

A morphometric study on *Anthyllis vulneraria* (Fabaceae) from Poland and its taxonomic implications

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Abstract: The paper presents results of morphometric analysis of *Anthyllis vulneraria* L. in Poland based on 828 herbarium specimens. This study investigates five taxa which have been recognized in Poland: *Anthyllis vulneraria* L. ssp. *vulneraria*, *Anthyllis vulneraria* ssp. *polyphylla* (DC.) Nyman, *A. vulneraria* ssp. *maritima* (Schweigg.) Corb., *A. vulneraria* ssp. *alpestris* Asch. et Graebn. and *A. vulneraria* ssp. *carpatica* (Pant.) Nyman as well as three intermediate taxa of presumably hybrid origin. Multivariate statistical analyses of 12 quantitative and 10 qualitative characteristics revealed conspicuous variation within *A. vulneraria*. Analysis of variance (ANOVA), principal coordinate analysis (PCoA), correspondence analysis (CA) and discriminant analysis (DA) proved the taxonomic usefulness of quantitative characteristics such as number of rosette leaves, calyx length, inflorescence length, the ratio of leaf length/width, bract length as well as qualitative characteristics such as stem hairiness, calyx colour, calyx indumentum, distances between stem leaves and form of rosette leaves. The issues concerning intermediate morphotypes occurring in Poland and their status are discussed. Finally, a key for determination of taxa within *A. vulneraria* in Poland and distribution maps based on the material examined are provided.

Key words: *Anthyllis vulneraria*; biometry; Fabaceae; morphological variability; multivariate statistics; Poland; systematic key; taxonomy

Introduction

Anthyllis L. belongs to the tribe Loteae (Polhill 1981; Allan & Porter 2000) within the family Fabaceae. *Anthyllis* L. is taxonomically very complex. It consists of numerous taxa wherein the status of most of them remains questionable. The number of species within the genus has been previously reported to range from 25 (Cullen 1986) up to 60 (Minjaev & Akulova 1987).

Anthyllis vulneraria L. is a very polymorphic species, consisting of 30 intraspecific taxa (many of them frequently recognized as species), between which intermediates occur, often over a large area (Cullen 1968). Some researchers distinguish only one polytypic species – *A. vulneraria* (Stankov & Taliev 1949; Jalas 1950; Jalas 1952). On the other hand, Rothmaler (1941) and Juzepczuk (1945) claim that there are several separate and more or less independent species distinguishable in Europe. These inter-grading variants might be, to some extent, ecologically and geographically separated (Cullen 1986); however, their classification is complicated. It should be noted here that in spite of wide variability within *A. vulneraria*, chromosome numbers of all the taxa examined in this study are the same ($2n = 12$) (Bolkhovskikh et al. 1969; Van Loon & Kieft 1980; Yeh et al. 1986; Dempsey et al. 1994; Lövkvist & Hultgörd 1999), which means they are diploid. Furthermore, *A. vulneraria* consists of local morphotypes in limited areas, which are of

ten morphologically indistinct (Juzepczuk 1945; Puidet et al. 2005). Most taxa are morphologically similar, and some of them have presumably hybrid origin. The wide variation in morphology observed nowadays suggests that there are a few taxa within *A. vulneraria* and intermediates, which could be commonly found on the border ranges. The species was probably divided into several taxa with distinct geographical ranges in the past (Jalas 1950). This differentiation has not, so far as is known, led to complete genetic isolation (Marsden-Jones & Turrill 1933). As a result of many factors such as climate change and the ability to occupy disturbed habitats, there was a partial blurring of the primary border-lines of particular morphotypes as well as diagnostic morphological differences (Jalas 1950). Therefore, in spite of the fact that it is not so widespread to recognize *A. vulneraria* as species complex, it should be considered in such a way, because the complexity of this taxon is evident (Krall 1983; Köster 2010).

Anthyllis vulneraria has wide distribution throughout most of Europe (Becker 1911) from the Atlantic coast to the Baltic region; south to the Mediterranean Basin as well as in North Africa (Algeria, Morocco, Ethiopia) (Bennett et al. 2001); east to SW Asia – mainly Turkey (Cullen 1976) and the Caucasus and Iran (Marsden-Jones & Turrill 1933). It has also been introduced into North America and New Zealand (Hultén & Fries 1986).

There are many studies concerning morphological variability and taxonomic problems of different *A. vulneraria* taxa (e.g. Sagorski 1908; Cullen 1976; Łukaszewska et al. 1983; Akeroyd 1988; Cagiotti et al. 1990; Gonzalez 1998; Rich 2001; Puidet et al. 2005; İlçim et al. 2008). Cullen (1976) presented a biosystematic study of *A. vulneraria* in Europe with particular emphasis on Western Europe. The taxa included there were treated as one species divided into a number of subspecies and varieties. This treatment is also adopted here. Furthermore, Cullen (1976) distinguished two aggregates – *Vulneraria* and *Alpestris* aggregate – into which subspecies are grouped. These subspecies form fairly natural groups corresponding to aggregates. But these aggregates have no nomenclatural standing and intermediates do occur between them. Cullen (1976) discussed division of the group and tried to evaluate taxonomic characteristics of the species. In addition, he provided distribution maps for each taxon in Europe.

Puidet et al. (2005) analyzed morphological variability of eight taxa of *A. vulneraria* in Estonia. Most of the characteristics examined were statistically significant in species determination. This investigation resulted in distinction of three species groups: *Vulneraria*, *Macrocephala* and *Coccinea*. In Poland, the study of morphological variability of *A. vulneraria* was carried out in northern Poland on populations from the Baltic coast (Łukaszewska et al. 1983). Multivariate statistical analyses confirm the differences between populations primarily on the basis of fertile parts of the plants, but they also considered vegetative characteristics (Łukaszewska et al. 1983).

Several characteristics which distinguish the taxa within *A. vulneraria* were given in different publications. The most distinct and well-defined features which differentiate the examined taxa are: the type of stem hairiness, the distribution of leaves on the stem, the abundance of rosette leaves, calyx colour and length. *Anthyllis vulneraria* ssp. *maritima* (Schweigg.) Corb. can be distinguish by silky and dense calyx hairiness (Kostrakiewicz 1959), as well as by some inflorescences with a few flowers (sometimes not fully developed) (Krall 1983). *Anthyllis vulneraria* ssp. *polyphylla* (DC.) Nyman is most readily distinguished by its patent hairs on the stems and petioles (Kostrakiewicz 1959; Cullen 1968; Jasičová 1988; Akeroyd 1991). In addition, it is a particularly important feature in relation to Polish material, because the other taxa with patent hairiness (e.g. *A. × polyphyloides* Juz., *A. colorata* Juz., *A. vulneraria* ssp. *vulnerarioides* (All.) Arcang.) have not been recorded in the country. A bi-coloured calyx is a characteristic feature of *A. vulneraria* L. ssp. *vulneraria* (Schlechtendal & Langethal 1885; Kostrakiewicz 1959; Cullen 1968); however, sometimes the calyx remains uni-coloured. *Anthyllis vulneraria* ssp. *alpestris* Asch. et Graebn. and *A. vulneraria* ssp. *carpatica* (Pant.) Nyman are considered as mountainous species in Poland (Kostrakiewicz 1959). Cullen (1976) included these taxa in the *Alpestris* aggregate. Their distinctive feature is the presence of many rosette

leaves often reduced to a single leaflet (Cullen 1976). *Anthyllis vulneraria* ssp. *alpestris* has a distinctly different calyx from the other analyzed taxa. Its specific villous to hirsute hairiness (Cullen 1968; Jasičová 1988) gives it a unique, smoky-greyish colour (Kostrakiewicz 1959). The length of the calyx is relatively high, ranging on average from 1.4 to 1.6 cm. While *A. vulneraria* ssp. *carpatica* has sparse sericeous and a usually yellowish calyx (Cullen 1968; Jasičová 1988), which differs this taxa from the previous one.

Anthyllis vulneraria has been genetically investigated. Several studies have been made in order to determine molecular traits that would resolve the complex (Köster 2010). However, the results of different molecular analysis are quite contentious (Akeroyd 1988; Kropf et al. 2002; Allan et al. 2003; Nanni et al. 2004; Honnay et al. 2006; Van Glabeke et al. 2007; Degtjareva et al. 2008; Köster et al. 2008). An issue concerning *A. vulneraria* was included in the study of molecular phylogeny of the genus *Anthyllis*. The internal transcribed spacers ITS1 and ITS2 of the nuclear ribosomal DNA and additional polymorphic chloroplast SSR of 10 *Anthyllis* species, including 11 subspecies of *A. vulneraria* were analyzed (Nanni et al. 2004), of which 3 are considered in this study. The other investigation used the amplified fragment length polymorphism (AFLP) analysis to find differentiation in the species complex (Köster et al. 2008). However, the study showed that the taxa of *A. vulneraria* cannot be differentiated by AFLP patterns, because the analyzed specimens of 7 taxa belonging to *A. vulneraria* did not comprise groups correlated with the subspecies (Köster et al. 2008).

In Poland, some examinations concerning differentiation within *A. vulneraria* were also conducted. Kalinowski et al. (1983) analyzed the geographic impact on the isoenzymatic variability of *A. vulneraria* populations in Poland to determine whether there exists any differentiation between populations of inland and coastal areas. The study confirmed significant differences among populations depending on geographic distance (Kalinowski et al. 1983).

In conclusion, different investigations concerning intraspecific taxa of *A. vulneraria* based on molecular studies have not resolved the problem of distinguishing some taxa. Thus, taxonomic analyses based on morphological features seem to be an essential element when it comes to sensitive taxonomy.

