

University of South Bohemia in České Budějovice

Faculty of Science



Diversity, variability and distribution of polyploid groups of ferns in Central Europe

Diverzita, variabilita a rozšíření polyploidních skupin kapradin ve střední Evropě

Summary of PhD. Thesis

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Annotation

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Diversity, morphological and cytogeographic variability and distribution of polyploid groups of ferns in the central part of Europe (especially in the Czech Republic) were examined. Particularly taxonomical critical taxa of genera Asplenium and Dryopteris were investigated in a more detailed. Cytotaxonomical variation (estimation DNA ploidy level and genome size) was studied using the methods of flow cytometry. Consequential study of morphological variation was investigated by multivariate morphometric analyses. Significance of individual morphological characters for the determination of species complexes is evaluated and some determination keys was compiled as same as the treatment of some taxa/groups to the local floras/identification keys (Czech Republic, Slowakia, Austria) is presented. Distribution of particular taxa in the Czech Republic was studied based on of revised herbarium specimens and own field research. New taxa for the Czech republic/Bohemia were recently confirmed (Dryopteris remota, D. cambrensis) during this study.

Key words:

Asplenium, Central Europe, Czech Republic, distribution, DNA ploidy level, Dryopteris, ferns, flow cytometry, genome size, multivariate morphometrics, Pteridophyta, taxonomy

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I would like to give the warmest thank to my wife as same as my parents and mother-in-law for their understanding and patience to my continual running to ferns. Despite they did not know, why are ferns so important they always remembered me that not only ferns are part of the life.

Introduction

Systematic research based on cytological examination (number of chromosomes, pairing of bivalents etc.) in ferns was started in Europe by Manton (1950), who first reported on complexity of fern polyploid groups. After this, number of other studies has followed. Conventional chromosome counts provided robust evidence for heteroploid hybridization, while studies of chromosome pairing during meiosis in both natural and artificial hybrids shed some light on species relationships (Manton & Walker 1953, Walker 1955, 1961). In Europe, systematic research in majority of large fern genera, such as *Asplenium*, *Cheilanthes*, *Dryopteris* or *Polystichum*, was conducted simultaneously with study of mechanisms and modes of sexual and asexual speciation (see paragraphs above). Species varying not only in their morphology but also in ploidy level and evolutionary history were recognized notably in species rich genera of *Asplenium* and *Dryopteris* (Lovis

1973, Fraser-Jenkins 1980, Gibby 1983, Dostál et al. 1984, Reichstein 1984, Fraser-Jenkins 1986, 1987, 1993, Viane et al. 1993, Frey et al. 2006, Fraser-Jenkins 2007).

There has been strong tradition of research in the field of cytology, chemotaxonomy and distribution of critical fern groups in North, West or South Europe and Macaronesia (Widén et al. 1967, Sorsa & Widén 1968; Widén & Sorsa 1968, Widén et al. 1970, Brownsey 1976, Gibby & Walker 1977, Piękoś-Mirkowa 1979, Nyhus 1987, Benl & Eschelmüller 1983, Gibby 1983, Bennert & Fischer 1993, Steinecke & Bennert 1993). However, these taxonomical studies have been mostly based on restricted datasets and modern formalized statistical methods have not been used for data analysing. On the other hand, large number of species/groups have not yet been studied and distinguished in some countries of Central and East Europe, e.g. in the Czech Republic, Slovakia, Poland or Romania (Hejný & Slavík 1988, Mirek et al. 1995, Marhold & Hindák 1998, Ciocârlan 2000, Kubát et al. 2002).

Important and excellent studies of last decade are focused on genetic structure of populations, migration routes and reticulate evolution, notably in *Asplenium* genus, using chloroplast DNA markers (Vogel et al. 1996, 1998, 1999a, 1999b, 1999c, Trewick et al. 2002).

This PhD thesis deals with systematics of selected fern groups in Central Europe. The general aim was to extend knowledge from those parts of Europe, where similar issues were intensively studied. This thesis brings formalized taxonomic revision of some fern groups from cytological and morphological point of view. In comparison to antecedent studies, large datasets were usually analysed. In addition, presented studies widely use methods of flow-cytometry, indicating usefulness of these methods in systematic research of ferns. For comprehensive overview of problems in particular taxonomic groups see introductions of the papers presented thereinafter.

