Zeitschrift: Botanica Helvetica
Herausgeber: Schweizerische Botanische Gesellschaft
Band: 92 (1982)

Artikel: Cytological examination of Dryopteris villarii (Bell.) Woynar ex Schinz et Thellung from the locus classicus (type locality)

Autor: Rasbach, Helga / Reichstein, Tadeus / Schneller, Jakob

DOI: https://doi.org/10.5169/seals-64640

Botanica Helvetica *92:* 33-40 (1982)

Cytological examination of *Dryopteris villarii* (Bell.) Woynar ex Schinz et Thellung from the *locus classicus* (type locality)

Helga Rasbach[1], *Tadeus Reichstein*[2] and *Jakob Schneller*[3]

Manuskript eingegangen am 23. April 1982

Zusammenfassung

Der *locus classicus* von *Dryopteris villarii* am Südfuß des Mont Cenis wurde besucht. Die Pflanze wächst dort noch reichlich und produziert große, breite Wedel, die auf Grund der Morphologie schwer von *D. submontana* zu unterscheiden sind. Die Population ist aber einheitlich, und zwei ausgewählte Stichproben erwiesen sich als diploid. Die bisher vorgeschlagene Nomenklatur der europäischen Vertreter der *D. villarii*-Gruppe ist demnach richtig.

Summary

The *locus classicus* of *Dryopteris villarii* was visited. The fern is still growing there abundantly. The population is homogeneous, producing large wide fronds difficult to distinguish from tetraploid *D. submontana,* but two selected specimens were found to be diploid. This shows that the present nomenclature of the members of the *D. villarii* group in Europe is correct.

1. Purpose of this investigation

Much has been written about the correct spelling of *Dryopteris villarii* (versus *D. villarsii,* see under 2) but, as far as we are aware, nobody has investigated the ploidy of the type specimen (or material from the type locality). Obviously it was assumed to be diploid. This, in our opinion, is by no means self-evident. Should it turn out to be tetraploid, it would upset the present day nomenclature (see under 3).

[1] Kurklinik Glotterbad, Gehrenstraße 12, D-7804 Glottertal
[2] Institut für Organ. Chemie der Universität, St. Johanns-Ring 19, CH-4056 Basel
[3] Institut für System. Botanik der Universität, Zollikerstraße 107, CH-8008 Zürich

2. Correct spelling

Dominique Villars (1745-1814) was born as Villar but all through his life (long before 1793), he wrote his name as Villars. On the isotype (FI-W, see Nardi 1976:9) Bellardi clearly wrote *Polypodium villarsii,* but in the publication (1793) he gives twice *P. villarii.* According to Professor K.U. Kramer (in litt.), one can assume that he latinized the name (as Villarius) and that the spelling *P. villarii* was intentional and not a printing error and has to be accepted.

3. Taxonomy and nomenclature

The *Dryopteris villarii* aggregate (a natural group of several taxa) is represented in Europe by three main taxa: the alpine diploid *D. villarii* (Bell.) Woynar, the Mediterranean diploid *D. pallida* (Bory) Fomin, and a tetraploid, first discovered in Great Britain by Manton (1950:65-7), later by Vida (1969) in Rumania, today known to occur from Spain through southern Europe to Turkey. It is usually easy to distinguish the two diploids on gross morphology; they are also ecologically and geographically relatively well separated. The tetraploid, however, is intermediate in many respects and its differentiation from both diploids can become difficult, and in certain cases, when only single fronds are available, is impossible even for the expert. This may be the reason why many workers including Heywood (1964:21 in Flora Europaea) treated these three taxa on a subspecific level as: *D. villarii* subsp. *villarii, D. villarii* subsp. *pallida* (Bory) Heywood and mentioned the tetraploid but without suggesting a name for it. A valid name for this taxon as *D. villarii* subsp. *submontana* Jermy & Fraser-Jenkins was published in 1977, but only a few days later Fraser-Jenkins (1977) suggested treating all these taxa as species as follows:
D. villarii (Bell.) Woynar ex Schinz et Thellung, Vierteljahresschr. Naturf. Ges. Zürich *60:*339 (1915), based on *Polypodium villarii* Bellardi (1793);
D. submontana Fraser-Jenkins, Candollea *32:*311-312 (1977);
D. pallida (Bory) Fomin, Věstn. Tiflissk. Bot. Sada *20:*32 (1910) based on *Nephrodium pallidum* Bory, Expéd. Sci. Moréc. *32:* 287 (1832).
The latter can further be subdivided into several subspecies of which only subsp. *balearica* (Litard.) Fraser-Jenkins (1977) is found in Europe (endemic in Mallorca).

