

**International Master Programme at
the Swedish Biodiversity Centre**

**Master theses
No. 33
Uppsala 2007
ISSN: 1653-834X**

Research on conservation possibilities for *Astragalus dasyanthus* Pall. in the Republic of Moldova.

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Abstract

The present study is concentrated on *Astragalus dasyanthus* Pall., which was used as a model specie to test *ex-situ* conservation possibilities and create a proposal for *in-situ* conservation.

The research consisted of several stages. At first, the information (in publications and other sources) about known occurrences and its range was collected. There were 55 accessions found in herbarium and 56 mentions in literature. After that, three of the identified habitats were checked for current status of *A. dasyanthus* using transect line method. Two of the selected plots were within protected areas (Bugeac and Codru) and one ordinary sloped area with steppe vegetation type close to Ghidighici. Analysis of selected sites for the research specie showed absence of *A. dasyanthus* in the first two and presence in Ghidighici region. During the field survey additional information was collected, namely anthropogenic effects, species that grow in association and landscape description. Analysis of this information showed negative correlation between number of individuals in the area and presence of hay cutting. On the other hand, there was no correlation found between grazing and number of individuals.

Next step included interviewing people to identify the effect of grazing and hay-cutting. Questionnaire showed that only 8.8% of interviewees recognized *A. dasyanthus*. A significant difference was found between the regions by the number of respondents who could recognize the plant. The highest number (10% of all interviewees) was observed in Ghidighici region, which matches field survey results.

Subsequently collected seeds were tested for ability to be preserved in cold storage. After extraction from cold storage 74% of seeds germinated, which demonstrates the efficiency of the selected method for *ex-situ* conservation. Information and results collected during this research show the need for improvement of existing *in-situ* conservation system and introduction of successful *ex-situ* conservation method.

Key words: *Astragalus dasyanthus*, *in-situ*, *ex-situ*, conservation, Moldova, Bugeac, Codru, Genebank, Cold Storage.

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Introduction

Priorities for conservation

Conservation of species diversity is one of the main goals of the 2010 Biodiversity Target (UNEP/CBD 2002). Human activities and use of natural resources induce changes in the global environment (Sala et al. 2000), while biological diversity is declining at an unprecedented speed (Pimm et al. 1995). Wild plant genetic resources, as all genetic resources in general, constitute resources important for food and livelihood of a still growing world population and for crop improvement (Laliberté and V. Negri 2000).

Each country has its own unique set of species and genetic varieties which have to be assessed, preserved and restored. Many environmental organizations and research institutions are trying to find ways to monitor and control species diversity and ecosystem health (Rapport et al. 1998). A critical part of the research connected to biological diversity problems is concentrated on conservation of species that are considered strategically important for agriculture or ones that maintain ecosystem functions (Hector et al. 2001). An urgent goal is to conserve, maintain and restore the full range of biological diversity present in an area (Chapin III et al. 2000). Furthermore there is a whole variety of species that are considered as ones of great importance. Some of them are part of ecosystem services production, others are the elements of significant habitats, natural weed competitors, or ones containing medical substances (UNEP/CBD 2002).

Concentrating research on the aforementioned ecosystem elements will help to conserve significant ecosystem units. In addition there is a necessity to elaborate and research various methods that could be applicable for wild plants conservation (Meilleur and Hodgkin 2004). Different characteristics of some plants can be discovered later, and consequently will turn them into research objects such as key species, indicator species or species which could be used as sources of genes (Simberloff 1998). Such discoveries can represent great value for science, which makes the conservation of individual species a vexed problem.

Biggest issues which complicate this process are the lack of information, shortage in practical experience and need for better funding. These problems slow down the process of plant genetic resources (PGR) conservation development.

Nature conservation in Moldova

Because of the lack of attention and resources, very little has been done in relation to wild plants conservation (Schoen and Brown 2001). According to

the Third National Report on Biological Diversity (Government of Rep. of Moldova 2000), a number of *in-situ* conservation projects have been started. However this process still requires big improvement. The number of protected areas present in the Republic of Moldova is insufficient (Andreev 2002). Areas are poorly managed, with only a small number of research projects taking place. In addition there is a big deficiency of practical experience and personnel (Chemonics International Inc. and Environment International Ltd. 2001). The Biodiversity Conservation Strategy and Action Plan was elaborated in Moldova, however its implementation is just in the starting stage. The Centre for Plant Genetic Resources, founded in 1999, is basically directed towards conservation of crops and their close relatives (Ganea and Gacota 1996).

