

## The endemic plant species *Pietrosia levitomentosa*, a real conservation challenge

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**Abstract.** The current paper presents a study upon the scientific knowledge on the conservation status and future perspectives of the endemic species *Pietrosia levitomentosa* Nyárády ex Sennikov. The scope of the paper is to improve our understanding about this endemic species and to underline its importance. 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* Nyárády ex Sennikov, endemic plant species, field investigations, morphology.

**Resumen.** El trabajo presenta un estudio sobre el conocimiento científico, el estado de conservación y las futuras perspectivas de las especies endémicas *Pietrosia levitomentosa* Nyárády ex Sennikov. El artículo subraya también la importancia de esta especie endémica. En el mismo tiempo, el estudio de caso se utiliza para destacar las principales características del proceso de conservación de la biodiversidad en Rumanía, un país con un alto número de especies endémicas.

**Palabras clave:** *Pietrosia levitomentosa*, Nyárády ex Sennikov, especies de plantas endémicas, investigaciones de campo, nuevo disco, morfología.

**Rezumat.** Această lucrare este un studiu științific ce are în vedere perspectivele conservării speciei endemice *Pietrosia levitomentosa* Nyárády ex Sennikov. Lucrarea are ca țintă relevarea importanței acestei specii endemice. Acest studiu își propune și trecerea în revistă a principalelor caracteristici ce țin de procesul conservării în România, o țară cu un număr mare de specii endemice.

**Cuvinte cheie:** *Pietrosia levitomentosa* Nyárády ex Sennikov, specie endemică, investigație de teren, date noi, morfologie.

**Introduction.** *Pietrosia levitomentosa* Nyárády ex Sennikov is a plant species belonging to the Family Asteraceae, is known as endemic for Romania (Nyárády 1963) and for Europe (Sell 1976). *P. levitomentosa* is known in the world from only two geographical locations, the first one is a mountain area Pietrosul Bistriței - Bogolin (Figure 1 - b, d), an area of about 400 ha in Eastern Carpathians - Romania and the second one appears to be in the Ukrainian Carpathians (Kricsfalusy & Budnikov 2007).

This species is listed as strictly protected by the Bern Convention and in various databases, but on the grounds of old data. We suspect that it already became extinct, because mature seeds have never been recorded to germinate, the species reproduces itself only vegetatively, this fact raise many questions upon the species long-term populational and genetical viability in such a small habitat area, and with current fast environmental changes. We report that we have vegetatively reproduce individuals of the species outside its location of the 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. In this communication, we confirm the recent observations (Sârbu & Ștefan 2000) that the species is not extinct, and report a number of locations where the species has been found since the latter study.

The species has also been referred to: *Pietrosia levitomentosa* (Nyárády 1963, 1965; Ștefureac 1968; Sennikov 1999; Negrean 2004), *Andryala levitomentosa* (Nyárády 1963, 1965; Sell 1976ab), or *Hieracium levitomentosum* (Soo 1968). If the name of *Pietrosia levitomentosa* Nyárády ex Sennikov were to prefer, then the taxon would also be a generic endemism (Ștefureac 1968; Negrean 2004).

We also present below some facts regarding the taxonomy, ecology, phylogeny and the nomenclature of this plant species.

It is possible that this species is growing also in the mountain areas of the Tisza River Basin - Ukraine (Kricsfalusy & Budnikov 2007), 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 (Ștefureac 1968).

Some preliminary genetic studies provided evidence for intergeneric hybridization between genera *Hieracium* (subgenera *Hieracium* and *Pilosella*) and *Andryala*. It is true that some initial genetic analyses revealed an early phylogenetic branching of *Pietrosia levitomentosa* Nyárády ex Sennikov (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 2007ab).

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 & 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 (Goldstein et al 2000), are too fundamental to be circumvented. There will always be an information gap between fine scale taxonomic information and conservation planning (e.g., Lozano et al 2007).

*Pietrosia levitomentosa* Nyárády ex Sennikov 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 *Pilosella* (Gaskin & Wilson 2007).

The genus *Andryala* in the European Flora has only six known species, in general from the Mediterranean areas, the most common is *Andryala integrifolia* L. which grows in Spain and Italy. *Andryala* and *Pietrosia* are genera also related to *Hieracium*, *Hispidella*, *Hypochaeris*, *Crepis*, *Lactuca* and *Sonchus*.

The habitat of *P. levitomentosa* was known as small as only about 150 m<sup>2</sup> on a few inaccessible rocks. The species *P. levitomentosa* has been also keyed and mentioned in recent times by Ciocârlan (2000). The early branching shown by genetic analysis for *P. levitomentosa* Nyárády ex Sennikov 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.

In Flora Europaea the species is named *Andryala levitomentosa* (Sell 1976ab), but throughout the paper we will name it as *Pietrosia levitomentosa*

Nyárády ex Sennikov because of the reestablishing of the genus *Pietrosia* by Sennikov (1999).

The main conclusion come out of this section is that *P. levitomentosa* Nyárády ex Sennikov 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 (1999) and Negrean (2004) on the basis of minute and variable morphological features, is more probable and necessary.