This study investigates five taxa which have been recognized in Poland: *Anthyllis vulneraria* ssp. *vulneraria*, *A. vulneraria* ssp. *polyphylla*, *A. vulneraria* ssp. *maritima*, *A. vulneraria* ssp. *alpestris* and *A. vulneraria* ssp. *carpatica* as well as three intermediate morphotypes. *Anthyllis vulneraria* is widespread in Poland (Zajac & Zajac 2001). The most frequent taxon in Poland is *A. vulneraria* ssp. *vulneraria*, which is distributed throughout the country with the exception of coastal areas. It is also quite rare in the Carpathians as it occurs only at low altitudes in the Carpathians and Carpathian Foothills (Szafer et al. 1976). On the other hand, the distribution of *A. vulneraria* ssp. *mar-*

Table 1. List of qualitative and quantitative characteristics used in the present study. Name of the characteristic, corresponding abbreviations and measure units or scale are given.

No	Character	Abb.	Unit/Scale
1	Habit	HB	1 – ascending to erect; 2 – decumbent
2	Form of rosette leaves	FRL	1 – simple leaves; 2 – pinnate
3	Form of leaves	FL	1 – all leaves with equal leaflets; 2 – lower leaves with unequal leaflets, upper leaves with equal leaflets; 3 – all leaves with unequal leaflets
4	Distances between stem leaves	DSL	1 – equal; 2 – unequal
5	Hairiness of the upper surface of leaf blade	HL	1 – glabrous; 2 – single hairs; 3 – many hairs
6	Stem hairiness	HS	1 – glabrous; 2 – appressed; 3 – patent
7	Petiole hairiness	HP	1 – glabrous; 2 – appressed; 3 – patent
8	Shape of bract	SB	1 – sharp-ended, gradually narrowed; 2 – blunt-ended, with convex edges
9	Calyx colour	CC	1 – bi-coloured with purple or red teeth; 2 – whitish to yellowish; 3 – greenish; 4 – greyish to ashen
10	Calyx indumentum	CI	1 – appressed; 2 – semi-patent to patent; 3 – thick and silky; 4 – villous to hirsute
11	Number of rosette leaves	NRL	discrete cardinal
12	Number of stem leaves	NSL	discrete cardinal
13	Number of inflorescence on the top of sprout	NI	discrete cardinal
14	Plant height	PH	Cm
15	Leaf length	LL	Cm
16	Leaf width	WL	Cm
17	Ratio leaf length/width	LL/WL	Ratio
18	Inflorescence length	LI	Cm
19	Bract length	LB	Cm
20	Calyx length	LC	Cm
21	Calyx width	WC	Cm
22	Ratio calyx length/width	LC/WC	Ratio

itima is restricted to sand dunes on the Baltic coast (Lukaszewska et al. 1983). *Anthyllis vulneraria* ssp. *carpatica* is regarded as a mountain subspecies, which occurs in the Carpathian Foothills and in the mountains up to 1000 meters above sea level (Kostrakiewicz 1959). The localities of *A. vulneraria* ssp. *alpestris* in Poland are limited to the Tatra Mountains. This subalpine species is distributed only in high mountains ranging from 1050 meters above sea level and reaches up to 2050 meters (Kostrakiewicz 1959). The distribution of *A. vulneraria* ssp. *polyphylla* in Poland remains indeterminate; a lot of data is still required for confirmation and verification (Szafer et al. 1976).

No morphometric studies on *A. vulneraria* as a whole have been carried out in Poland so far. In the present study, I was searching for morphological characteristics which could provide more precise and objective determination of the taxa within *A. vulneraria*. I have tried to evaluate the efficacy of qualitative and quantitative traits related to morphology to identify the taxa. The main aim of the study, which is an introduction for further investigations and should be an important reference for existing and subsequent studies (molecular, karyological, biochemical and taxonomic), is to analyze in detail the morphological variability of *A. vulneraria* in Poland in respect to different qualitative and quantitative characteristics. Furthermore, I have tried to point out diagnostic characteristics which distinguish the taxa within the complex in the most reliably way. The intermediates which I recorded in Poland were also included in the analysis. The issues concerning their distribution, frequency and status are discussed. In ad-

dition, a key and distribution maps based on the material examined for the taxa of *A. vulneraria* in Poland are provided.

Material and methods

Morphological analysis

The study comprised herbarium material (1018 individuals) from several herbaria in Poland: KRAM, KRA, of which 828 individuals (complete, undamaged and properly labelled) were selected for detailed morphometric analysis. The number of specimens for each taxon examined ranged from 8 to 328, depending upon availability (*A. vulneraria* ssp. *vulneraria* – 328, *A. vulneraria* ssp. *polyphylla* – 156, *A. vulneraria* ssp. *carpatica* – 90, *A. vulneraria* ssp. *alpestris* – 74, *A. vulneraria* ssp. *maritima* – 60). In addition, the study included three intermediate taxa: *A. vulneraria* ssp. *vulneraria* × *A. vulneraria* ssp. *carpatica* (92 individuals), *A. vulneraria* ssp. *polyphylla* × *A. vulneraria* ssp. *carpatica* (20 individuals) and *A. vulneraria* ssp. *vulneraria* × *A. vulneraria* ssp. *polyphylla* (8 individuals); however the rank of these taxa remains undefined. Due to the fact that only one intermediate taxon – *A. vulneraria* ssp. *vulneraria* × *A. vulneraria* ssp. *carpatica* – is considered to be distributed over a large area in Poland, I decided to include it in a separate statistical analysis. As a result, all data were gathered in two separate matrices (data sets). Morphological research was carried out to investigate detailed biometric features. In order to evidence differences among the taxa, I explored the variability range of selected discrete cardinal and continuous characteristics as well as essential qualitative features. Twenty-two diagnostic characteristics (including 7 related to floral structures and 15 to vegetative parts) were selected for examination (Table 1). These characteristics were chosen mainly on the basis of taxonomy literature included in

Table 2. Main characteristics of taxa within *Anthyllis vulneraria* L. occurring in Poland according to different authors.

Characteristic	<i>Anthyllis vulneraria</i> L. ssp. <i>vulneraria</i>	<i>Anthyllis vulneraria</i> ssp. <i>polyphylla</i> (DC.) Nyman	<i>Anthyllis vulneraria</i> ssp. <i>carpatica</i> (Pant.) Nyman	<i>Anthyllis vulneraria</i> ssp. <i>alpestris</i> Asch. et Graebn.	<i>Anthyllis vulneraria</i> ssp. <i>maritima</i> (Schweigg.) Corb.
Plant height (cm)	(15-) 20-40 (-50)	(12-) 20-40 (-65)	(10-) 12-25 (-30)	(10-) 15-40 (-45)	(10) 20-60
Stem hairiness	appressed	patent, especially in the lower part of stem and petioles	appressed	appressed	appressed
Stem leaves	4-5; evenly distributed	(3-) 4-6; evenly distributed	3-5; unequally distributed, leaves concentrated in the lower part of stem	1-3; unequally distributed, leaves concentrated in the lower part of stem	3-4; evenly distributed
Rosette leaves	few, pinnate	few, pinnate	many, sometimes reduced to a single leaflet	many, often reduced to a single leaflet	few, sometimes reduced to a single leaflet
Form of leaves	lower leaves with unequal leaflets, upper leaves with equal leaflets	lower leaves with unequal leaflets, upper leaves with equal leaflets	all leaves with unequal leaflets	all leaves with unequal leaflets	lower leaves with unequal leaflets, upper leaves with equal leaflets
Inflorescences	2-4; 1.5-2.5 cm in diameter	2-3; (2.5-) 3-4 cm in diameter	2-3; 3-4cm in diameter	3-4; 3.5-4cm in diameter	2-5; 2-2.5cm in diameter
Calyx (length/width)	10-12/4-4.5 (mm)	(10-)11-13/3-5 (mm)	(10-) 11-12/4.5-(5-7) (mm)	(13-) 14-16 (-17)/5-7 (mm)	8-11/3.5-5 (mm)
Calyx indumentum	semi-patent	strongly patent	sparse, patent	patent, villous to hirsute	sericeous, silky
Calyx colour	usually bi-coloured, with a red apex; whitish to yellowish	uni-coloured; whitish to yellowish	uni-coloured; whitish to yellowish	uni-coloured; greyish to ashen	uni-coloured; whitish to greenish

keys and diagnoses of the various taxa. Furthermore, I also tested the characteristics which have not been used so far.

Characteristics considered useful and diagnostic by other authors (Schlechtendal & Langethal 1885; Schintz & Keller 1914; Juzepczuk 1945; Kostrakiewicz 1959; Cullen 1968; Szafer et al. 1976; Jasičová 1988; Eglite & Krall 1996; Puidet et al. 2005; Rutkowski 2008; Sell & Murrell 2009) were selected for reliable identification (Table 2). These characteristics were used for initial examination in order to establish taxonomic status of specimens reliably.

The measurements were performed on herbarium material. On each herbarium sheet, measurements were made on two individuals when available. Morphological characteristics were counted, measured or estimated with a ruler and stereoscopic zoom microscope. The status and size of each characteristic were determined on mature plants. For accurate measurement, a white thread was placed every time on a line ruler when necessary. The height of the plant was recognized as the length of the longest stem on the top of which was inflorescence. The length and width of the leaf blade as well as its hairiness was measured/estimated on the biggest rosette leaf (if absent, on the biggest stem leaf). The length of inflorescence was measured horizontally in the widest part of inflorescence. The length of bract was measured from the base of inflorescence to the end of the

bract. The length of bract and calyx was measured on the same inflorescence. Corolla colour was not included in the analysis because it was not possible to evaluate this characteristic reliably from herbarium specimens. In addition, for each specimen, the habitat type was recorded as indicated on the herbarium label, if available.

A total of 828 specimens were analyzed, with each specimen representing an Operational Taxonomic Unit (OTU) in accordance with the methods used in numerical taxonomy (Sokal & Sneath 1963). OTU's were grouped in relation to characteristics selected in the present study, which shows the relationships between individuals.

Statistical analyses

Multivariate methods were used for statistical analyses. Data analyses were performed using the Statistica 9.1 (Stat-Soft Inc. 2010) and MVSP 3.1 (Multivariate Statistical Package; Kovach 1999).

