New diploid species in the Ranunculus auricomus complex (Ranunculaceae) from W and SE Europe

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New diploid species in the Ranunculus auricomus complex (Ranunculaceae) from W and SE Europe

Abstract: The Euro-Siberian complex of Ranunculus auricomus (Ranunculaceae) consists of about 800 mainly apomictic and polyploid taxa. So far, only four sexual species have been described (R. carpaticola Soó, R. cassubicifolius W. Koch, R. notabilis Hörandl & Gutermann and R. marsicus Guss. & Ten.). With the exception of R. marsicus and rare autotetraploids of R. cassubicifolius, sexuality seems to be linked to diploidy. Using flow cytometric ploidy estimation, flow cytometric seed screen and pollen quality, six new diploid sexual species have been revealed in C France, C and SE Slovenia and Croatia: Ranunculus austroslovenicus Dunkel, R. calapius Dunkel, R. cebennensis Dunkel, R. mediocompositus Dunkel, R. peracris Dunkel and R. subcarniolicus Dunkel. Here, they are described and depicted in detail. Sexual reproduction has been verified for R. austroslovenicus, R. cebennensis and also for the previously described R. envalirensis Grau and R. flabellifolius Heuff. ex Rchb.

Key words: Balkans, diploidy, France, new species, Ranunculus auricomus, sexuality, taxonomy

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Introduction

The Ranunculus auricomus species complex belongs to R. sect. auricomus and comprises about 800 taxa distributed throughout Greenland, Europe, W Siberia, Central Asia and Alaska, and from the arctic zone to the Mediterranean region (Soó 1965; Meusel 1965; Grau 1984; Jalas & Suominen 1989; Hörandl & al. 2009; Dunkel 2015). In recent years, the complex has been used as a model to study the mechanisms and evolutionary relevance of apomixis (e.g. Hörandl 2008, 2009; Hörandl & al. 2008; Hörandl & Temsch 2009; Hörandl & Hojsgaard 2012; Pellino & al. 2013). Hybridization and polyploidyization are strongly related to the evolutionary history of the buttercups (Paun & al. 2006b; Hojsgaard & al. 2014a, 2014b) and most of the known cytotypes are polyploid with aposporous and pseudogamous apomictic reproduction mode (Häfliger 1943; Rutishauser 1954a, 1954b; Rousi 1956; Izmailov1967, 1970, 1996; Nogler 1971, 1984, 1995).

In contrast to the great majority of apomictic taxa, only four sexual species have so far been described: Ranunculus carpaticola Soó, R. cassubicifolius W. Koch, R. marsicus Guss. & Ten. and R. notabilis Hörandl & Gutermann (Hörandl & Gutermann 1998b; Dunkel 2011). Ranunculus carpaticola is fairly widespread in beech, hornbeam and oak forests in the N and S Carpathians (Paun & al. 2006a). Diploid R. cassubicifolius is distrib-
uted disjunctly in wet meadows, swampy and riverine forests from Switzerland, Central Europe to Slovenia and Croatia (Hörandl 2002; Dunkel in prep.). Autotetraploids of R. cassubicus are reported for Austria (Hörandl & Greilhuber 2002; Hörandl & Gutermann 1998b). Ranunculus marsicus is a sexual species of the Apen-
nines with variable cytotypes (4x, 5x, 6x, 7x) (Spinosi 1998; Paule & al. 2018; Masci & al. 1994; Sgorbati & al. 1989). Triploid plants of R. marsicus var. incisior Dunkel in Pescoostanzo, Abruzzo (Italy), indicate either current or past presence of diploid cytotypes in the Abruzzo (Paule & al. 2018). Another diploid species, R. notabilis, has been detected in SE Austria (Hörandl & Gutermann 1998b) and has been hypothesized as a sexual progenitor of the whole complex (Hörandl & al. 2000). A diploid genome has further been reported for R. enva-
liensis Grau, a species of subalpine meadows from the W and C Pyrenees, and an unknown morpho-
type of C France (Diosdado & Pastor 1996; Nogler 1982 – sub “R. auricomus”). Sexual reproduction of these two taxa may be assumed, but information is only available for R. enva-
liensis.

Due to lack of clear boundaries between the main morphotypes, Marklund’s concept of four distinct species (Ranunculus auricomus L., R. cassubicus L., R. fallax (Wimm. & Grab.) Slob. and R. monophyllus Ovcz.) with numerous subspecies (subspecies apomicticae) has been abandoned in recent years (Marklund 1961, 1965; Eric-
sen 1992, 2001; Hörandl 1998; Hörandl & Gutermann 1998a). Without presenting monophyletic clades (Hörandl & al. 2009), but for practical reasons, the complex is still subdivided into two morphological collective groups: the “R. auricomus group” (thereto R. notabilis, R. enva-
liensis) and the “R. cassubicus group” (thereto R. car-
pticola, R. cassubicolius) (Hörandl & al. 2005). They differ strikingly in size and leaf shape (Löhwaasser 2001) as well as in relative genome size of diploids (Paule & al. 2018). The R. cassubicus group has basal sheaths with-
out leaf blades (cataphylla), large undivided basal leaves and normally broad cauline leaves with a toothed margin. The R. auricomus group is generally smaller, lacks basal sheaths and presents heterophyllous leaves. Depending on the individual taxon, basal leaves vary in shape: they can be deeply divided, while the upper stem leaves are linear with predominantly entire segments. Using isozyme data, the separation of sexual diploids from these two groups has been estimated at 900 000 years ago (Hörandl 2004).

For the understanding of the evolution of this variable apomictic species complex, detailed knowledge of the distribution and identity of diploid sexual taxa is crucial. In nutrition-poor humid meadows and moist forests of the Slovenian Prealps, as well as in floodplain forests close to Karlovac, Croatia, the first author found five new diploid morphotypes. Additionally, the diploid plants, already found by Nogler in the French Massif Central (1982) were categorized as Ranunculus auricomus and were not attrib-
uted to any of the known species. Here, we describe new-
ly discovered morphotypes as new species and compare them with previously known diploids from adjacent areas.

### Material and methods

Material was collected in France, Slovenia and Croatia from 2007 to 2017. For morphological analysis 10–30 plants of each site were investigated. One to eight plants of each taxon were transplanted and cultivated in the experimental garden (Karlstadt, Germany) and two to ten plants were collected as herbarium specimens for further investigation. Additionally, specimens from the herbaria Dresden (DR), Geneva (G), Göttingen (GOET), Jena (JE), Ljubljana (LJU), Munich (M) and Zurich (Z + ZT) were examined. All specimens collected are deposited in M and partly in other public herbaria as well as in the private herbarium of F. G. Dunkel (“Du”; collection number and herbarium number are identical). Collection details of transplanted plants are given in Table 1.

Geographical reference is given by coordinates or de-
dined grid fields in the form of quadrants of topographic maps. A quadrant encounters a latitude of 3° and a longi-
ditude of 5° and refers to the number of the official topo-
graphic maps of Germany with extrapolation to adjacent countries. For floristic purposes this reference system is widely used in Central Europe (e.g. Jogan 2001).

Fresh leaves from cultivated plants were used for flow cytomertic ploidy estimation using the standard two-step Otto protocol with AT-specific fluorochrome 4',6-diamidino-2-phenylindole (DAPI) as fluorescence stain (Otto 1990; Doležel & al. 2007). Pisum sativum L. ‘Citrad’ (Doležel & al. 1998) served as internal standard (Paule & al. 2018). Flow cytometric ploidy estimations (DNA-ploidy; Suda & al. 2006) were calibrated using a chromo-
some count of an individual: Ds-30442 (Paule & al. 2018).

Pollen quality was determined by carmine acetic staining according to Hörandl & al. (1997; see Fig. 4: 203). Two hundred pollen grains per specimen were in-
vestedigated.

For determination of the reproduction mode, several single mature seeds or 5–10 pooled seeds per plant were analysed by means of flow cytomertic seed screen (PCSS) according to Matz & al. (2000) using the two-step Otto protocol as outlined by Dobeš & al. (2013). For five out of the eight studied species, ripe fruits were available in cultivation (Table 1). The measurements were carried out both with and without the internal standard (Pisum sati-
vum) in order to determine also the ploidy of the embryo. The reproduction mode was assessed based on expected fluorescence ratios of embryo and endosperm nuclei as reviewed for a pseudogamous apomictic system (Dobeš & al. 2013) considering the threshold of 1.65 for discrimi-
ination between sexual and apomictic seeds (Schinkel & al. 2017).

