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Asplenium woronowii Christ (Aspleniaceae, Pteridophyta), a diploid ancestral fern new to Turkey, and the status of *Asplenium pseudolanceolatum* Fomin
Studies in *Asplenium* for "Flora Iranica" 1.

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ABSTRACT

DEMIRIZ, H., C. R. FRASER-JENKINS, J. D. LOVIS, T. REICHSTEIN, J. J. SCHNELLER & G. VIDA (1981). *Asplenium woronowii* Christ (Aspleniaceae, Pteridophyta), a diploid ancestral fern new to Turkey, and the status of *Asplenium pseudolanceolatum* Fomin. Studies in *Asplenium* for "Flora Iranica" 1. *Candollea* 36: 181-193. In English, German abstract.

Asplenium woronowii Christ (1906) is native to N.E. Turkey and was found to be a diploid sexual species, i.e. a potential ancestor of allopolyploids. *A. pseudolanceolatum* Fomin (1908) is conspecific with *A. woronowii* and is treated as a synonym. A new description with figures is given which covers the range of variation.

ZUSAMMENFASSUNG

DEMIRIZ, H., C. R. FRASER-JENKINS, J. D. LOVIS, T. REICHSTEIN, J. J. SCHNELLER & G. VIDA (1981). *Asplenium woronowii* Christ (Aspleniaceae, Pteridophyta), ein diploider Farn neu für die Türkei und der Status von *Asplenium pseudolanceolatum* Fomin. Untersuchungen an *Asplenium* für die "Flora Iranica" 1. *Candollea* 36: 181-193. Auf Englisch, deutsche Zusammenfassung.

Asplenium woronowii Christ (1906) ist heimisch in der N.E. Türkei. Es ist eine diploide Sippe und daher ein möglicher Vorfahre von allopolyploiden Arten. *A. pseudolanceolatum* Fomin (1908) erwies sich als konspezifisch mit *A. woronowii* und ist als Synonym zu behandeln. Eine neue Beschreibung von *A. woronowii* mit Figuren wird gegeben, welche die Variationsbreite der Arte zeigen soll.

1. *Asplenium woronowii* in Turkey and its cytology

During his visit to Turkey in September-October 1968 the senior author (TR) was shown an *Asplenium* new to him. It had been collected in N.E. Turkey by Kuru (ISTF-22722)¹ (see Fig. 1). Only after successfully raising living progeny from spores could it be identified as *A. woronowii* Christ (1906). It matched well the figure given in FOMIN (1908, tabl. II) (see Fig. 5, IIa, b) and the isotype specimen (in P!). This species was described from the Caucasus (Abchasia) and was until now not known from outside that region (KOMAROV, 1934; GROSSHEIM, 1939; TAKHTAJAN, 1954; KETSKHOVELI, 1971), but had nevertheless been collected previously in Turkey, though unidentified or sub *A. pseudolanceolatum* Fomin (see below). *A. woronowii* is poorly represented in herbaria and is unknown to most botanists outside Russia. We therefore give an emended description (see below) and Figs. 1-3 show the approximate range of variation in its morphology. Acquisition of living material made it possible to study cytology (previously unknown). It proved to be a sexual diploid (Fig. 7), i.e. a potential ancestor of polyploid species, perhaps including a newly discovered tetraploid taxon (see below).

2. The status of *Asplenium pseudolanceolatum* Fomin (1908)

When examining and cultivating *A. woronowii* from different origins during several years, we had increasing difficulty to differentiate it from *A. pseudolanceolatum*, and on reading the description by FOMIN (1908, 1912), we had the impression that he had also experienced similar difficulties.

2.1. Typification

FOMIN (1912) did not designate a type for *A. pseudolanceolatum* but "Flora of the USSR" (1934), in which Fomin was responsible for the Filicales, states: "Type in Tiflis". On 21.8.1976 one of us (CRFJ) could examine the following two specimens in TGM and on 24.6.1980 two others in P, all four mentioned in FOMIN (1912) and therefore syntypes:

1. Borshom 18th June. Coll.: Smirnov (TGM);
2. Borshom June 1867. Coll.: G. Radde (TGM);
3. Transcaucasia, Borshom in rupibus. Leg. Misczenko 5.6. 1905 (P).
4. Transcaucasia occidentalis, prov. Batum, distr. Artvin prope pagum Nakeraw ad rupes. Leg. Woronow 8.6.1907 (P).

¹Abbreviations for herbaria according to HOLMGREN & KEUKEN: *Index Herbariorum* I (Ed. 6), Utrecht 1974. TR means herbarium T. Reichstein Basel. All specimens collected by CRFJ (with his numbers) are deposited in BM.

