REDISCOVERY OF *PIETROSIA LEVITOMENTOSA* E. I. NYÁRÁDY EX SENNIK., AN ENDEMIC AND ENDANGERED PLANT SPECIES FROM PIETROSUL BISTRIȚEI MOUNTAIN, ROMANIA

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This paper presents a study upon the scientific knowledge of the conservation status and perspectives of an endemic Carpathian plant. The paper aims at improving our understanding of the extinction risk of the endemic species *Pietrosia levitomentosa* E. I. Nyárády *ex* Sennik. Further, the present study case is being used to highlight the main features of biodiversity conservation in Romania, a country with a high number of endemic species.

Key words: Pietrosia levitomentosa E. I. Nyárády *ex* Sennik., endemic plant, field investigations.

INTRODUCTION

Pietrosia levitomentosa E. I. Nyárády *ex* Sennik. (*Fam. Asteraceae*) is known as an endemic plant species from the Carpathians, growing only in the Carpathian Mountains Bistritei-Bogolin, northern Romania (Fig. 1). This species was listed as stricly protected by the Bern Convention and in various databases, but on the gounds of old data (from the sixties). Many began to suspect that it already became extinct. Because mature seeds have never been recorded to germinate, the species reproduces itself only vegetatively. This fact continue to raise many questions upon the species long-term populational and genetical viability in such a small habitat area, and with current fast environmental changes. In this communication, we comfirm the recent observations (Sarbu and Stefan, 2000) that the species is not extinct, and report a number of locations where the species has been found since the latter study. Moreover, we report that we have vegetatively reproduced individuals of the species outside its location of natural occurrence. Further, we discuss the extinction risk posed by climate changes, and possible ways to conserve this species, ranging from nature reserves to *in vitro* reproduction followed by subsequent reintroduction in its natural habitat.

At this point we present some facts regarding the taxonomy, ecology, phylogeny and the nomenclature: *Pietrosia levitomentosa* E. I. Nyárády *ex* Sennik. is a plant species belonging to the family *Asteraceae*, known as endemic for

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ROM. J. BIOL. – PLANT BIOL., VOLUME 54, No. 1, P. 101–114, BUCHAREST, 2009

Romania (Nyarady, E.I., 1963) and for Europe (Sell, 1976). The species is known in the world from only two geographical locations, the first one is a mountain area Pietrosul Bistritei (Fig. 1, 2c and 2d), an area of about 400 ha in Eastern Carpathians Mountains – Romania and the second one appears to be in the Ukrainian Carpathians (Kricsfalusy V. and Budnikov G., 2007). It is possible that this species is growing in the mountain areas of the Tisza River Basin – Ukrain, but we will confirm or infirm this fact in other papers. Due to its morphological and reproductive particularities, the species was described as an orophyte, Carpathian endemism, which appeared probably during the Tertiary period, 2–62 million years ago.

