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# Asplenium cuneifolium Viv. (diploid) from the type locality (Aspleniaceae, Pteridophyta) with an appendix on related plants from other places in south-western and central Europe

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## ZUSAMMENFASSUNG

RASBACH, H., J. SCHNELLER, M. GIBBY & T. REICHSTEIN (1986). *Asplenium cuneifolium* Viv. (diploid) vom locus classicus (Aspleniaceae, Pteridophyta) mit einem Anhang, in welchem nahe verwandte Pflanzen von anderen Fundorten in Südwest- und Mitteleuropa angegeben sind. *Candollea* 41: 219-244. Auf Englisch, deutsche Zusammenfassung.

Der locus classicus von *Asplenium cuneifolium* VIVIANI (1808) befindet sich auf dem Monte Ramazzo bei Sestri Ponente. Diese diploide Sippe, der man gewohnt ist, diesen Namen zu erteilen, wächst auch heute noch dort. Daneben wachsen dort reichlich auch das tetraploide *A. adiantum-nigrum* L., die triploide Hybride *A. × centovallense* D. E. Meyer (= *A. adiantum-nigrum* × *A. cuneifolium*) sowie wenig *A. onopteris* L. (diploid). Der Platz ist heute stark verwüstet und es ist möglich, dass *A. cuneifolium* dort früher häufiger war. An diesem Fundort, wie in anderen Serpentinegebieten des westeuropäischen Mittelmeerraumes, zeigt das tetraploide *A. adiantum-nigrum* (gleichgültig ob es mit *A. cuneifolium* zusammen oder allein wächst) oft eine "besondere Form", die dem *A. cuneifolium* äusserlich täuschend ähnlich sein kann, die einen besonderen Namen (als Unterart oder Varietät) erhalten sollte. Sie produziert meistens etwas grössere Sporen als *A. cuneifolium* und die genaue Sporenmessung erlaubt es oft (aber nicht immer), diese Sippe von diploidem *A. cuneifolium* zu unterscheiden. Eine relativ zuverlässige Unterscheidung erlaubt die Perispor-Architektur bei Raster Elektronen Mikroskop (REM)-Aufnahmen. Für die völlig eindeutige Bestimmung ist die Zählung der Chromosomen die zuverlässigste Methode. Der Typus von *A. cuneifolium* (früher in GE) ist während des letzten Krieges verbrannt. In BOLO fand PICHİ SERMOLLI (1976) noch weiteres Originalmaterial von Viviani, das er als Lectotypus bezeichnete. Es handelt sich um unreif gepresste, sehr dunkle Belege, die keine Sporen enthalten. Nach unseren Befunden ist es durchaus möglich, dass es sich um diploides *A. cuneifolium* handelt; es ist aber unmöglich, das Vorliegen der "speziellen Serpentineform" des tetraploiden *A. adiantum nigrum* auszuschliessen. Wir glauben, dass es trotzdem richtig ist, den fest eingebürgerten Namen *A. cuneifolium* Viv. beizubehalten.

## ABSTRACT

RASBACH, H., J. SCHNELLER, M. GIBBY & T. REICHSTEIN (1986). *Asplenium cuneifolium* Viv. (diploid) from the type locality (Aspleniaceae, Pteridophyta) with an appendix on related plants from other places in south-western and central Europe. *Candollea* 41: 219-244. In English, German abstract.

The diploid taxon, to which the name *Asplenium cuneifolium* Viviani (1808) is attached, still grows in the type locality, Monte Ramazzo above Sestri Ponente (Genova). Together with it, and more abundantly, we found the tetraploid *A. adiantum-nigrum* L. and the triploid hybrid *A. × centovallense* D. E. Meyer (= *A. adiantum-nigrum* × *A. cuneifolium*) as well as a few diploid *A. onopteris* L. The area is badly disturbed today and it is possible that *A. cuneifolium* was more frequent there in Viviani's time. In this place, as in other serpentine regions of the western European Mediterranean area, the tetraploid species *A. adiantum-nigrum* can occur in a "special serpentine form", which approaches or even completely imitates the diploid *A. cuneifolium* in colour and gross morphology. It can often (but not always) be distinguished from the diploid by its bigger spores and by examination of the perispor architecture by means of the scanning electron microscope (SEM). The counting of the chromosomes is, however, the most reliable method for final identification. We believe this "special serpentine form" of *A. adiantum-nigrum* should be given a proper name on subspecific or varietal level. Suitable names can be found in the literature but it is difficult

to determine the valid one. The type of *A. cuneifolium* (formerly in GE) was destroyed during the last world war. PICHI SERMOLLI (1976) found further original material of Viviani in BOLO which he designated as lectotype. This consists of very dark specimens pressed in an unripe state, without spores. Our results show that although this material may well be the diploid *A. cuneifolium*, it is impossible to exclude the possibility that it could also be the "special serpentine form" of tetraploid *A. adiantum-nigrum*. We nevertheless wish to retain the well-established name *A. cuneifolium* Viv. for the diploid taxon.

## 1. Introduction

### 1.1. *The Asplenium adiantum-nigrum* complex

According to MANTON (1950, 1953) and SHIVAS (1969) this group of plants comprises three main species, *A. cuneifolium* Viv. (1808) (diploid), *A. onopteris* L. (diploid) and *A. adiantum-nigrum* L. (tetraploid). The latter was shown by SHIVAS (1969) to be an allotetraploid, once formed by chromosome doubling from a diploid hybrid between *A. cuneifolium* and *A. onopteris*. Two other rather rare plants also belong to the complex: *A. balearicum* SHIVAS (1969), an allotetraploid (LOVIS & al., 1972; see also NARDI, 1983) and *A. woronowii* Christ (1906), a diploid (see DEMIRIZ & al., 1981). The last two species are not further discussed here.

*A. cuneifolium* has been reported from Europe (JALAS & SUOMINEN, 1972, map 92) and Turkey (HENDERSON, 1965: 50), where it is found almost exclusively on serpentinite, magnesite and similar ultrabasic rocks and scree. Plants from Zöblitz (Erzgebirge, DDR) (MEYER, 1952: 31; 1957: 58) and Kraubath (Austria) (MANTON, 1953: 182; MEYER, 1957: 58; SHIVAS, 1969: 71) were found to be diploid. The distribution of the other ancestral diploid, *A. onopteris* L. is centred in Macaronesia and the Mediterranean basin, but extending north to warmer parts of western European coastal regions and east to Anatolia and probably further. The allotetraploid *A. adiantum-nigrum* has a much wider distribution including N.-America, Macaronesia, South Africa, Ethiopia, Yemen, the Mediterranean basin, the warmer parts of Europe, western Asia to the Himalaya and China (teste R. C. Ching in litt., 19.3.1979) and Australia.

### 1.2. Difficulties in identification of the three species

The three main species of the complex, i.e. *A. cuneifolium*, *A. onopteris* and *A. adiantum-nigrum*, although genetically clearly distinct (SHIVAS, 1969) show many similarities in gross morphology. In addition to this, each shows great variation, sometimes producing forms in which many characters may overlap from one species to the other. Correct identification of the members of this complex may therefore become extremely difficult, and in certain cases, even impossible for the expert. This can occur when no spores are available, as, for example, in old herbarium specimens. For a long time, many workers have treated the two diploids as subspecies of *A. adiantum-nigrum*. This has the practical advantage that it allows the worker in the field to attach a valid name (the name of the complex) to his specimen. In our view and in the light of present knowledge, we believe it would be, however, a too simple means to return to this procedure. We prefer to treat the three taxa as the distinct species they obviously are (SHIVAS, 1969); modern workers usually distinguish them as species in spite of occasional difficulties with regard to correct identification.

### 1.3. Use of microcharacters and cytology

For correct identification of the three main species of the complex, it is essential to note the growing conditions (type of rock, altitude, exposure, together with the accompanying other ferns and phanerogams in the vicinity), collect and press a mature clean frond (without soil) and ensure that the spores are not lost (see REICHSTEIN, 1981: 91 under 4.1.). Checking of the sporangial content will then allow recognition of hybrids and careful measurement of spore size will often permit identification of the species (see Table 1 with comments). In particular cases, the identification of *A. cuneifolium* may require cytological examination as a final proof, and for this living plants are necessary.

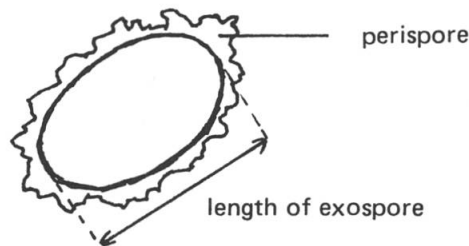


Fig. 1. — Drawing of a spore showing the exospore with sharp  $\pm$  even boundary as seen in translucent illumination under the microscope. The shape of the irregular perispore is highly characteristic for each species but is less suitable for measuring the size.

For comparing spore sizes in *Asplenium* we measure the length of the exospore of spores embedded in balsam (Fig. 1).

As can be deduced from Table 1, the examination of exospore length will nearly always allow a confident differentiation of *A. onopteris* from *A. adiantum-nigrum*, provided good spores are available. This is in agreement with former reports (REICHSTEIN & al., 1973: 150; ROBERTS, 1979; BADRÉ & PRELLI, 1980; PRELLI, 1985; BENNERT & al., 1982 and also FERNANDES, 1984 in spite of different absolute values). Spore measurements may sometimes also be helpful in differentiating *A. cuneifolium* from *A. adiantum-nigrum*, but, in our opinion, often cannot give unequivocal proof as the figures partly overlap. This result is in contradiction to BENNERT & al. (1982) who give figures for *A. cuneifolium* corresponding to *A. onopteris*. We have no explanation for this difference. BENNERT & al., however, found that examination of the perispore architecture with the scanning electron microscope (SEM) is a reliable tool with which to differentiate *A. cuneifolium* from *A. adiantum-nigrum*. This is in agreement with results of R. Viane (publ. in preparation) and may be the method of choice in suitable cases.

Species	Ploidy level	Length of exospore for spores embedded in balsam in $\mu m$
<i>A. cuneifolium</i>	diploid	(27-)30-36(-39)
<i>A. onopteris</i>	diploid	(24-)27-32(-33)
<i>A. adiantum-nigrum</i>	tetraploid	(30-)33-42(-45)

Table 1. — Spore size of the three main species of the complex. The figures without brackets give the range for at least 90% of the spores from populations of different countries. The first figure (in brackets) giving the approximate lower limit is not significant, as it may include values for misshapen spores etc. The last figure (in brackets) giving the highest limit is highly significant provided no foreign spores are present as contamination.

Note. — The above given figures for *A. adiantum-nigrum* are the result of measuring the spores of more than 300 specimens from nearly the whole range: N. America, S. Africa, Ethiopia, Europe (Spain to Caucasus) and Asia (Anatolia to Kashmir). We had no material from Australia but a specimen from Hawaii. The much higher and more irregular figures found by SHIVAS (1969) and by FERNANDES (1984) are obviously from spores measurement based on the perispore.

#### 1.4. The form of *A. adiantum-nigrum* which simulates *A. cuneifolium*

*A. adiantum-nigrum*, as other ferns, can show a great range of variation in its gross morphology. Some forms approach *A. onopteris* (one ancestor), while other forms are similar to *A. cuneifolium* (the other ancestor). DESCHATRES & al. (1978) described plants from Corsica which were undistinguishable in gross morphology from *A. cuneifolium*, but which were tetraploid and produced bigger spores. They assumed that these could be autotetraploid *A. cuneifolium*. Similar tetraploid plants were reported from Scotland and Ireland (SLEEP & al., 1978). Using experimental methods, involving hybridization with an unrelated species [*A. castaneoviride* Baker = *A. kobayashi* Tagawa, see CHING & IWATSUKI (1982) from Japan and *Phyllitopsis hybrida* (Milde) Reichstein (1981: 107) = *Phyllitis hybrida* (Milde) C. Chr.], followed by the examination of meiosis in the resultant hybrid, SLEEP (1980, 1983) showed that the plants from Scotland were not *auto*- but *allotetraploid*. She concluded that they are an extreme form of *A. adiantum-nigrum*

and provided further evidence by crossing them with an authentic specimen of this species. Meanwhile she also made a cross of the Corsican plants (TR-3839) with *A. castaneoviride*. This experimental hybrid behaved in the same way, showing only (or nearly only) univalent chromosomes at meiosis (SLEEP, 1983 and personal communication). There can therefore be little doubt that the Corsican plants, like those from Scotland, are extreme forms of *A. adiantum-nigrum* growing on serpentinite or other ultrabasic rock and scree. This form (Fig. 7) reproduces  $\pm$  true from spores when cultivated on normal, lime-free soil (SLEEP, 1985 and many plants mentioned in this paper) and deserves recognition at subspecific level. Appropriate names may exist already in the literature e.g. *A. lamotteanum* HÉRIBAUD (1880: 6-7); see also LEGRAND (1883: 74-75); *A. adiantum-nigrum* var. *corunnense* Christ in MERINO (1909). It is however not easy to select the valid one. Dr. Anne Sleep is currently working on this problem and until the correct name is found, we refer to such plants as the "special serpentine form" of *A. adiantum-nigrum*. These plants are found occasionally on ultrabasic rock, and in gross morphology are very close to and in some cases even indistinguishable from *A. cuneifolium*. The recognition of this "special form" of *A. adiantum-nigrum* has two important consequences which are discussed under 1.5 and 1.6.

