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

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PhD. thesis

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

Diverzita, variabilita a rozšíření polyploidních skupin kapradin ve střední Evropě

PhD. Thesis
(shortened electronic version)

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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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Declaration – Prohlášení

I hereby declare that this PhD. thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning except where due acknowledgements has been made in the text.

I declare that in accordance wit the Czech legal code § 47b law No. 111/1998 in its valid version, I consent to the publication of my PhD. thesis (in an edition made by removing marked parts archived by the Faculty of Science) in an electronic way in the public access to the STAG database run by the University of South Bohemia in České Budějovice on its web pages.

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“Nature formed ferns only like leaves to demonstrate its skills in this field.”

“Příroda vytvořila kapradiny jako pouhé listí, aby ukázala, co v tomto oboru dovede.”

H. D. Thoreau

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Author contribution statement

Libor Ekrt, author of this PhD. thesis, is the first author of all papers (manuscripts) and wrote the substantial part of them. Major part of the field sampling and raw data processing as well as majority of the statistical analyses and discussions the results with literature were performed by him. Pavel Trávníček performed important part of flow cytometric analyses, interpretation and data processing in Paper 6 (contribution of 25% of work). Renata Holubová collected and measured important part of *Dryopteris* samples in Paper 6 (contribution of c. 25% of work). Contributions of other co-authors do not exceed more than 20% of work on other presented papers. All co-authors hereby consent to the publication of the papers in the PhD. thesis of Libor Ekrt and support it by their signatures:

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Thesis content

General introduction

What are ferns – diversity and phylogenetic background of extant ferns

Recent phylogenetic analyses revealed two main lineages of vascular plants – lycophytes (*Lycopodiophyta*) and euphyllophytes (*Euphyllophyta*), which includes all other vascular plants (Kenrick et Crane 1997). Two monophyletic groups were distinguished in this clade recently (Pryer 2001). Monilophyta[1], which includes all non-seed-producing lineages of *Euphyllophyta* and are named often as ferns s. l. today, are sister to seed plants. Extant members of monilophytes (more than 11 500 species) belong to five major lineages. Most basal, small and old-time clade is represented by whisk ferns (*Psilotales*) and ophioglossoid ferns (*Ophioglossales*), which form a group *Psilotopsida.* Relationships among remaining three monophyletic clades are still not clearly resolved. While leptosporangiate ferns (*Polypodiopsida*) is probably the most derived group, positions of marattioid ferns (*Marattiopsida*) and horsetails (*Equisetopsida*) are rather ambiguous (Pryer et al. 2001, 2004, Smith et al. 2006, Schuettpelz et al. 2006). The leptosporangiate ferns are highly diversified and species-rich group which represent ca 3% of the total plant species richness in the world and they are the second most diverse group of vascular plants (after angiosperms), including about 11 000 species (Smith et al. 2006). Orders *Osmundales*, *Hymenophyllales*, *Schizeales* and *Gleicheniales* represents basal lineages of leptosporangiate ferns, while a group of „core leptosporangiates“ includes heterosporous (water) ferns (*Salviniales*), tree ferns (*Cyatheales*), and polypods (*Polypodiales*), each of which is clearly monophyletic (Smith et al. 2006). Polypods comprise from 15–30 families (depending on the classification followed) and account for more than 80% of extant fern species diversity (Pryer et al. 2001, 2004).

[1] The monilophytes (*Monilophyta*) share a distinctive vasculature, having protoxylem confined to lobes of the xylem strand, therefore the latin *moniliformis* appellation for „necklace-like“. Monophyly of this clade has been inferred from cladistic analyses of morphology including fossil taxa (Kenrick & Crane, 1997), studies of sperm ultrastructure (Renzaglia et al. 2001, 2002), and analyses of DNA sequence data (Nickrent et al. 2000; Pryer et al. 2001).

Arise of ferns in shadow of angiosperms through history

Diversity and distribution of ferns during the historical times was always an important question and source of speculations and theories. Over the course of some 80 million years during the Cretaceous period (i.e. from 145.5 Ma to 65.5 Ma; Gradstein et al. 2004), the Earth's vegetation changed dramatically from a landscape populated by gymnosperms and seed-free vascular plants to one dominated by angiosperms. As flowering plants rose to dominance, other vascular plant lineages were largely sidelined, if not driven completely to extinction. This concept lead to widespread opinion that ferns, as the most abundant part of terrestrial ecosystems, were surpassed in competition by angiosperms and recently represent relicts from late paleozoicum or early mezozoicum (Crane et al. 1995, Lupia et al. 1999, Nagalingum et al. 2002).