Species concept, definition of characters and depic-
tion follow Hörandl & Gutermann (1998a) and Dun-
Table 1. Analysed material and results of different techniques. A microscopic chromosome count of *Ranunculus austroslovenicus* (Du-30442) served for calibration (2n = 16) for flow-cytometric ploidy estimation. In the column “herbarium”, an asterisk (*) indicates the holotype.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Taxon</th>
<th>Sample / standard fluorescence ratio</th>
<th>DNA ploidy (FCM)</th>
<th>Pollen stainability (%)</th>
<th>FCS (number of analysed single seeds / pooled seeds)</th>
<th>FCS (embryo ploidy)</th>
<th>FCSS (mean embryo fluorescence ratio)</th>
<th>Locality (country, ISO code 3166-2)</th>
<th>Collections date</th>
<th>Altitude (m)</th>
<th>Latitude (°N)</th>
<th>Longitude (°E)</th>
<th>Herbarium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Du-19642</td>
<td><em>R. austroslovenicus</em></td>
<td>0.68 2x</td>
<td></td>
<td></td>
<td></td>
<td>1.50</td>
<td></td>
<td>Lipje (SI)</td>
<td>2 May 2007</td>
<td>425</td>
<td>45.849444</td>
<td>14.257533</td>
<td>M</td>
</tr>
<tr>
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<td><em>R. austroslovenicus</em></td>
<td>0.67 2x</td>
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<td></td>
<td></td>
<td>1.50</td>
<td></td>
<td>Lipje (SI)</td>
<td>24 Apr 2012</td>
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<td>45.849517</td>
<td>14.258833</td>
<td>M</td>
</tr>
<tr>
<td>Du-30443</td>
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<td>0.69 2x</td>
<td></td>
<td>1/3 2x</td>
<td></td>
<td>1.50</td>
<td></td>
<td>Mrtvice (SI)</td>
<td>22 Apr 2013</td>
<td>470</td>
<td>45.677134</td>
<td>14.826326</td>
<td>M</td>
</tr>
<tr>
<td>Du-30441</td>
<td><em>R. austroslovenicus</em></td>
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<td>87.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Srobočnik (SI)</td>
<td>23 Apr 2013</td>
<td>250</td>
<td>45.462527</td>
<td>14.817019</td>
<td>LJU*, B, M</td>
</tr>
<tr>
<td>Du-30442</td>
<td><em>R. austroslovenicus</em></td>
<td>0.69 2x</td>
<td>0/10 2x</td>
<td>1.48</td>
<td></td>
<td></td>
<td></td>
<td>Nove Ložine (SI)</td>
<td>22 Apr 2013</td>
<td>465</td>
<td>45.684671</td>
<td>14.810336</td>
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</tr>
<tr>
<td>Du-34889</td>
<td><em>R. calapius</em></td>
<td>0.68 2x</td>
<td>96.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Orlovač (HR)</td>
<td>10 Apr 2017</td>
<td>141</td>
<td>45.518944</td>
<td>15.570917</td>
<td>ZA*, B, FR, M, WB, ZT</td>
</tr>
<tr>
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<td><em>R. calapius</em></td>
<td>0.68 2x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Orlovač (HR)</td>
<td>10 Apr 2017</td>
<td>141</td>
<td>45.518944</td>
<td>15.570917</td>
<td>ZA, B, FR, M, WB, ZT</td>
</tr>
<tr>
<td>Du-33349</td>
<td><em>R. cebernensis</em></td>
<td>0.67 2x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Saignes (FR)</td>
<td>6 May 2016</td>
<td>1060</td>
<td>45.603096</td>
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<td>94.2</td>
<td>7/3 x 5 2x</td>
<td></td>
<td>1.58</td>
<td></td>
<td>La Chalèlle (FR)</td>
<td>6 May 2016</td>
<td>910</td>
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<td>2.656944</td>
<td>LY*, B, M,</td>
</tr>
<tr>
<td>Du-29983</td>
<td><em>R. envaliensis</em></td>
<td>0.69 2x</td>
<td>1/3 2x</td>
<td>1.52</td>
<td></td>
<td></td>
<td></td>
<td>Solden (AD)</td>
<td>23 May 2013</td>
<td>1750</td>
<td>42.581466</td>
<td>1.662778</td>
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<td>98.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Eynre (FR)</td>
<td>23 May 2013</td>
<td>1600</td>
<td>42.463966</td>
<td>2.074866</td>
<td>M</td>
</tr>
<tr>
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<td><em>R. flabellifolius</em></td>
<td>0.70 2x</td>
<td>0/5 2x</td>
<td>1.50</td>
<td></td>
<td></td>
<td></td>
<td>Ciclova montană (RO)</td>
<td>21 Apr 2010</td>
<td>870</td>
<td>45.049444</td>
<td>21.771306</td>
<td>M</td>
</tr>
<tr>
<td>Du-28639</td>
<td><em>R. mediocompositus</em></td>
<td>0.69 2x</td>
<td>94.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lipje (SI)</td>
<td>24 Apr 2012</td>
<td>425</td>
<td>45.849444</td>
<td>14.257533</td>
<td>LJU*, B, M</td>
</tr>
<tr>
<td>Du-33260</td>
<td><em>R. peracris</em></td>
<td>0.68 2x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sajevce (SI)</td>
<td>25 Apr 2016</td>
<td>152</td>
<td>45.862778</td>
<td>15.438472</td>
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</tr>
<tr>
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<td><em>R. peracris</em></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Sajevce (SI)</td>
<td>25 Apr 2016</td>
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<td>15.438417</td>
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<td>96.2</td>
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<td></td>
<td></td>
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<td>Gajma (SI)</td>
<td>23 Apr 2013</td>
<td>153</td>
<td>45.891185</td>
<td>15.370462</td>
<td>LJU*, B, M</td>
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<tr>
<td>Du-28640</td>
<td><em>R. subcarniolicus</em></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Lipje (SI)</td>
<td>24 Apr 2012</td>
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<td>M</td>
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<tr>
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<td><em>R. subcarniolicus</em></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Grospulje (SI)</td>
<td>26 Apr 2016</td>
<td>325</td>
<td>45.944056</td>
<td>14.653083</td>
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<tr>
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<td>0.68 2x</td>
<td></td>
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<td></td>
<td></td>
<td>Grospulje (SI)</td>
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<td>14.652833</td>
<td>M</td>
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<tr>
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<td></td>
<td></td>
<td>Grospulje (SI)</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>Sajevce (SI)</td>
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<td>15.438417</td>
<td>M</td>
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<tr>
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<td>0.68 2x</td>
<td>97.6</td>
<td>4/5 2x</td>
<td></td>
<td>1.52</td>
<td></td>
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<td>8 Apr 2017</td>
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<td>14.648317</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Gospic (HR)</td>
<td>10 Apr 2017</td>
<td>571</td>
<td>44.529389</td>
<td>15.375083</td>
<td>M</td>
</tr>
</tbody>
</table>
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Kel (2010). All data sheets (Fig. 2, 4, 6, 8, 10, 12, 14) present the most important and specific characters of a species: the basal leaf sequence from the initial leaves (normally no. 1 and 2), the spring leaves evolving during the flowering period (no. 3–5) and the final leaves developing during the fruiting period (no. 6 and 7). The small letters next to each basal leaf denote the individual of a population. Furthermore, the lowermost cauline or stem leaf with its specific number and form of segments is characteristic. Values of the largest segment are indicated in the description. In all diploid (and sexual) species the flowers consist of five (rarely six or seven) well-developed petals. Finally, form and hairiness of the receptacle is illustrated at the bottom of the right column of the data sheet.

Results and Discussion

Twenty-three transplanted individuals were investigated by flow cytometry, 2–7 per newly described species. All studied accessions revealed the same sample/standard fluorescence ratio class as the chromosome counted individual *Dur-30442* (2n = 16) (Table 1). Hence, all six novel morphotypes as well as *Ranunculus emovaliensis* and *R. flabellifolius* are considered diploid. Interestingly, all newly studied accessions fall into the relative genome size range (i.e. sample / standard fluorescence ratio) of the “*R. auricomus* group” as published previously (Paule & al. 2018).

Fluorescence ratios of embryo and endosperm nuclei for seven studied individuals ranged from 1.48 to 1.63 (mean 1.53) and measured embryos were revealed to be diploid. Hence two individuals of *Ranunculus austroslovenicus* sp. nov., *R. cebennensis* sp. nov. as well as one individual of *R. emovaliensis*, *R. subcarinolius* sp. nov. and *R. flabellifolius* can be considered for sexual embryo development and meiotic origin of female gametophyte (Table 1).