4. The type of *Polypodium villarii* Bell.

The holotype of this taxon is probably in TO; we could not see it, but a good isotype is in FI-W (see Nardi 1976:9), which we were able to examine. It is our opinion, for morphological and geographical reasons, that it is not the Mediterranean diploid *(D. pallida).* On the other hand, it is not possible to state certainly whether it is a large, broad form of the alpine diploid or a normal form of the tetraploid, which is known to occur on other parts of the southern slope of the Alps.

Careful measuring of the size of the spores can sometimes be helpful in differentiating the two taxa, but even with clean fresh material the results are not very reliable, as the ranges tend to overlap (see table 1). Efforts to make such measurements in old herbarium

*Table 1.*Length of exospore of untreated spores embedded in balsam. The figures which are not in brackets mean that at least 90% of the spores show this size.

Taxon	Length of exospore
Alpine diploid *(D. villarii* s. str.)	(27–)30–36(–39) μm
Tetraploid *(D. submontana)*	(27–)34–42(–45) μm

samples are normally of no use. The good spores are mostly lost and contamination with foreign spores is a serious source of additional error.

As no methods of counting chromosomes in herbarium material are known, the best approach to solve our problem was to search for living material at the *locus classicus*. If this can be located with precision, and if the plants are still growing and the population is well circumscribed and cytologically homogeneous, examination of this material is probably the best way to find out what the type really is. We therefore concentrated our efforts in this direction.

5. The *locus classicus* of *Polypodium villarii* Bellardi (1793)

Bellardi gives: «Reperi inter diffractos lapides a planitie *S. Nicolai* ad scalam montis *Cenisii*».

On the map available to us (1:100 000) we could not find the «Piano S. Nicolao» but it is clearly indicated on the old 1:25 000 map (ed. 1934. Tav. 55. IV SO-Novolesa) of which Dr. E. Nardi kindly sent us a copy. A little higher up, in the curves of the road, also «le Scale» are indicated. The place is on the southern slope of the Colle del Montecenisio (Col du Mt. Cenis) formerly on Italian, today on French territory near the present motor road from Susa to Lanslebourg, only about 0.7 km NNW of the present Italian custom-house (see fig.1). We visited the location on 3. Sept. 1979 and found *D. villarii* growing as a large colony (c. 500 m long) between limestone rocks and bolders, mainly along the old decaying road leading to an abandoned quarry of black marble a little above (WSW) the present road at c. 1750 m alt.

The population looked rather homogeneous and most plants were very big with wide fronds like *D. submontana* (see fig. 2). Fronds of a number of plants were pressed in order to check for hybrids, but none were found. In addition two big representative plants (TR-5092 and 5093) with wide fronds were selected for cytological examination. We were confident that unequivocal identification of these two plants would be possible by counting their chromosomes, and would prove whether or not the tetraploid was present. Root were fixed in the field and living specimens of both plants, together with a third one, were taken to Basel as precaution in the event that the fixations would give no result. But fortunately they allowed unequivocal counts.