Astragalus dasyanthus

Astragalus dasyanthus Pall. is spread all over the territory of the Eastern European steppes and appears in Moldova (Heywood and Ball 1968, Nikolaeva 1963, Mirza 1971). *A. dasyanthus* is a rare and vulnerable plant in the Republic of Moldova. It has been included in the Red List Europe as endangered since 1991 (Ganea and Gacota 1996). *A. dasyanthus* is a perennial herb with green compound leaves, average of 35 cm long (fig. 1), flowering in July and fruiting in August. A detailed description can be found in the botanical identification guide (Geideman 1986). In nature the research specie grows on sandy, rocky or calcareous soil types with low or average-low humus content. It is resistant to low levels of phosphorus and potassium. In Moldova it is mostly found in virgin, steppe and newly formed steppe areas. It prefers slopes with South, South-West or West exposure.



Fig.1 *Astragalus dasyanthus* in nature.

A. dasyanthus grows in association with different species. Often those are *Festuca sulcata* (Hack.) Beck, *Stipa ucrainica* P. A. Smirn., *Poa bulbosa* L. and *Bothriochloa ischaemum* (L.) Keng. (Mirza 1975). In Moldova *A. dasyanthus* is settled on the edge of its habitat and can grow and reproduce only in selected areas because it is very fastidious to different aforementioned conditions. Moreover only 1.92 percent of areas can fit as suitable steppe ecosystem (Mirza and Boichenko 1979, Government of Rep. of Moldova 2000). Based on these facts it could be used as an indicator specie for certain biomes (i.e. steppe) in future research.

A. dasyanthus has medicinal properties due to the presence of glycosides and flavonoids (Khoron'ko 1975, Khoron'ko and Glyzin 1975, Mirza 1975, Kudrin et al. 1987, Rios and Waterman 1998). Preparation of *A. dasyanthus* has been approved for use in case of cardiovascular collapse and hypotonic disease. It has general anti inflammation and diuretic effect and can be used for treatment of kidney vascular disorders (Korpusenko 1956, Shadrenko 1959, Stepashkina 1957, Zhang et al. 1990).

Like other species of genus *Astragalus*, *A. dasyanthus* has small dry seeds. This specie has difficulties in reproduction and propagation (Mirza and Boichenko 1979). It is necessary for many conditions to be respected for seeds to germinate in nature, namely moisture content and amount of nutrients in soil and sufficient sunlight. Moreover seeds of *A. dasyanthus* are subject to fungal and insect larvae attacks (Mirza 1971). Vegetative propagation has low probability of success for this specie even in laboratory environment (Harkevich et al. 1972).

Research design

For this study *A. dasyanthus* was selected as a model because of its special characteristics. It has basic traits for *Astragalus* group, it is limited in reproduction and spread, it has medicinal effects, it is endangered and an emergency conservation plan is required. These and other specific properties described before make this specie an object of interest for conservation research.

This study involves different methods for field, laboratory and sociologic research. It is essential to analyze previous publications, herbaria and field notes to obtain information about its range in the past to observe dynamics in changes, be able to predict future spreadof the specie and produce an action plan for its *in-situ* conservation. In addition, available sociological information should be collected and analyzed. This project applies questionnaire method to understand possible interactions of human activities in the area with range pattern of the research specie. GIS mapping helped to understand the pattern and region preferences.

If there is no possibility for effective *in-situ* conservation, *ex-situ* conservation should be considered (Schoen and Brown 2001). This study involves cold storage with preliminary drying *ex-situ* conservation method (WHO et al. 1993).

This research is aimed at investigating the current status of *A. dasyanthus* populations in some known habitats both within protected areas and areas with human interaction, to analyze the success of *in-situ* conservation, assess human impact on these populations and produce advice for future conservation of the given specie. The other part of this research is devoted to laboratory tests for possible ways of *ex-situ* conservation.