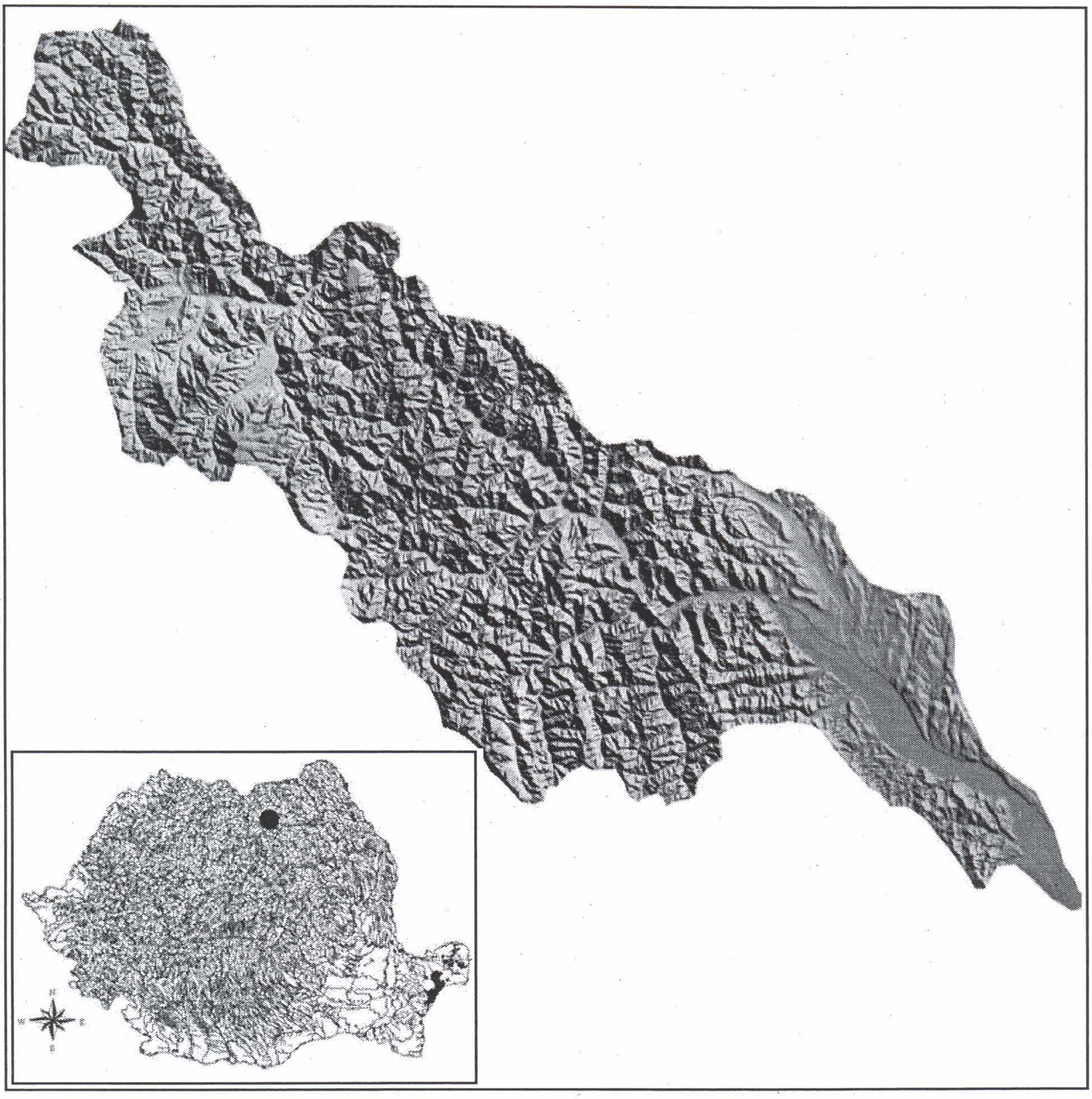

Fig. 1 The site with the Pietrosul Mountain in the North of Romania, left-below; and the site of *Pietrosia levitomentosa* on the Bistrita river basin, above (Original).

