Alchemilla mollis (*Rosaceae*) – a critically endangered species in Bulgaria

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Abstract. The present study discusses the current state of the only known so far population of *Alchemilla mollis* in Bulgaria. The species is a critically endangered on the territory of Bulgaria. The population is situated at the foot of peak Botev, the Central Balkan National Park. For the preservation of the species, some studies of the species population were initiated in the period 1990–2010. In this period the territory of the population has increased from 150 m^2 to 200 m^2. It is surrounded by a beech forest and harbours 65 vascular plants, mostly perennials (86.2 %), belonging to 57 genera and 24 families. In terms of age structure, young plants prevail. However, the ongoing process of erosion of the terrain and the anthropogenic impact are significant threats. Also the dryer and hot weather during the last few summers was affecting the phenological spectrum of *A. mollis* and could hamper the seed formation. On the basis of this, steps for *in situ* and *ex situ* conservation of the species are suggested.

Key words: *Alchemilla mollis*, Bulgaria, conservation, monitoring

Introduction

The species *Alchemilla mollis* (Buser) Rothm. is distributed in Europe. Outside Europe it is present in N. Anatolia, the Caucasus and N. Iran (Kurtto & al. 2007). On the territory of the Balkan Peninsula it occurs in Romania, Bulgaria and Greece. It is included in *Red Book of PR Bulgaria* with the status "Endangered Species" (Assenov 1984), as well as in the list of protected species, Supplement 3 to the Biological Diversity Act (2002) and the Act on Amending and Supplementing the Biological Diversity Act (2007). In 2006 it was assessed as "Critically Endangered" according IUCN (2001) and also included in the *Red List of Bulgarian vascular plants* (Vitkova 2009) and the *Red Book of R Bulgaria* (Vitkova in press).

In Bulgaria *A. mollis* was first reported from the vicinity of Rai chalet (1500 m a.s.l.) in the Central Balkan Range (Assenov 1973). In the respective communication, the author stated that the species could be found in humid streamside habitats in the beech belt, between 1600 m and 1700 m a.s.l. However, no plant specimens were deposited in any of the official Bulgarian Herbaria.

In 1990, in the same part of the Central Balkan Range, in the Kamenlivitsa locality, the first author confirmed the only known so far clone-population of the species in Bulgaria (Vitkova 1996, 1997, in press). Plant specimens were deposited in the Herbarium of the Institute of Botany, BAS (SOM 159980, AV, 27.08.1990) (Vitkova 1996). Until then, the size of the locality was only some 150 m^2 and the individuals of the species were in evidently good condition. Live plant material was taken to the experimental fields of the Institute of Botany, BAS in order to determine the dynamics in accumulation of flavonoids and tannins (Vitkova 1996) and the ontogenesis, phenological rhythm, reproduction and biological productivity of the species in field conditions (Vitkova 1997).

Alchemilla mollis is a medicinal and decorative plant (Pawłowski 1968; Vitkova 1997; Delipavlov & Cheshmedzhiev 2003). In modern medicine, the above-ground parts and rhizomes of the plant are used. The main biologically active substances in *Herba Alchemillae* are galotannins, flavonoids and saponins. Drugs have a rapidly regenerating skin epithelium impact, styptic and anti-inflammatory action (Nikolov 2006).

The aim of the presented study is to characterize the only known locality of *A. mollis* for the territory of Bulgaria and to name measures for conservation of the species *in situ* and *ex situ,* as part of floristic biodiversity of the country. On the other hand, creation of collections and research of the species in field conditions will contribute to its sustainable use.

Material and methods

The locality of *A. mollis* was visited in July 2007. The chorology was presented according to Kozhuharov & al. (1983), with a UTM-map of distribution of the species. The exact GPS position of the locality, its size, altitude, exposition, and basic rock type were determined.

An inventory list of the floristic composition of the community, where *A. mollis* was present, is made. The taxonomic status of the species in the investigated plant phytocenoses is given according Jordanov (1963–1979), Velchev (1982–1989), Kozhuharov (1992, 1995), and Delipavlov & Cheshmedzhiev (2003). The floristic composition and some phytosociological indices of the community are presented in Table 1. For every species is given the floristic element according Walters, in the Bulgarian adaptation of Assyov & Petrova (2006); biological type, according to Delipavlov & Cheshmedzhiev (2003); live forms, according to Raunkiaer (1934); and abundance-dominance and grouping, according to Braun-Blanquet (1964).

