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## ***Dryopteris affinis* subsp. *borreri* (Pteridophyta: *Dryopteridaceae*) in the Bulgarian flora**

### **Abstract**

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In the present paper some preliminary results from the biosystematic investigations on Bulgarian *Dryopteris* are reported. Differences between *D. affinis* (Lowe) Fraser-Jenk. subsp. *borreri* (Newman) Fraser-Jenk. and *D. filix-mas* (L.) Schott as well as illustrations showing some morphological characters of leaves and perispore sculpture are presented. Plants of *D. affinis* subsp. *borreri* from 6 localities were checked cytologically. All specimens investigated turned out to be triploid with  $2n = 123$ . This is the first count for the taxon from Bulgaria. *D. affinis* subsp. *borreri* in Bulgaria is much rarer than *D. filix-mas*. A map of distribution is also given. *D. affinis* subsp. *borreri* was always found in the presence of *D. filix-mas*. The habitat requirements are noted.

### **Introduction**

The pioneer cytotaxonomic work of Manton (1950) has been followed by floristic, cytological, chemotaxonomic and other modern studies, which have provided numerous taxonomic data for many fern genera. The pteridological science has more rapidly developed in the West and North European countries. In contradistinction the fern flora of the Balkans has been very poorly studied so far. In Bulgaria biosystematic investigations on ferns have started only several years ago with emphasis on the genus *Dryopteris*.

The recent systematic revision of the genus *Dryopteris* covering the whole area of its distribution has contributed to the better understanding of its taxonomy. The apomictic golden-scaled male-fern *D. affinis* (Lowe) Fraser-Jenk. is among the most problematic taxa from both nomenclatural and taxonomic points of view.

Jessen (1985) and later Derrick & al. (1987) and Widén & al. (1996) included Bulgaria within the area of distribution of *D. affinis*. However, in Atlas Florae Europaeae (Kozhuharov 1972) and the Med-Checklist (Greuter & al. 1984) the species was not mentioned for the Bulgarian flora. In the Bulgarian botanical literature, including the most recent field guide of vascular plants of the country (Andreev 1992), *D. affinis* also was not reported.

In 1987 C. R. Fraser-Jenkins revised *Dryopteris* specimens in the Bulgarian herbaria, where he recognized some individuals of *D. affinis* subsp. *borreri*. Later, in the second revised edition of *Flora Europaea* (Fraser-Jenkins 1993), he included Bulgaria in the area of its distribution. Our current biosystematic studies also confirm the presence of this subspecies in the Bulgarian flora.

### Current views on the taxonomic treatment of *D. affinis*

The most elaborate classification of genus *Dryopteris* on a world-wide base was proposed by Fraser-Jenkins (1986). About 225 species, grouped into 4 subgenera and 16 sections have been listed in this classification. One of the sections - sect. *Fibrillosae* - consists mainly of apomictic ferns, either diploids or higher polyploids. Members of the section were previously often referred to the "*Dryopteris filix-mas* group" (belonging to sect. *Dryopteris*) because of the morphological resemblance. Being apomictic, several of the species are notably variable and have been divided into subspecies and varieties in different combinations by different authors.

One of the taxa, which possess most taxonomic difficulties and problems is the golden-scaled male-fern *Dryopteris affinis* (Lowe) Fraser-Jenk. Its systematics is complex but it seems that it has arisen by hybridisation between, probably, the pan subtropical diploid *D. wallichiana* (Spreng.) Hyl., with the diploid *D. oreades* Fomin and/or *D. caucasica* (A. Br.) Fraser-Jenk. & Corley (Fraser-Jenkins 1982).

*Dryopteris affinis* is a taxon known for more than 100 years but treated by the earlier authors as a variety of *D. filix-mas* (L.) Schott. Only after the recognition of *D. affinis* as a separate species its morphology, size of variation, reproductive biology, distribution, cytogenetics etc. have become a subject of scientific interest. The species is an obligate apomict, markedly variable, it has different ploidy levels, can hybridize with species from other sections (because of the ability to function as sexual male in inter-specific hybridisation). This, as well as the long list of synonyms, often leads to serious problems in the taxa interpretation.

The great morphological variation of *D. affinis* is due to the presence of different genome combinations. Some of the variants deserve higher taxonomic rank than that of variety or forma. This is the "hot spot" of taxonomic discussion!

Some pteridologists recognise these variants at subspecific rank, a treatment suggested for any similar apogamous complexes. At present the subspecies in Europe are considered to be 3 or 4 according to different authors: subsp. *affinis*, subsp. *borreri* (Newman) Fraser-Jenk., subsp. *cambrensis* Fraser-Jenk. /and subsp. *persica* Fraser-Jenk., or subsp. *pseudodisjuncta* (Fraser-Jenk.) Fraser-Jenk./ (e.g. Stace 1991; Fraser-Jenkins 1993, 1996; Hutchinson & Thomas 1996; Widén & al. 1996).

Other pteridologists disagree with the interpretation of *D. affinis* as described above. Rather than confuse the existing nomenclature by re-combining epithets formally, for purposes of identification they use the term "morphotype" (which has no nomenclatural significance) to describe the variation of *D. affinis* (e.g. Jermy & Camus 1991; Pigott 1997; Krause 1998).

There exists a third point of view - treatment of the three subspecies as species on the

theoretical basis of their being biologically separate (e.g. Beitel & Buck 1988; Jermy, *personal communication*). Fraser-Jenkins decisively made a stand against the last two approaches and defended the “subspecies” view (for details see Fraser-Jenkins 1996).

All things considered, it is obvious that more investigations on morphology, size of variation, reproductive biology, molecular genetics, biochemistry of this complex fern species are still needed to clarify and resolve definitely the aggregate taxon “*Dryopteris affinis*”.

## Material and methods

Our studies are based on herbarium specimens from all Bulgarian herbaria, as well as personal collections of *D. affinis* subsp. *borreri* from different natural habitats in Bulgaria (see Appendix). Specimens of this taxon were detected only in SOM (Herbarium of the Institute of Botany, BAS). A corresponding map of distribution based on the 10 km UTM-grid is presented (Fig. 1).

Specimens of *D. filix-mas* used in spore studies as well as for morphological comparison of pinna-segments are listed in Appendix. All collections of the author have been deposited in SOM.

