

**Morphological and micromorphological characterization of achenes in *Ranunculus* species in Iran**

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In this study, the morphology and micromorphology of achenes in the species of *Ranunculus*, the largest genus in the *Ranunculaceae* in Iran, were examined for the first time. Fifty-eight species were studied through herbarium samples using a stereomicroscope, and micrographs of the surface of the pericarp were taken with a Dino-Lite Digital Microscopy (DM) system. The characters studied included the number, size, and shape of the achene, as well as the shape and size of the beak and surface ornamentation of the achene. The fruit shapes ranged from circular, oval, and egg-shaped to triangular. Surface ornamentations varied from smooth and glossy to hairy, warty, and punctate. In addition, the length of the beak was found to be an important trait for distinguishing species groups. Based on these features, species groups were identified, and a key for identifying species of *Ranunculus* based mostly on achene characteristics considering leaves and life form characters was provided. In addition, considering diagnostic characteristics, differences and relationships among species were discussed here.

**Keywords:** Achene ornamentation, beak shape, micromorphology, *Ranunculaceae*, species group**بررسی ریخت‌شناسی و ریزریخت‌شناسی فندقه در گونه‌های آلاله در ایران**

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در این مطالعه، ریخت‌شناسی و ریزریخت‌شناسی میوه در گونه‌های آلاله، بزرگ‌ترین جنس آلاله‌ایان در ایران برای نخستین بار مورد بررسی قرار گرفت. میوه‌های ۵۸ گونه از نمونه‌های هرباریومی با استفاده از استریومیکروسکوپ مطالعه و به کمک سیستم عکس‌برداری میکروسکوپ دیجیتالی، میکروکروگراف‌هایی از سطح پریکارپ تهیه شد. صفات مورد مطالعه شامل تعداد، اندازه و شکل میوه، شکل و اندازه منقار و تزیینات سطح میوه بود. شکل‌های میوه از دایره‌ای، بیضوی، تخم‌مرغی تا مثلثی مشاهده شد. تزیینات سطح میوه از صاف و صیقلی تا کرک‌دار، زگیل‌دار و منقوط متفاوت بود. همچنین، طول منقار از صفات مهم جهت تعیین گروه‌های گونه‌ای در نظر گرفته شد. بر این اساس، گروه‌های گونه‌ای مشخص شد و کلید شناسایی نیز با کمک ویژگی‌های میوه برای گونه‌های جنس آرایه شد. به علاوه، با استفاده از صفات تشخیصی، اختلاف و روابط گونه‌ها مورد بحث قرار گرفت.

**واژه‌های کلیدی:** آلاله‌ایان، تزیینات فندقه، ریزریخت‌شناسی، شکل منقار، گروه گونه‌ای

## Introduction

The *Ranunculus* L. is one of the largest genera in the *Ranunculaceae*, with approximately 600 species worldwide (Shehata & Turki 2001). Recently, Pakravan & Assadi (2024) reported 59 species of this genus in Iran. It has a cosmopolitan distribution but prefers damp habitats. Different species of *Ranunculus* occupy a wide range of habitats, from shallow waters to mountainous elevations and lowland plains (Pakravan & Assadi 2023). Based on habitat diversity, many structural adaptations can be observed in these plants, which enable them to grow in various environments. These adaptations result in significant variation in different plant organs, such as roots, leaves, and achenes. *Ranunculus* species exhibit growth forms ranging from annuals to perennials, with roots that can be simple, erect, fibrous or tuberous. The leaves vary from simple to palmate and pinnate in different species. The achene is an achene, arranged on a conical-shaped receptacle, and exhibits a wide range of sizes and shapes (Pakravan & Sharifinia 2023).

The first systematic review of the genus *Ranunculus* in Southwest Asia was conducted as a monograph by Boissier (1867). De Candolle (1818) also conducted studies on this family and used floral characteristics, underground organs, and achenes for taxonomic identification. Later, Cook (1966) carried out extensive studies on various characteristics of the genus. Tamura (1995) also made a comprehensive review of the *Ranunculus* genus. Several different classifications have been proposed for the genus, including Tamura's separation of several small genera from *Ranunculus* and the division of the species into three tribes (Tamura *l.c.*). Iranshahr *et al.* (1992) carried out extensive studies of *Ranunculaceae* for the Flora Iranica. Following this, molecular systematics and phylogenetic studies were also conducted by several scientists (Johansson 1998, Hörandle *et al.* 2005, Lehnebach *et al.* 2007, Gehrke & Linder 2009, Emadzadeh *et al.* 2010, 2011, Hörandle *et al.* 2012, Rastipisheh *et al.* 2011). Bidarlord *et al.* (2016) based on the ecological conditions and micromorphology of the fruit, reported *Ravnunculus polyrhizos* Stephan ex

Willd. from Iran. Several botanists have used multiple traits to group and identify species of *Ranunculus* (De Candolle 1817, Gray 1821, Benson 1940, Hörandl & Emadzadeh 2011, 2012, Emadzadeh *et al.* 2011, Pakravan & Sharifinia 2023, Pakravan & Assadi 2024). Some botanists have classified species based on the shape of the basal leaves and the type of root. For example, Boissier (1867) identified species groups based on the shape of the sepals, root type, and leaf shape. Iranshahr *et al.* (1971) artificially classified species into groups based on root and leaf shape. One group, with fibrous roots, was placed in the rhizomatosa and praemorsa groups, while another, based on tuberous roots, was classified under the "Gromorza" group. Morphological characters of nectar scales have also been used by several botanist as an important character for distinguishing the *Ranunculus* species (Cook 1966, Nemati *et al.* 2009, Emady *et al.* 2010). However, ultimately, achene characteristics should be used for accurate species identification.

Weigand (1895) was the first to study the achene structure in the *Ranunculaceae* and provided morphological and anatomical traits for various genera within the family. Following this, Cook (1963) examined the anatomical structure of the achene in the subgenus *Batrachium* and used achene characteristics for species identification. Salim *et al.* (2016) investigated the morphology of several taxa within the *Ranunculaceae*. Jung *et al.* (2017) examined the achene and seeds of some genera within this family as well.

Trzask (1999), in his study of the wood structure in *Ranunculaceae* achenes, identified it as a useful trait for species identification. Mourad *et al.* (2000) also studied the morphology and anatomy of the achene in this family. Fruit micromorphological studies in *Thalictrum* L. species of Iran, showed significant differences between species (Pakravan *et al.* 2021). Morphometry geometric studies based on fruit were useful in distinguishing the varieties of *Ceratocephala falcata* L. (Alirezaei *et al.* 2023). Shehata & Turki (2001) examined the external structure and micromorphology of the achene in *Ranunculus* species of Egypt. They investigated nine species of

*Ranunculus* from the said country, seven of which also grow in Iran. They primarily used patterns of pericarp cells and surface ornamentation of the pericarp to develop a species identification key for Egyptian *Ranunculus*.