**Material and Method.** The habitat of *Pietrosia levitomentosa* Nyárády ex Sennikov (see Figure 1) is made up by potassium-rich, rocky alpine grasslands, around 1700 meters altitude. Soils are skeletal and semi-skeletal, fixed by *Festuca sp.* and other grasses and herbs, as well as by scattered individuals of *Juniperus communis ssp. sibirica*.

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

Aiftimie-Păunescu & Vântu (2002) after they had obtain plantlets *in vitro* from callus, had concluded that micropropagation is a valuable method for the multiplication of *P. levitomentosa* Nyárády ex Sennikov, offering the possibility to preserve this unique and endangered species in the Romanian flora.

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 levitomentosa* Nyárády ex Sennikov 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 stared trichomes, with high density on the leaves, upper and lower *epidermis* (*herba* in general), mentioned in different key's for species identification (Beldie 1979; Ciocârlan 2000), was illustrated, this is a valid character and also the leaf morphology (Figures 1 and 2). The trichomes were mounted in glycerol-gelatin and observed with an optical microscope.

The closest climatic description of the area is provided by the Rarău mountain meteorological station (Rusu 2002), which is at slightly lower altitude (1536 m), but also about ca three 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.

**Results and Discussion.** We had counted a number of six populations, each population made up of several patches, with a total cover surface of about 45 square meters, and a conservatively estimated-total number of about 3000 rosettes (for all six populations).

In the current paper, we confirm recent observations (Sârbu & Ştefan 2000), that the species is not extinct. In 1968, only two locations were mentioned, we recently identified six populations, concentrated in six main locations in the studied area, we also show the habitat of this species, the leaf morphology, the biometry and the cultivation process.

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 three accessible locations, and estimate the minimum number of rosettes at three less accessible (very steep) locations, between 1676 - 1739 meters altitude. We assume that one location is occupied by one population.

We had also count the leaves/plant, the mean is about 15 leaves, with a maximum of 30 leaves/plant and a minimum of seven leaves/plant (this statistical data was collected from 100 plants, see Figure 1).

We had also measured the mature leaves, and compared the leaves - length/wide: the maximum length was about 6.5 cm and the minimum was 3.2 cm, the mean of the length was about 4.73; the maximum wide was 1.8 cm and the minimum was 0.9; the mean of the length/wide was 3.45. The data was collected from the measurements of 30 mature leaves. The leaf shape is spatulated and is compared whit an atypical leaf of *Hieracium aurantiacum* (Figure 2).

*P. levitomentosa* Nyárády ex Sennikov (Figure 1) 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 (Sârbu & Ştefan 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 it 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* Nyárády ex Sennikov mention a small population of cca 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 (Figure 1). This is an adaptation to the high-mountain environment, and is also related to its vegetative way of reproduction.

We have studied the flora, our own sampling in the habitat of *Pietrosia levitomentosa* Nyárády ex Sennikov indicate the presence of 21 species. The only species co-occurring in all samples with *P. levitomentosa* Nyárády ex Sennikov 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. Beside *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 six samples, 12 are able to reproduce via clones – present in the clonal base, database maintained by Klimesova & Klimes (CPD 2007): *Pietrosia levitomentosa* Nyárády ex Sennikov, *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 are both the most frequent species in our samples, and the most phylogenetically related taxa, the *Hieracium* species.