The sporophytic chromosome number was counted in squashed root-tip meristem. Root tips were pretreated with 0.5% colchicine for 3 to 5 hours, fixed in 96 % ethanol/glacial

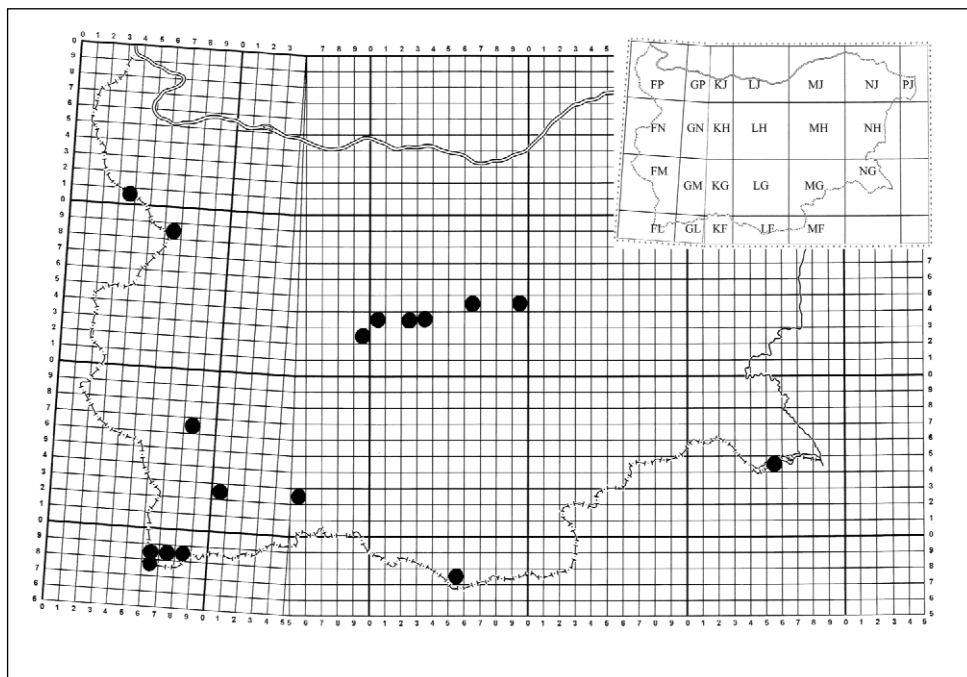


Fig. 1. Distribution of *Dryopteris affinis* subsp. *borreri* in Bulgaria presented on the UTM map (10 x 10 km).

acetic acid (3:1) and stored at -6 °C in a refrigerator for more than 24 h. Hydrolyzation was carried out with 1N hydrochloric acid for about 40 minutes at 60 °C. Root tips were stained in haematoxylin after Gomori (Melander & Wingstrand 1953) at 60 °C.

For an accurate spore study the light microscope as well as the scanning electron microscope had been used. For the LM investigation spores were mounted directly in Euparal on glass slides, without any chemical treatment. Fifty mature spores from every sample were measured. Measurements of spore length and width were made at the largest diameter, without perisporium.

For SEM investigation spores were air dried, attached to SEM stubs with double-sided scotch tape, coated with gold, and the spore sculpture was observed with a JEOL JSM-5300 SEM at 25 kV, at the magnification of 350-1500 x. The description terminology follows Ferrarini & al. (1986), Tryon & Lugardon (1991), and Punt & al. (1994).

## Results and discussion

### Range of *D. affinis* subsp. *borreri*

*Dryopteris affinis* subsp. *borreri* is a taxon of European sub-Atlantic and sub-palaeo-Mediterranean distribution. It occurs in Europe from Great Britain, Ireland and SW Norway to the Mediterranean (except Macaronesia), and from Portugal to SW Ukraine, Crimea, N Turkey, the Caucasus, Transcaucasia and the Caspian region of Iran, also in NW Africa (Morocco). It is absent from the far North and North-East of Europe and dies out in the far South Mediterranean region (Fraser-Jenkins 1980, 1982; Fraser-Jenkins & Reichstein 1984).

### Chorology and habitat of *D. affinis* subsp. *borreri* in Bulgaria

In comparison to the widespread in Bulgaria fern *D. filix-mas*, *D. affinis* subsp. *borreri* is a much rare taxon distributed in Western and Central Balkan Range, Southern Strouma valley, Mt Belasitsa, Pirin Mts, Rila Mts, Mt Sredna Gora, Western and Eastern Rhodopes and Mt Strandzha (Fig. 1, Appendix). From the map of distribution it can be seen that *D. affinis* subsp. *borreri* occurs rather irregularly throughout Bulgaria. In 6 floristic regions this taxon was found for the first time (see Appendix).

Ecological observation of the habitats of *D. affinis* subsp. *borreri* found till now indicates that the populations of this rare subspecies consist only of very few individuals in Bulgaria. The plants prefer moist and shady places, in wet gulches, along streamsides, on slopes and ditches along forest paths. The taxon appears mainly in beech forests. Less often it can be found in *Platanus orientalis* forests, *Castanea sativa* forests, *Picea abies* forests or mixed *Fagus sylvatica* - *Picea abies* - *Pinus silvestris* forests, or in open stony places, with no definite preference for calcareous or acidic substratum. *D. affinis* subsp. *borreri* always grows together with *D. filix-mas*. The vertical distribution varies from 200 to 1950 m a.s.l.