Staining by carmine acetic revealed 87.7–97.6% well-developed pollen in seven studied individuals, representing all novel morphotypes. Pollen quality is considered in line with regular meiotic (i.e. sexual) pollen development if the pollen stainability is above 80% (Hörandl & al. 1997).

Species characteristics of members of the *Ranunculus auricomus* complex are best demonstrated by comparative illustrations, especially of the basal leaf cycle and the cauline leaves, which are illustrated in Fig. 2–15. Detailed descriptions of the new species are below.

As indicated above, two of the four known diploid species, i.e. *Ranunculus carpathicus* and *R. cassubicifolius* belong to the “*R. cassubicus* group” and differ strikingly by undivided leaf shape and size from the new diploid species. Diploid taxa studied here all belong to the “*R. auricomus* group” due to divided leaf shape, which is also confirmed by the relative genome size. *Ranunculus notabilis*, a member of the “*R. auricomus* group” from SE Austria, is characterized by a conspicuous heterophyllous basal leaf cycle and can be discerned from all of the new diploid species by long rounded teeth of the leaf edge, especially of the spring leaves (Hörandl & Gutermann 1998c).

1. *Ranunculus austroslovenicus* Dunkel, sp. nov.

Holotype: Slovenia, Preddinarsko Območje, 0554.2, Kočejev, an der Kolpa 1 km vor Srobotnik, [on the rivulet Kolpa 1 km before Srobotnik], 45°29′43″N, 14°48′14″E, 250 m, Gebüscheid, Waldrand, [shrubbery, forest edge], 23 Apr 2013, F. G. Dunkel 30441 (LJU; isotypes: B, M, herb. Dunkel). – Fig. 1–3, Table 1.

Description — Flowering shoot gracile to slim, 11–28 cm tall, stalk 0.7–2.2 mm in diam., suberect to patent, angle between main and secondary axis 20–70°, flowers 1–6, enrichment shoots 0 or 1(0–2); basal leaves 3–5 per rosette. Basal leaf cycle: leaf edge (coarsely) crenate in first and second leaf, crenate to serrate-serrate in following leaves. First basal leaf 13–18 mm long, second basal leaf 15–20 mm long, with narrow-angled to V-shaped base (50–100°), main incision absent or up to 60% and 50%, respectively. Middle segment absent, or rectangular to broadly deltoid with 3(–5) in first, or 3–5 crenate teeth in second basal leaf; lateral segment undivided. Third basal leaf 15–30 mm long, with narrow-angled base (10–60°), divided to dissected by main incision (98–100%), middle lobe up to 3 mm stalked, deltoid to spatulate, occasionally triletted with incisions up to 40%, with 7–11 crenate teeth, lateral edge concave to convex; lateral segment stalked up to 3 mm, cleft to divided by first lateral incision (40–85%), second lateral incision absent or up to 50%. Fourth basal leaf 18–26 mm long, narrow- to wide-angled base (20–135°), divided by main incision (90–98%), middle lobe deltoid, occasionally trilobed with incisions up to 32%, with 3–5 crenate teeth, lateral edge concave to convex; lateral segment cleft by first lateral incision (32–60%). Fifth to seventh basal leaf 22–28 mm, 22–32 mm and 16–30 mm long, respectively, with narrow-angled (to V-shaped) base (10–60°, 20–100° and 20–60°, respectively); main incision absent or up to 80% in fifth and up to 45% in sixth basal leaf; if middle lobe present, in fifth leaf deltoid to broadly deltoid with straight to concave lateral edge and with 5–7 crenate teeth, in sixth leaf middle lobe trapezoid with straight lateral edge and mostly 5 crenate teeth; lateral segment undivided. Lowermost stem leaf divided into 5–7 segments, largest segment 18–52 mm long, 2.5–7 mm wide, occasionally linear, mostly narrowly lanceolate, undivided or with up to 4 small teeth, rarely with 1 or 2 patent, up to 11 mm long teeth. Petals 5, 10–12 mm long, 7–9 mm wide; androclinium 0.8–1.2 mm long, filaments up to 5 mm long; receptacle globose to ellipsoid, 1.5–4.3 mm long, 1.4–1.7 mm
wide, glabrous, invervallum occasionally up to 0.5 mm long (or 15 %), carpellophores 0.05–0.2 mm long; fruits 0.8–2.0 mm long, beak 0.4–0.8 mm long, uncinate.

**Pollen quality** — 87.7 % well developed (Du-30441, Table 1)

**DNA-ploidy** — 2x (Table 1).

**Chromosome number** — 2n = 16 (Du-30442, Table 1).

**Distribution** — From the Planinsko Polje SW of Ljubljana to the Kočevsko region in SE Slovenia, and adjacent Croatia (Fig. 1).

**Ecology** — Meadows, brushes, riparian forests, beech and hornbeam forests.

**Etymology** — The epithet refers to the main distribution in S Slovenia.

**Taxonomy** — Although the basal leaf sequence is heterophyllous, the round undivided leaves are prominent. The lateral segments of leaf no. 1, 2, 5–7 are undivided, occasionally the first lateral incision amounts up to 60 %. In general, 2–4(or 5) basal leaves are undivided, the blade at the base is mostly narrow-angled. In contrast to the most species of the complex, the third and not the fourth or fifth basal leaf is mostly divided.

Similar species are *Ranunculus glechomoides* (Markl.) Ericsson, *R. subglechomoides* Dunkel and *R. suborbicularis* Dunkel. *Ranunculus austroslovenicus* is readily distinguished by complete flowers with five petals; the basal apertures of the final leaves of *R. glechomoides* and *R. suborbicularis* are closed, the basis even overlapping in contrast to an angle of 20–60° in *R. austroslovenicus*.

The form of the middle lobes of the spring leaves is variable but smaller, only deltoid to broadly deltoid and not spatulate as in *Ranunculus subglechomoides*. For *R. austroslovenicus*, sexual reproduction is suggested (Table 1).

