Ranunculus pindicola sp. nov., the only species of the R. auricomus complex (Ranunculaceae) in Greece

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Source: Willdenowia, 45(2) : 223-230

Published By: Botanic Garden and Botanical Museum Berlin (BGBM)

URL: https://doi.org/10.3372/wi.45.45208

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Ranunculus pindicola sp. nov., the only species of the *R. auricomus* complex (*Ranunculaceae*) in Greece

Abstract

Dunkel F. G.: *Ranunculus pindicola* sp. nov., the only species of the *R. auricomus* complex (*Ranunculaceae*) in Greece – Willdenowia 45: 223–230. 2015. – Version of record first published online on 16 July 2015 ahead of inclusion in August 2015 issue; ISSN 1868-6397; © 2015 BGBM Berlin.

DOI: http://dx.doi.org/10.3372/wi.45.45208

Ranunculus auricomus s.l. (*Ranunculaceae*) has been found only a few times in Greece since its first finding there by Haussknecht in 1893, all records coming from the area of the Katara pass in the Pindos mountains. All populations seem to be identical, and the Greek plants are described and named here as *R. pindicola* sp. nov. The new species is geographically isolated; the nearest localities of other members of the *R. auricomus* complex are in the Rila mountains of Bulgaria and the Sila mountains of Calabria in S Italy. According to present knowledge, *R. pindicola* is a rare endemic species of C Greece. It is threatened by eutrophication or abandonment of its meadow sites.

Additional key words: apomictic, new species, Pindos mountains, pseudogamous

Introduction

Within the genus *Ranunculus* L., the *R. auricomus* complex comprises a rather well-defined group of about 800 mostly apomictic and pseudogamous Eurasiatic species (Ericsson 2001; Hörandl & al. 2009). It is characterized by a more or less heterophyllous leaf cycle, generation of carpellophores and haired nutlets. The number of petals varies from one to five – only characteristic for each species – and they may be completely absent.

After 1932, following the discovery of the pseudogamous reproduction mode by Rozanova (1932), the taxa were treated as a single species by most of the central European specialists. In contrast, the Scandinavian authors divided the complex into hundreds of (apomictic) subspecies und grouped them into four major "collective species" (*Ranunculus auricomus*, *R. cassubicus* L., *R. fallax* (Wimm. & Grab.) Sloboda and *R. monophyllus* Ovcz.). These four "species" are polyphyletic with ill-defined limits and transitions. Although the grouping and species concept is still under debate (Ericsson 1992; Hörandl 1998), all microspecies were recently treated unanimously as species as in other agamic complexes (Hörandl & Gutermann 1998; Dunkel 2010, 2014).

The presence of *Ranunculus auricomus* s.l. in Greece has been known for 130 years (Greuter & al. 1989; Dimopoulos & al. 2013). Haussknecht reported a collection of "*Ranunculus auricomus* L. β. *binatus* W. K." from subalpine spring communities at the Zygos pass during his excursion to Greece in 1885 ["in scaturiginosis jugi subalpini Zygos" (Haussknecht 1898: 98)]. Since then, only very few reports and collections have been added, all in the same area around the Katara pass of the Pindos mountains (Quézel & Contandriopoulos 1965; collections *A. Strid 24571* & *A. Strid 28937*). The assumption that *R. auricomus* "probably occurs in the forest zone elsewhere in N Greece" (Strid 1986) has not been confirmed. In 2011, during a visit to Metsovo, two populations of *R. auricomus* were found and have been studied more intensely. Morphologically, all populations

