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POLYPODIUM × *MANTONIAE* (*P. INTERJECTUM* × *P. VULGARE*) NEW HYBRID IN ROMANIA, CONFIRMED USING FLOW CYTOMETRY

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Abstract: Flow cytometry measurements confirmed the occurrence of *Polypodium* × *mantoniae* (*P. interjectum* × *P. vulgare*) in the Cheile Turzii Nature Reserve (Apuseni Mts., Cluj County). The relative 2C DNA content of this pentaploid hybrid was detected in relation to that of tetraploid *Polypodium vulgare* and hexaploid *P. interjectum*. *Polypodium interjectum* was found in the Bigăr Nature Reserve (Semenic Mts., Caraş-Severin County), close to its known locality in Cheile Nerei. Anatomical and morphological features (number of thick-walled cells in the annulus, spore length and stoma length) of all three Romanian *Polypodium* taxa are discussed and a brief survey of the distribution of these taxa in other European countries is included.

Introduction

The genus *Polypodium* L. s. str. includes approximately 100 species [6]. The *Polypodium vulgare* complex is a group of species which have primarily temperate Holarctic distributions, while the center of species diversity for the genus as a whole is situated in the tropical parts of America. Three species of the *P. vulgare* complex are native to Europe: *Polypodium cambricum*, *P. vulgare*, and *P. interjectum*.

The diploid (2n=74) *Polypodium cambricum* L. [Syn.: *P. australe* Fée, *P. vulgare* subsp. *serratum* (Willd.) H. Christ] occurs mainly in the southwest and south of Europe along the Atlantic and Mediterranean coasts. Its range stretches from central Scotland through W England, Wales, Ireland, France, SW Switzerland, Portugal, Spain, the Balearic Islands, Corsica, Sardinia, the Apennine Peninsula, Sicily, SW Slovenia, NW and SE Croatia, Montenegro, Herzegovina, Albania, Corfu, S Greece, Crete and Cyprus. It is also found in NE Africa in the Atlas Mts., in the E Mediterranean up to the islands of Ikaria and Samos, and in Lebanon, W Syria and coastal regions of Turkey. The easternmost localities are along the Russian and Georgian shores of the Black Sea. Some authors regard populations from the Azores Islands, the Canary Islands, Madeira and Porto Santo as the subspecies *P. c*. subsp. *macaronesicum* [incl. *P. c*. subsp. *azoricum* (Vasc.) E. Nardi].

The tetraploid (2n=148) *P. vulgare* L. is widely distributed throughout Europe from the upper Scandinavian Peninsula and Iceland to the Azores, the Canary Islands, Madeira and N Africa; in E Europe and W Asia it is distributed mainly in the mountains.

The hexaploid (2n=222) *P. interjectum* Shivas [Syn.: *P. vulgare* subsp. *prionodes* (Asch.) Rothm.] occurs from SW Norway through Denmark, Belgium, Luxembourg, the Netherlands, Great Britain, Ireland, Portugal, Spain and the Balearic Islands, rarely also in Madeira, France, Switzerland, the Apennine Peninsula, Sicily, Elba, Slovenia, Croatia, S and E Austria, Germany, the Czech Republic, S Slovakia, W and N Hungary, and SW Romania. It has not been reported from Serbia or Bulgaria, but its occurrence in these countries is probable. Disperse easternmost localities are near Kaliningrad, SE of Moscow, near Rakhov in the W Ukraine on the Crimean Peninsula, near Bursa in NW Turkey, near Bahçe in S Turkey (near the Iskenderun Gulf of the Mediterranean Sea) and in N Iran. From Romania *P. interjectum* is reported by Schrott (1968) from the Cheile Nerei Nature Reserve (Banat province, Caraş-Severin County) [2, 4]. Beldie & Váczy in [21] have also reported this species from Valea Cosustiei (Mehedinţi County), but in later floras [2, 4] this locality is not mentioned.

Hybrids are known from some areas where both parental species occur. Triploid *Polypodium* × *font-queri* Rothm. in Cadevall & Font Quer (= *P. cambricum* × *P. vulgare*) is known from Great Britain, France, Switzerland, Italy, and Spain. Tetraploid *Polypodium* × *shivasiae* Rothm. (= *P. cambricum* × *P. interjectum*) occurs in Great Britain, France, Germany, Spain, Italy, Slovenia, and the European part of Turkey.

The pentaploid hybrid *Polypodium* × *mantoniae* Rothm. in Rothm. & U. Schneid., Kulturpflanze Beih. 3: 245, 1962, is a hybrid of *P. interjectum* and *P. vulgare*. This hybrid was first found in Great Britain, Switzerland, and Italy [24], then in Germany [9, 13, 20], Hungary [25], Austria [10], France (Shivas & Walker 1970 in [1]), Slovakia [12], Slovenia [11], the Netherlands and Bulgaria [13], Spain [17, 18], Croatia [7], and the Czech Republic [3]. It had not been previously found in Romania [2, 4, 16]. The DNA content of *P.* × *mantoniae* was previously estimated to be exactly halfway between *P. vulgare* and *P. interjectum* [3].

