Polypodium interjectum and *P.* ×*mantoniae* (*Polypodiaceae*: *Pteridophyta*), new to the Bulgarian flora

Daniella Ivanova

Institute of Botany, Bulgarian Academy of Sciences, Acad. G. Bonchev St., bl. 23, 1113 Sofia, Bulgaria, e-mail: dani@bio.bas.bg

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Abstract. There are three closely related species of *Polypodium* in Europe: *P. cambricum*, *P. vulgare*, and *P. interjectum*. Although the first two species have been reported in the Bulgarian botanical literature, only *P. vulgare* s.str. has been so far confirmed. At present there is no evidence on the occurrence of *P. cambricum* in Bulgaria. This paper reports two taxa of *Polypodium* as new to the Bulgarian flora. These are *P. interjectum* and the natural hybrid *P.* ×*mantoniae* (*P. interjectum* × *P. vulgare*). Plants from several sites were subjected to morphological, cytological and spore analyses. The mitotic chromosome counts revealed a pentaploid number $2n = 185$ for the hybrid individuals, and a hexaploid $2n = 222$ for *P. interjectum*. A morphological comparison between Bulgarian *P. interjectum* and *P. vulgare* was made. The frond morphology of the hybrid *P.* ×*mantoniae* was intermediate between the two parents, and the spores were abortive, greatly varying in shape and size. Spore characteristics of *P. interjectum* and *P. vulgare* are noted. Distribution maps of the studied taxa in Bulgaria are presented. *Polypodium interjectum* and *P.* ×*mantoniae* are known to exist in many European countries. These records of the two taxa in Bulgaria extend the knowledge on their distribution.

Key words: Bulgaria, chorology, cytology, ferns, morphology, *Polypodium interjectum*, *P.* ×*mantoniae*, spore analysis

Introduction

Three species of the *Polypodium vulgare* complex are known to occur in Europe. These are the diploid *P. cambricum* L., the tetraploid *P. vulgare* L. s.str., and the hexaploid *P. interjectum* Shivas. The last species has never been recorded in the Bulgarian botanical literature. Until 1963, only *P. vulgare* had been recognized in the different editions of the *Flora of Bulgaria* (Stojanov & Stefanov 1924, 1933, 1948; Stojanov & al. 1966). Achtarov (1963) first mentioned *P. vulgare* var. *serratum* Willd. (= *P. cambricum*) for the northern part of the Valley of Strouma River (NW of Boboshevo village, Kyustendil district). Based on this record, Andreev (1992) and Assyov & al. (2002) also reported *P. cambricum*. Unfortunately, all attempts to locate either a more detailed literature record or a herbarium specimen to document the presence of this species in Bulgaria were unsuccessful.

During a field trip in 1995, looking for *P. cambricum*, the author collected a "strange-appearing" *P. vulgare* near Pastouh village in the Valley of Strouma River. Some plants were taken for cytological investigation. The mitotic chromosome counts revealed a pentaploid level, $2n = 185$, suggesting a hybrid nature. Surprisingly, one plant showed $2n = 222$ corresponding to *P. interjectum*. In 1998 the author revisited the same region together with Clive Jermy and Patrick Acock and more specimens were collected. They all were hybrids and were deposited in the herbaria of the Institute of Botany in Sofia (SOM) and

the Natural History Museum in London (BM). These finds provoked a search at other sites in Bulgaria during the following years. The plants were mostly genuine *P. vulgare*, but some turned out to be *P. interjectum,* or the natural hybrid *P.* ×*mantoniae* (Rothm.) Shivas. Their identity was confirmed by several methods.

Material and methods

This study was based on personal collections of *Polypodium* from different natural habitats in the period 1991–2004. Localities of the newly recorded taxa *P. interjectum* and *P.* ×*mantoniae* are given in Table 1. Distribution maps of the two taxa based on the 10 km UTM grid are presented (Maps 1, 2). All collections of the author are deposited in the herbarium at the Institute of Botany, Bulgarian Academy of Sciences (SOM).

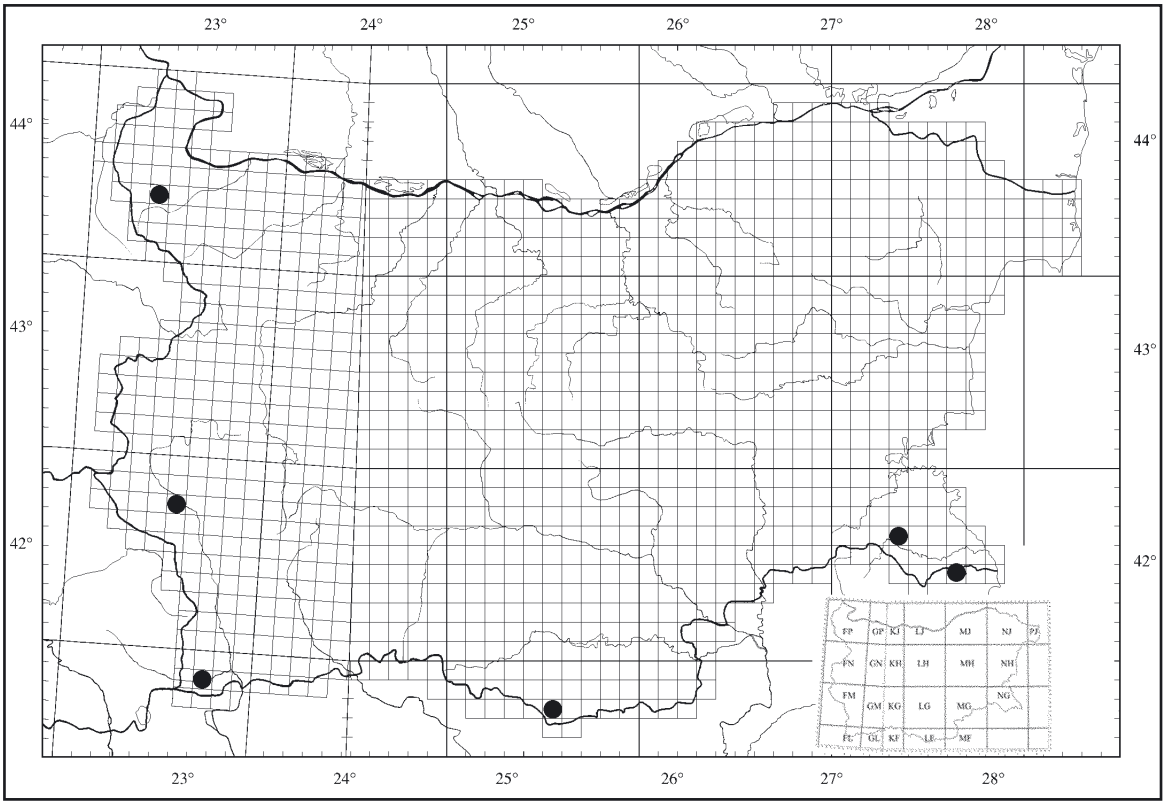
Morphological, cytological and spore analyses were carried out. The sporophytic chromosome number was counted in squashed root-tip meristem, following the method described in Ivanova (2004).

