Dryopteris ×*ambroseae* (*Dryopteridaceae*: *Pteridophyta*), a hybrid new to Bulgaria

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Abstract. The natural fern hybrid *Dryopteris* ×*ambroseae* is reported as new to the Bulgarian flora. Single scattered plants were found in mixed populations of both parents. Confirmation of their hybrid nature was accomplished by morphological, cytological and spore analyses. The frond morphology of the hybrid individuals was intermediate between the two parents, the mitotic chromosome number was triploid ($2n = 123$), and the spores, varying in shape and size, were entirely abortive.

Key words: Bulgaria, chorology, cytology, *Dryopteris* ×*ambroseae*, fern hybrid, morphology, spore analysis

Introduction

The inter-relationships of species within the *Dryopteris carthusiana* complex were studied by investigating chromosome pairing during meiosis in both wild and synthesised hybrids (Walker 1955, 1961). Since then many researchers have studied the morphology, cytology and chemotaxonomy of representatives of the complex in different countries.

Within the *Dryopteris carthusiana* complex in Bulgaria there are two allotetraploid species – *D. carthusiana* (Vill.) H.P. Fuchs and *D. dilatata* (Hoffm.) A. Gray, and one diploid species – *D. expansa* (C. Presl) Fraser-Jenk. & Jermy. These species are closely related. Experimental hybridisation followed by cytogenetic studies and chemical investigations have shown that *D. expansa* is one of the diploid ancestors of the allotetraploid *D. dilatata,* while *D. dilatata* and *D. carthusiana* share the genome of a member of *D. intermedia* complex. Consequently, the species of the *D. carthusiana* complex are very similar in morphology and have often been confused and misidentified.

Hybridisation among species of *D. carthusiana* complex is well known and the wild hybrids between the three above-mentioned species have been reported to occur rather frequently in areas where the parental species grow side by side.

No hybrids in genus *Dryopteris* have been previously identified within the Bulgarian flora. However, during an excursion in the Rila Mts in 1993, the author together with Ronnie Viane (from Gent University, Belgium) collected a frond of *Dryopteris* that could not be identified immediately as *D. expansa* or *D. dilatata*. More careful investigation showed that the spores of the plant were abnormal, suggesting a hybrid nature. This find provoked a search at other sites in Bulgaria and the collected material was identified through different analyses.

Material and methods

The studies were based on personal collections of *Dryopteris* specimens from different natural habitats, and the distribution map of *D.* ×*ambroseae* based on the 10 km UTM grid is given here as Fig. 1. The author's collections of *Dryopteris* have been deposited in SOM (the herbarium of the Institute of Botany, Bulgarian Academy of Sciences).