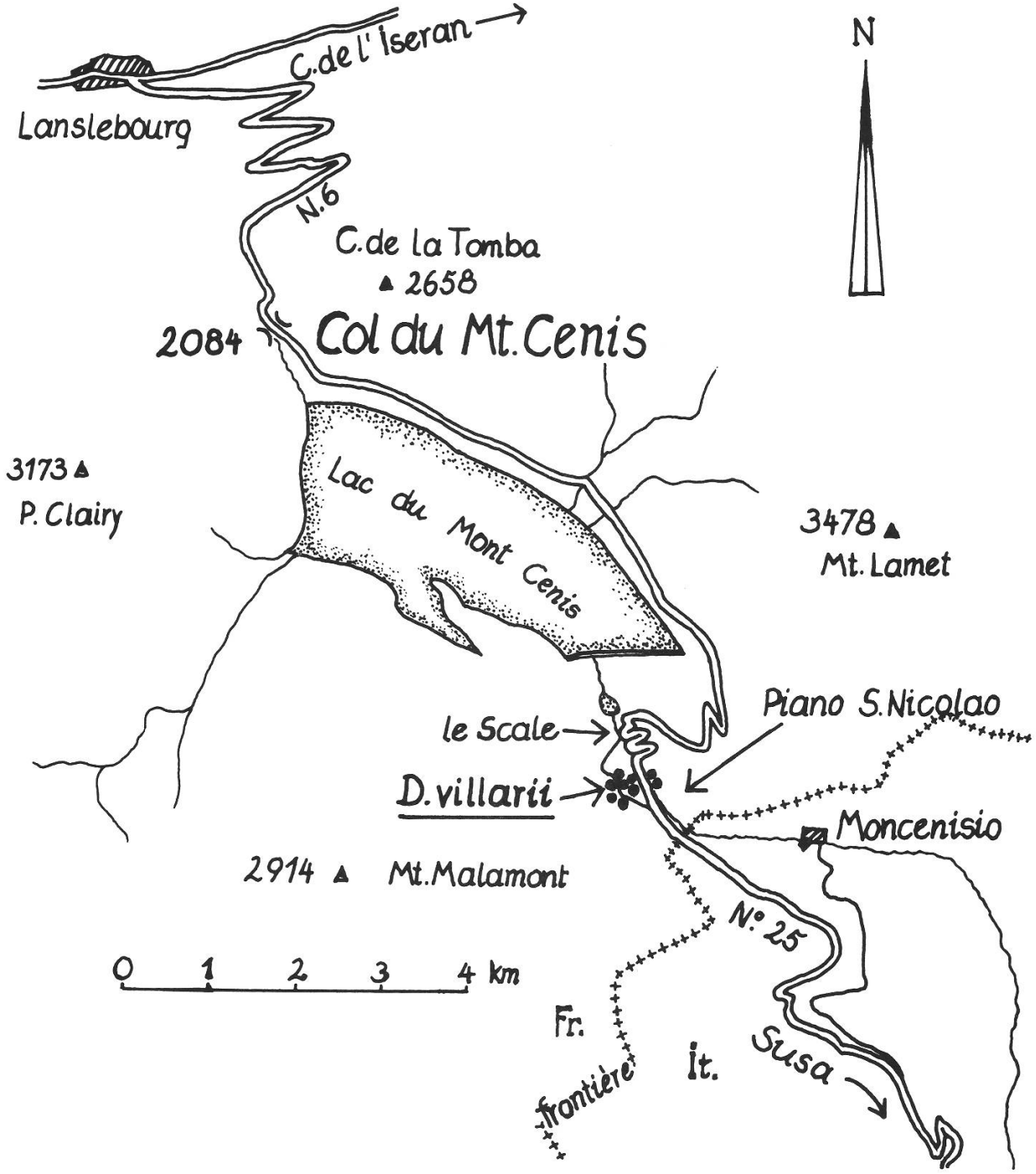

Fig. 1 The *locus classicus* of *Polypodium villarii* Bell.; the black dots show the locality.

6. Methods

Meristematic root tips were put immediately into a 0.1% aqueous colchicine solution. After c. 4 hours excess liquid was removed from the tips using blotting paper and the tips were then put into a mixture of one part glacial acetic acid and three parts abs. ethanol

*Fig. 2.*Silhouettes of fronds. A = *D. submontana* (tetraploid), TR-3568, Caussols, France; B = *D. villarii* (diploid), TR-5096, big frond from *locus classicus;* C = *D. villarii* (diploid), TR-1758, two fronds from Switzerland, Ct. Nidwalden, near Trübsee above Engelberg.