Material and Methods

Herbarium analysis

Available herbarium information from Moldova State University Museum of Nature and Codru Scientific Reserve was reviewed for *A. dasyanthus* presence. All available data from labels was added to a data base and corresponding GIS points were added to GIS Map.

Data Matrix was created to store obtained information. Variables used are presented in table 1.

Literature search

Available literature and publications referring to *A. dasyanthus* appearance in the territory of Republic of Moldova were reviewed. Obtained information was adapted to the information acquired from herbaria analysis and was added to Table 1 to complement the Data Base.

Table 1. List of variables for Database and their description.

Variable	Description
Number	Herbarium registration number
City	The closest inhabited point to the place where individual was located
Region	The Region where city is located
GPS	GPS coordinates
Collector	Name of the person who collected the specimen (if available)
Taxonomist	Name of the person who identified the specie (if available)
Year	The year when specimen was added to collection
Info	Additional information to help find the specimen location from the inhabited point (if available)

Field analysis

For detection of *A. dasyanthus* and registration of anthropogenic interaction, three areas were analyzed using Line Transect method (Thomas et al. 2002, Buckland et al. 1993).

Two protected areas were visited to examine existing populations and to evaluate the success of *in-situ* conservation for *A. dasyanthus*. One plain area close to a populated locality was selected as well (fig. 2).

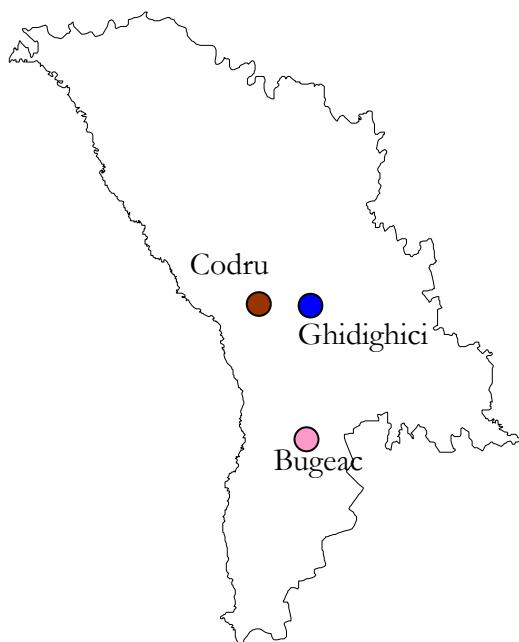


Fig. 2. Three research plots selected for current study.

Transects of 2 to 4 meters were used to analyze selected areas for desired species detection. Two observers checked the same plots independently. GPS device was used to map every recognized individual to prevent repetitions. The area within the buffer zone of Codru National Reserve of 111998.011 sq. meters at the forest edge (fig. 3) (central point 47°5'19.90"N, 28°23'26.52"E) was divided onto 73 transects, parallel to the road line (47° 5'7.50"N, 28°23'20.98"E and 47° 5'27.17"N, 28°23'31.94"E).

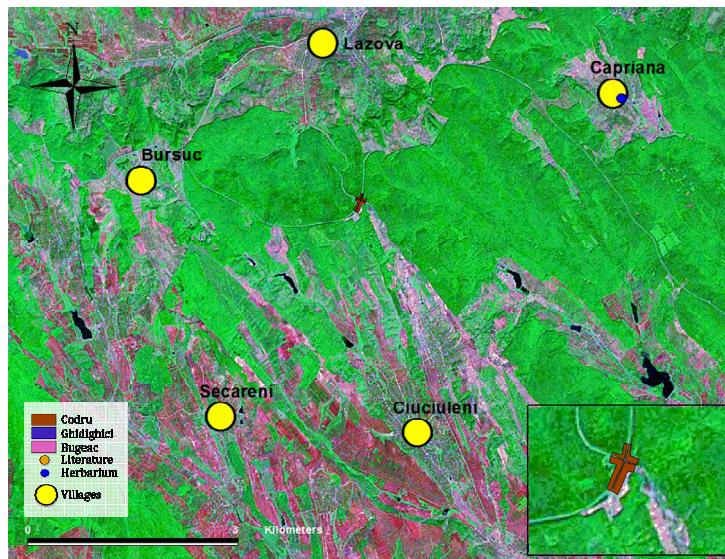


Fig. 3 Research plot in Codru Reserve.