Leptosporangiate ferns originated over 300 million years ago well before the evolution of angiosperms and, based on the fossil record, are thought to have undergone three successive radiations (Lovis 1977, Rothwell 1987). The first radiation occurred in the Carboniferous and gave rise to several now-extinct families. The second radiation took place in the late Paleozoic and early Mesozoic, resulting in several families with extant representatives. The third radiation has been running since the Cretaceous and has been still active – particularly within the so-called polypod fern clade. Main diversification within the polypod clade actually took place in the Late Cretaceous and Cenozoic after emergence of angiosperms. Subsequently, integration of data on fossil and living representatives confirmed that the third radiation was the most important in relation to recent fern flora (Pryer et al. 2004, Schneider et al. 2004), giving arise to so-called polypods – modern types of recent ferns. Due to great diversification rate, species richness of recent polypods exceeded many times those of other lineages of ferns and lycophytes both on the world-wide and regional scale (see tab. 1).

This relatively recent diversification was not restricted to polypods, but was also evident in several of the early diverging leptosporangiate orders (Pryer et al. 2004). This suggests that the remarkable diversity of leptosporangiate ferns is not simply the result of their ability to survive after expansion of flowering plants. Instead, it is probable that ferns were able to somehow capitalize dominance of angiosperms.

One rather plausible explanation for the success of leptosporangiate ferns involves an ecological opportunistic response. The proliferation of angiosperms across the landscape, and the ensuing establishment of more complex ecosystems, undoubtedly resulted in the formation of a plethora of new niches into which leptosporangiate ferns could penetrate. Although many of these novel ecospaces were evidently on shady forest floors, many others were actually within the angiosperm-dominated canopies (Smith 1972, Schneider et al. 2004).

Tab. 1. Overview of species richness in main lineages of seed-free vascular plants in the world (Pryer et al. 2004), Europe including adjacent islands (Frey et al. 2006) and Czechia (Kubát et al. 2002), respectively.

Numbers of particular species and subspecies are included in the total number of taxa in Europe and Czechia. Only indigenous species were included in the summary.

	seed-free lineages	world	Europe	Czechia
lycopods *Lycopodiophyta*	*Isoëtopsida*	150	18	2
	Lycopodiopsida	380	22	10
	Selaginellopsida	700	4	2
ferns *Monilophyta*	*Psilotales*	12	1	0
	Ophioglossales	15	9	5
	Equisetopsida	80	13	9
	Marattiopsida	150	0	0
	Leptosporangiate ferns	11000	159	46

Modes of speciation of extant ferns

Three different speciation modes are recognized in extant ferns and lycophytes – primary, secondary and tertiary (Haufler 1996, 2008). Primary speciation means divergence of a single lineage, usually at the diploid level. Such divergence is usually allopatric, induced by spatial isolation of formerly interbreeding populations. Gene flow among distant populations is less probable or even impossible, which offers potential for occurrence and accumulation of apomorphies in particular populations. There are different ways leading to effective spatial separation: expansion of species distribution range and formation of peripheral populations lacking contact with the central ones (Moran & Smith 2001), fragmentation of continuous distribution due to local extinctions or geological events (continental drift, orogenetic folding – Kato 1993). Among ferns and lycophytes, comparing current distributions of extant species and identifying „centers of species diversity“ can be used to support the importance of allopatry in speciation (Barrington 1993). Whereas an allopatric model is consistent with primary speciation of ferns in temperate regions. Other evidence suggests that, especially in tropical forests, speciation is linked with responses to ecological and geological variation (Tuomisto et al. 2003). This situation reflects very well the rate of genetic variation. In contrast to the pattern of low genetic identities among cryptic or nearly cryptic congeneric fern species in temperate regions (Haufler 1987), research in tropical regions showed that some morphologically distinct species have very high genetic identities (Ranker et al. 1996, Hooper & Haufler 1997).

Secondary speciation can occur through genome multiplication – polyploidization. Systematic research of polyploidization in ferns was started by Manton (1950), who first reported on intricacy of fern polyploid groups (complexes).

There are three types of genome multiplication in ferns – autopolyploidy, allohomoploidy, and allopolyploidy (Haufler 2008). Autopolyploidy – speciation by chromosome doubling within a species has been largely overlooked as a significant mechanism (Gastony 1986). In origin of angiosperms, autopolyploidy is not as common as allopolyploidy (Soltis et al. 2003). In some fern groups, however, autopolyploidy may be a frequent, rapid and important speciation mechanism, especially when accompanied by apomixis (Windham & Yatskievych 2003).