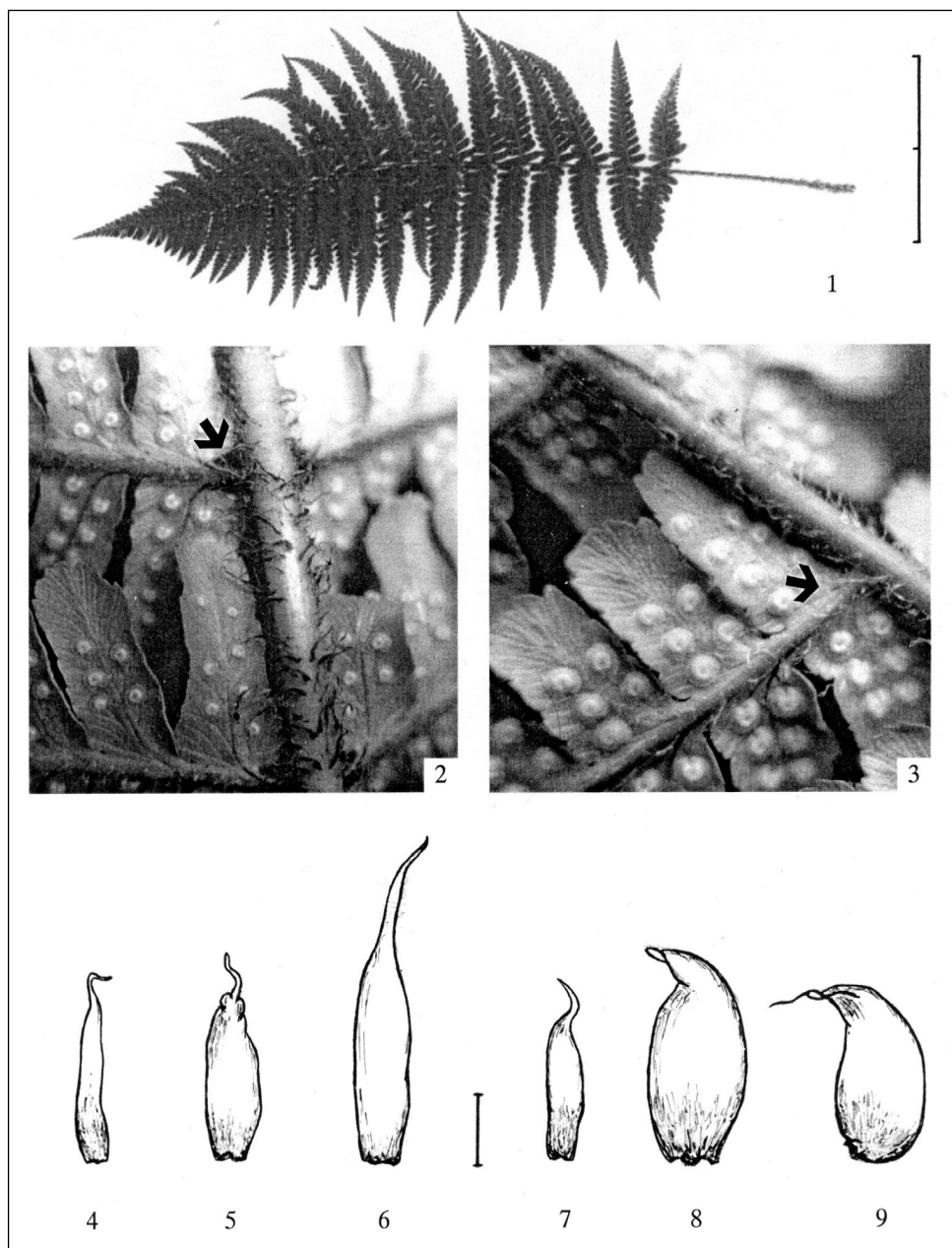


Plate I: 1. Fertile frond of *Dryopteris affinis* subsp. *borrieri* (DI-74.96). Scale bar = 10 cm; 2-3. Junction of the pinna midrib and the rachis: 2. *D. affinis* subsp. *borrieri*. Arrow shows the dark spot on underside at the junction; 3. *D. filix-mas* - there is no dark spot; 4-9. Form and size of scales of *D. affinis* subsp. *borrieri* and *D. filix-mas*: 4. Scale of *D. affinis* subsp. *borrieri* from the rachis; 5-6. Scales of *D. affinis* subsp. *borrieri* from the stipe; 7. Scale of *D. filix-mas* from the rachis; 8-9. Scales of *D. filix-mas* from the stipe. Scale bar = 5 mm.

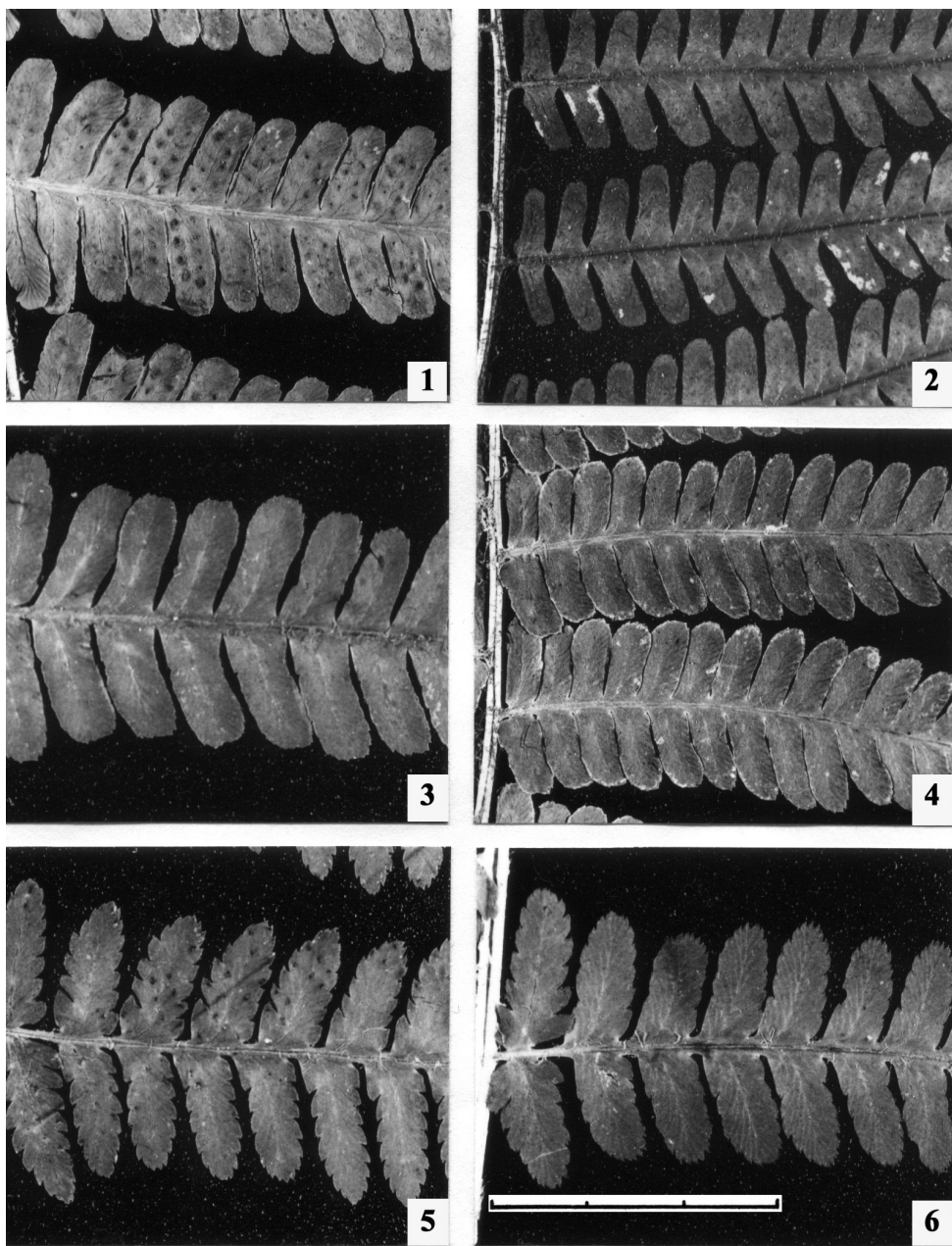


Plate II. 1-4. Variation of shape, margins and teeth of pinnules from the middle parts of fronds of *Dryopteris affinis* subsp. *borreri*: 1. DI-135.94, frond collected in the field; 2. Frond from the same plant, grown in a greenhouse, collected in 1998; 3. DI-63.98; 4. DI-167.94; 5-6. Variation of shape, margins and teeth of pinnules from the middle parts of fronds of *D. filix-mas*: 5. DI-153.98(A); 6. DI-62.98(A). Scale bar = 3 cm.