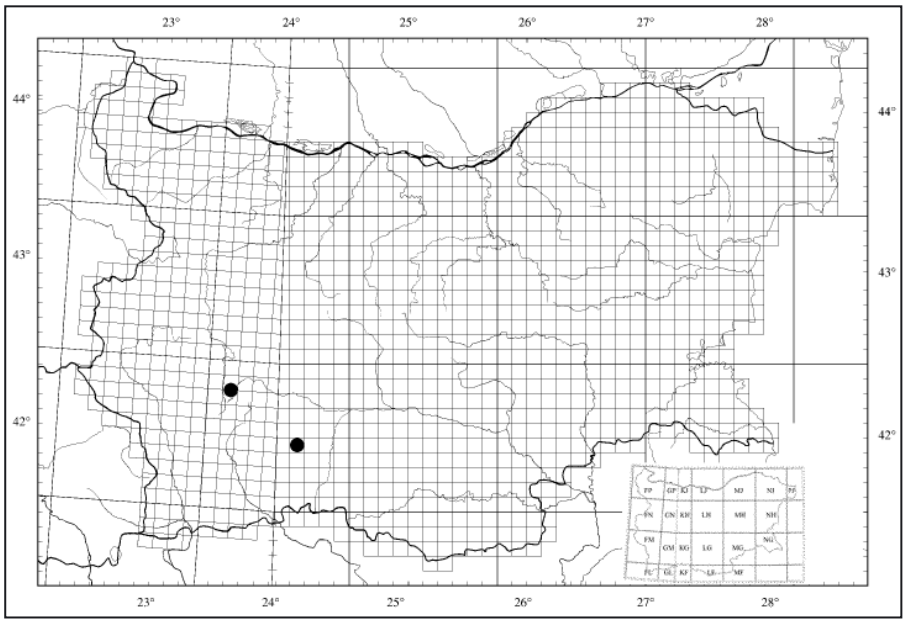


Fig. 1. UTM grid map of the distribution of *D.* ×*ambroseae* in Bulgaria.

Localities of the hybrids:

Rila Mts: along the path from Mousala chalet to Borovets, 1750 m alt., 25.07.1993, coll. *D. Ivanova* (DI-27.93).

Rila Mts: along Mousalenska Bistritsa river above Borovets, 1600 m alt., 02.09.1998, coll. *D. Ivanova* (DI-170.98).

Rhodopi Mts (*Western*): by a forest path, 1-2 km off the road fork from Beglika to Teheran chalet, 1550 m alt., 05.08.1998, coll. *D. Ivanova* (DI-79.98).

Morphological, cytological and spore-analyses techniques were utilised. The sporophyte chromosome number was counted in squashed root-tip meristem, following the method given in Ivanova (2004). For the spore and sporangia study a light microscope (LM) was used. Spores were mounted in Euparal on glass slides, without chemical treatment.

Results and discussion

Chorology and habitat preferences in Bulgaria

Dryopteris ×*ambroseae* Fraser-Jenk. & Jermy is known to occur in many European countries (e.g., Fraser-Jenkins 1982; Fraser-Jenkins & Reichstein 1984; Derrick & al. 1987). The record of the taxon in Bulgaria extends its range.

Dryopteris expansa in Bulgaria is mostly an alpine plant, growing in open habitats in the alpine and subalpine zone, but it can be found also in wet gulches and along the streams in coniferous forests above 1200 m alt. *Dryopteris dilatata* is a montane species growing in wet to moderately dry deciduous and coniferous forests, in wet stony places, in thickets, gulches, along the streams, and also in open habitats in the subalpine zone. Occasionally their ranges overlap in Bulgaria.

At present, hybrid individuals have been found in only three localities in the Rila Mts and the Western Rhodopes (Fig. 1), in coniferous (mainly spruce) forests – nearby a river (high humidity), or by forest paths (not very high humidity). Single scattered individuals have been found at sites where both parents grow together.

Quite probably the hybrids are overlooked in the field as a form of one of the parents.

Morphology

A number of authors have emphasized the considerable variability of the morphological characters traditionally used for differentiating the members of *D. carthusiana* complex. A single feature is insufficient to distinguish the taxa. Identification of the species of the complex is further complicated by the existence of the hybrids *D.* ×*ambroseae* (*D. dilatata* × *expansa*, triploid), *D.* ×*deweveri* (*D. carthusiana* × *dilatata*, tetraploid), and *D.* ×*sarvelae* (*D. carthusiana* × *expansa*, triploid), frequently overlooked and/or confused with their parents even though they can be distinguished from them by their spores.

Morphologically, *D.* ×*ambroseae* is more or less intermediate in general appearance between the two parents *D. dilatata* and *D. expansa* (Plate I). As parental species are morphologically variable, the variability of hybrids is also considerable. Plants mainly differ from *D. expansa* in having a slightly firmer and darker blade, and scales that are less gingery and more frequently have a darker central area that often becomes a dark median stripe, as in *D. dilatata*. They differ from *D. dilatata* in having a more triangular blade that is more finely dissected and appears more delicate and lace-like, with flatter, less downcurving pinnule margins, as in *D. expansa*.

