

RANUNCULUS POLYRHIZOS AS A NEW RECORD FOR IRAN, WITH ECOLOGICAL AND MICROMORPHOLOGICAL EVIDENCE

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Abstract. *Ranunculus polyrhizos* is reported as a new noteworthy record for the flora of Iran. This species was collected from alpine dry gravelly slope in Talesh Mountains. Taxonomic remarks and notes geographical distribution and habitat for this species are provided. Moreover nectar scale, pollen and achene micromorphological characters of the species are added and compared with related species.

Key words: *Ranunculus polyrhizos*, Talesh Mountains, new record, flora, autoecology

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Introduction

Genus *Ranunculus* L. (Ranunculaceae Juss.) with about 60 taxa is one of the largest genera in the flora of Iran (IRANSHAHR *et al.* 1992; NAQINEZHAD *et al.* 2016). This genus is distributed in different habitats including forests, dry and wet meadows, flood plains, lakes, rivers, and in alpine regions (JOHANSSON 1998). Recently, studies have been carried out in taxonomy (PAKRAVAN 2010, 2012), palynology (PAKRAVAN *et al.* 2010, 2014), phylogeny (RASTIPISHE *et al.* 2011) and morphology of nectaries (EMADY *et al.* 2010; NEMATI *et al.* 2009) of some Iranian *Ranunculus* species.

Pollen morphology of Ranunculaceae was investigated by various authors (WODEHOUSE 1936; KUMAZAWA 1936; SANTISUK 1979; HAMILTON 1976; PETROV *et al.* 1981). CLARKE *et al.* (1991) described three different pollen types of *Ranunculus* viz *R. acris*, *R. arvensis* and *R. parviflorus* types. In particular, *R. acris* type he divided onto nine groups which are

commonly characterized by their tricolpate (rarely pentacolpate) apertures.

BABINGTON (1856) described different shape of the nectaries of *Ranunculus*. Cook (1966) introduced three main nectary types: lunate, circular and pyriform. Subsequently, DAHLGREN (1992) described eight types of nectaries for subgen. *Batrachium*.

The main classifications (DE CANDOLLE 1818; TAMURA 1995; DAVIS & COOK 1965; IRANSHAHR *et al.* 1992) showed that characters of achenes are suitable in separation of taxa in *Ranunculus*. *Ranunculus* achenes are usually with a persistent glabrous beak, without distinct longitudinal wrinkles, rarely faintly wrinkled on lateral faces, pericarp with sclerenchymatous layer, have important role in infrageneric classification.

The aim of the current paper is to introduce *Ranunculus polyrhizos* as a new record from Iran. Additionally, some ecological and micromorphological characters of the species are represented.

