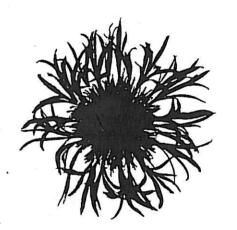


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Taxonomic study of Central European taxa of *Centaurea* sect. *Jacea*

[in Czech with English summary]



Mgr. Petr Koutecký Summary of Ph.D. Thesis

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Annotation:

Taxonomy of Centaurea sect. Jacea was studied in Central Europe (especially Czechia, Slovakia, and parts of the Ukraine and Romania). Morphological variation of C. phrygia agg. and C. jacea agg. was investigated by multivariate morphometric analysis. In C. phrygia agg. karyological variation was also studied. Distribution of all taxa of the section (except for C. jacea agg.) in Czechia and Slovakia was estimated on a basis of revised herbarium specimens. Some questions of nomenclature (identity of C. subjacea, names in C. phrygia agg.) were solved. Hybridization was studied in detail using hybridization experiments, flow cytometry and isozyme analysis. As a result, determination key of all taxa was compiled.

Key words:

Centaurea, Centaurea sect. Jacea, Centaurea jacea agg., Centaurea phrygia agg., Central Europe, Czechia, Slovakia, taxonomy, morphometric analysis, chromosome number, distribution, hybridization, flow cytometry, isozymes, nomenclature.

1. Introduction

The section Jacea of the genus Centaurea is known as a taxonomically critical group of plants. A need for revision of this group for the Flora of the Czech Republic and Determination key to ferns and seed plants of Slovakia have initiated my master and later Ph.D. studies focused on Centaurea phrygia agg. and the whole section, respectively.

The section *Jacea* can be divided into several morphologically quite well defined species groups/aggregates. The taxonomic problems, which were needed to solve, differ within particular groups. Each group was therefore studied separately, some of them (namely *Centaurea jacea* agg. and *C. phrygia* agg.) more in detail than the others. One of the main problems in the section is hybridization, which was therefore studied as a special topic. It all mirrors in structure of this Ph.D. Thesis: chapters 1–4 are a general part (introduction, description of the genus and the section *Jacea*, main taxonomic problems, and methods common to all groups), chapters 5–8 contain description of each species group (aims of study, details of methods, results and discussion), chapter 9 lists all known or reported hybrids, chapters 10–11 are focused on two experimental case studies of hybridization, and chapter 12 compiles all results as a determination key to the taxa and descriptions and nomenclature of all taxa and hybrids.

Since many "regional" topics are included (especially detailed distribution of individual taxa in Czechia and Slovakia for the purposes of the Flora and the Key, as described above) and the geographic focus includes mainly Czechia and Slovakia, this Ph.D. thesis is written in Czech with extensive English summary. Separate publication of the main results in English will follow.

2. The genus Centaurea and the section Jacea

The genus *Centaurea* L. s. l. is very large and comprises between 400 and 700 species, depending on taxonomic concepts. They are distributed mainly in Eurasia. Modern phylogenetic studies show that the genus should be divided into several smaller more natural genera. The genus *Centaurea* s. str. then only include the most derived members of the group.

The section *Jacea*, which is this Ph.D. thesis focused on, is recognized as a phylogenetically primitive group within the genus *Centaurea* s. str. Its typical morphological features are undivided leaves (sometimes lower leaves lobate to pinnatifid), middle-sized capitula (involucre ca. 1–2 cm in diameter), involucral bracts bearing a scarious appendage usually not decurrent on a bract and never spiny, purple florets, outer florets usually strongly radiating, and pappus of achenes short or absent. The section *Jacea* is characterized by the base chromosome number x=11, and includes diploid (2n=22) and tetraploid (2n=44) taxa. The majority of ca 50 species of the section inhabit mountains of the Mediterranean and south-western Asia, some reaching Central Europe and rarely as far as Scandinavia, Finland, or north-western Russia.

3. Sources of taxonomic and determination problems Morphological variation and plasticity

Centaurea sect. Jacea is morphologically quite homogenous. All taxa are of the same growth form (perennial herbs with erect stem) and they have similar leaves, indumentum, capitula and flowers. These structures are therefore of little use as determination characters within the section.