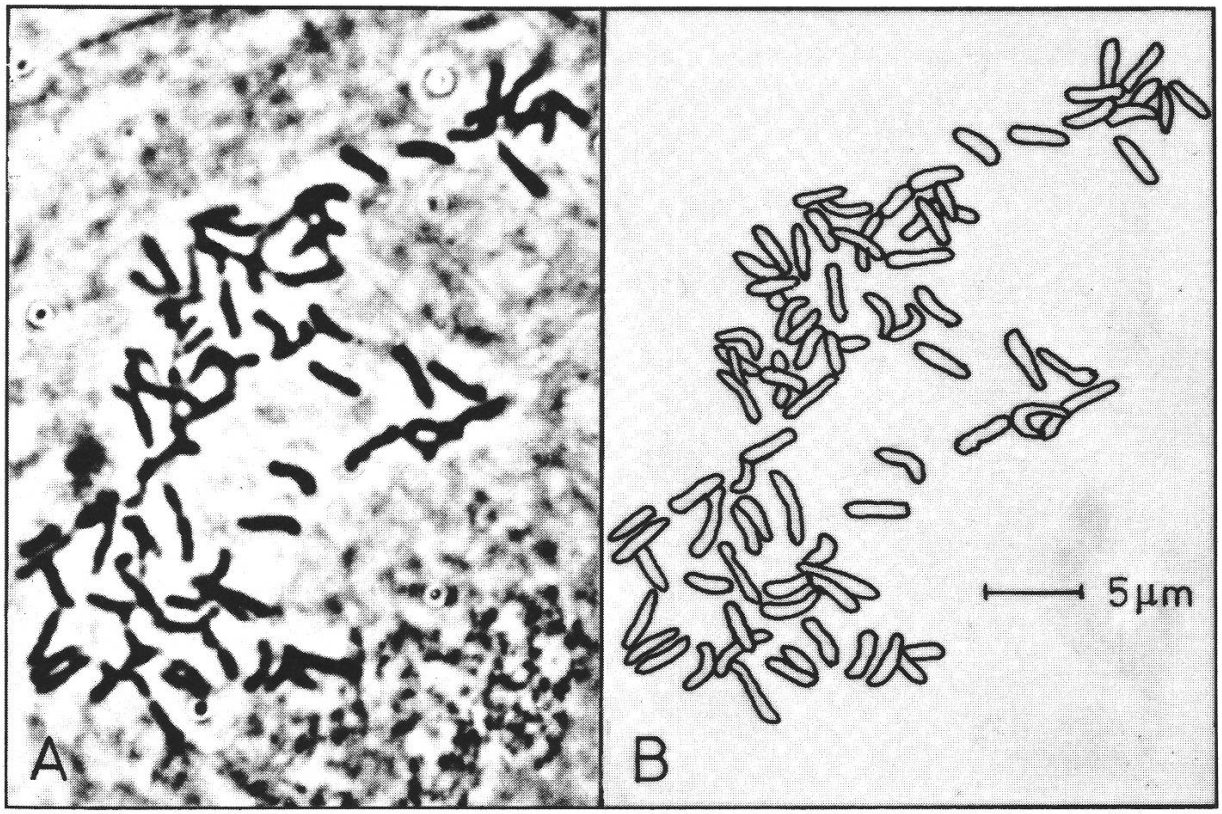

Fig. 3. Cytology. *Dryopteris villarii.* TR-5093 from the *locus classicus.* Cell of a root tip at mitosis showing c. 82 chromosomes. A = photograph; B = explanatory diagram. Plant TR-5092 gave a similar result.

(freshly mixed). Glass tubes with tight plastic stoppers were used. These tubes were brought to the Institute in Zurich within two days and kept there at –15 °C until examination was possible. The tips were then softened by enzymatic degradation in a 2% solution of cellulase CP_1 (a commercial product of the Schweizerische Ferment AG, Basel) in 1% aqueous acetic acid for 1 1/2 hours at 25 °C. After washing and fixing again in acetic-ethanol for 1/2 hour the roots were stained in acetocarmine and squashed. The preparations were made permanent and examined with a phase contrast microscope.

7. Results

Fig. 3 shows a cell of TR-5093 at mitosis with 82 ± 2 chromosomes. Root tips of TR-5092 gave a similar result. There is no doubt that the plants are diploid.

8. Examples of other collections of *D. villarii* with wide fronds

Big plants of *D. villarii* with large, broad fronds, easily confused with *D. submontana,* were found in other places, particularly on the southern slopes of the European mountains. We give here a few examples for which chromosome counts have been made.

Italy

1. Monte Baldo (E. of Lago di Garda), along path from S. Zeno to Costabella 1550 m, 14. VII. 1969, leg. E. Hauser, G. Vida, K. Vida & T. Reichstein, TR-2729, cult. in Basel, 2n = c. 80 (det. G. Vida in litt. 18 VIII. 1970).

2. Monte Bondone (S.W. of Trento) near road to the mountains at c. 1600 m, 15. VII. 1969, leg. E. Hauser, G. Vida, K. Vida & T. Reichstein, TR-2743, cult. in Basel, n = 41 (det. G. Vida in litt. 28. V. 1970).

3. Valley of T. Cimolina, N. of Cimolais, dolomitic scree at c. 900 m, E. side of the river where road passes over a bridge, 4. VIII. 1969, leg. H. Melzer, H. Kunz & T. Reichstein, TR-2799. On the E. side of the river *Arenaria huteri, Heliosperma glutinosa* and *Asplenium seelosii* were growing. The shape of the fronds of this plant approaching *D. carthusiana.* Cult. in Basel, n = 41 (det. G. Vida in litt. 28. V. 1970).

4. Monti Lessini (N.W. of Vicenza). Dolomitic scree above the Rifugio Cesare Battista along the path to the Passo di Lora at c. 1400 m alt., 20. VIII. 1978, leg. H. Rasbach, J. Schneller & T. Reichstein), J.S. – 23E 4764, living progeny (TR-4764) was raised from spores in Basel, 2n = c. 82 (det. J. Schneller, in litt. 16. VI. 1981).