The protected area Bugeac (central point coordinates $46^{\circ}25'59''N$, $28^{\circ}41'52''E$) situated in the forest-steppe district between inhabited localities Bugeac, Dezghingea, Ciucur-Mingir and Topala (fig. 4) is in the southern part of Moldova, Anenii Noi region, close to the city Comrat. The area of 1137640000 sq. meters was divided into 317 transect lines (parallel to the line with coordinates $46^{\circ}26'9.77''N$, $28^{\circ}42'9.44''E$ and $46^{\circ}25'58.73''N$, $28^{\circ}42'15.03''E$).

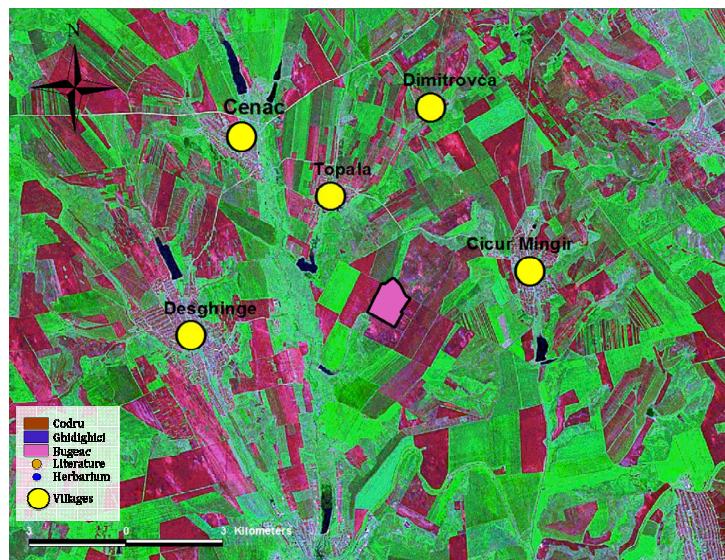


Fig. 4 Research plot in Bugeac.

An area close to Ghidighici (steppe slope along the railroad, central point $47^{\circ}5'20.04''N$, $28^{\circ}44'35.20''E$) of 605510000 sq. meters (fig. 5) was divided into 47 transects (parallel to the line with coordinates $47^{\circ}5'13.05''N$, $28^{\circ}44'30.23''E$ and $47^{\circ}5'55.03''N$, $28^{\circ}44'21.68''E$).

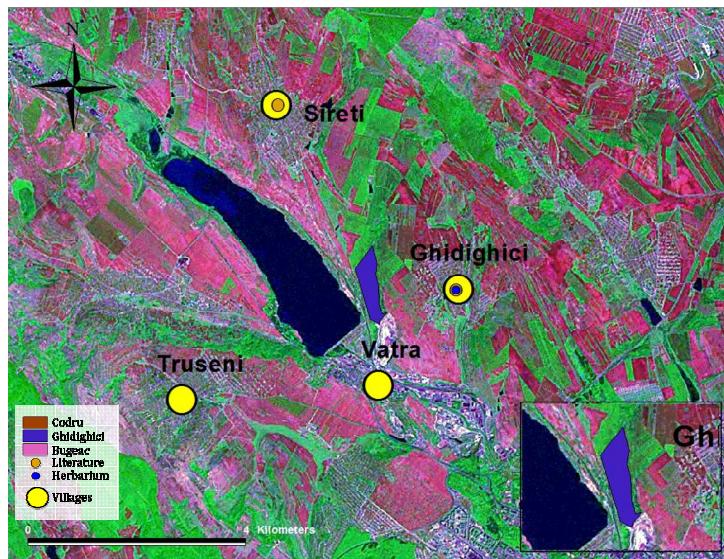


Fig. 5 Research plot in Ghidighici.