Probably not very frequent and still poorly studied mechanism is allohomoploidy. This process comes up in diploid species which may be ecologically isolated, but not always reproductively isolated. Hybrid swarms may then arise. Examples of allohomoploidy were found in genus *Polystichum* in western North America (Mullenniex et al. 1998) or in tree ferns from *Cyatheaceae* family (Conant & Cooper-Driver 1980).

Much widely recognized is the process of allopolyploidy. In this case, progenitors of hybrid-derived species are not interfertile and form sterile offspring when they cross (Manton 1950). Pteridophytes are generally prone to formation of allopolyploid complexes (Haufler 1987, 2002). It seems that barriers to development of hybrid zygotes are weak and field studies have demonstrated high frequency of vigorous but sterile hybrids in some complexes (Reichstein 1981, Petit et al. 1999). Primary interspecific hybrids are usually sterile due to unballanced meiosis: pairing chromosomes (each coming from distinctly different genome) are not actually homologous, resulting spores then usually lack necessary portion of genetic information and are aborted. Polyploidization event restores possibility of homologous chromosome pairing and correct transfering of genetic information into spores. Possessing redundant copies of genes, polyploid gametophytes are more tolerant to intragametophytic selfing than their diploid progenitors. Thus, normal viable sporophytes may then develop. However, even though allopolyploidy is generally accepted as frequent mode of speciation in ferns, there are still many open questions about so-called species complexes that involve allopolyploids (Haufler 2008).

Relatively high basal chromosome numbers and large genome sizes of extant ferns have probably been derived from lower ones by (paleo)polyploidy (Walker 1979). Therefore species recently representing diploids are considered as ancient polyploids whose ancestors became extinct. Although it was initially thought that paleopolyploidy is restricted to ferns only, it has recently been recognized in numerous families of angiosperms as well (Soltis et al. 2009).

Tertiary speciation is a major discovery of the past decade. It resides in rapidly reorganization of genome in fern polyploids (Soltis et al. 2003). This process could result in genetically isolated populations whose separation is maintained by reciprocal gene silencing (Werth & Windham 1991). Main portion of fern biodiversity in the wild could be probably generated through this mechanism.

Non-sexual modes of reproduction

In ferns we can find other important mechanisms generating wide scale of variability. It is estimated that about 10% of ferns are without capability of sexual reproduction. Their reproduction depends on non-sexual processes such as apospory, apogamy or agamospory (Walker 1979).

Apospory represents the formation of gametophytes from sporophytic tissue in the absence of meiosis or spore formation (Raghavan 1989; Grossniklaus et al. 2001). Apospory occurs only sporadically in ferns, is probably of little significance in the wild but can be induced artificially (Sheffield & Bell 1981). For example Ward (1963) reported that *Phlebodium aureum* readily produced prothalli from petiole segments or Munroe & Sussex (1969) found that roots of *Pteridium aquilinum* can be source of gametophyte formation. Aposporous prothalli customarily bear normal sex organs and therefore have the same chromosome number as the parental sporophytic tissue. This process of induced apospory provides means of artificially inducing (auto)polyploid series (Walker 1979). This technique has been used in experimental studies of Bouharmont (1972a) who induced apospory in diploid *Asplenium ruta-muraria* subsp. *dolomiticum* in order to compare such artificial autotetraploid with wild *A. ruta-muraria* subsp. *ruta-muraria*. Induced apospory in a normally sexual species cannot be repeated indefinitely from generation to generation because this would involve doubling of the chromosome number at the each fertilization (Walker 1979).

The other important non-sexual process is apogamy. Apogamy involves the production of a sporophyte from a prothallus without the intervention of oogenesis or fertilization. Apogamy may be induced on normally sexual prothalli by watering from below to avoid the formation of a water film in which the spermatozoids may move and carry out fertilization. Sporophytes will then sometimes develop autonomously from the gametophytic cells and –because no gametic fusion has occurred – the developing plant has the same number of chromosomes as the gametophyte. In some species, apogamy can be induced artificially – this provides a very convenient way by which the chromosome number of a fern can be halved and can be used to determine the type of polyploidy (auto- vs. allo-) present in a species based on genome homology through (non-)pairing bivalents (Manton 1950, Walker 1979). *Dryopteris filix-mas* and *D. dilatata* were analyzed at first when the question of a possible allo-/autopolyploid origin was raised (Manton 1950, Manton & Walker 1954). Similarly, autopolyploid origin of tetraploid taxa *Asplenium trichomanes* subsp. *quadrivalens* and *A. septentrionale* subsp. *septentrionale* was revealed by induced apogamy (Bouharmont 1972b, 1972c).