Aims of the thesis

- Study of taxonomically critical groups of ferns primarily from genera Asplenium and Dryopteris in Central Europe
- Applications of flow cytometry to fern systematics
- Can genome size be used as an informative marker for taxonomic decision-making?
- What is the level of genome size variation in the group?
- Evaluation of morphological variability of selected groups based on multivariate morphological analysis
- What are the species/hybrid-specific morphological characters?
- Study of distribution of taxonomically critical fern taxa in the Czech Republic

What is the abundance and distribution of particular species/hybrids in the area studied?

Abstracts

Paper 1: A morphometric study and revision of the Asplenium trichomanes group in the Czech Republic

Ekrt L. & Štech M. (2008) Preslia 80(3): 325-347.

A detailed cytogeographic and morphometric study of the Asplenium trichomanes group in the Czech Republic is presented. We detected diploid (2n = 72), tetraploid (2n = 144) and hybrid triploid plants (2n = 108). Based on the morphometric study, four intraspecific taxa are recognized. These taxa correspond to the four subspecies of A. trichomanes $(A.\ t.\ subsp.\ trichomanes,\ A.\ t.\ subsp.\ quadrivalens,\ A.\ t.\ subsp.\ pachyrachis\ and\ A.\ t.\ subsp.\ hastatum)$ distinguished in the floras of western, southern and northern Europe. Triploid plants were determined as A. t. nothosubsp. lusaticum $(A.\ t.\ subsp.\ trichomanes \times A.\ t.\ subsp.\ quadrivalens)$. The individual morphological characters used for determining subspecies are evaluated and a determination key presented.

Paper 2: Rozšíření a problematika taxonů skupiny Asplenium trichomanes v České republice [Distribution and problematic of taxa of Asplenium trichomanes group in the Czech Republic]

Ekrt L. (2008) Zprávy České Botanické Společnosti 43(1): 17-65.

The distribution of taxa of the Asplenium trichomanes group in the Czech Republic was studied. Collections of 32 public herbaria were visited and a total of 1477 specimens examined. The four taxa A. trichomanes subsp. trichomanes, A. trichomanes subsp. quadrivalens, A. trichomanes subsp. pachyrachis, A. trichomanes subsp. hastatum and four hybrid combinations were recorded from the Czech Republic. An overview of morphological characters, distribution maps and a brief summary of habitat preferences and total distribution of the taxa are presented.

Paper 3: Asplenium trichomanes. - In: Online-Flora von Österreich

Ekrt L. (2007–2009) In: Fischer M. A., Willner W., Niklfeld H. & Gutermann W. [eds]

http://flora.vinca.at, http://f

[paper without abstract; compilation of determination key of Asplenium trichomanes group for Austrian Flora]

Paper 4: Asplenium trichomanes. – In: Určovací kľúč paprad'orastov a semenných rastlín Slovenska [Identification key of ferns and flowering plants of

the Slovak Republic]

Ekrt L. In: Marhold K., Feráková V., Goliašová K., Grulich V., Hodálová I., Hrouda L., Kochjarová J., Mártonfi P., Mereďa P. jun. [eds], VEDA, Bratislava [submitted]

[paper without abstract; compilation of determination key of Asplenium trichomanes group for Slovak Flora]

Paper 5: Revize rozšíření sleziníku střídavolistého (Asplenium ×alternifolium) v České republice [Revision of geographical distribution of Asplenium ×alternifolium in the Czech Republic]

Ekrt L. (2008) Zprávy České Botanické Společnosti 43(2): 231–250.

The distribution of Asplenium × alternifolium nothosubsp. alternifolium and Asplenium × alternifolium nothosubsp. heufleri was studied in the Czech Republic. A revision of 33 public herbarium collections was carried out and a total of 442 specimens were examined. Asplenium × alternifolium nothosubsp. alternifolium was recorded scattered throughout the Czech Republic. The studied taxon occurs mainly on siliceous rocks, only occasionally (1 % of known localities) was recorded from secondary habitats (walls). Therefore, the taxon is missing in the Polabí lowland and in the south, east and northeast of Moravia. Distribution dot maps, a list of recorded localities and overview of habitat preferences are presented. According to field knowledge and herbarium revisions it seems that recent localities are much rarer than historical ones. Asplenium x alternifolium nothosubsp. heufleri is very rare on the territory of the Czech Republic. The historical localities in Josefovské údolí valley near the town of Adamov, and in valleys near the village of Kamenný Újezd and the village of Černolice near Jíloviště town, and at Staré vinice near the town of Znojmo have not been verified recently. Two new localities were discovered and revised recently in the field. One is situated in castle ruins at Litice nad Orlicí in the .ambersko region and the other one on rocks above the Vltava river near the town of Kamýk nad Vltavou in the Střední Povltaví region.