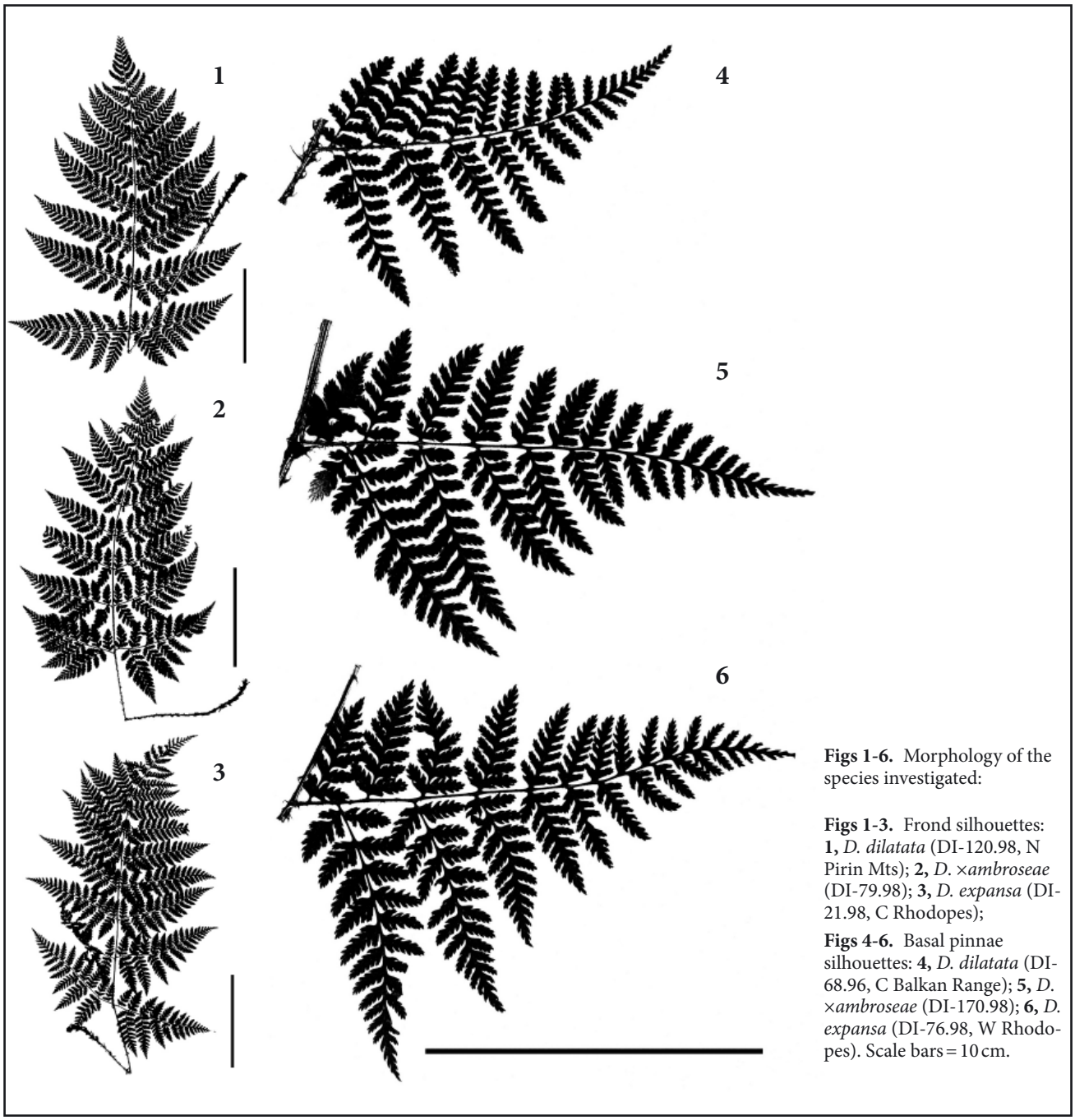
However, it is often difficult to differentiate the plants with certainty from either parent, without examining the spores, which are abortive. In the field, the whole sporangia often appear sparse and abortive too.