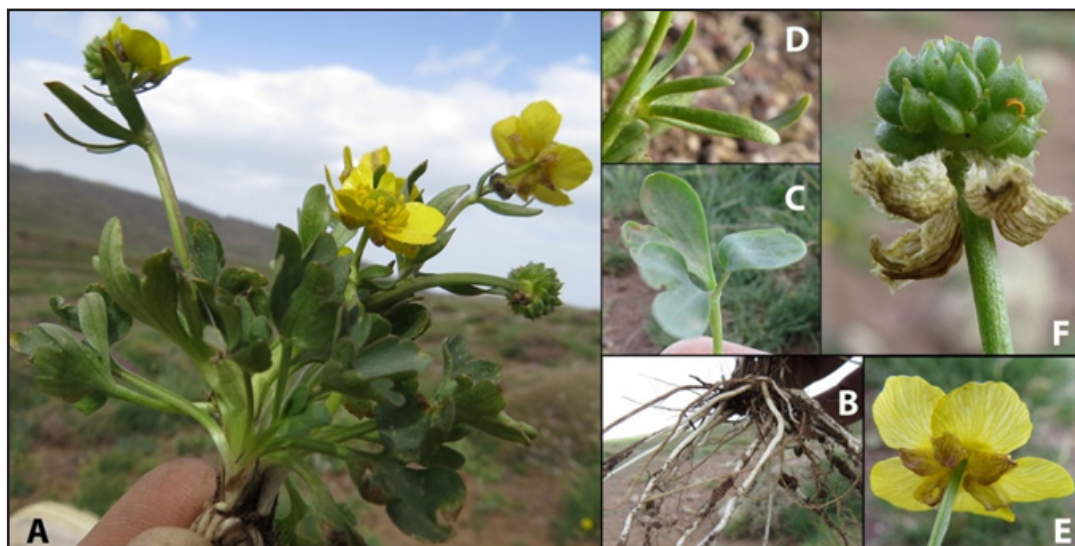


Fig. 1. *Ranunculus polyrhizos*: **A** – habit; **B** – root; **C** – basal leaf lamina; **D** – stem leaf lamina; **E** – flower (sepals and petals); **F** – fruit.

Material and methods

During the field work in Talesh mountains some interesting *Ranunculus* specimens were collected. Later these specimens were crosschecked with Floras (BOISSIER 1867; OVCHINNIKOV 1937; DAVIS & COOK 1965; IRANSHAHR *et al.* 1992; WANG & GILBERT 2001) and then, on the base of inspection of herbarium collections at T, FAR, TARI and W, the specimens have been identified as *R. polyrhizos* Stephan ex Willd. Recorded material was deposited at FAR, T and W herbaria.

Some morphological characters were measured in the field on living plants, while others were analyzed on herbarium specimens by using stereomicroscope Zeiss Stemi SV 6. Micromorphological analyses were carried out on scanning electron microscope KYKY-EM 3200. Soil samples were taken from the center of the population to 30 cm depth. Measured soil variables include physical and chemical properties. Soil texture was determined by the hydrometric method (BOUYOUCUS 1951). Soil pH and soil electrical conductivity (EC) were determined by pH-meter with glass electrode and EC-meter respectively. Organic matter (OM)

was estimated by Walkley and Black method (NELSON & SOMMERS 1996).

Results and discussion

Ranunculus polyrhizos Stephan ex Willd., Sp. Pl., ed. 4 [von Willdenow.] **2** (2): 1324, 1799; Ovchinnikov, Flora of USSR 7: 301, 1937; Davis, Flora of Turkey **1**: 170, 1965; Wang, Flora of China **6**: 282, 2001 (Fig. 1).

Specimens examined. IRAN: Ardabil province, 43 km on the road of Ardabil to Khalkhal, Neor, Lissar protected area, Bacrodagh mountain, 2800-2900 m a.s.l., 37° 58' N, 48° 36' E, 03 May 2014, *Bidarlord 15887* (FAR, T, W).

Additional specimens examined. CHINA: Manchuria, Tigrowe Prope Schi-touhodse. 5.6.1928, N. Kozlow, *W 12438*, 1940. **RUSSIA:** *W 9910*, 1964; *W 21992*, 1974; *W 12892*, 1992; *W 09052*, 1991. **TURKEY:** B8 Erzurum, Palandoken Dag Gebirgs steppe, 2900 m, 1978, *W 12892*.

Morphological remarks. Perennial glabrous plants, 5-17 cm high. Roots fasciculate, slender. Collar fibrous. Stems 1-3, usually ascending or erect, distally branched, mostly 2-5-flowered. Basal leaves petiolate, petioles 1.5-5 cm, glabrous; blade 0.7-1.8×1-3.2 cm, more or less reniform or rounded-reniform in outline, deeply tripartite or trisected, the segments tapering to a petiole like base, the middle segment oblong

