Plantago sempervirens (Plantaginaceae): a dwarf shrub new for the Bulgarian flora

Stoyan Stoyanov & Kiril Vassilev

Department of Plant and Fungal Diversity and Resources, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Acad. Georgi Bonchev St., bl. 23, 1113 Sofia, Bulgaria, e-mail: stoyanov@bio.bas.bg (corresponding author), kiril5914@abv.bg

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Abstract. *Plantago sempervirens* is reported for the first time for the Bulgarian flora. It was found near the Skrino Gorge of River Struma, in the vicinities of Vukovo village (Boboshevo district), in the floristic region of the Valley of River Struma (*Northern*). The article presents data on the specificities of the habitat, population structure and dispersal modes of this species. The determined chromosome number is 2n = 12.

Key words: Bulgaria, chorology, habitat, karyology, Plantago sempervirens, population

Introduction

Plantago L. has been so far represented by 17 species in the Bulgarian flora, 15 of which known for a long time (Petrova 1995) and two recently discovered (Dimitrov & Trifonov 2006; Tzonev & Karakiev 2007). During field studies in the hilly vicinities of Vukovo village (Boboshevo district), situated above the Skrino Gorge of River Struma, an uncommon suffrutescent Plantago species was discovered. According to Chater & Cartier (1976), among the European representatives of the genus only two species have a characteristic dwarf shrub habit: P. asperri*ma* Hervier (endemic to East and South Spain) and P. sempervirens Crantz (widely distributed in Southwest Europe). One of these, namely *P. sempervirens*, to which the collected plant belongs, is reported as a new species for the Bulgarian flora. It favours dry habitats, as well as anthropogenically affected terrains. The vitality and high density of the population testified that it is stable and approximately of long standing.

Results and discussion

Plantago sempervirens belongs to subgenus *Psyllium* (Mill.) Harms & Reiche, to which traditionally are referred all species of *Plantago* with characteristically branched stems, opposite leaves and elongated internodes. So far this subgenus has been represented by the two annual species in Bulgaria: *P. arenaria* Waldst. & Kit. and *P. afra* L. *Plantago sempervirens* distinctly differs from them by its woody stems.

Subgenus *Psyllium* was treated as a monophyletic group already by Pilger (1937), who offered one of the first complete classification schemes of genus *Plantago*, mainly based on morphological characters. The substance and limits of this classification were retained in the taxonomic development of Rahn (1996), in which he relied both on traditional morphology and on embryological data. On the basis of molecular investigations, Rønsted & al. (2002) suggested the inclusion of subgenus *Albicans* sensu Rahn (1996) which, according to them, is a paraphyletic of subgenus *Psyllium* s.str., into a wider subgenus *Psyllium*. Nevertheless, subgenus *Psyllium* sensu Pilger (1937) and sensu Rahn (1996) forms a distinct monophyletic subclade and retains its independence in subgenus *Psyllium* s.l. at section level.

Besides morphologically, subgenus *Psyllium* s.str. is clearly differentiated geographically. It includes 16 species which inhabit some dry areas of the Mediterranean and Macaronesia. Its suffrutescent representatives are concentrated in the Western Mediterranean (the Iberian Peninsula, Northwest Africa and Macaronesia). Of these, only *P. sempervirens* has gone outside these limits, reaching the southeastermost parts of the Balkan Peninsula.

Plantago sempervirens Crantz, Inst. Rei Herb. 2: 331 (1766) (Fig. 1)

Dwarf shrub, 10-30(40) cm, with usually strongly branched, shortly pubescent stems. Young shoots 5-15 cm; internodes 2-15 mm. Leaves opposite $10-60 \times ca$. 1 mm, linear or linear-subulate, entire, scabrid. Peduncles 2-8 cm,