All four with a determination by A. Fomin in his handwriting as "*Asplenium pseudolanceolatum*". CRFJ could also see a duplicate of the Radde specimen on 6.1.1976 in LE, which was subsequently sent to Basel and examined by TR. It had the following label: "86. *Asplenium Adiantum nigrum* L. Leg. G. Radde m. Junio 1867. Borshom Transcaucasiae." With a second label in Fomin's hand: "*Asplenium pseudolanceolatum* Fomin" with his signature (in Russian). Of these specimens we designate Radde 1867 in TGM as lectotype and the duplicate in LE as isolectotype. Another syntype is in S. "In angustiis Krabisschevi, prope Borshom. 5.6.1901." CRFJ could see a photograph in B.

2.2. Identification of *A. pseudolanceolatum* with *A. woronowii*

The above mentioned lectotypes and syntypes had the correct morphology of *A. woronowii*. It was possible to examine the spores of the isolectotype (LE) and they were found to be small, corresponding with those of *A. woronowii*. In addition one of us (CRFJ) was able to collect a living plant (CRFJ-5560 G) and fronds with ripe spores (CRFJ-5560 A-F) from a population at (or in the close vicinity of) the locus classicus (above Borshomi, Transcaucasia). The morphology of this material (Fig. 4) correspond well with Fomin's description and figure (1908, tab. I) (see Fig. 5, IIIa) of *A. pseudolanceolatum*. The living material of this plant (original plant CRFJ-5560 G, cult. as TR-4028 and plants TR-4639 obtained as progeny from spores of

Fig. 1. — *A. woronowii*, photographs of fronds of wild collections from small plants. **A** = ISTF-22722; **B** = CRFJ-4066 a (BM), natural size.

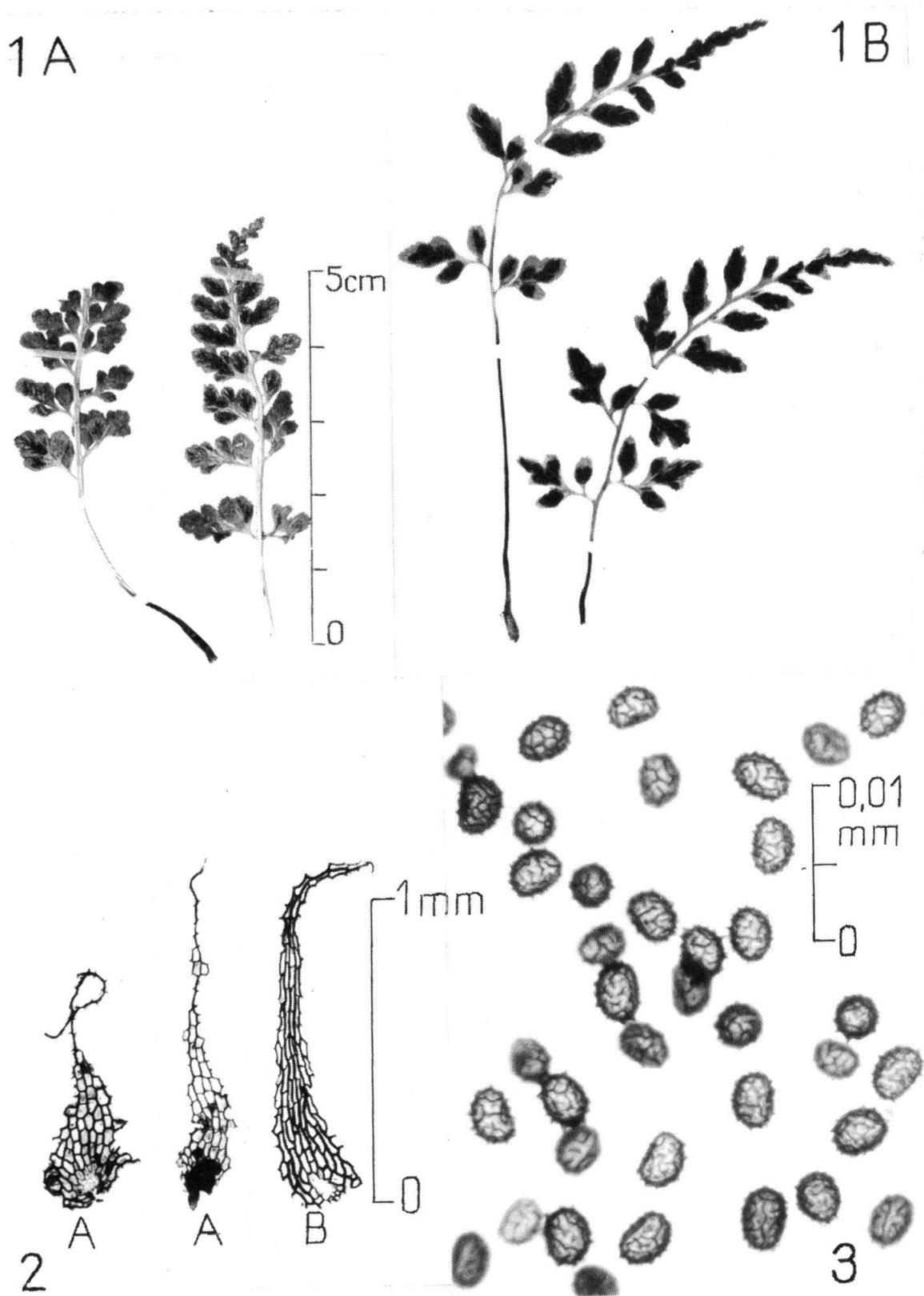
Fig. 2. — Rhizome scales enlarged $\times 20$. **A** = from a small plant TR-2344; **B** = from a bigger plant TR-3643.