In the period 2008–2010 the locality was visited again and the effect of the very dry summer weather in 2008 and 2009 was studied. The collected data were included in fact sheets, according to Gussev & al. (2008). During field work were measured the size of the clone population, projection coverage of the entire vegetation and particularly of the investigated species. In 2009, the spatial and age structure of the clone population was determined. The natural restoration abilities of the clone population were considered: number of seedlings, virginal and generative plants. Biometric measurements of the adult generative plants (clones) were taked: area of the clones, number and height of flowering stems, length of petioles, length and width of leaf laminas.

Table 1. Species diversity and indices characterizing the locality of *A. mollis*.

	Biological type	Life form	Floristic element	Altitude	Abundance-dominance	Grouping
1	2	3	4	5	6	7
Shrubs						
Bruckenthalia spiculifolia (Salisb.) Rchb.	sh	Ch	subMed	1000–2250	1	1
Juniperus pygmaea C. Koch	sh	Ph	Med	1500–1700	r	1
Rosa canina L.	sh	Ph	subMed	0–2000	1	1
Rubus caesius L.	sh	Ph	Eur-As	0–1600	+	1
Vaccinium myrtillus L.	sh	Ch	Boreal	1000–2200	+	1
Grasses						
Agrostis capillaris L.	p	H	Boreal	0–2000	1	3
Anthoxanthum odoratum L.	p	H	Eur-As	0–2300	+	2
Brachypodium sylvaticum (Huds.) P. Beauv.	p	H	Eur-As	0–1600	+	2
Briza media L.	p	H	Eur	0–1800	+	2
Calamagrostis arundinacea (L.) Roth	p	H	subBoreal	300–2000	1	3
Dactylis glomerata L.	p	H	Eur-As	0–1500	+	2
Deschampsia flexuosa (L.) Trin.	p	H	Boreal	800–2500	1	1
Festuca dalmatica (Hack.) K. Richt.	p	H	subMed	1000–2000	1	2
Nardus stricta L.	p	H	Arct-Alp	1200–2400	1	2
Poa compressa L.	p	H	Eur-subMed	0–1500	1	1
Secale strictum (C. Presl) C. Presl	p	H	Med	1500–2500	+	1
Leguminous						
Dorycnium herbaceum Vill.	p	H	Eur-Med	0–1700	+	2

Table 1. Continuation

1	2	3	4	5	6	7
Lotus corniculatus L.	p	H	Eur-Med	0–2300	+	2
Trifolium medium L.	p	H	Eur-As	0–1800	1	1
T. ochroleucon Huds.	p	H	Eur	0–1600	1	1
Herbs						
Achillea crithmifolia Waldst. & Kit.	p	H	Pann-Bal	0–1500	r	1
Ajuga laxmannii (L.) Benth.	p	H	SSib	0–1800	+	1
Alchemilla mollis (Buser) Rothm.	p	H	Carp-Bal-Cauc-Anat	1600–1700	1	2
Allium flavum L.	p	Cr	Med	0–2900	+	1
Asplenium trichomanes L.	p	H	Kos	0–1700	+	1
Campanula rapunculoides L.	p	H	Eur	0–1900	r	1
C. scheuchzeri Vill.	p	H	Eur	1300–2000	r	1
C. sparsa Friv.	p	H	Eur	1300–2000	+	1
Carlina acanthifolia All.	p	H	Eur	0–2000	r	1
C. vulgaris L.	p	H	Eur-Med	0–1500	+	1
Centaurea splendens L.	b-p	H	subMed	0–1500	r	1
Clinopodium vulgare L.	p	H	subBoreal	0–2000	+	1
Cichorium intybus L.	p	H	Eur-Sib	0–1000	+	1
Dianthus petraeus Waldst. & Kit.	p	H	Bal-Dac	0–2000	+	1
Digitalis grandiflora Mill.	p	H	Eur-Sib	500–1800	+	1
Euphorbia cyparissias L.	p	H	Eur	0–2200	+	1
Fragaria vesca L.	p	H	subBoreal	0–2000	+	2
Galium lucidum All.	p	H	subMed	250–1900	+	1
Helleborus odorus Waldst. & Kit.	p	H	Eur-sMed	0–1500	+	1
Helianthemum nummularium (L.) Mill.	p	Ch	Alp-Med	0–2600	+	1
Hieracium pilosella L.	p	H	Eur-Med	0–2000	+	1
H. sparsum Friv.	p	H	subMed	2000–2800	+	1
Hypericum maculatum Crantz	p	H	Boreal	0–2000	+	1
H. perforatum L.	p	H	Kos	0–2000	+	1
Knautia midzorensis Form6nek	b-p	H	Bal	500–2900	+	1
Leontodon crispus Vill.	p	H	Pont-Med	0–2800	+	1
Linum catharticum L.	a	Th	subBoreal	200–2600	+	1
Mentha longifolia (L.) Huds.	p	H	Eur-Sib	0–1200	+	1
Plantago lanceolata L.	p	H	Kos	0–2000	+	1
P. major L.	p	H	Boreal	0–2000	+	1
Potentilla ternata K. Koch	p	H	Carp-Bal	1400–2600	+	1
Primula veris L.	p	H	Eur-Med	0–2500	+	1
Prunella laciniata (L.) L.	p	H	Eur	0–1000	+	1
Sanguisorba minor Scop.	p	H	subBoreal	0–1200	+	1
Satureja pilosa Velen.	p	H-Ch	Bal	200–1500	+	1
Scabiosa columbaria L.	b-p	H	Eur-Sib	1000–2900	+	1
Sedum album L.	p	H	subMed	0–2500	+	3
Sempervivum erythraeum Velen.	p	H	Bal	1000–2700	+	1
Seseli libanotis (L.) W.D.J. Koch	p	H	Eur-Sib	500–1600	+	1
S. peucedanoides (M. Bieb.) Koso-Pol.	p	H	Med-OT	900–1700	+	1
Silene roemeri Friv. subsp. *roemeri*	p	H	Bal	300–2700	+	1
Solidago virgaurea L.	p	H	Boreal	500–2800	+	1
Teucrium chamaedrys L.	p	H	subMed	0–1500	+	1
Thymus vandasii Velen.	p	H	Eur-Med	900–2900	+	2
Vincetoxicum hirundinaria Medik.	p	H	Eur-Sib	0–1500	+	1