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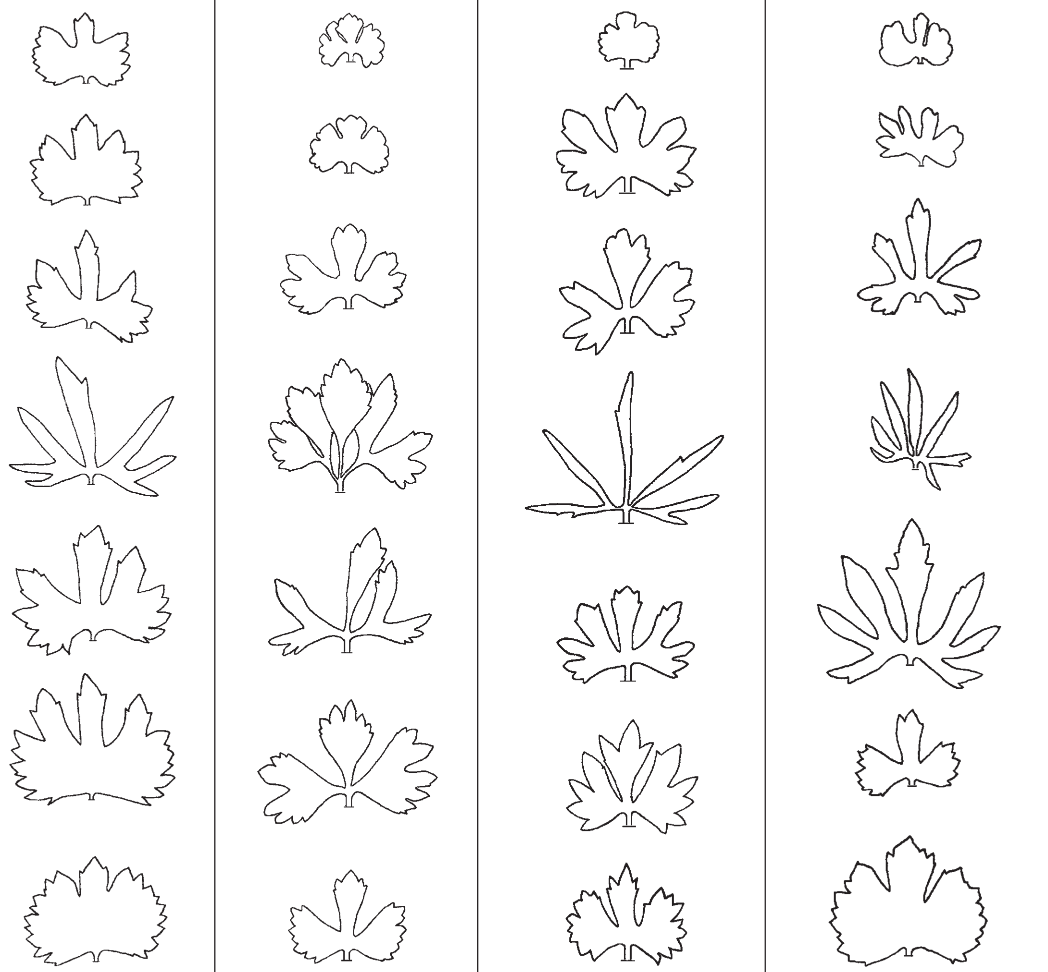
Table 1. Characters of *Ranunculus pindicola* and morphologically similar species in S and SE Europe.

Species	***R. pindicola***	***R. silanus***	***R. binatus***	***R. pseudobinatus***
Based on	isotypes	Du-23699	holotype	holotype
Basal leaf cycle first leaf second leaf third leaf fourth leaf fifth leaf sixth leaf seventh leaf				
middle segment	± parallel margins	deltoid to spatulate	cuneiform to deltoid	(narrowly elliptic) deltoid
middle segment of fourth leaf	unstalked	petiolate	small cuneiform, but unstalked	occasionally petiolate
main incision of final leaf	40–65 %	90–95 %	80–90 %	66–90 %
leaf margin	deeply crenate-serrate	crenate	crenate-serrate	crenate-serrate
Stem leaves	linear, entire	(linear) small deltoid, with ≤5 teeth	linear, entire	linear, entire
Gynoclinium				
pilosity	glabrous	glabrous	pilose	glabrous to scarcely haired
length	2.5–5.2 mm	2.5–4 mm	1.5–2.5 mm	2–3 mm
intervallum	<10 %	≤25 %	≤15 %	<10 %
Distribution area	Greece: Pindos mountains	Italy: Calabria, Sila mountains	Slovakia: Tatra	Romania: Transylvania

are identical and they are close but different to *R. binatus* Kit. ex Rchb., recently lectotypified (Dunkel 2011a). Due to their geographic isolation and morphological characters it seems justified to describe the Greek populations as a new species of the *R. auricomus* complex.