**Specimens seen** — SLOVENIA: Dinarsko Območje, 0151.4, Logatec, Planinsko Polje, 500 m nordöstlich Liplje, beidseits der Straße Planina-Laze, 100 m südlich P. 446, 45°51’00"N, 14°14’59"E, 445 m, magere Wirtschaftswiese, Flachmoor, 2 May 2007, F. G. Dunkel 19642 & 19645 (herb. Dunkel); ibidem, O Liplje, W der Straße Planin-
Fig. 2. Data sheet (basal leaf cycle, stem leaves, flower, fruits, receptacle) of *Ranunculus austroslovenicus* (for explanation see under Material and methods). Scale bars in detail photographs = 2 mm.
Laze, 300 m S P. 446, 45°50'44"N, 14°15'19"E, 430 m, magere Wirtschaftswiese, Flachmoor, 24 Apr 2012, F. G. Dunkel 28608 (herb. Dunkel); ibidem, W der Straße Planin-Laze, etwa auf Höhe P. 446, 200–300 m S Flüsschen Unica, 45°50'54"N, 14°15'27"E, 445 m, magere Wirtschaftswiese, Flachmoor, 24 Apr 2012, F. G. Dunkel 28627 (herb. Dunkel); Preddinarsko Območje, Kočevja, Kočevsko polje, 0354.2, Nove Ložine, 200 m severno od Novih Ložin, 45°41'17"N 14°48'54"E, 470 m, ob gozdni poti, ki pa je velikokrat poplavljena, 17 Apr 1999, M. Zupančič (LJU-10046603), det. F. G. Dunkel; Nove Ložine, gegenüber dem Ort, SW SS 106, 45°41°04"N,
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14°48′47″E, 465 m, Waldchen, 22 Apr 2013, F. G. Dunkel 30442 (herb. Dunkel); ibidem, cultivated, 22 Apr 2015, F. G. Dunkel 32593 (herb. Dunkel); ibidem, Mrtvice, 45°40′40″N, 14°49′35″E, 460 m, gozd doba belega gabra, [forest of Quercus robur and Carpinus betulus], stadium ćrno jelša, [stadium with Alnus glutinosa], 10 May 1981, M. Accetto (LIU-10120552), det. F. G. Dunkel; ibidem, Mrtvice, O der SS 106, c. 200 m N des nördlichen Ortsrandes, 45°40′37″N, 14°49′35″E, 470 m, Laubwald, 2 Apr 2013, F. G. Dunkel 30443 & 30444 (herb. Dunkel); ibidem, Mrtvice, O der Straße, N Tankstelle, 45°40′37″N, 14°49′34″E, 470 m, feuchter Laubwald, 23 Apr 2013, in cultivation 3 May 2013, F. G. Dunkel 29818 (herb. Dunkel); ibidem, 0454.1, in cultivation, 20 Apr 2016, F. G. Dunkel 33175 (herb. Dunkel); 0454.4, Mirtoviči, ob poti proti Sv. Ani, 45°30′25″N, 14°47′50″E, 260 m, Laubwald, 10 May 1981, I. Štirme (LIU-10046607), det. F. G. Dunkel; ibidem, Sروبnik ob Kolpi, doline zgozuje črno jelša, [stadium with moderately divergent (patent), angle between main and lateral segment undivided or cleft by first lateral incision (up to 60%)]. Second basal leaf 22–34 mm long, narrowly angled at base (40–80%), divided by main incision (95–100%), middle lobe deltoid with 3–5 crenate teeth, lateral edge straight to slightly concave; lateral segment undivided or cleft by first lateral incision (26–65%), second lateral incision absent (or rarely up to 40%). Third basal leaf 26–37 mm long, narrowly to wide-angled at base (60–110%), dissected by main incision, middle lobe deltoid to cleft at 4 mm, deltoid to broadly deltoid, tricleft with incisions up to 50%, with 3–7 crenate teeth, lateral edge straight (to slightly concave); lateral segment divided by first lateral incision (70–85%), cleft by second lateral incision (33–45%), lobed to cleft by third lateral incision (25–40%). Fourth basal leaf 34–44 mm long, wide-angled to truncate at base (130–180°), dissected by main incision, middle lobe up to 10 mm long stalked, narrowly deltoid to lanceolate, integer or with 2–4 teeth, lateral edge straight to concave; lateral segment divided by dissected by first lateral incision (70–100%), cleft by second lateral incision (32–65%), third lateral incision absent or up to 30%. Fifth basal leaf 36–54 mm long, narrow- to wide-angled at base (35–130°), divided by main incision (95–100%), middle lobe up to 4 mm long stalked, rhomboid to deltoid with 5–7 teeth, lateral edge straight; lateral segment divided by first lateral incision (80–95%), cleft by second lateral incision (50–66%), third lateral incision absent or up to 37%. Sixth basal leaf 27–44 mm long, narrow-angled to V-shaped at base (45–100°), divided by dissected by main incision (95–100%), middle lobe deltoid to rectangular with 3–5 teeth; lateral segment undivided. In robust plants sometimes intermediate leaves developed: 40–50 mm long, closed to narrowly-angled at base (10–30°), dissected by main incision, middle lobe deltoid to tripartite with incisions up to 77%; lateral segment divided by first lateral incision (90–95%), divided by second lateral incision (66–75%), cleft by third lateral incision (33–60%); leaf edge crenate-serrate. Lowermost stem leaf divided into 7–9 segments, largest segment 33–58 mm long, 3–6 mm wide, narrowly lanceolate, occasionally stalked, with 0–2 slightly patent teeth. Petals 5, 10–12 mm long, 7–8 mm wide; androclinium 0.8–1.1 mm long, receptacle obovate, 2.4–3.2 mm long, 1.6–2.2 mm wide, (glabrous to scarcely pilose, short involuvalum occasionally present, carpellophores 0.25–0.5 mm long; fruits 1.7–2.2 mm long, beak 0.6–1.0 mm long, straight to uncinate.

2. Ranunculus calapius Dunkel, sp. nov.
Holotype: Croatia, Turopolje, 0459.3, Karlovac, Orlovačke oblasti, 1.1 km SE des Ortes [of the village], W Sisačka ulica, 45°31′08.2″N, 15°34′15.0″E, 115 m, feuchter Bachauenwald, 23 Apr 2013, F. G. Dunkel 32736 & S. Wittwer (herb. Dunkel), det. F. G. Dunkel; Croatia, Primorje-Gorski Kotar, 0555.1, Brod na Kupi, 1.3 km SW Friedhof und Kirche Vinica, nahe N-Ufer der Kupa, 45°27′08″N, 15°14′30″E, 1 Jun 1981, D. Kordiš (LIU-10046609), det. F. G. Dunkel; Karlovac, Orlovačke oblasti, 1.1 km SE des des Ortes [of the village], W Sisačka ulica, 45°31′08.2″N, 15°34′15.0″E, 115 m, feuchter Bachauenwald, 23 Apr 2013, F. G. Dunkel 32736 & S. Wittwer (herb. Dunkel), det. F. G. Dunkel. – Fig. 1, 4, 5, Table 1.

Description — Flowering shoot slim to robust, 22–40 cm tall, stalk 1.0–3.0 mm in diam., suberect to moderately divergent (patent), angle between main and secondary axis (15–55°), flowers (1 or)2–5–7, enrichment shoots 0–2; basal leaves 2–5(or 6) per rosette. Basal leaf cycle: leaf edge of first basal leaf crenate, of following ones irregularly crenate-serrate. First basal leaf 16–26 mm long, blade at base closed to narrowly-angled (5–50°), divided by dissected by main incision (90–100%), middle lobe deltoid to spatulate with 3–5 crenate teeth, lateral edge straight to slightly concave; lateral segment undivided or cleft by first lateral incision (up to 60%). Second basal leaf 22–34 mm long, narrowly-angled at base (40–80°), divided by dissected by main incision (95–100%), middle lobe deltoid with 3–5 crenate teeth, lateral edge straight (to slightly concave); lateral segment undivided or cleft by first lateral incision (26–65%), second lateral incision absent (or rarely up to 40%). Third basal leaf 26–37 mm long, narrowly to wide-angled at base (60–110°), dissected by main incision, middle lobe deltoid to cleft at 4 mm, deltoid to broadly deltoid, tricleft with incisions up to 50%, with 3–7 crenate teeth, lateral edge straight (to slightly concave); lateral segment divided by first lateral incision (70–85%), cleft by second lateral incision (33–45%), lobed to cleft by third lateral incision (25–40%). Fourth basal leaf 34–44 mm long, wide-angled to truncate at base (130–180°), dissected by main incision, middle lobe up to 10 mm long stalked, narrowly deltoid to lanceolate, integer or with 2–4 teeth, lateral edge straight to concave; lateral segment divided by dissected by first lateral incision (70–100%), cleft by second lateral incision (32–65%), third lateral incision absent or up to 30%. Fifth basal leaf 36–54 mm long, narrow- to wide-angled at base (35–130°), divided by main incision (95–100%), middle lobe up to 4 mm long stalked, rhomboid to deltoid with 5–7 teeth, lateral edge straight; lateral segment divided by first lateral incision (80–95%), cleft by second lateral incision (50–66%), third lateral incision absent or up to 37%. Sixth basal leaf 27–44 mm long, narrow-angled to V-shaped at base (45–100°), divided by dissected by main incision (95–100%), middle lobe deltoid with 3–7 crenate teeth, lateral edge straight; lateral segment undivided. In robust plants sometimes intermediate leaves developed: 40–50 mm long, closed to narrowly-angled at base (10–30°), dissected by main incision, middle lobe deltoid to tripartite with incisions up to 77%; lateral segment divided by first lateral incision (90–95%), divided by second lateral incision (66–75%), cleft by third lateral incision (33–60%); leaf edge crenate-serrate. Lowermost stem leaf divided into 7–9 segments, largest segment 33–58 mm long, 3–6 mm wide, narrowly lanceolate, occasionally stalked, with 0–2 slightly patent teeth. Petals 5, 10–12 mm long, 7–8 mm wide; androclinium 0.8–1.1 mm long, receptacle obovate, 2.4–3.2 mm long, 1.6–2.2 mm wide, (glabrous to scarcely pilose, short involuvalum occasionally present, carpellophores 0.25–0.5 mm long; fruits 1.7–2.2 mm long, beak 0.6–1.0 mm long, straight to uncinate.
Fig. 4. Data sheet (basal leaf cycle, stem leaves, flower, fruits, receptacle) of Ranunculus calapius (for explanation see under Material and methods). Scale bars in detail photographs = 2 mm.
Fig. 5. Holotype of Ranunculus calapius Dunkel, F. G. Dunkel 34889 (ZA).
Pollen quality — 96.8% well developed (Du-34889, Table 1).

DNA-ploidy — 2x (Table 1).

Distribution — Known only from the type locality near Karlovac in the alluvial zone of the river Kulpa (Fig. 1).

Ecology — Softwood floodplain forest together with *Fritillaria* sp. and *Leucojum aestivum* L., deciduous forest.

Etymology — The epithet derives from Calapius, the Latin name for the river Kolpa, which is situated close to the type locality.