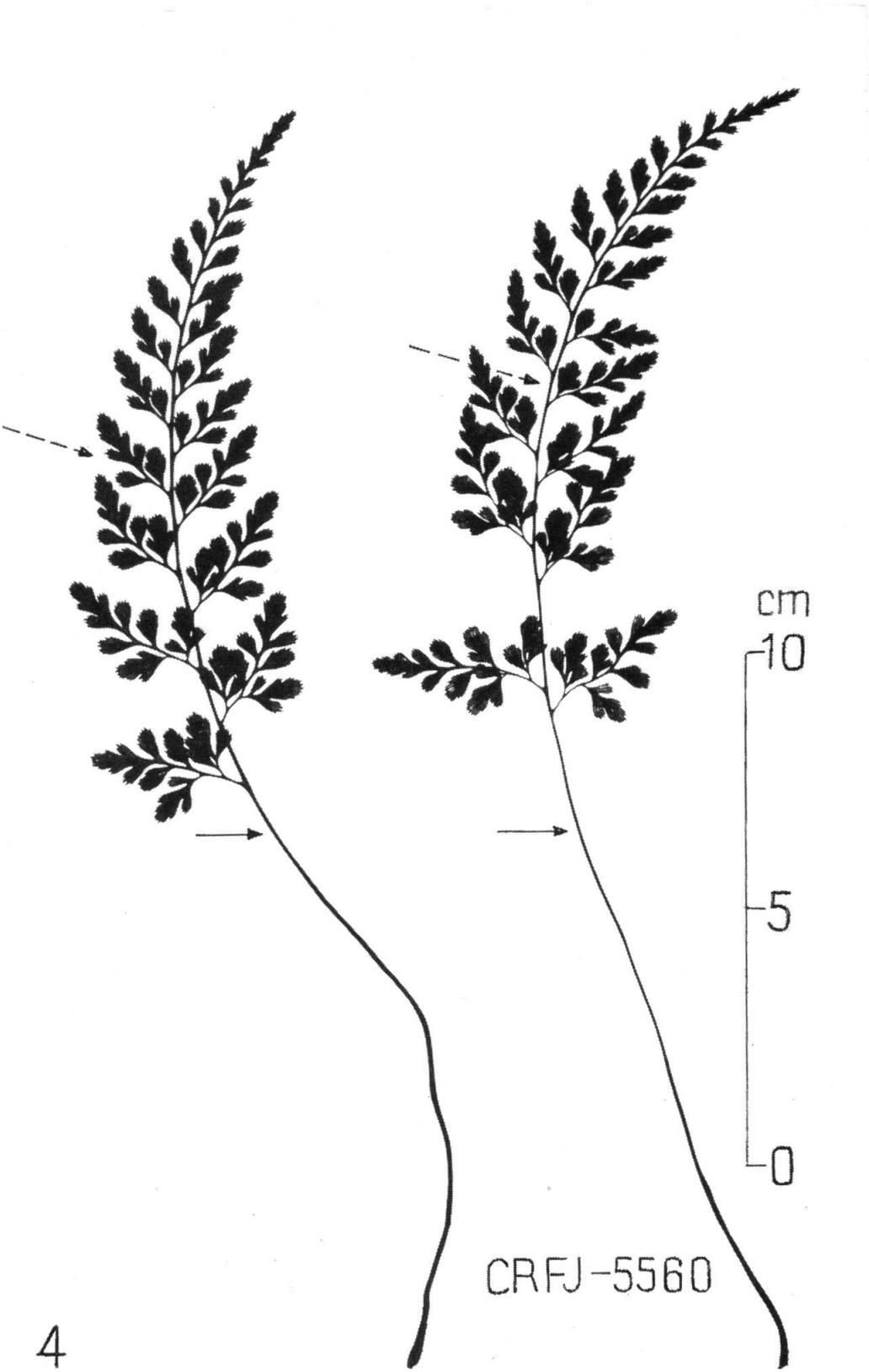
Fig. 3. — Spores enlarged $\times 200$ from TR-2344.

Fig. 4. — *A. woronowii*, silhouettes of fronds of wild collection from a big plant CRFJ-5560 reduced $2/3$. Arrows = upper limit of brown colour on adaxial side (solid line) and on abaxial side (broken line).

