

## *Dactylorhiza maculata* subsp. *transsilvanica* (Orchidaceae): new for the Bulgarian flora

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Received: November 10, 2009 ▷ Accepted: November 20, 2009

**Abstract.** *Dactylorhiza maculata* subsp. *transsilvanica* was recorded for the first time in Bulgaria. The taxon grows in wet meadows in the Western Balkan Range, NW Bulgaria. Data about the morphology, habitats and populations are presented and illustrated with colour photographs. Tetraploid chromosome number ( $2n = 4x = 80$ ) for the taxon was obtained, congruent with the earlier published data from elsewhere.

**Key words:** Bulgaria, chromosome number, *Dactylorhiza maculata* subsp. *transsilvanica*, orchids

### Introduction

*Dactylorhiza* is a monophyletic genus spread in the boreal and temperate zones of Europe and Asia and reaching up to North Africa and the Himalayas in the south. It is one of the most taxonomically intricate European orchid genera, mainly due to extensive hybridization and morphological variation caused by differences in environmental conditions (Delforge 2006). Due to taxonomic and nomenclatural difficulties and conceptual changes, there are differences in the Bulgarian botanical literature too. According to Assyov & Petrova (2006), the genus is represented by eight species in the Bulgarian flora: *D. baumanniana* J. Hölz. & Künkele, *D. cordigera* (Fries) Soó, *D. incarnata* (L.) Soó, *D. kalopissii* E. Nelson, *D. pindica* B. Willing & E. Willing, *D. romana* (Sebast. & Mauri) Soó, *D. saccifera* (Brongn.) Soó and *D. sambucina* (L.) Soó.

Recently, a new taxon – *Dactylorhiza maculata* subsp. *transsilvanica* (Schur) Soó – has been recorded.

### Material and methods

Plant material was collected by the authors from a locality in NW Bulgaria. Morphological characters were recorded from this plant material and relevant literature sources (Paučá & Beldie 1972; Delforge 2006). Herbarium specimens were deposited in the Herbarium (SOM) of the Institute of Botany, BAS.

Two living specimens were collected from the field and brought into cultivation to obtain the chromosome number. Counting of the chromosomes was done on metaphase plates from actively growing root meristem. Root tips were collected and pre-treated with 0.01 % colchicine for 90 min, fixed in ethanol:glacial acetic acid (3:1) for at least 2 h at room temperature and stored in 96 % ethanol until needed. Hydrolization was conducted in 1n HCl at 60 °C for 25 min. Then the root tips were treated with HCl:ethyl ether (1:1) for 8 min at 60 °C, rinsed in distilled water and stained with haematoxylin after Gomori (Melander & Wingstrand 1953) for 30 min, at 60 °C. Final-

ly, the root tips were squashed in a drop of 45% acetic acid and observed with a light microscope.

## Results and discussion

***Dactylorhiza maculata*** (L.) Soó subsp. ***transsilvanica*** (Schur) Soó, Nom. Nov. Gen. *Dactylorhiza* (1962) 7 (Fig. 1)

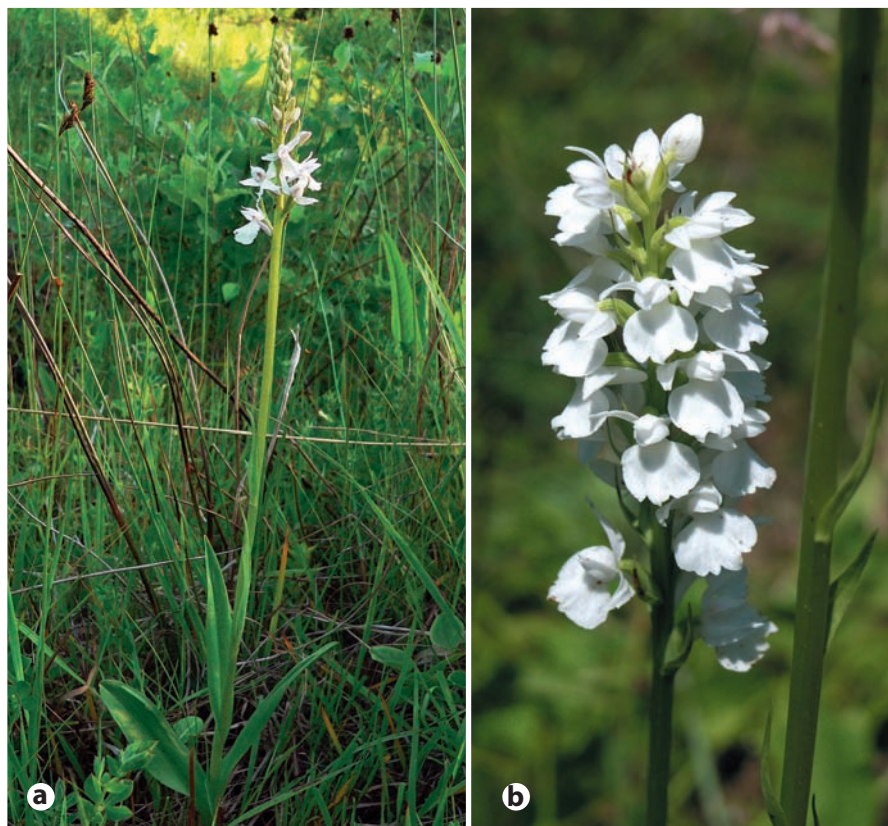
Perennial herb, with 2 deeply digitate root tubers. Stems (20)30–60(70) cm, solid, leaves 4–8, green, unspotted, oblong-lanceolate, the middle 10–12×1.5–3 cm, widest in the lower half, the upper ones bract-like. Inflorescence 6–10 cm, conical (later to cylindrical), dense, with 20–50 flowers, bracts as long as ovaries. Flowers white, pink-tinted, without spotting. Sepals 7–11×2–3.5 mm, laterally spread, dorsal sepal forming a hood with petals, lip wider than high, (8)9–12×12.5–16 mm, slightly convex, 3-lobed, median lobe narrower than lateral ones, those rounded or slightly pointed, spur 10–12 mm, parallel to ovary. Fruit a pod. Flowering in June, fruiting in (June) July.

