (×400).

Soil tests

The soil results are shown in tables 1-2-3.

Comparison of Methods in this research

Observation of spores by optical microscope through fixation by Hoyer's Medium was more transparent; however, observation of rhizoid and somatic cells by fixation in formaldehyde and invert microscope was even more transparent.

DISCUSSION

According to Ros & *al*. (2007) report, *Riccia huebeneriana* Lindenb. is the synonym of *Riccia pseudo-frostii* (Schiffn.) Müll. Frib.

The scientific name of the subject plant was recognized *Riccia pseudo- frostii* as named in Flora of North Africa (Ros *et al*. 1999), Hungary (Erzberger and Papp 2004), Mediterranean region (Ros & al. 2007), Britain (Watson 2013) and some other countries. This plant is also known as *Riccia hoebeneriana* in India (Pande and Udar 1958; Manju & al. 2008), China (Yu & al. 2002),West Bangal (Singh & al. 2010), Hungary (Erzberger and Papp 2004), Russia (Dulin & al. 2009), UK (Riley & al. 2003).

In this research *R. pseudo-frostii* was found in Tonekabon Iran and its density and vegetative coverage was determined. This species with density of 60% and vegetative coverge 42.66% formed communities with *Funaria* sp. and *Marchantia polymorpha*.

The edaphic analysis in this research showed the average soil pH was (7.57). Felle (1988) reported that *Riccia fluitans* cytoplasmic pH may be alkaline or acidic depending on its metabolic activity type. As suggested by Dia (1992), the soil pH ranges from 4.5 to 7.5 for *Riccia bicarinata* and from 6.5 to 7.8 for *Riccia covernosa*. Therefore, it seems that genus *Riccia* can grow in the soils ranging from acidity to alkalinity. Also, the percentage of the organic matters were remarkable and macroelements (Ca, Na, Cl, Mg, etc) were seen at the high extent. Microelements (Fe, Cu, Mn, etc) except Zn had high density (tables 1-2-3).

R. pseudo-frostii is highly close to *R. frostii* in terms of thallus appearance and status. However, *R. frostii* is dioecious, heterothallic and unisextual and is dichotomized only once i.e. thallus is only placed on a circle; while *R. pseudo-frostii* is monoecious, homothallic and bisexual and is dichotomized twice to three times i.e. thalli may be formed several times around a center. Diameter of spores was determined averagely 70 µm in *R. pseudo-frostii* and about 40 µm in *R. frostii*. So the sample was identified *R. pseudo-frostii*, as the pseudosample of *R. frostii.*

Table 1- Percentage of organic matters, minerals, saturation moisture, calcium corroborate, pH, electrical conductivity (EC), and type of soil texture.

Composite sample	Saturation %age	EC (dS/m)	pH	Equivalent Calcium carbonate (%TNV)	Organic matter (%OM)	Total Nitrogen (%N)	Available k (ppm)	Available p (ppm)	Particle size analysis			Texture
									Sand (%)	Silt (%)	Clay (%)	
Soil	49.71	2.17	7.57	12.92	3.66	0.37	144.65	56.14	50	35	15	L
Optimal range	>40	<1.5	6.5-8.2	0	>0.2	>0.3	200.220	15.20	-	-	-	-

Table 2- Density of some macroelements of soil solution (mg/l).

Ca (me/l)	Ca (mg/l)	Mg (me/l)	Mg (mg/l)	Na (me/l)	Na (mg/l)	Cl (me/l)	Cl (mg/l)
20.36	407.2	1.36	16.32	2.36	54.28	4.96	176.08

Table 3- The mass of oligoelements in one kilogram of the area soil and their optimal ranges (mg/kg).

Composite sample	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
Soil	30	12.4	1.6	1.82
Optimal range	6.0-6.5	3-3.5	0.8-1.2	2.0-2.5

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