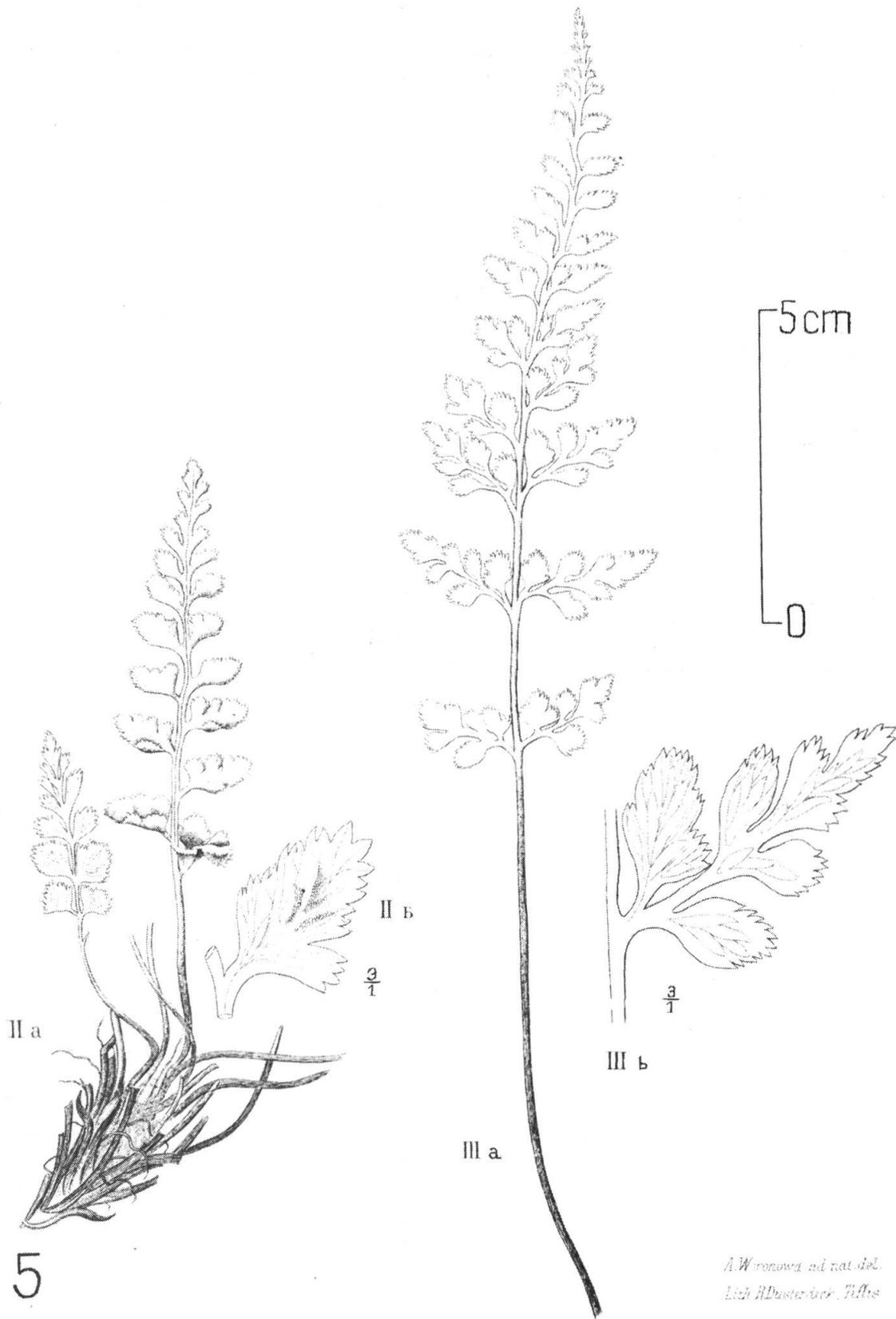
Fig. 5. — *A. woronowii*, drawings reproduced from FOMIN (1908, Tab. I). **IIa** = a small singly pinnate plant; **IIIa** = a big bipinnate plant. No scale is given in the original publication, but is given here assuming the original to be natural size. **IIb** and **IIIb** = details with sori $\times 3$ in relation to the fronds.

Fig. 6. — *A. woronowii*, silhouettes of fronds, natural size, and detail enlarged $\times 4$ from cultivated plants to show variation. **A** = TR-2344-o; **B** = TR-2344-c; **C** = TR-3314-c; **D** = pinna of TR-3314-c from middle of frond. Arrows as in Fig. 5. The shape of **B** is the most common in young plants both wild (see Fig. 1 B) and cultivated.