Material and methods

Material collected in Greece in 2011 was studied. Additionally, specimens from the herbaria Copenhagen (C) and Jena (J), including the collection by Haussknecht 1885 (J), were examined (herbarium codes according to Thiers 2015+). The private herbarium of F. G. Dunkel is indicated by the prefix "Du-" followed by the collecting number and herbarium number (which are the same).

Cultivated plants from collections by F. G. Dunkel were used for flow-cytometric analysis of DNA content (Dunkel, Paule & Gregor in prep.). DNA-ploidy levels were estimated by flow-cytometric analyses of fresh leaves using a Partec CyFlow space (Partec, Germany) fitted with a high-power UV LED (365 nm). Leaf tissues of the analysed sample and internal standard *Pisum sativum* (Doležel & al. 1992) were co-chopped using a razor blade in a plastic Petri dish containing 1 ml of ice-cold Otto I buffer [0.1 M citric acid, 0.5 % Tween

20 (Otto 1990; Doležel & al. 2007)]. The suspension was filtered through Partec CellTrics® 30 µm (Partec, Germany) to remove tissue debris and incubated for at least 10 min at room temperature. Isolated nuclei in filtered suspension were stained with 1 ml of Otto II buffer (0.4 M $Na_2HPO_4 \times 12H_2O$) containing the AT-specific fluorochrome 4´,6-diamidino-2-phenylindole (DAPI; 4 $\mu g \cdot ml^{-1}$) and β-mercaptoethanol (2 $\mu g \cdot ml^{-1}$). The relative fluorescence intensity was recorded for 3000 particles. Sample/standard ratios were calculated from the means of fluorescence histograms visualized using the FloMax v2.4d software (Partec, Germany). Only histograms with coefficients of variation (CVs) <5 % for the G_0/G_1 peak of the sample were considered.

Stained by carmine acetic, the pollen quality was determined according to Hörandl & al. (1997).

Species concept, definition of characters and depiction broadly follow Hörandl & Gutermann (1998) and Dunkel (2010).

Results

Species characteristics of the *Ranunculus auricomus* complex are best demonstrated and mediated by comparative illustrations, especially of the basal leaf cycle (Fig. 1–3). *Ranunculus pindicola* differs from all other similar species (*R. binatus*, *R. pseudobinatus* Soó and *R. silanus* Pignatti) by the almost parallel margins of the middle segment. The main incisions of the final leaves amount to 40–65 % compared to 66–90 % in *R. pseudobinatus*, 80–90 % in *R. binatus*, and 90–95 % in *R. silanus*.

The type population of *Ranunculus pindicola*, sample no. 030_RAN21B_01_Pisum300.fcs, collected at 39°47'11.9"N, 21°09'30.8"E, was investigated by flow cytometry. CVs for the G0/G1 peak of the samples were 1.35 % and 1.78 %, respectively. Two distinct classes of sample/standard ratios were identified: 0.66 (±0.04) and 1.33 (± 0.05) (Dunkel, Gregor & Paule in prep.). Cultivated plants from the type localities of *R. carpaticola* Soó [2*n* = 16 (Hörandl & Greilhuber 2002); 007_RAN25_01_Pisum300.fcs, Slovakia: Banská Bystrica Region, 1 km NW Revúca, 46°41'21.1"N, 20°07'28.6"E] and *R. vindobonensis* Hörandl & Gutermann [2*n* = 32 (Hörandl & al. 1997); 008_RAN26_01_Pisum300.fcs, Austria: Lower Austria, 0.3 km N Schwarzensee, 48°00'46.1"N, 16°03'43.6"E] served for calibration. The obtained values were 0.59 and 1.26, respectively. The ratio of 1.29 for *R. pindicola* indicates tetraploidy.

Staining by carmine acetic revealed 53 % normal pollen morphology.