## Morphology

Mature specimens of *D. affinis*, growing in reasonably normal environmental conditions, are relatively easy to distinguish from *D. filix-mas* by their morphology. But the species is a morphologically variable obligate apomict, with very complex genomic origin, and this might pose sometimes problems of identification. It can also hybridize with *D. filix-mas*, which makes the identification of the taxon even more difficult.

In the same time, due to the agamic reproduction and absence of gametic fusion and in consequence of gene exchange minor local morphological variations are retained throughout the succeeding generations. It is important to remember this fact when identifying plants of *D. affinis*.

Morphological analysis of *D. affinis* specimens listed in the Appendix revealed that all Bulgarian materials collected till now belong to *D. affinis* subsp. *borreri*.

Jessen (1985) reported two subspecies of *D. affinis* from Bulgaria - subsp. *borreri* and subsp. *cambrensis*. We have explored several times the cited locality in Pirin Mts looking for subsp. *cambrensis*, but so far we have not found plants morphologically corresponding to this subspecies.

The basic diagnostic morphological characters distinguishing *D. affinis* subsp. *borreri* from *D. filix-mas* are presented in Table 1.

Table 1. Comparison of morphological features of *Dryopteris affinis* subsp. *borreri* and *D. filix-mas*.

Features	<i>D. affinis</i> subsp. <i>borreri</i>	<i>D. filix-mas</i>
Lamina	somewhat coriaceous	herbaceous, soft
Color of lamina	pale- to mid-green	dark- to pale-green or grey-green
Stipe	± short (1/5 to 1/3 leaf length), densely scaly; scales up to 5 mm broad (Plate I-5, 6)	medium length (1/4 to 1/3 leaf length), stipe-base moderately to densely scaly, scales becoming more scattered above; scales up to 12 mm broad (Plate I-8, 9)
Rachis	moderately densely scaly; scales up to 2.5 mm broad (Plate I-4)	sparsely scaly, or sometimes without scales; scales up to 4 mm broad (Plate I-7)
Form of scales	narrow, linear-lanceolate to ovate-lanceolate, acuminate (Plate I-4, 5, 6)	setiform or linear-lanceolate to ovate-lanceolate, cordate, hair pointed (Plate I-7, 8, 9)
Color of scales	pale-yellowish, reddish, ginger to brown, often with ± dark-brown basal regions	pale- to yellow-brown

Table 1. Continued.

Pinna midrib	(when living) pinnae with a dark brown or black spot on underside at junction of pinna and rachis (Plate I-2). In herbarium specimens this spot tends to disappear	without a dark spot on underside at junction of pinna and rachis (Plate I-3)
Pinna-segments (pinnules)	incompletely separated at the base (Plate II-1, 2, 3, 4), almost all adnate to the costa	completely separated at the base (Plate II-5, 6), almost all adnate to the costa except the lowest basiscopic one on lower pinnae, which may become stipitate
Apices of pinna-segments	varying from markedly squarely-truncate to somewhat pointed, usually at least somewhat obtuse (Plate II-1, 2, 3, 4)	slightly ovately rectangular-lanceolate, obtusely or acutely pointed (Plate II-5, 6)
Lateral margins of pinna-segments	variable but often straight- and noticeably parallel-sided (Plate II-1, 2, 3, 4); the innermost pair of pinnules of lower pinnae sometimes slightly auriculate or with a $\pm$ large, rectangular basal lobe	serrate (rarely deeply so or even lobed) (Plate II-5, 6)
Teeth of pinna-segments	very few or no teeth at the sides, $\pm$ scattered, wide based, but acutely pointed teeth at the apices (Plate II-1, 2, 3, 4)	acute teeth, pointing slightly inwards towards a point beyond the pinnule-apex (Plate II-5, 6)
Sori	small, (2-)4-5 on each side of the midrib of the pinna-segment (Plate I-2)	relatively large, forming 2 lines near and parallel with the midrib of the pinna-segment (Plate I-3)
Indusium	pale- to orange-brown, turned down at their sides and loosely surrounding the sorus when young, shrivelling and lifting (but not as much as <i>D. filix-mas</i> ) to a small inverted cone on ripening	white to pale-grey, slightly curved over the top of the sorus when young, but not turned down at the sides so as to surround the sorus, lifting and shrivelling markedly on ripening
Spores	dark good viable spores (Plate IV) (maximum 32 per sporangium), as well as a small proportion of abortive spores (Plate III-3, 4); ripening July - October	dark regular viable spores (Plate V) (64 per sporangium); ripening July - September



Table 1. Continued.

Spore size	42,5-67,5 x 25-37,5 $\mu\text{m}$	37,5-50 x 22,5-32,5 $\mu\text{m}$
Ploidy level and chromosome number	triploid, $2n = 123$ (Plate III-1, 2)	tetraploid, $2n = 164$
Breeding system	apomict	sexual

## Cytology

Two different ploidy levels ( $2n = 2x = 82$  and  $2n = 3x = 123$ ) are known to occur throughout the range of *D. affinis*. Nine individuals from six Bulgarian localities (see Appendix) have been studied cytologically for the first time. All accessions turned out triploid ( $2n = 123$ , Plate III-1, 2). Our result confirms reports by Manton (1939, 1950), Manton & Sledge (1954), Döpp (1955), Döpp & al. (1963), Vida (1963), Corley (1967), Schneller (1975), Nardi & Mori (1977), Heckmann & al. (1989), Bär & Eschelmüller (1989), Jäger & Leonhards (1995), Rasbach (1998) from other European countries.

## Spores

This is the first comparative study of the morphology (size variation and surface sculpture) of the spores of Bulgarian *D. filix-mas* and *D. affinis* subsp. *borreri* providing for additional distinguishing features. It is easy to determine *D. affinis* subsp. *borreri* with light microscope: it shows high proportion of viable good spores, but also a small quantity (not as many as in a typical hybrid) of sterile, “abortive” spores due to the apomictic nature of the species (Plate III-3, 4).