For the spore and sporangia studies a light microscope (LM) was used. Spores and sporangia were mounted in Euparal on glass slides, without chemical treatment. Abortive spores of *P.* ×*mantoniae* were not measured. Seven plants from *P. interjectum* and five plants from *P. vulgare* were selected, and 50 well-developed spores from each plant were measured. Measurements of exospore length and width were made at the largest diameter, and not including the perispore. Only spores whose long axis was perpendicular to the line of sight were measured. The average values, as well as the standard deviation and the coefficient of variation for every sample are presented in Table 3. Table 2 shows the minimum, grand mean, standard deviation, and maximum values of spore measurements for all plants studied. The terminology follows Ferrarini & al. (1986), Tryon & Lugardon (1991), and Punt & al. (1994).

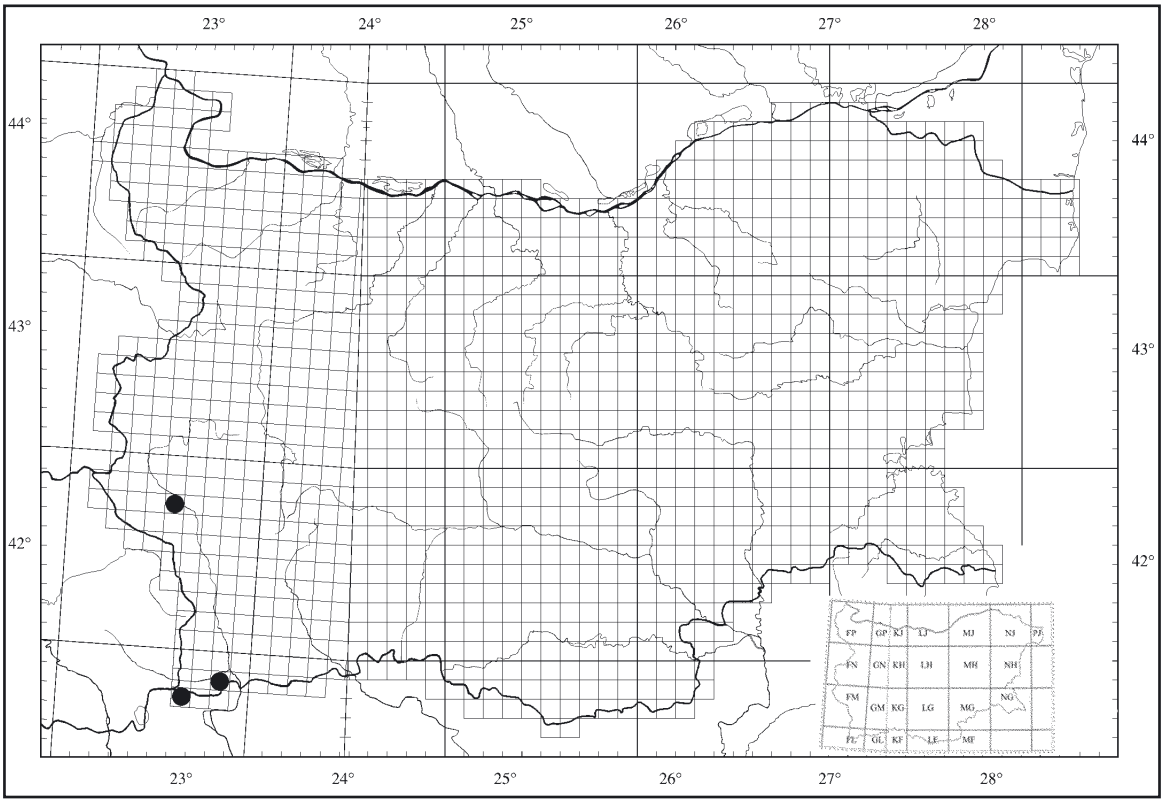
The numbers of the thick-walled cells and basal cells of 50 sporangia from seven plants of *P. interjectum*, 17 plants of *P.* ×*mantoniae* and five plants of *P. vulgare* were counted. The ranges for these characters, as well as the average number of indurated cells are presented in Table 3.

Table 1. Localities of the studied *Polypodium* taxa.