Taxonomy — The sequence of the basal leaves is heterophyllous and characterized by three different types of basal leaves. The initial leaves (no. 1–3) possess a broadly deltoid to spatulate middle lobe, and the leaf edge has obtuse crenate teeth or it is only crenate. The spring leaves (no. 4 and 5) present often stalked middle lobes in narrowly deltoid to lanceolate form with deep and wide lateral incisions. In contrast, the lateral segments of the final leaves (no. 6 and 7) are undivided. In the last leaf of the cycle the main incision is reduced to at most 65% or even lacking. In robust plants, sometimes intermediate leaves (Lohwasser 2001) are developed, obviously between basal leaves no. 4 and 5 of the cycle. They have a dissected blade by the main incision and three mostly deep lateral incisions, resembling the basal leaf of *Ranunculus acris* L.

In the adjacent alluvial forests around Karlovac similar populations grow with a more homophyllous leaf sequence (Dunkel in prep.) They differ by more obtuse teeth of the basal and cauline leaves. In these populations, no diploid plants could be found, and they are therefore not a subject of this paper. The trisection of the leaf cycle is similar in *Ranunculus pedemontanus* Dunkel (Dunkel 2011) and maybe a characteristic feature of the species at the S edge of the distribution area. *Ranunculus calapius* differs from *R. pedemontanus* by even smaller middle and lateral lobes of leaf no. 4, stem leaves with some teeth and longer carpellophores. In this group of species the whole leaf sequence is needed for determination (Hörandl & Gutermann 1998c).

Specimens seen — CROATIA: Turopolje, 0459.3, Karlovac, 1.1 km SE Orlovac, W Sisačka ulica, 45°31’08.5”N, 15°34’15.3”E, 115 m, feuchter Hainbuchen-Auenwald [swampy hornbeam alluvial forest], 10 Apr 2017, F. G. Dunkel 33354 (herb. Dunkel), det. F. G. Dunkel. – Fig. 7, Table 1.

Description — Flowering shoot gracile, 12–22(–26) cm tall, stalk 0.4–1.5 mm in diam., flexible, angle between main and secondary axis 30–45°, i.e. plant moderately divergent, number of flowers 1( or 2), enrichment shoots 0 or 1( or 2), cataphylls absent, basal leaves 2–5(–7) per rosette. Basal leaf cycle divided into three parts: first to third basal leaf similar with lateral segments almost undivided, fourth leaf deeply divided and pedate. Fifth to seventh leaf again similar and lateral undivided segments. *First to third basal leaf* 5–12 mm, 8–12 mm and 9–14 mm long, respectively, with closed to narrow-angled base (0–50%), first and second basal leaf cleft to split by main incision (50–90%), third basal leaf dissected by main incision; middle segment deltoid to spatulate, lateral edge of third leaf slightly concave; lateral segment undivided or at third leaf occasionally cleft by first lateral incision (5–62%), lobed by second lateral incision (up to 33%); leaf edge of first and second leaf crenate, at following leaves irregularly crenate-serrate.

*Fourth basal leaf* 14–22 mm long, with narrow-angled (to V-shaped) base (30–80[–90]°), divided or dissected by main incision (90–100%), middle lobe stalked up to 5 mm, deltoid, partly tripartite with incisions up to 25%, with (2 or)3–5 crenate teeth, lateral edge straight to convex; lateral segment divided by first lateral incision (75–90%), cleft by second lateral incision (40–60%), third lateral incision absent or up to 33%; *Fifth and sixth basal leaf* 10–23 mm long, with narrow-angled to V-shaped base (40–70° and 50–110°, respectively), divided by main incision (75–95% and 66–80%, respectively), middle lobe deltoid, with (4 or)5–7 crenate teeth, lateral edge slightly concave, occasionally at sixth basal leaf middle lobe broadly deltoid with straight leaf edge; lateral segment undivided or cleft by first lateral incision (up to 50% or 45%, respectively). *Seventh basal leaf* 8–20 mm long, with narrow-angled base (40–70°), main incision absent or leaf cleft by main incision (up to 60%), middle lobe absent, rectangular or deltoid with 3–5 crenate teeth; lateral segment undivided. *Lowermost stem leaf* divided into 7–9 segments, largest segment 10–28 mm long, 1–3 mm wide, mostly undivided, rarely with 1 or 2 short teeth or trifid and narrowly deltoid. *Petals* 5, 7–11 mm long, 6–9 mm wide; *androcliniun* 0.7–1.0 mm long; *receptacle* globose (or ellipsoid), 1.5–2.6 mm long, 1.4–2.1 mm wide, glabrous, involvallum absent, carpellophores 0.2–0.4 mm long; *fruits* 1.5–2.4 mm long, beak 0.5–0.8 mm long, uncinate to involute.

Pollen quality — 94.2% well developed (Du-333349, Table 1).

DNA-ploidy — 2x (Table 1).

3. *Ranunculus cebennensis* Dunkel, sp. nov.

Holotype: France, Auvergne, Puy-de-Dôme, Verbins; [connecting road between D 29 and D 203, S La Chaleille], 45°33’38”N, 02°39’25”E, 910 m, Hangweide, [steep meadow], 6 May 2016, F. G. Dunkel 333354 (LY; isotypes: B, M, herb. Dunkel), det. F. G. Dunkel. – Fig. 1, 6, 7, Table 1.
Fig. 6 – Data sheet (basal leaf cycle, stem leaves, flower, fruits, receptacle) of *Ranunculus cebennensis* (for explanation see under Material and methods). Scale bars in detail photographs = 2 mm.

<table>
<thead>
<tr>
<th>Fig. 6</th>
<th>1–7: Du-33354-2</th>
<th>1,2: Du-33354-3; 3: 33354-5; 4: 33341-1; 5–7: 33349-5</th>
<th>1,2: Du-33354-5; 3–7: 33354-2</th>
<th>cauline leaves: Du-33354-1; 33354-6; 33354-3</th>
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*Ranunculus cebennensis*
Fig. 7. Holotype and isotypes of *Ranunculus cebennensis* Dunkel, F. G. Dunkel 33354 (holotype at LY; isotypes at M).
**Distribution** — Massif Central: around the Monts Dore, at the Monts Vivarais, at Margeride (La Chapelle-Laurent) (Fig. 1).

Populations of the *Ranunculus auricomus* complex are rather common in the Auvergne (Antonetti & al. 2006). Therefore, indications of the occurrence are rather preliminary. Specimens originating from Ardèche and Lozère were not sufficient for secure determination, but the occurrence in these departments is probable. On the other hand, at the Margeride (Chapelle-Laurent) only tetraploid plants were found morphologically rather similar to *R. marsicus*.

**Ecology** — Nutrient-poor to mesotrophic meadows over basalt, talus; from 1050–1220 (–1400) m.

**Etymology** — The epithet derives from the Gaulish name “Cebenna”. It signifies “round mountain” or “back of a mountain”. The Cévennes are the SE part of the Massif Central.

**Taxonomy** — *Ranunculus cebennensis* belongs to a group of morphologically related species of meadows of the subalpine or montane zone, normally above 1000 m. These taxa are characterized by almost undivided initial and final leaves with crenate or broadly crenate-serrate leaf edge. This group, provisionally named the *R. marsicus* group (Dunkel 2012), consists besides *R. cebennensis* of *R. degenii* (Albania, Macedonia and Serbia), *R. envalirensis* Grau (Andorra, France and Spain: Pyrenees) (Grau 1984), *R. marsicus* Guss. & Ten. (Italy: Abruzzo) and *R. subtubatricus* Jasiewicz (Poland: Tatra).

*Ranunculus cebennensis* represents a strongly heterophyllous leaf cycle. Unique to the species is the fourth basal leaf: deeply divided in a pedate manner with a second lateral incision (Fig. 6). In contrast to *R. envalirensis*, the lateral segments of the fifth to seven basal leaf of *R. cebennensis* are almost undivided and similar to *R. marsicus*.


**Pollen quality** — 98.2% well developed (Du-29988-3, Table 1).

**DNA-ploidy** — 2x (Table 1)

**Distribution** — *Ranunculus envalirensis* seems to be restricted to the C and E Pyrenees (Diosdado & Pastor 1996: 169) (Fig. 1).

**Ecology** — Subalpine meadows.

**Etymology** — The epithet refers to the type locality at Puerto de Envalira.

**Taxonomy** — *Ranunculus envalirensis* represents a species consisting of plants with a more divided blade of the basal leaves and a tendency to develop more than two enrichment shoots with intermediate leaves presenting deep incisions of middle and lateral lobes (Fig. 8). In contrast, within populations of *R. envalirensis*, plants with less divided blades of the basal leaves sometimes occur. These are characterized by the main incisions being up to 85% instead of 100% and mostly lacking lateral incisions (Fig. 8) [leaves h–i, 9]. This demonstrates a broader morphological variability than in almost any apomictic taxon.