Firstly, all twenty-two characteristics have been analyzed using cluster analysis to illustrate the general relationships and similarities between OTU's. The analysis was performed in order to obtain results which give only a general view. This helped me to find out whether different morphological groups (representing taxa) are separated into distinct clusters and to check the diversity within the groups. Before

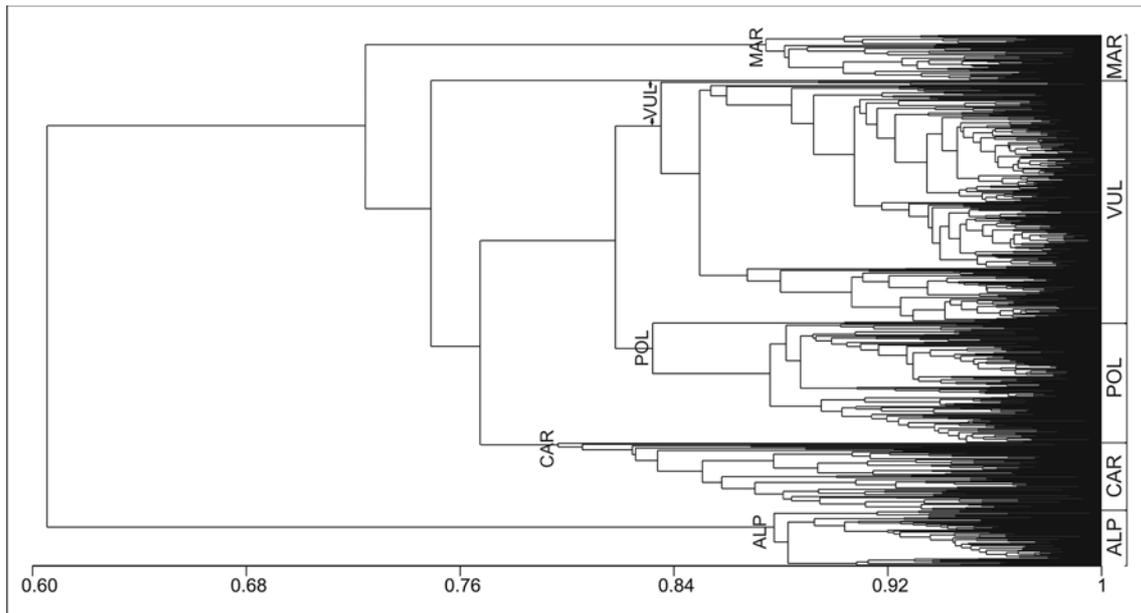


Fig. 1. Dendrogram made on the basis of cluster analysis (UPGMA method of classification and Gower's General Similarity Coefficient) showing relationships between examined taxa. Main clusters are marked with abbreviation: VUL – *A. vulneraria* L. ssp. *vulneraria*; POL – *A. vulneraria* ssp. *polyphylla* (DC.) Nyman; MAR – *A. vulneraria* ssp. *maritima* (Schweigg.) Corb.; CAR – *A. vulneraria* ssp. *carpatica* (Pant.) Nyman; ALP – *A. vulneraria* ssp. *alpestris* Asch. et Graebn..

performing the analysis, all the data was standardized. The similarity between two OTU's was calculated on the basis of Gower's General Similarity Coefficient. The dendrogram was prepared using UPGMA method.

One-way analysis of variance (ANOVA) was used (after using Lilliefors test to check if the data are normally distributed) to compare differences among means of characteristics and also to analyze each metric continuous and discrete cardinal characteristic for its mean, range and standard deviation (SD) with particular taxa as the grouping factor. Prior to the analysis of mean variability, all measurements were log-transformed. Finally, Tukey's HSD test was used to estimate which taxa differ significantly in relation to all continuous and discrete cardinal characteristics. Qualitative characteristics were tested with the nonparametric Kruskal-Wallis ANOVA test. Next, multiple comparisons of average ranks for all the tests were performed in order to determine which means differ.

The results of cluster analysis gave a clear reason for applying the next fundamental statistical methods – principal coordinate analysis (PCoA), correspondence analysis (CA) and finally discriminant analysis (DA). The goal of PCoA was the positioning of objects (individuals) in a space of reduced dimensionality while preserving their distance relationships. PCoA includes all quantitative and qualitative features. CA was performed on all qualitative binary-coded characteristics to check how particular variables (features) differentiate individuals and to what extent. DA was conducted separately on two previously defined data matrices. First, each specimen was determined on the basis of diagnostic qualitative characteristics. Then, standard discriminant analysis was applied in order to check which quantitative characteristics discriminate the investigated taxa in the most remarkable way. The analysis was carried out on the matrix of all quantitative characteristics for the first data set. For the second one, the analysis was based on qualitative and quantitative features which distinguished pairs of parent taxa from each other. These features were selected

from the results of Tukey's HSD test and multiple comparison test. The measurements were standardized before the analysis in order to avoid the effect of different scales of measurement. The discriminatory power is presented by Wilkis' *lambda* statistics values. Subsequently, canonical discriminant analysis was performed.

Results

In the first step, a cluster analysis was performed in order to establish morphological relationships among OTU's and separate distinct OTU groups. The dendrogram of the entire data set, based on results from the cluster analysis, shows five distinct clusters (Fig. 1). After comparing the OTUs with my indications from the herbarium specimens, it turned out that each individual classified as a concrete taxon corresponded to an appropriate cluster. This shows a clear distinction between the examined taxa. In addition, high variation within *A. vulneraria* ssp. *vulneraria* can be observed. Whereas, *A. vulneraria* ssp. *alpestris* proved to be the most isolated.

The results of the one-way ANOVA revealed differences in all characteristics of examined taxa. The values of F and H statistics obtained in the ANOVA and Kruskal-Wallis test for all examined characteristics were higher than the critical F value (Table 3). This indicates significant differences between taxa. The most important for discriminating the studied taxa qualitative features were: calyx colour, calyx indumentum, stem hairiness and distances between stem leaves; whereas calyx length, number of rosette leaves, inflorescence length, calyx width and bract length proved to be the most essential quantitative features. Variability ranges for quantitative characteristics are pre-

Table 3. Results of variance analysis ANOVA ($p < 0.05$): F and p values for each continuous and discrete cardinal characteristic; Results of Kruskal-Wallis test ($p < 0.05$): H and p values for each qualitative characteristic. Characteristics as well as corresponding values for which F/H values are the highest are given in bold. See Table 1 for abbreviations of characteristics.

Model	Characteristic	H	F	p value
Qualitative	HB	427.3899		<0.0001
	FRL	270.6946		<0.0001
	FL	560.3228		<0.0001
	DSL	664.6308		<0.0001
	HL	396.3012		<0.0001
	HS	668.3991		<0.0001
	HP	579.5212		<0.0001
	SB	435.7000		<0.0001
	CC	707.0000		<0.0001
	CI	707.0000		<0.0001
Discrete cardinal	NRL		215.6282	<0.0001
	NSL		72.0979	<0.0001
	NI		9.1480	<0.0001
Continuous	PH		48.3072	<0.0001
	LL		12.0161	<0.0001
	WL		25.7041	<0.0001
	LL/WL		35.3647	<0.0001
	LI		150.7812	<0.0001
	LB		102.7528	<0.0001
	LC		377.4433	<0.0001
	WC		108.0162	<0.0001
	LC/WC		11.4664	<0.0001

Table 4. Twelve quantitative characteristics for each taxon within *Anthyllis vulneraria* L. in Poland. Abbreviations of particular characteristics as in Table 1. M – arithmetical mean; Min – minimum value; Max – maximum value; SD – standard deviation.

Number of individuals	<i>A. vulneraria</i> L. ssp. <i>vulneraria</i>				<i>A. vulneraria</i> ssp. <i>polyphylla</i> (DC.) Nyman				<i>A. vulneraria</i> ssp. <i>maritima</i> (Schweigg.) Corb.				<i>A. vulneraria</i> ssp. <i>alpestris</i> Asch. et Graebn.				<i>A. vulneraria</i> ssp. <i>carpatica</i> (Pant.) Nyman			
	328				156				60				74				90			
Characteristic	M	Min	Max	SD	M	Min	Max	SD	M	Min	Max	SD	M	Min	Max	SD	M	Min	Max	SD
PH	33.42	4.00	68.00	11.10	25.94	10.00	62.00	9.67	25.80	13.00	45.00	7.91	18.39	11.00	32.00	5.23	24.12	9.00	51.00	9.15
NRL	2.47	1.00	11.00	0.93	3.17	1.00	9.00	1.28	4.43	2.00	10.00	1.92	7.76	4.00	20.00	3.09	6.40	4.00	21.00	2.44
NSL	3.48	2.00	5.00	0.79	2.96	2.00	5.00	0.78	3.30	2.00	5.00	0.79	2.03	1.00	3.00	0.44	2.59	1.00	4.00	0.72
LL	3.60	1.60	6.80	0.98	3.70	1.20	6.90	1.25	3.37	1.70	5.10	0.88	4.47	2.70	7.90	1.24	3.48	1.90	7.60	1.23
WL	1.20	0.60	2.80	0.38	1.38	0.60	2.90	0.48	0.91	0.40	1.70	0.37	1.52	0.90	2.60	0.36	1.21	0.70	2.00	0.30
LL/WL	3.14	1.60	6.60	0.79	2.73	1.50	4.70	0.56	4.06	1.80	8.40	1.32	2.95	2.10	3.80	0.46	2.88	1.80	4.90	0.63
NI	2.12	1.00	3.00	0.43	2.12	1.00	3.00	0.47	2.10	1.00	3.00	0.44	1.80	1.00	3.00	0.50	2.18	1.00	3.00	0.46
LI	2.34	1.50	3.40	0.28	2.53	1.60	3.90	0.40	2.24	1.50	3.10	0.30	3.36	2.50	4.50	0.40	2.71	2.00	4.10	0.39
LB	1.05	0.70	1.50	0.14	1.20	0.80	1.90	0.22	1.21	0.70	1.80	0.26	1.50	1.00	2.20	0.26	1.40	0.90	2.00	0.26
LC	0.94	0.80	1.10	0.08	1.02	0.90	1.20	0.09	0.91	0.80	1.10	0.08	1.39	1.10	1.70	0.14	0.99	0.80	1.50	0.12
WC	0.39	0.30	0.50	0.05	0.42	0.30	0.50	0.05	0.40	0.30	0.50	0.05	0.52	0.40	0.80	0.07	0.43	0.30	0.60	0.05
LC/WC	2.49	1.80	3.70	0.36	2.51	1.80	3.70	0.36	2.33	1.80	3.00	0.31	2.70	1.80	3.50	0.34	2.41	1.80	3.30	0.25

sented in Table 4 and Fig. 2 (for the biggest F values). Depending on the taxa different traits were found to be statistically significant. All considered quantitative characteristics were suitable to distinguish at least one taxa pair. The following features: number of rosette leaves, calyx length, inflorescence length and bract length, proved to be diagnostically important, because they differ all pairs of taxa from each other or with the exception of one. On the other hand, the number of inflorescences and leaf length failed to distinguish most taxa. These features should not be considered as diagnostically important, because the majority of taxa remained indistinguishable.