### 1.5. The type of *A. cuneifolium* must be examined

Viviani described his *A. cuneifolium* from northern Italy. If this plant were the "special serpentine form" of *A. adiantum-nigrum*, the diploid species which we know as *A. cuneifolium* would require another name. The first aim of the work described in this article was to investigate this problem.

### 1.6. The distribution of *A. cuneifolium* must be checked

The recognition of the "special serpentine form" of *A. adiantum-nigrum* made all former reports of assumed *A. cuneifolium* suspect until substantiated by cytological examination or very careful study of spores. Unfortunately most of the important reports (CRABBE & al., 1964: 16; JALAS & SUOMINEN, 1972, map 92; HENDERSON, 1965: 50) are deficient in this respect. As mentioned above (under 1.1.), the ploidy of *A. cuneifolium* has been checked (and found diploid) for only two localities (Zöblitz in East Germany and Kraubath in Austria). We have therefore attempted to examine cytological material from more western parts of Europe (see appendix).

## 2. The type of *A. cuneifolium*

According to PICHI SERMOLLI (1976: 39-41) the holotype of *A. cuneifolium* (originally in GE) was destroyed with the whole of Viviani's herbarium during the last world war but he was able to locate in Bologna (BOLO) material of Viviani with appropriate labels which he designated as lectotype and of which he gives a figure (slightly reduced). We examined this material (Fig. 2-3), which consists of two plants and three loose fronds. The gross morphology of the bigger plant with one frond (Fig. 2) corresponds to small specimens of *A. cuneifolium* but it is impossible to exclude with confidence the "special form" of *A. adiantum-nigrum*. No spores could be found. The material was obviously collected in an immature state. It is now very dark and brittle and we did not try to measure guard cells or other epidermal structures as they are of little value for distinguishing *A. adiantum-nigrum* from *A. cuneifolium* (R. Viane, publ. in preparation). We therefore decided to collect living material from the type locality, as we recently and successfully did in order to check the type of *Dryopteris villarii* (Bell.) Woyнар ex Schinz & Thellung (RASBACH & al., 1982). The outcome in this case proved, disappointingly, to be less conclusive (see below, section 3-5).

## 3. The locus classicus (type locality) of *A. cuneifolium*

VIVIANI (1808) gives the following information: "Reperi in fodinis subterraneis montis Ramazzo, prope Genuam supra *Sestri a Ponente*, in fissuris Serpentinae pyritiferi, unde sal catharticum extrahitur".

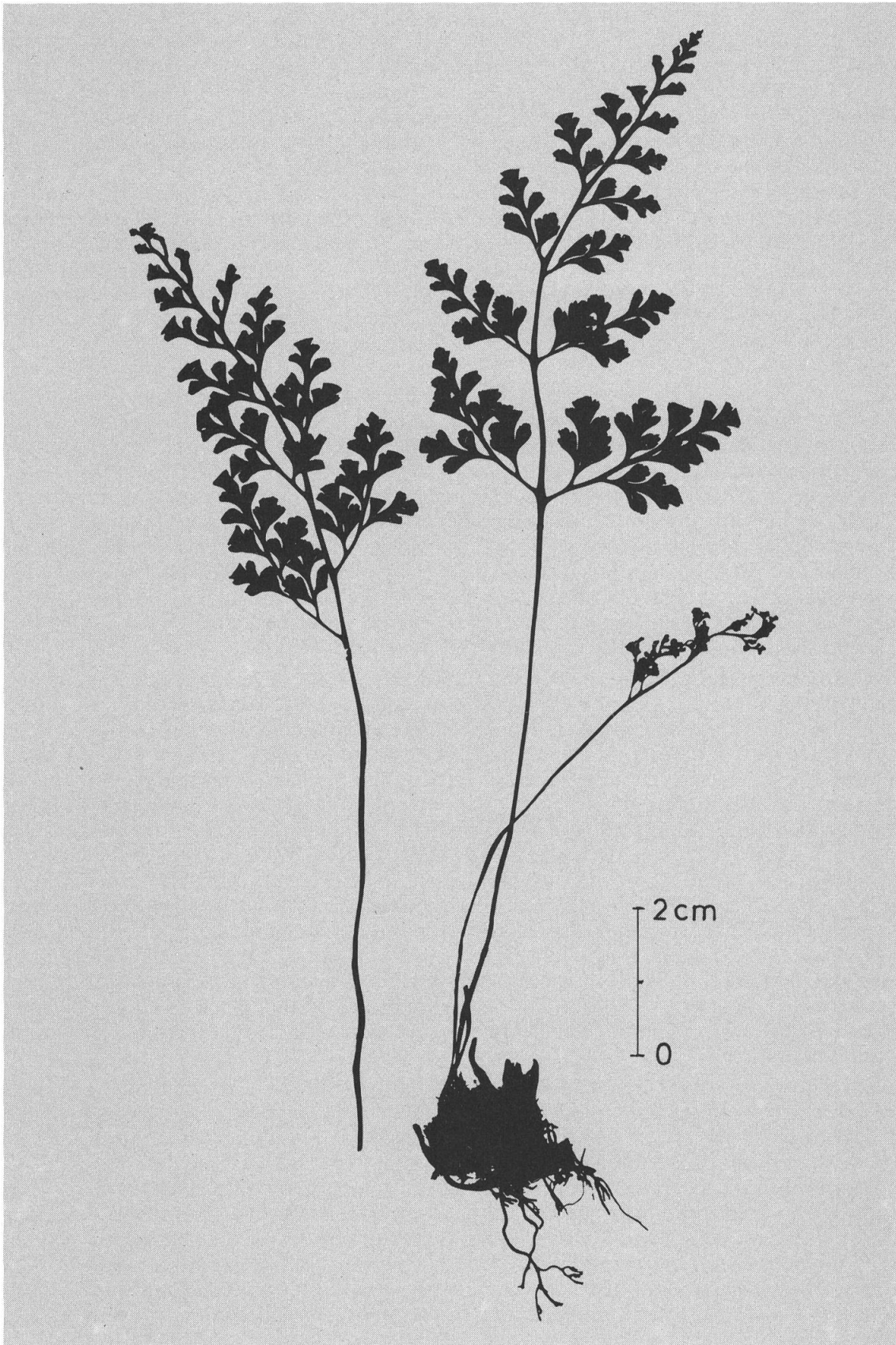


Fig. 2. — Photograph of part of Viviani's material of *Asplenium cuneifolium* in the Herb. Bertoloni in BOLO; the bigger plant and one loose frond (this was perhaps still attached when photographed by PICHI SERMOLLI, 1976). The material was obviously immature when collected, no trace of spores could be found.

We encountered serious difficulties in locating Mt. Ramazzo; it is not shown on any map. Professor R. E. G. Pichi Sermolli, now living near Florence but formerly living and working in Genova (GE) did not know it and recommended to us his former assistant Dr. Enrico Martini, who still works in GE and has lived in Sestri for many years. He also did not know Mt. Ramazzo but after much searching, and enquiring of many people living in the vicinity, in the hills above Sestri, he found a schoolboy, Giuseppe Barea, who could precisely indicate to him the hills which the inhabitants of the nearby houses still call Mt. Ramazzo (Dr. Martini in litt., 27.6.1978).

The region can be found on the old map 1: 25 000 (black and white): Carta d'Italia, Foglio 82 II N.E. (Sestri Ponente) ed. 1878 (Aggiornamenti 1930, Ricognizioni parziali 1940) centered at ca. 44°28'N.; 3°35'W. at ca. 500-590 m alt. The old-abandoned mine (at ca. 500 m alt.) for extracting "bitter salt" (magnesium sulfate), mentioned by Viviani, is given on this map as "Rud.<sup>ri</sup> Min.<sup>re</sup> Solf.<sup>o</sup> Magn." showing that it is exactly the locality where Viviani collected. The place includes ± steep slopes with poor vegetation, *Pinus spec.*, *Robinia pseudoacacia*, *Erica arborea*, *Daphne cneorum*, *Molinia coerulea*, *Schoenus nigricans* etc. among outcrops of serpentinite rocks (see Fig. 4).

Unfortunately the region has suffered in a horrible way since Viviani's time. The hills above the "miniera", which must have been a beautiful wild and interesting place in his time, are used today as a rubbish tip for the garbage of the big city of Genova. A new wide road ("strada militare", not open to the public on working days) leads to the top. On this road heavy lorries arrive regularly to unload their unpleasant content on Monte Ramazzo day after day. Evil smelling smoke escapes from the fires which are lit to burn paper and other easily combustible material, but the bulk rots. When we asked Dr. Martini to lead us to Mt. Ramazzo, he first refused, as he did not want to show us the worst side of his beautiful country which he likes so much. He also doubted whether the ferns would have survived. Only after explaining that we know much of his country and would not judge it from this unfortunate place, that it was the only means to find out what *A. cuneifolium* really is and that some plants may have survived, he finally agreed.

For the first visit three of us (HR, JS and TR) met on 29.5.1978 evening in Arenzano to discuss and spend the night. Next morning Dr. Martini led us to the place. It would have been very difficult to find a reasonable approach without his knowledge as local guide. He drove us on small steep roads to a place ca. 2 km W. of Mt. Ramazzo, from where we could reach it, walking in about 40 minutes. The hill consists of a huge area of serpentinite rock and scree and we were happy to see that some parts (including the "miniera") were nearly free of deposits. We could easily find the plants, which although in a good state for fixing were much too young for the species to be recognized. We took fixings of four plants (TR-4651 to TR-4654) at random and collected two of them living. They were all growing at ca. 590 m alt. in serpentinite rocks with small pines, just above, N.E. of the "strada militare", i.e. also above the "miniera". Fixing No. TR-4652 proved to be a triploid hybrid showing ca. 36<sup>II</sup> + 36<sup>I</sup> at meiosis (see Fig. 11A), while the other three TR-4651, 4653 and 4654) were tetraploid (with ca. 72<sup>II</sup>). The two living plants (4651 and 4653) after cultivation in Basel showed the morphology of *A. adiantum-nigrum*. This rather disappointing result showed, however, that different species are present at that place and that a much more careful investigation was required. The triploid hybrid was evidence that a diploid species of the group must be present.