The most important determination characters within the section Jacea include size, shape, and colour of appendages of involucral bracts, presence/absence of pappus of achenes, and shape of leaves (esp. length/width ratio). The section can be divided into several distinct species groups/aggregates, namely C. jacea agg., C. nigrescens agg., C. nigra agg., and C. phrygia agg., and few taxa that are intermediate between them. The aggregates are easy to recognize. However, within them often only minute quantitative features discriminate between taxa. The quantitative characters are highly variable and variation ranges of individual taxa overlap. The variation is also enhanced by hybridization and introgression. Some determination characters display certain level of morphological plasticity. They are influenced by light conditions, moisture and structure of the soil and also be management of a locality (esp. mowing). However, none of these problems is usually considered in literature, which only report small variation of diagnostic characters, resulting in ill-defined taxa and numerous misidentifications.

Polyploidy

Three ploidy levels are known within *Centaurea* sect. *Jacea*. Diploids and tetraploids are present within each species group. Intermediate triploid level rarely emerges in experimental crosses between diploids and tetraploids. Hybridization experiments have shown that the ploidy levels are reproductively well isolated. It means that it may be reasonable to distinguish taxa separated by chromosome numbers, even if there is little morphological differentiation.

Hybridization

Extensive hybridization occurs in the section *Jacea*. Numerous hybrids were described. In addition, some taxa originally described as non-hybrid proved to be hybrids, too. The hybrids are usually morphologically intermediate between the parental taxa. The extent of hybridization between particular taxa depends largely on their ploidy levels. Taxa of the same ploidy level can cross easily and their hybrids are often fertile and capable of backcrossing (formation of extensive hybrid swarms and introgression are possible). In opposite, taxa differing in ploidy levels hybridize rarely and their hybrids are almost sterile.

4. Methods

Morphometric analyses

Morphometric analyses were applied to (i) *C. jacea* agg. and (ii) *C. phrygia* agg. Samples of 25–30 individuals were collected in selected populations. The populations for analysis were selected to represent the whole range of morphological variation and all main areas of occurrence of respective group. The character set was chosen to include all characters used in determination keys and floras to distinguish the reported taxa. Several other characters were added based on field experience.

Correlation coefficients were calculated and subsequently, some selection of correlated characters expressing similar morphological features was done. Cluster analysis of populations and principal component analysis of populations and individuals were employed to provide an overall view of the structure present among the populations. Canonical discriminant analysis and classificatory discriminant analysis of both populations and individual were then used to test the groups ('taxa') defined on the basis of the cluster analysis and the principal component analyses.

Chromosome counting

Chromosome numbers were counted in root apices of seedlings that were germinated from the achenes collected *in situ*. At least three samples were analyzed for each population of *C. phrygia* agg. (the same populations as for morphometric analyses) and selected populations of *C. jacea* agg. A rapid squash method using lacto-propionic orcein as a stain was employed.

Flow cytometry

Flow cytometry was used to detect DNA ploidy level of offspring of hybridization experiments and of selected plants from populations studied by morphometric and karyological analyses. DNA ploidy levels were estimated using DAPI fluorochrome.

Hybridization experiments

Two experiments were carried out: (i) hybridization of *C. elatior* and *C. stenolepis* to confirm that these two taxa can cross and (ii) hybridization of *C. elatior* (diploid) and *C. jacea* (tetraploid) to study hybridization between the ploidy levels. Design of both experiments was similar. Three types of crosses were done within each experiment: (1) autogamy (control), (2) crossing within the same taxon (different individual), and (3) crossing between the taxa. Mature achenes were harvested and differences in seed-set between the individual types of crosses were tested.

Isozymes

Isozyme analysis was employed to check supposed hybrid origin of several population of *C. phrygia* agg. Five populations of *C. elatior*, three populations of *C. stenolepis*, and three populations thought to be their hybrids were studied. Six enzyme systems were studied. Basic population genetic parameters were computed for all populations and taxa, and differences among the taxa and populations were studied by analysis of molecular variance and principal coordinate analysis based on Nei's genetic distances.