However, Pakravan (2010) utilized achene structure to verify the identification of some *Ranunculus* species in Iran. Emadzade *et al.* (2010), through the study of achene morphology and anatomy in *Ranunculaceae*, and comparing their findings with molecular phylogenetic analyses, concluded that the achene structural features somewhat correspond with the placement of species on the phylogenetic tree, supporting their taxonomic classification. Seed micromorphological characters have studied in various plants families (Hoseini *et al.* 2017).

Since herbarium samples often do not include roots and sometimes even basal leaves, the aim of this research is to conduct a comparative study and identify achene traits in the *Ranunculus* species of Iran,

providing an identification key based on achene characteristics to facilitate species identification.

### Materials and Methods

In this study, herbarium samples from the Faculty of Biological Sciences, Alzahra University (ALUH), Forests and Rangelands Research Institute (TARI), Kharazmi University (T, FAR), and Herbarium of Forests and Rangelands Research Department, Kermanshah (RANK), Iran were used (Table 1) (Thiers 2021). Here, about 100 specimens of 58 *Ranunculus* species from Iran were examined.

For micromorphological studies, the achenes were used without any pre-treatment. After removing dust from the surface of the achenes, they were photographed using a Digital Microscopy. Forty-five micrographs, were then selected from the obtained images, are presented in this paper. The terminology used for description of the micromorphology of the pericarp surface, was based on Davis (1965).

**Table 1.** The list of studied Iranian taxa of *Ranunculus* along with related data

No.	Taxon	Locality, collector & voucher No.	Herbarium Name*
1	<i>R. sphaerospermus</i> Boiss. & Blanches in Boiss.	Mazandaran Prov.: Nodahak Village, Akbari 11056	ALUH
2	<i>R. trichophyllus</i> Chaix in Vill.	Ardebil Prov.: Neor Lake, 2500 m, Naqynejad & Akbari 1105	ALUH
3	<i>R. peltatus</i> Schrank	Ardebil Prov.: Aghadaghi, 2296 m, Ashrafi 2296	TARI
4	<i>R. rionii</i> Lagger.	Azarbaijan Prov.: Ardebil to Khalkhal road, Neor Lake, 2450 m, Zehzad, Taheri & Pakravan 70538	TARI
5	<i>R. ophioglossifolius</i> Vill.	Gilan Prov.: Bandar-e Anzali, Mozaffarian 65127	TARI
6	<i>R. scleratus</i> L.	Mazandaran Prov.: Sangdeh Village, 1500 m, Pakravan 4312	ALUH
7	<i>R. dolosus</i> Fisch. & C.A.Mey.	Gilan Prov.: 13 km Asalem to Khalkhal, 200–250 m, Zehzad, Taheri & Pakravan 67269	TARI
8	<i>R. meyerianus</i> Rupr.	Azarbaijan Prov.: Arasbaran protected area, S. of Kharil Mountain, 2000–2500 m, Assadi & Maasoumi 20266	TARI
9	<i>R. strigillosus</i> Boiss. & A. Huet.	Azarbaijan Prov.: 50 km W. of Khoy, near Turkish border, 2800 m, Assadi & Olfat 68801	TARI
10	<i>R. koeiei</i> Rech.f.	Kermanshah Prov.: Gahvareh, 1550–1650 m, Nemati & Roshanzadeh 37065	TARI
11	<i>R. oreophilus</i> M.B.	Azarbaijan Prov.: Arasbaran protected area, W of Makeidy, 2400 m, Assadi & Maasoumi 20211	TARI
12	<i>R. polyrhizus</i> Stephan ex Willd.	Ardebil Prov.: 43 km of Ardebil to Khalkhal, Lissar protected area, Bacrodagh Mountain, 2800–2900 m, Bidarlord 15887	T, FAR

Table 1 (contd)

13	<i>R. lateriflorus</i> DC.	Mazandaran Prov.: South of Ramsar, E. of Lapasar, 2950 m, Runemark & Maasoumi 21669	TARI
14	<i>R. cornutus</i> DC.	Khuzestan Prov.: Ramhormoz to Behbahan road, Soltan-abad, 100 m, Mozaffarian 63261	TARI
15	<i>R. chius</i> DC.	Khuzestan Prov.: Dezful, Sardasht to Ahmadfadaleh, 860 m, Maasoumi & Mahmoodi 100453	TARI
16	<i>R. lingua</i> L.	Gilan Prov.: Mordab-e Amir-Kolayeh, -25 m, Moradi 12	TARI
17	<i>R. cicutarius</i> Schlechtend.	Semnan Prov.: Shahrud, Kuh-e Abr, 2060 m, Foroughi 9818	TARI
18	<i>R. illyrichus</i> L.	Azərbayjan Prov.: Between Maku and Khoy, Arab Dizaji Village, 2150 m, Assadi & Mozaffarian 30267	TARI
19	<i>R. macropodioides</i> Briq.	Kerman Prov.: Lalehzar Mountain, Darreh-zard Village, 3350 m, Foroughi & Assadi 16337	TARI
20	<i>R. elymaticus</i> Boiss. & Hausskn.	Fars Prov.: Dena Mountain, Tange-ye Namaky, 3950 m, Safyani 230	TARI
21	<i>R. bulbilliferus</i> Boiss. & Hohen.	Zanjan Prov.: Mahnesan, Anguran, Belgheis Mountain, 2700–3200 m, Maasoumi 64852	TARI
22	<i>R. eriorrhizus</i> Boiss. & Buhse	Yazd Prov.: Shirkuh, 3400 m, Mirhosseini & Soltani 1580	TARI
23	<i>R. amblyolobus</i> Boiss. & Hohen.	Azərbayjan Prov.: Ardebil to Khalkhal, Neor Lake, 1450 m, Zehzad <i>et al.</i> 70539	TARI
24	<i>R. buhsei</i> Boiss.	Ardebil Prov.: Kaleybar, 1900–2500 m, Pakravan 23390	ALUH
25	<i>R. brachylobus</i> Boiss. & Hohen.	Hamedan: Alvand Mountain, 2700 m, Assadi & Mozaffarian 36834	TARI
26	<i>R. repens</i> L.	Mazandaran Prov.: Ramsar, Javaher-deh, 2000 m, Rastipisheh 3703	ALUH
27	<i>R. polyanthemos</i> L.	Azərbayjan Prov.: Arasbaran protected area, Makeidy, 2000 m, Pakravan 3513	ALUH
28	<i>R. bulbusus</i> L.	Gilan Prov.: Espili, Larestan, 1400 m, Saeedi 18583	ALUH
29	<i>R. merovenssis</i> Grossh.	Ardebi Prov.: 3 km from Ali-abad to Oskoloo, 1400 m, Pakravan 3521	ALUH
30	<i>R. aucheri</i> Boiss.	Yazd Prov.: Kuh-e Barfkhaneh, Assadi & Wendelbo 16481	TARI
31	<i>R. caucasicus</i> M.B.	Ardebil Prov.: Arasbaran, 17 km from Kaleibar to Vaighan, 1900 m, Pakravan 3518	ALUH
32	<i>R. sericeus</i> Banks & Soland.	Mazandaran Prov., Khoram-abad to Dohezar, Nemati & Mehrabian 2512	ALUH
33	<i>R. grandiflorus</i> L.	Azərbayjan Prov.: Arasbaran protected area, W. of Makeidy, 2500 m, Assadi & Maasoumi 2045	TARI
34	<i>R. kotschy</i> Boiss.	Zanjan Prov.: 45 km to Zanjan to Dandi, Marasa Village, 2532 m, Mahmudi 100097	TARI
35	<i>R. crymophilus</i> Boiss. & Hohen	Tehran Prov.: Damavand, Tar Lake, 2750–3750 m, Mozaffarian & Mohamadi 49292	TARI
36	<i>R. marginatus</i> d'Urv., Mem. var. <i>trachycarpus</i> (Fisch. & C.A.Mey.) Aznavour	Baluchestan Prov.: Rask, 30 km of Sarbaz road, 630 m, Runeemark <i>et al.</i> 22363	TARI
37	<i>R. zenjanensis</i> Iranshahr & Rech.f.	Azərbayjan Prov.: S.W. of Ahar, 15 km Mizanlu Village, 2500 m, Assadi & Shamsavari 65941	TARI
38	<i>R. procumbens</i> Boiss.,	Azərbayjan Prov.: Salmas, Zavieh-jik Village, Darreh Goli, 1559–1900 m, Alizadeh & Khodakarimi 44215	TARI
39	<i>R. sahandicus</i> Boiss. & Buhse	Azərbayjan Prov.: Near Maragheh, S slope of Sahand Mountain, 2200–2600 m, Assadi & Mozaffarian 30726	TARI
40	<i>R. microflorus</i> Pakravan	Hamedan Prov.: Road of Nahavand to Noor-abad, Above Gamasab, Garin Mountain, 2500–3400 m, Assadi 75125	TARI
41	<i>R. renzii</i> Iranshahr & Rech.f.	Hamedan Prov.: 8 km E of Ganjnameh, 2700 m, Assadi & Mozaffarian 36872	TARI