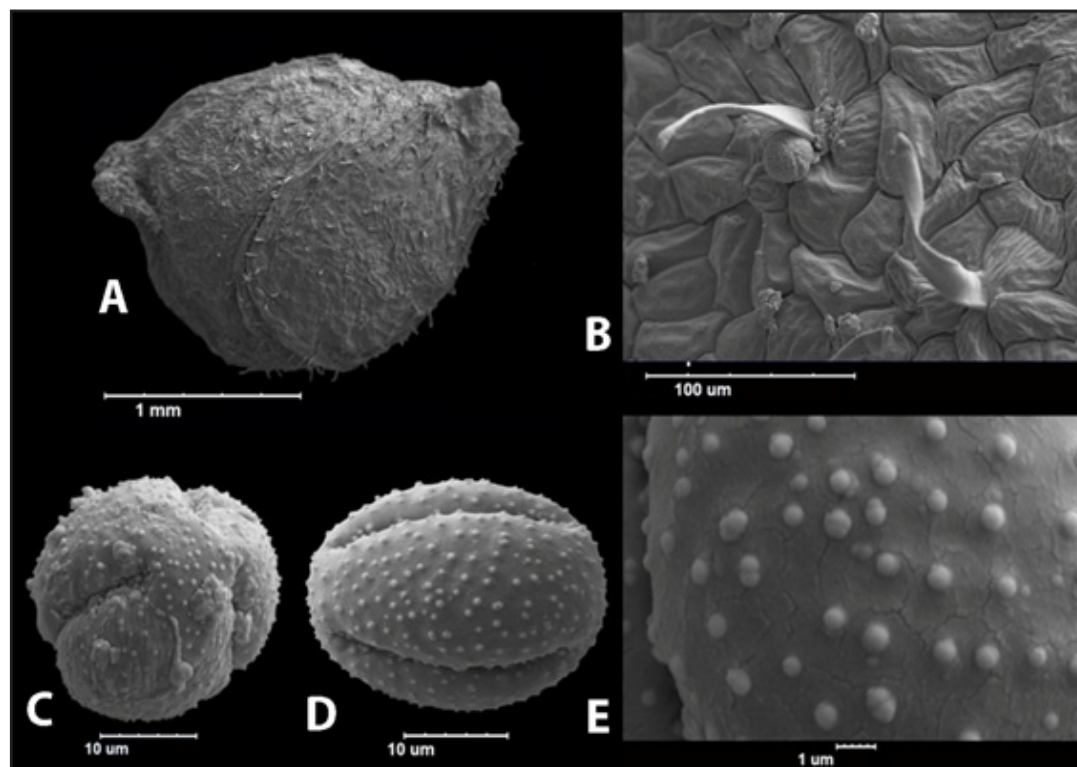


Fig. 2. SEM micrographs of *Ranunculus polyrhizos*: **A** – achene; **B** – achene surface; **C** – polar view of pollen grain; **D** – equatorial view of pollen grain; **E** – exin surface.

obovate, cuneate, with 3 rounded apical teeth or small lobes, the lateral segments broader than the middle one, bifid, with entire obtuse margin. Lower stem leaves similar to basal leaves. Upper stem leaves sessile, trisected, segments linear, bracts sessile, 2-3-partite, with linear lobes. Peduncles finely sulcate, often divaricate, in groups of 2 or 3, glabrous or minutely hairy distally. Flowers solitary, terminal, 1-2 cm in diameter. Receptacles puberulent. Sepals 5, as long as half of petals, more or less broad ovate, convex, with numerous prominent rather dark longitudinal partly branching veins, abaxially sparsely yellowish and puberulent; the edges whitish, scariosus, hairy. Petals 5-7, 5-10×5-8 mm obovate, with prominent veins on both surfaces, the margin rounded, with a few hairs near nectary, nectar scale sacate. Stamens numerous; anthers narrowly oblong. Achenes 2-2.5 mm long, *ca.* 1.5-2 mm wide, more or less obovate or oblong-obovate, turgid, covered with a short whitish-scarious hairs,

without transverse wrinkles, but pericarp often in lower seed-bearing part with two longitudinal spongy wings; the beak to 0.3 mm, glabrous, uncinata. Scanning electron microscope analysis of achene morphology showed that epidermal cells are imbricate, with strip-like hairs. Seeds light brown with dark brown longitudinal ribs, ellipsoid or triangular, 1-1.5 mm long and 0.8-1 mm wide, outline on the hilum face triangular (Figs 2 & 3).

Phenology. Flowering in April – May, fruiting in May – June.