Principal coordinate analysis (PCoA) was performed on all examined characteristics. According to the results of PCoA, the first three coordinates accounted for 54.0% of the total variance (Fig. 3). The first explains 31.4% of the variation, the second 12.8%, and the third one – 9.8%. A PCoA ordination diagram showed the groups of *A. vulneraria* ssp. *alpestris*, *A. vulneraria* ssp. *carpatica* and *A. vulneraria* ssp. *maritima* clearly separated from *A. vulneraria* ssp. *vulneraria* along the first axis. Accessions of *A. vulneraria* ssp. *alpestris* formed a single grouping in a most distant position. Consequently, this taxon proved to be the most isolated.

All qualitative characteristics were analyzed using

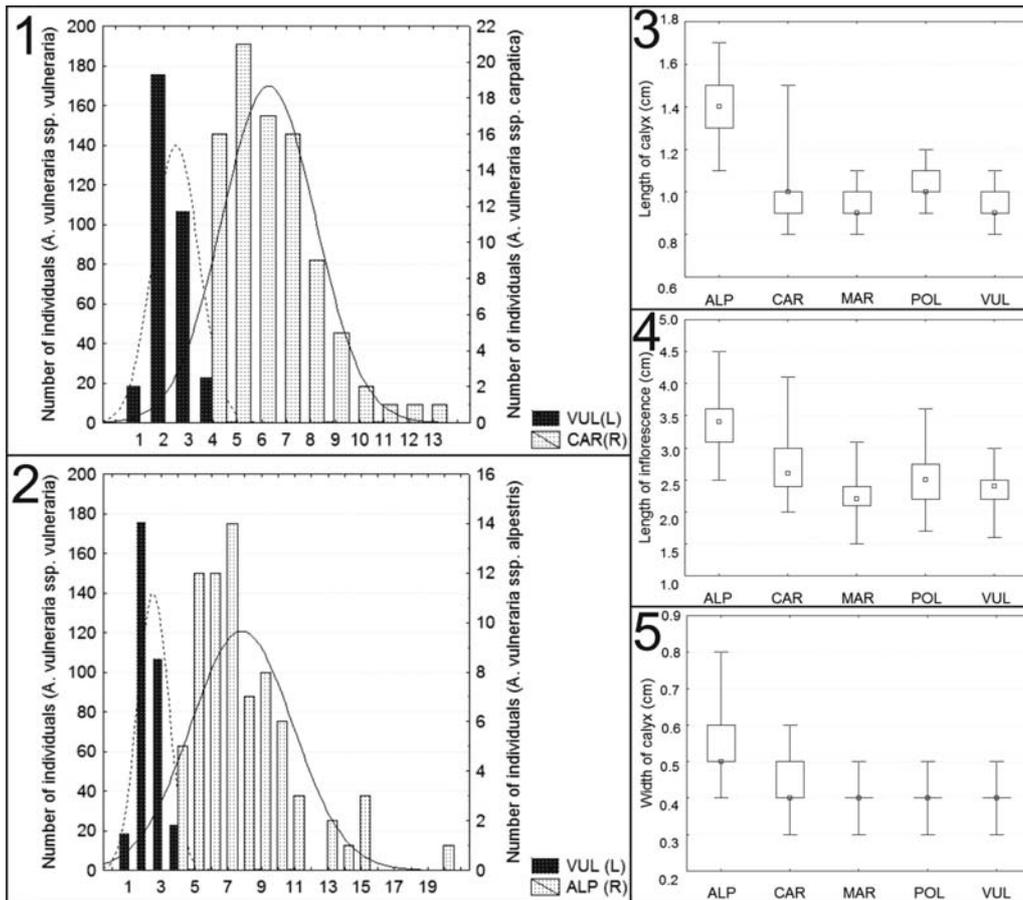


Fig. 2. Variability ranges for quantitative characteristics with the biggest F values. 1, 2 – Histograms presenting the variation in the number of rosette leaves in *A. vulneraria* ssp. *vulneraria* compared to *A. vulneraria* ssp. *carpatica* (1) and *A. vulneraria* ssp. *alpestris* (2). 3, 4, 5 – Box-and-whisker plots of selected characteristics with the biggest F values: calyx length (3), inflorescence length (4) and calyx width (5). Points indicate mean value, boxes represent 25% and 75% percentile, whiskers represent 1% and 99% percentile. See Fig. 1 for abbreviations.

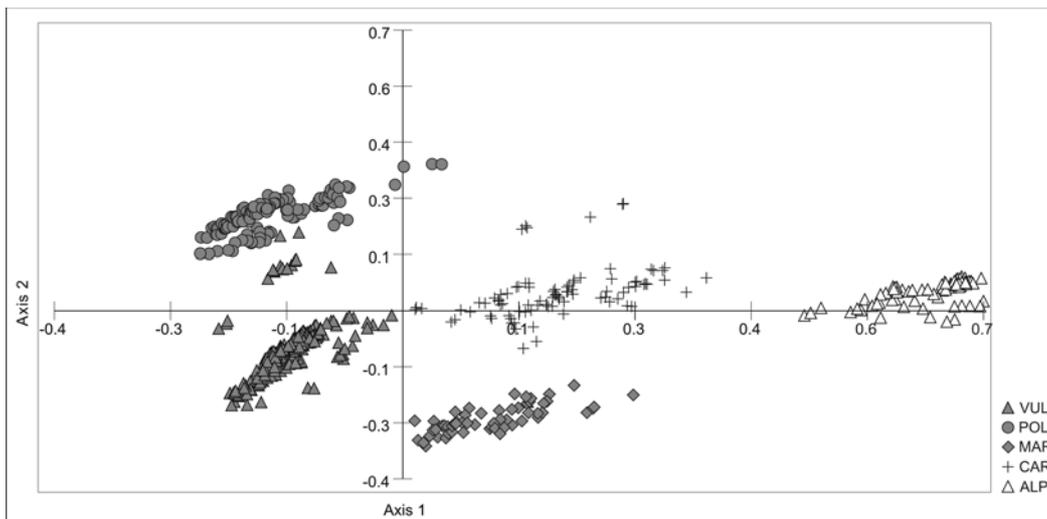


Fig. 3. Scatter diagram presenting the results of principal coordinate analysis (PCoA) of individual specimens of *Anthyllis vulneraria* L. along axis 1 and axis 2 based on quantitative and qualitative characteristics. See Fig. 1 for abbreviations.

CA. The eigenvalues of axis 1 and 2 are 0.72 and 0.53, respectively. The first component allows us to explain 35.77% of the variation in the matrix elements of the input data, the second 19.16% and the third 15.10%. The

plot showing the configuration of points representing particular individuals and validity for each characteristic in a two-dimensional space is presented in Fig. 4. The close location of the points representing individu-

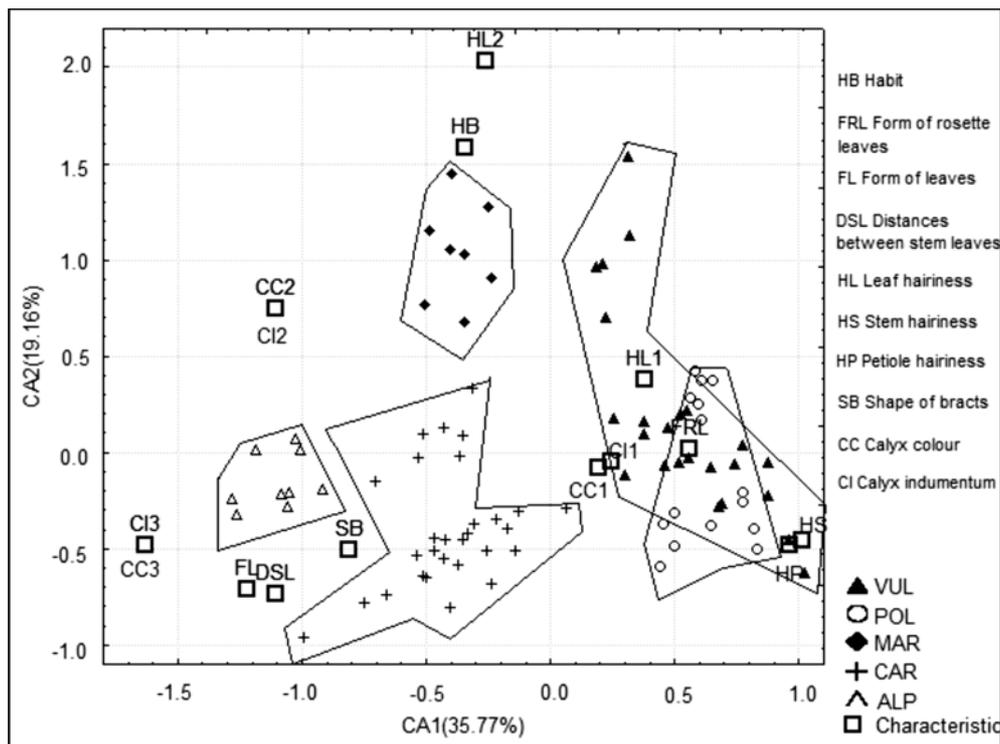


Fig. 4. Scatter diagram presenting the results of correspondence analysis (CA) of individual specimens of *Anthyllis vulneraria* L. along CA1 and CA2 based on all qualitative characteristics. See Fig. 1 for abbreviations.