*D. affinis* subsp. *borreri* has maximum 32 viable spores per sporangium, the remaining being abortive material of irregular and distorted shapes (Plate VI-1, 2). The viable spores are 42,5 - 67,5  $\mu\text{m}$  long, 25 - 37,5  $\mu\text{m}$  wide, monolete, heteropolar, bilaterally symmetrical with elliptic profile, concavely convex to piano-convex (Plate IV). The perisporium is ectovermiculate with elongated elements, forming sometimes a reticulum-like pattern (Plate VI-3, 4, 5, 6). The surface is irregularly scabrate under SEM.

*D. filix-mas* has regular viable spores, 64 per sporangium, 37,5 - 50  $\mu\text{m}$  long, 22,5 - 32,5  $\mu\text{m}$  wide, monolete, heteropolar, bilaterally symmetrical with short elliptic profile, piano-convex to slightly biconvex (Plate V). The perisporium is roundly tuberculate-ectovermiculate, with coarse short tubercles and irregular, short, inflated folds (Plate VI-7, 8). The surface is finely venulated.

## Conclusions

The C. R. Fraser-Jenkins’ visit to Bulgaria in 1987 and the followed revisions of materials in the herbarium SOM, as well as the investigations on genus *Dryopteris* started some years ago, turned our attention to the species *D. affinis*, which has not been mentioned up to that moment in the Bulgarian literature sources.

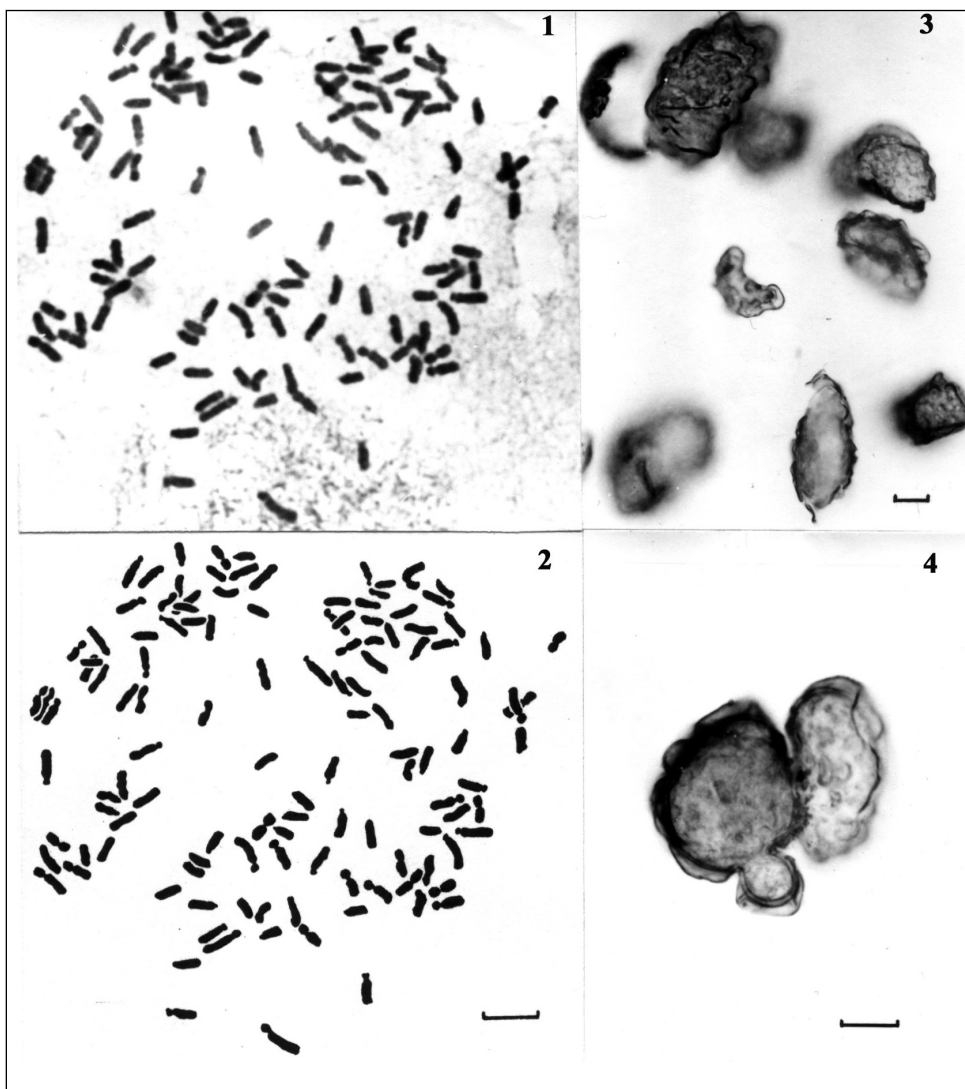


Plate III. 1-4. Chromosomes and spores of *Dryopteris affinis* subsp. *borreri*: 1. Microphotograph and 2. explanatory diagram of mitotic metaphase plate,  $2n = 123$  (DI-135.94); 3-4. LM microphotographs of abortive spores with irregular shape and size (DI-74.96). Scale bars = 10  $\mu\text{m}$ .

The current research has confirmed the distribution of *D. affinis* subsp. *borreri* in Bulgaria. It has been established that it occurs rarely and in various habitats, does not form big clone-populations, but only single individuals, growing always in the presence of *D. filix-mas*.

In spite of the great morphological variability mature, well-developed fronds of *D. affi-*