Distribution of taxa

The distribution of individual taxa was estimated for the Czechia and Slovakia based on the revision of ca 3600 herbarium sheets from major Czech and Slovak public herbaria.

5. Centaurea jacea agg.

Centaurea jacea agg. is recognized by rounded, entire appendages of involucral bracts. Morphometric analysis of populations from Czechia, Slovakia and western Ukraine revealed that there are two groups of populations, which roughly correspond to the traditionally distinguished taxa C. jacea subsp. jacea and C. jacea subsp. angustifolia. However, about one quarter of the populations could not be assigned to any group with confidence. These populations are of more or less intermediate character.

Other three taxa reported in literature were not identified by morphometric analysis: (1) Centaurea weldeniana is an East-Central Mediterranean taxon, which was reported from the very south of Slovakia. It likely never occurred there and the records are based on wrong determination of atypical C. jacea populations. Surprisingly, one population of C. weldeniana of most probably adventive origin was found near Vienna. (2) Centaurea bracteata was once published as alien from Czechia. However, voucher herbarium specimens were not found and this record is therefore doubtful. (3) Another taxon from C. jacea agg. was described from salty sites in southern Slovakia under an invalid name Jacea tomentosa. Although three populations from

salty sites of southern Slovakia were included in the morphometric analysis, no of them corresponded to *Jacea tomentosa*. Specimens corresponding to *Jacea tomentosa* from South-Slovakian salty sites were even not found in herbaria. In opposite, individuals resembling *Jacea tomentosa* as described in literature can occasionally be found in some otherwise normal populations of *C. jacea* from not salty sites. It can therefore be concluded that taxon *Jacea tomentosa* does probably not exist at all.

6. Centaurea subjacea

Centaurea subjacea is included at the specific or subspecific rank (C. jacea subsp. subjacea) in the majority of Central European floras and determination keys. It is usually described as a plant similar to C. jacea, from which it should differ by fimbriate appendages of involucral bracts. Surprisingly, no such taxon was found during revision of Centaurea sect. Jacea in Czechia and Slovakia. All specimens studied in herbaria that were determined as C. subjacea by their collectors belong to other taxa or to hybrids between C. jacea and other taxa of the section. Similar situation is also in Austria, where the taxon was described from.

The original description and type material were therefore checked. It seems that part of original description was overlooked by later authors and descriptions of the taxon in modern floras differ from the original one. The shape on appendages of involucral bracts referred in original description fits on hybrids between *C. jacea* and other taxa of the section very well. The search for original herbarium material yielded three syntypes. All of them are hybrids for sure, the most probably *C. jacea* × *C. macroptilon*. In conclusion, *Centaurea subjacea* does not exist as an autonomous non-hybrid taxon and should be excluded from Central European flora.

7. Centaurea phrygia agg.

Centaurea phrygia agg. is morphologically characterized by a special shape of appendages of involucral bracts. They are ovate to linear with a pectinate-fimbriate margin, attenuated into a filiform, laterally fimbriate acumen; terminal fimbriae are longer than lateral and the appendages are recurved from the involucre in the upper part forming a "sheath" around the involucre. The pappus of achenes is always present and is usually about 1 mm long. Up to six taxa are reported in literature from Central Europe, including both diploids and tetraploids.

Karyological analysis

Both diploids (2n=22) and tetraploids (2n=44) were identified among the studied populations of *C. phrygia* agg., but no population with the co-occurrence of both ploidy levels was found. The ploidy level generally agreed with literature and also with results of morphometric analysis. In three populations single individuals with triploid chromosome numbers were found. They possibly originated from hybridization of diploid and tetraploid plants (of other species groups), which occurs in the surroundings.

Morphometric analyses

Five taxa were identified by morphometric analysis: *C. phrygia* s.str. (diploids and tetraploids), *C. elatior* (= *C. pseudophrygia* auct.) (diploids), *C. stenolepis* (diploids), *C. carpatica* (tetraploids), and *C. oxylepis* (tetraploids; this taxon is usually not considered within *C. phrygia* agg., but was included into the analysis for its morphhological similarity). Another taxon,

C. indurata (diploid), was found in the field, but only one population is known from the studied area and could not be included in statistical analyses. Beside typical populations three groups of atypical populations of probably hybrid origin were found: populations intermediate between (i) C. elatior and C. stenolepis, (ii) C. oxylepis and C. phrygia s.str., and (iii) C. elatior and C. phrygia s.str.