No	Locality, collection date and collector's name	Voucher number
	P. interjectum	
1	The Rhodopes (*Eastern*), at frontier checkpoint above Chakalarovo village, on the western slope of peak Sredniya Koulei above Arabadzhiyskoto locality, 14.08.1991, D. Ivanova	DI-4.91(c)
2	Valley of Strouma River (*Northern*), near Pastouh village, by the road to Boboshevo, 19.07.1995, D. Ivanova	DI-217.95
3 4 5 6	Mt Belasitsa, southwestwards of Kolarovo village (Petrich district), by the river, on serpentine, c. 400-500 m, 31.08.1998, D. Ivanova	DI-137.98 DI-137.98(1) DI-137.98(2) DI-137.98(3)
7	Mt Strandzha, westwards of Mladezhko village, by a stream near the sources of river Mladezhka, 06.05.2004, leg. B. Assyov & A. Petrova, det. D. Ivanova	DI-15.04
8	Mt Strandzha, Ouzounboudzhak Reserve, near Maluk Boudzhak locality, 09.11.2004, D. Ivanova	DI-79.04
9	Forebalkan (*Western*), along the trail from Belogradchik town to Planinitsa chalet, c. 700 m, 13.07.1995, D. Ivanova	DI-196.95
	P.* ×*mantoniae	
10	Mt Belasitsa, above Skrut village (Petrich district), along the dirt road from the village to Partizanski Shtab locality, near a stream, c. 1150 m, 30.08.1994, D. Ivanova	DI-134.94
11 12 13 14 15 16	Valley of Strouma River (*Northern*), near Pastouh village, by the road to Boboshevo, 19.07.1995, D. Ivanova	DI-218.95 DI-218.95(1) DI-218.95(2) DI-218.95(3) DI-218.95(4) DI-219.95
17 18 19 20 21	Valley of Strouma River (*Northern*), near Pastouh village, by the road to Boboshevo, 01.09.1998, D. Ivanova, C. Jermy & P. Acock	DI-142.98(1) DI-142.98(2) DI-142.98(3) DI-142.98(4) DI-142.98(5)
22 23 24 25 26	Valley of Strouma River (*Northern*), between Boboshevo and Pastouh village, 6-7 km from Boboshevo, 01.09.1998, D. Ivanova, C. Jermy & P. Acock	DI-143.98 DI-144.98 DI-145.98 DI-146.98 DI-146.98(A)
27	Mt Belasitsa, at Vodopada locality above Belasitsa chalet, 11.07.2000, D. Ivanova	DI-6.00
	P. vulgare	
28	Mt Vitosha, near Boyana waterfall, c. 1200 m, 07.08.1997, D. Ivanova	DI-13.97(1)
29	Mt Sredna Gora (*Western*), Lozenska Mt, peak Visokata Ela, 20.07.1999, A. Vitkova & D. Tsvetanova	DI-19.99
30	Vitosha Region, Plana Mt, along the trail to Kokalyane Monastery, 05.05.2001, D. Ivanova	DI-5.01
31	West Frontier Mts, Mt Osogovska, valley of the river Glogozka above Zhilentsi village, 24.09.2003, D. Ivanova	DI-70.03
32	Mt Belasitsa, along the trail from Belasitsa chalet to Vodopada locality, 27.04.2004, D. Ivanova	DI-11.04



Map 1. UTM grid map of the distribution of *P. interjectum* in Bulgaria.



Map 2. UTM grid map of the distribution of *P.* ×*mantoniae* in Bulgaria.

Results and discussion

Chorology and habitat preferences of *P. interjectum* and *P. ×mantoniae* in Bulgaria

Polypodium interjectum and *P. ×mantoniae* occur in many European countries. A compilation of their distribution is given in Bureš & al. (2003). The present record of the two taxa in Bulgaria extends their range.

In comparison to the widespread *P. vulgare*, *P. interjectum* is much more rare in Bulgaria. It is distributed in the floristic regions of the Forebalkan (*Western*), the Valley of Strouma River (*Northern*), Mt Belasitsa, the Rhodopes (*Eastern*), and Mt Strandzha (Table 1, Map 1). Both species usually require different ecological conditions, but in some places they meet and form vigorous clones of sterile hybrids (Vida 1972). At present, the hybrid *P. ×mantoniae* was found only in several localities in the Valley of Strouma River (*Northern*) and on Mt Belasitsa (Table 1, Map 2).

Polypodium interjectum occurs in moist, shady habitats in beech, oak or mixed deciduous forests, or on sun-exposed rocks in open habitats. It seems to be more thermophilous than *P. vulgare*.

Polypodium ×mantoniae grows in moist habitats in *Castanea sativa – Fagus sylvatica – Quercus* sp. forests, or in dry places on exposed rocks and on rocks in *Carpinus betulus* shrubs. The hybrid is sterile but can spread vegetatively and forms vigorous clone-populations, occasionally occurring together with one of the parents. Some authors (e.g., Dostál & Reichstein 1984) note that there is a risk that *P. ×mantoniae* may reduce or displace the local populations of the less competitive *P. interjectum*.

Since most hybrids are formed where the two parents grow close to each other, hybrid plants would likely occur at other sites in Bulgaria, too.

Morphology

Identification of polypodies on the basis of field characters alone is unreliable. Morphological characters used in the identification keys can only give an indication of what the plant is most likely to be. Microscopic examination is necessary to confirm the identification. Sometimes many of the collected "interjectums" turned out to be *P. vulgare*.

Polypodium vulgare and *P. interjectum* can generally be distinguished by leaf and sorus shape. However, these are marginal differences that are not always conclusive. Sometimes both species are much more variable than indicated in the Floras. *Polypodium interjectum* has a hybrid origin, one of its parents being *P. vulgare*. Meinders-Groeneveld & Segal (1967) noted that most of the differential characters of both species reported in literature overlap rather broadly. Furthermore, there are forms of polypodies that cannot be identified as either of the two species. Most of these are hybrids that at closer examination show intermediate leaf characters between the two parental species. Therefore, it is often necessary to use a "syndrome" of characters for the correct determination of specimens as suggested by Meinders-Groeneveld & Segal (1967).