At all the mentioned field sites *A. dasyanthus* presence was sampled along the established transects. Transects were measured using a GPS device and measuring tape. Every appearing specimen was added to the table with variables established before (table 1). Supplementary descriptors were added to a separate table (table 2) and connected to the first one that is resulted from Herbarium and Literature review.

Table 2. Additional descriptors for previously created Database.

Variable	Description
Association	Plant species growing in association/ close to registered specimen (if available)
Habitat	Type of habitat (Steppe, Forest, Forest-Steppe)
Exposition	The direction of the slope (if available)
Effects	Observed anthropogenic effects (Hay-cut, Grazing, etc.)

Different factors were visually detected along transects. The effect of grazing was recorded based on remaining traces of browsing, footprints and presence of cattle. The effect of hay cut and recent hay cut was measured depending on remained grasses and cut-down grasses on the surface. Results were stored in aforementioned table in present or absent format. (table 1,2).

Interviews

A small questionnaire (see appendix) was elaborated for additional information collection about human interaction within the assigned area.

Questionnaire included basic requests for information about the person being questioned, and the knowledge about the area. Also the person was asked about the amount of cattle kept in the household and other practical information about grazing and hay cutting areas which they were asked to

show on the map (see appendix) Respondents were chosen randomly from villages close to the field site. Moreover some questions connected to *A. dasyanthus* usage were asked, namely if the respondent was able to recognize the plant and name it correctly. If the plant was used, information about that and sources of knowledge was also collected.

Different difficulties were met during interviewing. One of them was wittingly providing false answers (which were not expected while developing the research design); the other one was refusal to answer part of questions. These make questionnaire results more descriptive than statistical. Nevertheless Chi-square method was used to analyze the answer about species recognition. Individual interviews were used in the discussion section to describe practical plant usage.

GIS Map

For the current research it is important to use GIS modeling for human activities impact assessment, so a map of the research area was created using available paper maps, satellite images and GPS tracking device. All the data obtained during the project was stored in databases using Microsoft Access, for example results from the questionnaire and *A. dasyanthus* population description.

Seed collection

Seeds were collected from Ghidighici field site randomly from 15 plants in amount of 10 seeds from each plant. The collecting event took place in August 2006.

Cold storage conservation

As the *A. dasyanthus* seed is of orthodox type, it can be stored in low temperatures after being dried. To prepare seeds for cold storage they were dried with silica gel to moisture content around 5%, which was determined using silica gel colour change, together with measuring the weight changes of seeds. After drying seeds were stored at -5°C temperature for three months. After extraction from storage, seeds were brought to normal moisture content and tested for germination (Ellis et al. 1985a).

Germination tests

Germination tests were performed to determine the response of seeds for drying and cold storage, comparing to the control. Following conditions were used: 25°C and 100% humidity in germination chamber with no access to light. The percent of germination was determined by the number of seed germinated within 21 days from the total number of seeds in each of 5 replications (Ellis et al. 1985b).

Data analysis

Several methods of statistical analysis were involved to interpret or check the results of experiments. For data obtained from questionnaires Chi-square method (Mantel 1963) was used to define if there was a difference in number of interviewees who recognized the research specie between different villages and regions.

For transect line sampling analysis simple correlation method (Dytham 2003) was used to determine if there is a correlation between number of individuals of *A. dasyanthus* appearing in areas with presence/absence of grazing and hay cutting.

To check variation in groups and between groups during germination tests ANOVA method (Dytham 2003) was used.

All the statistical analysis was performed using SPSS and Minitab software.

Results and discussion

Based on data from different sources (Yakovlev et al. 1996, Mirza 1971, Geideman 1986, Nikolaeva 1963, Ganea and Gacota 1996) *A. dasyanthus* can be found in steppe zones of South-eastern Europe and partly Central Europe. Most of the available literature sources are from 1960-1970's but even then authors indicate reduction in amount of *A. dasyanthus*. Information about locations of populations in Moldova obtained from literature review was added to the data base and GIS map (fig. 5)

Besides the literature analysis available herbaria were analyzed. The Herbarium of Moldova State University's Museum of Nature has 55 accessions of *A. dasyanthus* (from 1960 to 1992). The Herbarium of Codrii National Reserve was searched but no accessions were found.