Some characters of *D. dilatata*, *D. expansa* and *D.* ×*ambroseae* from Bulgaria, which facilitate the identification of the hybrid in the field, are presented in Table 1.

Table 1. Comparison of some characters of *D. dilatata*, *D.* ×*ambroseae* and *D. expansa* in Bulgaria.

Features	*D. dilatata*	*D.* ×*ambroseae*	*D. expansa*
Rhizome	Large and stout, erect or ascending, densely scaly; frond bases forming a dense shuttlecock.	As in both parents; frond bases forming an open shuttlecock.	Large and stout, erect or ascending, densely scaly; frond bases forming an open shuttlecock.
Stipe	Thick, with wide base, densely scaly at the base, and somewhat less so higher up.	As in both parents.	Thick, with wide base, usually rather densely scaly at the base, and somewhat less so higher up.
Scales on the stipe	Long and narrow to lanceolate to broadly ovate-deltoid, bicolorous, ± light brown with dark brown or almost black central stripe. Balooned scales absent.	Long and narrow to broadly ovate, light brown (less gingery than in *D. expansa*) with darker brown or almost black central area or stripe.	Very variable in shape and size – from long and narrow to broadly ovate, pale brown to ± reddish-brown, both concolorous forms and those with dark-brown (never black) spot or median stripe being found. Some scales are blistered or baloon-like.
Fronds	15–85 (–100) cm, comparatively thick.	50–105 cm, almost erect, slightly tougher than in *D. expansa*.	14–95 (–110) cm, erect, lax, thin.
Frond blade	Oblong, lanceolate-ovate to broadly triangular-ovate, dark green to bluish (olive)-green, bi- or tripinnate-pinnatifid, not glandular or glandular only on the veins when young.	More triangular-outlined blade than in *D. dilatata* – triangular to triangular-ovate, sometimes bluer-green than in *D. expansa*, tripinnate.	Broadly triangular-ovate, ovate-lanceolate to almost linear-lanceolate, pale green, mid- to yellowish-green, tripinnate to tripinnate-pinnatifid (quadripinnate), ± not glandular or glandular.
Frond dissection	Sinus closed, cut 1/2 or 2/3 to the costule.	More finely cut than in *D. dilatata*.	Sinus open, cut ± to the costule.
Pinnae	Broadly lanceolate to triangular-lanceolate, ± equidistant. The lowermost pair of pinnae triangular-lanceolate to ovate-lanceolate, asymmetric (Plate I, Figs 1, 4), occasionally more distant than the upper ones. The remaining pinnae less unequal and more nearly lanceolate.	More like *D. expansa* (Plate I, Figs 2, 5).	Broadly lanceolate to triangular-lanceolate. The lowermost pair of pinnae triangular-ovate to broadly triangular, usually strongly asymmetric at the base (Plate I, Figs 3, 6), occasionally more distant than the upper ones. The remaining pinnae less unequal and more nearly lanceolate.
Pinnules	Oblong-ovate to oblong-lanceolate, the basal ones stalked, the rest sessile or decurrent. Length of the basal basiscopic pinnule of the lowermost pinna almost always less than half the total length of the pinna (Plate I, Fig. 4). First basiscopic pinnule of the basal pinna often equal or even shorter than the second one.	Basal basiscopic pinnule of the lowermost pinna long, as compared with the other pinnules (like in *D. expansa*) (Plate I, Fig. 5).	Oblong-ovate to oblong-lanceolate, almost all stalked. Length of the basal basiscopic pinnule of the lowermost pinna varying, usually more than half the total length of the pinna (Plate I, Fig. 6). Lowermost basiscopic pinnules often much longer than the rest.
Ultimate segments	Usually somewhat convex.	Flat.	Flat.
Spores	Rather large, ± dark chestnut to almost blackish-brown, with a shallowly folded perispore (Plate II, Figs 2, 5), bearing dense tubercles.	Normal spores totally lacking. Sporangia contain abortive spores mixed with brown (black) powdery mass (Plate II, Figs 3, 6, 8).	Rather large, pale brown, straw-coloured to reddish amber-brown, with a wide sparsely folded perispore (Plate II, Figs 4, 7), bearing widely spaced tubercles.
Chromosome number and ploidy level	$2n = 164$, tetraploid.	$2n = 123$, triploid (Plate II, Fig. 1a, b).	$2n = 82$, diploid.