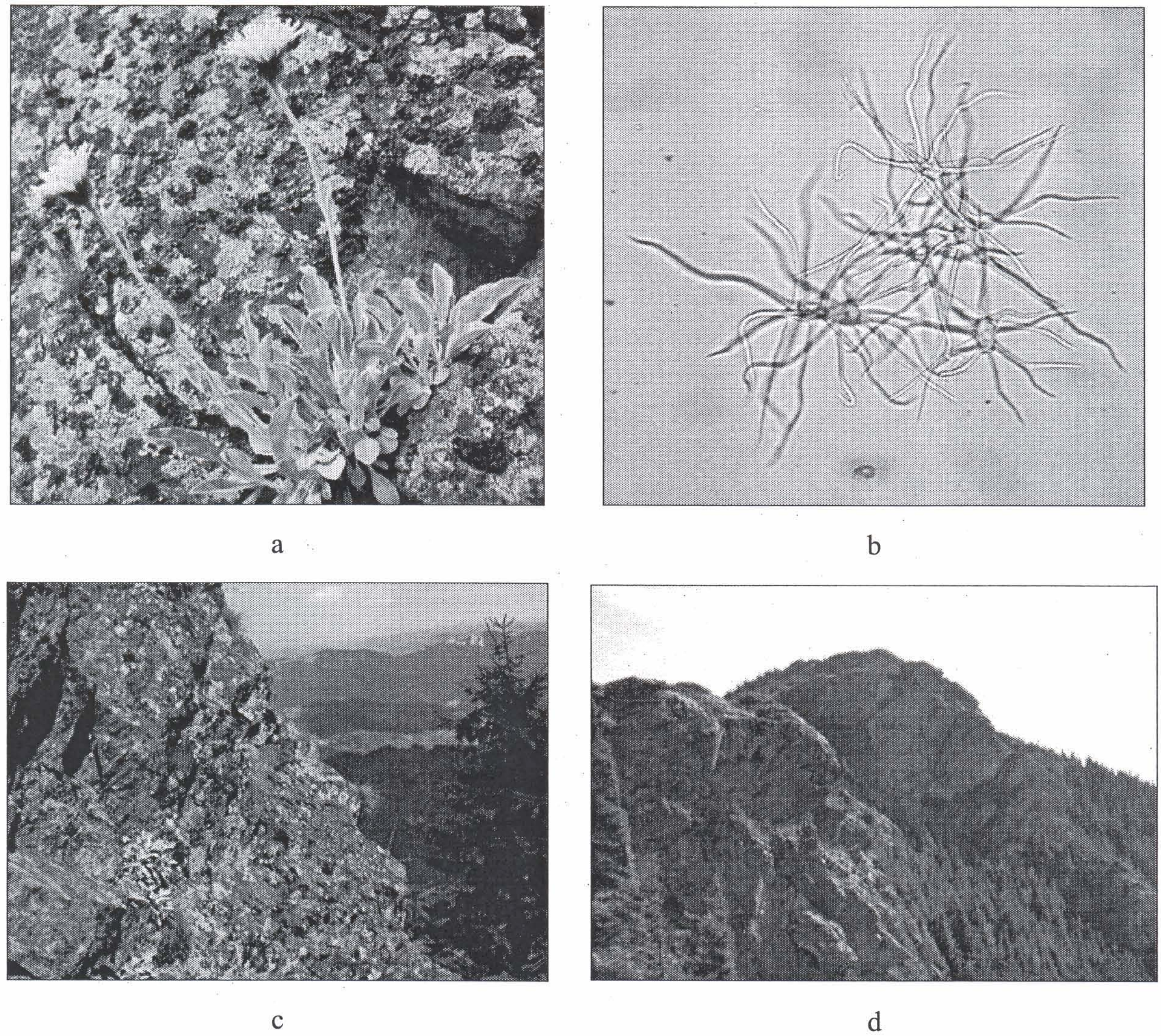

Fig. 2. Some characteristics regarding the endemic species *Pietrosia levitomentosa*: a – habitus of the living plants; b – starred trichomes from the upper epidermis – 200X; c and d – the natural habitat on the Pietrosul-Bogolin, Bistritei Mountains (Original).

The species has also been referred to: *Pietrosia levitomentosa* (Nyarady, E.I.), 1963, 1965; Stefureac, 1968; Sennikov, 1999; Negrean, 2004), *Andryala levitomentosa* (E.I. Nyarady) P.D. Sell or *Hieracium levitomentosum* (Soo, 1968). If the name of *Pietrosia levitomentosa* E. I. Nyárády *ex* Sennik. were to be preferred, then the taxon would also be a generic endemism (Stefureac, 1968, Negrean, 2004).

There will always be an information gap between fine scale taxonomic information and conservation planning (e.g., Lozano *et al.*, 2007). Studies of molecular genetics elucidating adaptive evolution in plants are at an incipient stage, still largely lacking sampling of local populations, explicit comparisons between *loci* and appropriate theoretical tools for tackling population issues (Wright and Gaut, 2004). The problems posed in biodiversity conservation by the frequent taxonomic changes and re-changes (Rojas, 1992; Mace, 2004), as well as the lack of genetic information (Soltis & Gitzendanner, 1999; Goldstein *et al.*, 2000), are too fundamental to be circumvented.