In contrast to the occasional and sporadic occurrence of the two above-mentioned modes of non-sexual reproduction in the wild, the most common natural apomictic alternation of generations is agamospory. The main principle of this process is development of unreduced spores during meiosis, resulting in the same chromosome number of sporophyte and gametophyte (Manton 1950, Lovis 1977). At first, agamospory was revealed and studied in *Dryopteris remota* (Döpp 1932, Manton, 1950). Walker (1979) found agamospory in a number of fern genera, e.g. *Adiantum*, *Asplenium*, *Diplazium*, *Dryopteris*, *Polypodium*, *Polystichum* or *Trichomanes*. In Central Europe the most common and well known agamosporous group is *Dryopteris affinis* complex. Bennert et al. (2005) have discovered it recently in *Equisetum*. The majority (50–70%) of agamosporous species is triploid, next 20–35% is diploid, and the rest is represented by tetraploids or higher polyploids (Lovis 1977). Spores from triploid plants may sometimes develop into either diploid or triploid gametophytes, which further apogamously produce diploid or triploid sporophytes, respectively. This mechanism could be important in ploidy reduction of agamosporous ferns (Lin et al. 1992).

From taxonomical point of view is remarkable the existence of so-called cryptic species in ferns. Application of current molecular methods (allozyme composition, nucleotide sequences of chloroplast DNA) led to discovery of series of cryptic species within widely distributed conventional morphological species. (Yatabe et al. 2001, Masuyama et el. 2002, Windham & Yatskievych 2003, Masuyama & Watano 2005). Subsequent focusing on these genetically defined groups revealed often quite subtle but consistent morphological characters and crossing tests correlated with the molecular data (Masuyama et el. 2002, Haufler 2008). In many cases, agamosporous groups are also cryptic in that they are triploid autopolyploid derivatives of otherwise "normal" diploid species (Gastony & Windham 1989).

Flow cytometry – a powerful tool in plant systematics

Since the half of 20th century, the polyploid fern groups has been studied based on chromosome counts provided by robust evidence for heteroploid hybridization and studies of chromosome pairing during meiosis in both natural and artificial hybrids. Those studies shed some light to relationships among taxa of various fern polyploid groups (Manton 1950, Manton & Walker 1953, and consecutively others). Limitations of the methods mentioned above were in examining only a few study plants and subsequent poor interpretation of whole of morphological variability of particular study taxa. In addition to classical karyological research, of polyploid fern groups was studied by means of chromatographic methods, particularly in Scandinavia (Widén et al. 1967, Widén & Sorsa 1968, Widén et al. 1970).

In the last two decades, methods of flow cytometry have been widely applied in plant biosystematics (Doležel 1991, 1997). The knowledge that genome size is usually constant within the same taxonomic entity (Greilhuber et al. 2005) but may vary tremendously even among closely related taxa (Bennett & Leitch, 2005) provides a foundation for employing genome size as an important taxonomic marker (Bennett & Smith 1991, Bennett & Leitch 1995, 2005, Burton & Husband 1999, Dimitrova et al. 1999, Suda et al. 2004, 2007a, 2007b, 2007c). Indeed, this character has proven successful in resolving complexity of polyploid groups, low-level taxonomies, delimiting species boundaries, evolution and population biology, and revealing cryptic taxa (Dimitrova et al. 1999, Mahelka et al. 2005, Kron et al. 2007, Suda et al. 2007b).

Applications of flow cytometry in current plant biosystematics have several important advantages over laborious chromosome counting. Sample preparation is very easy and convenient, lasting only few minutes. High accuracy of flow cytometry assays facilitates detection of minute variation in nuclear DNA amount. The method is non-destructive for the plant individual examined since only small quantities of plant tissue are required for the sample preparation. Wide variety of plant tissues can be utilized for flow cytometry, i.e. roots, stems, sepals, petals or seeds and low operating costs of analyses are also important. Easy analyses of numerous plant samples in a short time should be underlined above all (Suda 2004, 2005).

One can not claim that ferns are traditional, frequent and excessively studied objects of cytometrical research – rather the opposite is true. The Plant DNA C-values database (Bennett & Leitch 2005) still harbours only a relatively low number of estimates (87) of fern C-values (Bennett & Leitch 2001, Obermayer et al. 2002, Hanson & Leitch 2002). Despite its potential taxonomic value, genome size has been largely neglected in fern biosystematics in general (but see Bureš et al. 2003).

Applications of flow cytometry in study of polyploid fern groups together with multivariate morphometrics are key results of this PhD. thesis.

Systematic research of ferns in Europe

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?
- Compilation of determination keys and materials to local Floras/Keys

The thesis consists of ten original studies/papers (see below).