The most surprising result is identification of a new taxon for Central Europe: Centaurea carpatica. It was considered endemic of the East Carpathians (Romania). However, the populations from Slovakia are morphologically very close to the Romanian plants and they also do not differ in ecology (occurrence above timberline, the only within C. phrygia agg.).

Two taxa from six reported in literature were not identified in the studied material: C. melanocalathia and C. nigriceps. Single individuals that have some characters of C. melanocalathia as described by recent floras occur in populations of C. phrygia s.str., but no population corresponding to C. melanocalathia was found. It therefore seemed that this taxon does not exist. Search for a type of the name C. melanocalathia has shown that the name was misinterpreted by later authors and the it belongs to a hybrid C. jacea × C. phrygia s. str. Centaurea nigriceps is a very unclear taxon. Its description is based on single herbarium specimen from the East Carpathians (Ukraine). Six populations from the Ukrainian East Carpathians were studied in morphometric analysis. However, they did not substantially differ from populations of C. phrygia s.str. from other areas. It therefore seems that C. nigriceps might not be separate taxon and the name might be included into the synonymy of C. phrygia s.str.

Centaurea phrygia s.str. is the only species studied for which both cytotypes are reported. Nevertheless, the cytotypes seem to be separated geographically: the tetraploids are confined to mountains of the West Carpathians and neighbouring the East Sudetes, while diploids occur in the rest of the species area; both cytotypes meets only in a small area in the West Carpathians. Discriminant analysis focused on separation of the cytotypes of C. phrygia s. str. was therefore computed. It has shown that they are well separated at population level. At individual level there is also considerable separation, though not complete. In conclusion, these results indicate that it is possible to treat the cytotypes as separate taxa, the subspecific levels seem to be the most appropriate.

Distribution of the taxa

Distribution of individual taxa was estimated based on own collections and revision of ca 3600 herbarium sheets in 24 Czech and Slovak public herbaria and several private herbaria. The main differences from distributions reported in literature include: (i) absence of *C. elatior* in the West Carpathians and northern part of the East Carpathians, (ii) absence of *C. phrygia* s. str. in Bohemia except for northernmost part, (iii) occurrence of *C. oxylepis* in southern Slovakia.

Nomenclature

For naming of newly recognized subspecies of *C. phrygia* the interpretation of the name *C. phrygia* L. is crucial. This name was not typified yet. Since the end of 19th it is widely accepted for a taxon occurring in north and east part of Europe. The lectotype of *C. phrygia* L. should be selected to maintain the current use of the name.

The north-European plants should be named C. phrygia subsp. phrygia. They are identical with the diploid cytotype/subspecies involved in my morphometric study. A name is needed for the newly recognized tetraploid subspecies. Revision of available names has shown that the only name that can be adopted for this taxon is C. erdneri J. WAGNER. New combination is therefore proposed: Centaurea phrygia subsp. erdneri (J. WAGNER) KOUTECKÝ.

The name Centaurea carpatica (PORCIUS) PORCIUS is widely accepted for a taxon from C. phrygia agg.. However, this name is younger homonym of a name C. carpatica GENERS. and it is therefore illegitimate. The name C. carpatica GENERS. probably belong to some taxon from the genus Cyanus and is virtually unknown and was never used. The only name to replace C. carpatica (PORCIUS) PORCIUS is C. rodnensis SIMONK., which has virtually fallen to oblivion. It seems that best solution to stabilize the nomenclature is conservation of the name C. carpatica (PORCIUS) PORCIUS and rejection of the Genersich's name.

