



Are they different species or vicariant elevational races of the same species? The case of an Iberian endemic plant, *Ranunculus bupleuroides* (Ranunculaceae)

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Abstract

The infrageneric taxonomy and evolution of *Ranunculus* are still not well understood. In this regard, we investigated the origin and genetic diversity of the Iberian endemic plant *Ranunculus bupleuroides* s.l. by analysis of nuclear internal transcribed spacers (ITS) and plastid (*rpl32-trnL*, *rps16-trnQ*, *trnK-matK*, *ycf6-psbM*) sequence data. Phylogenetic analysis reveals two geographically well supported subclades: one formed by high mountain plants and another by more typical samples of *Ranunculus bupleuroides* from the lowlands of the western Iberian Peninsula. The subclade of montane plants includes both plants originating in Gredos and Béjar (*Ranunculus cherubicus* subsp. *cherubicus*) and those from Sierra Nevada (*R. cherubicus* subsp. *gibretal*). In the light of the new molecular results obtained, combined with an assessment of morphological, geographical and ecological characters, a new systematic treatment is proposed.

Key words: endemism, integrative taxonomy, Mediterranean, molecular markers, mountain flora, phylogeny, section *Ranuncella*, species complex

Introduction

Ranunculus Linnaeus (1753: 548) is the most diverse genus of Ranunculaceae (placed in tribe Ranunculeae) and comprises about 600 herbaceous species with a cosmopolitan distribution (Tamura 1995, Hörandl *et al.* 2005, Emadzade *et al.* 2010, Hörandl & Emadzade 2012). Modern systematics of the genus has substantially varied from those established in the nineteenth century (De Candolle 1824, Spach 1839, Freyn 1880, Prantl 1887). Current *Ranunculus* systematics is derived from Tamura (1991, 1992, 1995), who recognized seven subgenera and a large number of sections. The incorporation of molecular criteria alongside the traditionally used techniques (morphological, cytogenetic, phytogeography, etc.) has generated new taxonomic proposals, covering the definition of the genus (Emadzade *et al.* 2010) to its infrageneric taxonomy (Hörandl & Emadzade 2012), with 17 recognized sections divided into two subgenera.

Ranunculus section *Ranuncella* (Spach 1839: 204) Freyn (1880: 915) is one of these more generally accepted sections (Tutin & Cook 1964, 1993, Küpfer 1974, Cook *et al.* 1986, Hörandl *et al.* 2005, Hörandl & Emadzade 2012), albeit with some changes in its circumscription, such as the inclusion of sections *Acetosellifolii* Tutin (1964: 55) and *Chloeranunculus* Janehen ex Tamura (1991: 185, see Hörandl & Emadzade 2012), along with the exclusion of *R. kuepferi* Greuter & Burdet in Greuter & Raus (187: 452, see Huber 1988, Hörandl *et al.* 2005, Cosendai & Hörandl 2010, Cosendai *et al.* 2011). *Ranunculus* section *Ranuncella* is, according to Hörandl & Emadzade (2012), a monophyletic taxon, with a diversity mainly concentrated in the Iberian Peninsula and the Pyrenees (Jalas & Suominen 1989). The seven endemic species included in this section are: *Ranunculus abnormis* Cutanda & Willk. in Willkomm (1859-60: 83), *R. acetosellifolius* Boissier (1834: 5), *R. amplexicaulis* Linnaeus (1753: 549), *R. angustifolius* De Candolle (1808:10), *R. bupleuroides* Brotero (1805: 365) (Fig. 1), *R. cabrerensis* Rothmaler (1934: 148) and *R. pyrenaicus*

Habitat:—A species of dry meadows, areas of clear pine and open scrub, generally on siliceous and dry substrates (Franco 1971, Cook *et al.* 1986, Flora de Portugal Interactiva 2014). According to C. Aguiar (pers. comm.) and our observation, such grassland habitats could correspond to the alliance *Festucion merinoi* (*Agrostietalia castellanæ*, *Stipo giganteæ-Agrostietea castellanæ*) (Rivas-Martínez *et al.* 2011).

***Ranunculus cherubicus* (J.A.Sánchez Rodr., M.J.Elias & M.A.Martin) Fern.Prieto, Sanna, M.Pérez & Cires, comb. et stat. nov.**

Basionym:—*Ranunculus bupleuroides* subsp. *cherubicus* Sánchez Rodríguez *et al.* (2000: 402)

Holotype:—SPAIN. **Salamanca**: Sierra de Béjar, Collado Bonal, 6 June 1995, *J.A. Sánchez Rodríguez, SALA97639* (Sánchez Rodríguez *et al.* 2000).

Distribution:—see Fig. 1C.

Note:—This species includes two geographically isolated subspecies.

Ranunculus cherubicus* subsp. *cherubicus

Description:—referring to the original description of *Ranunculus bupleuroides* subsp. *cherubicus* by Sánchez Rodríguez *et al.* (2000; see Fig. 4).



FIGURE 4. *Ranunculus cherubicus* subsp. *cherubicus*; Collado Bonal, Sierra de Béjar, Candelario (Salamanca, Spain). Picture by Mauro Sanna.

Distribution:—Bejaran-Gredensean Sector [Carpetan-Leonese Subprovince, Mediterranean West Iberian Province, Mediterranean Region] (Sánchez Rodríguez *et al.* 2000, Rivas-Martínez 2007, Ramírez Rodríguez & Sánchez Rodríguez 2014).

Habitat:—This species occurs in oro-Iberian acidophilous stripped grasslands of the association *Agrostio rupestris-Armerietum bigerrensis* (*Minuartio bigerrensis-Festucion curvifoliae*; *Festucetalia curvifoliae*; *Festucetea indigestae*), that often grow in clearings of *Cytisus purgans* fields (*Cytision oromediterraneæ*; *Juniperetalia hemisphaericae*, *Junipero sabinæ-Pinetea Ibericae*; Rivas-Martínez *et al.* 2002, Rivas-Martínez *et alii* 2011, Sánchez Rodríguez *et al.* 2000, Ramírez Rodríguez & Sánchez Rodríguez 2014).

***Ranunculus cherubicus* subsp. *girelai* Fern.Prieto, Molero Mesa, Muñoz Díaz & Sanna, subsp. nov.**

Holotype:—SPAIN. **Granada**: Sierra Nevada, close to Puerto de La Ragua, the Morrón of the S. Juan slope, 2350 m, 37° 6' 12"N / 3° 2' 31"O, 25 June 2014, *J. Molero Mesa, M. R. González-Tejero & M. Ruiz Girela, GDA 61386*. Isotypes: *GDA 61387, GDA 61388, FCO 35282*.

Diagnosis:—Differs from the typical subspecies because of its larger size in all the parts, wider leaves and sepals partially or completely covered with floccose indumentum (Fig. 5).