Paper 1 EKRT L. & ŠTECH M. (2008): A morphometric study and revision of the *Asplenium trichomanes* group in the Czech Republic. – *Preslia* 80(3): 325–347.

Paper 2 EKRT L. (2008): Rozšíření a problematika taxonů skupiny *Asplenium trichomanes* v České republice [Distribution and problematic of taxa of *Asplenium trichomanes* group in the Czech Republic]. – *Zprávy České*

Botanické Společnosti 43(1): 17–65.

Paper 3 Ekrt L. (2007–2009): *Asplenium trichomanes.* In: Fischer M. A., Willner W., Niklfeld H. & Gutermann W. [eds], Online-Flora von Österreich, online aviable at <http://flora.vinca.at, http://62.116.122.153/flora/Asplenium_trichomanes>

Paper 4 Ekrt L. (xxxx) *Asplenium trichomanes*. In: Marhold K., Feráková V., Goliašová K., Grulich V., Hodálová I., Hrouda L., Kochjarová J., Mártonfi P., Mereďa P. jun. [eds], Určovací kľúč papraďorastov a semenných rastlín Slovenska [Identification key of ferns and flowering plants of the Slovak Republic]. VEDA, Bratislava. (*accepted*)

Paper 5 Ekrt L. (2008): Revize rozšíření sleziníku střídavolistého (*Asplenium ×alternifolium*) v České republice [Revision of geographical distribution of *Asplenium ×alternifolium* in the Czech Republic]. – *Zprávy České Botanické Společnosti* 43(2): 231–250.

Paper 6 Ekrt L., Holubová R., Trávníček P. & Suda J. (xxxx): Species boundaries and frequency of hybridization in the *Dryopteris carthusiana* (Dryopteridaceae) complex: a taxonomic puzzle resolved using genome size data. (*submitted* to *American Journal of Botany*)

Paper 7 Ekrt L., Trávníček P., Jarolímová V., Vít P. & Urfus T. (2009): Genome size and morphology of the *Dryopteris affinis* group in Central Europe. – (*Preslia – accepted*)

Paper 8 Ekrt L., Štech M., Lepší M. & Boublík K. (xxxx): Rozšíření a taxonomická problematika skupiny *Dryopteris affinis* v České republice [Distribution and taxonomical problems within *Dryopteris affinis* group in the Czech Republic]. – (*submitted* to *Zprávy České Botanické Společnosti*)

Paper 9 Ekrt L., Lepší M., Boublík K. & Lepší P. (2007): *Dryopteris remota* rediscovered for the flora of the Czech Republic. – *Preslia* 79: 69–82.

Paper 10 Ekrt L. & Štech M. (xxxx): *Asplenium trichomanes* L. – sleziník červený, *Dryopteris affinis* agg. – kapraď rezavá, *Dryopteris remota* (A. Braun ex Döll) Druce – kapraď tuhá, *Trichomanes* L. – vláskatec – In: Štěpánková J. [ed.], Květena ČR 9 [Flora of the Czech Republic 9], Academia, Praha. (*submitted*)

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Summary of Results

The first study is devoted to taxonomic revision of *Asplenium trichomanes* polyploid group in the Czech Republic. Using cytometric and morphometric methods, four subspecies were recognized and presence of their hybrids was detected. The important determination characters are discussed and compared with other papers and determination key was compiled (Paper 1). Detailed overview of distribution of particular taxa in the Czech Republic is presented in form of distribution maps and lists of examined specimens from the public Czech herbaria collections. Morphological descriptions of particular taxa are presented and notes on habitat preferences as well as on issue of hybridization are given (Paper 2). Finally, results of these studies has been consecutively used for compilation of determination key for the Flora of Austria (Paper 3), Identification key of ferns and flowering plants of the Slovak Republic (Paper 4) and the most comprehensive compilation was carried out for the additions to the Flora of the Czech Republic (Paper 10).

The fifth study presents the result of distribution and problems of *Asplenium ×alternifolium* in the Czech Republic. Historical and recent distribution of *Asplenium ×alternifolium* nothosubsp. *alternifolium* and *Asplenium ×alternifolium* nothosubsp. *heufleri* was examined based on revision of herbarium and recent data. Habitat and distribution preferences were specified and discrimination characters were presented in the Czech literature for the first time (Paper 5).

The sixth study is focused on cytometrical and morphometrical delimitation of species and hybrids of *Dryopteris carthusiana* group in Central Europe. Genome size was found as powerful criterion to determine taxa boundaries. Multivariate morphometrical analysis revealed the best discriminant characters among taxa and key to determination using quantitative characters was compiled. Based on analysis of population samples, the rate and frequency of hybridization in the wild is discussed (Paper 6).