The name Centaurea elatior (GAUD.) HAYEK is usually considered as a synonym of C. pseudophrygia C. A. MEYER ex RUPRECHT. The first name is based on plants from Switzerland; the latter is believed to be based on plants from South-West Russia. Small morphological differences are reported between plants from East and Central Europe and ecological and phytogeographical differences between the areas also suggest taxonomical differentiation. Therefore, it seems reasonable to treat Central European populations as separate taxon, for which the name C. elatior is correct, while the name C. pseudophrygia is to be used for East European plants. However, the problem requires further study. It is not clear if the name C. pseudophrygia C. A. MEYER ex RUPRECHT was published validly and if so, what its type should be. It cannot be even ruled out, that it will be necessary to typify this name by Central European plants and to find new name for the East European plants.

8. Other taxa of the section Jacea

Centaurea macroptilon and C. oxylepis

These two taxa differ in geographic distribution, but they are very similar morphologically and only minute quantitative differences between them are reported. Their morphological differentiation is unclear and should be revised. Both taxa are tetraploid and their origin is unknown. Morphologically they stand between C. jacea agg. and C. phrygia agg. and their hybrid (allopolyploid) origin can therefore be hypothesized and should be tested.

Centaurea nigrescens agg.

There are up to seven recognized within C. nigrescens agg. Majority of them are reported as native from South Europe. Both diploids and tetraploids are known, for some taxa chromosome counts are unknown. Three taxa are sometimes introduced to Central Europe north of their native distribution areas: C. nigrescens s. str. (ca 30 occurrences in Czechia and Slovakia over last 100 years), C. carniolica (1 occurrence), and C. transalpina (1 occurrence).

Centaurea nigra agg.

Centaurea nigra agg. has its distribution centre in West Europe. In Flora Europaea eight taxa are recognized within C. nigra agg.; both diploids and tetraploids are known. The variation of C. nigra agg. is probably increased by hybridization with C. jacea agg.; morphological plasticity seem to be important, too.

9. Hybrids

Hybridization is one of the main sources of variation and taxonomic and determination problems in Centaurea sect. Jacea. Thirteen hybrids within Centaurea sect. Jacea, two of which are triple hybrids, were identified with certainty in Central Europe. Three other hybrid combinations are likely to occur, though were not found neither in the field nor in herbaria. Two hybrids were also found between members of the section Jacea and section Acrolophus (C. jacea x C. stoebe, and C. oxylepis × C. stoebe). The hybrids in the section Jacea are generally intermediate between their parents.

10. Introgression in C. phrygia agg.

Populations exist within C. phrygia agg., which are morphologically intermediate between two taxa. Three types of the intermediate populations were identified; (i) between C. phrygia s.str. and C. oxylepis, (ii) between C. elatior and C. stenolepis, and (iii) between C. elatior and C. phrygia s.str. Variation within each particular intermediate population is similar to the variation within typical populations of either species but there is considerable variation among the intermediate populations. They differ slightly from one another, forming a more or less continuous transition from one species to the other, each of the intermediate populations being one "step". It is probable that this pattern originated from introgressive hybridization.

Apart from morphological variation, there are other facts that seem to accord with hybrid origin of the intermediate populations: (i) pattern of ploidy levels (always the same in putative hybrid and presumed parents), (ii) geographic distribution of the intermediate populations on a contacts of distributions of putative parents, (iii) intermediate position of the intermediate populations on climatic/altitudinal gradient "between" the putative parents.

The diploid taxa C. elatior and C. stenolepis and populations intermediate between them were studied more in detail. Crossing experiment confirmed that the two species can hybridize. Genetic variation of selected populations was studied using isozymes. There were no qualitative differences among the parental species, except for rare alleles. Quantitative differences in allele frequencies between the species were quite small, too; the largest part of variation was within populations. The results therefore do not support with certainty the presumed hybrid origin of intermediate populations, but they are certainly not in conflict with it.

Nomenclature of the hybrids was studied. The plants intermediate between C. elatior and C. stenolepis should be called Centaurea xcastriferrei BORBÁS et WAISB. For the other two hybrids no binomials are available.

11. Hybridization between ploidy levels

Previous studies of hybridization within the section *Jacea* have demonstrated strong reproductive barrier between diploids and tetraploids: hybridization of taxa sharing the same chromosome number is frequent and the hybrids are generally fertile, whereas hybridization between the ploidy levels is rare and the hybrids are usually sterile. In crossing experiments triploid hybrids between diploids and tetraploids formed by union of reduced gametes rarely appeared, but they were never found as mature plants in natural populations. Rare occurrence of unreduced gametes was also rarely detected in experiments (tetraploid progeny of diploid–tetraploid crosses).