Ranunculus pindicola Dunkel, **sp. nov.** – Fig. 1–3.
Holotypus: Greece, Ioannina, Metsovo, Zighos, 9–10 km vom Katara-Pass gegen Ioannina, W der Straße bei der Kapelle Profitis Ilias, 39°47'11.9"N, 21°09'30.8"E, 1335 m, Hochebene, Feuchtwiese mit *Narcissus radiiflorus*, 2 Jun 2011, leg. & det. *F. G. Dunkel* (B; isotypi: C, M, STR, Du-26710 [8 sheets], Du-26720 [7 sheets] [Du-26710 and Du-26720 belong to the same gathering despite the different collecting/herbarium numbers]).

Description — *Flowering shoot* (17–)22–45 cm tall, slender; *stalk* 1–2(2.9) mm in diam., suberect or moderately divergent (15–45°), with 1–5 flowers; *enrichment shoots* 0–2; *cataphylls* absent; *basal leaves* 1–4 per rosette, all apertures of basal leaves with wide-angled base ((100–)120–170°), occasionally fourth basal leaf truncate (170–190°) (Fig. 2). *First basal leaf* 11–14 mm long, cleft by main incision (55–60 %); middle lobe rectangular to deltoid, with 3 teeth; lateral lobes undivided or lobed (25–32 %); leaf margin irregularly crenate-serrate to serrate. *Second basal leaf* 16–22 mm long, cleft to divided by the main incision (55–75 %); middle lobe rectangular to deltoid, mostly with 3 teeth; lateral lobes lobed or cleft by the first lateral incision (25–45 %); leaf margin irregularly serrate. *Third basal leaf* 18–24 mm long, divided by main incision (66–93 %); middle lobe rectangular to deltoid, with 3–7 teeth; lateral lobes cleft by first lateral incision (35–65%); leaf margin deeply and irregularly serrate. *Fourth basal leaf* 28–40 mm long, foot-like divided or dissected by main incision (95–100 %); middle lobe lanceolate to cuneate, petiolate to 2 mm, undivided or with 2–5 teeth; lateral lobes petiolate to 10 mm, cleft or divided by first lateral incision (45–75 %, with angle up to 60°), lobed to cleft by second lateral incision (25–50 %); leaf margin deeply and irregularly crenate-serrate. *Fifth basal leaf* 24–30 mm long, divided by main incision (75–90 %), middle lobe rectangular to deltoid with 5–7 teeth; lateral lobes cleft by first lateral incision (40–60 %), rarely divided to 75 %; second lateral incision absent or to 37 %; leaf margin deeply and irregularly (crenate-serrate or) serrate. *Sixth basal leaf* 24–32 mm long, cleft to divided by wide main incision (50–75%); middle lobe rectangular to narrowly deltoid with 3–7 teeth; lateral lobes lobed or cleft by first lateral incision (25–45 %), second lateral incision absent or rarely to 35 %; leaf margin deeply and irregularly crenate-serrate or serrate. *Seventh basal leaf* 20–33 mm long, cleft by main incision (40–65 %); middle lobe rectangular or deltoid with 3–7 crenate teeth; lateral lobes undivided; leaf margin deeply and coarsely crenate-serrate or serrate. *Lowest stem leaf* divided into 7–9 segments, largest segment 24–55 mm long, 2.5–4 mm wide, narrowly lanceolate, undivided (Fig. 3). *Flowers: petals* 0–2(or 3), 6–9 mm long, 5–7 mm wide; *androclinium* 0.3–0.4 mm long; *gynoclinium* (ellipsoid to) cylindric, 2.5–5.2 mm long, 1.5–2 mm wide, glabrous, *intervallum* absent, *carpellophores* medium-sized, 0.05–0.2 mm long. *Fruits* 2–2.7 mm long; *beak* 0.4–0.6 mm long, uncinate.

Chromosome number — 2*n* = 32 (derived from C value of *Ranunculus pindicola* compared to *R. vindobonensis*; Dunkel, Paule & Gregor in prep.).

Fig. 1. *Ranunculus pindicola* – A: holotype; B: flower (from Du-26720-7); C: fruit (from Du-26720-7); D: receptacle (from holotype). – Scale bars: A: 5 cm; B, C, D: 2 mm.