Table 5. Values of Wilkis' *lambda*, partial Wilkis' *lambda* for twelve characteristics as a result of discriminant analysis (DA) of the specimens examined. Wilkis' and partial Wilkis' *lambda* values with the highest discriminatory power are given in bold. Correlation coefficients of morphological characteristics and canonical axes as a result of canonical discriminant analysis. Values for which discriminant functions are most weighted are given in bold. See Table 1 for abbreviations of characteristics.

Characteristic	Wilkis' <i>lambda</i>	partial Wilkis' <i>lambda</i>	Root 1	Root 2
PH	0.098855	0.916277	0.236091	-0.187304
NRL	0.121778	0.743800	-0.502496	0.611651
NSL	0.094902	0.954437	0.313546	-0.144576
LL	0.094564	0.957850	-0.111337	-0.177132
WL	0.094172	0.961837	-0.133529	-0.271567
LL/WL	0.101453	0.892813	0.076762	0.187598
NI	0.091387	0.991150	0.094520	0.117740
LI	0.098331	0.921157	-0.464209	-0.079185
LB	0.100337	0.902741	-0.354429	0.384801
LC	0.105206	0.860965	-0.710360	-0.523717
WC	0.094682	0.956664	-0.395124	-0.055598
LC/WC	0.094865	0.954817	-0.088288	-0.235449
Eigenvalue	-	-	3.905542	0.59824
Cumulative proportion	-	-	0.799802	0.92231

als of one morphological group in relation to the point representing the feature indicates that mainly because of this characteristic, the current group of points differs from the other groups.

The results of discriminant analysis (DA) showed that 11 of the analyzed characteristics are statistically significant (Wilkis' *lambda* 0.0905785, $F(48.2667) = 48.09217$, $p < 0.0001$). The characteristics that discriminate the taxa within *A. vulneraria* in the clearest way are: number of rosette leaves, calyx length, ratio leaf length/width, bract length, plant height and inflorescence length. Table 5 presents values of Wilkis' *lambda*, partial Wilkis' *lambda* of discriminant analy-

sis for twelve characteristics. Considering canonical discriminant analysis, the result of the chi-square test for all canonical roots for the data matrix proved their statistical significance. Correlation coefficients of morphological characteristics and canonical axes are presented in Table 5. The first function is most influenced by: calyx length, number of rosette leaves, inflorescence length and calyx width, whereas number of rosette leaves, calyx length and bract length were the most important for the separation of the individuals along the second axis. A scatterplot of the canonical variables shows five distinctive clusters formed by the examined taxa; however, cluster edges overlap to some

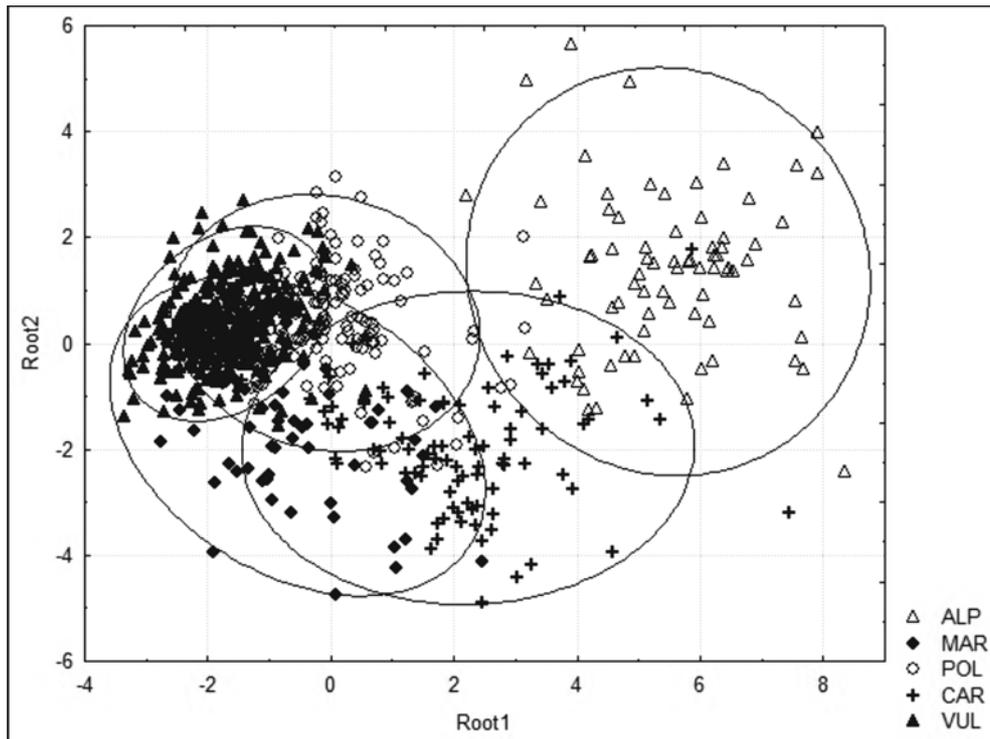


Fig. 5. Scatterplot presenting the result of canonical discriminant analysis for the first data set conducted on the basis of twelve characteristics. See Fig. 1 for abbreviations.

extent (Fig. 5). The group of *A. vulneraria* ssp. *alpestris* proved to be the most isolated. The result of canonical discriminant analysis of intermediate taxa in comparison to the parent taxa is presented in the form of scatterplots of canonical values (Fig. 6). This method was used to show the continuity of characteristics between parent taxa and putative hybrid. The results of discriminant analysis have confirmed the existing differences between parent taxa, while intermediates overlap with both of them (see Fig. 6). However, the discrimination of all three taxa is highly significant (Wilks' λ 0.09701; $F(24.992) = 91.372$; $p < 0.0001$).

Discussion

This study of *A. vulneraria* in Poland is based on the results of statistical analyses of morphological characteristics. The investigation resulted in determining characteristics that can be helpful in the diagnosis of the examined taxa. The taxa differ significantly in respect to all analyzed characteristics (Table 3). According to the results of different statistical analyses (cluster analysis, analysis of variance (ANOVA), Kruskal-Wallis ANOVA test, PCoA, CA and canonical discriminant analysis) the characteristics that discriminate taxa within *A. vulneraria* in the most remarkable way are: calyx length, number of rosette leaves, stem hairiness, distances between stem leaves, inflorescence length, shape of bracts, calyx indumentum and colour. In addition, it should be noted that when determining the taxa, each characteristic is important to varying degrees in relation to different taxa. For this reason, I recommend examining the greatest possible number of quantitative and

qualitative characteristics in order to avoid taxonomic confusion during diagnosis.

Several authors have suggested that the analyzed taxa within *A. vulneraria* can be distinguished by distances between stem leaves, stem hairiness, calyx length and form of rosette leaves (Juzepczuk 1945; Kosttrakiewicz 1959; Cullen 1968; Cullen 1976; Jasičová 1988; Eglite & Krall 1996). These characteristics proved to also be statistically important according to this study (Tables 3, 5). The number of rosette leaves also reliably distinguishes the examined taxa (see Fig. 2), although it has not been previously included in literature in relation to investigated taxa. This study provides concrete information on this characteristic, expressed by an exact number instead of the less precise terms "many" or "few" that exist in literature (Jasičová 1988). The length of bracts proved to be another diagnostically important feature, although it was mentioned only in Puidet et al. (2005). However, that study did not show the relevance of this characteristic for identifying (Puidet et al. 2005), probably as other taxa were examined as well as *A. vulneraria* ssp. *alpestris* was not included in the study. The other noteworthy characteristic is calyx colour in relation to Polish material. Bi-coloured calyxes with red/purple tips are characteristic only for *A. vulneraria* ssp. *vulneraria* in Poland. Another feature that has proved to be useful for identification of specimens is the ratio of leaf length/width in relation to the Polish material. It turned out that *A. vulneraria* ssp. *maritima* has much higher values than the other taxa. Form of leaves is also a useful feature, which was also mentioned by Cullen (1976), who defined this characteristic as a distinctive feature of two

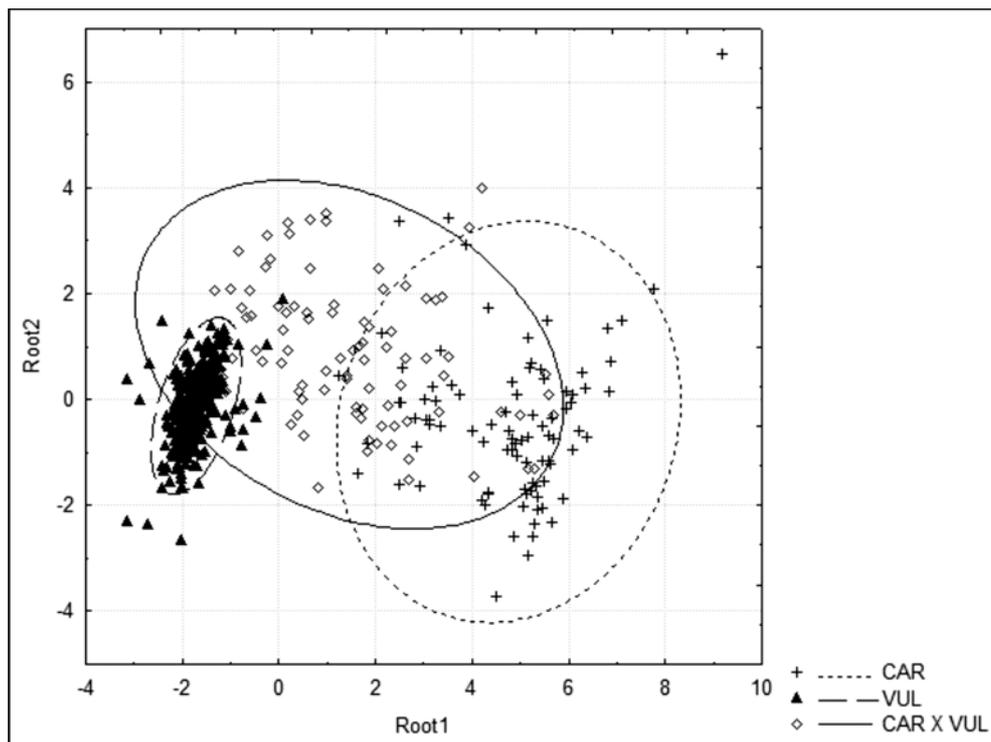


Fig. 6. Scatterplot of canonical scores as a result of canonical discriminant analysis for the second data set. CAR – *A. vulneraria* ssp. *carpatica* (Pant.) Nyman; VUL – *A. vulneraria* L. ssp. *vulneraria*; CAR X VUL – *A. vulneraria* ssp. *carpatica* (Pant.) Nyman × *A. vulneraria* L. ssp. *vulneraria*.