Surprisingly, one mixed populations of *C. elatior* (diploid) and *C. jacea* (tetraploid) containing hybrids was found in Czechia. The hybrids in the population are highly fertile it also seems that the hybrids back-cross with *C. jacea*. However, it should not be possible theoretically. The population was therefore examined more in detail. Analysis of DNA ploidy levels by flow cytometry has shown that all plants of *C. elatior* were diploid and all plants of *C. jacea* were tetraploid (as expected) and all hybrids were tetraploid. It means that unreduced gametes from diploid had to be involved in their formation. The fact that hybrids are tetraploid could explain observed fertility of hybrids and possible back-crossing of the hybrid with tetraploid *C. jacea*.

Pilot hybridization experiment confirmed reproductive barrier between the ploidy levels: the seed set in inter-ploidy crosses was about 5-times reduced in comparison to within-ploidy crosses. Flow cytometric analysis of progeny of the inter-ploidy crosses has shown that hybrids were formed both by fusion of two reduced gametes (triploids) and by fusion of unreduced gamete of diploid and reduced gamete of tetraploid (tetraploids). Tetraploid hybrids were about 4-times more common than triploid hybrids. No higher ploidy level was detected, i.e. unreduced gametes of tetraploid were not involved. Cultivation of a part of the progeny from the experiment suggests that morphology of the progeny may depend on the mother plant – it seems that it is shifted towards the mother plant instead of being exactly intermediate. The hybrids C. elatior $Q \times C$. jacea C are of particular interest: they are usually tetraploid and they are sometimes morphologically similar to a taxon C. oxylepis, which is morphologically "intermediate" between C. jacea agg. and C. phrygia agg. and its hybrid origin is hypothesized.

The main difference between the natural population and the experiment is very rare occurrence of triploids in the former (only a single triploid seeds, no mature plants). It can be caused by competition of pollen. In an experiment pollen of one taxon/ploidy level is only delivered on particular capitulum/floret and pollination by this pollen or autogamy is only possible. In opposite, in a large mixed population probably mixture of different pollen is delivered by insects, which allows to "choose" pollen of "correct" ploidy level/taxon for pollination.

12. Conclusions

In the last chapter determination key to the taxa is compiled and information on individual taxa (description, nomenclature, distribution) is summarised. Unresolved subjects requiring further study are listed.

Curriculum vitae

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· Education and qualifications:

1997–2000: University of South Bohemia, Faculty of Biological Sciences, bachelor study; Bc. Thesis: Regeneration of meadows in the Morava River floodplain after catastrophic flooding in 1997

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- Employment: since November 2004 University of South Bohemia, Faculty of Biological Sciences, Department of Botany researcher (part time), since 2007 full-time
- Teaching: Biostatistics (practical lessons), Systematics of higher plants (practical lessons),
 Determination course of vascular plants, various botanical excursions (incl. abroad), supervisor
 of diploma theses of undergraduate students, one of the authors of 'Biological Olympiad' (a
 competition in biology for secondary school students).
- Research activities: Taxonomy of vascular plants, floristics, nature conservation. Ruderal flora
 of villages and its changes and relation to environmental factors. Water and wetlands plants.
 Vegetation mapping and floristic inventory and monitoring in protected areas. Determination
 and preparation of plants for the herbarium of Faculty of Sciences.
- Membership in scientific associations: Czech botanical society (since 1997)

· Conferences:

12. Österreichisches Botanikertreffen, Kremsmünster, Austria (2006)

Hybridization in *Centaurea* sect. *Jacea*: *Centaurea subjacea* and other hybrids from Austria (lecture); Factors affecting synanthropic flora of villages in Blanský les, South Bohemia (poster; together with J. Těsitel, F. Kolář, and M. Kubešová)

Conference of the Czech Botanical Society, Prague, Czechia (2006)

Water and wetland plants of the district of Karviná (poster; in Czech; together with V. Koutecká and T. Koutecký).

XVIIth International Botanical Congress, Vienna, Austria (2005)

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