Plate I



Figs 1-6. Morphology of the species investigated:

Figs 1-3. Frond silhouettes: **1,** *D. dilatata* (DI-120.98, N Pirin Mts); **2,** *D. ×ambroseae* (DI-79.98); **3,** *D. expansa* (DI-21.98, C Rhodopes);

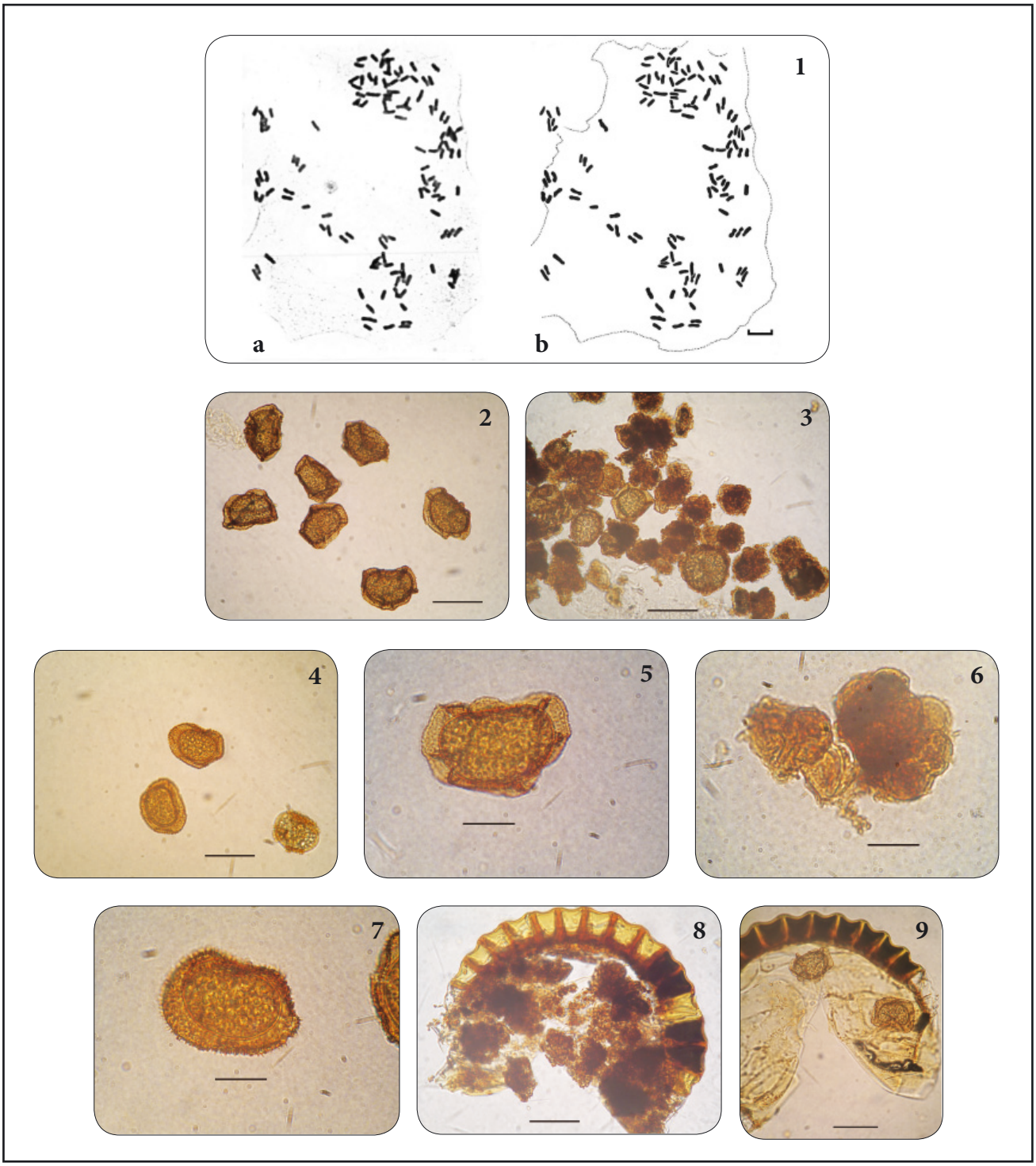
Figs 4-6. Basal pinnae silhouettes: **4,** *D. dilatata* (DI-68.96, C Balkan Range); **5,** *D. ×ambroseae* (DI-170.98); **6,** *D. expansa* (DI-76.98, W Rhodopes). Scale bars = 10 cm.