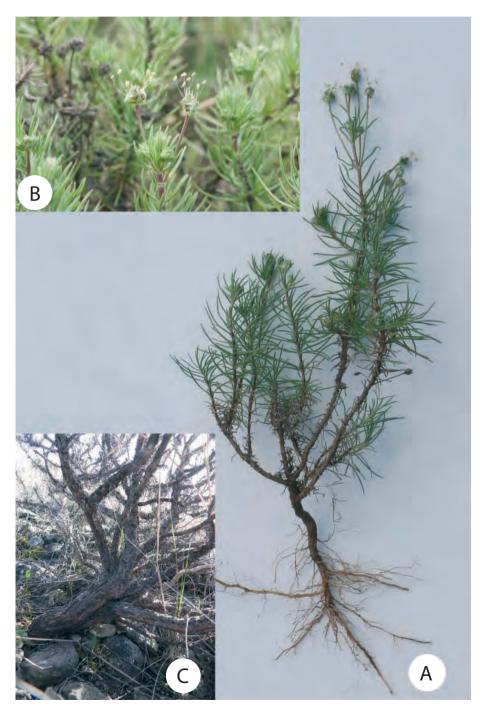


Fig. 1. *P. sempervirens*: **A** – habit; **B** – young shoot with flowering spikes; **C** – woody stem.

hirsute; spikes 5–15 mm, ovoid-globose, with 4–10 flowers. Lower bracts 5–10 mm, ovate to ovate-orbicular with a wide membranous margin, abruptly contracted into a linear apex or acute; upper bracts ovate to lanceolate. Sepals 5–6 mm, unequal. Corolla tube 4–5 mm; lobes 2–2.5 mm, ovate-lanceolate, acuminate. Capsule 2.5–3.5 mm; seeds 2, 2–3 mm, cymbiform, ovate to oblong-lanceolate in outline, brown.

Phenology

Flowering in the second half of June to August, fruiting from September to November.

Distribution in Bulgaria

The species has been found in the Valley of River Struma (*Northern*) floristic region, to the N and SE of Vukovo village, Boboshevo district, at altitudes of 650–950 m, FM-67, 16.06.2010 & 04.07.2010, coll. *S. Stoyanov* & *K. Vassilev* (SOM 166567–166569). GPS data on the more important and on peripheral points of the population of *P. sempervirens* are given in Table 1.

General distribution

Plantago sempervirens is a widely distributed species, considered autochtonous (Pignatti 1982) in the European West Mediterranean (incl. the Apennine Peninsula). According to Greuter & al. (1989), it occurs in Portugal, Spain, France, Italy and European Turkey. The species is regarded as naturalized in Austria, Switzerland and Germany (Chater & Cartier 1976; Oberdorfer 2001), and recently has been reported for Romania, with isolated distribution in the centre of the Transylvanian Plain (Puşcaş & al. 2003). On the Balkan Peninsula, *P. sempervirens* was collected for the first time in 1913 by the Bulgarian botanist Todor Nikolov (during the Balkan War) in the vicinities of the

Turkish town Kumbağ (Tekirdağ Province). Its herbarium specimen deposited in the Herbarium of Sofia University (SO 69072, sub *P. cynops* L.) had remained unknown until present day. Much later the species was found in some other places of European Turkey (Table 2). There are five known localities of *P. sempervirens* in the Balkans (including that in Bulgaria) (Fig. 2). The latest discovered Turkish locality (added after Tutel 1982), the one closest to Bulgaria and

 Table 2. Localities of *P. sempervirens* in the Balkans according to revised material.