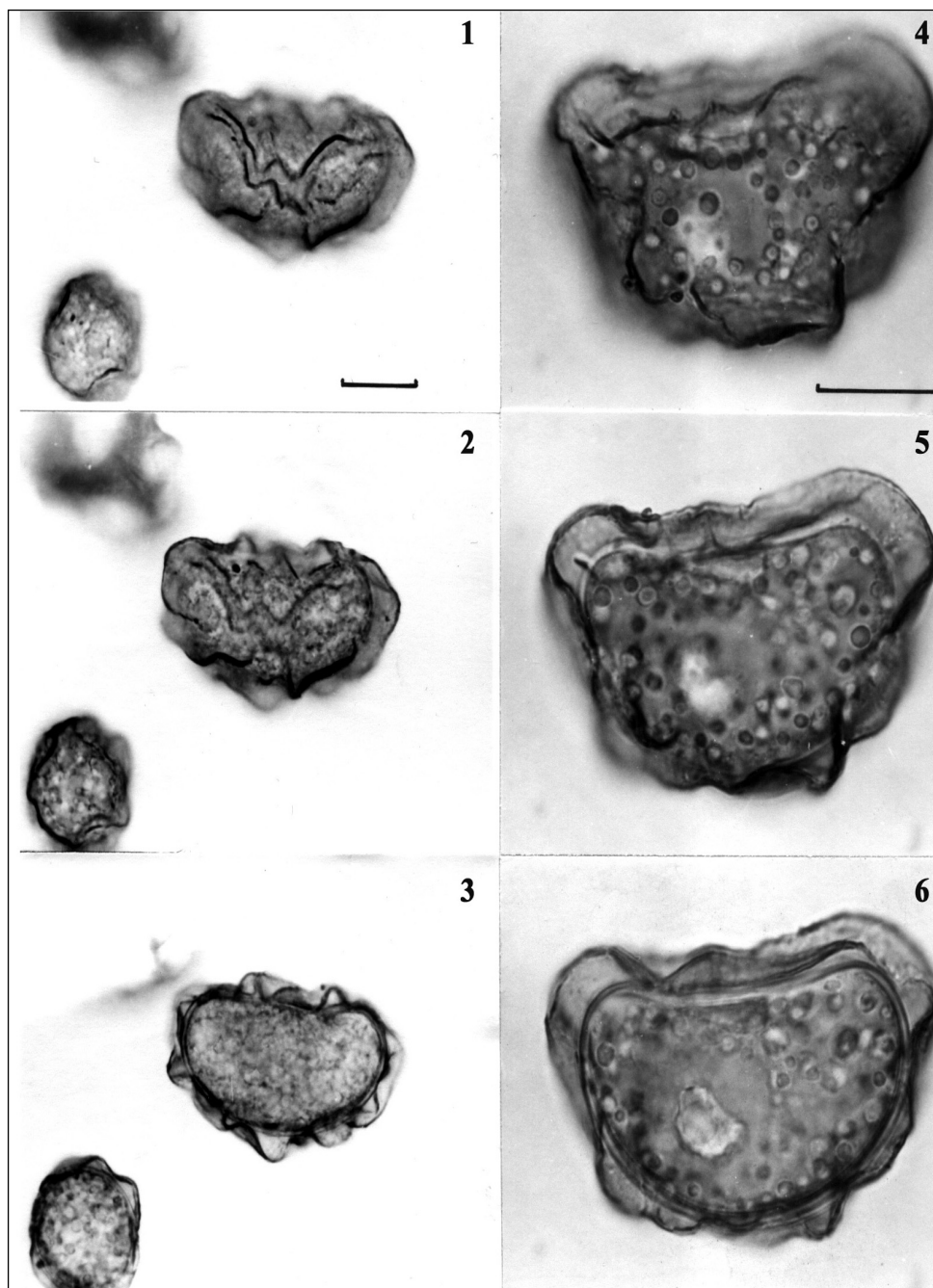


Plate IV. 1-6. LM microphotographs of spores of *Dryopteris affinis* subsp. *borrieri*: 1-3. Two spores of *D. affinis* subsp. *borrieri* (DI-167.94) are illustrated, the upper is good and viable, the lower is abortive; 4-6. Spore of *D. affinis* subsp. *borrieri* (DI-135.94). Scale bars = 20  $\mu$ m.

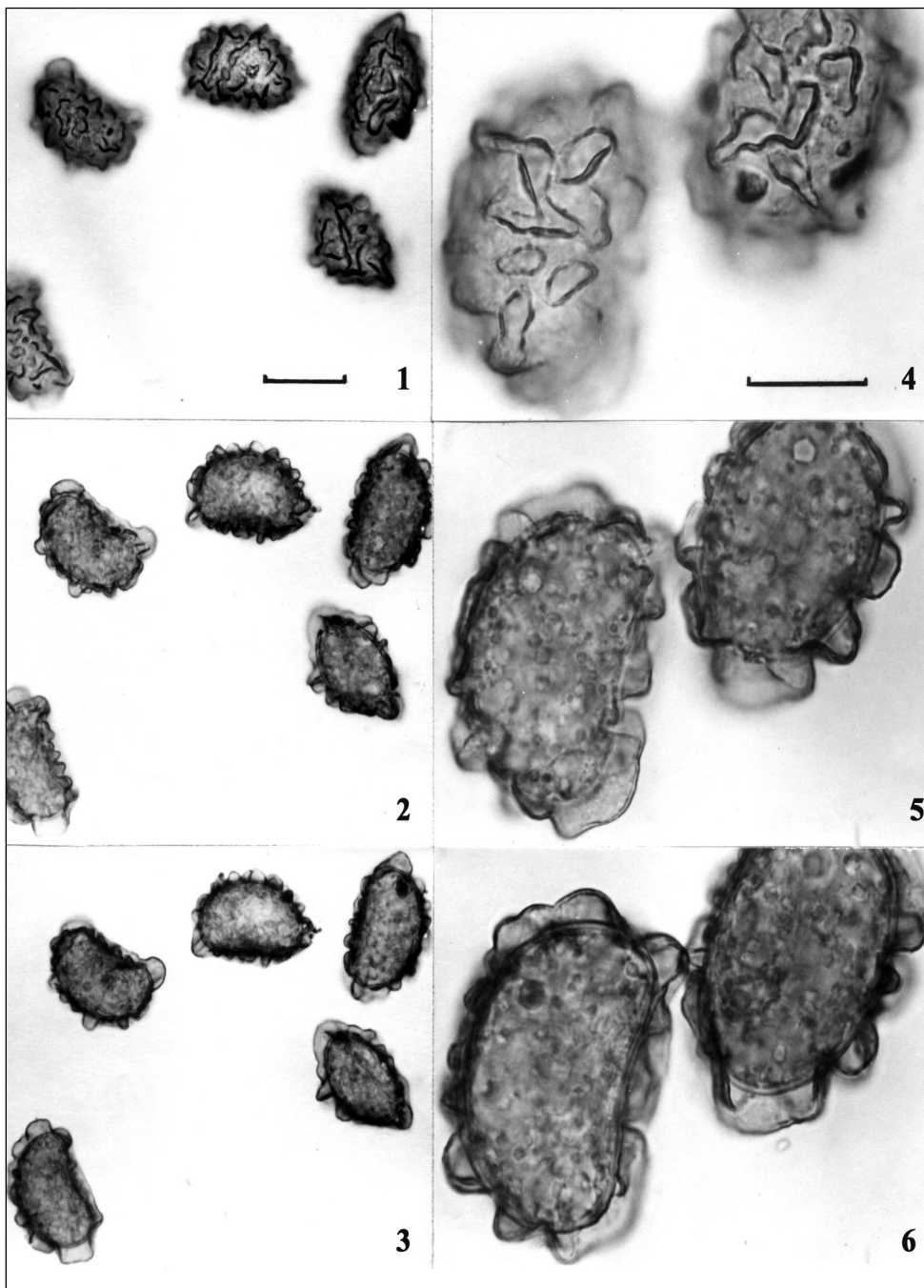


Plate V. LM microphotographs of spores of *Dryopteris filix-mas*: 1-3. DI-72.98(A); 4-6. DI-72.98(A). Scale bars = 20  $\mu$ m.