Table 1 (contd)

42	<i>R. oxyspermus</i> Willd.	Kermanshah Prov.: Bisotoon to Songhor, 1700 m, Assadi & Hamzeh'ee 87923	TARI
43	<i>R. damascenus</i> Boiss. & Gaill.	Kurdistan Prov.: Baneh, Armardeh to Belaki, 700 m, Maroofi & Kargar 9882	TARI
44	<i>R. asiaticus</i> L.	Khuzestan Prov.: 15 km Bagh-malek to Izeh, 700 m, Assadi & Abuhamez 38805	TARI
45	<i>R. straussii</i> Bornm.	Charmahal-o-Bakhtiari Prov.: Zard-kuh, Kuhrang Tunnel, 2600–3200 m, Mozaffarian 57694	TARI
46	<i>R. afghanicus</i> Aitch. & Hemsl.	Khorasan Prov.: 45 km Shirvan, Gulul-sarani protected area, 300–2300 m, Assadi & Maasoumi 50430	TARI
47	<i>R. termei</i> Iranshahr & Rech.f.	Charmahal-o-Bakhtiari Prov.: Shehrekord, Baba Heydar, 2150–2500 m, Mozaffaria 54814	TARI
48	<i>R. muricatus</i> L.	Khuzestan Prov.: Izeh, wetland around Kuhbad Morady, 830 m, Mozaffarian 70214	TARI
49	<i>R. pinardii</i> Boiss.	Kurdistan Prov.: Mahmara Village, 20 km Marivan, 1520 m, Fattahi & Khaledian 599	TARI
50	<i>R. millefolius</i> Banks & Sol.	Kurdistan Prov.: 20 km N.W. Marivan, Mahmara Village, 1520 m, Fattahi & Khaledian 594	TARI
51	<i>R. brutius</i> Ten.	Ardebil Prov.: Arasbaran protected area, Doghroon Mountain, 2200–2250 m, Jamzad <i>et al.</i> 70390	TARI
52	<i>R. constantinopolitanus</i> (DC.) d'Urv.	Mazandaran Prov.: 10 km from Khorram-abad to Dohezar road, Mehrabian & Nemati 2506	ALUH
53	<i>R. arvensis</i> L.	Khuzestan Prov.: Bagh Malek, 750 m, Mozaffarian 53572	TARI
54	<i>R. sojakii</i> Iranshahr & Rech.f.	Tehran: Ghazvin, Alamut, above Evan Village, 3300–3700 m, Assadi & Maasoumi 61130	TARI
55	<i>R. tricarpus</i> Boiss. & Kotchy	Tehran Prov.: Between Taleghan and Alamut, Alborz Mountain, 3400 m, Mirfakhrae 20112	TARI
56	<i>R. leptorrhynchus</i> Aitch. & Hemsl.	Khorasan Prov.: 74 km from Kalat-e Naderi to Mashhad, 950 m, Assadi & Maasoumi 55863	TARI
57	<i>R. macrorrhynchus</i> Boiss.	Kermanshah Prov.: Bisoton to Songhor, 1820–2000 m, Hamzeh'ee & Asri 87921	TARI
58	<i>R. dalechanensis</i> Iranshahr & Rech.f.	Kermanshah Prov.: Sonqhor, Dalakhani Mountain, 3100 m, Nemati 10372	RANK

\* ALUH: Herbarium of Alzahra University; TARI: Herbarium of Forests and Rangelands Research Institute; T, FAR: Herbarium of Kharazmi University; RANK: Herbarium of Forests and Rangelands Research Department, Kermanshah (Iran)

## Results and Discussion

Based on the results obtained from the achene morphology studies, the number of achenes in each flower is an important trait for species identification. The lowest number of achenes (4–6) was observed in *R. arvensis*, while the highest number (50–80) was found in *R. sphaerospermus*. However, the results achieved here, is in agreement with Davis description in Flora of Turkey (1965).

Achene size is another significant trait in *Ranunculus* species. Among the Iranian *Ranunculus* species, *R. sphaerospermus* exhibited the smallest achene, measuring approximately 4 mm in length, while

*R. pinardii* had the largest achene, measuring 15 mm in length, which is here in agreement with Iranshahr *et al.* (1992).

The shapes of the achenes in the *Ranunculus* species were observed in the following forms: circular, ovate, ovate-oblong, triangular, and spherical.

The beak of the achene varied in shape between different species and were observed in the following forms: short and straight, short and curved, long and straight, long and curved, and long and circinate. Based on this characteristic, the species can be grouped into the following categories:

## - Group 1

Twelve species fall into this category, mostly annuals or aquatic having a beak less than 0.5 mm length or without beak, including the following species: *R. sphaerospermus*, *R. trichophyllus*, *R. peltatus*, *R. rionii*, *R. ophioglossifolius*, *R. scleratus*, and *R. dolosus*; and

species that grow in alpine and cold regions, such as *R. meyerianus* (Arasbaran Mountain), *R. strigillosus* (Slopes of Sahand Mountain), *R. koeiei* (Gahvareh Mountain), *R. oreophilus* (Damavand and Sahand Mountains), and *R. polyrhizus* (Bakrodagh Mountain, Ardebil) (Table 2 & Fig. 1).