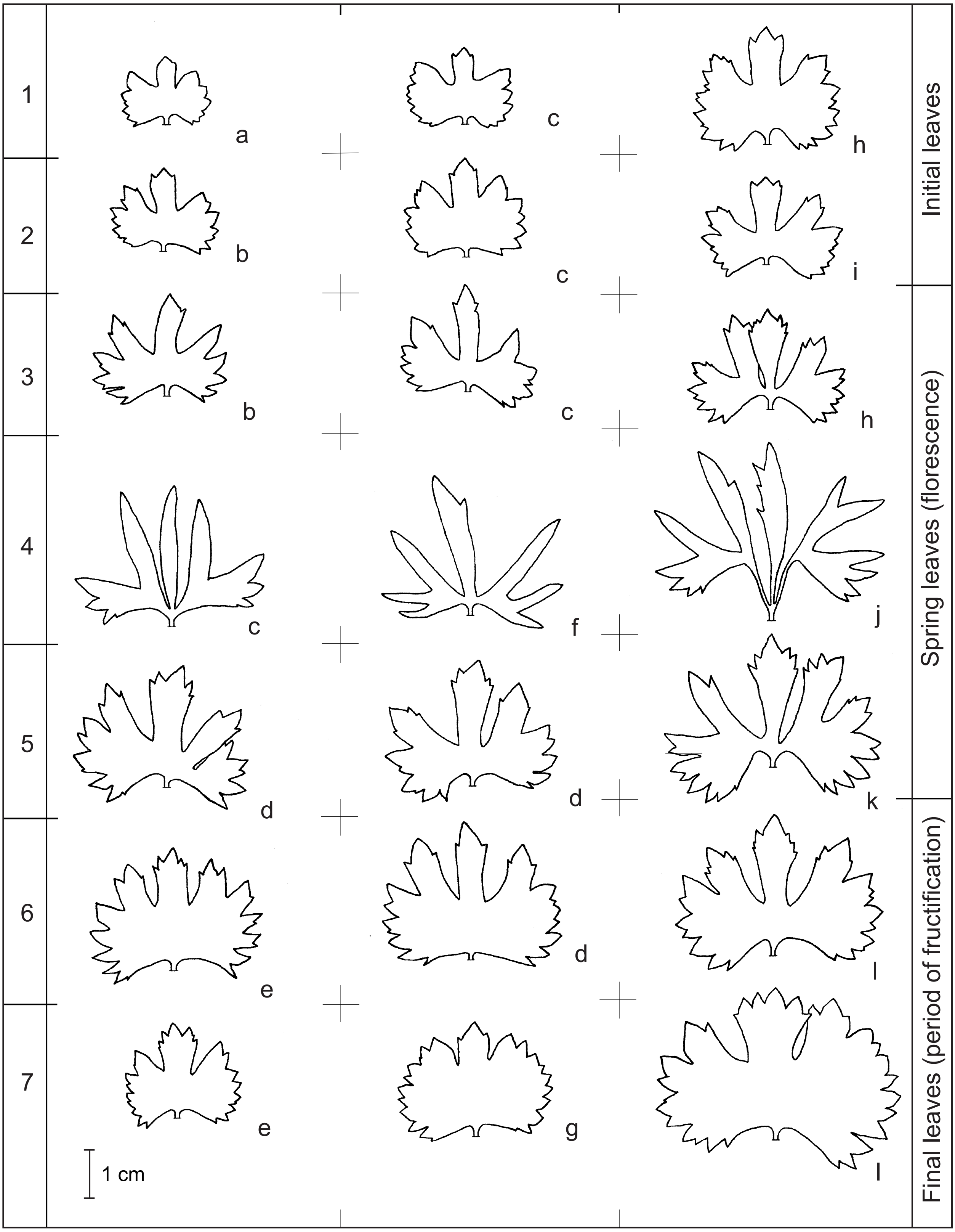


Fig. 2. *Ranunculus pindicola* basal leaf cycle; rows 1–7 represent first to seventh basal leaves; letters a–l represent different individual plants from specimens as follows: a, g: Du-26710-3; b: Du-26720-9; c: Du-26720-12; d: Du-26720-10; e: Du-26720-11; f: Du-26710-2; h & i: Du-26708-2; j: Du-26708-9; k: Du-26708-12; l: Du-26708-4.