Again the three of us revisited the place with Dr. Martini on 23.7.1978, a Sunday, which enabled us to drive directly to the hill on the "strada militare". The plants were in excellent condition, mostly bearing ripe spores, and we immediately realized that a complicated mixture of taxa was present. With the help of a field microscope we were able to identify many hybrids. Typical *A. adiantum-nigrum* was recognized by its morphology and glossy green colour. Other plants showed the grayish-green colour, dull surface and shape of *A. cuneifolium* but from brief examination in the field it was not possible to decide whether they were this species or the "special serpentine form" of *A. adiantum-nigrum* mentioned under 1.4. One plant (TR-4694) still carried a frond with unripe sori, a fixing was taken which subsequently proved to be tetraploid. After some searching, we also found two plants of *A. onopteris*. For further study we collected (at the miniera and its immediate vicinity) mature fronds bearing ripe spores from 29 plants (TR-4674 to TR-4702) more or less at random but including the two *A. onopteris* (TR-4676 and 4692 growing a little further off) and at least a few extreme forms of putative *A. cuneifolium* and *A. adiantum-nigrum* and pressed them immediately in the field. The sporangial content was examined at home. The

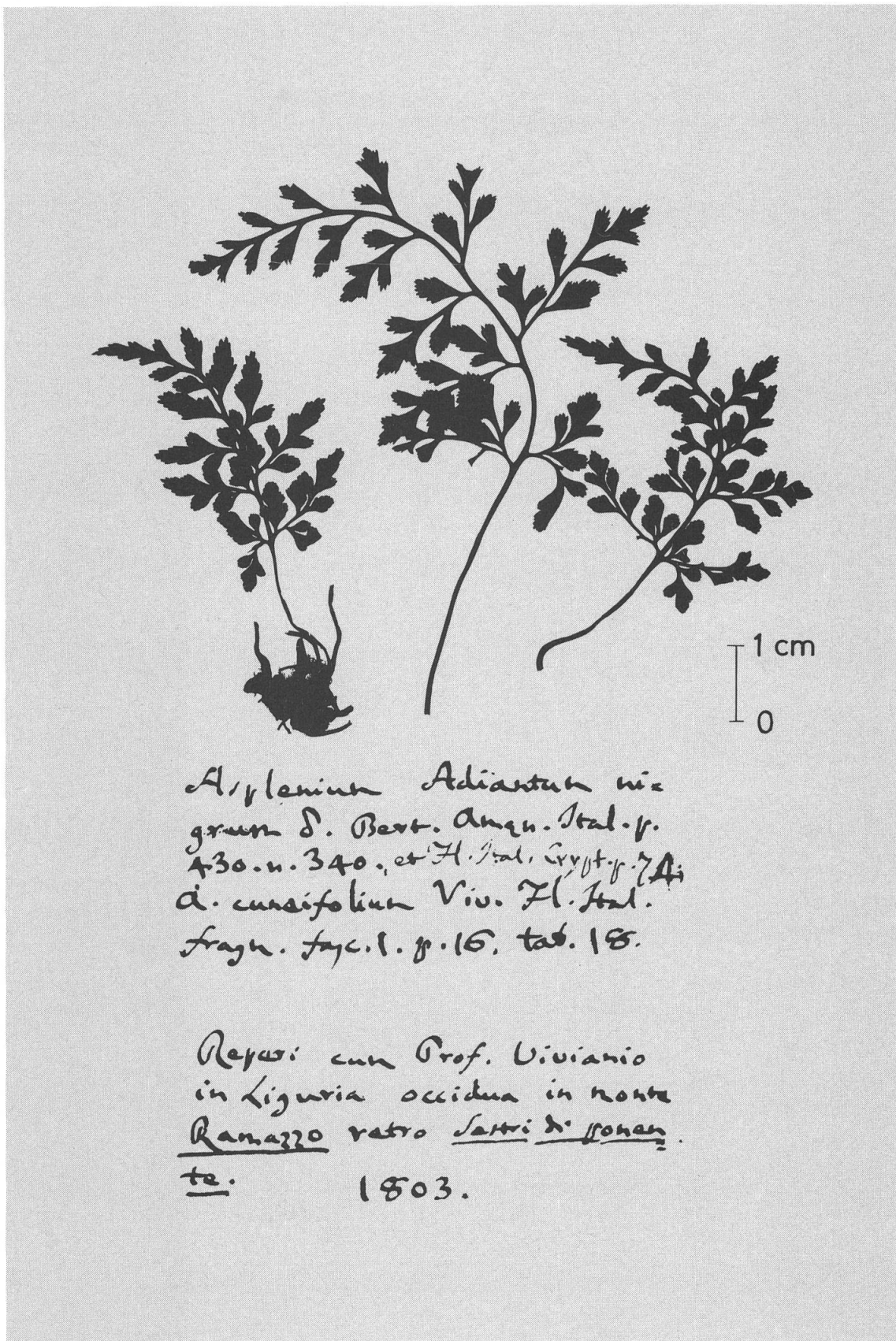


Fig. 3. — Photograph of the rest of Viviani's material, the smaller plant and two loose fronds and the label (transcription of the latter, see PICHI SERMOLLI, 1976: 39). This material is obviously not from the same plant as that of Fig. 2.



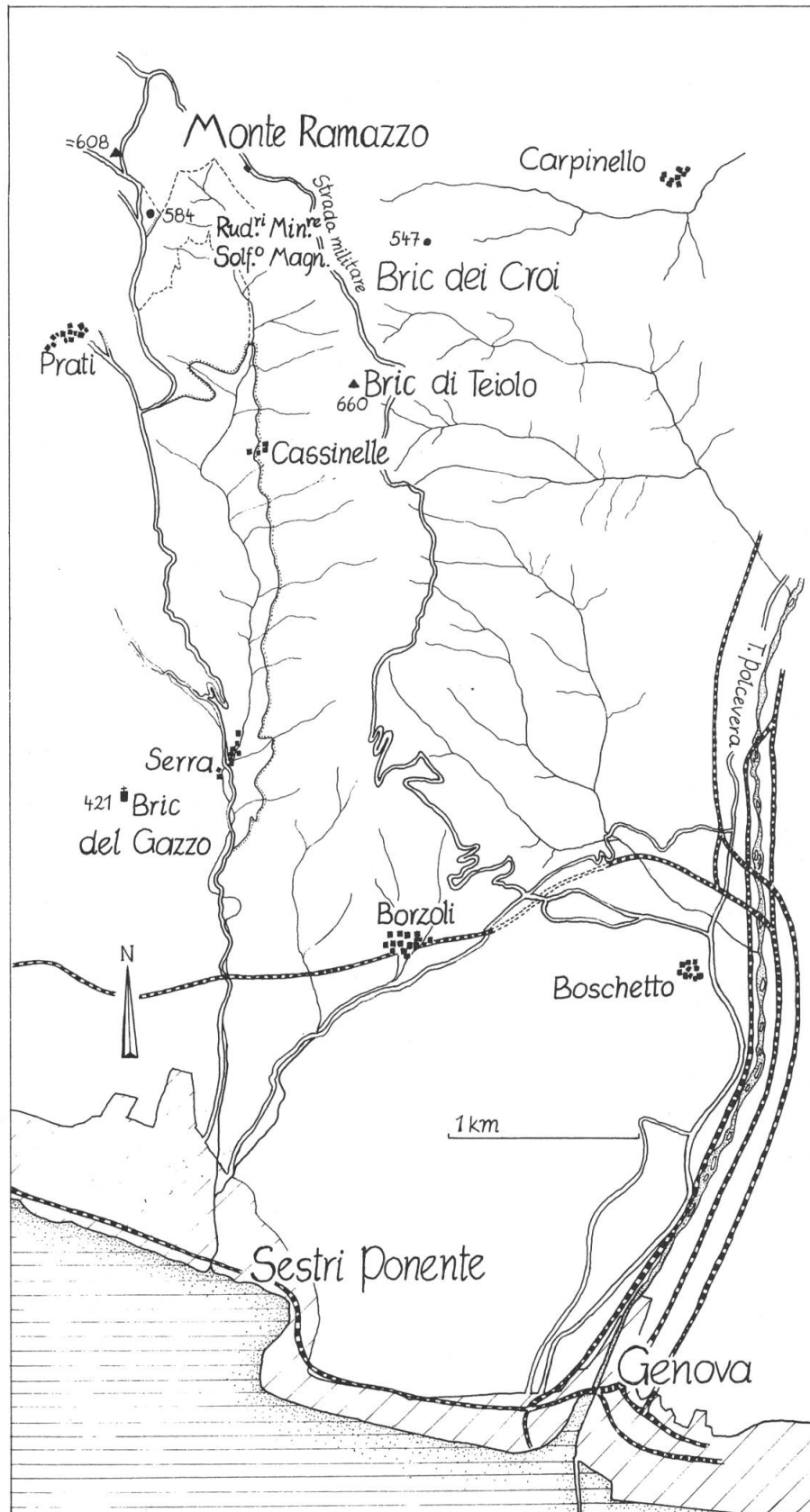


Fig. 4. — Sketch of Monte Ramazzo and the “miniera” at the type locality with local roads leading to it (as seen in the year 1978). Railways are given but autostradas are not included (drawing by H. R.). The name Monte Ramazzo given on this sketch is not be found on any map.

specimens TR-4674, 4675, 4677, 4678, 4679, 4680, 4682, 4684, 4685, 4691, 4693, 4696, 4697, 4698 and 4699 (total 15) turned out to be hybrids with abortive spores, and the spores of the remaining collections measured carefully. The two *A. onopteris* showed the expected size of the exospore to be (27-)30(-33)  $\mu\text{m}$  long. From the spores of one of them (TR-4676) progeny were raised and found to be diploid with  $n = 36^{\text{II}}$  (Fig. 12D). Ten plants (4681, 4683, 4687, 4688, 4689, 4694, 4695, 4700, 4701 and 4702) were identified as *A. adiantum-nigrum* partly by gross morphology and the shining surface of the fronds, those with a more grey-green colour and morphology approaching the "special serpentine form" by spore size with an exospore (27-)33-39(-42)  $\mu\text{m}$  long. From the spores of five of them (TR-4681, 4687, 4689, 4695 and 4700) living progeny was raised in Basel and found to be tetraploid. A fixing of plant TR-4694 was also taken in the field, and proved to be tetraploid. Only two plants (TR-4686 and 4690) turned out to be correct *A. cuneifolium*. They had the appropriate morphology (see Fig. 5 and 6), dull surface and relatively small spores with exospore (27-)30-36(-39)  $\mu\text{m}$  long (see Fig. 10D of TR-4686). Progeny raised from No. 4686 turned out to be diploid ( $n = 36^{\text{II}}$ , see Fig. 11B). Plant TR-4690 was further identified by a SEM photograph of spore architecture (det. by R. Viane); living progeny was also raised and found to be diploid,  $2n = 72$  (det. H. R. 30.10.1984). Cytological examination of these two plants is an unequivocal proof that the diploid *A. cuneifolium* is still growing at the *locus classicus*. It grows there with much *A. adiantum-nigrum* (which in morphology partly approaches or completely matches the "special serpentine form"), a few *A. onopteris*, and many hybrids. We strongly suspect that all the hybrids which we have seen are *A. \times centovallense* D. E. Meyer = *A. adiantum-nigrum*  $\times$  *A. cuneifolium*. The morphology fits well as does the cytological result for TR-4652 (see Fig. 11A). This hybrid is known to form with great ease and is nearly always found when the parents grow together. The other triploid hybrid which theoretically could be present and would show similar behaviour at meiosis is *A. \times ticinense* D. E. Meyer (1960: 391-2) = *A. adiantum-nigrum*  $\times$  *A. onopteris*. This hybrid forms much less readily, is relatively rare and in morphology is much closer to *A. onopteris* (see Fig. 7 in REICHSTEIN, 1981: 119). Among the hybrids which we have seen on Mt. Ramazzo none corresponded with this cross.

The remarkable fact that so many hybrids were found on Mt. Ramazzo is surely not only due to the ease of the formation of *A. \times centovallense* but also to its vigorous nature. One of us (TR) tried to establish both *A. adiantum-nigrum*, *A. cuneifolium* and the hybrid *A. \times centovallense* in an artificial rockery in his garden in Basel. The two species (raised from spores) survived usually 2-4 years while the hybrid was still in good condition after many years.

#### 4. Conclusions

Neither the examination of Viviani's material in BOLO (Fig. 2 and 3), nor the study of the plants still growing on the *locus classicus* give a full answer to the question as to whether or not Viviani's material is the diploid species. All we can say is that it could well be correct. The morphology, at least of the bigger plant (Fig. 2), is  $\pm$  consistent with the diploid species which we know as *A. cuneifolium* and the diploid still grows at the *locus classicus*. But at the same time *A. adiantum-nigrum* also grows there, even more abundantly, and some specimens (e.g. TR-4700) approach the "special serpentine form" mentioned under 1.4. Conditions in the place may have changed since Viviani's time but hardly very much, if they have changed then surely not in favour of the more delicate *A. cuneifolium*. In our opinion Viviani's name should be retained for the diploid.