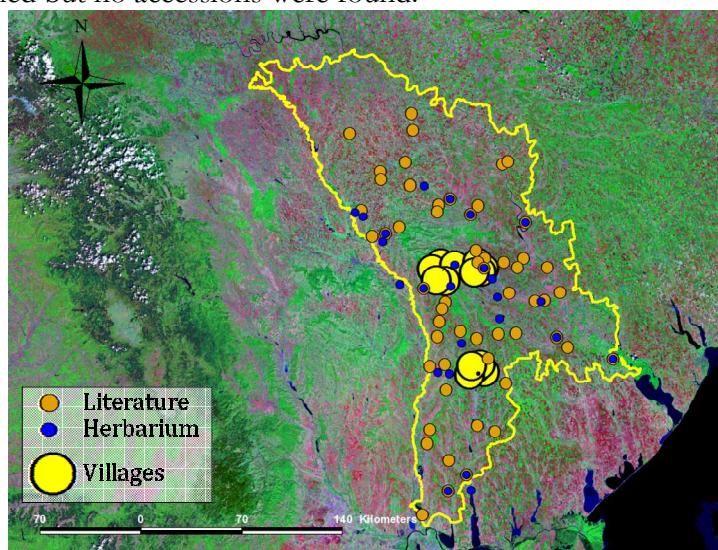


Fig. 4. *A. dasyanthus* location Information found in different sources (1841-1970). Blue – published literature. Orange – herbarium.

In the Scientific Reserve Codrii a meeting with the reserve's botanist took place and field notes from the last 10 years were reviewed to check *A. dasyanthus* appearance within this period. No records were found.

Obtained pattern shows that *A. dasyanthus* is spread unevenly not depending on type of region (fig. 5). Considering Moldova's general landscape, most remaining natural steppe habitats are those which could not be used for agriculture.

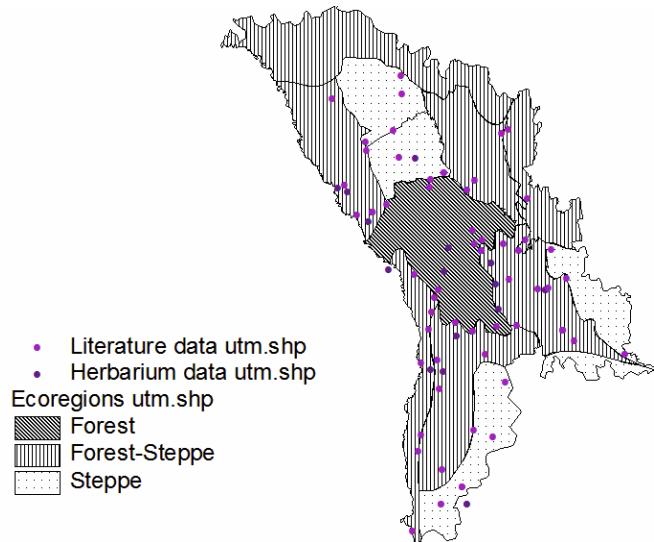


Fig. 5: Range of *A. dasyanthus* in Moldova comparing to main ecological regions.

These areas include edge of forest, slopes and hills, rocky areas, ravines and others. Usually these regions are exploited for grazing or hay-cutting by communities of villages nearby.

Field research

Based on information obtained from literature, three territories were selected for sampling of *A. dasyanthus*. All of them are previously mentioned in literature for the presence of the research specie (Geideman 1986, Mirza 1971, Nikolaeva 1963).

The first one was Bugeac and there were no representatives of *A. dasyanthus*. One plain region to the south-west was also analysed. That was a slope where only hay cutting and grazing have been taking place, nevertheless the research specie was not detected in surrounding areas as well. The last mention of the research specie in nature reserve was in a book by Nikolaeva L.P. (1963). There could be several reasons for the disappearance of this specie from Bugeac but the most significant one is isolation. The habitat is very fragmented and there was not enough shifting space for species to adapt and survive in changing conditions (Andreev 2002). Other possible reasons could be connected to human activities, though not taking place at Bugeac Nature Reserve but close by, like usage of herbicides on the neighbouring fields.