Some preliminary genetic studies provided evidence for intergeneric hybridization between genera *Hieracium* (subgenera *Hieracium* and *Pillosella*) and *Andryala*. It is true that some initial genetic analyses revealed an early phylogenetic branching of *Pietrosia levitomentosa* E. I. Nyárády *ex* Sennik. (Fehrer *et al.*, 2007a).

The nomenclature is derived from that of the origin of the species, namely whether *P. levitomentosa* is a relict species (paleoendemism) not a newly emerged one (neoendemism) (Engler, 1982). Current knowledge can sort this out, at least the basics (Fehrer *et al.*, 2007a, b).

Pietrosia levitomentosa E. I. Nyárády *ex* Sennik. is geographically isolated from all present *Andryala* species, but show clear evidence for a common ancestor with them. The data with nuclear DNA (nuclear ribosomal DNA internal transcribed spacer – ITS) is believed to reflect true phylogenetic relationships, as reflected by the nomenclature from Flora Europaea. Also, the chronology of hybridization events (capture of chloroplast DNA), shown by data with chloroplast trnT-trnL intergenic spacer and with chloroplast matK gene, indicates close phylogenetic links with the south-eastern European main lineage of *Pilosella*. This and other studies in the literature would even support that the entire genus *Andryala* should be placed under genus *Hieracium*, subgenus *Pillosella* (Gaskin and Wilson, 2007).

The early branching shown by genetic analysis for *P. levitomentosa* E. I. Nyárády *ex* Sennik. indicates that this is an old species. Together with *Andryala agardhii* Haensel *ex* DC. from Spain, our species is one of the oldest within its genus.

The main conclusion that comes out of this section is that *P. levitomentosa* E. I. Nyárády *ex* Sennik. is a glacial relict species (Fehrer *et al.*, 2007b). Second, in the context of such genetic advances during recent years, changing names, as proposed by Sennikov and Negrean on the basis of minute and variable morphological features, is more probable and necessary.

In Flora Europaea the species is named *Andryala levitomentosa* (Sell, 1976a, b), but throughout the paper we will name it as *Pietrosia levitomentosa* E. I. Nyárády *ex* Sennik., because of the reestablishing of the genus *Pietrosia* by Sennikov (1999).

MATERIALS AND METHODS

The studied area is in the Bistritei Mountains, the soils are districambisoils formed on metamorphic crystalline schists, with pH values usually around

5.5. Mountain crests are made up of porphyritic gneisses. The habitat of *Pietrosia levitomentosa* E. I. Nyárády *ex* Sennik. (Fig. 2c, 2d) is made up by potassium-rich, rocky alpine grasslands, around 1700 meters altitude. Soils are skeletic and semi-skeletic, fixed by *Festuca sp.* and other grasses and herbs, as well as by scattered individuals of *Juniperus communis ssp. sibirica* Lodd. in Burgsd.

The climate of the area is moderate continental temperate, on to which superimposes a high-mountain climate.

The closest climatic description of the area is provided by the Rarau mountain meteorological station (Rusu, 2002), which is at slightly lower altitude (1536 m), but also about ca 3 km to the east (25°27' E, 47°27' N). According to these data, the average annual temperature is 2.2°C. Monthly average above-zero maximum/minimum daily temperatures are between end-March – mid-November /end-April–mid-October. Monthly average maximum temperature reaches +11.2 to +11.4°C between mid-July – mid-August. Average minimum temperature reaches -7.1 to –7.4°C between mid-January – mid-February. Extreme temperatures records are: -28.4°C in January, and 29.0°C in July. The annual number of days with frost can go up to 200, and the frost can also occur during summer. The average annual precipitations amount to *ca* 902 mm, most of it during spring and summer. The month with the highest/lowest number of rainy days, 11.7/0.3, is June/February. The month with the highest/lowest amount of precipitation, 147/39 mm, is June/November-December. Snow can fall in any season. Sunny days are most frequent during October. The fog is present 201 days per year. Dominant winds are from south-east (44.8%), with wind speeds between 2.1–4.5 m/s during summer and 3.2–3.9 m/s during winter.