Populations of white flowering plants of *D. maculata* group in SE Europe, mainly in the Carpathi-

an Mts, have been known for a long time. However, there has been no agreement on their taxonomic status. Delforge (2006) has treated them as *D. maculata* var. *transsilvanica* (Schur) P. Delforge and has stated that there are many intermediates with *D. m. maculata*. Averyanov (1988, 1989) accepted a specific rank: *D. transsilvanica* (Schur) Aver. We prefer to use the subspecific rank in accordance with the treatment of many other authors, e.g. in Flora Europaea (Soó 1980), and in Baumann & Künkele (1988), Buttler (1991) and as accepted in the flora of Serbia (Diklić 1976) and Romania (Ciocârlan 2000). Although the name *Orchis maculata* L. was used in the past in the Bulgarian botanical literature (Stojanov 1964; Stojanov & al. 1966), it was clearly stated that the plants belonged to *O. m.* subsp. *macrostachys* (Ten.) Rohl., which is a synonym of the currently accepted *Dactylorhiza saccifera* (Brong.) Soó. In more recent publications (Andreev 1992; Delipavlov 2003) *D. saccifera* is the only member of the “*maculata*” group.

Irrespective of the taxonomic rank, the Bulgarian populations differ from the typical *D. maculata* by their unspotted leaves, usually white, unspotted flowers, lip up to 16 mm wide (9–13.5 mm in the typical *D. maculata*) and spur up to 12 mm long (6–10.5 mm in the typical *D. maculata*).

Plants in the observed populations in Bulgaria are fairly uniform, both in terms of vegetative and generative characters. According to most above-cited sources, the flowers are white or greenish-yellow. In Bulgaria, they are white with a distinct pink tint, especially at the base of the flowers.



**Fig. 1.** *Dactylorhiza maculata* subsp. *transsilvanica*: **a** – whole plant (photo Ya. Stoyanov); **b** – inflorescence (photo V. Vladimirov).

## Distribution in Bulgaria

**Balkan Range (Western):** Mt Chiprovska, wet meadows in the valley of river Lopushanska Ogosta, above Kopilovtsi village, Montana district, 43°20'01.7"N, 22°51'57.6"E & 43°20'02.7"N, 22°51'56.3"E, FN-59, 24.06.2008, coll. V. Vladimirov, A.S. Petrova & Y. Stoyanov (SOM; Fig. 1b), first observed in the same locality by VV in July 2003; in wet meadow in the Chuprene Nature Reserve, along the trail to the Gorski Rai shalet, FP-31, 18.06.2006, observed by Y. Stoyanov (Fig. 1a).

## Habitat and population data

The larger observed population was that above Kopilovtsi village (Fig. 2). Here the mountain valley was locally slightly wider (up to 70 m) and with only small inclination – about 5°. Wet meadows developed along the river and an adjacent brook, and were rich in species dominated by *Briza media*, *Holcus lanatus*, *Anthoxanthum odoratum*, *Agrostis capillaris*, and *Cynosurus cristatus*. *Eriophorum latifolium* dominated in more depressed spots. Other common species were *Potentilla erecta*, *Prunella vulgaris*, *Succisa pratensis*, *Juncus articulatus*, *Juncus conglomeratus*, *Carex muricata*, *Carex lepidocarpa*, *Carex pallescens*, *Crepis paludosa*, *Lychnis flos-cuculi*, and *Galium palustre*. Patches of *Scirpus sylvaticus* and *Lythrum salicaria* have developed locally. A rich moss cover was found too: *Hamatocaulis vernicosus* [National IUCN category: Vulnerable (Natcheva & al. 2006); included in Annex IIB

of the Directive 92/43/EEC], *Aulacomnium palustre* and *Calliergonella cuspidata*. The other orchid species were *Epipactis palustris*, with an abundant population; about 30–35 plants of *Dactylorhiza cordigera* and single individuals of *Platanthera bifolia*.