There could be several reasons for the disappearance of the research specie within the protected area, namely soil composition changes or loss in competition with more aggressive species.

In contrast adjacent to protected area slopes which are not suitable for agricultural use, had obvious traces of hay cutting. If hay cutting would have taken place at the time right before reproduction or during the reproduction period, then full disappearance of *A. dasyanthus* would be expected after several years, as it was observed. According to the results from Moldova State University herbarium analysis there are some more accessions of the research specie around villages close by; however, these have to be checked for authenticity in future projects.

The second protected area was Scientific Reserve Codru. There is one source referring to *A. dasyanthus* presence by Geideman T.S. (1986). The author indicates that the research specie can be found at the edge of the forest and at clearings in the woods. Though not a single accession of *A. dasyanthus* was found it was advisable to sample the one area of Codru forest edge. After sampling there were no representatives found. Hay cutting is allowed in this area. The forest edge is the limit of distribution for *A. dasyanthus* from one side and the road or agricultural land limits its spreading from the other side.

Finally, a third area was sampled. A plain area with recent accessions of *A. dasyanthus* was selected (according to Moldova State University herbarium). There were 38 individuals detected, two groups of 24 and 5 individuals each and the rest were spread as standalones. A certain amount of human impact is present in this area. Mostly grazing and hay cutting occur at random places where the access is easy.

All transect lines were divided onto sectors 100m length which resulted in 542 plots. The number of individuals per plot, presence of recent hay cutting and grazing were measured. Collected data was checked for correlation which resulted in the following table (table 3).

Table 3. Correlations between Number of observed individuals, Grazing and Hay Cutting presence.

		Number of individuals
Recent Hay Cutting	Pearson corelation	-0.121
	P-Value	0.005
Grazing	Pearson corelation	-0.012
	P-Value	0.771

Test results show P-value lower than 5% for negative correlation between Number of individuals and Recent Hay Cutting which proves the hypothesis that hay cutting is reflecting negatively on the amount of individuals in the area. On the other hand there was no correlation found between grazing and number of species. This can be explained by the fact that during hay cut all the grasses are removed and there is not enough time for Astragalus to recover.

Then again grazing is a selective process which is actually improving the amount of sunlight and nutrients, but nevertheless no correlation was found.

Questionnaire results.

There were 182 forms with questions filled. Interviewing took place in 3 regions. Regions were chosen in coordination with field plots assigned for *A. dasyanthus* sampling.

Most of the interviewing took place in Ghidighici Region (65.9%) because unlike the other two field sites (Codru Region 17.6%, Bugeac Region 16.5%) there *A. dasyanthus* presence was detected.

Frequency analysis of obtained results showed that only 8.8% of interviewees could recognize *A. dasyanthus* by picture. Even less knew its name (2.7%). The highest number of cases when the plant was identified by pictures was observed in Ghidighici Region (10%), and less in Bugeac and Codru Regions (6.7% and 6.3% correspondingly)

Chi-square test was applied to check the significance of the hypothesis, that cases when *A. dasyanthus* was recognized, were unevenly distributed between different villages. Chi-square test result for villages was 13.000, df=7 and Asymp. Sig.=0.072. The result of Asymp. Sig.=0.072 (significance value) is greater than .05 which disproves the hypothesis that the average rate of positive answers differ by each village. This test showed that there is no significant difference between the number of individuals who could recognize *A. dasyanthus* in different villages. Based on this result answers were grouped to check the same hypothesis in three regions.

After grouping villages by regions and running the same test again the following result was obtained Chi-Square=12.500, df=2, Asymp. Sig.=0.002. The .002 Asymp. Sig. for villages grouped by regions shows greater difference between Ghidighici and other two regions. This result shows that significantly less individuals could recognize *A. dasyanthus* in Codru and Bugeac regions.

Aforementioned field analysis results display the absence of the research specie in same areas.