Morphologically, *Ranunculus envalirensis* is close to *R. cebennensis* and due to a modificative variability
Fig. 8. Data sheet (basal leaf cycle, stem leaves, flower, fruits, receptacle) of *Ranunculus envalirensis* (for explanation see under Material and methods). Scale bars in detail photographs = 2 mm.

<table>
<thead>
<tr>
<th>1. holotype: 2, 3, 6, 7; Du-29983-4, 4, 5: holotype</th>
<th>1–6: Du-29983-6; 7: 29983-5</th>
<th>1–6: Du-29984-2; 7: 29987-3</th>
</tr>
</thead>
</table>

- **1**: holotype
- **2, 3, 6, 7**: Du-29983-4, 4, 5: holotype
- **1–6**: Du-29983-6; **7**: 29983-5
- **1–6**: Du-29984-2; **7**: 29987-3

- **1 cm**

**Ranunculus envalirensis**

- Flower: holotype; fruits, receptacle: Du-29813-1;
- Petals: 5; beak of fruit: erect to uncinate;
- Receptacle: glabrous

**Intermediate leaves**: Du-29985-1

**Cauline leaves**: Du-29983-6; 29987-2; 29983-1

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Fig. 9. Specimens of *Ranunculus envalirensis*. – Upper three plants and lower left plant: *F. G. Dunkel* 29983 (herb. Dunkel); lower right plant: *F. G. Dunkel* 29987 (herb. Dunkel).
both species are sometimes difficult to differentiate, if the complete basal leaf sequence is not developed or not available. Further investigations, especially in the S parts of the Massif Central, must elucidate whether *R. envalensis* extends its geographical range to C France.

**Specimens seen** — FRANCE: Languedoc-Roussillon, Pyrénées-Orientales (66), Eyne, SSW Eyne am Bach, W D33 (Voie Communale Carretera d’Eina), 42°27’50”N, 02°04’29”E, 1580 – 1610 m, Feuchtwiese, Bachbett [moist meadow, streambed], 23 May 2013, *F. G. Dunkel* 29987 (herb. Dunkel [Fig. 9]). — ANDORRA: C Pyrenees, Soldeu, W Soldeu, in der großen Schleife der CG-2, W Callissa de l’Asô, 42°34’53”N, 01°39’46”E, 1750 m, subalpine Wiesen, 23 May 2013, *F. G. Dunkel* 29983 (herb. Dunkel [Fig. 9]): ibidem, *F. G. Dunkel* 29984 (herb. Dunkel); ibidem, Soldeu, an der Cami Pont d’Incles, am Riu d’Incles, Stichstraße, 42°35’02”N, 01°39’49”E, 1755 m, Wiese, 23 May 2013, *F. G. Dunkel* 29985 (herb. Dunkel).

5. *Ranunculus mediocompositus* Dunkel, sp. nov.

Holotype: Slovenia, Predalpsko Območje, 0151.4, Logatec, 500 m nordöstlich Lipje, beidseits der Straße Planina-Laze, 0151.3, Logatec, 500 m nordöstlich Lipje, beidseits der Straße Planina-Laze, 100 m südöstlich von P. 446, 50 – 100 m S Flüsschen Unica, [E of road Planinsko Polje, E Liplje, E der Straße Planina-Laze, N of road Planinsko Polje, 0151.4, Logatec, 500 m nordöstlich Lipje, beidseits der Straße Planina-Laze, 100 m südöstlich von P. 446, 50 – 100 m S Flüsschen Unica, W Soldeu, in der großen Schleife der CG-2, W Callissa de l’Asô, 42°34’53”N, 01°39’46”E, 1750 m, subalpine Wiesen, 23 May 2013, *F. G. Dunkel* 29983 (herb. Dunkel [Fig. 9]): ibidem, *F. G. Dunkel* 29984 (herb. Dunkel); ibidem, Soldeu, an der Cami Pont d’Incles, am Riu d’Incles, Stichstraße, 42°35’02”N, 01°39’49”E, 1755 m, Wiese, 23 May 2013, *F. G. Dunkel* 29985 (herb. Dunkel).

**Description** — Flowering shoot gracile to slim, 14–26 cm tall, stalk 1.0–1.8 mm in diam., suberect to moderately divergent, angle between main and secondary axis 10–50°, flowers 2–5, enrichment shoots (0 or)1 or 2; cataphylls absent; basal leaves 3–6(or 7) per rosette. *First basal leaf* 9–14 mm long, *second basal leaf* 11–17 mm long, narrow-angled at base (30–50° and 20–30°, respectively), divided to dissected by main incision (80–100% and 92–100%, respectively), middle lobe deltoid up to 2 mm stalked, deltoid, lateral edge straight; lateral segment undivided, leaf edge leaf coarsely crenate-serrate. *Second basal leaf* 16–22 mm long, narrow-angled at base (10–40°), dissected by main incision, middle lobe stalked up to 2 mm long, deltoid (to broadly deltoid) with 5–7 crenate teeth, lateral edge straight to convex; lateral segment divided by first lateral incision (70–90%), cleft to divided by second lateral incision (35–75%), cleft by third lateral incision (35–50%); leaf edge crenate-serrate. *Third basal leaf* 22–30 mm long, narrow-angled to V-shaped at base (20–90°), divided by main incision (90–98%), middle lobe deltoid, partly tricleft with incisions up to 42%, with 5–7 crenate teeth, lateral edge straight to concave; lateral segment undivided or first lateral incision up to 62%, leaf edge leaf coarsely crenate-serrate. *Sixth basal leaf* 22–30 mm long, narrow- to wide-angled at base (40–120°), divided by main incision (80–90%), middle lobe deltoid with 5–7 crenate teeth, lateral edge concave; lateral segment undivided or first lateral incision up to 40%, leaf edge leaf coarsely crenate-serrate. *Seventh basal leaf* 16–22 mm long, at base narrow- to wide-angled (10–120[–140]°), main incision absent or main incision up to 75%, middle lobe broadly deltoid, lateral edge straight; lateral segment undivided, leaf edge coarsely crenate-serrate. *Lowermost stem leaf* divided into 5–7 segments, largest segment 15–54 mm long, 1.8–6 mm wide, narrowly lanceolate to lanceolate, undivided or with 1 or 2 short teeth. *Petals* 5, 10–12 mm long, 7–10 mm wide; *androclinium* 0.5–0.7 mm long; *receptacle* globose to obovate, 1.7–2.2 mm long, 1.7–2.0 mm wide, glabrous, invervallum absent, carpellophores 0.15–0.3 mm long; *fruits* 1.0–1.7 mm long, beak 0.5–0.7 mm long, straight to uncinate.

**Pollen quality** — 94.3% well developed (*Du-28639, Table 1*).

**DNA-ploidy** — 2x (Table 1)

**Distribution** — Endemic to Planinsko Polje SW of Ljubljana (Fig. 1).

**Ecology** — Nutrient-poor anthropogenic meadows.

**Etymology** — The epithet refers to the median morphological position between *Ranunculus austroslovenicus* and *R. peracris*.

**Taxonomy** — Hybridization experiments in *Ranunculus auricomus* have demonstrated that it is almost impossible to derive the morphology of the parents from the hybrid progeny (Hodač & al. 2014). However, the morphology of *R. mediocompositus* is strikingly intermediate between *R. austroslovenicus* and *R. peracris*. The middle lobes of the spring leaves resemble that of *R. peracris* with petiolate, deep incisions and concave lateral edges; the initial leaves with crenate or obtuse crenate-serrate leaf edge and mostly undivided final leaves are closer to the morphology of *R. austroslovenicus*.

**Specimens seen** — SLOVENIA: Predalpsko Območje, Planinsko Polje, 0151.3, Logatec, 500 m nordöstlich Lipje, beidseits der Straße Planina-Laze, 100 m süd-
Fig. 10. Data sheet (basal leaf cycle, stem leaves, flower, fruits, receptacle) of *Ranunculus mediocompositus* (for explanation see under Material and methods). Scale bars in detail photographs = 2 mm.
Fig. 11. Holotype and isotype of *Ranunculus mediocompositus* Dunkel, F. G. Dunkel 28639 (both at LJU).
lich P. 446, 45°51′00″N, 14°14′59″E, 445 m, magere Wirtschaftswiese, Flachmoor, 2 May 2007, F. G. Dunkel 19642 (herb. Dunkel); 0151.4, ibidem, O Lipiže, O der Straße Planina-Laže, N. P. 446, 50–100 m S Flüsschen Unica, 45°50′58.2″N 14°15′31.8″E, 445 m, magere Wirtschaftswiese, Flachmoor, 24 Apr 2012, F. G. Dunkel 28638 & 28640 (herb. Dunkel).