#### 5. Typification

As mentioned above (section 2), the holotype of *A. cuneifolium* no longer exists. PICHI SERMOLLI (1976) suggested that Viviani's material in Bertoloni's herbarium (BOLO) be accepted as lectotype. This was at a time before the "special serpentine form" of *A. adiantum-nigrum* (section 1.4 and Fig. 7) was known and before the complicated conditions at the type locality were recognized. We should have preferred to reject the material in BOLO as lectotype and to designate a frond of plant TR-4686 (Fig. 5) as a neotype for the following reasons:

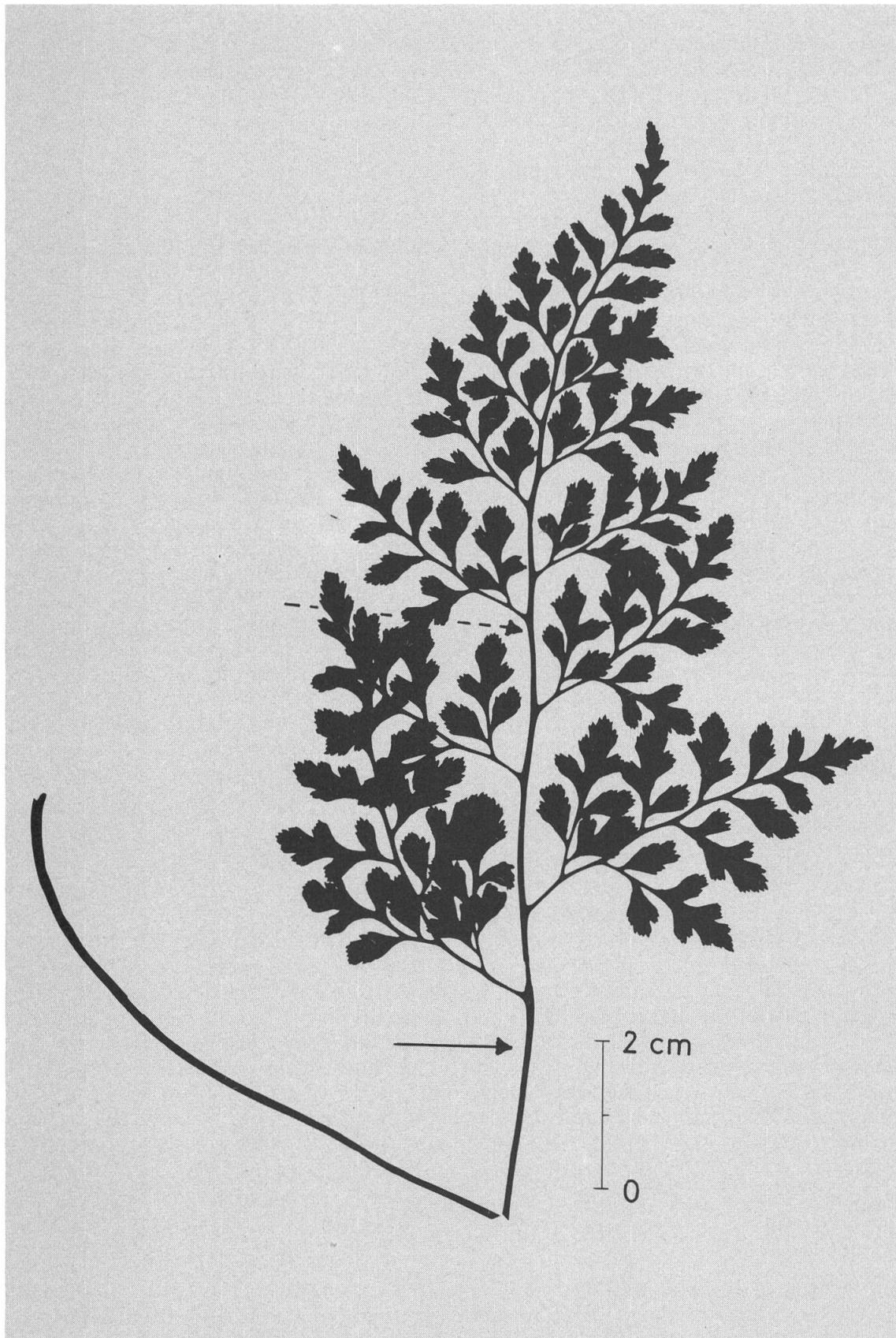


Fig. 5. — Silhouette of *Asplenium cuneifolium* Viv. (diploid) from Mt. Ramazzo, vicinity of the "miniera", TR-4686, 23.VII.1978 (G). The stipe on the original specimen is undamaged, it is only folded in this photograph. The limit between the blackish and the green part of the stipe or the rachis on the upper (adaxial) side is indicated on this and the followings figures 6-9 and 14 by a full arrow, on the lower (abaxial) side by a broken arrow.

1. With present knowledge it is impossible to identify the BOLO material correctly i.e. to establish whether it is the diploid species or a form of *A. adiantum-nigrum* or a hybrid.
2. None of the fronds of the BOLO material (Fig. 2 and 3) matches the illustration which VIVIANI (1808) gives in tav. XVIII of his original description and therefore none of these fronds could have been used as a model for drawing this figure (tav. XVIII).
3. It is by no means certain and even improbable, that the BOLO material is part of the same plant as the holotype which was destroyed (in GE).
4. It is highly probable that Viviani's original collections were heterogeneous.

In our opinion these facts would allow the designation of a neotype even if this procedure is not in full agreement with Art. 7.8 of the Code (VOSS & al., 1983). It would fix the identity of the name *A. cuneifolium* in an unequivocal way but it could only be done with agreement of Professor R. E. G. Pichi Sermolli. Unfortunately he does not share our view about the doubtful nature of the BOLO material and wants to have it stay as lectotype (in litt. 27.2.1986). We therefore accept it as such.

## 6. Appendix. Checking of some collections of putative *A. cuneifolium* from other areas

### 6.1. Portugal

PINTO DA SILVA (1965: 10; 1970: 191-193, 256, 271, 287-292, 333, 348-351) reported *Asplenium cuneifolium* from N.E.-Portugal. He sent us pressed material for inspection. At that time (March 1970) one of us (TR) agreed with the determination, but this became questionable after the discovery of the "special serpentine form" of *A. adiantum-nigrum*. Prof. Pinto da Silva therefore sent us the following living material (with letter of 23.6.1980): *Asplenium* spec., Prov. Tras-os-Montes et Alto Douro, Bragança Donai, Serro 3°, in fissuris rupium serpentinosarum, 900 m.s.m., 19.6.1980., leg. A. N. Teles No. 2249. There were several plants in two clumps each containing several rhizomes with interwoven roots. The first clump was carefully separated into the two individual rhizomes numbered as TR-5192a and b. Fronds of both were slightly shiny. Both plants were potted and, after cultivation, tentatively identified as *A. adiantum-nigrum*, later verified by chromosome counts: both were tetraploid (det. J. S. 7.10.1980). The second clump, which was treated in the same way, gave five plants, TR-5192a-e, all with dull surface, tentatively identified as *A. adiantum-nigrum* but close to the "special serpentine form"; after cultivation two were counted and proved to be tetraploid.

Our result by no means proves that diploid *A. cuneifolium* does not grow in N.E.-Portugal but it does show that much more careful search will be necessary to find it, if in fact it really exists there.

### 6.2. Spain

JALAS & SUOMINEN (1972, map 92) give six dots for *A. cuneifolium* in northern Spain. We have not been able to check the cytology of plants from these localities but we have, however, obtained some results for southern Spain where *A. cuneifolium* has been reported by RIVAS-MARTÍNEZ & al. (1973).

On 16.4.1976 when searching for *Cheilanthes* (see RASBACH & al., 1977: 529 under section 2), H. & K. Rasbach, H. L. & T. Reichstein collected two living plants which were just starting to grow and which we assumed to be *A. cuneifolium*, from the following locality: Prov. Malaga, 36°37'40"N.; 23°5'W. (Spanish map 1:50 000, Ed. Militar, Marbella No. 1065) on serpentinite rocks in a ravine E. of the road from Ronda to San Pedro de Alcántara at ca. 22-23 km from Ronda, eastern spur of the Sierra Bermeja at ca. 1100 m alt., together with *Cheilanthes guanchica* Bolle. The two plants were cultivated in Basel.

Plant No. TR-3968-A was a rather small specimen which succumbed before a cytological count could be made. The bigger specimen, TR-3968, grew well outdoors in Basel in an artificial rockery of tufa in a pocket with some serpentinite chips added. It was still in good condition in the spring of 1983 and it proved to be a hybrid with abortive spores and morphology corresponding to *A. × centovallense* D. E. Meyer (see Fig. 14). A fixing taken 30.9.1976 was triploid with ca. 36<sup>II</sup> + 36<sup>I</sup> at meiosis (see Fig. 13F, F'). The plant succumbed after the very hot summer of 1983.

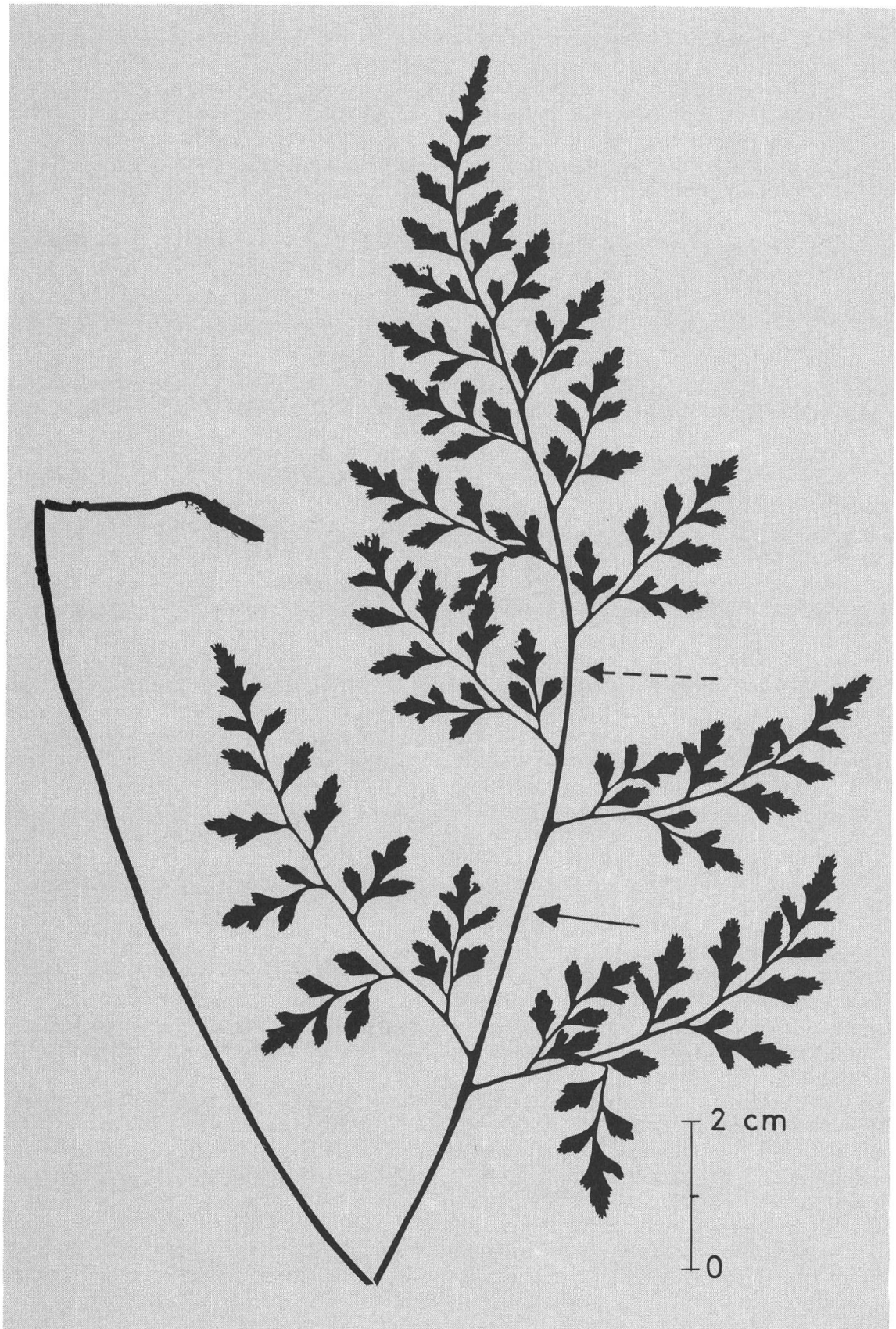


Fig. 6. — Silhouette of *Asplenium cuneifolium*, TR-4690, second plant from Mt. Ramazzo, vicinity of the "miniera", to show part of the range of morphology.