Distribution and habitat. *R. polyrhizos* has been collected from alpine dry gravelly slope of the Backrodagh mountain in the Talesh mountains, ranging from altitudes of 2800-2900 m a.s.l. Accordingly to conducted analysis, this species grows on the loamy soils (sand 48, clay 20, silt 32) with pH 7, soil EC – 670 μSiemens/cm, and organic matter near 4.5%. This species was accompanied by such alpine species as *Allium derderianum* Regel,

Alopecurus aucheri Boiss., *Artemisia melanolepis* Boiss., *Astragalus aureus* Willd., *Campanula stevenii* M. Bieb., *Colchicum raddeanum* (Regel) K. Perss., *Festuca rupicola* Heuff., *Ficaria kochii* (Ledeb.) Iranshahr & Rech. f, *Jurinea monocephala* Aitch. & Hemsl., *Minuartia recurva* (All.) Schinz & Thell., *Onobrychis cornuta* (L.) Desv., *Poa bulbosa* var. *vivipara* Koch, *Scutellaria pinnatifida* A. Ham., *Tanacetum chiliophyllum* (Fisch. & E. Mey. ex DC.) Sch. Bip, *Thymus kotschyanus* Boiss. & Hohen., *Valeriana leucophaea* DC., and *Veronica kurdica* Benth.

R. polyrhizus is an Euro-Siberian element. It was firstly described from Siberia (WILDENOW 1799). It is distributed from Turkey, Transcaucasia, Central and South Russia, Siberia, Kazakhstan to China (Xinjiang). This species grows in alpine screes, steppes, meadows, among scrubs, sometimes on abandoned fields, dry gravelly slopes in altitude from 1200 to 3000 m a.s.l. (OVCHINNIKOV 1937; DAVIS & COOK 1965; WANG & GILBERT 2001).

According to molecular results (EMADZADE *et al.* 2010), the Central Asian specimens of *R. polyrhizus* were nested within North American clade. However, some previous investigations (NEMAT *et al.* 2009; EMADY *et al.* 2010) showed that characters of nectar scale have taxonomical value and separate these *Ranunculus* species.

In *R. polyrhizus* nectar is excreted by nectar scale at the base of petal on the yellow claw. Nectar scale is about 1×0.8 mm. It arises directly from the petal to which it is laterally attaching in the whole of its length and forming a sack at the petal bottom. At the top it is hairy and sometime dentate (Fig. 3). Basing on NEMAT *et al.* (2009) *R. polyrhizus* nectar scale shape is similar to such in *R. asiaticus*, but in color it is golden-yellow instead of red-purple.

The pollen grains of *R. polyrhizus* are triporate, radically symmetrical, and heteropolar. The length of polar axis (P) is 29.5 µm and equatorial length (E) is 22.01 µm, ±P/E = 29.5/22.1. Pollen shape is prolate. Ornamentation is verrucate. Pollen characters *R. polyrhizus* is similar to *R. glacialis* (HALBRITTER *et al.* 2011). These pollen

characters occurred in the *R. acris* type in accordance to CLARKE *et al.* (1991).