Field investigations have been carried out during summers of 2000 until 2008. During the summer of 2007, vegetation samples (2m x 2m) have been taken in the entire alpine store of the Bogolin Mountain, and presence-frequency tables have been realized using the standard Braun-Blaquet method (Leps & Handicova, 1992; Van der Maarel, 2005). Vascular plants have been recorded, and ferns, mosses and lichens have also been noted. *Pietrosia levitomentoa* E. I. Nyárády *ex* Sennik. has been particularly searched for within the expected area, as to be able to confirm its presence. Visual inspections have been carried out in the area in order to identify any sign of human activity in the habitat of the species. During the 2007 summer, the population of *P. levitomentosa* has been inventoried: number of locations, patch sizes and number of rosettes. Locations have been established using GPS. One of the main micro-morphological characters of this species are the starred trichomes, with high density on the leaves, upper and lower *epidermis* (*herba* in general), mentioned in different keys for species identification (Beldie, 1979; Ciocarlan, 2000), was illustrated, this is a valid character (Fig. 2b). The trichomes ware mounted in glycero-gelatin and observed with an optical microscope.

After Aiftimie-Paunescu and Vantu (2002) they had obtained plantlets *in vitro* from the callus, they concluded that micropropagation is a valuable method for the multiplication of *P. levitomentosa* E. I. Nyárády *ex* Sennik., offering the possibility to preserve this unique and endangered species in the Romanian flora.

RESULTS AND DISCUSSION

In this communication, we confirm recent observations (Sarbu and Stefan, 2000), that the species is not extinct. In 1968, only two locations were mentioned; we recently identified 12 populations, concentrated in 6 main locations in the studied area (Table 1).

Table 1

The six main locations of *P. levitomentosa*

	Locations	Geographical coordinates
I	Pietrosul Bistritei, Pietrosul Bogolin versant, the South-East slope,	N 47° 23' 19" / E 25 ° 32' 12,5". Altitude 1680 m
II	Pietrosul Bistritei, Pietrosul Bogolin versant, the South-East slope,	N 47° 23' 16.8" / E 25 ° 32' 12,6" Altitude 1676 m
III	Pietrosul Bistritei, Pietrosul Bogolin versant, the South-East slope,	N 47° 23' 08.2" / E 25 ° 32' 19,3" Altitude 1739 m
IV	Pietrosul Bistritei, Pietrosul Bogolin versant, the South slope	N 47° 22' 31.2" / E 25 ° 32' 29.1". Altitude 1783 m
V	Pietrosul Bistritei, Pietrosul Bogolin versant, the South-East slope,	N 47° 22' 19.7" / E 25 ° 32' 37" . Altitude 1706 m
VI	Pietrosul Bistritei, Pietrosul Bogolin versant, the South-East slope	N 47° 22' 20.6" / E 25 ° 32' 32.8" Altitude 1713 m

Therefore, for practical reasons it is more appropriate to count the number of rosettes, and temporarily assume it equal to the number of individuals. In July 2007, we were able to count rosettes at 3 accessible locations, and estimate the minimum number of rosettes at 3 less accessible (very steep) locations, between 1676–1739 meters altitude. We assume that one location is occupied by one population.

We have also studied the flora, our own sampling with 2m x 2m plots (4 m^2), in the habitat of *Pietrosia levitomentosa* E. I. Nyárády *ex* Sennik., indicate the presence of 21 species. The only species co-occurring in all samples with *P. levitomentosa* E. I. Nyárády *ex* Sennik. is the tufted fescue, *Festuca airoides* Lam. (=*F. supina* Schur.), a perennial graminoid spread into Central, Southern and Eastern Europe but also in Western Asia. Besides *P. levitomentosa*, another species found in our samples is strictly protected by the Bern Convention, this is