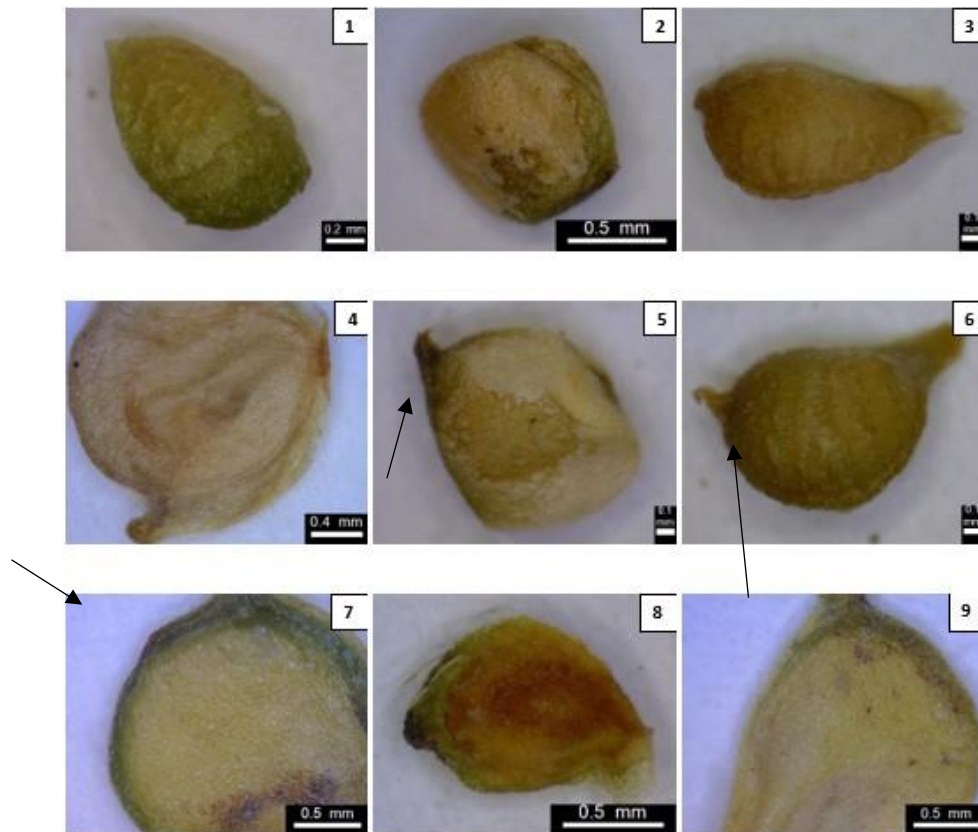
**Table 2.** *Ranunculus* species without beak or with a beak less than 0.5 mm length

Taxon	Achene shape	Length width (mm)	Beak shape	Beak length	Surface sculpturing	No. of achene
<i>R. sphaerospermus</i> Boiss. & Blanches in Boiss.	± Spheroidal	0.85–0.95	-	-	With marginal vein, striate	50–80
<i>R. trichophyllus</i> Chaix in Vill.	Elliptic	1.3–1.5	-	-	Striate	15–30
<i>R. peltatus</i> Schrank	± Elliptic	1.8–2	Without beak, with sharp tip	-	Striate	30–40
<i>R. rionii</i> Lagger.	± Elliptic	1–1.4	Without beak, with sharp tip	-	With marginal vein, striate	60–80
<i>R. ophioglossifolius</i> Vill.	Ovate	1–1.5	Erect	-0.4	With marginal vein, hairy	60–80
<i>R. scleratus</i> L.	Ovate-semi circular	1–1.3	Erect	-0.4	With marginal vein, wrinkled	
<i>R. dolosus</i> Fisch. & C.A.Mey.	Ovate-semi circular	1	Without beak, with sharp tip	-	With marginal vein, glabrous to ± faveolate	50–60
<i>R. meyerianus</i> Rupr.	Ovoid-oblong	2.5–3.5	Beak broad, triangular	0.2–0.4	With marginal vein, glabrous	30–40
<i>R. strigillosus</i> Boiss. & A. Huet.	Obovate	2–2.4	Short conical	-0.2	With marginal vein, glabrous	40–50
<i>R. koeiei</i> Rech. f.	± Orbicular	1.5–2	Straight, with ± curved tip	0.5	Appressed hairy	
<i>R. oreophilus</i> M.B.	Ovate-semi circular	1.8–2	Uncinate	0.4	With marginal and median veins, glabrous	30–38
<i>R. polyrhizus</i> Stephan ex Willd.	Obovate	2–2.5	Uncinate	0.3	Marginal winged, hairy	-

## - Group 2

Twenty-four species belong to this group, most of which grow in humid and mountainous regions having beak length between 0.5–1.5 mm including the following species: *R. lateriflorus* (Slopes of Sahand Mountain), *R. cicutarius*, *R. illyrichus*, *R. macropodioides*, *R. elymaticus*, *R. bulbiferus* (Manishan Mountain), *R. eriorrhizus* (Lalezar Mountain), *R. amblyolobus* (Kandovan), *R. buhsei* (Northern Alborz forests), *R. cornutus* (Silvana in Zagros), *R. chius* (Poshteh Kuh in

Lorestan), *R. lingua*, *R. brachylobus* (Sabalan Mountain), *R. repens* (Kelardasht forests), *R. polyanthemus* (Arasbaran and Javaherdeh forests), *R. bulbosus* (Anbarlu forests), *R. merovens.* (Arasbaran), *R. caucasicus* (Asalem forests), *R. sericeus* (Taleghan, Peshteh Kuh), *R. grandiflorus* (Ramsar forests), *R. kotschyi* (Rudbar), *R. crymophilus* (Varosht Mountain), *R. marginatus*, and *R. zenjanensis* (Kamar and Mahneshan Mountains) (Table 3 & Fig. 2).



**Fig. 1.** Achenes of the Iranian taxa of *Ranunculus*: 1. *R. trichophyllus*, 2. *R. dolosus*, 3. *R. sphaerospermus*, 4. *R. macropodioides*, 5. *R. scleratus*, 6. *R. rionii*, 7. *R. bulbusus*, 8. *R. ophioglossifolius*, 9. *R. amblyolobus* (arrows indicate the beak).