6. Ranunculus peracris Dunkel, sp. nov.

Holotype: Slovenia, Unterkrain (Dolenjska), 0158.1, 1.6 km S Gmajna an der Straße nach Zameško, O der Straße, [on the road to Zameško, E site], 45°53′24″ N, 15°22′09″E, 155 m, Wiese, Waldbrand und angrenzender Hainbuchen-Wald, [meadow, edge of the forest and adjacent hornbeam forest], 23 Apr 2013, leg. & det. F. G. Dunkel 30446 (LIJ; isotypes: B, LJU, M, WB, herb. Dunkel). – Fig. 1, 12, 13, Table 1.

Description — Flowering shoot gracile to slim, 13–28 cm tall, stalk 1.0–2.0 mm in diam., suberect to mediums to moderately divergent, angle between main and secondary axis 20–50°, 2 or 3 (or 4) flowers, enrichment shoots 0 or 1 or 2; basal leaves (3 or) 4–6 (or 7) per rosette. Basal leaf cycle: leaf edge coarsely crenate, in seventh basal leaf coarsely crenate-serrate. First basal leaf 13–18 mm long, narrow- to wide-angled at base (50–125°), divided by main incision (85–95%), middle lobe deltoid with 3–5 teeth, tricleft with incisions up to 45%, lateral edge straight to concave; segment cleft by first lateral incision (40–50%), second lateral incision absent or up to 45%. Second basal leaf 11–19 mm long, V-shaped at base (80–100%), (divided) to dissected by main incision (90–100%), middle lobe stalked up to 2 mm, deltoid to 3–5 crenate teeth, tricleft with incisions up to 50%, lateral edge straight to concave; segment cleft to divided by first lateral incision (45–75%), cleft by second lateral incision (33–50%), third lateral incision absent or up to 33%. Third basal leaf 13–22 mm long, wide-angled at base (100–140°), dissected by main incision, middle lobe stalked up to 4 mm long, deltoid (to broadly deltoid), tricleft with incisions up to 55%, with 3–5 crenate teeth, lateral edge straight to concave; segment cleft to divided by first lateral incision (55–80%), cleft by second lateral incision (33–45%), lobed to cleft by third lateral incision (30–42%). Fourth basal leaf 18–26 mm long, (narrow-angled) V-shaped to wide-angled at base (165–190°), dissected by main incision, middle lobe up to 2 mm long stalked, deltoid, tricleft with incisions up to 55%, with 5–7 crenate teeth, lateral edge straight to concave; segment cleft by first lateral incision (70–85%), cleft by second lateral incision (40–65%), lobed to cleft by third lateral incision (30–55%). Fifth and sixth basal leaf similar: 18–30 mm long, narrow-angled to V-shaped at base (55–90° and 60–100°, respectively), dissected in fifth, divided to dissected in sixth basal leaf (95–100%), middle lobe stalked up to 4 mm and 3 mm, respectively, deltoid (to broadly deltoid), tricleft with incisions up to 60% and 55%, respectively, and 5–7 and 4 or 5 crenate teeth, respectively, lateral edge concave; segment cleft by first lateral incision (70–95%), cleft by second lateral incision (50–65%), cleft by third lateral incision (40–60% and 33–55%, respectively), fourth lateral incision absent or up to 30% and 50%, respectively. Seventh basal leaf 18–26 mm, narrow-angled to V-shaped (wide-angled) at base (70–100–105°), divided by main incision (90–95%), middle lobe deltoid, tricleft by incisions up to 37%, with 5 crenate teeth, lateral edge concave; segment cleft to divided by first lateral incision (45–80%), lobed to cleft by second lateral incision (30–40%), third lateral incision absent or up to 33%. Lowermost stem leaf divided into (5–) 7–9 segments, largest segment 17–52 mm long, 2–4 mm wide, linear, mostly undivided, occasionally with two short, rarely with 1 or 2 up to 12 mm long patent teeth. Petals 5, 7–9 mm long, 6–8 mm wide; androclinium 0.7–0.9 mm long; receptacle globose to ellipsoid, 1.4–2.2 mm long, 1.4–1.7 mm wide, glabrous to scarcely pilose, intervalum absent, carpellophores 0.05–0.2 mm long; fruits 1.4–2.0 mm long, beak 0.5–0.8 mm long, uncinate.

Pollen quality — 96.2% well developed (Du-30446, Table 1).

DNA-ploidy — 2x (Table 1).

Distribution — A Slovenian endemic scattered from C Slovenia around Ljubljana to SE Slovenia around Novo Mesto (Fig. 1).

Ecology — Humid oak-hornbeam forests, forest edges, shrubbery, humid nutrient-poor meadows.

Etymology — The epithet alludes to the strongly divided basal leaves, which are reminiscent of the basal leaves of Ranunculus acris L.

Taxonomy — Ranunculus peracris is characterized by a strongly divided blade with a leaf edge of obtuse crenate teeth. Normally, five basal leaves are dissected, i.e. divided completely, by the main incision. The middle lobe is deeply trifid with incisions up to 55%, the basal leaves no. 3–6 possess at least a third lateral incision. Occasionally, the basal leaf blades are less divided but still with obtuse leaf segments or laciniulae. Species with such strongly divided basal leaf blades are especially found in Scandinavia, e.g. R. acidotus (Markl.) Ericsson, R. defectus (Markl.) Ericsson or R. rotundidens (Julin) Ericsson, but they differ by incomplete flowers, acute stem leaf segments and/or less incised middle lobes of the basal spring leaves (Julin 1965, 1980; Marklund 1961, 1965).

Similarly as in Ranunculus subcarniolicus, R. peracris is conspicuous by an early flowering period at the beginning of April.
Fig. 12. Data sheet (basal leaf cycle, stem leaves, flower, fruits, receptacle) of *Ranunculus peracris* (for explanation see under Material and methods). Scale bars in detail photographs = 2 mm.
Fig. 13. Holotype and isotype of *Ranunculus peracris* Dunkel, *F. G. Dunkel 30446* (both at LJU).
**Description** — **Flowering shoot** gracile to slimm, 12–25 cm tall, stalk 0.6–2.0 mm in diam., (suberect to) moderately divergent (to patent), angle between main and secondary axis 25–55°, flowers 1–4, enrichment shoots 0–2; basal leaves 2–5 per rosette. Sometimes **basal leaves** of previous year present (indicated as “−1” in Fig. 14), yellowish, 8–10 mm long, always larger than first basal leaf, V-shaped at base (80–100%), divided by main incision (70–90%), middle lobe deltoid to broadly deltoid with 5–7 crenate teeth; **segment undivided** or lobed by first lateral incision (0–30%); leaf edge crenate. **First basal leaf** 6–10 mm long, narrow-angled to V-shaped at base (70–90%), divided by main incision (70–90%), middle lobe deltoid to broadly deltoid with 3–5 crenate teeth, lateral edge slightly convex to slightly concave; **segment undivided** or lobed by first lateral incision; leaf edge (coarsely) crenate. **Second basal leaf** 8–15 mm long, blade with V-shaped to wide-angled base (85–125°), divided by main incision (80–98%), middle lobe deltoid to broadly deltoid (spatulate) with 3–5 crenate teeth, lateral edge convex; **segment undivided** or cleft by first lateral incision (10–50%); leaf edge (coarsely) crenate. **Third basal leaf** 13–18 mm long, narrow- to wide-angled at base (60–110°), divided to dissected by main incision (92–100%), middle lobe deltoid, tricleft with incisions up to 28%, with 3–5 crenate teeth, lateral edge straight to concave; **segment cleft** by first lateral incision (40–65%), second lateral incision (20–42%); leaf edge crenate to crenate-serrate. **Fourth basal leaf** 10–24 mm long, wide-angled to truncate at base (130–180°), dissected by main incision, middle lobe up to 2 mm long stalked, deltoid, tricleft with incisions up to 30%, with (3−)5–7 crenate teeth, lateral edge straight to concave; **segment cleft** by first lateral incision (60–88%), lobed by second and third lateral incision (25–33% and 20–30%, respectively); leaf edge coarsely crenate to crenate-serrate. **Fifth basal leaf** 15–25 mm long, blade at base V-shaped to wide-angled (90–135°), divided by main incision (90–95%), middle lobe deltoid with 5 crenate teeth, lateral edge straight; **segment cleft** by first lateral incision (25–66%); leaf edge coarsely crenate to coarsely crenate-serrate. **Sixth basal leaf** 14–28 mm long, blade at base V-shaped (80–100%), divided by main incision (85–98%), middle lobe deltoid with 4–9 crenate teeth; **segment cleft** by first lateral incision (32–40%); leaf edge irregularly and coarsely crenate-serrate. **Seventh basal leaf** 14–30 mm long, blade at base wide-angled (100–130°), cleft to divided by main incision (60–75%), middle lobe deltoid with 3–5(−7) (crenate) teeth; **segment undivided** or cleft by first lateral incision (50–70%); leaf edge irregularly and coarsely crenate-serrate. **Lowermost stem leaf** divided into 5(−7) segments, largest segment 18–50 mm long, 1.5–6 mm wide, linear to nar-
Fig. 14. Data sheet (basal leaf cycle, stem leaves, flower, fruits, receptacle) of *Ranunculus subcarniolicus* (for explanation see under Material and methods). Scale bars in detail photographs = 2 mm.