Biological types: a – annual herbaceous plant; **b** – biennial; **p** – perennial; **sh** – shrub; **Life form: Ph** – phanerophytes; **Ch** – chamaephytes; **H** – hemicryptophytes; **Cr** – cryptophytes; **Th** – therophytes; **Floristic element: Alp** – Alpine; **Anat** – Anatolian; **Arct** – Arctic; **As** – Asian; **Bal** – Balkan; **Boreal** – Boreal; **Carp** – Carpathian; **Cauc** – Caucasus; **Dac** – Dacian; **Eur** – European; **Kos** – Cosmopolitan; **Med** – Mediterranean; **OT** – Oriental-Turanian; **Pann** – Pannonian; **Pont** – Pontic; **Sib** – Siberian; **prefixes: sub-**; **S** – south.

Results and discussion

The locality of *A. mollis* is situated on the southern slopes of the Central Balkan Range, on the territory of Dzhendema Reserve, in a place known as Kamenlivitsa – 42°41'N, 24°57'E, 1158–1162 m a.s.l. (Fig. 1). Evidently, the investigated locality is significantly lower than the vertical distribution of the species in Bulgaria reported by Assenov (1973) at 1600–1700 m a.s.l. and later cited by Assyov & Petrova (2006). The lower altitude, along with the dry and rugged terrain gives reason to assumptions that this is a secondary locality. It probably originated from seeds of the plants growing on the humid, steep and hard for access slopes high above the described locality, driven down by rain or snow-melting water. A probable reason for the existence of the species at this altitude is that it is surrounded and sheltered by a beech forest. Attempts are under

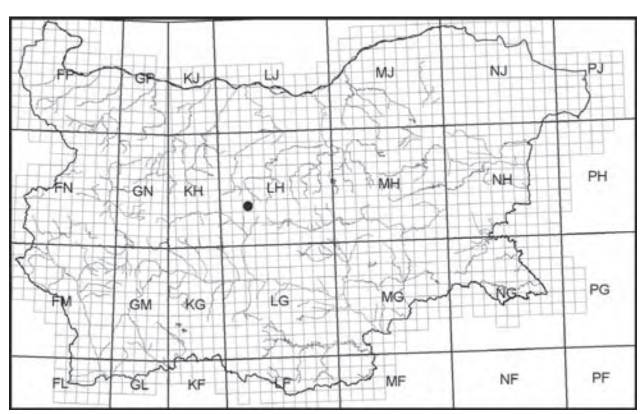

Fig. 1. Distribution map of *A. mollis* in Bulgaria.

way to find other localities of *A. mollis* across the entire territory of the Central Balkan National Park. While revising the materials of genus *Alchemilla* in the Herbarium of the Agricultural University in Plovdiv (SOA), we came across a specimen of *A. mollis* collected in the surroundings of peak Vezhen (Central Balkan Range) and mistaken for *A. catachnoa* Rothm. It gave rise to hope that the species is distributed also elsewhere in the Central Balkan Range. However, in our field studies in the area of Vezhen peak *A. mollis* was not confirmed.