Examination of sporangia under the microscope is usually required for accurate identification. The exam-

Table 2. Comparison of some discriminating features between *P. interjectum* and *P. vulgare* in Bulgaria.

Features	*P. interjectum*	*P. vulgare*
Frond shape	(narrowly) ovate, ovate-lanceolate to oblong-lanceolate (Plate I, Figs 1–3)	linear, lanceolate to oblong (Plate I, Figs 8, 9)
Apices of pinnae	acuminate to acute, occasionally rounded (Plate I, Figs 1–3)	rounded, obtuse or occasionally acute (Plate I, Figs 8, 9)
Basal pair of pinnae	generally projecting forward	generally not projecting forward
Immature sori	initially mostly elliptic (oval); usually ochreous, more or less suffused with orange	mostly orbicular (round), rarely broadly elliptic; frequently reddish-brown
Colour of ripe annulus	quite variable in different sporangia: pale buff, pale yellow, orange-yellow, golden brown or pale-brown	dark reddish-brown
Number of indurated cells per annulus	(6) 9–14 (20) (Plate II, Figs 1, 2, 6, 7)	(9) 11–13 (14) (Plate II, Fig. 4)
Number of basal cells per sporangium	1–3 (exceptionally 4) most often 2 (Plate II, Figs 6, 7)	0–2 most often 1 (Plate II, Fig. 4)
New fronds produced	in summer and autumn	in spring and early summer
Spores ripe	summer to autumn	in summer
Spore length	(59.1) – 75.58 ± 6.58 – (104.7) µm	(51.2) – 60.71 ± 4.06 – (74.3) µm
Spore width	(35.6) – 47.56 ± 5.12 – (77.9) µm	(30.1) – 39.34 ± 3.36 – (54.5) µm
Chromosome number	2n = 222 (Plate IV, Fig. 1)	2n = 148 (Plate IV, Fig. 3)

iner needs mature sporangia, though old fronds from the previous year may provide the material necessary to obtain the microscopic data. Fern leptosporangia have a row of thick-walled (indurated) cells forming the annulus, which upon drying helps open the sporangium and eject the spores. The number of indurated cells varies slightly within each European species of *Polypodium*, while the ranges partly overlap between the species. Another feature that facilitates the distinction of the species of *Polypodium* is the number of non-indurated cells between the base of the annulus and the top of the sporangium stalk.

A comparison of some discriminating features between *P. interjectum* and *P. vulgare* in Bulgaria is presented in Table 2. Macromorphological analysis of Bulgarian plants showed that *P. vulgare* and *P. interjectum* are very variable. Variation of some fronds is shown in Plate I (Figs 1–3, 8, 9). The frond morphology of the hybrid *P.* ×*mantoniae* is intermediate between that of the two parents (Plate I, Figs 4–7). A study of the sporangial contents coupled with a cytological analysis is necessary to detect hybrids.

Some authors (e.g., Bureš & al. 2003) claim that one of the most useful characters for identification of the taxa of *Polypodium* is the number of indurated annulus cells. Mean values for *P. interjectum* given in various papers and Floras are 5–9, but seldom higher. The results obtained in the present analysis are different in this respect. The number of cells per annulus varied considerably, ranging from 6 to 20 cells (Plate II, Figs 1, 2), frequently 9–14, and with an average of 12 cells. Mean values for individual plants varied from 9.3 to 14.4 (Table 3).

The results obtained for *P. vulgare* did not differ significantly from the data given in earlier publications and Floras: a higher proportion of sporangia had 11–13 cells, seldom 9–10 or 14, with a mean number of 11.8 per annulus. Individual plants showed low variation of mean values: from 11.1 to 12.3 (Table 3).

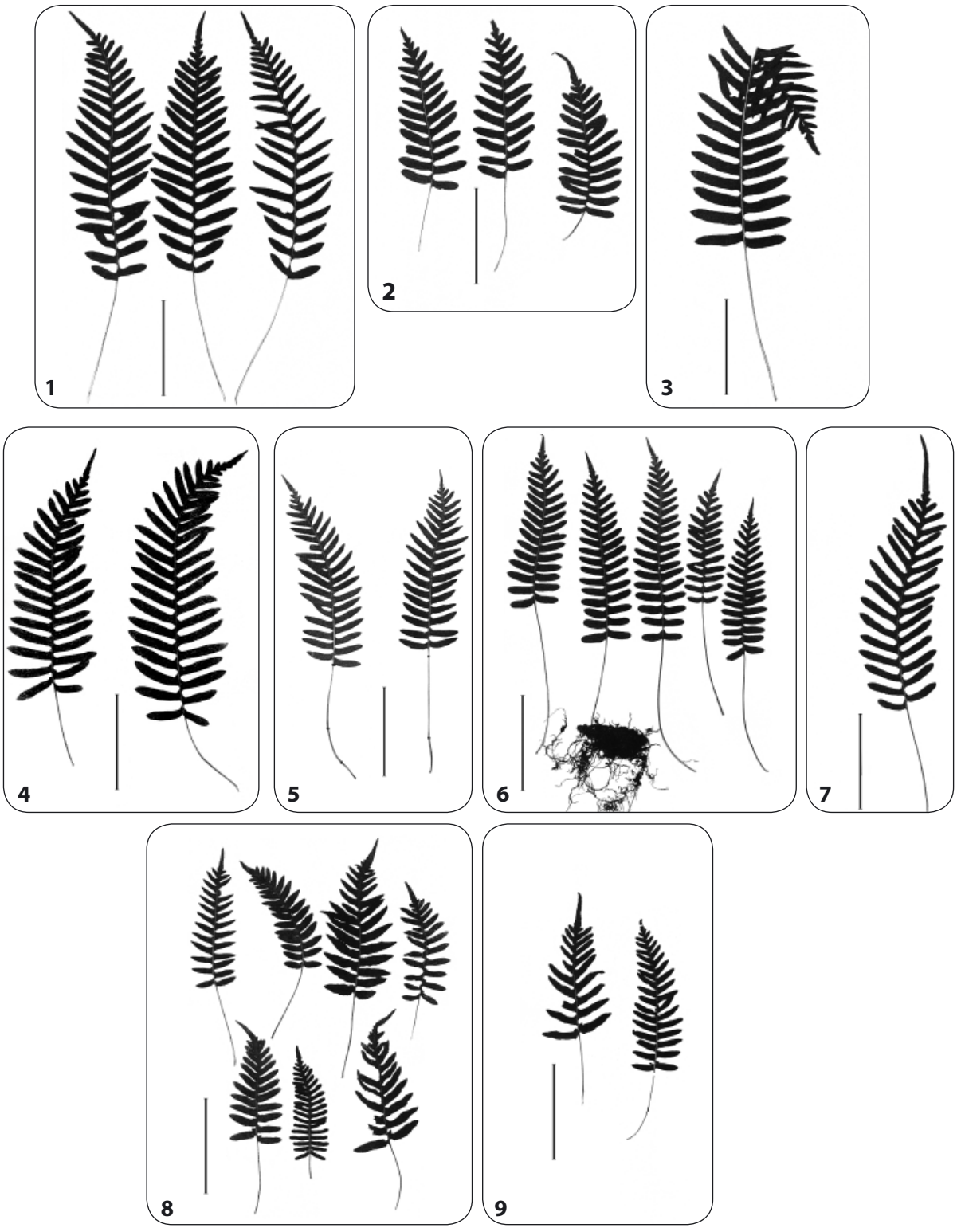
The number of indurated cells per annulus of the hybrid *P.* ×*mantoniae* was (7) 10–13 (17), with an average of 11.8, and individual mean values varying from 10.4 to 13.9 (Table 3); the number of basal cells per sporangium was (0) 1–2 (very seldom 3). Basal cells of the three taxa are shown in Plate II (Figs 3–7).