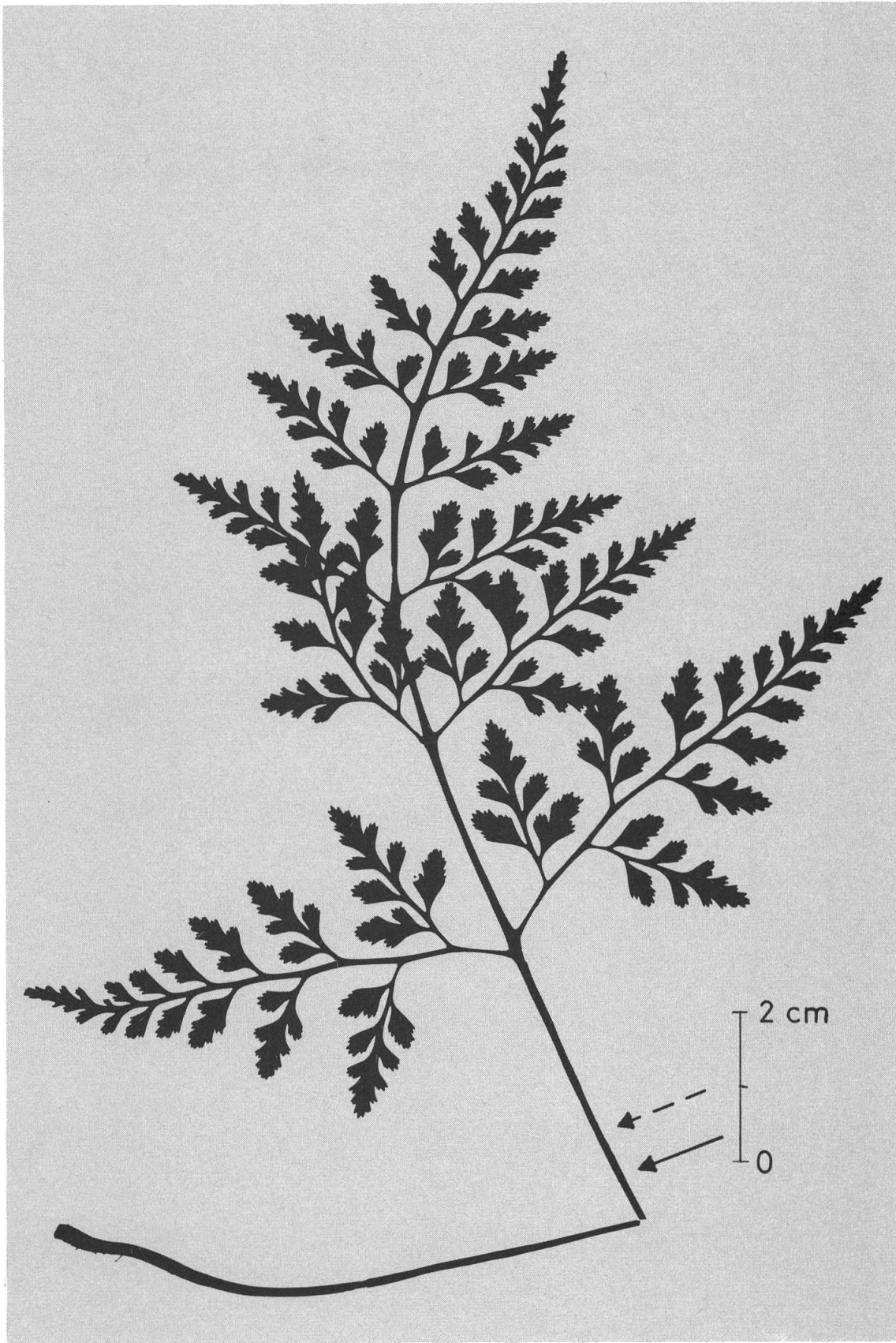


Fig. 7. — Silhouette of the “special serpentine form” of *Asplenium adiantum-nigrum* L., TR-4700 from Mt. Ramazzo, vicinity of the “miniera”, 23.VII.1978, showing how it imitates *A. cuneifolium* in morphology.

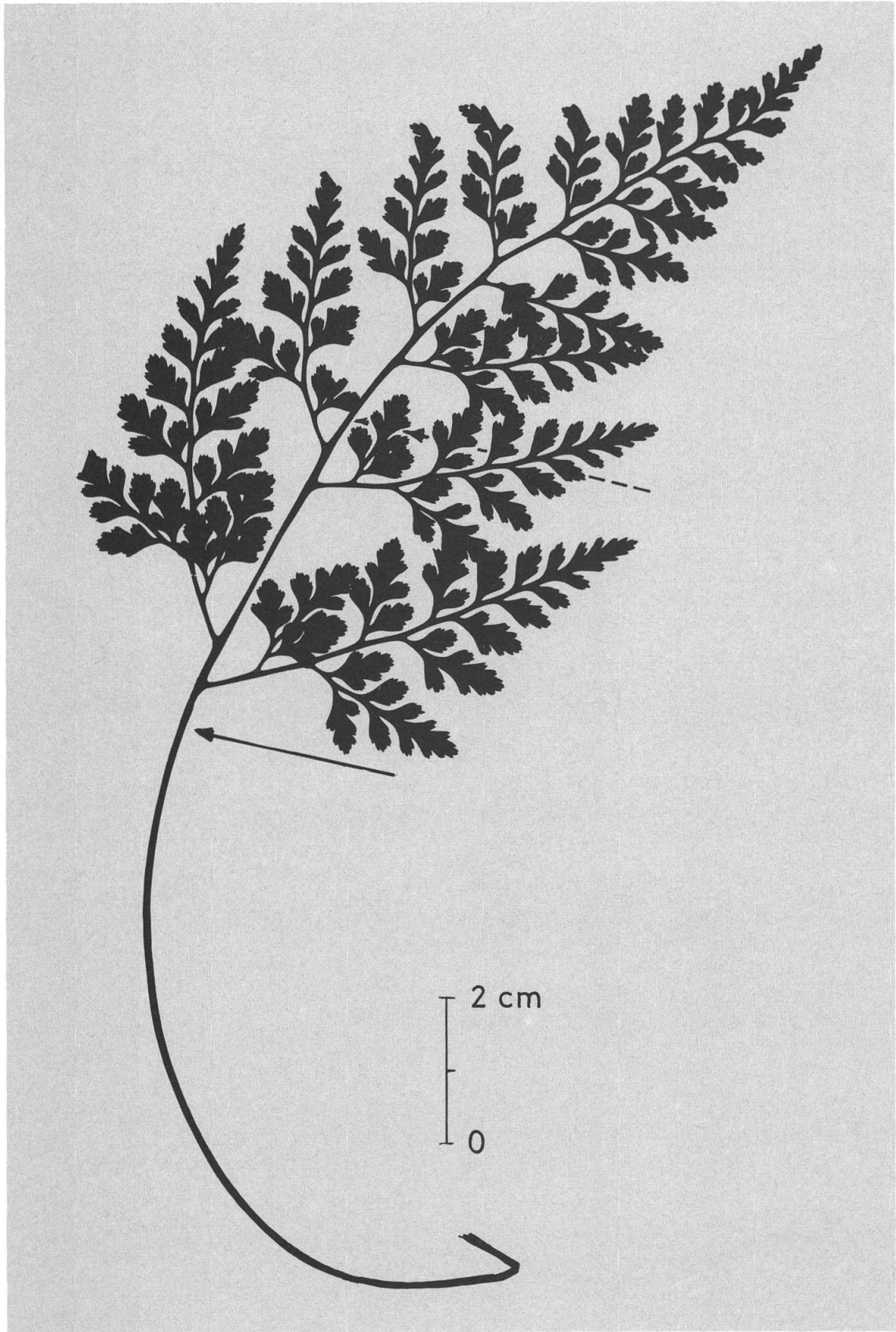


Fig. 8. — Silhouette of *Asplenium* × *centovallense* D. E. Meyer, TR-4675 from Mt. Ramazzo at ca. 590 m alt., 23.VII.1978.  
This hybrid is rather polymorphic and can become much bigger.

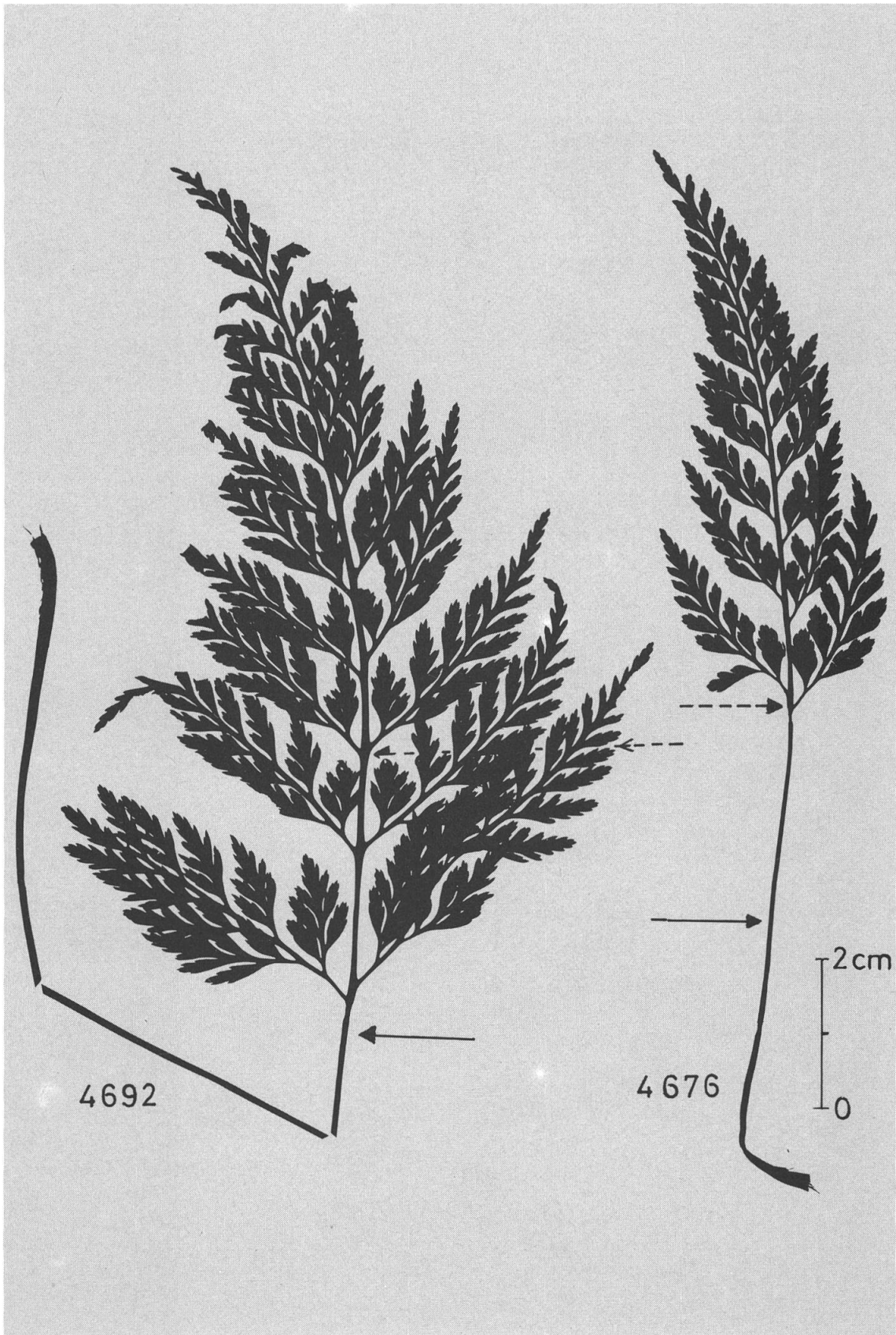


Fig. 9. — Silhouettes of *Asplenium onopteris* L., TR-4692 from Mt. Ramazzo at ca. 530 m, 23.VII.1978 (spores small) and TR-4676 (small plant) from Mt. Ramazzo at ca. 560 m alt., 23.VII.1978. Progeny of TR-4676 was counted and found diploid (Fig. 12D).