Pollen viability — Low; 53% well developed.

Distribution — Greece, a local endemic of the Pindos mountains around the Katara pass (Fig. 4).

Ecology — All populations grow in light-exposed meadow communities, one in a damp fallow meadow along a small brook, partly enriched by *Juncus effusus* L., the other in winter-wet or winter-flooded meadows dominated by *Alopecurus rendlei* Eig with concomitant *Narcissus radiiflorus* Salisb. (*Alopecurion utriculati* Zeidler 1954). An exact analysis of the inhabited communities is still lacking. *Ranunculus pindicola* occurs fom 1180 m to 1700 m above sea level.

Conservation status — Endangered (EN B1ab(iii,v)+2ab (iii,v); D), according to IUCN (2012) criteria, due to a small and fragmented extent of occurrence and area of occupancy, with fewer than five subpopulations and an overall population of fewer than 250 mature individuals. The habitat of *Ranunculus pindicola* is severely endangered by risk of eutrophication or abandonment of damp meadows.

Etymology — The specific epithet means dweller in the Pindos mountains.

Additional specimens seen (paratypi) — Greece, Epirus (Ioannina regional unit), Pindos mountains, Metsovo, Pindus Tymphaeus: in summo montis Zygos (Lakmon veter.) supra Metzovo, substrato silicico-serpentino, 1370–1523 m, Jul 1885, *C. Haussknecht s.n.* (J), rev. O. Schwarz 1950 sub *Ranunculus binatus*, rev. F. G. Dunkel 2012 sub *R. pindicola* [with fruits, no basal leaves; 2 specimens]; ibid., Metsovo, Katara pass, 9–10 km from summit along road to Ioannina, meadows in opening of *Fagus* forest, gregarious in damp meadow, 1350 m, 18 May 1985, *A. Strid 24571* (C-8/2009-2), det. A. Strid sub *R. auricomus*, rev. F. G. Dunkel 2012 sub *R. pindicola*; ibid., Metsovo, 3 km from the village of Milea along road to Katara pass, meadows in opening of *Fagus* forest, gregarious in damp meadow, 1400 m, 39°50'N, 21°15'E, 7 May 1989, *A. Strid & al. 28937* (C-68/2009-1), det. A. Strid sub *R. auricomus*, rev. F. G. Dunkel 2012 sub *R. pindicola*; ibid., Metsovo, Milia, 1.5 km SW Milia, Talsenke, Talverebnung, Wiesengraben, Binsensumpf, 39°50'44"N, 21°12'56"E, 1180 m, 2 Jun 2011, *F. G. Dunkel* (Du-26708), det. F. G. Dunkel.

Reported localities in the literature — Greece, Pinde, bord des eaux: pozzines de Metzovon, vers 1700 m (Quézel & Contandriopoulos 1965); in scaturiginosis jugi subalpini Zygos P. T. [Pindus Tymphanos = Katara pass] (Haussknecht 1898).

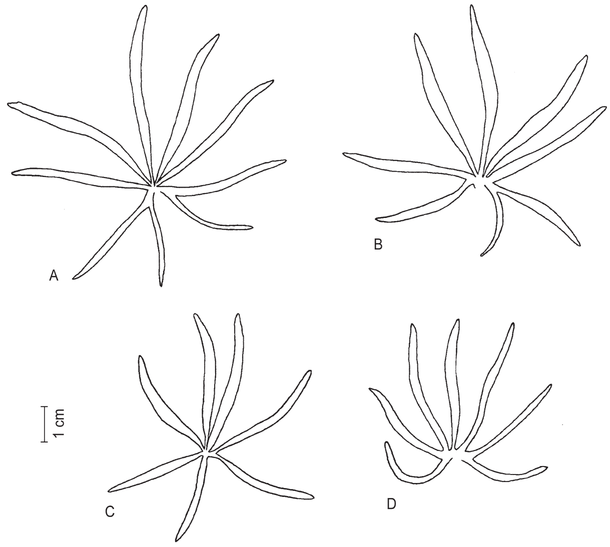


Fig. 3. Stem leaves of *Ranunculus pindicola* – A, D: holotype; B: isotype (Du-26720-6); C: isotype (Du-26710-7).