9. Some specimens of *D. submontana* of which the chromosomes have been counted

Spain

1. Sierra Nevada: North slope of the Dornacho, near Granada, limestone rocks c. 2050 m alt., 26. VII. 1969, leg. H. Metlesics, progeny (TR-3023) raised from spores in Basel, 2n = c. 164 (det. G. Vida in litt. 29.III. 1972).

2. Ovieto province, south of Covadonga, slopes of Peña Santa, above Los Lagos, on limestone, 6500 ft. alt., leg. H.A. McAllister, 2n = 164 (det. M. Gibby, June 1973).

France

3. Pyr. Atlantiques, vallée d'Aspe, Lescun, montée au lac de Lhurs (1691 m), dans le défilé calcaire, 1500 alt., 10. X. 1973, leg. J. Vivant, progeny (TR-3668) raised from spores, 2n = c. 80 (det. J. Schneller in litt. 16. VII. 1976).

4. Pyr. Atlantiques (d'Ossau), Pé-de-Hourat, haut-bassin calcaire du torrent: Le Baset. Cirque creusant le flanc nord du Pic Durban. Chaos de rochers, éboulis grossiers vers 1600 m alt., leg. J. Vivant, 26. VIII. 1975. Progeny (TR-3932) raised from spores in Basel, 2n = c. 164 (det. J. Schneller in litt. 30. IV. 1982).

5. Alp.-Mar., N. of Grasse, Caussols, limestone pavement, S. of the road, c. 1150 m alt. (E. and W. of the village Caussols), 1. VII. 1973, leg. H.L. & T. Reichstein, TR-3568 and 3569, since then cultivated in Basel, 2n = c. 160 (det. G. Vida in litt. 26. X. 1973).

Yugoslavia

6. Velebit, near the road from Obrovoc in N. direction (to Zagreb), above the Mali Alan pass at. c. 1200 m, shaded limestone rocks, 13. VII. 1971,leg. E. Mayer, M. Mayer, V. Ravnik, H. Melzer, H. Kunz & T. Reichstein, TR-3241. Progeny raised from spores in Basel, 2n = c. 164 (det. G. Vida in litt. 29. IX. 1972). At the same place in sunny position *D. pallida* was growing, 2n = c. 82 (det. G. Vida in litt. 29. IX. 1972) for TR-3242 and 3243.

Greece

7. Oxio Ori. S.E. of Karpension Aitolia, Sterea, 4. IX. 1973, leg. C R. Fraser-Jenkins 4101 under *D. pallida.* Progeny (TR-3680) raised in Basel, 2n = c. 160 (det. J. Schneller in litt. 16. VI. 1981).

Turkey

8. Antalya Villayet, N. facing shady calcareous cliffs, N. side of Geyik Dag, above shephards' village, N. of Alanya, 2050 m alt., 12. IX. 1979. C.R. Fraser-Jenkins & S. Coombe No. 9809, plant cult. in Basel under TR-5121, 2n = c. 164 (det. M. Gibby in litt. 22. V. 1980).

Acknowledgements. We express our gratitude to the following persons: Dr. E. Nardi (FI) for his help in locating the *locus classicus* of *Polypodium villarii* Bell., Dr. Mary Gibby and Professor G. Vida for counting the fixations which we sent them and for allowing us to quote their results, Mrs. Mary Fallen (Z) for linguistic corrections of the manuscript, Mr. C. R. Fraser-Jenkins, Mr. H. Metlesics and Mr. J. Vivant for plant material or spores.

Literature

Bellardi L. 1973. Appendix ad floram pedemontanam, Mém. Acad. Roy. Sci. Turin: 255 (1790-91).

Fraser-Jenkins C.R. 1977 (16. Dez.). Three species in the *Dryopteris villarii* aggregate (Pteridophyta). Candollea *32:* 305-319.

Fraser-Jenkins C.R. and Jermy A.C. 1977 (12. Dez.). Nomenclatural notes on *Dryopteris:* 2. Fern Gaz. *11:* 338-340.

Heywood V.H. 1964. *Dryopteris* in Tutin T.G. et al. (ed.), Flora Europaea. I. Cambridge, Univ. Press.

Manton I. 1950. Problems of Cytology and Evolution in the Pteridophyta. Cambridge, Univ. Press.

Nardi E. 1976 La distribuzione italiana di «*Dryopteris pallida*» (Bory) Fomin. Webbia *30:* 3-32.

Vida G. 1969. Tetraploid *Dryopteris villarii* (Bellardi) Woynar ex Schinz et Thell. in Rumania. Bot. Közlem. (Budapest) *56:* 11-15.