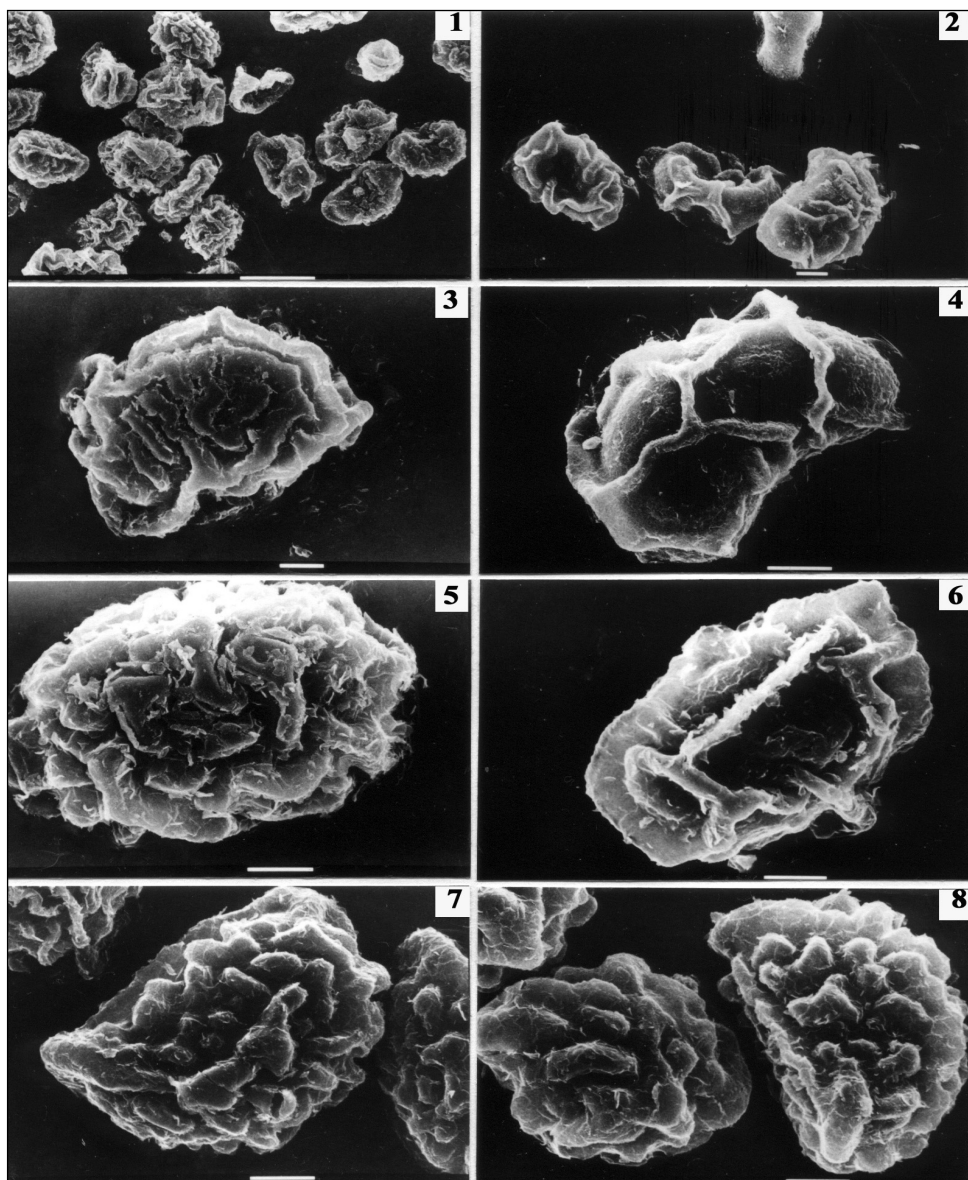


Plate VI. 1-2. SEM microphotographs of abortive spores of *Dryopteris affinis* subsp. *borrieri*: 1. DI-135.94, scale bar = 50 µm; 2. DI-63.98, scale bar = 10 µm; 3-6. SEM microphotographs of normal spores of *D. affinis* subsp. *borrieri*: 3. Spore in lateral view, ectovermiculate perisporium, DI-74.96; 4. Spore in lateral view, ectovermiculate perisporium with elongated elements, forming a reticulum-like ornamentation, DI-63.98; 5. Spore in distal view, ectovermiculate perisporium, DI-74.96; 6. Spore in proximal-lateral view, showing the laesura, DI-167.94; 7-8. SEM microphotographs of spores of *D. filix-mas*: 7. Spore in lateral view, tuberculate-ectovermiculate perisporium, DI-84.98; 8. Spore on left in distal view, spore on right in lateral-proximal view, tuberculate-ectovermiculate perisporium, DI-118.98(A). Scale bars = 10 µm.

*nis* can be distinguished comparatively easy from those of *D. filix-mas*. The variation of Bulgarian materials falls into the range of *D. affinis* subsp. *borreri* variation in other parts of its area. In this article the basic morphological features for distinguishing *D. affinis* subsp. *borreri* and *D. filix-mas* are shown.

When macromorphological characters of the collected material are not enough distinct and reliable, additional microscopic methods, e.g. cytological (if live material is available), as well as spore analysis can also lead to good identification. At the current research the differences of spore size and surface sculpture between *D. filix-mas* and *D. affinis* subsp. *borreri* have been established. It has been found out as well that only the triploid race of the apomict fern is distributed in Bulgaria. The chromosome count alone is quite enough to distinguish *D. affinis* from the tetraploid *D. filix-mas* or from any other tetraploid taxon. However, this is not enough to determine the intra-specific taxa, because there exist 3 triploid subspecies in Europe (Widén & al. 1996). Therefore, the application of morphological, cytological and spore analyses is desirable for the right determination of specimens.