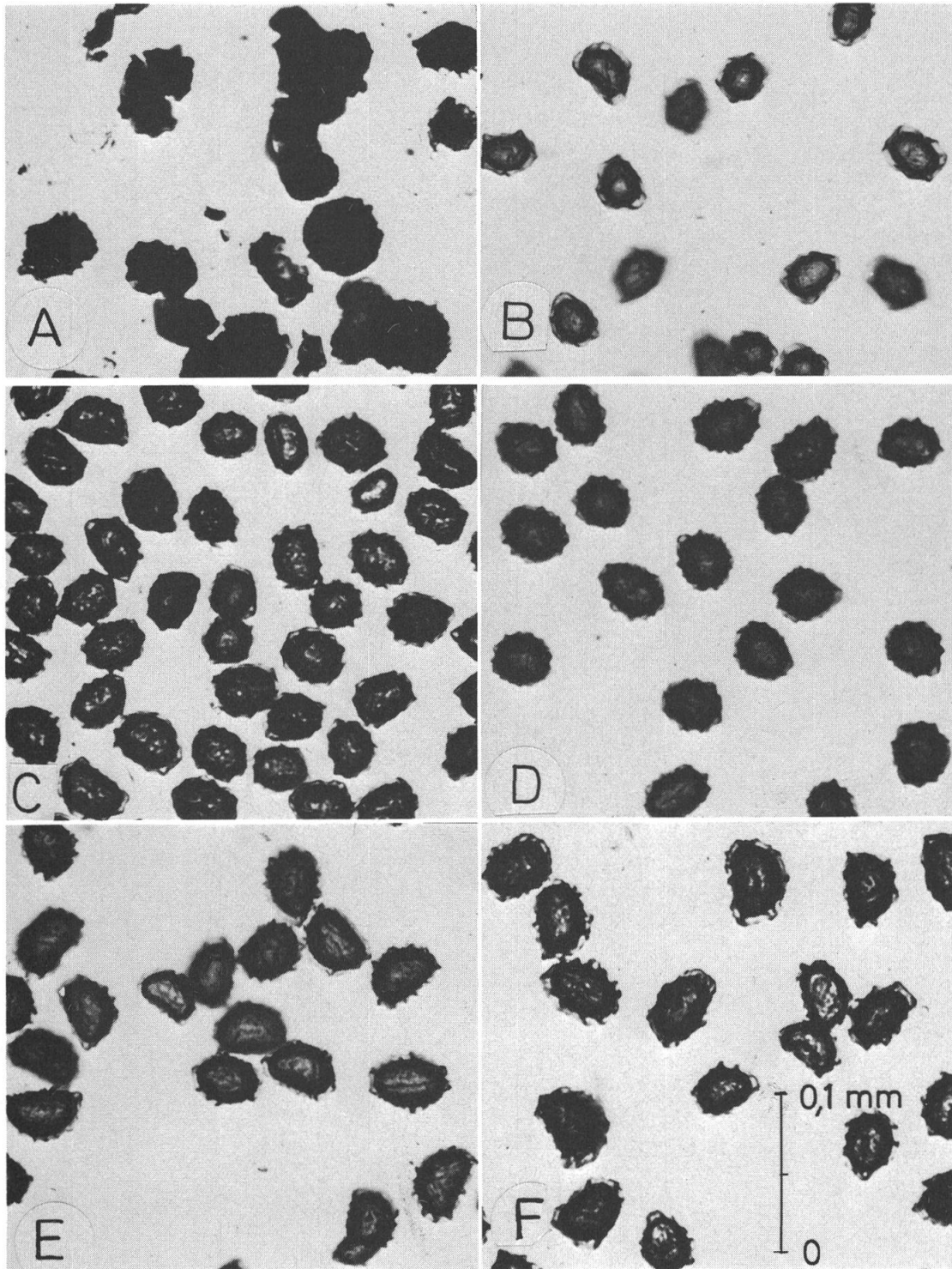


Fig. 10. — Sporangia content (200 ×).

**A** = TR-4684, *A. × centovallense*, only aborted spores visible; **B** = TR-4692, *A. onopteris* L.; **C** = TR-5525, *A. cuneifolium*, Laret (Switzerland), diploid; **D** = TR-4686, *A. cuneifolium*, Mt. Ramazzo, diploid; **E** = TR-3839, *A. adiantum-nigrum* "serpentine form", Corsica, tetraploid, **F** = TR-4700, *A. adiantum-nigrum* "serpentine form", Mt. Ramazzo, tetraploid.

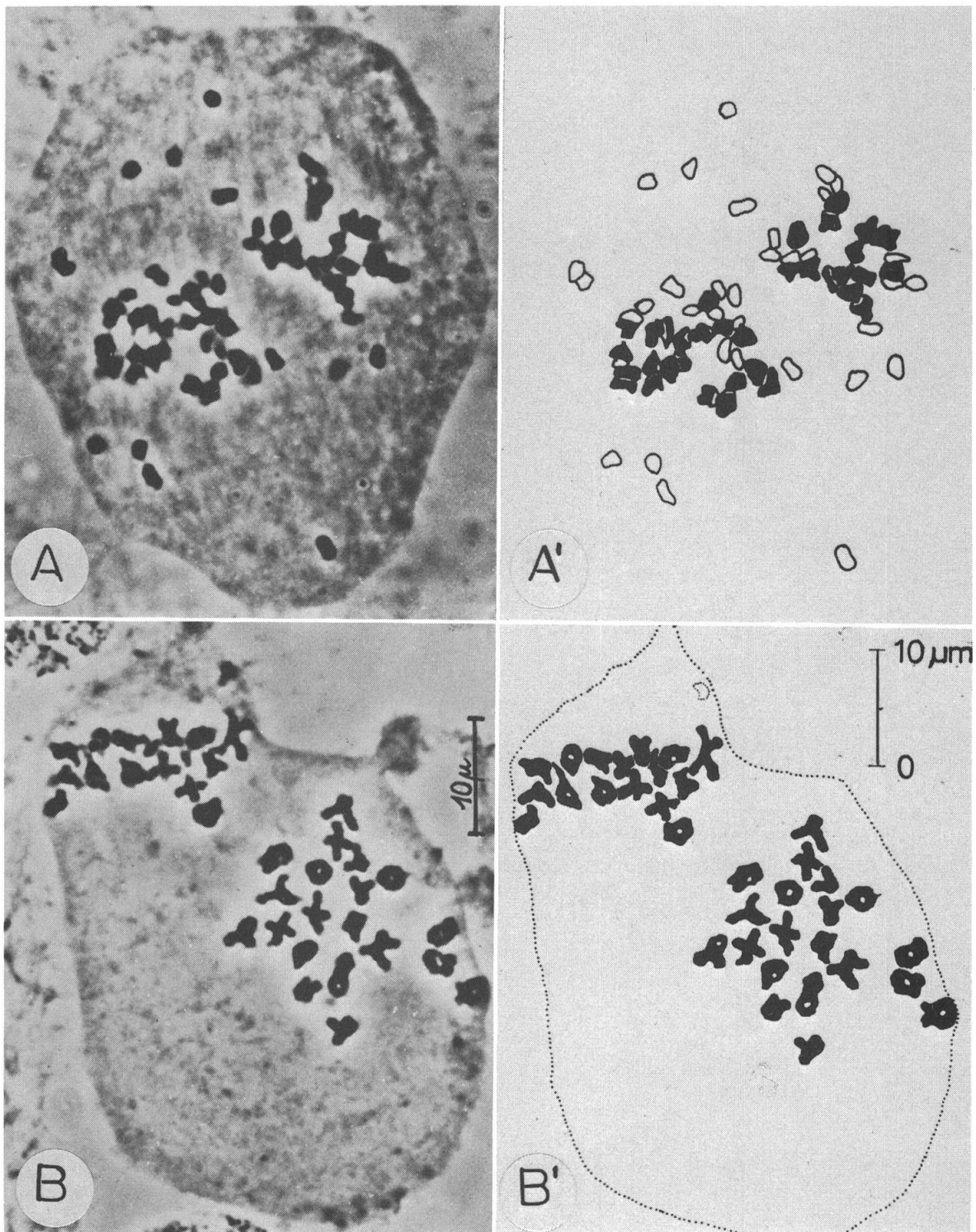


Fig. 11. — Cytology of some plants from Monte Ramazzo.

**A** and **B** = photographs; **A'** and **B'** = explanatory diagrams. **A, A'** = TR-4652 = *A. centovallense* fixed in the field with ca. 36 bivalents (black) and ca. 36 univalents (in outline) at meiosis (diakinesis) (J. S. 2. VII.1978). **B, B'** = TR-4686 prog. = *A. cuneifolium* progeny raised from spores with 36<sup>II</sup> at meiosis (diakinesis) (H. R. 7.VII.1982).

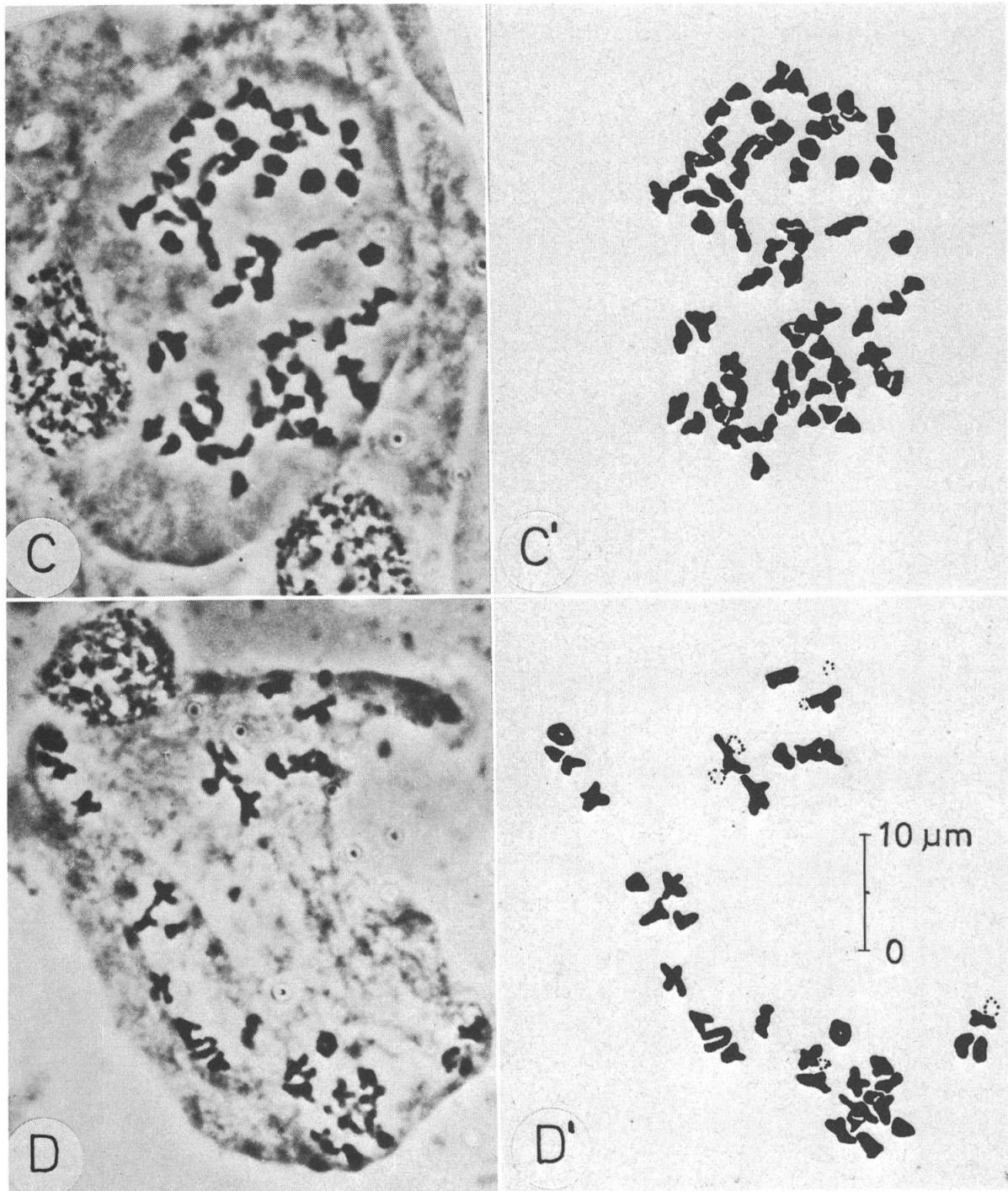


Fig. 12. — Cytology of some other plants. Presentation as in Fig. 11.