aggregates. *Anthyllis vulneraria* ssp. *alpestris* and *A. vulneraria* ssp. *carpatica* can be reliably distinguished by having all leaves with unequal leaflets (inequifoliate), in contrast to the rest of the examined taxa, which have at least upper stem leaves with equal leaflets (equifoliate).

According to Cullen (1968) and Clapham et al. (1987), the width of the calyx is an important diagnostic feature which distinguishes *A. vulneraria* ssp. *carpatica* from *A. vulneraria* ssp. *polyphylla* and *A. vulneraria* ssp. *vulneraria*. In spite of the fact that this characteristic proved to be a statistically important trait for discrimination of all taxa (see Tables 2, 5), its variability range suggests that it should not be regarded as a diagnostic characteristic when considering these taxa in Poland (see also Jasičová 1988).

In some keys the type of calyx indumentum is regarded as an important diagnostic characteristic which distinguishes *A. vulneraria* ssp. *vulneraria* from *A. vulneraria* ssp. *polyphylla* (Kostrakiewicz 1959; Cullen 1968; Szafer et al. 1976), wherein the calyx of *A. vulneraria* ssp. *vulneraria* is characterized as weakly semi-patent hairy and the calyx of *A. vulneraria* ssp. *polyphylla* as strongly-patent hairy. In addition, the calyx of *A. vulneraria* ssp. *polyphylla* is believed to have densely arranged hairs. I have noted the varying levels of intensity and arrangement of hairs on the calyx in *A. vulneraria* ssp. *vulneraria*. Moreover, the type of hairiness of this taxon depends largely on the nature of habitat and therefore also on geographical localization in Poland. As a result, some correlations between habitat conditions and hairiness intensity, density and arrange-

ment of the calyx's hairs can be found. This also refers to *A. vulneraria* ssp. *carpatica*. According to my observations, these taxa have denser and more patent calyx indumentum in dry, thermophilic habitats (e.g. grasslands); while in moister habitats, the hairiness is sparser and rather semi-patent or even appressed. Therefore, in drier habitats, the appearance of *A. vulneraria* ssp. *vulneraria* calyx resembles the typical calyx of *A. vulneraria* ssp. *polyphylla*. These various phenotypes, which can be frequently found in the field, should ultimately be regarded as ecotypes. Taking this into account, I do not consider calyx indumentum as a diagnostic characteristic when considering these three taxa. However, *A. vulneraria* ssp. *alpestris* and *A. vulneraria* ssp. *maritima* have a specific type of calyx indumentum that makes them relatively easy to distinguish from the other subspecies.

In addition, the type of habitat proved to be an important factor during recognition of specimens. Therefore, information on habitat is extremely important, especially for the taxa whose distribution ranges overlap. In Poland, it is helpful to distinguish *A. vulneraria* ssp. *vulneraria* from *A. vulneraria* ssp. *polyphylla*. It is related to the fact that *A. vulneraria* ssp. *polyphylla* was recorded mainly in highly thermophilic and well-insolated habitats. This taxon is apparently an element of Pannonian flora (Juzepczuk 1945), and so it is associated with dry grasslands, mainly those resembling the steppe grasslands in Poland. Consequently, it occurs only in south-eastern and eastern Poland. Moreover, it is worth noting that *A. vulneraria* ssp. *maritima* is confined to coastal sand dunes and does not

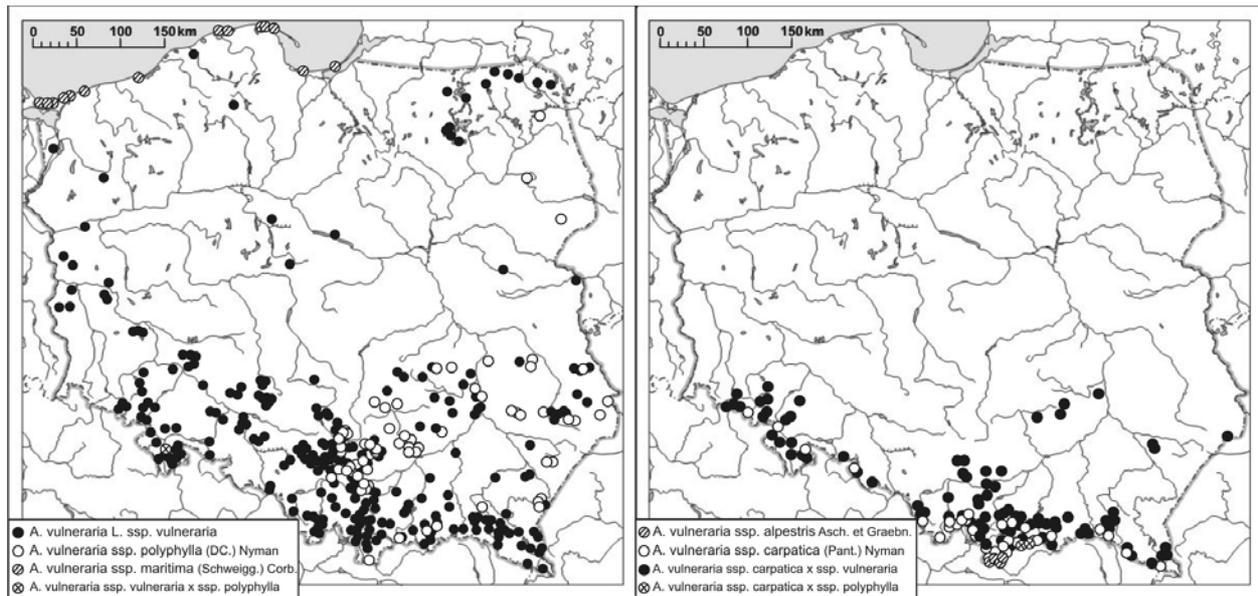


Fig. 7. Distribution of *Anthyllis vulneraria* L. in Poland. Left – *Vulneraria* aggregate; Right – *Alpestris* aggregate.

occur outside the Baltic Sea coast. Whereas *A. vulneraria* ssp. *alpestris* is associated with the subalpine calcareous grasslands in Poland. The distribution maps of investigated taxa prepared on the basis of the material examined are presented in Fig. 7.

Taking into consideration the results of all analyses, *A. vulneraria* ssp. *alpestris* differs greatly from the other taxa in terms of most characteristics, which explains it being distinguished as a separate species by some authors (e.g. Fritsch 1897; Becker 1911; Kostakiewicz 1959; Mirek et al. 2002). On the other hand, the results also indicate that this taxon is similar to *A. vulneraria* ssp. *carpatica* regarding some features (e.g. number and form of rosette leaves, distances between stem leaves, form of leaves and shape of bracts). This might suggest that they are closely related. In addition, both taxa belong to the same *Alpestris* aggregate (Cullen 1976). Consequently, some of the taxa within *A. vulneraria* may deserve species status, but this will need to be resolved on the basis of further molecular studies.

According to the results of different molecular studies (based on ITS sequences and AFLP data), genetic differentiation is not related to the extant taxonomic information, based only on morphological characteristics (Nanni et al. 2004; Köster et al. 2008). Therefore, some individuals classified as different subspecies of *A. vulneraria* with different geographical distribution show no significant differences in the ITS sequences or AFLP data (Nanni et al. 2004; Köster et al. 2008). There were no studies concerning all subspecies of *A. vulneraria* so far. Consequently, further molecular and morphological studies, as well as their comparison, are required to revise the current classification of taxa within the species.

The study has shown that there are also intermediates in Poland, which was not surprising, because ranges of *A. vulneraria* ssp. *carpatica*, *A. vulneraria*

ssp. *polyphylla* and *A. vulneraria* ssp. *vulneraria* overlap to some extent (Fig. 7). Morphologically intermediate taxa between *A. vulneraria* ssp. *carpatica* and *A. vulneraria* ssp. *vulneraria* were found to be widespread in southern Poland (Sudety Mountains, lower altitudes in the Carpathians and Carpathian Foothills, also a few localities within the uplands zone, including Świętokrzyskie Mountains). Similar morphotypes were also recorded on the south side of the Western Carpathians in Slovakia (see Jasičová 1988). I have classified to this group individuals which differ from *A. vulneraria* ssp. *carpatica* with evenly distributed leaves at least to the middle part of the stem and usually \pm equifoliate upper stem leaves. There were also a high proportion of specimens with a bi-coloured calyx with a red tip. The species from NW Europe, morphologically intermediate between these taxa are regarded as *A. pseudovulneraria* Sagorski (later synonymized with *A. vulneraria* ssp. *carpatica*) (Cullen 1968; Sell & Murrell 2009). The native distribution of *A. vulneraria* var. *pseudovulneraria* (Sagorski) Cullen has perhaps been obscured by a history of cultivation (Cullen 1976). Therefore, it is difficult to compare specimens from different regions of Europe (taking into account the possibility of hybridization with local taxa), even if at present all British and Irish material of *A. vulneraria* ssp. *carpatica* is referable to *A. vulneraria* var. *pseudovulneraria* (Cullen 1976; Akeroyd 1991; Akeroyd 1998). Consequently, I cannot suggest any taxonomic status for this intermediate morphotypes recorded in Poland at this time. This problem may be explained only by comparing material from Poland with material from nearby countries (e.g. Slovakia, Czech Republic, Germany) and from NW Europe, but at present, I recommend the continuation of referring these specimens to *A. vulneraria* ssp. *carpatica*. I have studied the variability between *A. vulneraria* ssp. *vulneraria* and to *A. vulneraria* ssp. *carpatica*. These intermediates occur on the border of

two parent species' geographical ranges in Poland. This prompted me to study morphological variability among the three taxa, treating transitional specimens as a distinct taxon. The result of canonical discriminant analysis revealed the continuity of characteristics between parent taxa and their putative hybrids. Because of the transitional character of different features which occur in these taxa, sometimes it is difficult to assign a particular specimen to a concrete taxon. Therefore, correct diagnosis requires a comparison with herbarium specimens. It is possible that some molecular or biochemical studies may help in solving this problem.