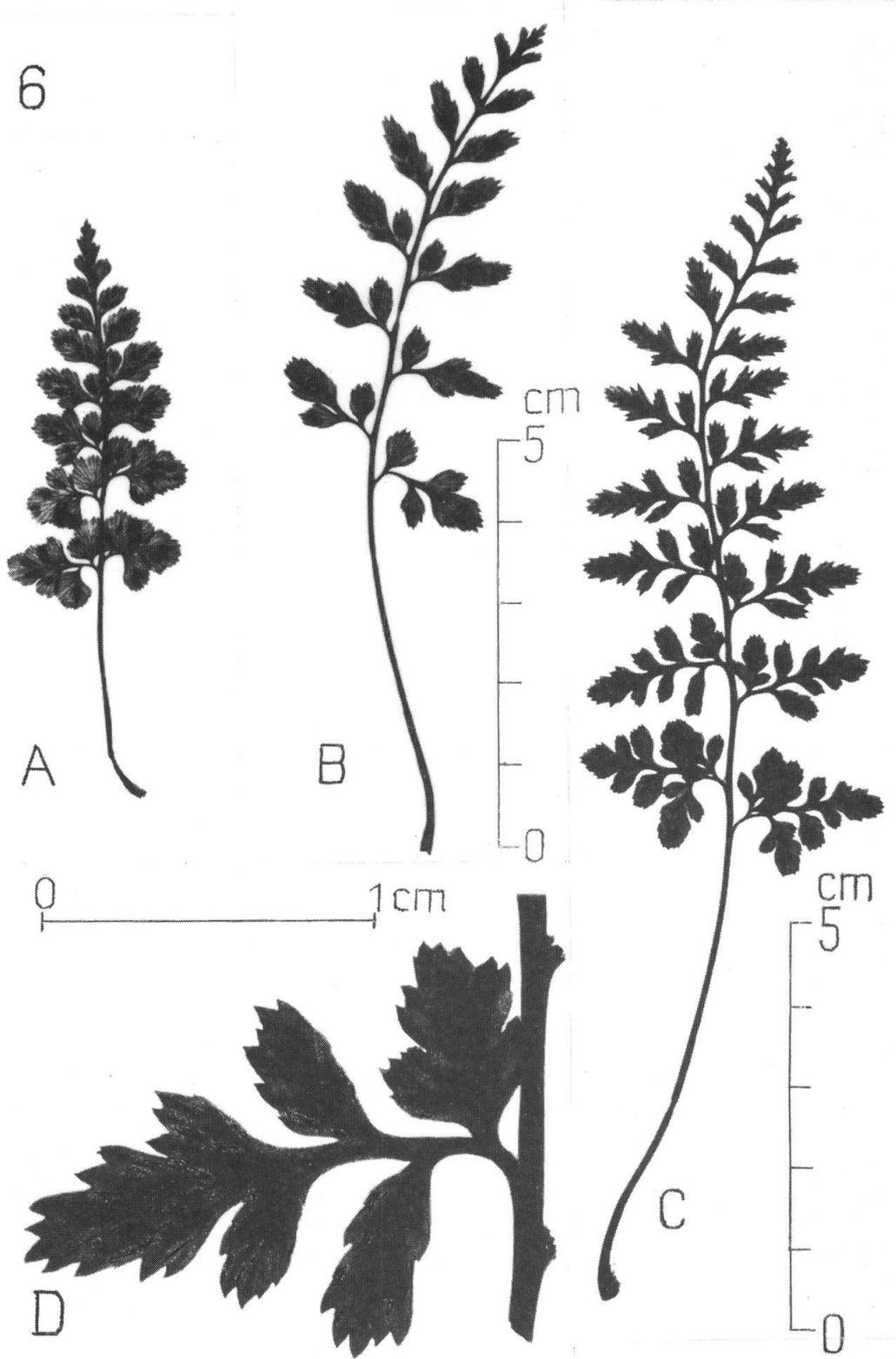




4



A. Wronowa ad nat. del.
Lich. H. Dastoevsk. Tiflis



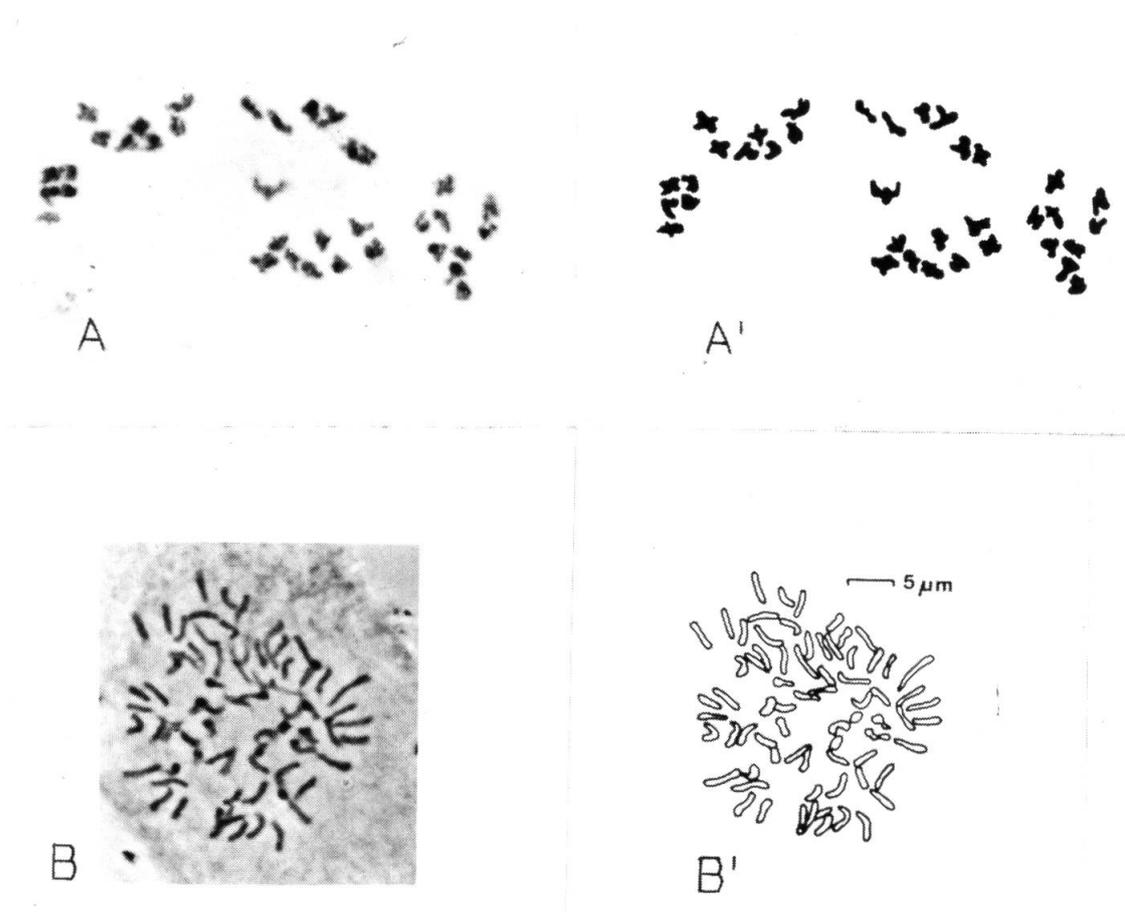


Fig. 7. — Cytology of *A. woronowii*, all $\times 1000$. **A** = photograph of first meiotic division in spore mother cell of TR-3314-b; **A'** = explanatory diagram showing 36 pairs of chromosomes, preparation by G. Vida; **B** = photograph of mitosis in root tip of TR-4639 (progeny of CRFJ-5560); **B'** = explanatory diagram showing 72 chromosomes, preparation by J. J. Schneller.

CRFJ-5560 C) not only made it possible to establish that it was diploid, but also that it was truly conspecific with *A. woronowii*. The two plants, Kuru (ISTF-22722) and CRJF-5560, though rather different in their original state gave progeny of very similar morphology when cultivated under identical conditions.

We were also able to examine the following sheet deposited in Z: "Grossheim A. et Schischkin B. *Plantae orientalis exsiccatae* Fasc. VII 151 *Asplenium pseudolanceolatum* Fomin. Transcaucasia, pr. Tiflis distr. Gori, in m-te Mitarba, in silva, 19.VIII.23. Leg. W. Kozlowsky." We have seen sheets of the same collection in B and K and it was obviously distributed to several big herbaria and accepted as being representative of *A. pseudolanceolatum*. The morphology of CRJF-5560 corresponds well this specimen. As it was over 50 years old, we did not try to raise progeny, but were able to check the spore size. The spores (TR-5184) were good and small with exospore (27-)30-33 μm in length, corresponding with those of *A. woronowii* and excluding not only *A. adiantum-nigrum*, but also the unnamed tetraploid

taxon (see below) with which big specimens of *A. woronowii* may be confused. We are therefore confident that this representative sample is also a big specimen of *A. woronowii*.

We confess that it took us some time to accept the idea that such an eminent expert as Fomin could have published a taxon as new which had already been described two years before by Christ from a closely adjacent area. The main reason for this error may be that Christ had to describe his *A. woronowii* from a single, relatively small specimen, while Fomin had relatively big material in hands. This is also the reason why both descriptions are partially wrong in important details. According to Christ the lowest pair of pinnae is the biggest, while Fomin emphasizes that it is shorter than the second. In reality one can find fronds of both types on the same plant. In the following we give a new description.