**C, C'** = TR-4700 prog. = *A. adiantum-nigrum* "special serpentine form" progeny raised from spores of TR-4700 with 72<sup>II</sup> at meiosis (metaphase I) (J. S. 12.VI.1980). **D, D'** = TR-4676 prog. = *A. onopteris* from Monte Ramazzo, progeny raised from spores of TR-4676 with 36<sup>II</sup> at meiosis (diakinesis-metaphase I) (J. S. 12.VI.1980).

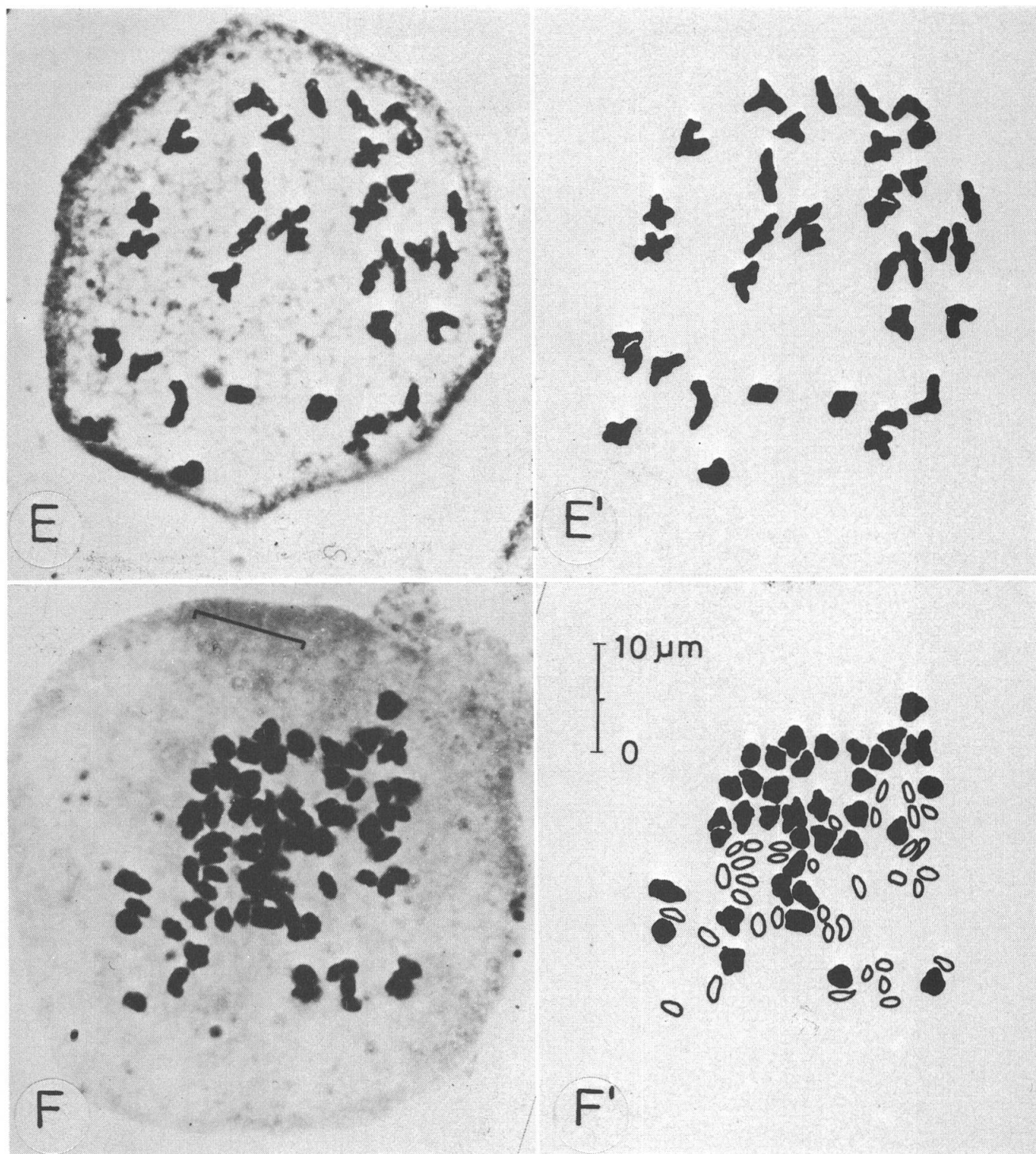


Fig. 13. — Cytology of further plants. Presentation as in Fig. 11.

E, E' = TR-4656 = *A. cuneifolium* from Valle Cannobina, ca. 850 m alt., cult. in Basel with  $36^{II}$  at meiosis (metaphase I) (J. S. 12.VI.1978). F, F' = TR-3968 = *A. × centovallense* from Spain, between Ronda and San Pedro de Alcantara at ca. 1120 m alt., cult. in Basel with ca.  $36^{II} + 36^I$  at meiosis (metaphase I) (M. G. 4.X.1976).

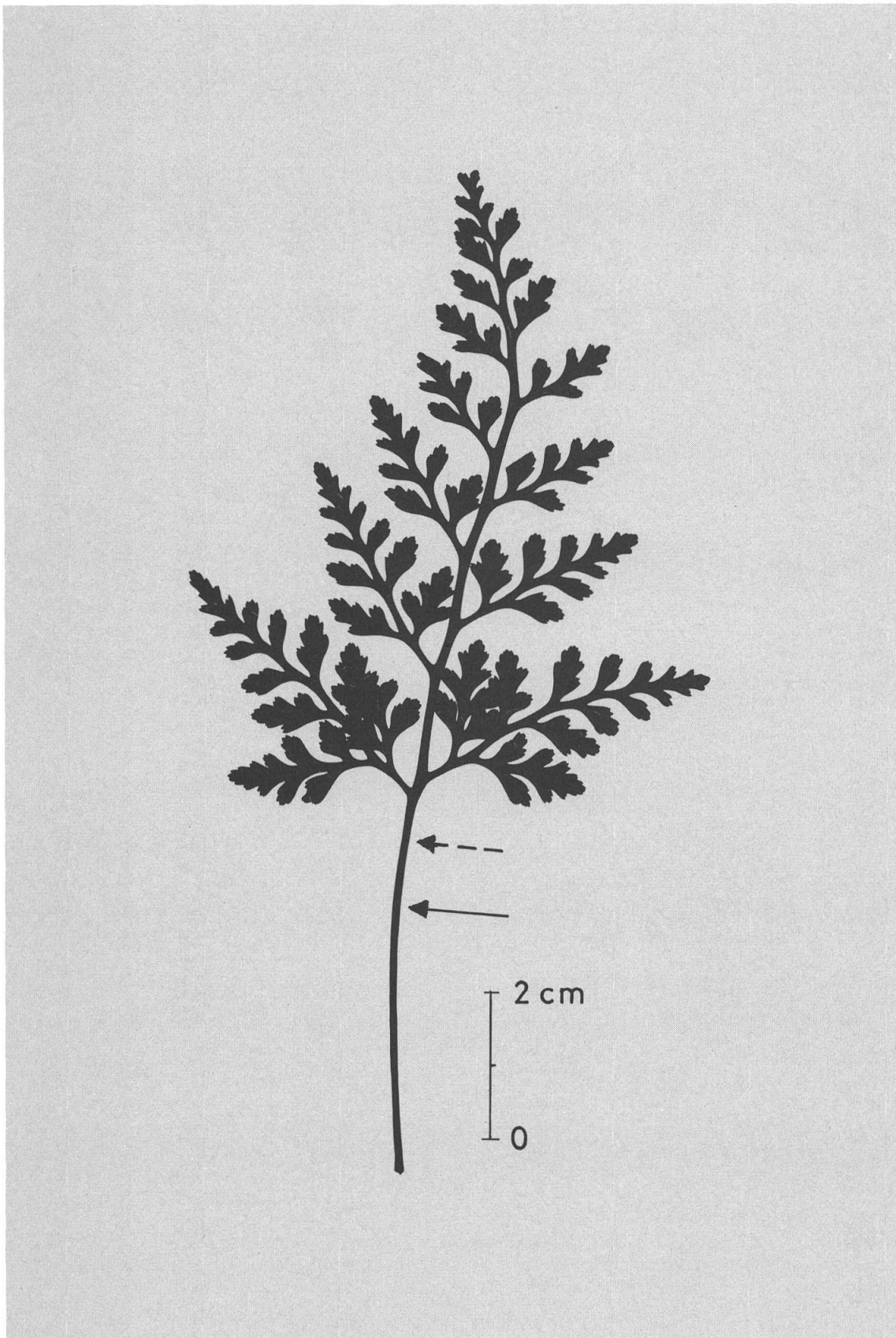


Fig. 14. — Putative *A. × centovallense* D. E. Meyer from Spain, E. spur of the Sierra Bermeja (see 6.2.), TR-3968 coll. 16.IV.1976, pressed 30.IX.1976 after short cultivation in Basel. Triploid, cytology see Fig. 13F, F'.

The results for this hybrid provide good evidence that diploid *A. cuneifolium* must have been growing in the vicinity. It must, however, be rare because when revisiting the place on 16.5.1980 (R. E. Holttum, H. Rasbach, T. Reichstein & A. Sleep), we found only *A. adiantum-nigrum* (TR-5248-5251) of which four were counted and found to be tetraploid (two, J. S. 9.7.1981, two, A. Sleep 1983).

Similar plants of *A. adiantum-nigrum* which partly approached *A. cuneifolium* in their gross morphology, were detected by Mrs. B. Molesworth-Allen in an extensive area with serpentinite rocks, accessible by the new road leading from Estepona in a northern direction to the Sierra Bermeja, between 890 and 1150 m alt., and were examined by H. & K. Rasbach & T. Reichstein on 24.4.1981. They had just produced young growth. Five living plants (TR-5321-5) were collected: three of them were checked after cultivation and found to be tetraploid ( $n = ca. 70^{II}$ , det. J. S. 18.6. and 9.7.1981).

On evidence of our single triploid hybrid, we conclude that diploid *A. cuneifolium* is probably growing in the Sierra Bermeja but that it must be rather rare. Careful and extensive search will be necessary to check whether this assumption is correct.

*Note.* — RIVAS MARTÍNEZ & al. (1982: 91 and 92) report on whole populations of *A. × centovallense* in the Sierra Bermeja. This is a misinterpretation because *A. × centovallense* is a triploid hybrid never forming populations, but always found as single individuals, formed *de novo* at the place where it grows. The two fronds which the authors illustrate are at least approaching the "special serpentine form" of *A. adiantum-nigrum*, and the authors admit that they are tetraploid without giving cytological evidence. The above mentioned plant (TR-3968) is, in our opinion, the only specimen of *A. × centovallense* with checked cytology so far reported from Spain and correctly quoted by GREUTER (1980: 17).

### 6.3. France

Several localities are reported for *A. cuneifolium* in France (JALAS & SUOMINEN, 1972, map 92; BADRÉ & DESCHATRES, 1979: 387-8 but see PRELLI, 1985: 137) particularly for the Massif Central and the "Limousin" (Dep. Haute-Vienne). We examined material from the following places, checked spore size and, in some cases, chromosome number.

1. Pyrénées-Atlantiques. Montagnes d'Ossau, commune de Pé-de-Hourat. Rocher de basalte (pillow lavas, éruptions sous-marines dans les calcaires crétacés) au Pic Moncant, ca. 750 m alt., 26.8.1975, leg. J. Vivant. *A. adiantum-nigrum* approaching *A. cuneifolium*, spores with exospore (30-)33-36(-39)  $\mu\text{m}$  long. Sowings (TR-3937; 5303) gave few prothalli but no sporophytes.

2. Haute-Vienne. La Porcherie (carte Michelin 72, pli 17). A small hill of serpentinite rocks ca. 600 m alt., S.W. of the railway station. This place was revisited on 26.6.1981 by HR, TR, Anne Sleep, JS and Marianne Schneller. Many plants of *A. adiantum-nigrum* grow there, some approaching *A. cuneifolium* in colour and morphology. Three fixings (TR-5468, AS/81/1 and AS/81/2) were taken of three of the latter in the field, and proved to be tetraploid,  $n = ca. 72^{II}$  (det. J. S. 8.7.1981, A. S. 11.9.1981, A. S. 3.8.1981), the last one gave  $n = 72^{II}$  precisely).

La Roche l'Abeille (carte Michelin 72, pli 17). The serpentinite rocks ca. 1-2 km north of this place are also well known for putative *A. cuneifolium* growing partly together with *Notholaena marantae* (L.) Desv. We visited this place on 27.6.1981, large populations of *A. adiantum-nigrum* more or less approaching *A. cuneifolium* were present and Anne Sleep took two fixings in the field, both proved to be tetraploid (AS/81/3 with  $n = 72^{II}$  precisely and AS/81/4 with  $n = ca. 70^{II}$ ).