Table 3. List of the studied *Polypodium* individuals.

No.	Method applied	2n	Spore length			Spore width			Number of indurated cells per annulus		Number of basal cells per sporangium (range)
			Mean (µm)	SD	CV (%)	Mean (µm)	SD	CV (%)	Range	Mean	
	P. interjectum										
1	M, C, S	222	66.9	3.84	5.75	44.6	2.95	6.63	(8-) 13-18 (-20)	14.4	(1-) 2 (-3)
2	M, C, S	222	76.6	6.07	7.94	48.9	3.89	7.95	(7-) 10-11 (-14)	10.1	(1-) 2 (-3)
3	M										
4	M, C, S	222	75.1	4.33	5.77	45.2	2.69	5.95	(9-) 12-16 (-19)	13.2	(1-) 2-3 (4)
5	M, S		80.8	6.09	7.55	52.4	5.78	11.05	(7-) 11-14 (-17)	12.3	(1-) 2-3 (4)
6	M, C, S	c. 222	73.1	3.26	4.46	44.5	3.79	8.52	(7-) 10-12 (-15)	10.4	1-2 (-4)
7	M, C	222									
8	M, C, S	222	76.9	5.10	6.64	47.3	3.67	7.77	(8-) 14-17 (-20)	14.4	(1-) 2-3 (-4)
9	M, C, S	c. 222	79.6	5.81	7.30	49.9	6.27	12.56	(6-) 9-10 (-12)	9.3	(1-) 2-3
	P.* ×*mantoniae										
10	M, C, S	185	AS			AS			(9-) 10-12 (-15)	11.3	(0) 1-2
11	M										
12	M, C, S	185	AS			AS			(8-) 9-12 (-14)	10.8	(0-) 1-2
13	M, S								(9-) 10-13 (-16)	11.8	1-2 (3)
14	M, S		AS			AS			(9-) 11-13 (-16)	12.3	(0) 1-2 (3)
15	M, C, S	c. 185	AS			AS			(9-) 10-14 (-17)	12.0	(0-) 1-2
16	M, C, S	185	AS			AS			(9-) 11-13 (-15)	11.9	1-2 (-3)
17	M, C, S	185	AS			AS			10-12 (-16)	12.0	(0) 1-2
18	M, C, S	185	AS			AS			(8-) 10-13 (-14)	11.6	(0-) 1 (-2)
19	M, C, S	185	AS			AS			(9-) 10-13 (-16)	12.1	0-1 (-2)
20	M, C, S	185	AS			AS			(9-) 10-12 (-16)	11.7	(0-) 1-2 (3)
21	M, S		AS			AS			(10-) 12-13 (-15)	12.7	0-1 (2)
22	M, S		AS			AS			(10-) 13-14 (-17)	13.9	(0-) 1-2
23	M, S		AS			AS			(9-) 11-13 (-15)	11.8	(0-) 1-2 (-3)
24	M, S		AS			AS			(10-) 11-14 (-16)	12.4	0-1 (-2)
25	M, C, S	c. 185	AS			AS			(8-) 10-13 (-15)	11.5	0-1 (-2)
26	M, S		AS			AS			(9-) 10-12 (-15)	11.4	0-1
27	M, C, S	185	AS			AS			(9-) 10-11 (-14)	10.4	1-2 (-3)
	P. vulgare										
28	M, C, S	148	61.9	4.14	6.70	42.0	3.54	8.43	(10-) 11-13 (-14)	12.1	(0) 1 (-2)
29	M, C, S	148	56.4	2.55	4.53	38.4	2.78	7.24	(10-) 11-13 (-14)	12.0	(0) 1
30	M, C, S	148	59.7	3.16	5.30	36.8	2.51	6.84	(9-) 11-12 (-14)	11.5	(0) 1 (-2)
31	M, C, S	148	62.6	3.04	4.86	39.2	2.45	6.25	(9-) 10-12 (-13)	11.1	(0) 1 (-2)
32	M, C, S	148	62.9	3.33	5.29	40.3	3.04	7.57	11-13 (-14)	12.3	(0-) 1

SD – standard deviation; **CV** – coefficient of variation; **M** – morphological analysis; **C** – cytological analysis; **S** – spore and sporangial analysis; **AS** – abortive spores.