Paper 6: Species boundaries and frequency of hybridization in the *Dryopteris* carthusiana (Dryopteridaceae) complex: a taxonomic puzzle resolved using genome size data

Ekrt L., Holubová R., Trávníček P. & Suda J. [submitted to *American Journal of Botany*]

Dryopteris carthusiana agg. is a taxonomically intricate group of temperate ferns composed of one diploid (D. expansa) and two allotetraploid (D. carthusiana and D. dilatata) species in Central Europe. Overall phenotypic similarity, large plasticity, and the incidence of putative interspecific hybrids have led to a continuous dispute concerning species circumscription and delimitation. We used

flow cytometry and multivariate morphometrics to assess the level of phenotypic variation and the frequency of hybridization in a representative set of Central European samples covering all recognized species and hybrids. Flow cytometric measurements revealed unique genome sizes in all species/hybrids, allowing easy and reliable (notho)taxa identification. A set of taxonomically informative morphological characters was then selected based on the results of morphometric analyses. While determination of D. carthusiana usually did not pose much of a problem, differentiation between D. expansa and D. dilatata was more challenging. Different species often formed mixed populations, providing opportunity for interspecific hybridization. The frequency of particular hybrid combinations differed dramatically and depended primarily on evolutionary relationships whereas ploidy level had only a little effect. Collectively, our study introduces a new and robust character (genome size) for taxonomic decisionmaking in the D. carthusiana complex, and thus represents a significant step forward in resolving taxonomic complexities in this important component of the temperate fern flora.

Paper 7: Genome size and morphology of the *Dryopteris affinis* group in Central Europe

Ekrt L., Trávníček P., Jarolímová V., Vít P. & Urfus T. [Preslia – accepted]

The agamosporous and taxonomically critical *Dryopteris affinis* group was investigated as part of a cytogeographic and morphometric study of ferns in Central Europe. Material from 27 localities in the Czech Republic, Slovakia, Poland and Austria was sampled and evaluated using both morphometric multivariate and karyological approaches. Chromosome counts and flow cytometric analyses revealed the existence of two distinct triploid taxa (2n = 123) of differing genome size, which correspond to *D. borreri* and *D. cambrensis*, and of a rare pentaploid hybrid (2n = 205) *D.* ×critica $(D. borreri \times D. filix-mas)$. Morphometric analyses confirmed a clear separation between both triploid taxa. New quantitative characters were selected according to the results of discriminant analyses, and a key to their identification is presented.

Paper 8: Rozšíření a taxonomická problematika skupiny *Dryopteris affinis* v České republice [Distribution and taxonomical problems within *Dryopteris affinis* group in the Czech Republic]

Ekrt L., Štech M., Lepší M. & Boublík K. [submitted to *Zprávy České Botanické Společnosti*]

The distribution of taxa of *Dryopteris affinis* group in the Czech Republic was studied. Collections of 21 Czech public herbarium were revised and a total of 257 specimens was examined. Two species *D. borreri* and *D. cambrensis* and one hybrid *D.* \times critica (*D. borreri* \times *D. filix-mas*) were recorded in the Czech Republic. *D. cambrensis* was recently rediscovered after more than 30 years for

the flora of the Czech Republic and recently was found as a new species for Bohemia. 16 localities of rare *D. cambrensis* are known for the Czech Republic. A single locality of *D. cambrensis* was found in Poland in surroundings of village of Pstrążna near border with the Czech Republic. Review of the morphological characters, distribution maps and review of habitat preferences, total distribution of the taxa and determination key is presented.

Paper 9: Dryopteris remota rediscovered for the flora of the Czech Republic

Ekrt L., Lepší M., Boublík K. & Lepší P. (2007) Preslia 79: 69-82.

Until now, Dryopteris remota was only recorded in the Czech Republic from the Moravian Karst, ca 70 years ago. This record is mentioned in some studies, but references to the data's origin have always been missing. For this reason itwas uncertain whether D. remota was still present in the Czech Republic. Recently, the records from the Moravian Karst were verified by re-examination of original herbarium specimens. In 2002 a specimen of D. remota was found for the first time in Bohemia, close to the village of Ktiš, on a slope of Malý Plešný hill in the foothills of the Bohemian Forest (S Bohemia). At this locality only one plant occurred on the boundary between Lonicera nigra-shrub and spruce-beech-fir forest, on a gneiss outcrop. Determination of the Czech specimens of D. remota was based on comparisons with macro- and micromorphological characters of both Alpine (Upper Austria) and Carpathian (West Ukraine) specimens, as well as descriptions in the literature. A detailed morphological description and comparison with similar taxa are included. A map of its distribution within the Czech Republic as well as a map of the distribution of D. remota worldwide is also presented. It is suggested that D. remota be designated a critically endangered plant species in the Czech Republic.