3. Description

Asplenium woronowii Christ. Perennial herb. Rhizome short erect to ascending when young, branching and becoming caespitose later, its tip, as well as the stipe bases, clad with small (2-3 mm long) clathrate brown scales without dark middle stripe. Fronds numerous (4-)5-25(-35) cm long, 1.5-5(-6) cm wide, soft or subcoriaceous. Stipes \pm as long as the blade, not or only slightly (up to 2-3 mm) swollen at base, ca. 1 mm (or less) thick above, and like the rachis sparingly beset with suberect small scales, at least the lower half of the stipe brown on the adaxial side, green higher up, on the abaxial side often the whole stipe and lower half of rachis brown; stipe and rachis \pm flat with elevated borders along both margins on adaxial side. Lamina long deltate-lanceolate in outline, varying from bipinnatifid to tripinnate in lower part, pinnate higher up, becoming confluent near the tip and often producing a 0.5-1.5 cm long nearly linear tip with wavy or obliquely incised margins and acute or obtuse apex. Pinnae 5-10(-12) pairs subopposite to alternate, lowest or second pair the biggest, stipitate with stalk of ca. 1-5 mm. Lowest pair of pinnae entire-tripartite in small specimens, distinctly anadromically pinnatifid or pinnate with only the innermost acroscopic segment stipitate (with a stalk of ca. 0.5-1 mm) and with a wide end-segment of ca. three confluent lobes in specimens of medium size, or becoming fully bipinnate in big specimens; second pair of pinnae often similar, other pinnae with shorter stipes or sessile. Ultimate segments suboval-obovate with a cuneate base and distal margin sharply toothed. Teeth ca. 0.2-1 mm long. Sori ca. 1-4 per segment at its base and near the midrib 1-5(-6) mm long. Spores bean shaped, small with the exospore (27-)30-33(-37) μ m long and brown reticulate, rugose, perispore with knobs and folds protruding up to ca. 6-7 μ m. Sexual, diploid chromosome number $2n = 72$ with 36 regular pairs at meiosis. Often reported from limestone rocks, from ca. (800-)1200-2000(-3000) m alt.

4. Systematic position

We consider *A. woronowii* to be an ancient ancestral member of the *A. adiantum-nigrum* group easy to distinguish from the other three members of this group, viz: *A. adiantum-nigrum* L. (tetraploid), *A. cuneifolium* Viv. (diploid) and *A. onopteris* L. (diploid), which are known to grow in Turkey (HENDERSON, 1965, in DAVIS (ed.), "Flora of Turkey" 1). However another so far unnamed tetraploid taxon also grows in Turkey, the Caucasian and Transcaucasian region of USSR and in Iran. In morphology it is \pm intermediate between *A. woronowii* and *A. adiantum-nigrum* and can be and often has been confused with both these species. Differentiation of doubtful specimens from *A. woronowii* by counting chromosomes is unequivocal, but measuring spore size (if adequate material is available) is also highly reliable. On the other hand differentiation of this taxon from *A. adiantum-nigrum* is difficult and can become impossible in critical cases when only one or few doubtful fronds are available in herbarium material. Raising living progeny was necessary in such cases. We shall describe this taxon as soon as experimental work, still in progress, enables us to settle its real status.

HENDERSON in "Flora of Turkey" (1965: 50) stated: "*A. pseudolanceolatum* Fomin in Flora Cauc. Crit. 1(1): 137 (1912) seems scarcely distinguishable from *A. cuneifolium* and *A. adiantum-nigrum*; recorded from A8 & A9 (Grossheim 1: map 5). No specimens have been seen." This is, as mentioned above, at least partially true for the unnamed tetraploid taxon, but not for *A. woronowii* (= *A. pseudolanceolatum*).

5. Methods and material

Raising of plants from spores was done on agar nutrient, followed by transplanting young prothallia onto soil as described by REICHSTEIN & al. (1973). Fixing of unripe sporangia for examining meiosis was in abs. ethanol-glacial acetic acid-(3:1), staining with acetic acid carmine and squashing according to MANTON (1950: 293-299). Root tips for counting mitosis were pretreated for 8 hours in 0.1% aqueous colchicin solution at +4°C. and, after removing the last drops of this liquid on blotting paper, were fixed in ethanol-acetic acid as above. Before squashing they were softened either by acid hydrolysis (see below) or enzymatically in a mixture of 2% each of cellulase ("CP-1", an experimental product produced with *Corticium rolfsii*) and pectinase ("Pectinex 3× Super L", a commercial product from *Aspergillus niger*) in 1% acetic acid in water for 2 hours at +22°C. We are grateful to "Ferment AG Basel" for a gift of these two highly active enzymes. Roots so treated were stained with acetic carmine. For acid hydrolysis the roots were warmed for 10 min. at +60°C. in 1N aqueous HCl, stained with Feulgen reagent (see DARLINGTON & LA COUR, 1963) and after squashing were examined under phase contrast in the microscope.

6. Specimens seen

Isotype (P). USSR. G. Woronow, *Plantae caucasiae* No. 16. *Asplenium Adiantum nigrum* L. var. *Abchazia*. In convalle ad pedem N. montis Arbika. In rupestribus calcareis. 7500' ca. 11. Augusto 1905 Leg. G. Woronow. (NB Christ 1906 says "pedem S"). Spores were checked (TR-5174), and found to have an exospore (27-)30-33 μm long.

USSR. Transcaucasus, Georgia. Grossheim A. et Schischkin B., leg. W. Kozlowsky 1923 (Z) see text, spores checked. Same collections seen in B & K.

USSR. Transcaucasus, Georgia, Adsharia, Herb. Bornmüller. G. Woronow, *Iter adžaro-lacium* 1910 No. 60 (3 plants). *Asplenium pseudolanceolatum* Fomin. Circa Bodyš (Adžaria superior ad fines Guriae). Ad rupes reg. alp. (B).