**Table 3.** *Ranunculus* species with a beak length of 0.5–1.5 mm

Taxon	Achene shape	Length (mm)	Beak shape	Beak length	Surface sculpturing	No. of achene
<i>R. lateriflorus</i> DC.	Ovate-circular	2.7–3	Straight to slightly curved	1.45	With marginal vein, papillate	25–35
<i>R. cornotus</i> DC.	Ovate-elliptic	3–6 (without beak)	Straight, with curved tip	1.2–1.4	Acutely muriculate, winged marginally	4–9
<i>R. chius</i> DC.	Ovate-circular	3.5–4.5	Broad triangular, curved	1	Papillate	8–16
<i>R. lingua</i> L.	Ovate-oblong	1.5–3	Slightly curved	0.7	With marginal vein glabrous	
<i>R. cicutarius</i> Schlechtend.	Ovoid-triangular	3–4	Straight to $\pm$ curved	1.4	With marginal vein, Punctate -alveolate	30–40
<i>R. illyrichus</i> L.	Ovate-circular	2–2.5	Straight with $\pm$ curved tip	1	Punctate, laterally winged	
<i>R. macropodioides</i> Briq.	Orbicular	2–2.3	Straight with $\pm$ curved tip	1.3	With marginal vein, glabrous	40–50
<i>R. elymaticus</i> Boiss. & Hausskn.	Orbicular	2–2.3	Straight	0.8	Inconspicuous vein, glabrous	40–50
<i>R. bulbiferus</i> Boiss. & Hohen.	Orbicular	2–2.5	Straight with $\pm$ curved tip	1	Inconspicuous vein, glabrous	–
<i>R. eriorrhizus</i> Boiss. & Buhse	$\pm$ Orbicular	2–3	Curved	1	With marginal vein, glabrous	–
<i>R. amblyolobus</i> Boiss. & Hohen.	Ovoid-orbicular	1.5–2	Uncinate	0.75–1	With marginal vein, $\pm$ reticulate	25–35

**Table 3 (contd)**

<i>R. buhsei</i> Boiss.	Orbicular	2.5–3	Straight with ± curved tip	0.5–1	With marginal vein, glabrous	29–40
<i>R. brachylobus</i> Boiss. & Hohen.	Rounded- obovate	1.4–2.6	Uncinate	0.5–1	glabrous	20–40
<i>R. repens</i> L.	Rounded- obovate	3–3.5	Straight	0.7–1	With marginal vein, ± punctate	20–40
<i>R. polyanthemos</i> L.	Orbicular	3.5–4.5	Uncinate	0.5–0.75	With marginal vein, glabrous	20–38
<i>R. bulbosus</i> L.	Orbicular	2.5–4	Uncinate	0.5	With marginal vein, glabrous	20–30
<i>R. merovensis</i> Grossh., Beih.	Obovate-oblong	3–3.5	Straight with ± curved tip	0.6–1	Glabrous	–
<i>R. caucasicus</i> M.B.	Obovate	2.5–3	Uncinate	0.75–1.2	With marginal vein, glabrous	20–38
<i>R. sericeus</i> Banks & Soland.	Orbicular with triangle beak	3–6	Triangle, straight	0.75–1	With marginal vein, reticulate	20–40
<i>R. grandiflorus</i> L.	Rounded- obovate	2.5–3	± Curved	0.5–0.7	With marginal vein, ± reticulate	20–30
<i>R. kotschyi</i> Boiss.	Obovate	2–3	Uncinate	0.5–1.5	With marginal vein, ± punctate	20–30
<i>R. crymophilus</i> Boiss. & Hohen	Rounded- obovate	3	Uncinate	1	With marginal and lateral veins, glabrous	10–20
<i>R. marginatus</i> d'Urv., Mem. var. <i>trachycarpus</i> (Fisch. & C.A.Mey.) Aznavour	Rounded- obovate	3–6	Straight-triangular	1–1.5	With marginal vein, with tubercles	15–20
<i>R. zenjanensis</i> Iranshahr & Rech.f.	Semi orbicular	3–4	Uncinate	0.8	With marginal and lateral veins, glabrous	–

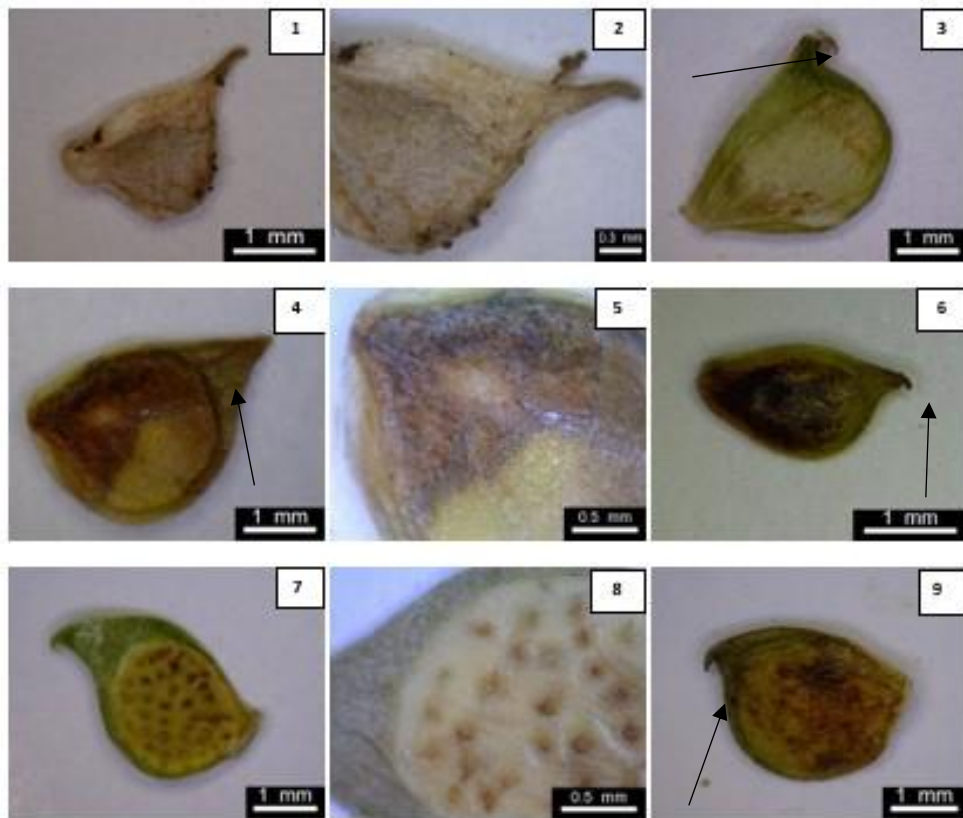
## - Group 3

Twenty-three species has achenes with beaks longer than 1.5 mm (Table 4). Among these species, nine have achenes with beaks longer than 2 mm, including the following species: *R. pinardii*, *R. muricatus*, *R. dalechanensis*, *R. macrorrhynchus*, *R. leptorrhynchus*, *R. sojakii*, *R. constantinopolitanus*, *R. millefolius*, and *R. procumbens*. These species mainly grow in tropical regions, except for *R. pinardii*, *R. sojakii*, and *R. constantinopolitanus*, which are found in Azarbaijan, Mazandaran, Kermanshah, and Hamedan provinces. The other species in this group have tuberous roots, indicating an adaptation to tropical and subtropical habitats. As noted

by Emadzade *et al.* (2010), in the dry habitats, the long, curved beaks and the presence of protuberances on the surface of the achene serve as effective tools for achene dispersal by animals.