Other intermediate morphotypes within *A. vulneraria* were also recorded in Poland. The intermediates between *A. vulneraria* ssp. *carpatica* and *A. vulneraria* ssp. *polyphylla* were firstly classified as *A. affinis* A. Kern. (Cullen 1968), later synonymized with *A. vulneraria* ssp. *carpatica* (Pant.) Nyman. However, Cullen (1976) believes that this taxon is quite widespread in eastern and central Europe and has probably been introduced into some areas by cultivation as fodder plants. Due to the fact that the specimens representing these intermediates cover the whole morphological range between the two parent species, he proposes to treat such specimens as this subspecies, which they more closely resemble (Cullen 1976). As Cullen (1976) cites no specimens from the area of Poland, I singled out a few populations as a typical representative of such morphotype. These individuals could be easily diagnosed by identifying features characteristic for *A. vulneraria* ssp. *polyphylla* (strongly patent hairs on the lower part of stem and petioles) and the remaining features typical for *A. vulneraria* ssp. *carpatica*.

Poland: Pieniny Mountains, Ostra Skała near Sromowce Niżne village, limestone scree, S slope, 500 m a.s.l., 06.07.1983, *A. Jasiewicz* (KRAM-430856). Pieniny Mountains, the trail from Krościenko village to Trzy Korony peak, grassland, 25.06.1964, *H. Piękoś* (KRAM-205241). Pieniny Mountains, Stolarzówka near Krościenko, 620–640 m a.s.l., 02.06.1981, *H. Piękoś*, *B. Bzowska* (KRAM-228388)

I can also distinguish intermediates between *A. vulneraria* ssp. *vulneraria* and *A. vulneraria* ssp. *polyphylla* with only one locality in Poland. These individuals have patent hairiness in the lower part of the stem and petioles as a distinctive characteristic of *A. vulneraria* ssp. *polyphylla*, while the remaining features are typical for *A. vulneraria* ssp. *vulneraria*, including the red-tipped calyx. This population is located in the Eastern Sudetes:

Poland: Sudety Mountains, Śnieżnik Mountains, Wapniarka Mountain, E slope, 420 m a.s.l., 12.06.1990, *Z. Szela* (KRAM-446489)

This seems to be an interesting issue because of the fact that I did not record *A. vulneraria* ssp. *polyphylla* in this region (Fig. 7). The status of these specimens

in Poland is difficult to determine. It should be noted that there is a taxon of apparently hybrid origin, which has already been described as *Anthyllis colorata* Juz. The Polish material is consistent with the description of *A. colorata* (Juzepczuk 1945). Until now this taxon was considered endemic to Estonia (Minjaev & Akulova 1987; Puidet et al. 2005). Nevertheless, the status and exact distribution range require a comparison with material from Estonia as well as further taxonomic studies.

Other intermediate morphotypes within *A. vulneraria* are often observed in other parts of Europe. Some of them were described as hybrids in a separate taxonomic rank. For example, *Anthyllis baltica* Juz. (probably *A. vulneraria* ssp. *vulneraria* × *A. vulneraria* ssp. *maritima*) is considered endemic to the Baltic region (Juzepczuk 1945; Minjaev & Akulova 1987). On the other hand, some are simply described as intermediate morphotypes between the distribution ranges of the parent species. In addition, these specific transitional morphotypes resemble in their characteristics this parent species, whose geographical range is closer (e.g. intermediates between *A. vulneraria* ssp. *alpestris* and *A. vulneraria* ssp. *vulneraria*; Jasičová 1988). The standing of such intermediates has not been determined yet and can only be ascertained by further observations in situ.

Different multivariate statistical analyses result in five groups, which are related to five taxa occurring in Poland. Based on the results of these analyses and according to my observations, the taxonomy of *A. vulneraria* is presented in a form of the following classification. This work should help in bringing out the characteristics that contributed greatly in differentiating individual taxa from each other.

Key for determination of *Anthyllis vulneraria* in Poland

- 1 Distances between stem leaves definitely unequal. Leaves concentrated in the lower part of the stem, one exceptionally in the upper part. All stem leaves with unequal leaflets (inequifoliate). Many rosette leaves (4–) 5–9 (–12), often reduced to a single leaflet. Mountain plant 2
- 1* Distances between stem leaves ± equal. Leaves evenly distributed along the stem. Lower stem leaves with unequal leaflets (inequifoliate), upper with ± equal leaflets (equifoliate). Usually a few rosette leaves 2–4 (–6), generally pinnate, very rarely reduced to a single leaflet. Lowland plant 3
- 2 Calyx smoky-greyish to ashen, (1.2–) 1.3–1.5 (–1.6) cm with ± patent, villous to hirsute hairs. Inflorescence (2.6–) 3.1–3.6 (–4.3) cm in diameter ..
.. *Anthyllis vulneraria* ssp. *alpestris* **Asch. et Graebn.**
- 2* Calyx whitish to yellowish, (0.8–) 0.9–1.1 (–1.3) cm with ± semi-patent sericeous, rather sparse hairs. Inflorescence (2.0–) 2.4–3.0 (–3.5) cm in diameter ...
... *Anthyllis vulneraria* ssp. *carpatica* (**Pant.**)
Nyman

- 3 Lower parts of the stem and petioles with dense, patent hairs. Calyx (0.9–) 1.0–1.1 (–1.2) cm
 . . . *Anthyllis vulneraria* ssp. *polyphylla* (DC.)
 Nyman
- 3* Stems and petioles with appressed hairs. Calyx 0.8–1.0 (–1.1) cm 4
- 4 Plant of coastal sand dunes, usually decumbent, covered with thick and silky hairs with silvery-white shine. Usually a lot of hairs on the upper surface of leaf blade. Highest leaflet of the rosette leaf (or simply rosette leaf when single) lanceolate (ratio leaf length/width 2.9–5.0). Calyx always uni-coloured, greenish to whitish with ± patent, thick and silky indumentum. *Anthyllis vulneraria* ssp. *maritima* (Schweigg.) Corb.
- 4* Plant ascending to erect covered by inconspicuous and usually sparse hairs, not silky. Upper surface of leaf blade usually glabrous or sometimes with single, sparsely hairs. Highest leaflet of the rosette leaf (or simply rosette leaf when single) cuneate to elliptic (ratio leaf length/width 2.4–3.6). Calyx often bi-coloured with a red tip, usually yellowish with ± semi-patent, not silky indumentum.
 *Anthyllis vulneraria* L. ssp. *vulneraria*