The genome size and DNA ploidy levels were important criteria also for cytogenetic recognition of two similar triploid agamosporous taxa *Dryopteris borreri* and *D. cambrensis* from *D. affinis* group. Multivariate morphometrical analysis detected the best morphological characters suitable for field recognition and determination key was compiled. Genome size was compared with other records from C-value DNA database and results are discussed. Rate and frequency of natural hybridization was specified (Paper 7). Detailed morphological description of taxa the *D. affinis* group was comprehensively compiled to the additions to the Flora of the Czech Republic (Paper 10). Detailed actual distribution of species of *D. affinis* group in the Czech Republic was compiled to Paper 8.

The nineth study presents rediscovering of rare *Dryopteris remota* for the flora of the Czech Republic. *D. remota* was still considered as uncertain species until now in the Czech Republic. The species has been known ca 70 years ago from the Moravian Karst only. New locality on a slope of Malý Plešný hill in the foothills of the Bohemian Forest is described as same as the ecological preferences, detailed morphological description and comparison with similar taxa are included. A map of its distribution within the Czech Republic based on revised herbarium data is presented as well as a map of the worldwide distribution (Paper 9).

The last paper brings additions to the Flora of the Czech Republic on some new fern taxa discovered recently in the Czech Republic (*Dryopteris remota*, *Trichomanes speciosum*) and new upgrade of critical fern groups (*Asplenium trichomanes*, *Dryopteris affinis* agg.) from the first book of the Flora of the Czech Republic from 1988. Especially compilation of *Trichomanes speciosum* represents the first comprehensive summary of morphological characterization of independent gametophyte stage in the Central European floras based on own characters measurement and review of literary data (Paper 10)

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).

Future perspectives

Some of the studies presented raise further questions for future research. Especially further cytometrical or molecular research could explain many following questions and issues.

What is the genome size of particular taxa of *Asplenium trichomanes* group? Previous study used relative DNA ploidy level only. Is the close morphology of *A. t.* subsp. *hastatum* and *A. t.* subsp. *pachyrachis* based on historical allo-/auto- polyploid events of common ancestral taxa? Is it possible to recognize these two close tetraploid species and their hybrids according to genome size?

Asplenioid ferns were recently studied intensively by molecular studies but the second large european fern genus – *Dryopteris* was still poorly studies through whole Europe. Molecular and cytometrical methods may be helpful for solving the issues of systematics and evolution of the genus in Europe. Such understanding may elucidate also the width of variability and rate of hybridization among related species.

Detailed study is needed to clarify the rates of hybridization of some other groups in the wild. For example, there is nothing exactly known about the rate of natural hybridization between *Athyrium filix-femina* vs. *A. distentifolium*, *Dryopteris cristata* vs. *D. carthusiana* or *Polystichum aculeatum* vs. *P. braunii*.

Ferns are very poorly represented in the Plant DNA C-values database. Further research focused on genome size assessment may also fill the gaps and enable comparison with angiosperms abundantly represented there.

Genome size estimation of extant ferns would also allow us to address question on the evolution of genome size in ferns and on its correlation to the variation in chromosome numbers or other features. Are there any correlations between genome size in ferns from latitudinal or altitudinal gradients through Europe? Is increase in genome size linear through polyploidization events? Is genome size constant in gametophytes and sporophytes of agamosporous ferns vs. equally separated in sexually reproduced ferns?

A detailed revision of distribution of some taxonomically critical fern species is necessary for modern overview of current distribution of pteridophytes in the Czech Republic. Distribution of some *Dryopteris* species is now in progress.

Abstracts/Abstrakty

Paper 1/Článek 1

A morphometric study and revision of the *Asplenium trichomanes* group in the Czech Republic
[Morfometrická studie a revize skupiny *Asplenium trichomanes* v České republice]

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* × *A. t.* subsp. *quadrivalens*). The individual morphological characters used for determining subspecies are evaluated and a determination key presented.

V příspěvku je prezentována detailní cytogeografická a morfometrická studie taxonů z okruhu sleziníku červeného (*Asplenium trichomanes*) v České republice. Byly detekovány diploidní (2n = 72), tetraploidní (2n = 144) a hybridní triploidní rostliny (2n = 108). Na základě morfometrické studie byly rozpoznány čtyři intraspecifické taxony. Tyto taxony korespondují se čtyřmi poddruhy v rámci druhu *A. trichomanes* (*A. t.* subsp. *trichomanes*, *A. t.* subsp. *quadrivalens*, *A. t.* subsp. *pachyrachis* and *A. t.* subsp. *hastatum*), které jsou rozpoznávány v květenách západní, jižní a severní Evropy. Triploidní rostliny byly určeny jako *A. t.* nothosubsp. *lusaticum* (*A. t.* subsp. *trichomanes* × *A. t.* subsp. *quadrivalens*). Jednotlivé morfologické znaky použitelné k určení jednotlivých poddruhů byly statisticky ohodnoceny a dále je prezentován klíč k určení jednotlivých taxonů.