Another important feature of the achene is its surface ornamentation. The achene surface can be smooth and glossy, striated, wrinkled, hairy, spiny, warty or perforated. Achene with hairy surfaces were observed in the following species: *R. pinardii*, *R. macrorrhynchus*, *R. sojakii*, *R. damascenus*, *R. straussii*, *R. afghanicus*, *R. termei*, *R. aucheri*, *R. tricocarpus*, *R. dalechanensis*, and *R. renzii*. Spiny achenes were observed in *R. muricatus*, *R. arvensis*, and *R. cornutus*.





**Fig. 2.** Achenes of the Iranian taxa of *Ranunculus*: 1. *R. oreophilus*, 2. *R. straussii*, 3. *R. zenjanensis*, 4, 5. *R. sericeus*, 6. *R. strigillosus*, 7, 8. *R. chius*, 9. *R. grandiflorus* (arrows indicate the beak).

Tuberculated achenes were found in the following species: *R. lateriflorus*, *R. chius*, *R. marginatus*, *R. procumbens*, and *R. arvensis*. Punctate achenes were observed in *R. cicutarius*, *R. repens*, *R. kotschyi*, *R. aucheri*, and *R. millefolius*. Striated achene surfaces were observed in *R. sphaerospermus*, *R. trichophyllus*, *R. peltatus*, and *R. rionii*. The results obtained in this study are consistent with the findings of Shehata & Turki (2001) on *Ranunculus* species from Egypt.

Another characteristic of *Ranunculus* achenes is the presence of a prominent ridge along the achene's margin, composed of sclerenchyma cells. This feature is observed in most *Ranunculus* species. In the samples studied here, the marginal ridge was not distinguishable in *R. bulbiferus*, *R. elymaticus*, and *R. merovensis* due to immature achenes, and no marginal ridge was found in *R. brachylobus*.

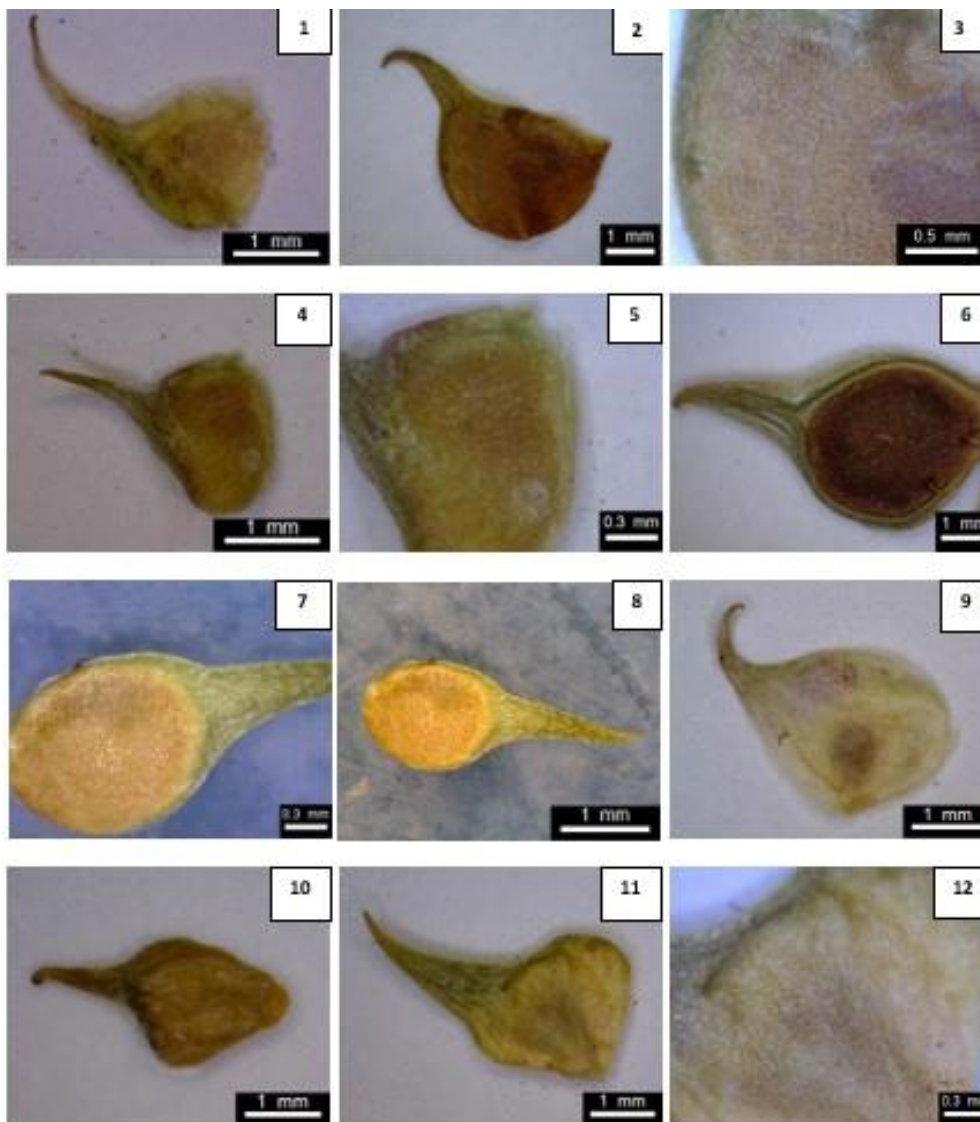
*Ranunculus* achenes show specialized structures related to their dispersal mechanisms. For example, aquatic *Ranunculus* species (*R. trichophyllus*, *R. peltatus*, *R. rionii*, and *R. sphaerospermus*) have smooth surfaces

and are nearly beakless. The prominent marginal ridge is the area where the achene splits, allowing water to enter the achene, which facilitates seed germination (Emadzade *et al.* 2010). In contrast, in xerophytic species, where seed dispersal occurs via epizoochory, the achenes have beaks and often possess rough surfaces, such as hairs, spines or protuberances, which enhance dispersal by animals (Emadzade *et al. l.c.*).

Achene characteristics are valuable tools for distinguishing closely related species. For example, *R. pinardii*, with its 5–8 large achenes and a very long, curved beak, is easily distinguishable from other species. *R. termei* and *R. macropodioides*, both of which have tuberous roots and divided leaves, can only be differentiated by the achene surface ornamentation: *R. macropodioides* has smooth, swollen achenes, while *R. termei* has hairy and flat achenes. Additionally, the two aquatic species *R. sphaerospermus* and *R. rionii*, both of which are submerged aquatic plants with divided leaves, can be differentiated by the presence of hairs on the achenes of *R. sphaerospermus* and the absence of hairs in

*R. rionii*. The two species of *R. zenjanensis* and *R. renzii*, which grow in mountainous regions and have short stems and pinnately divided leaves, can be distinguished by the length and shape of the achene beak (straight in *R. renzii*

and curved in *R. zenjanensis*), and the presence or absence of hairs on the achene (hairless in *R. zenjanensis* and hairy in *R. renzii*).