Localities	Collected by/ date	Determined by/ Revised by	Herbarium sheet No
Turkey-in-Europe			
Rodosto (= Tekirdağ) Province: Kumbağ, roadsides	T. Nikolov 05.1913	 N. Stojanov as <i>P. cynops</i> L. S. Stoyanov as <i>P. sempervirens</i> 	SO 69072
Tekirdağ Province: Tekirdağ to Barbaros	A. Baytop 25.03.1968	 A. Baytop as <i>P. cynops</i> L. B. Tutel as <i>P. sempervirens</i> 	ISTE 12438
Kırklareli Province: 3 km from Pınarhisar to Kırklareli, open ridges	A. Baytop 21.05.1974	 A. Baytop as <i>P. cynops</i> L. B. Tutel as <i>P. sempervirens</i> 	ISTE 28213
Tekirdağ Province: Kumbağ to Manastır, roadsides	N. & E. Özhatay 22.05.1974	 A. Baytop as <i>P. cynops</i> L. B. Tutel as <i>P. sempervirens</i> 	ISTE 28359
Kırklareli Province: Kırklareli-Dereköy, 1km after Demircihalil village, stony places	K. Alpınar & H. t'Hart 21.08.1988	– K. Alpınar as P. sempervirens	ISTE 59680
Bulgaria			
Kyustendil Region: Boboshevo district, North of Vukovo village, abandoned lands and stony pastures	S. Stoyanov & K. Vassilev 16.06.2010 & 04.07.2010	– S. Stoyanov as P. sempervirens	SOM 166567 SOM 166568 SOM 166569

Table 1. GPS data on the population of P. sempervirens in the vicinities of Vukovo village.

No	Decalities	Ν	Ε	Altitude, m
1.	First largest subpopulation, abandoned fields 1 km N of Vukovo, E of the dirt road Vukovo-Lokvata	42.20499°	22.97419°	830
2.	Second largest subpopulation, abandoned fields 1 km N of Vukovo, N slope of Kalenitsa hill	42.20576°	22.96596°	800
3.	Westernmost point of population, stony pastures near the summit of Kalenitsa hill	42.20155°	22.96393°	845
4.	Easternmost point of population, along the dirt road near the military object of Pogled hill	42.22113°	22.98134°	950
5.	Northernmost point of population, along the dirt road Vukovo-Lokvata	42.22180°	22.96970°	835
6.	Southernmost point of population, SE of Vukovo, along the dirt road towards St. Spas chapel	42.19318°	22.97683°	685
7.	Central point of population, along the dirt road Vukovo-Lokvata, at the fork of the road to the military object at the Pogled hill.	42.20874°	22.97092°	800
8.	Nearest to the village subpopulation, along the dirt road at the north end of Vukovo	42.19785°	22.97157°	715

Fig. 2. Distribution of *P. sempervirens* in the Balkans (▲ – new locality; ● – localities in European Turkey).

situated within the boundaries of the Turkish part of Mt Strandzha (ISTE 59680), is 350 km off the locality near Vukovo village and at a distance of only 20 km from the Bulgarain-Turkish frontier. Similarly to the Romanian locality, the Bulgarian locality of *P. sempervirens*, which is nearly in the centre of the Balkan Peninsula, is distanced from the main area by more than 600 km.

Habitat and population characteristics

The locality of P. sempervirens in the vicinities of Vukovo village occupies an area of approximately 4 km² and most of the population is concentrated in the two main vast stretches of abandoned agricultural lands (north of the village) and the stony pastures and sparse bushes bordering on them (Fig. 3A). To the north and northeast of the ex-arable fields, in a strip of nearly two kilometres, the population follows the tracks of dirt roads (or occurs up to 15–20 m away from them) to Lokvata village and a military site (on the Pogled Hill). Isolated individuals are found at these two destinations, as well as small groups and strips of dense groups (along the road periphery and in between the tire ruts). The largest patch covers a road strip about 50 m long (Fig. 3B). The southernmost fragments of the population of P. sempervirens are located about 500 m southeast of the village, along a dirt road to the St. Spas Chapel, where several small groups of about ten individuals are located presently.

In the former agricultural fields the density of the population in relatively stony slightly to moderately inclined parts of the slopes is greater than in almost levelled bottom parts, where the soil is deeper, with higher clay content, poorly aerated and occupied by caespitose grasses, which suppress the development of *P. semper-virens*. Within the framework of the first largest subpopulation (Table 1, GPS No 1) a patch of approximately 0.5 ha is discovered, where density reached as many as 30 individuals per m², and the species coverage exceeded 70% (Fig. 4). Within the second largest subpopulation, on the Kalenitsa Hill (Table 1, GPS No 2), the individuals are evenly distributed (less than one individual per m²), or form small groups.