Half of interviewees (50%) who identified *A. dasyanthus* are using infusions of leaves (25%), roots (18.8%) or flowers (6.3%) together with other herbs and compounds for treatment of heart kidney and liver disorders. The fact that flowers and roots are used shows a certain level of impact made by collectors. This reduces the number of individuals within remaining populations of *A. dasyanthus*.

All the respondents who recognized the research specie were asked to comment on the number of individuals they met in the past few years. 50% of interviewees noticed reduction in population, 6.3% observed increase and 43.8% have seen no change. When frequency analysis was narrowed down to respondents who used the plant, an even more dramatic figures were obtained: 62.5% noticed reduction, 25% noticed no change and 12.5% noticed increase correspondingly. This result can not bring any conclusion because it is based

on very few interviewees who recognized *A. dasyanthus*, however it can add more confidence to other results obtained in this study.

During interviews all respondents were asked if they were ready to participate in taking measures (reduce grazing/ hay cutting to some extent and restrict access to some areas) for conservation of research specie. 89% disagreed, 6.1% of interviewees agreed to control grazing/ hay cutting in some areas, 4.9% agreed to any measures. This result shows lack of awareness about the necessity to preserve biological diversity and its importance.

Cold Storage Tests.

In case of impossible or insufficient *in-situ* conservation possible *ex-situ* conservation alternatives should be considered. The most simple and inexpensive method for orthodox seed *ex-situ* conservation is cold storage (Scarascia-Mugnozza and Perrino 2002). The possibility of this method application was tested for *A. dasyanthus*.

There were three germination tests accomplished, each of them involved 50 seeds. The first one was made right after collection, the second one after drying the material to humidity content of 5% and the third one after cold storage. Germination rate before treatments was 88%, after drying – 76% and after cold storage – 74%. These values were checked using ANOVA test which had P-value=0.012 and proved the significance of obtained results.

The final germination rate of 74% is high enough (Ellis et al. 1985b, Cromarty et al. 1982) to approve the Cold Storage method for *ex-situ* conservation of *A. dasyanthus*.

Conclusions

Continuous monitoring and attempts to find the best way to preserve endangered species can solve many problems facing biodiversity conservation. This research shows that both Codru and Bugeac protected areas were insufficient to preserve the rare specie *A. dasyanthus* *in-situ* and their improvement should be considered.

Bugeac Natural reserve restricts any human activities and this helps to preserve many steppe species, however *A. dasyanthus* has disappeared from this site. Some of the possible reasons were mentioned before. One of the other reasons could be the complete absence of grazing, which would remove some part of the vegetation giving a chance for other species to grow. It is therefore important to have buffer zones around protected areas and let some amount of controlled human activities take place there. Furthermore there should be enough of extra buffer zone space for species shift, in case of environmental condition changes.

Scientific reserve Codru has a forest as the main object to be preserved. The area is not exactly suitable for *A. dasyanthus*, as it can only grow on the edge of forest or within forest clearings. In Codru the forest edge is the same as the

boundary of the reserve's buffer zone, so there is no special management taking place.

The area next to Ghidighici village has naturally maintained human activities, which are limited only by landscape and possibility to access the area. The status of *A. dasyanthus* is showing aggravation of conditions because the numbers of individuals have reduced lately. This could be caused by the open mine which is constantly enlarging and taking territory used by the research specie to grow. Besides that *A. dasyanthus* could have been subject to global disadvantageous environmental conditions, i.e. affected by climate change and lacking the possibility to adapt because of low reproduction rate and high level of fragmentation.

The general public lacks awareness about the importance of conservation. Special education, introduction of managerial practices and *on-farm* conservation of important and disappearing species can improve the current conservation status in Moldova.

Analyzing data obtained by this project, the following conclusion can be drawn: The best way to manage protected areas for *A. dasyanthus* conservation is to study the wild population's ecology and use it as a model. The current status of wild population is showing reduction or complete disappearance of *A. dasyanthus*, therefore *ex-situ* conservation and restoration of natural populations should be implemented.

Acknowledgements

I would like to thank my supervisors Eva Thörn (CBM) and Maria Duca (USM) for their great help. I would also like to thank Mihail Mîrza (USM) and the persons who helped me with advice and discussion for this project.

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