Paper 2/Článek 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.

Bylo studováno rozšíření taxonů stupiny *Asplenium trichomanes* v České republice. Revidováno bylo 32 veřejných herbářových sbírek a celkově 1477 herbářových položek bylo studováno. V České republice byly zaznamenány čtyři taxony: *A. trichomanes* subsp. *trichomanes*, *A. trichomanes* subsp. *quadrivalens*, *A. trichomanes* subsp. *pachyrachis*, *A. trichomanes* subsp. *hastatum* a čtyři hybridní kombinace. V příspěvku je prezentován celkový přehled rozšíření jednotlivých taxonů stejně jako přehled morfologických znaků, mapky rozšíření a stručné shrnutí stanovištních nároků.

Paper 3/Článek 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://62.116.122.153/flora/Asplenium_trichomanes>.

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

[příspěvek bez abstraktu; zpracování určovacího klíče pro skupinu sleziníku červeného (*Asplenium trichomanes*) pro rakouskou Květenu]

Paper 4/Článek 4

***Asplenium trichomanes*. – In: Určovací kľúč papraďorastov 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]

[příspěvek bez abstraktu; zpracování určovacího klíče pro skupinu sleziníku červeného (*Asplenium trichomanes*) pro slovenský Klíč]

Paper 5/Článek 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* × *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.

Na území České republiky bylo sledováno rozšíření *Asplenium* × *alternifolium* nothosubsp. *alternifolium* a *Asplenium* × *alternifolium* nothosubsp. *heufleri*. Revidováno bylo 33 veřejných herbářových sbírek a celkově 442 herbářových položek. *Asplenium* × *alternifolium* nothosubsp. *alternifolium* byl zaznamenán roztroušeně na celém území České republiky. Vyskytuje se zejména na silikátových skalách a na sekundárních stanovištích (zdi) se vyskytuje pouze ojediněle (1% známých lokalit). Proto tento taxon chybí v nížinách jako je Polabí a v oblasti jižní, východní a severovýchodní Moravy. Prezentovány jsou bodové mapy rozšíření, seznam zaznamenaných lokalit a přehled stanovištních nároků. Na základě poznatků z terénu a revizí herbářů se zdá, že současné lokality jsou mnohem vzácnější než v minulosti. *Asplenium* × *alternifolium* nothosubsp. *heufleri* je velmi vzácným taxonem v České republice. V současné době nebyly potvrzeny historické lokality v Josefovském údolí u Adamova, v údolí nedaleko Kamenného Újezdu a u Černolic nedaleko Jíloviště a u Starých vinic nedaleko Znojma. Objeveny a v současné době v terénu revidovány byly dvě nové lokality. První se nachází na zřícenině hradu Litice nad Orlicí v oblasti Žamberska a druhá se nachází na skalách nad Vltavou nedaleko Kamýka nad Vltavou ve Středním Povltaví.

Paper 6/Článek 6

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

[Vymezení druhů a frekvence hybridizace u komplexu *Dryopteris carthusiana* (Dryopteridaceae): taxonomické puzzle vyřešeno pomocí analýzy velikosti genomu]

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 decision-making 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.

Dryopteris carthusiana agg. je taxonomicky problematická skupina temperátních kapradin, která se na území střední Evropy skládá z jednoho diploida (*D. expansa*) a dvou allotetraploidů (*D. carthusiana* a *D. dilatata*). Celková fenotypická podobnost, značný podíl plasticity a domnělý výskyt intraspecifické hybridizace vedla k neustálým polemikám ohledně vymezení jednotlivých druhů. Za použití průtokové cytometrie a mnohorozměrných morfologických analýz jsme stanovili stupeň morfologické variability a frekvenci hybridizace u reprezentativního středoevropského souboru set vzorků u všech známých druhů a hybridů komplexu. Průtoková cytometrie odhalila unikátní velikosti genomu u všech druhů/kříženců a umožnila jednoduchou a spolehlivou identifikaci (notho)taxonů. Jako výsledek morfometrických analýz byl vybrán soubor taxonomicky významných morfologických znaků. Zatímco determinace *D. carthusiana* obvykle nečiní závažné problémy, odlišení *D. expansa* a *D. dilatata* je více komplikovaná. Různé druhy často tvoří smíšené populace s předpokladem interspecifické hybridizace. Frekvence výskytu jednotlivých hybridních kombinací se dramaticky liší, což záleží v první řadě na evolučních vztazích jednotlivých taxonů a ploidní úroveň tu má zřejmě jen malý efekt. Naše studie představuje nový a robustní charakter (velikost genomu) pro taxonomické rozřešení komplexu *D. carthusiana*, který představuje významný krok vpřed v řešení této taxonomicky složité a významné skupiny temperátních kapradin.