**Ranunculus subcarniolicus**

flower, fruit, receptacle: *Du*-34772-5 (holotype); petals: 5; beak of fruit: uncinate; receptacle: glabrous

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Fig. 15. Holotype and isotypes of Ranunculus subcarniolicus Dunkel, F. G. Dunkel 34772 (all at LJU).
rowly oblanceolate, mostly undivided, occasionally with up to 4 short teeth. Petals 5, 6–9 mm long, 5–6 mm wide; *androclinium* 0.7–0.9 mm long; receptacle globose to ellipsoid, 1.0–1.5 mm long, 0.7–1.4 mm wide, glabrous, invervallum absent or short (<10%), carpellophores 0.1–0.25 mm long; fruits 1.2–2.0 mm long, beak 0.4–0.8 mm long, unicinate to involute.

**Pollen quality** — 97.6% well developed (Du-34772, Table 1).

**DNA-ploidy** — 2x (Table 1).

**Distribution** — From C Slovenia S of Ljubljana to SE Slovenia around Šentjernej, S border at the N Velebit, region of Lika, close to Gospić in Croatia (Fig. 1).

**Ecology** — Nutrient-poor meadows, hornbeam and alder forests alongside brooks.

**Etymology** — The epithet refers to Lower Carniola, the SE part of the historical Carniola region.

**Taxonomy** — *Ranunculus subcarniolicus* possesses a rather homophyllous cycle, it is characterized by basal leaves with a V-shaped to wide-angled base, the fourth basal leaf is often even truncate at the base. The main incision is, with the exception of the fourth leaf, wide-angled, up to 30°. In contrast to the other diploid species, the lowest cauline leaf consists of five oblanceolate segments and resembles the *R. palaeoeuganeus* group but with a completely different ecology (Dunkel 2011). The carpellophores are rather long (0.1–0.25 mm).

At the type locality, a nutrient-poor humid meadow, *Ranunculus subcarniolicus* grows together with two other taxa of the *R. auricomus* complex. There, *R. subcarniolicus* is easily recognizable not only by its small size but also by its earlier flowering period at the beginning of April.

**Specimens seen** — **Slovenia**: Preddinarsko Območje, 0053.2, Gropsulje, ob potoku Bičje, nasproti tovarne Black & Decker, 340 m, na vlažnem travniktu, Aluvialni nanosi rek in potokov, 15 May 1998, D. Simonić (LJU-10046600), det. F. G. Dunkel; ibidem, Gropsulje SW des Ortes, 100 m SW der Straße Ponova Vas, 50–100 m W der Brücke über die Bičje, O des bebuschten Grabens, 45°56'38.6"N, 14°39'11.1"E, 325 m, Grauweidengebüscht, 26 Apr 2016, *F. G. Dunkel* 33266 (herb. Dunkel); ibidem, in cultivation, 27 Apr 2017, *F. G. Dunkel* 34158 (herb. Dunkel); ibidem, W des bebuschten Grabens, 45°56'38.6"N, 14°39'10.2"E, 325 m, Grauweidengebüscht, 26 Apr 2016, *F. G. Dunkel* 33268 (herb. Dunkel); ibidem, W Gropsulje, c. 150 m N der Ponova Vas, 250 m W des Flüsschens Bičje, 45°56'46"N, 14°38'54"E, 325 m, magere Wirtschaftswies, 26 Apr 2016, *F. G. Dunkel* 33277 (herb. Dunkel); Predalpsko Območje, 0151.4, Logatec, Planinsko Polje, O Lipljje, W der Straße Planina-Laze, 300 m S P. 446, 45°50'44"N, 14°15'20"E, 430 m, magere Wirtschaftswies, Flachmoor, 24 Apr 2012, *F. G. Dunkel* 28610 (herb. Dunkel); ibidem, O der Straße Planina-Laze, N P. 446, 50–100 m S Flüsschen Unica, 45°50'58"N, 14°15'32"E, 445 m, magere Wirtschaftswies, Flachmoor, 24 Apr 2012, *F. G. Dunkel* 28640 (herb. Dunkel); ibidem, Planina, prope vicum Planina, 45°50'10"N, 14°15'30"E, 450 m, in pratis paludosis, 24 May 1976, T. Wraber (LJU-10046571), det. F. G. Dunkel; Preddinarsko Območje, 0158.2, Sajevec, 500 m NNO Sajevec, an der Schottersstraße in den Wald, 150 m, Hainbuchen-Wald, 12 Apr 2017, F. G. Dunkel 34490 (herb. Dunkel). — **Croatia**: Lika, Gospić, 1458.3, Waldgebiet Jasikovac, 380 m, vom O-Rand, 450 von der S-Spitze, 44°31'45.8"N, 15°22'30.3"E, 570 m, feuchter Quellbereich im Hainbuchen-Wald, 10 Apr 2017, *F. G. Dunkel* 34886, 34887 & 34930 (herb. Dunkel); ibidem, Waldgebiet Jasikovac 1.6 km S Brücke, 350 m vom O-Rand, 44°31'47.5"N, 15°22'32.4"E, 570 m, Eichen-Hainbuchen-Wald mit Kräutern, 10 Apr 2017, *F. G. Dunkel* 34880 (herb. Dunkel).


*Ranunculus flabellifolius* is a conspicuous taxon already described in the *Flora germanica excursoria* by Heuffel in 1832 (Reichenbach 1830–1833: 723). It is unmistakable by its fan-shaped cauline leaves. We can confirm *R. flabellifolius* as a diploid species (Milošević & al. 1977). Our results of FCSS suggest a sexual mode of reproduction for the species.

Although specimens of *Ranunculus flabellifolius* are scattered throughout all larger European herbaria, *R. flabellifolius* itself seems to be restricted to a few places in SW Romania and adjacent Serbia (Nyárády 1933; Josipović 1977) (Fig. 1). With the exception of the single population outside Romania, all collections date from the 19th century up to 1914. On a specimen label (B 10 034860), W. Seymann annotated: “Banatus. In silvis montis „Kasan“ prope pagum Plavisevicza. Locus lectotypicus.”. The population of *Ranunculus flabellifolius* at Mount Simion was refound by the first author. Here the species hybridizes readily with other species of the *Ranunculus auricomus* complex. Such transitional forms were already mentioned by Reichenbach: “planta speciosa […] cuius forte cum *R. auricomus* hybrida proles” (1832: 732). Therefore, as far as no molecular biological data are available, *R. flabellifolius* should still be taxonomically included in the *R. auricomus* complex.
Fig. 16. Lectotype of *Ranunculus flabellifolius* Heuff. ex Rchb., J. A. Heuffel (JE00007661).
Fig. 17. Specimen of *Ranunculus flabellifolius*, F. G. Dunkel 25795-1 (herb. Dunkel).
Specimens seen (selection) — Romania [“Hungary”]: Banat, Komitat Krassó-Szőrény, in valle Gracka prope pagum Plaviszecza. Locus unicus!”, 27 Apr 1913, W. Seymann; “Banatus. In silvis montis “Kasan” prope pagum Plaviszecza. Locus unicus!”, 27 Apr 1913, W. Seymann; “Hungary”, Banat, Banater Gebirge, Oraviţa, an schattigen Waldstellen des Berges Simion, 1 May 1841, P. Wierzbicki, Flora exsiccata Romanae Nr. 2270 (DR-041269); Banat, Komitat Krassó-Szőrény, Bázíás, ad pag. Bázíás, c. 120 m, in dumetis, 7 Apr 1914, S. Jávorka & G. Timkó, Flora Hungarica exsiccata no. 169 (G00303321, LJU-10102804, ZT), det. S. Jávorka; Banat, in monte lapidoso “Csukar” infra pag. Slavische Buchen-Hainbuchen-Wald, 21 Apr 2010, F. G. Dunkel 25795 (herb. Dunkel [Fig. 17]).

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