Material and Methods

A PA-I ploidy analyzer (Partec GmbH, Münster, Germany) equipped with an HBO-100 mercury arc lamp was used for the ploidy level determination. Sample preparation was carried out in a *two-step procedure* [5, 15] in the Laboratory of Flow Cytometry, Department of Botany, Masaryk University Brno. Tetraploid *Polypodium vulgare* from the valley Suchý Žleb (Moravian Karst, Czech Republic) was chosen as a reference standard. Young leaf samples (0.5 cm^2) from a measured individual and a standard were chopped with a new razor blade for about 20 s in a Petri dish containing 0.5 ml of ice-cold Otto I buffer (4.2 g citric acid monohydrate + 1 ml 0.5% Tween 20 adjusted to 200 ml and filtered through 0.22 μm filter), then 0.5 ml more Otto I buffer was added. The solution was filtered through nylon cloth (50 μm mesh size). For DNA staining, 2 ml of Otto II buffer (0.4 M disodium hydrogenphosphate dodekahydrate) including DAPI (4′,6-diamidino-2-phenylindole; 4 μg/ml final concentration) was used. Voucher specimens are deposited in the herbarium of the Department of Botany, Masaryk University, Brno (BRNU).

Results and Discussion

Live samples of *Polypodium* plants were collected by the first and third authors in rocky habitats of the nature reserves Cheile Turzii and Bigăr during an investigation of Romanian xerothermic flora in 2003. They were determined as hexaploid *P. interjectum* and pentaploid hybrid *P.* × *mantoniae* using flow-cytometry (Fig. 1).

Polypodium × *mantoniae* was found in the Cheile Turzii Nature Reserve (Cluj County, Apuseni Mts.), cca. 3 km SE of the village Petreşti de Jos. The locality is situated in the eastern part of the gorge Cheile Turzii (46°33'34"N; 23°41'04"E). Individual plants of *Polypodium* were scattered on the south-facing slope, 2 m above the path on a rather dry limestone rock. *Festuca pallens* and *Thymus* sp. grew nearby. *Polypodium interjectum* was found in the Bigăr Nature Reserve (Caraş-Severin County, Semenic Mts.), 10 km N of the village Bozovici by the road (45°00'13"N; 21°57'30"E). An extensive population of *Polypodium* grew together with *Ceterach officinarum* on a shadowed limestone rock in an oak-hornbeam grove.

The micromorphological data in table 1 are based on a synthesis of literature data [6, 7, 8, 11, 12, 14, 19, 23, 26]. Determination can be seriously hampered by the considerable morphological variation of both hybrids and parental species. Dependable determination requires either chromosome counting or DNA content estimation (e. g. by flow-cytometry).

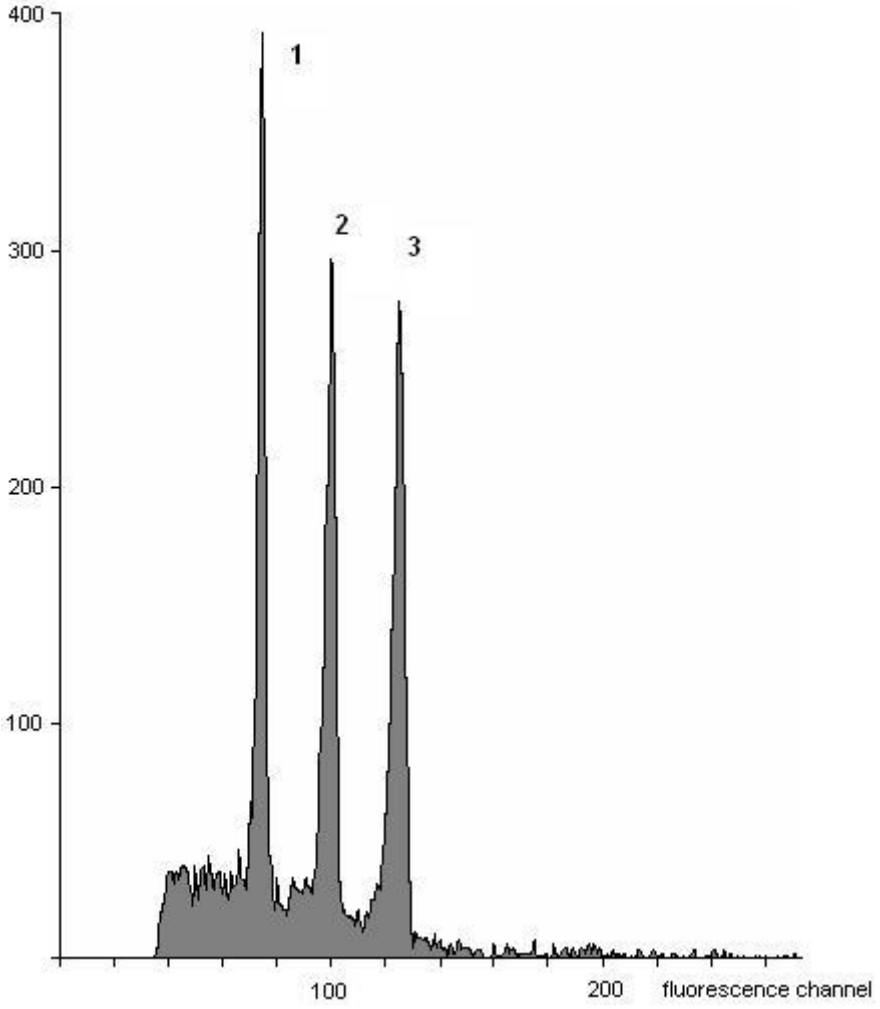
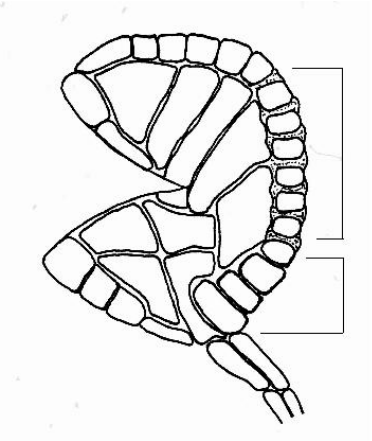