Plate I



Morphological variation of fronds of *Polypodium* taxa:

Figs 1-3. *P. interjectum*: **1,** DI-137.98(1); **2,** DI-137.98(3); **3,** DI-137.98;

Figs 4-7. *P.* ×*mantoniae*: **4,** DI-134.94; **5, 7,** DI-144.98; **6,** 142.98;

Figs 8-9. *P. vulgare*: **8,** DI-5.01, DI-13.97(1); **9,** DI-70.03. Scale bars = 10 cm.

Plate II

Number of indurated and basal cells (per sporangium) of *Polypodium* taxa:

Figs 1-2. Indurated cells of *P. interjectum*: **1,** low number of cells in DI-196.95; **2,** high number of cells in DI-79.04;

Figs 3-7. Basal cells of *Polypodium*: **3,** *P.* ×*mantoniae* (0 cells), DI-142.98(5); **4,** *P. vulgare* (1 cell), DI-13.97(1); **5,** *P.* ×*mantoniae* (2 cells), DI-218.95(1); **6, 7,** *P. interjectum* (3 and 4 cells), DI-79.04. Scale bars = 50 µm.

Spores

The exospore of both *P. interjectum* and *P. vulgare* is relatively thin and covered by a very thin perispore; the laesura is linear. The LM examination of spores of Bulgarian samples did not reveal significant differences in perispore ornamentation. As it was already noted by other authors (e.g., Nardi & Tommei 1976), the spore morphology does not seem to have diagnostic importance.

The present data about the spore size (Tables 2, 3) coincide with the data of previous authors (Rothmaler & Schneider 1962; Meinders-Groeneveld & Segal 1967; Nardi & Tommei 1976; Ferrarini & al. 1986; Hutchinson & Thomas 1996; Nielsen & Johnsen 2000; etc.).

The spores of *P. interjectum* are regularly developed, (59.1)–75.58–(104.7) µm long, (35.6)–47.56–(77.9) µm wide, monolete, bilaterally symmetrical, elliptic in outline, piano-convex to concavely convex (Plate III, Figs 1, 5, 6). The ornamentation is tuberculate to verrucate.

The spores of *P. vulgare* are regularly developed, (51.2)–60.71–(74.3) µm long, (30.1)–39.34–(54.5) µm wide, monolete, bilaterally symmetrical, elliptic in outline, piano-convex to slightly concavely convex (Plate III, Figs 2, 9). The ornamentation is tuberculate to verrucate.

Given the difficulty in identifying the parents, it is essential that putative hybrids are checked up for abortive sporangia (i.e. that remain small and indehiscent – see Plate III, Fig. 10) and spores. *Polypodium* ×*mantoniae* forms abortive spores with irregular and distorted shapes, greatly varying in size, usually shrivelled or abnormally large and pale (Plate III, Figs 3, 4, 7, 8). In some spores the protoplast is absent. In herbarium specimens the empty spores are commonly filled with air and this is evidenced optically (in LM) by their black appearance (Plate III, Figs 3, 4, 8). *Polypodium* ×*mantoniae* is perhaps not completely sterile, since at least some spores seemed to be normal.

Cytology

The interrelationships and reticulate evolution of the *P. vulgare* complex have been clarified using cytological, as well as chloroplast DNA methods (Manton 1947, 1950; Shivas 1961a, b; Haufler & al. 1995a, b). Three different ploidy levels have been distinguished in Europe representing three species: diploid *P. cambricum*, allotetraploid *P. vulgare* s.str. and allohexaploid *P. interjectum*. The allohexaploid *P. interjectum* results from ancient hybridisation, followed by chromosome doubling and has been shown to contain the genomes of the other two taxa (Manton 1950; Shivas 1961a, b).

The basic chromosome number in *Polypodium* is x = 37 (Manton 1950; Shivas 1961a, b).

Three ploidy levels occur in Bulgaria. *Polypodium interjectum* is known to be hexaploid (Manton 1950; Shivas 1961a). Our plants also showed $2n = 222$ (Plate IV, Fig. 1), corresponding to reports by a number of authors from other countries (Vida 1963; Nardi & Tommei 1976; Murín 1982; Murray 1985; Manton & al. 1986; Queirós 1998; see also refs. in Löve & al. 1977). *Polypodium vulgare* is tetraploid (Plate IV, Fig. 3), with $2n = 148$ chromosomes (see refs. in Ivanova 1998).

The natural hybrid *P.* ×*mantoniae* is known to be pentaploid (Manton 1950; Shivas 1961a). The mitotic chromosome counts we made also showed the same pentaploid number $2n = 185$ (Plate IV, Fig. 2), confirming the report of Murín (1982).