Plantago sempervirens grows on dry and shallow Chromic Cambisols (CMx) and Lithic Leptosols (LPq) (the soil classification is according to Ninov 2002), rich in calcareous pebbles, on slopes of various exposition. It prefers disturbed habitats like eroded terrains (along the dirt roads), livestock trails in grazing and abandoned fields. In the Mediterranean basin this species colonizes road embankments, but it is not considered suitable for erosion control along the roads (Tormo & al. 2006; Bochet & al. 2009).

In Bulgaria, *P. sempervirens* takes part in open to semi-close grassland communities, with total vegetation cover from 60% to 85%. Species with southern distribution (Euro-Mediterranean, Submediterranean, and Mediterranean floristic elements) predominate in the community. Some grasses, such as *Stipa eriocaulis* Borbás, *Dichantium ischaemum* (L.) Roberty, *Festuca oviniformis* J. Vetter, but also *Thymus callieri* Borbás ex Velen., and *Fumana procumbens* (Dunal) Gren. & Godr., are with higher abundance and cover in the area.

Communities of *P. sempervirens* in Bulgaria belong to the class *Festuco-Brometea* Br.-Bl. & Tüxen ex Soó 1947. Puşcaş & al. (2003) referred its communities from Romania to the same class, with three associations: *Botriochloetum ischaemi* Pop 1977, *Stipetum lessingianae* Soó (1927) 1947 and *Festucetum valesiaco-rupicolae* Csűrös & Kovács 1962. This species is seldom found on roadsides in Germany as a pioneer species of *Agropyretalia* and *Xerobromion* (Oberdorfer 2001). On the other hand, Molina & al. (2004) refer its communities to chasmophytic vegetation of the class *Thlaspietea rotundifolii* Br.-Bl. 1948.

A small part of the population (on the westernmost periphery) of *P. sempervirens* falls into the Natura 2000 network in Bulgaria in the Skrino Protected Zone (BG 0001013).

Part of the locality of *P. sempervirens*, in whose boundaries a small population of the extremely rare



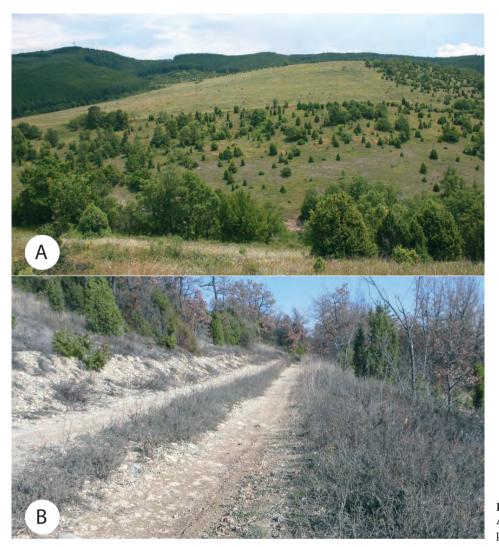
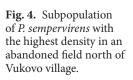


Fig. 3. Habitats of *P. sempervirens*: **A** – abandoned fields and stony pastures; **B** – dirt roads.



species *Verbascum anisophyllum* Murb. (a Balkan endemic and target species of the project "A pilot network of small protected sites for plant species in Bulgaria using the plant micro-reserve model") has been identified, shall be suggested for protection within the framework of a protected area.

Dispersal modes

Plantago sempervirens acts as a pioneer species. It is able to colonise new lands and open spaces quickly and successfully, thanks to a combination of different modes of dispersal of its numerous seeds: anemochorous, zoochorous and hemerochorous (anthropochorous). These types of dispersal are analysed below.