The investigated clone population covers an open, dry and erosive terrain, with inclination of 25° and southwestern exposition. The basic rock consists of shale and the soil type is Oxysols. The soil was with low humus content (1.44 %), poor in total nitrogen (0.068 %) and phosphorus (0.048 %), with pH 6.2–6.5 (Vitkova 1997). As a result of the snow cover and melting snow, the soil remains fresh and moist in winter and spring. In the other seasons the moisture is scarce and depends on the rainfall.

The approximate size of the locality of *A. mollis* has increased since 1990 from 150 m^2 to 200 m^2. A hiking trail from Kalofer town to Rai chalet (in the vicinity of peak Botev) crosses it. The locality is surrounded by a beech forest and single trees of *Acer campestre* L., *Carpinus orientalis* Mill., *Crataegus monogyna* Jacq., *Fraxinus ornus* L., *Ostrya carpinifolia* Scop., *Pyrus pyraster* Burgsd., and *Sorbus aucuparia* L.

A floristic inventory of the community with presence of *A. mollis* listed 65 vascular plant species, included in 57 genera (Table 1). The families *Poaceae* (11) and *Asteraceae* (9) have the highest number of genera. Distribution of the species into biological types is as follows: the group of perennials show the highest numbers (86.2 %), followed by shrubs (7.7 %), biennials (4.6 %) and annuals (1.5 %). According to the Raunkiaer's classification (1934), hemicryptophytes (H) are dominant in the plant community, with 87.8 %. The other groups have symbolic presence: chamaephytes (Ch) 4.6 %, phanerophytes (Ph) 4.6 %, cryptophytes (Cr) 1.5 %, and therophytes (Th) 1.5 %. Most of the species are widely distributed on the territory of Bulgaria and only three are local for the Central Balkan Range: *Alchemilla mollis*, *Campanula scheuchzeri* and *Centaurea splendens*. *Agrostis capillaris*, *Alchemilla mollis* and *Teucrium chamaedrys* have the highest projection coverage in the community, the rest of the species are presented with few or single individuals (Table 1).

Analysis of the floristic elements has shown that the elements with European component common for the beech belt of most European countries prevail – 43.1 %. Second rank the elements with a Mediterranean component – 20 %. Their significant number could be explained with the medium altitude of the locality, its position on the southern slopes of the Balkan Range and the Continental-Mediterranean climatic influence along the rivers in that part of the mountains. There is also a considerable number of elements with a Boreal component (Boreal – 9.2 % and Subboreal – 7.7 %), which is in line with the mountain nature of the community (Fig. 2).

The vegetation coverage in the locality is 35–40 %. The projection coverage of *A. mollis* is 2 % and the individuals are distributed single, in small groups or in patches with prevalence of small groups. This type of "episodic mosaic pattern" is defined by the chances of dissemination and survival of the seedlings (Pavlov 2006). Most *A. mollis* groups are observed on the steep and erosive slope and few single plants grow in the border areas of the beech forest (Fig. 3).

The age structure of the population was studied in July 2009. The analysis has shown dominance of virginal plants (85 %); the generative plants comprised only 15–20 %. In the virginal group were counted 33 seedlings and 18 juvenile and immature plants. There were nine mature generative clones. According to Rabotnov (1983), this is a "normal type" of population, independent of the inward transport of seeds and able to reproduce sexually or vegetatively. As a result of the high summer temperatures during 2008–2009, some of the flowering stems had withered in the phase of full blossom. This suggests that a few more very hot

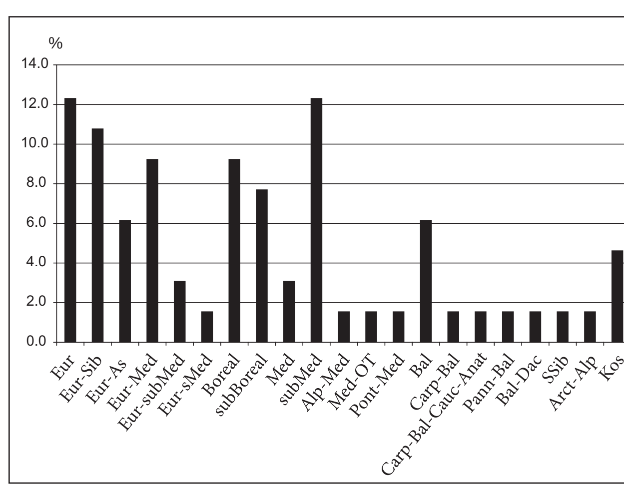

Fig. 2. Quantitative participation of floristic elements in the plant community of *A. mollis* (for abbreviations see Table 1).