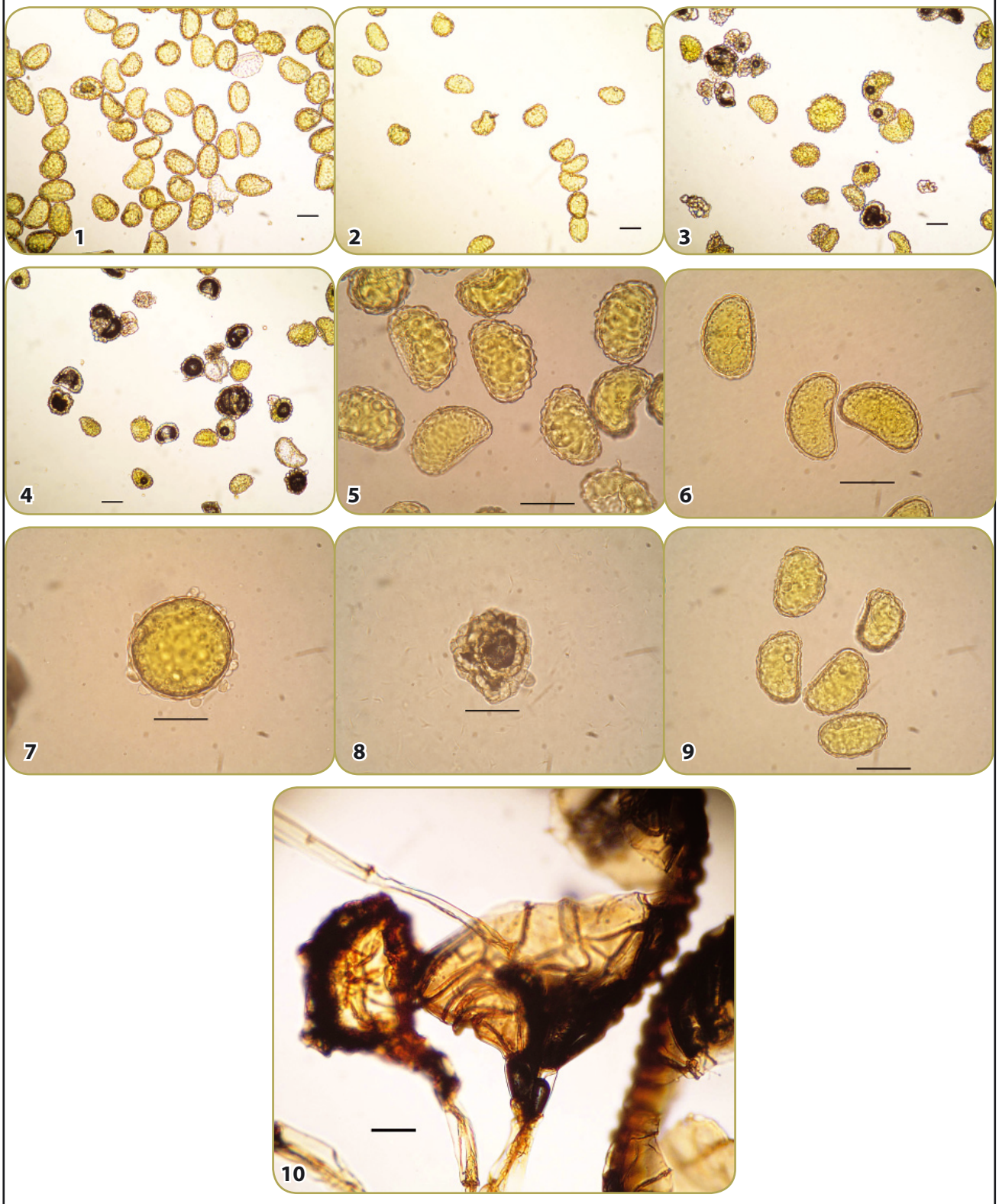
Conclusions

This study establishes the presence and distribution of *P. interjectum* and *P.* ×*mantoniae* in Bulgaria. Characteristics of the habitats are also noted. Three ploidy levels occur in Bulgarian *Polypodium*: tetraploid ($2n = 148$), pentaploid ($2n = 185$) and hexaploid ($2n = 222$), confirming the results of a number of authors from other countries.

Due to variability and reticulate relationships between the taxa in the *P. vulgare* complex, specimens often cannot be identified in the field. The chromosome number remains the most reliable character for correct determination. Furthermore, certain micromorphological characters of sporangia and spores are reliable. They are much less variable than the macromorphological characters and therefore allow determination of taxa, although values do overlap. Taking a larger number of characters into consideration gives more satisfying results in the identification of these taxa (Jäger & al. 1994).

In this article we managed to confirm that some characters discriminate *P. interjectum* and *P. vulgare* rather well. We also confirmed that frond morphology