The testa of many *Plantago* seeds becomes sticky when moistened, thanks to the swelling of polysaccharides it contains (Pilger 1937). Owing to that property, the seeds may stick to the feet of birds and thus could be transported to great distances. Considerable distantiation of the population of P. sempervirens at Vukovo village from the main area of the species gives us grounds to assume that it has resulted from ectozoochorous seed dispersal by birds. There is little probability that the locality is of relict nature. It is impossible to determine when the species has first appeared there, but it is quite obvious that some 20 years ago it has rapidly started to grow in numbers and to colonise new territories. After 1989 and the political changes in Bulgaria, a process of denationalization of agricultural lands started and most of them switched over from cooperative to private management. As a result of that and of the gradual migration of village population towards the big cities, many arable lands were abandoned, especially those in the hilly regions, where the soils were stony and of low fertility. Such was the destiny of lands in the territory of Vukovo village. After a span of 20 years, in the result of succession, vegetation in the former arable fields has started to resemble that in the adjacent stony pastures. Presently, the population of Vukovo village amounts approximately to 50 persons, mainly old people, whose livelihood, although on a smaller scale, is related to pasture rearing of small livestock, while in the past farming of cereals and tobacco was practiced too. From talks with the local people we learned that P. sempervirens became a habitually occurring plant only after 1990, when it settled in mass numbers and quite durably in the abandoned fields. Probably by that time P. semper*virens* already was present in the composition of the adjacent xerothermic grassy and shrub communities and, thanks to its high seed production and anemochorous dispersal, it rapidly colonised the newly freed spaces. Now its two most numerous and densest subpopulations occupy the two largest and most compact stretches of land (each with an area of approximately 10 ha) of the former arable lands, situated 1 km northwards of Vukovo village, on both sides of the dirt road to Lokvata village.

One of the biological specificities of P. sempervirens is that it develops young shoots already in autumn and they remain green during the winter months. The local shepherds, who use the former arable fields as pastures now, said that these fresh shoots are grazed by the goats and sheep chiefly in the autumn-winter season, when there is no green grass. The livestock probably also grazes out the ripe spikes in the upper part of the old branches, which reach the level of the young shoots with their long peduncles. Along with this, when the soil is muddy, the capsules and seeds that have fallen on the ground may stick to the animals' feet. While endozoochorous seed dispersal is only an assumption, the presence of numerous individuals of P. sempervirens on and along the livestock trails and along the dirt roads proves ectozoochorous dispersal.

As important is the role of man as an agent of seed dispersal of seeds of *P. sempervirens*. They disperse indirectly via the mowing machines. Presently, the dirt roads from Vukovo to Lokvata village and to the military site on the Pogled Hill are rather deserted, while in the past they were quite intensively used, especially by the agricultural machines. These roads ensure open terrains for settling of the species and at the same time serve as a road-bed for its spreading.

Chromosome number

The karyotype was studied on somatic metaphase plates, using the squash technique. Seeds were collected and germinated in Petri dishes to obtain chromosome counts. Root tips were cut and pre-treated with colchicine (0.01 %) for 1 h at room temperature. Then the roots were fixed in ethanol/glacial acetic acid (3:1) for 24 h in a refrigerator, hydrolyzed in 1N HCl for 15 min at 60 °C, transferred in HCl/ethyl ether (1:1) for 15 min at room temperature, stained with Gomori's haematoxylin for 40 min at 60 °C (Melander & Wingstrand 1953), and finally squashed in 45 % acetic acid.

The chromosome number of 2n = 2x = 12 was constant in the examined cells of five root tips. It confirms the earlier counts of Natarajan (1978) from France, Elena Rosselló & al. (1985), Luque & Díaz Lifante (1991), and Parra & al. (1998) from Spain. The karyotype consists of meta- and submetacentric chromosomes, which are relatively equal in size. Two pairs of submetacentric chromosomes being SAT (Fig. 5).

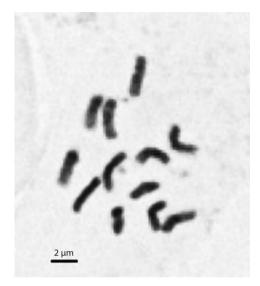


Fig. 5. Microphotograph of a metaphase plate of *P. sempervirens*, 2n = 12.

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