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References

- BABINGTON C.C. 1856.** On the *Batrachium ranunculi* of Britain. *Trans. Bot. Soc. Edinburgh* **5**: 65–84.
- BOISSIER E. 1867.** Flora orientalis: Sive, enumeratio plantarum in Oriente a Graecia et Aegyptoad Indiae fines hucusque observatarum, **1**: 20–57. H. Georg, Genevae.
- BOUYOCUS G.J. 1951.** A recalibration of the hydrometer for making mechanical analysis of soils. *Agron. J.* **43**: 434–438.
- CLARKE G.C.S., PUNT W., HOEN P.P. 1991.** The Northwest European pollen flora. 51. Ranunculaceae. *Rev. Palaeobot. Palynol.* **69**: 117–271.
- COOK C.D.K. 1966.** A monographic study of *Ranunculus* subgen. *Batrachium* (DC.) A. Gray. *Mitt. Bot. Staatssamml. Münch.* **6**: 47–237.
- DAHLGREN G. 1992.** *Ranunculus* subgenus *Batrachium* on the Aegean Islands and adjacent areas: Nectary types and breeding system. *Nordic J. Bot.* **12** (3): 299–310.
- DAVIS P.H., COOK C.D.K. 1965.** *Ranunculus*. In: Davis P.H. (ed.), *Flora of Turkey and the East Aegean Islands*. **Vol. 1**: 146–197. Edinburgh University Press, Edinburgh.
- DE CANDOLLE A. 1818.** Regni vegetabilis systema naturale, sive ordines, genera et species plantarum secundum methodi naturalis normas digestarum et descriptorum. Vol. 1. Sumptibus sociorum Treuttel et Würtz, Parisiis.
- EMADY N.S., PAKRAVAN FARD M., AMINI T. 2010.** Study of nectar scale characters in annual *Ranunculus* from Ranunculaceae in Iran. *Taxonomy Biosystematics* **2** (4): 25–32.
- EMADZAD K., LEHNEBACH C., LOCKHART P., HÖRANDL E. 2010.** A molecular phylogeny, morphology and classification of genera of Ranunculaceae (Ranunculaceae). *Taxon* **59**: 809–828.
- HALBRITTER H., AUERAA., KOHLER R. 2011.** *Ranunculus glacialis*. In: PALDAT (2011-10-17), A palynological database. https://www.paldat.org/pub/Ranunculus_glacialis/203506. Accessed 21 April 2016.
- HAMILTON A.C. 1976.** Identification of East African Urticales pollen (Ranunculaceae). *Pollen et Spores* **18** (1): 27–66.
- IRANSHAH M., RECHINGER K.H., RIEDL H. 1992.** *Ranunculus*. In: RECHINGER K.H. (ed.), *Flora Iranica*. **Vol. 171**: 127–194. Akademik Druck-und Verlagsanst, Graz.

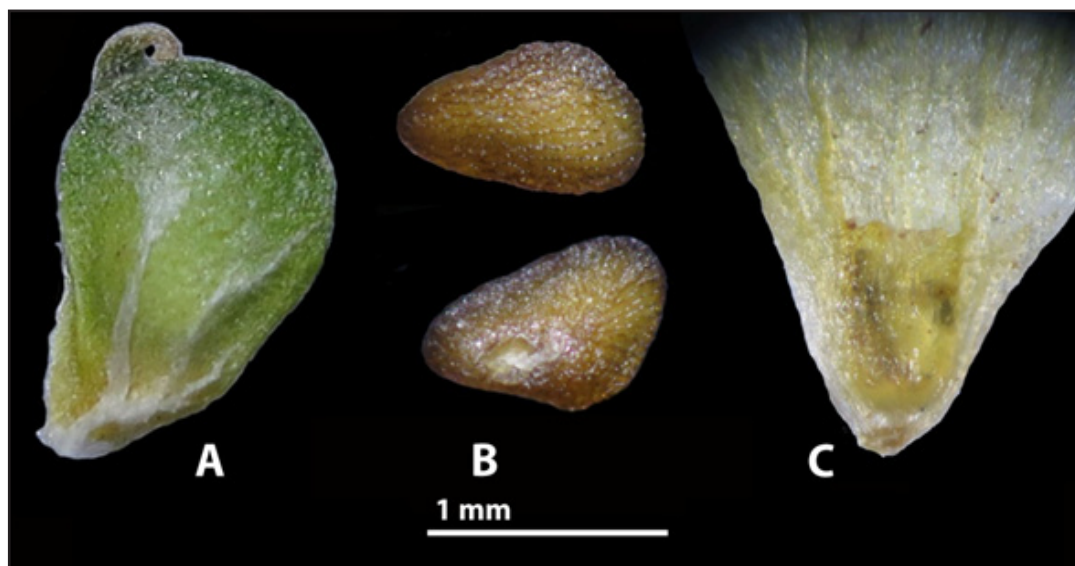


Fig. 3. LM micrographs of *Ranunculus polyrhizos*: **A** – achene; **B** – seed in abaxial and adaxial view; **C** – nectar scale.