Paper 7/Článek 7

Genome size and morphology of the *Dryopteris affinis* group in Central Europe
[Velikost genomu a morfologie skupiny *Dryopteris affinis* ve střední Evropě]

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* × *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.

V rámci střední Evropy byla zkoumána jako součást cytogeografické a morfologické studie taxonomicky kritická a agamosporická skupina *Dryopteris affinis* agg. Materiál z 27 lokalit z České republiky, Slovenska, Polska a Rakouska byl sebrán a ohodnocen pomocí morfologických mnohorozměrných metod a metodami karyologickými. Počet chromosomů a výsledky průtokové cytometrie odhalili existenci dvou zřetelných triploidních taxonů (2n = 123) o různé velikosti genomu, které korespondují s *D. borreri* a *D. cambrensis,* a vzácnějšího pentaploidního křížence (2n = 205) *D.* ×*critica* (*D. borreri* × *D. filix-mas*). Morfometrické analýzy potvrdily zřetelné diferenciace mezi oběma triploidníma taxonama. Nové kvantitativní znaky byly vybrány podle výsledků diskriminační analýzy a zároveň je zde prezentován klíč k jejich identifikaci.

Paper 8/Článek 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.* ×*critica* (*D. borreri* × *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.

Bylo studováno rozšíření taxonů stupiny *Dryopteris affinis* v České republice. Celkem bylo revidováno 21 českých veřejných herbářových sbírek a celkem 257 herbářových položek. V České republice byly zaznamenány dva druhy *D. borreri*, *D. cambrensis* a kříženec *D.* ×*critica* (*D. borreri* × *D. filix-mas*). *D. cambrensis* byla v současné době znovuobjevena po více než 30 letech pro květenu České republiky a zároveň byla nalezena jako nový druh pro Čechy. V České republice bylo zaznamenáno 16 lokalit vzácné *D. cambrensis*. Ojedinělá lokalita *D. cambrensis* byla zaznamenána v Polsku v okolí obce Pstrążna nedaleko státní hranice s Českou republikou. V příspěvku je přezentován přehled morfologických znaků, mapky rozšíření v ČR, stanovištní preference a celkový areál druhů.

Paper 9/Článek 9

***Dryopteris remota* rediscovered for the flora of the Czech Republic**
[*Dryopteris remota* znovuobjevena pro květenu České republiky]

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.

Do současné doby byla *Dryopteris remota* zaznamenána v České republice před 70 lety pouze v Moravském krasu. Tento údaj je zmíněn v řadě publikací, ale obvykle bez odkazu na originální zdroj. Z tohoto důvodu nebylo jasné, zda-li *D. remota* dosud roste v České republice. V současné době byly revidovány a ověřeny originální herbářové doklady z Moravského krasu. V roce 2002 byla *D. remota* nalezena na první lokalitě v Čechách blízko obce Ktiš na svazích kopce Malý Plešný na úpatí Šumavy (Jižní Čechy). Na této lokalitě byla nalezena pouze 1 rostlina, která se nacházela na rozmezí křovité vegetace s *Lonicera nigra* a smrko-buko-jedlového lesa na rulových výchozech. Determinace rostliny *D. remota* z nové české lokality bylo provedeno na základě porovnání makro- and mikromorfologických znaků s rostlinami z Alp (Horní Rakousko) a Karpat (Západní Ukrajina) stejně jako porovnání popisu rostliny v literatuře. V příspěvku je zpracován detailní morfologický popis a porovnání s podobnými taxony. Dále jsou zde prezentovány mapy rozšíření druhu v České republice a celková mapa areálu druhu. Dryopteris remota je navržena jako kriticky ohrožený druh České republiky.

Paper 10/Článek 10

***Asplenium trichomanes* L. – sleziník červený, *Dryopteris affinis* agg. – kapraď rezavá, *Dryopteris remota* (A. Braun ex Döll) Druce – kapraď 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]

[příspěvek bez abstraktu; zpracování morfologie, variability, ekologie a rozšíření vybraných taxonů pro Květenu ČR]