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References

- Akeroyd J.R. 1988. Variation in *Anthyllis vulneraria* in Sicily and South Italy. *Notes Roy. Bot. Gard. Edinburgh* **45**: 369–74.
- Akeroyd J.R. 1991. *Anthyllis vulneraria* L. subsp. *polyphylla* (DC.) Nyman, an alien kidney-vetch in Britain. *Watsonia* **18**: 401–403.
- Akeroyd J.R. 1998. *Anthyllis vulneraria* L., pp. 45–46. In: Rich T.C.G. & Rich M.D.B. (eds), *Plant Crib*, London.
- Allan G.J. & Porter J.M. 2000. Tribal delimitation and phylogenetic relationships of Loteae and Coronilleae (Faboideae: Fabaceae) with special reference to *Lotus*: evidence from nuclear ribosomal ITS sequences. *Am. J. Bot.* **87**: 1871–1881.
- Allan G.J., Zimmer E.A., Wagner W.L. & Sokoloff D.D. 2003. Molecular phylogenetic analysis of tribe Loteae (Leguminosae), implications for classification and biogeography, pp. 371–393. In: Klitgaard B.B. & Bruneau A. (eds), *Advances in Legume Systematics. Part 10. Higher Level Systematics*. Royal Botanic Garden, Kew.
- Becker W. 1911. Bearbeitung der *Anthyllis*-sektion *vulneraria* DC., pp. 256–287. In: Verlag von G. Thieme (ed.), *Beihefte zum botanischen Centralblatt. Erste Abteilung, Anatomie, Histologie, Morphologie und Physiologie der Pflanzen*. Band XXVII. Dresden-N.
- Bennet S.J., Francis C. & Reid B. 2001. Minor and under-utilised legumes, pp. 207–209. In: Maxted N. & Bennett S.J. (eds), *Plant genetic resources of legumes in the Mediterranean*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Bolkhovskikh Z., Grif V., Matvejeva T. & Zakharyeva O. 1969. Khromosomnuie chisla tsvetkovuikh rastenii. Nauka, Leningrad, 926 pp.
- Cagiotti M.R., Poceschi N., Ranfa A., Romano B., Fornaciari M. & Miniati E. 1990. Morphologic and phytochemical investigation of the genus *Anthyllis* L. in Italy. *Annali della Facolta di Agraria Universita degli Studi di Perugia* **44**: 773–96.
- Clapham A.R., Tutin T.G. & Moore D.M. 1987. *Flora of the British Isles*. 3rd edn. Cambridge University Press, Cambridge, 1232 pp.
- Cullen J. 1968. *Anthyllis* L., pp. 179–181. In: Tutin T.G. (ed.), *Flora Europaea Vol.2*. Cambridge University Press, Cambridge.
- Cullen J. 1976. The *Anthyllis vulneraria* complex: a resume. *Notes Roy. Bot. Gard. Edinburgh* **35**: 1–38.
- Cullen J. 1986. *Anthyllis* in the British Isles. *Notes Roy. Bot. Gard. Edinburgh* **43**: 277–281.
- Degtjareva G., Sokoloff D., Samigullin T. & Valiejo-Rom C. 2008. Molecular phylogeny of the genus *Anthyllis* (Leguminosae: Loteae): evidence for long-branch attraction in parsimony analysis of nrITS data, pp. 52. In: Gradstein S.R., Klatt S., Normann F., Weigelt P., Willmann R. & Wilson R. (eds), *Systematics 2008 Programme and Abstracts Göttingen 7–11 April 2008 10th Annual Meeting of the Gesellschaft für Biologische Systematik 18th International Symposium “Biodiversity and Evolutionary Biology” of the German Botanical Society*, Universitätsverlag Göttingen.
- Dempsey R.E., Gornall R.J. & Bailey J.P. 1994. Contributions to a cytological catalogue of the British and Irish flora, 4. *Watsonia* **20**: 63–66.
- Eglite Z. & Krall H. 1996. *Anthyllis* L., pp. 128–132. In: Kuusk V., Tabaka L. & Jankeviciene R. (eds), *Flora of the Baltic countries 2*. Eesti Loodusfoto, Tartu.
- Fritsch K. 1897. *Excursionsflora für Oesterreich, mit Ausschluss von Galizien, Bukowina und Dalmatien. Mit theilweiser Benützung des “Botanischen Excursionsbuches” von G. Lorinser*. Verfasst von Karl Fritsch, Wien, 664 pp.
- Gonzalez C.B. 1998. Notes on the genus *Anthyllis* L. (Loteae-Leguminosae) and its treatment for the Flora iberica. *Anales del Jardin Botanico de Madrid* **56**: 279–303.
- Honnay O., Coart E., Butaye J., Adriaens D., Van Glabeke S. & Roldán-Ruiz I. 2006. Low impact of present and historical landscape configuration on the genetics of fragmented *Anthyllis vulneraria* populations. *Biol. Conserv.* **127**: 411–419.
- Hultén E. & Fries M. 1986. *Atlas of North European vascular plants. North of the tropic cancer. Vol.3*. Koeltz Scientific Books, Königstein, Federal Republic of Germany, 204 pp.
- İlçim A., Çenet M. & Dadandi M.Y. 2008. Notes on *Anthyllis vulneraria* L. *KSU Journal of Science and Engineering* **11**: 10–13.
- Jalas J. 1950. Zur Kausalanalyse der Vorbereitung einiger nordischen Os- und Sandpflanzen. *Ann. Bot. Soc. Zool.-Bot. Fenn. “Vanamo”* **24**: 1–362.
- Jalas J. 1952. *Anthyllis kuzenevae* Juz. and the borealis group of *Anthyllis vulneraria* L. *Arch. Soc. “Vanamo”*. **7**: 49–52.
- Jasičová M. 1988. *Anthyllis* L., pp. 336–344. In: Bertová L. (ed.), *Flóra Slovenska 4(4)*, Veda, Bratislava.
- Juzepczuk S.V. 1945. *Anthyllis* L., pp. 262–276. In: Komarov V. (ed), *Flora SSSR 11*, Moskva-Leningrad.
- Kalinowski A., Szweykowski J. & Kaczmarek Z. 1983. Analysis of the variability of natural *Anthyllis vulneraria* s. l. populations from Baltic coast. Patterns of three enzyme systems following electrophoretic separation. *Bulletin de la Societe des Amis des Sciences et des Lettres* **23**: 67–78.
- Köster E. 2010. Morphological and genetical variation within species complexes: *Anthyllis vulneraria* s. l. and *Alchemilla vulgaris* (coll.). Tartu University Press. University of Tartu, Estonia, 101 pp.
- Köster E., Bitocchi E., Papa R. & Pihu S. 2008. Genetic structure of the *Anthyllis vulneraria* L. s. l. species complex in Estonia based on AFLPs. *Cent. Eur. J. Biol.* **3**: 442–450.
- Kostrakiewicz K. 1959. *Anthyllis* L., pp. 79–83. In: Szafer W. & Pawłowski B. (eds), *Flora Polska. Rośliny naczyniowe Polski i ziem ościennych*, Vol.8, Państwowe Wydawnictwo Naukowe, Warszawa.
- Kovach W.L. 1999. MVSP – A Multivariate Statistical Package for Windows, ver. 3.1. Kovach Computing Services, Pentraeth, UK.

- Krall H. 1983. Koldrohud (Genus *Anthyllis*). Eesti Loodus **6**: 388–393.
- Kropf M., Kadereit J.W. & Comes H.P. 2002. Late Quaternary distributional stasis in the submediterranean mountain plant *Anthyllis montana* L. (Fabaceae) inferred from ITS sequences and amplified fragment length polymorphism markers. Mol. Ecol. **11**: 447–458.
- Lövkvist B. & Hultgørd U.M. 1999. Chromosome numbers in south Swedish vascular plants. Opera Bot. **137**: 1–42.
- Lukaszewska K., Kalinowski A., Kaczmarek Z. & Szweykowski J. 1983. Analysis of the variability of natural *Anthyllis vulneraria* s. l. populations from Baltic coast. Variability of morphological traits on natural stands. Bulletin de la Societe des Amis des Sciences et des Lettres **23**: 45–55.
- Marsden-Jones E.M. & Turrill W.B. 1933. Studies in variation of *Anthyllis vulneraria*. J. Genet. **27**: 261–285.
- Minjaev N.A. & Akulova Z.F. 1987. *Anthyllis* L., pp. 98–103. In: Fedorova V.A. (ed), Flora of the European part of USSR, Nauka, Leningrad.
- Mirek Z., Piękoś-Mirek., Zajac A. & Zajac M. 2002. Flowering plants and pteridophytes of Poland. A checklist. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, 442 pp.
- Nanni L., Ferradini N., Taffetani N. & Papa R. 2004. Molecular phylogeny of *Anthyllis* spp, Plant Biol. **6**: 454–464.
- Polhill R.M. 1981. Lotae, pp. 371–375. In: Polhill R.M. & Raven P.H. (eds), Advances in legume systematics. Part 1, Royal Botanic Gardens, Kew.
- Puidet E., Liira J., Paal J., Pärtel M. & Pihu S. 2005. Morphological variation of eight taxa of *Anthyllis vulneraria* L. s. l. (Fabaceae). Ann. Bot. Fenn. **42**: 293–304.
- Rich T.C.G. 2001. What is *Anthyllis vulneraria* L. subsp. *corbierei* (Salmon & Travis) Cullen (Fabaceae)? Watsonia **23**: 469–480.
- Rothmaler W. 1941. Westmediterrane arten der sektion vulneraria DC. der gattung *Anthyllis* L. Repert Spec. Nov. Regnii Veg. **50**: 177–192.
- Rutkowski L. 2008. Klucz do oznaczania roślin naczyniowych Polski niżowej. Wydawnictwo naukowe PWN, Warszawa, 814 pp.
- Sagorski E. 1908. Ueber den formenkreis der *Anthyllis vulneraria* L., pp. 40–43. In: Kneucker A. (ed.), Allgemeine botanische zeitschrift für systematik, floristik, pflanzengeographie etc. Jahrg. 14. Karlsruhe, J.J. Reiff.
- Schintz H. & Keller R. 1914. Flora der Schweiz 11. Teil: Kritische Flora. Verlag von Albert Raustein, Zürich, 264 pp.
- Schlechtendal D.F. & Langethal L.E. 1885. Flora von Deutschland. Revidirt, verbessert und nach den neusten wissenschaftlichen Erfahrungen bereichert von Ernst Hallier. Drei- und zwanzigster und vierundzwanzigster. Band: 101. Papilionaceae (Leguminosae). Erster und zweiter Theil. 5. Auflage, Eugen Köhler, Gera-Untermhaus, pp. 149–153.
- Sell P. & Murrell G. 2009. Flora of Great Britain and Ireland. Volume 3: Mimosaceae – Lentibulariaceae. Cambridge University Press, Cambridge, UK, 624 pp.
- Sokal R.R. & Sneath P.H.A. 1963. Principles of numerical taxonomy. W.H. Freeman, San Francisco, 359 pp.
- Stankov S.S. & Taliev V.I. 1949. Opredelitel' vuisshikh rasteniï Evropejskoï chasti SSSR. Sovetskaja Nauka Press, Moscow, 1151 pp.
- StatSoft Inc. 2010. STATISTICA (data analysis software system), version 9.1. www.statsoft.com.
- Szafer W., Kulczyński S. & Pawłowski B. 1976. Rośliny polskie. Wydawnictwo naukowe PWN, Warszawa, 1019 pp.
- Van Glabeke S., Coart E., Honnay O. & Roldán-Ruiz I. 2007. Isolation and characterization of polymorphic microsatellite markers in *Anthyllis vulneraria*. Mol. Ecol. Notes **7**: 477–479.
- Van Loon J.C. & Kieft B. 1980. In chromosome number reports LXVIII. Taxon **29**: 538–542.
- Yeh M.S., Yuasa H. & Maekawa F. 1986. Chromosome numbers in the Leguminosae. Sci. Rep. Res. Inst. Evol. Biol. **3**: 57–71.
- Zajac A. & Zajac M. (eds). 2001. Atlas rozmieszczenia roślin naczyniowych w Polsce. Laboratory of Computer Chorology, Institute of Botany, Jagiellonian University, Kraków, 715 pp.

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