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## Appendix

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### *Dryopteris affinis* subsp. *borreri*

#### List of herbarium specimens used

##### For morphological investigation:

**\*Western Balkan Range** - southwestward of Berkovitsa town, Haidoushki Vodopadi locality, along river Golema Reka, 500 m a.s.l., 14/06/1996, leg. *M. Nikolova & G. Terziyski*, det. *D. Ivanova* *DI-8.96* (SOM-155408), *DI-9.96* (SOM-155409); above Gorni Lom village, along the trail to peak Midzhour, by river Burza Reka, 800 m a.s.l., 29/07/1993, leg. *V. Vladimirov*, det. *D. Ivanova* *DI-10.93* (SOM-155411);

**Central Balkan Range** - Troyan mts: Yumrouk-Chal, 1899, leg. *I. Urumov*, det. *B. Davidov* (SOM-236); Yavorova Luka chalet (Troyan district), 10/08/1978, *N. Andreev* (SOM-136207); Troyan mts, leg. *St. Grancharov*, det. *B. Davidov* (SOM-235); ad rivulum Sjunkevitzta ad urbem Gabrovo, 400 m alt., 06/06/1907, *B. Davidov* (SOM-204); ad rivulum Sjunkevitzta prope urbem Gabrovo, 450 m alt., 06/06/1907, *B. Davidov* (SOM-153311); between Tuzha chalet and Apriltsi town, 1550 m a.s.l., 31/07/1996, *D. Ivanova* *DI-71.96* (SOM-155418), *DI-74.96* (SOM-155419); between Tuzha chalet and Apriltsi town, 1200 m a.s.l., 31/07/1996, *D. Ivanova* *DI-74.96(A)* (SOM-155420); above Troyan: Kozya Stena chalet, 21/07/1998, leg. *D. Dimitrova & V. Vladimirov*, det. *D. Ivanova* *DI-12.98(A)* (SOM-155421); Elena mts: Savchov Chair Reserve, 13/07/2000, leg. *Ch. Gousev*, det. *D. Ivanova* *DI-32.00* (SOM 157735);

**\*Southern Strouma valley** - between Klyuch village and river Stroumeshnitsa (Petrich district), along the stream, 420 m a.s.l., 09/05/2003, *D. Ivanova* *DI-12.03* (SOM 159683);

**\*Mt Belasitsa** - between Petrich town and Belasitsa village, 400 m a.s.l., 29/08/1994, *D. Ivanova* *DI-116.94* (SOM-155413); between Skrut village and Partizanski Shtab locality, 1150 m a.s.l., 30/08/1994, *D. Ivanova* *DI-135.94* (SOM-155185); southwestward of Kolarovo village, Petrich district, 450 m a.s.l., 01/09/1994, *D. Ivanova* *DI-166.94* (SOM-155414), *DI-167.94* (SOM-155415);

**Pirin Mts** - in front of Vihren chalet, 1950 m a.s.l., 17/08/1994, *D. Ivanova* *DI-107.94* (SOM-155412);

**Rila Mts** - along river Rilska, Veli Lak locality, 26/09/1978, *P. Panov* (SOM-142503);

**Mt Sredna Gora** - peak Bogdan, *A. Toshev* (SOM-212);

**\*Western Rhodopes** - above Surnitsa village, 1250 m a.s.l., 16/09/1994, *D. Ivanova* *DI-198.94* (SOM-155416); above Surnitsa village, 1250 m a.s.l., 04/08/1998, *D. Ivanova* *DI-63.98* (SOM-155184), *DI-64.98* (SOM-155422);

**\*Eastern Rhodopes** - above Chakalarovo village, on the western slope of peak



Sredniya Koulei above Arabadzhyskoto locality, 14/08/1991, *D. Ivanova* *DI-4.91(a)* (SOM-155410);

**\*Mt Strandzha** - along the road ca. 6 km before Slivarovo village, ca. 200 m a.s.l., 21/06/1995, *D. Ivanova* *DI-121.95* (SOM-155417).

(\*Floristic regions for which *Dryopteris affinis* subsp. *borreri* is reported for the first time).

#### **For cytological investigation:**

**Central Balkan Range** - between Tuzha chalet and Apriltsi town, 1550 m a.s.l., 31/07/1996, *D. Ivanova* *DI-71.96* (SOM-155418), *DI-74.96* (SOM-155419);

**Mt Belasitsa** - between Skrut village and Partizanski Shtab locality, 1150 m a.s.l., 30/08/1994, *D. Ivanova* *DI-135.94* (SOM-155185); southwestward of Kolarovo village, Petrich district, 450 m a.s.l., 01/09/1994, *D. Ivanova* *DI-166.94* (SOM-155414), *DI-167.94* (SOM-155415);

**Pirin Mts** - in front of Vihren chalet, 1950 m a.s.l., 17/08/1994, *D. Ivanova* *DI-107.94* (SOM-155412);

**Western Rhodopes** - above Surnitsa village, 1250 m a.s.l., 04/08/1998, *D. Ivanova* *DI-63.98* (SOM-155184), *DI-64.98* (SOM-155422);

**Mt Strandzha** - along the road ca. 6 km before Slivarovo village, ca. 200 m a.s.l., 21/06/1995, *D. Ivanova* *DI-121.95* (SOM-155417).

#### **For spore investigation:**

**Central Balkan Range** - between Tuzha chalet and Apriltsi town, 1550 m a.s.l., 31/07/1996, *D. Ivanova* *DI-74.96* (SOM-155419);

**Mt Belasitsa** - between Skrut village and Partizanski Shtab locality, 1150 m a.s.l., 30/08/1994, *D. Ivanova* *DI-135.94* (SOM-155185); southwestward of Kolarovo village, Petrich district, 450 m a.s.l., 01/09/1994, *D. Ivanova* *DI-167.94* (SOM-155415);

**Western Rhodopes** - above Surnitsa village, 1250 m a.s.l., 04/08/1998, *D. Ivanova* *DI-63.98* (SOM-155184).

#### ***Dryopteris filix-mas***

#### **List of selected herbarium specimens used**

#### **For morphological investigation:**

**Rila Mts** - between Rila Monastery and Kirilova Polyana locality, along river Rilska, 1250 m a.s.l., 01/09/1998, *D. Ivanova* *DI-153.98(A)* (SOM-155426);

**Western Rhodopes** - Dospat dam, along the southern road from Dospat to Surnitsa, 1200 m a.s.l., 04/08/1998, *D. Ivanova* *DI-62.98(A)* (SOM-155190).

**For spore investigation:**

**Pirin Mts** - near lake Bezbog, c. 2260 m a.s.l., 29/08/1998, *D. Ivanova* DI-118.98(A) (SOM-155425);

**Rila Mts** - Gyolechitsa locality, by river Cherni Iskur, c. 1300 m a.s.l., 09/08/1998, *D. Ivanova* DI-84.98 (SOM-155424);

**Western Rhodopes** - by the Vassil Kolarov dam, c. 1500 m a.s.l., 04/08/1998, *D. Ivanova* DI-72.98(A) (SOM-155423).