Plate III

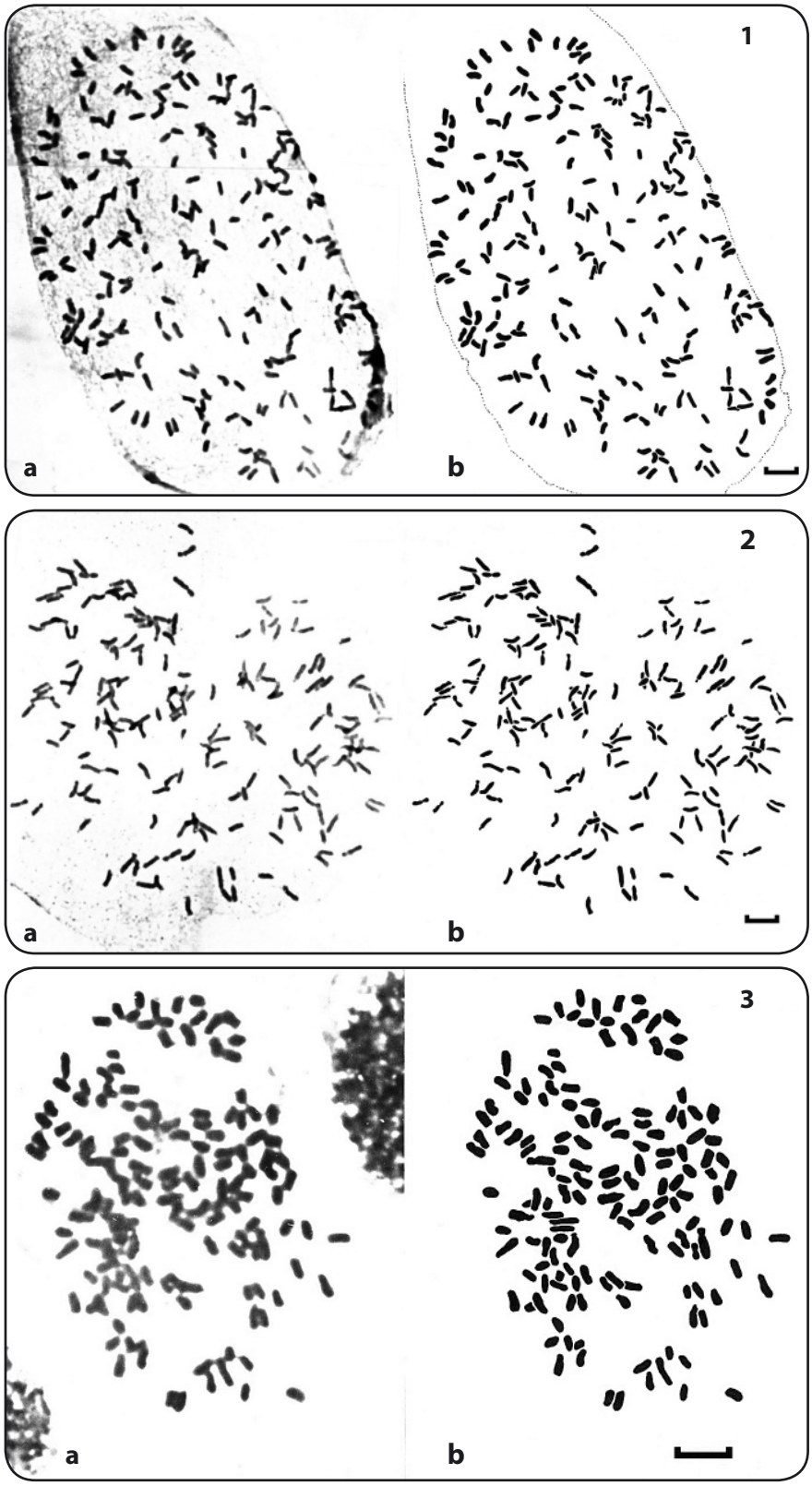


LM microphotographs of spores and sporangia of *Polypodium* taxa:

Figs 1-9. Spores of *Polypodium*: **1,** *P. interjectum*, DI-79.04; **2, 9,** *P. vulgare*, DI-70.03; **3, 4,** abortive spores of *P.* ×*mantoniae*, DI-6.00; **5, 6,** *P. interjectum*, DI-137.98(3), DI-196.95; **7, 8,** abortive spores of *P.* ×*mantoniae*, DI-142.98(1), DI-142.98(2);

Fig. 10. Normal and abortive sporangia of *P.* ×*mantoniae*, DI-142.98(1). Scale bars = 50 μm.

Plate IV



Figs 1-3. Microphotographs (a) and explanatory diagrams (b) of mitotic metaphase plates of *Polypodium* taxa: **1,** *P. interjectum*, $2n = 222$, DI-217.95; **2,** *P.* ×*mantoniae*, $2n = 185$, DI-142.98(3); **3,** *P. vulgare*, $2n = 148$, DI-5.01. Scale bars = 10 μm.

of the hybrids is generally intermediate between that of the two parents. However, the macromorphological features of the Bulgarian plants studied were extremely variable, and in most cases did not yield trustworthy results, if considered on their own. European *Polypodium* specimens can be recognized best using micromorphological features, e.g., the number of indurated annulus cells, the number of basal cells, exospore size, and the presence of aborted spores and sporangia. Though Bureš & al. (2003) claimed that the number of indurated annulus cells is the most useful character, this study did not correspond fully to this statement. The grand means for the three taxa were almost identical (11.8 for *P. vulgare*, 11.8 for *P.* ×*mantoniae*, and 12 for *P. interjectum*), although there was variation of sample means of *P. interjectum* and *P.* ×*mantoniae*. However, only few authors give a maximum number of indurated cells higher than 13 for *P. interjectum* (e.g., Dostál & Reichstein 1984 – up to 20). About 21 % of our counts showed 15 to 20 indurated cells.

The number of basal cells turned out to be more reliable in the present investigation: in tetraploid *P. vulgare* 88.8 % of the sporangia possessed one basal cell, in hexaploid *P. interjectum* 78.3 % of the sporangia had two or three basal cells, and in pentaploid *P.* ×*mantoniae* 86 % had one or two basal cells. These results agree well with the data given by a number of authors and Floras and confirm the statement of Lenski (1964) that the number of basal cells nearly always correlates well with the ploidy level in European *Polypodium*.

Spore morphology does not have significant diagnostic value, as the spores of both *P. vulgare* and *P. interjectum* are very similar. The mean spore size, however, showed that the species are distinguishable and confirmed that generally exospore size is positively correlated with ploidy level in ferns (Barrington & Paris 1986).

Until now there is no evidence to confirm the occurrence of *P. cambricum* in Bulgaria. The report of Achtarov (1963) probably relates to *P.* ×*mantoniae* or *P. interjectum*. These taxa proved to be widely spread (especially the first one) in the Valley of Strouma River, northwest of Boboshevo village, although *P. vulgare* can be found in the region, as well. Nevertheless, the search of *P. cambricum* will continue.

The presence of *P. interjectum* and *P.* ×*mantoniae* in Bulgaria is not surprising, considering their general distribution. They have not been recorded until now because Bulgarian botanists have not applied the modern taxonomic treatment and have not used cytology to identify the members of *P. vulgare* complex. Careful revision of specimens in the Bulgarian herbaria, as well as further field studies will undoubtedly provide more data on the distribution of *P. vulgare*, *P. interjectum* and *P.* ×*mantoniae* in Bulgaria.

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