The population of *D. m.* subsp. *transsilvanica* was large (Fig. 3), with more than 600 plants at the first location cited above, and another 200–250 plants higher in the valley (second sited coordinates). As mentioned above, *Dactylorhiza cordigera* was growing there usually close to the brook. A few plants of apparently hybrid origin with other *Dactylorhiza* species and intermediate characters were also observed.

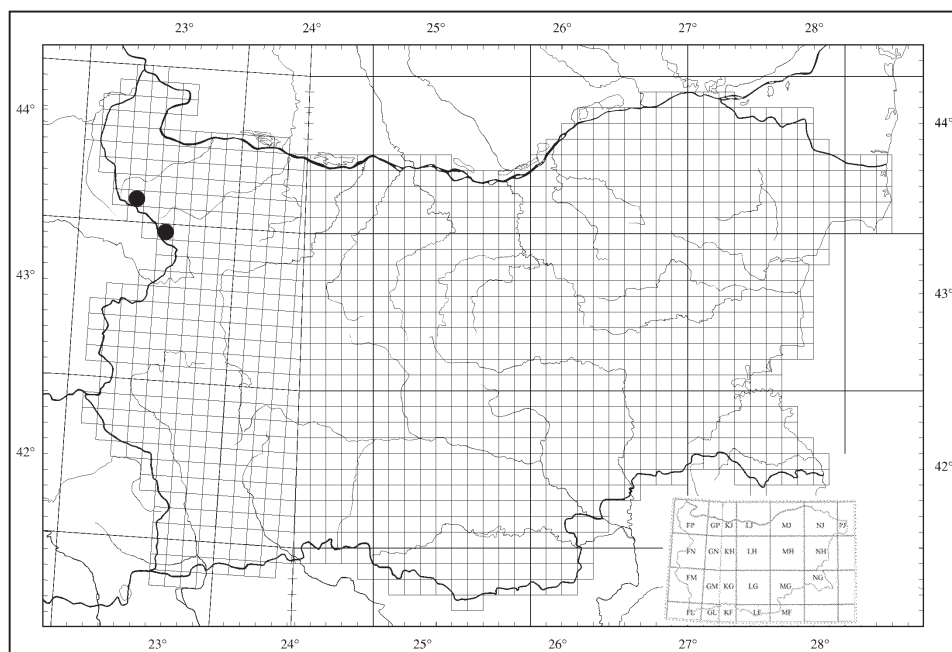
The habitat of the population in the Chuprene Nature Reserve was fairly similar, also rich in bryophytes. It was observed at very early stage of flowering, with 15 flowering plants counted, as well as some non-flowering ones. Here the earlier-flowering *D. cordigera* had a larger population. Only one individual of hybrid origin was observed.

## Chromosome number

Tetraploid chromosome number  $2n = 4x = 80$  was established in both living plants (Fig. 4), which confirms the data on this species from elsewhere (Klein & Deutisch 2005; Delforge 2006).

## Conservation status

Most species of *Dactylorhiza* known under the vernacular name of Marsh Orchid grow in wet places and occasionally form abundant populations. In Bulgaria, due to the climatic conditions, such habitats are relatively rare and rather fragmented. Therefore, some of the species are of conservation concern: *D. kalopissii*



**Fig. 2.** UTM-distribution map of *D. maculata* subsp. *transsilvanica* in Bulgaria.

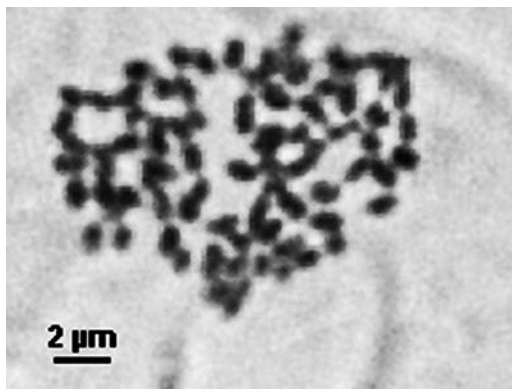


(National IUCN category – Critically Endangered), *D. incarnata* (Endangered), *D. romana* (Vulnerable) (Petrova 2009). It seems that *D. maculata* subsp. *transsilvanica* is also rather rare in Bulgaria and confined to the Western Balkan Range, NW Bulgaria. Both known localities fall into the Natura 2000 Zone **BG 0001040 Zapadna Stara Planina & Predbalkan** (Western Stara Planina and Forebalkan).

**Acknowledgements.** Financial support of the Bulgarian Science Fund under projects B 1503/2005 and IFS–605/07 is gratefully acknowledged. The authors are indebted to Dr Rayna Natcheva (Sofia) for determination of the bryophyte species and to the anonymous reviewer for the critical notes.



**Fig. 3.** Part of the population of *D. maculata* subsp. *transsilvanica* above Kopilovtsi village (photo V. Vladimirov).



**Fig. 4.** Metaphase plate of the karyotype of *D. maculata* subsp. *transsilvanica* ( $2n = 80$ ).

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