Paper 10: Asplenium trichomanes L. – sleziník červený, Dryopteris affinis agg. – kaprad' rezavá, Dryopteris remota (A. Braun ex Döll) Druce – kaprad' tuhá, Trichomanes L. – vláskatec in Květena ČR – dodatek [Flora of the Czech Republic – Additamenta]

Ekrt L. & Štech M. In: Štěpánková J. [ed.], Academia, Praha [submitted] [paper without abstract; compilation of morphology, variation, habitat preferences and distribution of selected taxa for the Flora of the Czech Republic]

Conclusions

Diversity and variability of several Central European fern groups from the genera Asplenium and Dryopteris were evaluated. Especially more detailed taxonomical revisions of following groups were carried out: Asplenium trichomanes group, Dryopteris carthusiana group and Dryopteris affinis agg. (Papers 1, 6, 7). Flow cytometry was found to be a powerful tool for delimitation of particular taxa in all the study cases. It should be emphasized that detection of different genome

size was successful not only between representatives of different ploidy levels (Paper 1, 6) but also between taxa of the same ploidy level (Paper 6, 7). Delimitation of studied taxa and detection of hybrids is a step necessary for consequent successful morphological definition of taxa.

Based on cytometrical screening of larger population samples from *Dryopteris carthusiana* group, unexpectedly high frequency of hybridization between *D. dilatata* and *D. expansa* was revealed, whereas relatively low frequency of hybridization between *D. carthusiana* and *D. dilatata* was found (Paper 6). Surprisingly low frequency of hybridization was found in *D. affinis* group. The pentaploid hybrids were rarely found at three localities only (Paper 7). A hypothesis – the frequency of particular hybrid combinations (among sexually reproducing taxa) differed dramatically and depended primarily on evolutionary relationships whereas ploidy level had only a little effect is presented.

First chromosome counts from the Czech Republic are presented for Asplenium trichomanes subsp. trichomanes, Dryopteris expansa, Dryopteris borreri, Dryopteris cambrensis and Dryopteris filix-mas (Papers 1, 6, 7).

The particular papers identify the boundaries between studied taxa within the studied groups to find the best diagnostic characters based on multivariate morphometrical analyses. Finally a determination keys were compiled (Paper 1, 6, 7). Estimation of the frequency of hybridization within the study groups in Central Europe (the Czech Republic and adjacent countries) was carry out. In the case of *Asplenium trichomanes* group, determination key was compiled for the local floras/identification keys of the Czech Republic (Paper 9), Austria (Paper 3) and Slovakia (Paper 4).

Rare fern species were newly revealed or rediscovered after a longer time for the flora of the Czech Republic. *Dryopteris remota* was recognized after more than 70 year in the Czech Republic (Paper 8). *Dryopteris cambrensis* was discovered as a new taxon for Bohemia and rediscovered for the Czech Republic after more than 30 years. This taxon still has not been included in the Flora of the Czech Republic/Key to the flora of the Czech Republic; however, its occurence in the Czech Republic is generally known from other European compilations on *Dryopteris* by C. Fraser-Jenkins. Finally, recent rediscovery of *Asplenium* ×alternifolium nothosubsp. heufleri was revealed in several localities (Paper 5).

Overall distribution of some taxonomically critical fern taxa in the Czech Republic was compiled, based on study of herbarium specimens and field research (Paper 2, 5, 8). A map of the worldwide distribution of *Dryopteris remota* is presented for the first time (Paper 8).

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Professional Curriculum Vitae

Personal data

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2001 Landscape Management Services, The Groundskeeper Corp., Tucson, Arizona, USA 2002–2004 Natura 2000 habitat mapping, Agency of Nature Protection of the Czech Republic, Prague.

2003–2004 Administration of the Broumovsko Protected Landscape Area (part-time-job)

2005–2009 Research and Nature Protection Department, Šumava National Park (parttime-job)

since 2007 Natura 2000 habitat updating, Agency of Nature Protection of the Czech Republic, Prague.

Publication

Papers in journals with impact factor

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