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Micromorphology, chromosome numbers and phloroglucinols of *Arachniodes foliosa* and *A. webbiana* (Dryopteridaceae, Pteridophyta)

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Abstract

Gibby M., Rasbach H., Reichstein T., Widén C. J. and Viane R. L. L. 1992. Micromorphology, chromosome numbers and phloroglucinols of *Arachniodes foliosa* and *A. webbiana* (Dryopteridaceae, Pteridophyta). Bot. Helv. 102: 229–245.

Arachniodes foliosa from Kenya, the only representative of the genus in continental Africa, was found to be a tetraploid sexual species, while the closely related *A. webbiana*, an endemic of the island of Madeira, turned out to be a diploid sexual taxon. Chemical analysis by thin layer chromatography showed that both species contain phloroglucinols but, like other members of the genus, in much smaller amounts than most representatives of *Dryopteris*. The chemical composition is very similar in both species. The only clear difference was the absence of trispara-aspidin in *A. foliosa* and its presence in relatively large amounts in *A. webbiana*. This relatively small difference in chemical composition alone cannot be taken as valid support for treating these two taxa as different species, and morphological differences hardly exist, even the perispore structure (spiny and distinct from all American and Asiatic species examined so far) is the same. The important difference in ploidy level (coupled, as usual, with spore size and size of guard cells) shows, however, that they represent different taxa. The very close morphological similarity makes it reasonable to assume that *A. foliosa* is an auto-tetraploid species arisen by chromosome doubling from *A. webbiana*. We therefore reduce it to subspecific level as *Arachniodes webbiana* subsp. *foliosa* (C. Chr.) Gibby et al. stat. nov. It was our intention to check this hypothesis by experimental hybridisation. We were not able to perform such experiments through lack of time and appropriate spore material.

Key words: Arachniodes, cytology, chemotaxonomy, phloroglucinols, micromorphology.

1. Introduction

"Acylphloroglucinols" (or just "phloroglucinols") is the name for a particular group of phenolic compounds among the secondary products found in the rhizomes and stipes bases of most representatives of the genus *Dryopteris* Adans. (1763); see reviews by v. Euw et al. (1981), Widén et al. (1991), and literature cited there. In a given species a mixture of a few (out of a total of c. 28 known) compounds is usually present. They are produced and stored in the rhizomes and stipe bases of the plants in internal (Harada 1951 a, b; 1952 a–c, Harada & Saiki 1956 a–c, Mittal 1959, Mehra & Mittal 1961, Huurre et al. 1979) or external secretary glands (Widén et al. 1976; 1978; 1981 see also review by Widén et al. 1983 and other lit. mentioned there). Their biosynthesis via tetra-oxo-decanoic acid was elucidated by Penttilä (1967), see also Berti & Bottari (1965) and Geissmann (1967). Chemical analysis of their composition can often provide a useful basis for recognition or identification of a species and also for checking the relations between different species. Outside *Dryopteris* such compounds have been observed in some (often only few) representative of several closely related genera within the family Dryopteridaceae Ching (1965). These include: *Acrophorus nodosus* Presl (see Widén et al. 1978), several species of *Arachniodes* Blume (1829: 241) (see below), several species of *Ctenitis* (C. Chr.) C. Chr. in Verdoorn (1938) (see Mittal 1959, Mehra & Mittal 1961, Nico et al. 1961, Widén & Puri 1979), two (out of six) species of Australian *Lastreopsis* Ching (1938): *L. decomposita* (R. Br.) Tindale (1957: 184) and *L. marginans* (F. v. Muell) D. A. Smith & Tindale in Tindale (1957: 182) (see Tindale et al. quoted by Widén et al. 1983, never published), *Pleocnemia conjugata* (Blume) Presl (1836: 182) and *P. irregularis* (C. Presl) Holttum (see Widén et al. 1981), *Polybotrya caudata* Kunze (see Widén et al. 1983), only two species from the large genus *Polystichum* Roth (1799): *P. tsus-simense* (Hook.) S.-J. Smith and *P. rigens* Tagawa (see Widén et al. 1978), and *Rumohra adiantiformis* (G. Forster) Ching (1934: 70). In all these genera the content of phloroglucinols is very much smaller than in *Dryopteris*. This may be the reason why first reports on different members of *Arachniodes* were negative (see Harada 1951–52, Harada & Saiki 1956, Inagaki et al. 1961). Meanwhile it became evident that rather many representatives of *Arachniodes* contain small amounts of phloroglucinols, see Widén et al. (1976; 1978; 1981), Tanaka et al. (1979), Tindale et al. (1983) and Widén et al. (1983).