- JOHANSSON J.T.** 1998. Chloroplast DNA restriction site mapping and the phylogeny of *Ranunculus* (Ranunculaceae). *Plant Syst. Evol.* **213**: 1–19.
- KUMAZAWA M.** 1936. Pollen grain morphology in Ranunculaceae, Lardizabalaceae and Berberidaceae. *J. Jpn. Bot.* **8**: 19–46.
- NAQINEZHAD A., NOROOZI J., BIDARLORD M., ENGLMAIER P.** 2016. First evidence of a heterophyllous water crowfoot (*Ranunculus peltatus*, Ranunculaceae) in Iran, its phytogeographical implications and a new determination key for Iranian *Batrachium*. *Ann. Naturhist. Mus. Wien B* **118**: 135–145.
- NELSON D.W., SOMMERS L.E.** 1996. Total carbon, organic carbon and organic matter. In: SPARKS D.L., PAGE A.L., HELMKE P.A., LOEPPERT R.H., SOLTANPOUR P.N., TABATABAI M.A., JOHNSTON C.T., SUMNER M.E. (eds), *Methods of soil analysis. Part 3. Chemical Methods. SSSA Book Series No. 5*: 961–1010.
- NEMATI S., PAKRAVAN M., TAVASSOLI A., ZARRE S.** 2009. A review on the nectar scale characters in some species of *Ranunculus* in Iran. *Rostaniha* **10** (2): 193–202.
- OVCHINNIKOV P.N.** 1937. *Ranunculus* L. In: KOMAROV V.L., SHISHKIN B.K. (eds), *Flora of SSSR. Vol. 7*: 271–509. Botanical Institute of the Academy of Sciences of USSR, Moscow – Leningrad.
- PAKRAVAN M.** 2010. A new record and a synonym in the genus *Ranunculus* (Ranunculaceae) from Iran. *Rostaniha* **11** (1): 107–109.
- PAKRAVAN M.** 2012. A new species of the genus *Ranunculus* from Iran. *Science Asia* **38**: 419–421.
- PAKRAVAN M., JAMSHIDNEJAD AVVAL A., TAVASSOLI A.** 2014. Palynological study of some species in grumorsae group of the genus *Ranunculus* in Iran. *Taxonomy Biosystematics* **6** (20): 73–84.
- PAKRAVAN M., RASTIPISHEH S., EMADI N., NEMATI S.** 2010. Study of pollen grains characters in the genus *Ranunculus* L. (Ranunculaceae) from Iran. *Iran. J. Biol.* **23** (1): 1–8.
- PETROV S., BORRISOVA-IVANOVA O.** 1981. Palynomorphological characteristics of the Bulgarian representative of the family Ranunculaceae Juss. VI. *Ranunculus* L. *Fitologia, Sofia* **16**: 5–40.
- RASTIPISHE S., PAKRAVAN M., TAVASSOLI A.** 2011. Phylogenetic relationships in *Ranunculus* species (Ranunculaceae) based on nrDNA ITS and cpDNA *trnL-F* sequences. *Prog. Biol. Sci.* **1** (1): 41–47.
- SANTISUK T.** 1979. A palynological study of the tribe Ranunculeae (Ranunculaceae). *Opera Botanica* **48**: 1–74.
- TAMURA M.** 1995. Angiospermae. Ordnung Ranunculales. Fam. Ranunculaceae. II. Systematic Part. In: HIEPKO P. (ed.), *Die Natürliche Pflanzenfamilien. 2nd ed. 17aIV*: 223–519. Duncker Humblot, Berlin.
- VON WILLDENOW C.L.** 1799. *Species plantarum. Editio Quarta. Tomus II. Pars II*: 1324–1325. Impensis G.C. Nauk., Berolini.
- WANG W.T., GILBERT M.G.** 2001. *Ranunculus* L. In: WU Z.Y., RAVEN P.H., HONG D.Y. (eds), *Flora of China. Vol. 6*: 391–431. Science Press & Missouri Botanical Garden Press.
- WODEHOUSE R.P.** 1936. Pollen grains in the identification and classification. *Bull. Torrey Bot. Club* **63** (9): 495–514.