Cytology and spores

Several authors (e.g., Crabbe & al. 1970; Piękoś-Mirkowa 1979) stressed that the most reliable characters for identification of the taxa of *D. carthusiana* complex are the chromosome number, spore colour and perispore sculpture.

Dryopteris expansa is a diploid, with $2n = 82$ chromosomes, and *D. dilatata* is a tetraploid, with $2n = 164$ (Ivanova 1997, 1999). The two studied individuals (DI-79.98 and DI-170.98) of the natural hybrid *D. ×ambroseae* in Bulgaria revealed a chromosome number $2n = 123$ at mitosis (Plate II, Fig. 1a, b). The same mitotic number or the meiotic number n = c. 41 bivalents + 41 univalents have been reported earlier by many authors (Walker 1955; Döpp & Gätzi 1964; Sorsa & Widén 1968; Crabbe & al. 1970; Widén & al. 1970; Piękoś 1974; Gibby 1977; Benl & Eschelmüller 1983).

Plate II



Figs 1-9. Chromosomes, spores and sporangia of the species investigated:

Fig. 1. Microphotograph (a) and explanatory diagram (b) of mitotic metaphase plate of *D.* ×*ambroseae*, 2*n* = 123 (DI-79.98). Scale bar = 10 µm;

Figs 2-7. LM microphotographs of spores: **2, 5,** normal spores of *D. dilatata* (DI-58.96, C Balkan Range; DI-68.98, W Rhodopes); **3, 6,** abortive spores of *D.* ×*ambroseae* (DI-170.98); **4, 7,** normal spores of *D. expansa* (DI-82.94, Rila Mts). Scale bars on Figs 2-4 = 50 µm, on Figs 5-7 = 20 µm;

Figs 8-9. LM microphotographs of sporangia: **8,** *D.* ×*ambroseae* (DI-170.98); **9,** *D. expansa* (DI-56.98, W Rhodopes). Scale bars = 50 µm.

Spores and sporangia of the three taxa are illustrated by Plate II (Figs 2-9). However, because of the difficulty in identifying the parents, it is essential that putative hybrids are examined for abortive misshapen spores. *Dryopteris ×ambroseae* has deformed, always entirely abortive spores, i.e. the spore proper ceases to develop, while the perispore remains in the sporangia as a dark, granular mass (Plate II, Fig. 8), in which single irregular spores with distorted shapes and varying size can be found (Plate II, Figs 3, 6).

Conclusions

The wild fern hybrid *D. ×ambroseae* has been found for the first time in Bulgaria, occurring in mixed populations of parents *D. expansa* and *D. dilatata*. The chromosome number established in root-tip mitosis was $2n = 123$; spores were totally abnormal. Since hybrids are formed where the two parents grow close to each other, it seems likely that hybrid plants occur at other sites in Bulgaria too.