Fig. 3. General view of the population of *A. mollis*.

and dry summers could badly affect the age structure of the population and cause prevalence of senile plants, along with a cut-down in seedlings.

The measurement of area of the nine mature clones has shown that they cover 3.1 m^2, which comprise 1.6 % of the whole locality. The coverage of the separate clones varied from 0.1 to 1.26 m^2, which could be explained with difference in their age (Table 2). This assumption comes from the number of flowering stems per 0.100 m^2. The highest number of flowering stems 10–18 was observed in clones 3, 7 and 8. They cover the smallest area and probably are the youngest. Age increases the size of the clone, but reduces the number of flowering stems per unit of area. This is clearly evident in clone 6, which had the largest area but only three flowering stems per 0.100 m^2. There was significant variability in the height of flowering stems, length and width of the leaf laminas, and length of the petioles in the different clones. On the one hand, this may result from the plants age, but on the other, the local environmental conditions may be responsible.

On the basis of these observations is drawn the conclusion that the main negative factors for the population of *A. mollis* were erosion of the terrain and hot and dry weather during the last few summers. The hiking trail crossing the locality is a precondition for stamping and withering of juvenile plants and seedlings.

Some measures have been taken for preservation of the species. Live material was transported and planted in three experimental fields of the Institute of Biodiversity and Ecosystem Research (IBER) at different altitudes: West Rhodopi Mts, on the territory of the Beglika State Forestry Farm, at 1500 m a.s.l.; Mt Vitosha, in the Zlatnite Mostove locality, at 1500 m a.s.l.; and in Sofia, in the IBER glasshouse, at 500 m a.s.l. Studies conducted in the *ex situ* collections have shown that plants from the natural population of *A. mollis* in the Central Balkan Range develop successfully. This will allow the cultivation of species in field conditions and its sustainable use.

Successful experiments for *in vitro* propagation of *A. mollis* have been conducted in the Biotechnological Laboratory of IBER, BAS in 2010.

Seeds and herbarium materials are collected and transferred for conservation to the National Seed Bank with the Institute for Introduction and Plant Gene Resources, Sadovo.

Table 2. Morphometric characteristics of the mature generative clones in the population of *A. mollis*.

№ Clone	Length of the clones (cm)	Width of the clones (cm)	Area of the clones (m^2)	Number of flowering stems per 0.100 m^2	Height of flowering stems (cm) $M_1 \pm m_1$	Length of leaves (cm) $M_2 \pm m_2$	Width of leaves (cm) $M_3 \pm m_3$	Length of petioles (cm) $M_4 \pm m_4$
1.	46	40	0.184	7	28.75±1.25	5.75±0.59	9.00±0.20	8.13±1.60
2.	60	45	0.270	6	31.75±3.20	7.75±0.25	10.13±0.83	14.25±3.16
3.	70	22	0.154	10	25.25±1.93	7.13±0.32	7.50±0.35	8.00±1.24
4.	115	35	0.402	6	26.50±2.90	9.40±0.24	10.00±0.20	10.63±0.37
5.	60	37	0.222	9	28.50±1.26	8.00±0.41	9.38±0.47	10.00±0.70
6.	99	127	1.257	3	31.25±3.10	9.63±0.55	11.50±0.29	15.87±0.83
7.	40	25	0.100	18	21.50±2.10	7.00±0.20	7.62±0.38	10.00±0.50
8.	73	30	0.219	14	23.00±1.22	5.87±0.51	7.62±0.24	14.25±0.95
9.	65	40	0.260	4	30.00±1.10	7.12±0.24	8.38±0.32	12.00±0.67

M – arithmetic average; **m** – error of average

Further changes in the condition of the population of *A. mollis* will be thoroughly recorded by regular monitoring already initiated in 2009.

Conclusions

The results of this study show that the only population of *Alchemilla mollis* known so far from Bulgaria has increased in numbers, but its condition is still a matter for concern. The legislative measures (inclusion of the species in Supplement 3 of the Biological Diversity Act) taken so far have been insufficient. *Alchemilla mollis* should be entered in the List of Vascular Plants subject to the National Biodiversity Monitoring System in Bulgaria. Further active measures should be taken by the administration of the Central Balkan National Park for conservation of the population on its territory. Original live plant material grown in the available experimental fields should be used appropriately for restoration of the species in the area of the locality. Regular monitoring has to be carried out.

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