USSR, Georgia, Transcaucasus, above Borzhomi on Bakuriani road, W. of Tbilisi, shale at 1200 m alt., 23.8.1976, leg. C. R. Fraser-Jenkins No. 5560 (BM and TR). One plant (5560-G) was brought living to Basel, divided into 5 parts and cultivated as TR-4028 and found to be diploid (J.J.S. in litt. 23.6.1978). Six fronds (5560-A-F) had small spores with exospore (27-)30-36 μm long (very few reaching 37.5 μm) which were sown in Basel on 26.3.1978 and gave ample uniform progeny, TR-4639, which was diploid, $2n = 72$ (see Fig. 7B).

USSR, Caucasus, Abkhasia, calc. pavement between Gora Arabika & G. Mamdyschka above Goluboe Oz., 13 km N. of Bzib, Gagrinsky Khrebet, Sochi To Sukhumi, 2000 m, 26.8.1976, leg. C. R. Fraser-Jenkins No. 5652-3 (BM and TR). Spores small (TR-5181-2) with exospore (27-)30-33(-37.5) μm long.

Turkey A8. *Asplenium* spec. Prov. Çoruh (Artvin) Tiryal dağ above Murgul 2300 m. Igneous rocks 23.6.1957. Leg. Davis & Hedge No. 29944 (K).

Turkey A8, Artvin, Arhavi, Dikyamaç köyü yaylası, Küçük-çamlık yaylası, 3.9.1967, leg. A. Kuru (ISTF-22722), see Fig. 1A. Spores small with exospore (27-)30-33(-36) μm long, sown in Basel 13.10.1968 gave progeny, TR-2344 (Fig. 6A, B) which was diploid (det. J. D. L. in litt. 30.12.1970).

Turkey, A8, Rize, rocks ca. 5 km W. of the turning off from İkizdere-Ispir road, in the Khabakhor valley, S. of Rize, alt. ca. 1500 m, 8.9.1970, leg. C. R. Fraser-Jenkins No. 2371 (BM and TR). Spores small with exospore (27-)30-33 μm long, sown in Basel 23.12.1971, gave progeny TR-3314, (see Fig. 6C, D), which was diploid: $2n = \text{ca. } 70$ (G. V. in litt. 5.2.1974); $n = 36^{\text{II}}$ precisely (G. V. in litt. 25.5.1975).

Turkey, A7, Trabzon, N.-side of Soganlı Dağ (pass), above Çaykara, N. of Bayburt, alt. ca. 1500 m, 29.8.1973, leg. C. R. Fraser-Jenkins No. 4066a (BM and TR) (see Fig. 1B). Growing together with the new tetraploid taxon and

the triploid hybrid CRFJ-4050 (publ. in prep.). Spores small, exospore (27-)30-33 μm long, sown 6.7.1975 gave progeny, TR-3643, which was diploid: $n = 36^{\text{II}}$ precisely (J. J. S. in litt. 27.5.1975).

We have seen a number of other specimens in Russian herbaria, mainly sub *A. pseudolanceolatum*, mainly in LE, TBI and TGM, but quote here only the following, particularly in order to show the range of distribution (Russian names in transcription, text in translation).

Flora armeniaca No. 33 *Asplenium pseudolanceolatum* Fom. Lori Chain of Tshubuchlinkij mountains (?), Dash-Dashi on border of Rhododendron bushes 7500', 2.4.1922, leg. (?). W. Shelkovnikov, teste Shishkin (ERE). Spores small, exospore (28-)30-33 μm long. Another specimen from the same locality in LE.

Herb. Inst. Botan. AZ. Sc. Armeniae, s.n., indet. Arm. SSR, Region of Spitak, Spitak, east slope. rocks 3000 m, 27.VIII.1956. Leg. J. Mulkijanian (ERE). Good specimen, remarkable for the high altitude of this specimen in the Transcaucasus.

Botan. Inst. Acad. Nauk SSSR. Floristic Expedition to South Karabach 1948. *Asplenium pseudolanceolatum* Fomin. Azerbaidzhan, autonomic region of Karabach mountains, region of Gadрут, near Dommy, around 1100 m alt. on N.-exposed bushy slopes. L. A. Smoljaninowa. 26.5.1948 (LE). Small specimen from low altitude; Gadрут is only ca. 30 km from the Persian border on the Aras (= Araxas) river.

7. General distribution

Apparently endemic to the Caucasus and Transcaucasus. Turkey (Trabzon, Rize and Artvin) and USSR (Abkhasia, Georgia, Armenia and Azerbaidzhan). Could perhaps grow in N.-Iran and has been tentatively recorded by PARSÁ & MALEKI (1978: 376) from Iran under both: *A. woronowii* and *A. pseudolanceolatum*, but this is in common with many other species which also do not occur in Iran. Apart from this there are no other records from Iran and WENDELBO (1976) does not mention it, but the Karabach specimen comes from rather near to its border.

ACKNOWLEDGEMENTS

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