Fig. 1: Histogram of relative nuclear 2C DNA contents obtained by flow-cytometric analysis of DAPI stained nuclei, x axis – relative fluorescence channel; y axis – number of nuclei; peak 1 = G_0/G_1 nuclei of tetraploid *Polypodium vulgare*, loc. Czech Republic: Moravian Karst, valley Suchý Žleb (mean of peak = 74.06; *CV* = 1.01 %); peak 2 = G_0/G_1 nuclei of pentaploid *P.* ×*mantoniae*, loc. Rumania: Cluj County, Cheile Turzii Nature Reserve (mean of peak = 99.35; *CV* = 1.26 %); peak 3 = G_0/G_1 nuclei of *P. interjectum*, loc. Rumania: Caraş-Severin County, Bigăr Nature Reserve (mean of peak = 124.37; *CV* = 1.81 %). The ratio of peak means is 1 : 1.34 : 1.68 i. e. hybrid *P.* ×*mantoniae* is exactly halfway between *P. vulgare* and *P. interjectum.*

Table 1: Micromorphological determination features of Rumanian taxa of *Polypodium vulgare* agg.

	P. vulgare	*P. × mantoniae*	*P. interjectum*
spore length (µm)	(47−) 55 − 71(−76)	variable*	(63−) 70 − 87 (−97)
number of annulus cells	(9−) 12−16 (−20)	(6−) 8−13 (−16)	(5−) 6−10 (−12)
sorus shape	Round	Oval	oval
number of basal cells**	0 − 2 (average 1)	1 − 3 (average 2)	2 − 4 (average 3)
stoma length (µm)	(42−) 47 − 57 (−62)	(46−) 50 − 58 (−62)	(53−) 59 − 66 (−71)
chromosome number	2n = 148 (4x)	2n = 185 (5x)	2n = 222 (6x)
2C DNA content (pg)***	29.00 ± 0.32	37.18 ± 0.38	45.24 ± 0.31



* Spores abortive, not regularly bean-shaped as in the other species, of varying sizes (the majority of normal size for the parent species, a minority clearly smaller, and a few almost twice normal size) [14]. ** Basal cells see on fig. 2. *** According to Bureš et al. [3].

Fig. 2: Thick-walled annulus cells and basal cells on the sporangium of *Polypodium interjectum* (det. K. Helánová according to Zenner [26]).

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CONFIRMAREA PREZENȚEI ÎN FLORA ROMÂNIEI A HIBRIDULUI *POLYPODIUM* X *MANTONIAE* (*P. INTERJECTUM* X *P VULGARE*), UTILIZÂND CITOMETRIA ÎN FLUX

(Rezumat)

Pe baza datelor obținute prin utilizarea tehnicii citometriei în flux, lucrarea confirmă prezența în flora României, în rezervația naturală Cheile Turzii, a hibridului *Polypodium* x *mantoniae* (*P. interjectum* x *P. vulgare*). S-a detectat conținutul relativ al 2C ADN, al hibridului pentaploid, comparativ cu tetraploidul *Polypodium vulgare* şi, respectiv, hexaploidul *P. interjectum*. Acesta din urmă este prezent în rezervația naturală Bigăr (Munții Semenic. Județul Caraş-Severin), în apropierea localităților cu prezență confirmată din Cheile Nerei.

Pentru toți cei 3 taxoni de *Polypodium* prezenți în flora României, s-au analizat particularitățile anatomice şi morfologice (număr de celule din anulus, mărimea sporilor şi a stomei) şi s-a realizat o scurtă trecere în revistă a distribuției acestor taxoni în alte țări europene.