Campanula abietina Griseb. Out of the vascular plants in the 6 samples, 12 are able to reproduce *via* clones – present in the clonal base, database maintained by Klimesova and Klimes (CPD, 2007): *Pietrosia levitomaentosa* E. I. Nyárády *ex* Sennik., *Arnica montana* L., *Cystopteris fragilis* (L.) Bernh. (fern), *Festuca airoides* Lam., *Hieracium alpinum* L., *Hieracium aurantiacum* L., *Hypochoeris uniflora* Vill., *Juncus trifidus* L., *Polypodium vulgare* L. (moss), *Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L. and *V. uliginossum* L. Among clonal species they are both the most frequent species in our samples, and the most phylogenetically related taxa, the *Hieracium* species. Ferns, mosses and lichens are also quite frequent in this rocky habitat, we mention the species: *Cetraria islandica* L., *Xanthoria parietina* (L.) Th. Fr. and *Thamnolia vermicularis* (Sw.) Ach. ex Schaerer. We have to mention also other species co-occurring in this subalpine habitat: *Campanula carpatica* L., *Campanula kladniana* (Schur.) Witasek, *Dianthus tenuifoius* Schur., *Juncus trifidus* L., *Hypochoeris uniflora* Will., *Luzula luzuloides* (Lam.) Dandy & E. Willm. and *Juniperus communis* subsp. *sibirica* (L.) Lodd in Burgsd. We also have tried but we did not obtain plants from seeds.

Thus, we counted a number of six populations, each population made up of several patches, with total cover surface of about 44,5 square meters, and a conservatively estimated total number of 3310 rosettes (Table 2).

P. levitomentosa E. I. Nyárády *ex* Sennik. has been mentioned as strictly protected by the Bern Convention and in various databases, but on the grounds of old data, mainly from the sixties. The recent lack of visibility of the species in the research literature fuelled fears that it went extinct. Here by we confirm earlier conference notes (Sarbu and Stefan, 2000) that the species is alive. The inaccessibility of its natural habitat, in the rocky subalpine floor, and the species rarity, made the study of the species quite difficult. This may be the reason why the conservation status, along with the biology and the ecology of this species, resisted close inspection. In addition, the lack of information could explain the past and present hesitations upon which genus belongs to. We have shown that there are six main populations, with an estimated total number of rosettes above 3000. Most recent references to *P. levitomentosa* E. I. Nyárády *ex* Sennik. mention a small population of *ca* 200 individuals (Fehrer *et al.*, 2007b), but without indication on how the estimation has been made. In fact, the natural habitat, the real number of individuals is hard to count because of the vegetative (clonal) way of reproduction of the species. Individuals tend to be aggregated into more or less compact populations, forming pillow-like patches (Fig. 2,c). This is an adaptation to the high-mountain environment, and is also related to its vegetative way of reproduction.

Table 2

The inventory of *P. levitomentosa* from the Bistritei Mountains

Location (site) and altitude (meters)	*Patch number*	*Number of rosettes*	*Cover* (m^2)
I - 1680 m	1	168	1,5
	2	54	1
	3	161	1
	4	92	1.5
	5	33	1
	6	68	1
	7	37	0.5
	8	55	0.5
	9	39	0.5
	10	9	1
	11	20	0.5
	12	87	0,5
II – 1676 m	13	184	1
	14	56	0.5
	15	49	0.5
	16	161	1
	17	132	1
	18	50	0.5
	19	7	0.5
	20	118	1
	21	100	1
	22	93	0.5
	23	192	1
	24	85	0.5
	25	73	1
III – 1739 m	26	31	0,5
	27	35	1
	28	29	1,5
	29	32	1
IV – 1783 m	30	41	4,5
V – 1706 m	31	488	3,5
	32	44	2,5
VI – 1691 m	33	487	9,5
Total	33	3310	44,5

Indeed, clonal growth is also very frequent in the alpine environment. As noted, 70 % of species which are growing in the habitat of *P. levitomentosa* are capable of clonal growth. This is in agreement with the existent literature. Many plant species in high mountains combine sexual and clonal reproduction as a way to minimize the risks of flowering and seed production in these habitats. There are various strategies of clonal growth, like phalanx or guerrilla, which influence both the genetic structure and the ramets to genets proportion (Reisch *et al.*, 2007). Clonal growth allows plants to continuously increase their size, hence their capacity of sexual reproduction. This is thought to balance the negative effect of high adult mortality (when it occurs) and the negative effect of mortality on fitness (Franco and Silvertown, 1996). Clonal growth tends to be more frequent at higher altitudes, as a way to adapt to local climatic conditions (Stöcklin and Bäumler, 1996; Körner, 2003).