3. Corrèze. Roc de serpentine de Bettu (carte Michelin 75, pli 9), Exp. S., 5.9.1978, leg. R. Maisonneuve No. 2. Morphology and colour more like *A. adiantum-nigrum* but approaching *A. cuneifolium*. A sowing (TR-4952, 23.1.1979) gave living progeny, which was tetraploid,  $n = ca. 70^{II}$  (det. J. S. 20.8.1980).

Same place, Exp. E., 5.9.1978, leg. R. Maisonneuve No. 3. Morphology similar to above plant, sowing (TR-4953) gave tetraploid progeny,  $n = ca. 72^{II}$  (det. J. S. 20.8.1980).

Roc de serpentine de Reygade (carte Michelin 75, pli 19-20). 5.9.1978, leg. R. Maisonneuve No. 1. Frond more like *A. adiantum-nigrum*, but also approaching *A. cuneifolium*. A sowing (TR-4951) gave living plants of which one was counted and found tetraploid,  $n = ca. 72^{II}$  (det. J. S. 20.8.1980).

4. Haute-Loire. Rocher de serpentine à St. Prejet-Armandon, Est de Brioude (carte Michelin 76, pli 6), ca. 700 m alt., août 1972, leg. R. Prelli. Morphology approaching *A. cuneifolium* but spores with exospore (30-)31-36(-42)  $\mu\text{m}$  long. Sowing (TR-4465, 30.10.1977) gave living progeny which was tetraploid,  $n = \text{ca. } 70^{\text{II}}$  (det. J. S. 18.10.1978).

5. Aveyron. Puy de Volf, N. of Firmi (carte Michelin 80, pli 1). Sur serpentine ca. 500 m alt., 27.6.1972, leg. Christian Bernard communicated by R. Deschatres in litt. 27.11.1980) with two sheets (a and b).

- a. One plant with a ripe frond containing good spores but according to morphology and colour this was *A. adiantum-nigrum*. A sowing (TR-5769, 1.2.1981) did not germinate.
- b. A whole plant with two ripe fronds and one loose frond designated as: "*Asplenium cuneifolium* Viv. var. *lamotteanum* HÉRIBAUD (1880), vidit Callé, 1975". See the note on this taxon by LE GRAND (1883: 74-75). In morphology and colour this material matched well *A. cuneifolium* but no spores could be found.

The Puy de Volf was visited by HR, TR, Anne Sleep, JS and Marianne Schneller on 28.6.1981 but bad weather prevented us from climbing the hill. Above the fountain at ca. 290 m alt., only *A. adiantum-nigrum* was seen. The place was revisited on 20.6.1984 by one of us (H. R. together with the "Schweiz. Vereinigung der Farnfreunde", RASBACH, 1984) during good weather and the hill searched up to ca. 500 m alt. but no diploid *A. cuneifolium* could be found. Independently Anne Sleep got several living plants from the summit of the hill at Puy de Volf by a colleague, and cultivated them at Leeds for about two years. They showed the morphology of the serpentine form of *A. adiantum-nigrum* and they proved to be, like the previous collections, tetraploid.

So far we have not been able to find diploid *A. cuneifolium* in France; more careful searching is necessary to find out whether in fact it grows there.

#### 6.4. Italy

In addition to the *locus classicus* (see section 3), three of us (HR, TR and JS) visited the following places from which *A. cuneifolium* has been reported:

1. Monte Ferrato, N. of Prato (Firenze), see FIORI (1914), MESSERI (1936) E. & S. PIGNATTI (1977). We visited the serpentinite rocks at the base of Mt. Ferrato W. of Figline at ca. 140 m alt., 24.7.1978. In the vicinity of the quarry undamaged outcrops, covered with original vegetation and small trees, were still extant. Many *Asplenium* of the *A. adiantum-nigrum* group which approached *A. cuneifolium* to different degrees were present, partly together with *Notholaena marantae*. Due to lack of time, no careful investigation could be made. A single plant had fixable sori (TR-4709), and was found to be tetraploid (det. J. S.).

2. Alta Valle Tiberina (N.E. of Arezzo), see PICHI SERMOLLI (1948). In this extensive area, we visited two localities:

- a) The limestone and serpentinite rocks above the road from Tavernelle (W. of Anghieri) to Ponte alle Piera ca. 550 m alt., on 25.7.1978. Here we encountered again a similar situation as on Mt. Ramazzo, with many *Asplenium* growing. Although we did not see any *A. onopteris*, we had more luck with *A. cuneifolium*. Among eight collections (TR-4711-8) two were hybrids and one plant with fixable sori (TR-4717) proved to be diploid (det. J. S., 2.8.1978). Its spores had an exospore of (30-)33-36(-39)  $\mu\text{m}$  long. Among the remaining five plants probably 2-3 were also *A. cuneifolium* but no living progeny was raised.
- b) Monte Petroso, S.W. of Pieve S. Stefano at ca. 480 m alt. We took only fronds of three specimens (TR-4720-2). According to spore size, morphology and perispore architecture (SEM photogr. by R. Viane), two of them (TR-4721-22) were *A. cuneifolium*. Spores of TR-4722 were sown and gave diploid progeny,  $n = 36^{\text{II}}$  (det. H. R., 5.7.1985).

3. Piemonte. S. of Druogno in the Valle Vigezzo (E. of Domodossola) and E. of Finero in the Valle Cannobina (N.W. of Cannobio, Lago Maggiore). *A. adiantum-nigrum* and *A. cuneifolium*, *A. adulterinum* Milde with partly nearby *A. ruta-muraria* L., *A. septentrionale* (L.) Hoffm., *A. trichomanes* L. (both subspecies), *A. viride* Huds. and six different hybrids on

ultrabasite-containing mixed rocks, have been reported by BECHERER (1968) and RASBACH, RASBACH & REICHSTEIN (1969), but chromosome counts for the hybrids only were published (REICHSTEIN, 1981). *A. cuneifolium* was much easier to identify in these cooler and less exposed places than on the hotter and more sunny sites on Mt. Ramazzo and the localities mentioned above (1., 2.). Three of us (HR, TR and JS) revisited the area E. of Finero on 31.5.1978 and took two fixings of *A. cuneifolium* (TR-4656 and 4657) which could be identified with the naked eye. Both were diploid with  $n = 36^{II}$  (det. J. S., 12.6.1978 and 28.7.1978, see Fig. 12E).

4. Valtellina. *A. cuneifolium* is also known from the large serpentinite areas in this valley near Sondrio etc. but no counts have yet been reported.

### 6.5. Switzerland

1. Kt. Ticino. Serpentinite rocks above Verdasio in the Centovalli, detected by Becherer & Mokry (BECHERER, 1968). *A. adiantum-nigrum* and *A. cuneifolium* grow there together with *A. adulterinum*, *A. trichomanes* and hybrids, and also *Notholaena marantae* in more or less sunny position; some plants of *A. adiantum-nigrum* tend to approach the "special serpentine form", but *A. cuneifolium* can still be readily distinguished in most cases. This site is also the type locality of *A. × centovallense* and *A. × bechereri* D. E. Meyer = *A. adiantum-nigrum* × *A. adulterinum* (see REICHSTEIN, 1981: 133).

2. Another locality detected in 1969 by I. Ceschi (BECHERER, 1970: 306) is a small serpentinite outcrop above the road W. at "Tries" within the community of Borgnone at 550-570 m alt. *A. adiantum-nigrum*, *A. cuneifolium*, the hybrid *A. × centovallense* and *Notholaena marantae* grow there. Three of us (HR, TR and JS) revisited the place with the "Schweizerische Gesellschaft der Farnfreunde" guided by I. Ceschi on 6. Sept. 1979. The *A. cuneifolium* there can also readily be identified by morphology and spore measurements.

3. Monti di Ravecchia, E. above Giubiasco (S. of Bellinzona) at ca. 985 m alt. Serpentinite rocks along a small road, ca. 300 m N.E. of the parking place. The late Dr. R. Sutter found *A. cuneifolium*, *A. adulterinum* and *A. trichomanes* subsp. *quadrivalens* D. E. Meyer growing there without *A. adiantum-nigrum*. The place was revisited by R. Sutter & T. Reichstein on 17.9.1981. A living plant of *A. cuneifolium* was collected (TR-5560), and found to be diploid  $n = ca. 36^{II}$  (det. J. S., 22.9.1981).

4. Kt. Graubünden. Serpentinite rocks and scree along the Totalpbach W. above Wolfgang-Davos at ca. 1650 m alt. A fixing J. Sch. s.n. [pressed frond (Z)] taken 31.8.1980 was diploid with  $n = 36^{II}$ .

5. Serpentinite rocks and scree above Schwarzsee near Laret at ca. 1570 m alt. A fixing J. Sch.-291, [pressed frond (Z)] taken 31.7.1980 was diploid with  $n = ca. 36^{II}$ . No *A. adiantum-nigrum* is growing on either of these places (4 and 5).

### 6.6. Austria

1. Steiermark. In the surroundings of Kraubath (Murtal) is a wide area of serpentinite rocks and scree. *A. cuneifolium* grows there with *A. adulterinum*, *A. trichomanes* subsp. *quadrivalens*, *A. viride*, hybrids and *Notholaena marantae* but without *A. adiantum-nigrum*. This is one of the places from where the chromosome number of *A. cuneifolium* has already been reported (MANTON, 1953; MEYER, 1957; SHIVAS, 1969). We obtained a living plant collected by H. Melzer in the "Sommergraben" on 20.11.1962 which was cultivated in Basel and found diploid,  $n = 36^{II}$  (det. J. D. Lovis, 4.5.1963).

2. Burgenland. Kienberg above Bernstein. This hill of serpentinite rocks is another well known place for *A. cuneifolium*. It grows there again without *A. adiantum-nigrum*, and produces some very big fronds but no count from this area was previously been reported. A fixing, J. Sch.-6, 21.7.1975, was found to be diploid with  $n = 36^{II}$ , (pressed frond in Z).



### 6.7. Fed. Rep. Germany

Bavaria, see GAUCKLER (1954).

The following seven specimens of *A. cuneifolium* were collected living at two well known places on serpentinite scree and rocks. The distance from this area to Zöblitz (Erzgebirge) in Eastern Germany, where D. E. Meyer had counted two plants, is about 100 km. They were growing here without *A. adiantum-nigrum* and could easily be identified with the naked eye.

1. Fichtelgebirge, Oberfranken, Wojaleite, ca. 500 m alt., 21.9.1982, Ras-299, spores small with exospore (21-)30-33(-36)  $\mu\text{m}$  long, prep. TR-6002.

2. Fichtelgebirge, Oberfranken, near Wurlitz, ca. 510 m alt., 5.4.1984, two plants Ras-386 (cult. in Glottertal) and Ras-387 (cult. as TR-6004 in Basel), both were diploid with  $n = 36^{\text{II}}$  (det. H. R.).

3. Frankenwald, Peterlestein near Kupferberg, ca. 580 m alt., 20.9.1982, Ras-292, spores small with exospore (27-)30-36(-39)  $\mu\text{m}$  long, prep. TR-6003. Three more plants Ras-390, 391 and 392 were collected living and cultivated as TR-6005 and 6006 in Basel and Ras-392 in Glottertal. All three were diploid with  $n = 36^{\text{II}}$  (det. H. R.).

### 6.8. Other countries

We have not examined plants from Eastern Europe (Czechoslovakia, Poland, Jugoslavia, Albania, Greece, Roumania and Anatolia from where *A. cuneifolium* has also been reported. The situation may be less complicated there than in Italy, but the illustrations in NOVAK (1926) give an idea of the many forms he found in Serbia.

## Conclusions

*Asplenium cuneifolium* grows in Austria and in Germany where it is not in association with *A. adiantum-nigrum* and is relatively easy to identify. The same is true for most localities in northern Switzerland (Kt. Graubünden), while in southern Switzerland (Kt. Ticino) and in northern Italy (Piemonte) mixed populations exist and in such localities many hybrids (*A. × centovallense*) are usually present. Cytologically attested diploid material of *A. cuneifolium* has not yet been recorded from France or the Iberian peninsula, although a triploid hybrid found in southern Spain suggests that *A. cuneifolium* does grow there.

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