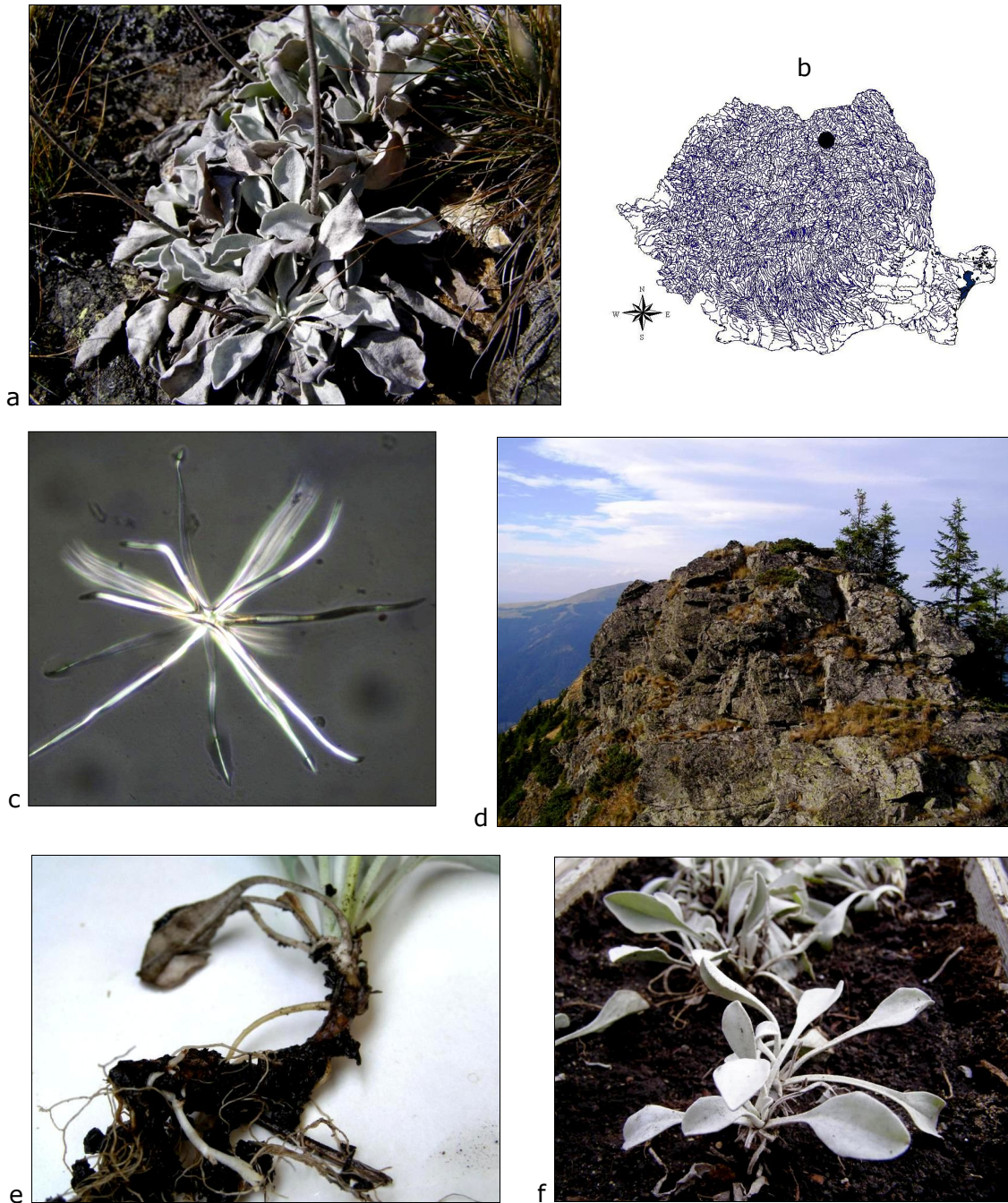


Figure 1. Some aspects regarding the plant species *Pietrosia levitomentosa*: a – the habitus of the living plants; b – the Pietrosul Mountain site - Northern part of Romania, c - stared trichome from the upper epidermis - 200X; d - the natural habitat on the Pietrosul-Bogolin, Bistritei Mountains, e – clonal growth (apomixis); f – the cultivation process from Dorna-Arini, Suceava county (original)

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 tenuifolius* 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.

Clonal growth is also very frequent in the alpine environment (Figure 1). 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 plant 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 & Silvertown 1996). Clonal growth tends to be more frequent at higher altitudes, as a way to adapt to local climatic conditions (Stöcklin & 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: *Asplenieta trichomanis* (Br.-Bl. in Meier & Br.-Bl. 1934) Oberd. 1977; *Potentilletalia caulescentis* Br.-Bl. 1926; *Gypsophilion petraeae* Borhidi & 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* (Sârbu & Ștefan 2000), which we have to confirm.

The species *P. levitomentosa* is vulnerable because of the 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 Ștefureac 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* Nyárády ex Sennikov 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). The species was also cultivated in Piatra Neamț (315 m elevation) but did not survive.

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.

From 2000 until now we 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* Nyárády ex Sennikov. Nevertheless, the genetic uniformity determined by the clonal reproduction makes the population particularly vulnerable to diseases (Silvertown & 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 growth. The risk is amplified by the small total population size of the species, known from this location.

All previous aspects are particularly important for *P. levitomentosa* Nyárády ex Sennikov, 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* Nyárády ex Sennikov 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.



Figure 2. Spatulated leaves: a – *P. levitomentosa*, b – *H. aurantiacum* (original)

Clonal growth can be a good strategy to survive in alpine environment, and trade offs are documented between sexual and clonal reproduction (Sutherland & Vickery 1988; Silvertown & Charlesworth 2001; Pluess & Stöcklin 2005). Phylogenetic constraints like genetic drift/bottleneck situations are thought to determine lower genetic diversity at higher altitudes (Peterson & 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 & Ide 2007).

Genetic diversity of a population depends on seedlings. Using computer simulations, Watkinson & 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 dominated 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 prerequisite for the conservation of clonal plants is to insure the protection of suitable habitats for these species.

The clonal way of reproduction of this species, we mention that it is a very high risk 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 & Kalinowski 2000; Willi et al 2006). 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 & Callaghan 1990).

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 add to the extinction risk of the 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.

**Conclusions.** The conservation of this species will be a complex matter given the biological peculiarities of the species, the current environmental changes and the Romanian socio-economic condition. We confirm earlier concerns exposed in Carpathian List of Endangered Species (2003) referring to *Pietrosia levitomentosa* Nyárády ex Sennikov as an endangered species are rightful. Further studies will be needed to elucidate the best ways to conserve this species. We propose that this endangered species should be protected by the designation of a special protection area – a reserve.

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