Fig. 4. Distribution of the *Ranunculus auricomus* complex in SE Europe: ■ = *Ranunculus pindicola*; ⬭ = *R. marsicus*; ▲ = *R. degenii*; ⬢ = *R. silanus*; ✸ = *R. pseudobinatus*.

Discussion

In contrast to other apomictic groups, e.g. *Alchemilla* and *Rubus*, many microspecies of the *Ranunculus auricomus* complex possess a small distribution area (Julin 1980; Jalas & Suominen 1989; Kurtto & al. 2007, 2010). At the S edge of the distribution area, the populations are geographically isolated (Grau 1984; Jalas & Suominen 1989; Dunkel 2011). The geographically closest populations of *R. auricomus* s.l. to *R. pindicola* represent undescribed lineages of the Rila mountains of S Bulgaria and *R. silanus* Pignatti from the Sila mountains of Calabria, S Italy, within a distance of c. 300 km and c. 400 km, respectively (Fig. 4; Dimitrov 2002; Dunkel 2011b).

Morphologically, *Ranunculus pindicola* differs from *R. silanus*, among others, by the parallel margins of the middle segments of the basal leaves, a smaller incision of the final leaves, and almost always entire stem leaves. The morphology and distribution area of *R. binatus* and *R. pseudobinatus* are poorly known. Since the publication by Soó (1964, 1965), there has been no progress in the knowledge of these taxa. The lectotype of *R. binatus* stems from the N parts of Slovakia (ancient region of Liptov) (Dunkel 2011a), the holotype of *R. pseudobinatus* (BP-62950) was collected in Transylvania, part of modern Romania. For Bulgaria, the taxa listed in Jordanov (1970) could not be attributed to some of the above-mentioned agamospecies; a modern investigation dealing with the *R. auricomus* complex and its distribution in Bulgaria is lacking.

Ranunculus binatus is characterized by a pilose receptacle, and *R. pseudobinatus* differs by its (mostly) petiolate middle segment of the fourth basal leaf and undivided lateral segments of the final basal leaves (for details, see Table 1).

Similar to the likely twin pair *Ranunculus degenii* Kümmerle & Jáv. from Macedonia and nearby Albania and S Serbia and *R. marsicus* Guss. & Ten. from the C Apennines, Italy, also *R. pindicola* and *R. silanus* may represent amphi-Adriatic relatives, originating during the Pleistocene with reduced sea level and a land bridge or at least islands between Italy and Greece. To prove this hypothesis, molecular research is strictly needed.

Although there may be similar morphotypes of *Ranunculus pindicola* in C Europe, including *R. variabilis* Hörandl & Gutermann, due to the geographic situation it is unlikely that these taxa are conspecific.

Over 95 % of all taxa of the *Ranunculus auricomus* complex s.str. screened for ploidy level are tetraploid (Rousi 1956; Lohwasser 2001; Dunkel & al. in prep.). Therefore, our results derived by flow-cytometric analysis and indicating tetraploidy for *R. pindicola* are not surprising. Together with poor pollen development (53 %) and only 0–2 petals per flower, *R. pindicola* should represent a newly described apomictic lineage with a small relict distribution area in damp meadows in the surroundings of the Katara pass (Strid 2002 sub *R. auricomus*). Even as an agamospecies, due to its rareness at the edge of the distribution area, *R. pindicola* deserves more attention, and it needs protection due to its occurrence in endangered habitats.

Acknowledgements

I would like to sincerely thank H.-J. Zündorf, curator of the Herbarium Haussknecht, Jena (J), and an unknown staff member of the Herbarium Copenhagen (C), for the opportunity of a loan. T. Gregor and J. Paule, both Frankfurt, kindly undertook the flow-cytometric measurement of cultivated material. I highly appreciate the assistance of J. Sandersson for improving the English text. I am also grateful for the helpful comments of two anonymous reviewers on an earlier version of the manuscript.

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