**Fig. 3.** Achenes of the Iranian taxa of *Ranunculus*: 1. *R. leptorrhynchus*, 2, 3. *R. constantinopolitanus*, 4, 5. *R. cicutarius*, 6. *R. marginatus*, 7, 8. *R. lateriflorus*, 9. *R. asiaticus*, 10. *R. oxyspermus*, 11, 12. *R. millefolius*.

**Table 4.** *Ranunculus* species with a beak length more than 1.6 mm

Taxon	Achene shape	Length (mm)	Beak shape	Beak length	Surface sculpturing	No. of achene
<i>R. procumbens</i> Boiss.	± Ovoid	6–7	Curved	3–4	With marginal vein, tuberculate	30–45
<i>R. sahandicus</i> Boiss. & Buhse	± Ovoid	4–5	Circinate	1.5–2	With marginal vein, glabrous	30–40
<i>R. microflorus</i> Pakravan	Obovate	3–4.5	Straight	1.5–2	With marginal vein, glabrous	20–30
<i>R. renzii</i> Iranshahr & Rech.f.	Rounded-obovate	4	Uncinate	2	With marginal vein, hairy	10–17

**Table 4 (contd)**

<i>R. oxyspermus</i> Willd.	Obovate	2.5–4	Straight	2	With marginal vein and winged, hairy	40–50
<i>R. damascenus</i> Boiss. & Gaill.	Obovate	2.5–4	Straight	2	With marginal vein and winged, hairy	40–50
<i>R. asiaticus</i> L.	Rounded-obovate	3–4	Straight curved at tip	1–5	With marginal vein and winged, glabrous	40–50
<i>R. straussii</i> Bormm.	Triangular-obovate	2.5–4	Straight	2	With marginal vein, reticulate, hairy	40–50
<i>R. afghanicus</i> Aitch. & Hemsl.	Rounded-obovate	3–4	Straight with $\pm$ curved tip	2	With inconspicuous marginal vein, hairy	30–40
<i>R. termei</i> Iranshahr & Rech.f.	Triangular-convex	4	Straight with $\pm$ curved tip	1.5–2	With marginal vein, hairy, reticulate	40–50
<i>R. aucheri</i> Boiss.	Broadly ovate to triangular	2–3.5	Straight with $\pm$ curved tip	1.5–2	With marginal vein, faveolated, hairy	30–40
<i>R. millefolius</i> Banks & Sol.	Triangular	2–4	Straight with $\pm$ curved tip	1.5–3	Triangular, humped at the base, with marginal vein, punctate	30–40
<i>R. brutius</i> Ten.	Orbicular	5	Circinate	2	With marginal vein, glabrous	20–25
<i>R. constantinopolitanus</i> (DC.) d'Urv.	Semi orbicular	3.5–5	<u>U</u> ncinate	1–3.5	With marginal vein, glabrous	20–25
<i>R. diversifolius</i> Gilib.	Semi orbicular	4	<u>U</u> ncinate	1.5–2	With marginal vein, glabrous	20–25
<i>R. sojakii</i> Iranshahr & Rech.f.	Rounded-obovate	3–6	Circinate	0.7–3.5	With marginal vein, hairy	12–20
<i>R. tricocarpus</i> Boiss. & Kotchy	Obovate	4	<u>U</u> ncinate	1.5–2	With marginal vein, hairy	
<i>R. leptorrhynchus</i> Aitch. & Hemsl.	Rounded-obovate	4–6	Straight curved at tip	2–3	Punctate, wrinkled in margin, glabrous, with marginal vein inflated	40–50
<i>R. acrorrhynchus</i> Boiss.	Semi orbicular	5.7–7	Straight curved at tip	3–4	With marginal vein, hairy	20–30
<i>R. dalechanensis</i> Iranshahr & Rech.f	Semi orbicular	3–4	<u>U</u> ncinate	2–4	With marginal vein, hairy	-
<i>R. arvensis</i> L.	Obovate	5–8	Without beak or with a lanceolate spine like beak	0–2	Smooth or spiny or wrinkled, tuberculate	4–6
<i>R. muricatus</i> L.	Rounded-obovate	3–8	Lanceolate-uncinate	1.5–3	With marginal vein, with sharp projection	15–20
<i>R. pinardi</i> Boiss.	Rounded-obovate	21–23 (including beak)	Straight curved at tip	15	With marginal vein, hairy, tuberculate	5–8



**Fig. 4.** Achenes of the Iranian taxa of *Ranunculus*: 1. *R. renzii*, 2. *R. leptorrhynchus*, 3. *R. sojakii*, 4, 5. *R. termei*, 6. *R. procumbens*, 7. *R. pinardii*, 8. *R. muricatus*, 9. *R. aucheri*, 10, 11. *R. tricocarpus*, 12. *R. afghanicus*.

Two alpine species of *R. kotschyi* and *R. polyanthemus*, with divided leaves and hairy surfaces, can be distinguished by the type of achene ornamentation while *R. kotschyi* has a punctate surface, and *R. polyanthemus* has a smooth surface. Additionally, *R. merovensisi* and *R. caucasicus*, both of which have divided leaves and a similar hairy indumentum, grow in alpine regions and can be distinguished by achene size and beak length. *R. caucasicus* has larger achenes with a long, sickle-shaped beak, while *R. merovensisi* has a straight

beak. *R. damascenus*, which shares a habitat with *R. oxyspermus* and is sometimes considered a subspecies of it by some botanists, can be differentiated from *R. oxyspermus* by its hairy achenes and curved beak. Therefore, based mostly on the traits derived from morphological and micromorphological studies of *Ranunculus* achenes considering leaves and life form characters, the following identification key is provided with the help of these features:

#### Diagnostic key to *Ranunculus* taxa in Iran

- |   |    |
|---|----|
| 1. Achene without beak or with a beak less than 0.3 mm long ..... | 2  |
| - Achene with conspicuous beak, more than 0.3 mm long .....       | 11 |
| 2. Achene without beak .....                                      | 5  |
| - Achene with a beak less than 0.3 mm length .....                | 3  |