Dryopteris ×ambroseae is difficult to identify because of the strong similarities of both parents. Hybrid individuals occur spontaneously in low numbers in the population and can be recognized initially (in the field) by their intermediate appearance and later on can be reliably identified by their abortive spores. *Dryopteris ×ambroseae* possesses intermediate morphological characters between those of the parents *D. dilatata* and *D. expansa*, but the resemblance of the Bulgarian material to *D. expansa* is by far more pronounced (Plate I). Plants are likely to be confused not only with both parents, but also with the tetraploid hybrid *D. ×deweveri*, which also has abortive spores. However, *D. ×deweveri* has not been found in Bulgaria yet.

It is, therefore, important to check for hybrids before identifying the specimens to species level and this can be done reliably only by examining the spore-samples. For this reason, it is important to collect specimens with ripe sporangia, in the act of dehiscing, or shortly afterwards, but not before the sporangia are mature, nor when they have lost all their spores (Fraser-Jenkins 1982).

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References

Benl, G. & Eschelmüller, A. 1983. Zur Vorkommen weniger bekannter *Dryopteris*-Sippen im bayerischen Alpen- und Voralpenraum. – Ber. Bayer. Bot. Ges., **54**: 77-102.

Crabbe, J.A., Jermy, A.C. & Walker, S. 1970. The distribution of *Dryopteris assimilis* S. Walker in Britain. – Watsonia, **8**: 3-15.

Derrick, L.N., Jermy, A.C. & Paul, A.M. 1987. Checklist of European Pteridophytes. – Sommerfeltia, **6**: i-xx, 1-94.

Döpp, W. & Gätzi, W. 1964. Der Bastard zwischen tetraploider und diploider *Dryopteris dilatata*. – Ber. Schweiz. Bot. Ges., **74**: 45-53.

Fraser-Jenkins, C.R. 1982. *Dryopteris* in Spain, Portugal and Macaronesia. – Bol. Soc. Brot., Sér. 2, **55**: 175-336.

Fraser-Jenkins, C.R. & Reichstein, T. 1984. *Dryopteris dilatata* x *expansa*. – In: **Kramer, K.U.** (ed.), Hegi, G., Illustrierte Flora von Mitteleuropa. Bd. **1**. *Pteridophyta*. Teil 1, pp. 168-169. Verlag Paul Parey, Berlin, Hamburg.

Gibby, M. 1977. The origin of *Dryopteris campyloptera*. – Canad. J. Bot., **55**: 1419-1428.

Ivanova, D. 1997. Reports (831-839). – In: **Kamari, G., Felber, F. & Garbari, F.** (eds), Mediterranean chromosome number reports – 7. – Fl. Medit., **7**: 225-235.

Ivanova, D. 1999. Reports (1058-1063). – In: **Kamari, G., Felber, F. & Garbari, F.** (eds), Mediterranean chromosome number reports – 9. – Fl. Medit., **9**: 346-356.

Ivanova D. 2004. *Dryopteris affinis* subsp. *borreri* (*Pteridophyta*: *Dryopteridaceae*) in the Bulgarian flora. – Fl. Medit., **14**: 201-218.

Piękoś, H. 1974. The hybrid *Dryopteris assimilis* × *dilatata* in Poland. – Fragm. Florist. Geobot., **20**(3): 333-338 (in Polish).

Piękoś-Mirkowa, H. 1979. The ferns of the *Dryopteris dilatata* complex in Poland. – Monogr. Bot., **59**: 1-75 (in Polish).

Sorsa, V. & Widén, C.-J. 1968. The *Dryopteris spinulosa* complex in Finland. A cytological and chromatographic study of some hybrids. – Hereditas (Lund), **60**: 273-293.

Walker, S. 1955. Cytogenetic studies in the *Dryopteris spinulosa* complex – I. – Watsonia, **3**(4): 193-209.

Walker, S. 1961. Cytogenetic studies in the *Dryopteris spinulosa* complex. II. – Amer. J. Bot., **48**(7): 607-614.

Widén, C.-J., Sorsa, V. & Sarvela, J. 1970. *Dryopteris dilatata* s. lat. in Europe and the island of Madeira. A chromatographic and cytological study. – Acta Bot. Fenn., **91**: 1-30.