As with species' nomenclature, hesitations occurred in respect to which plant alliance the species should be ascribed to. Thus, after the species was described, some authors placed it, quite arbitrarily, in some older plant alliances, like: *Asplenietea trichomanis* (Br.-Bl. in Meier et Br.-Bl. 1934) Oberd. 1977; *Potentilletalia caulescentis* Br.-Bl. 1926; *Gypsophilion petraeae* Borhidi et Pócs 1957. More recently, Seghedin placed *P. levitomentosa* in the plant alliance: *Sempervivo soboliferae-Andryaletum levitomentosae* Seghedin 1985 (Seghedin, 1986, 1989). The plant alliance *Sempervivo soboliferae-Andryaletum levitomentosae* Seghedin 1985 was mentioned also by Oprea (2007). As the nomenclature of the alliance suggests, *Andryala levitomentosa* occurs together with *Sempervivum soboliferum*. However, despite our 8-year long floristic investigations in the area, we have never found the later species. As a consequence, we cannot endorse the alliance proposed by Seghedin. Our current data rather supports an alliance between *P. levitomentosa* and *Festuca airoides*. Therefore, provisionally has been proposed an alliance called *Andryalo levitomentosae – Festucetum airoidi* (Sarbu and Stefan, 2000), which we have to confirm.

Wider ecological and conservational aspects regarding *P. levitomentosa* E. I. Nyárády *ex* Sennik. are beyond the aim and space of the present report. In the next paragraphs, we will only brief the factors that could potentially pose a risk to the existence of our species.

The species is vulnerable because of its small populations size, which poses serious survival problems, probably due to depletion of genetic diversity, which leads to lower fitness and ability to cope with environmental challenges (Parsons, 1989). Many rare perennials show lower genetic diversity or polymorphism in reintroduced populations than in source ones, due a founder effect (e.g., Smulders *et al.*, 2000). The possibility of cultivation in botanical gardens, for conservation purposes, has been suggested by Stefureac in his 1968 paper. However, he expressed the concern that the species might be bound to high amounts of potassium in soils, and possibly to some microelements. In another paper we will demonstrate that *P. levitomentosa* E. I. Nyárády *ex* Sennik. can be cultivated outside its natural environment. We mention that we have obtained plants *ex situ* cultivated at a lower altitude by vegetative ways (846 m. – in the locality Dorna – Arini, Suceava County, Romania). Small populations, particularly those restricted to a small area, are vulnerable to biotic and abiotic disturbances. If a disturbance is large enough to wipe out the entire area, no population will be left for recovery. Even when several populations are left, any destruction will enhance risks faced by small population species.

Given the habitat to human and domestic animals, the main risks for the species come from the fast environmental changes, notably climate changes and pollution. If climate changes are too fast or potential pollution too intense (Rusek, 1993), the species may not cope. Nevertheless, even in such remote and steep mountain areas like Pietrosul, the human threat is not to be ignored, given the transitional nature of Romanian society and economy from state-driven to private. This socio-economic situation is already known to be harmful to the environment in various and unexpected ways (Ioras, 2003).

Invasions by other plant species or surges of parasites occasioned by serious climate changes may also add to the extinction risk of the species. From 2000 until now we have observed that the population of *Juniperus communis subsp. sibirica* Lodd in Burgsd. tend to extend over the area covered by the species we studied. The cover of *J. communis subsp. sibirica* has increased by 5 to 6% over the surface covered by *P. levitomentosa* E. I. Nyárády *ex* Sennik. Nevertheless, the genetic uniformity of determined by clonal reproduction make the population particularly vulnerable to diseases (Silvertown and Charlesworth, 2001), unless the species developed some form of programmed ramet independence (Kelly, 1995). The later possibility is a subject for further study interest, as are the other aspects related to clonal growths. The risk is amplified by the small total population size of the species, known from this location.