3. Beak obtuse, plant perennial .....	41. <i>R. meyerianus</i>
- Beak acute .....	4
4. Plant perennial .....	56. <i>R. strigillosus</i>
- Plant annual .....	11. <i>R. scleratus</i>
5. Achene surface without prickle .....	6
- Achene surface with prickle .....	14. <i>R. arvensis</i>
6. Achene surface tuberculate .....	6. <i>R. ophioglossifolius</i>
- Achene surface without tubercule .....	7
7. Plant terrestrial .....	12. <i>R. dolosus</i>
- Plant aquatic .....	8
8. Leaves biform .....	3. <i>R. peltatus</i>
- Leaves uniform .....	9
9. Number of achenes more than 50 .....	10
- Number of achenes less than 50 .....	2. <i>R. trichophyllus</i>
10. Achenes glabrous, nectar scale pyriformis .....	1. <i>R. sphaerospermus</i>
- Achenes hairy, nectar scale lunate .....	4. <i>R. rioni</i>
11. Achene beak less than 1.5 mm length .....	12
- Achene beak more than 1.5 mm length .....	41
12. Annual .....	38
- Perennial .....	13
13. Perennial with fleshy root .....	32
- Perennial without fleshy root .....	14
14. Leaves simple .....	15. <i>R. lingua</i>
- Leaves compound with various shape .....	15
15. Leaves pinnately divided.....	16
- Leaves not pinnately divided.....	17
16. Stem glabrous .....	57. <i>R. crymophilus</i>
- Stem hairy .....	59. <i>R. zenjanensis</i>
17. Leaf segments sessile .....	18
- Leaf segments petiolated .....	24
18. Achene without beak or with a short and erect beak .....	19
- Achene with curved beak .....	22
19. Stem tuberously thickened proximally .....	43. <i>R. bulbosus</i>
- Stem never forming proximal tuberous thickenings .....	20
20. Plant glabrous. Achene with strip-like hairs .....	44. <i>R. polyrhizus</i>
- Plant with patule-pilose or villose indumentums. Achene glabrous .....	42. <i>R. polyanthemus</i>
21. Achene surface punctate. Plant tall, covered with long hairs. Petiole rather long .....	54. <i>R. kotschyi</i>
- Achene surface smooth. Plant dwarf, covered with short and scabrous hairs. Petiole short .....	39. <i>R. oreophilous</i>
22. Rhizome long. Stem ascending and glabrous. Calyx glabrous .....	37. <i>R. brachylobus</i>
- Rhizome short. Stem erect and hairy. Calyx hairy .....	35. <i>R. amblylobus</i>
23. Middle segments of basal leaves with a short-broadened petiole, lateral segments sessile .....	36. <i>R. buhsei</i>
- Middle segments of basal leaves with long petiole, lateral segments with short petiole or sessile .....	24
24. Stem creeping .....	40. <i>R. repens</i>
- Stem not creeping .....	25
25. Plant covered with silky hairs .....	51. <i>R. sericeous</i>
- Plant without silky hairs .....	26
26. Achene hairy .....	52. <i>R. grandiflorous</i>
- Achene glabrous .....	27
27. Flower small, less than 1 cm width .....	53. <i>R. microflorous</i>
- Flowers large, more than 1 cm width .....	28
28. Achene beak circinate, 2–4 mm long. Leaves large .....	45. <i>R. brutius</i>
- Achene beak curved but not circinate .....	29
29. Achene beak less than 1 mm long .....	46. <i>R. merovensis</i>
- Achene beak more than 1 mm long .....	30
30. Root not fleshy .....	31
- Root fleshy .....	32
31. Leaves 3–5 partite, middle segment sessile or with short stalk .....	47. <i>R. diversifolius</i>
- Leaves tripartite, middle segment long petiolate .....	48. <i>R. caucasicus</i>
32. Sepals recurved .....	20. <i>R. cicutarius</i>
- Sepals spreading or divergent .....	33
33. Leaf segments long petiolated .....	34

- Leaf segments sessile .....	21. <i>R. illyrichus</i>
34. Achene rounded, papery and flat .....	26. <i>R. macropodioides</i>
- Achene not rounded with convex surface .....	35
35. Leaves with a bulb at the axis (a swollen fleshy tissue) .....	31. <i>R. bulbiferous</i>
- Leaves without a bulb at the axis .....	36
36. Sepals persistent .....	27. <i>R. elymaticus</i>
- Sepals deciduous .....	37
37. Achene slightly swollen .....	34. <i>R. eriorrhizus</i>
- Achene compressed .....	28. <i>R. koeiei</i>
38. Leaves entire, dentate or undulate margin .....	5. <i>R. laterifolius</i>
- Leaves divided .....	39
39. Basal leaves circular or renal with shallow lobes and dentate margin. Flowers 5–7 mm in diam. Peduncles bent in fruiting stage .....	10. <i>R. chius</i>
- Basal leaves three parted, simple or compound. Flowers larger than above mentioned. Peduncle straight .....	40
40. Achene with acute projection .....	9. <i>R. cornatus</i>
- Achene with tubercles, with navicular margin and straight beak less than 1 mm long .....	7. <i>R. marginatus</i>
41. Achene with sharp projection .....	8. <i>R. muricatus</i>
- Achene without sharp projection .....	42
42. Achene with a beak less than 2 mm long .....	43
- Achene with a beak more than 2 mm long .....	53
43. Plant with fleshy root .....	46
- Plant without fleshy root .....	44
44. Leaves pinnately divided .....	58. <i>R. renzii</i>
- Leaves not divided pinnately .....	45
45. Achene glabrous .....	38. <i>R. sahandicus</i>
- Achene hairy .....	50. <i>R. tricocarpus</i>
46. Sepals recurved .....	47
- Sepals spreading or divergent .....	50
47. Leaves three parted up to near the base .....	48
- Leaves compound, segments long petiolate .....	49
48. Fruiting head oblong, 5–6 mm width. Achene with erect beak, hairy .....	16. <i>R. oxyspermus</i>
- Fruiting head oval shape, 6–7 mm width. Achene with bent beak .....	<i>R. damescens</i>
49. Achene rounded, papery and flat, with smooth surface. Beak up to 1.3 mm long .....	26. <i>R. macropodioides</i>
- Achene not rounded, with convex reticulate surface. Beak up to 2 mm long .....	24. <i>R. termei</i>
50. Flowers red .....	30. <i>R. asiaticus</i>
- Flowers yellow .....	51
51. Achene slightly swollen .....	33. <i>R. straussii</i>
- Achene compressed .....	52
52. Leaves simple with shallow lobes .....	22. <i>R. afghanicus</i>
- Leaves simple divided up to near the base of leaf or compound .....	23. <i>R. aucheri</i>
53. Plant annual .....	13. <i>R. pinardi</i>
- Plant perennial .....	54
54. Root fleshy .....	56
- Root not fleshy .....	54
55. Achene hairy .....	49. <i>R. sojakii</i>
- Achene glabrous .....	56
56. Leaves large, with petiolated segments. Achene beak circinate .....	45. <i>R. brutius</i>
- Leaves with sessile segments. Achene beak uncinated .....	55. <i>R. constantinopolitanus</i>
57. Achene hairy .....	58
- Achene glabrous .....	59
58. Achene beak erect. Stem glabrous .....	32. <i>R. dalechensis</i>
- Achene beak curved, with projection at the base. Stem hairy .....	18. <i>R. macrorrhynchus</i>
59. Achene obtusely obtriangular, with a short basal appendage .....	<i>R. millefolius</i>
- Achene not as above .....	60
60. Achene surface tuberculed, beak 3–4 mm long .....	<i>R. procumbens</i>
- Achene surface reticulated, beak 2–3 mm long .....	25. <i>R. leptorrhynchus</i>

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