A risk of introduction of a congener or related species with *P. levitomentosa* E. I. Nyárády *ex* Sennik., may hybridize (and so become instinct by loss of genetic identity) and exists in theory. Interestingly, hybridization may also be a way to protect against losses caused by extinction of rare species (Soltis & Gitzendanner, 1999).

All previous aspects are particularly important for *P. levitomentosa* E. I. Nyárády *ex* Sennik., because of its reproduction restricted to vegetative means (ramets) at the expense of seedling (genets). The species may have already lost the ability to reproduce sexually. We have made experiments with seeds of

P. levitomentosa E. I. Nyárády *ex* Sennik. but without success. As we observed in the natural populations, the species produces flowers and seeds, but every time we collected seeds we noticed they were not viable.

Clonal growth can be a good strategy to survive in alpine environment, and trade offs are documented between sexual and clonal reproduction (Sutherland and Vickery, 1988; Silvertown and Charlesworth, 2001; Pluess and Stöklin, 2005). Phylogenetic constraints like genetic drift/bottleneck situations are thought to determine lower genetic diversity at higher altitudes (Peterson and Jones, 1997; Van Goenendael *et al.,* 1997; Morris *et al*., 2004). Genetic diversity tends to decrease again at very high altitudes (>5000 m), due to the harsh conditions damaging ramet connections (Taira *et al*. 1997; Klimes, 2003; Ohsawa and Ide, 2007).

Genetic diversity of a population depends on seedlings. Using computer simulations, Watkinson and Powell (1993) demonstrated that the genetic structure of new clonal plant populations (colonisation of available space) is essentially determined by the seedling to ramet ratio. In the absence of new genets coming into populations (and such is the case with species which lost the ability to produce new genets, like *P. levitomentosa*), genetic diversity of the population decreases slowly, and becomes dominate by only few genets. This dynamics was confirmed empirically. For example, studying natural populations of *Paris quadrifolia* L., Jaquemyn *et al*. (2006) have concluded that stressful environmental conditions can make populations of clonal plants to gradually evolve into remnant populations with low genetic diversity and limited sexual reproduction. This happens because less adapted genotypes are out competed, as shown by the negative correlation between ramets diversity and density of shoots. They further conclude that the pre-requisiste for the conservation of clonal plants is to insure the protection of suitable habitats for these species.

The fact that, apparently, there viable seeds are not produced by *P. levitomentosa* anymore, may reflect a situation suggested already for other clonal species where flowers are not serving genet production anymore, but flowering is necessary for regulating the production of shoots (Carlsson and Callaghan, 1990). This is an aspect which is worth further attention.

For *P. levitomentosa*, it will be important to study the genetic diversity within the existing populations. Such studies will show the degree to which sub-populations from the recorded locations have local adaptations or are genetically similar. Potential reintroductions will be much easier and successful when the populations are genetically diverse. Given the above-discussed issues related to the clonal way of reproduction of the species, there is a very high risk in that genetic diversity may be low, in which case the long-term viability of the species is very questionable. Especially in plant populations which have gone through bottleneck and founder effects, the genetic drift effects in small populations are known to decrease the fitness of all individuals in rare species, which significantly increases the risk of extinction in changing environments, like climate changes or other disturbances (e.g., Ellstrand & Elam, 1993; Hedrick and Kalinowski, 2000; Willi *et al.*, 2006).

CONCLUSION

We confirm that earlier concerns exposed in Carpathian List of Endangered Species (2003) referring to *Pietrosia levitomentosa* E. I. Nyárády *ex* Sennik. as an endangered species are rightful. Initial population information is being provided to support this statement. Conservation of the species will be a complex matter given the biological peculiarities of the species, the current environmental changes and the Romanian socio-economic condition. Further studies will be needed to elucidate the very best ways to conserve this species. We propose that this endangered species should be cultivated for experimental and ornamental purpose.

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