Arachniodes is a genus mainly distributed in Asia, its delimitation from *Dryopteris* and other closely related genera was discussed by Iwatsuki (in Widén et al. 1976). Christensen (1905: 42) merged it with *Polystichum*, Tryon and Tryon (1982) with *Dryopteris*, while most other authors including A. Tryon & Lugardon (1991: 430-2) accept it as a distinct genus comprising c. 50 different species (Kramer 1990).

We were interested in comparing both chromosome numbers and chemistry of *A. foliosa* (C. Chr.) Schelpe (1967: 203), the only representative of the genus in continental Africa (see Schelpe 1970, Schelpe & Anthony 1986, Jacobsen 1983 and Burrows 1990), with *A. webbiana* (A. Br.) Schelpe (1967: 203-4) from Madeira. The two species are very similar in gross morphology. According to Schelpe (1967: 203) *A. webbiana* has "longer marginal spines on the pinnules and rather broader pinnules". Such small differences are spurious and can be observed in different leaves of the same plant in both taxa. In our opinion *A. foliosa* and *A. webbiana* cannot be distinguished by gross morphology. They differ in chromosome number, size of spores, and size of guard cells, see table 1. The geographical separation is no reason for absence of close relations, other elements of the African flora are known to be present in Madeira.

2. Material and methods

2.1 Arachniodes foliosa

TR-2980, 17-XI-1969. Kenya, Taita Hills, Mbombolo Forest, Ronge Ridge, c. 1700 m alt., near the top, among bushes in rocky open place partly shaded by trees, by P. R. O. Bally, Raffael Abdallah and T. Reichstein. Two small living plants were collected, cultivated at Basel in pots in a greenhouse in winter, outdoors in summer. Propagated from spores. Several plants also cultivated outdoors in Agarone (Ct. Ticino, S. Switzerland, above Cugnasco, at c. 250 m alt.) on natural, limefree soil near water courses where they survived the winters in spite of frost. Root tip fixings (22-VI-1978) and sporangia fixings (29-VII-1978) were sent to M. G. in London (see Fig. 8). A mature frond was pressed and deposited as voucher in H, others in Herb. T. R. Five rhizomes (fresh weight 42 g) were taken from small cultivated plants (5-VI-1975), washed and dried for a week on sieves in a stream of air at 40°. They yielded 17 g of dry material for analysis.

RV 287, Kenya, Mt. Kenya, N. side, E. side of Sirimon track at c. 2670 m alt. 25-VII-1975 (not for chemical analysis, Gent).

RV 395, Kenya, Mt. Kenya, E. side, Meru track, terrestrial c. 2300 m alt. 1-VIII-1975 (not for chemical analysis, Gent).

2.2 Arachniodes webbiana

Five collections were available: 1. TR-2603, 4-IV-1969. Madeira, Ribeira Funda, c. 300 m alt., c. 17°8'West, 32°49'North, leg C. J. de Joncheere, H. L. R. and T. R. Three living plants collected and several fronds, among these two fertile ones. The species was usually sterile when growing in shade. Fertile fronds (very large, c. 90–100 cm long, see Fig. 2) were found in a partly sunny place among shrubs. The plants were cultivated as mentioned for *A. foliosa* and propagated from spores (sowing 11-V-1969). – 2. C. Simon 76-95, 29-III-1976, Madeira, Ribeira Funda. The rhizome of a large plant was collected, washed and dried, yielding 27.3 g for analysis. – 3. TR-5228 raised from spores of Ras 96, 11-VI-1980. Madeira at c. 130 m alt. in a small Ribeira E. of Seixal, leg H. and K. Rasbach. Fragment of a leaf with mature spores. Sowing 14-VIII-1980 yielded good progeny. First root-tips fixed 3-VI-1982 were diploid, 2n = c. 82 (M. G. in lit. 13-XI-1982). The plants were further cultivated outdoors in Agarone. – 4. H. & K. Rasbach 128, 20-VI-1980, Madeira, same small Ribeira E. of Seixal, c. 100 m, only a fertile leaf was collected. – 5. R. Viane 3140, 18-VII-1985, 5 km W. of S. Jorge, 450 m alt. (not for chemical analysis). Later also cult. in GENT.

2.3 Microcharacters

Size of spores and guard cells in stomata was measured independently by two of us (H. R. and R. V.). Small accumulations of spores were embedded in balsam, no acetolysis or other pretreatment was used. H. R. measured the length of the exospore in c. 30 mature spores. For estimating the length of the guard cells a few pinnules or ultimate segments were cleared in chloralhydrate (80% crystals liquified with 20% water), washed and mounted in balsam. For measuring she used a light microscope with calibrated eye-piece and 400 × magnification, see details in Demiriz et al. (1990) and Rasbach et al. (1990). R. V. used Ruthenium red for staining epidermis preparations (Fig. 6); for measuring cell sizes a quicker, more precise and statistically more correct, computerized method (see Demiriz 1990; Viane 1990) was used. His results (given as mean ± standard

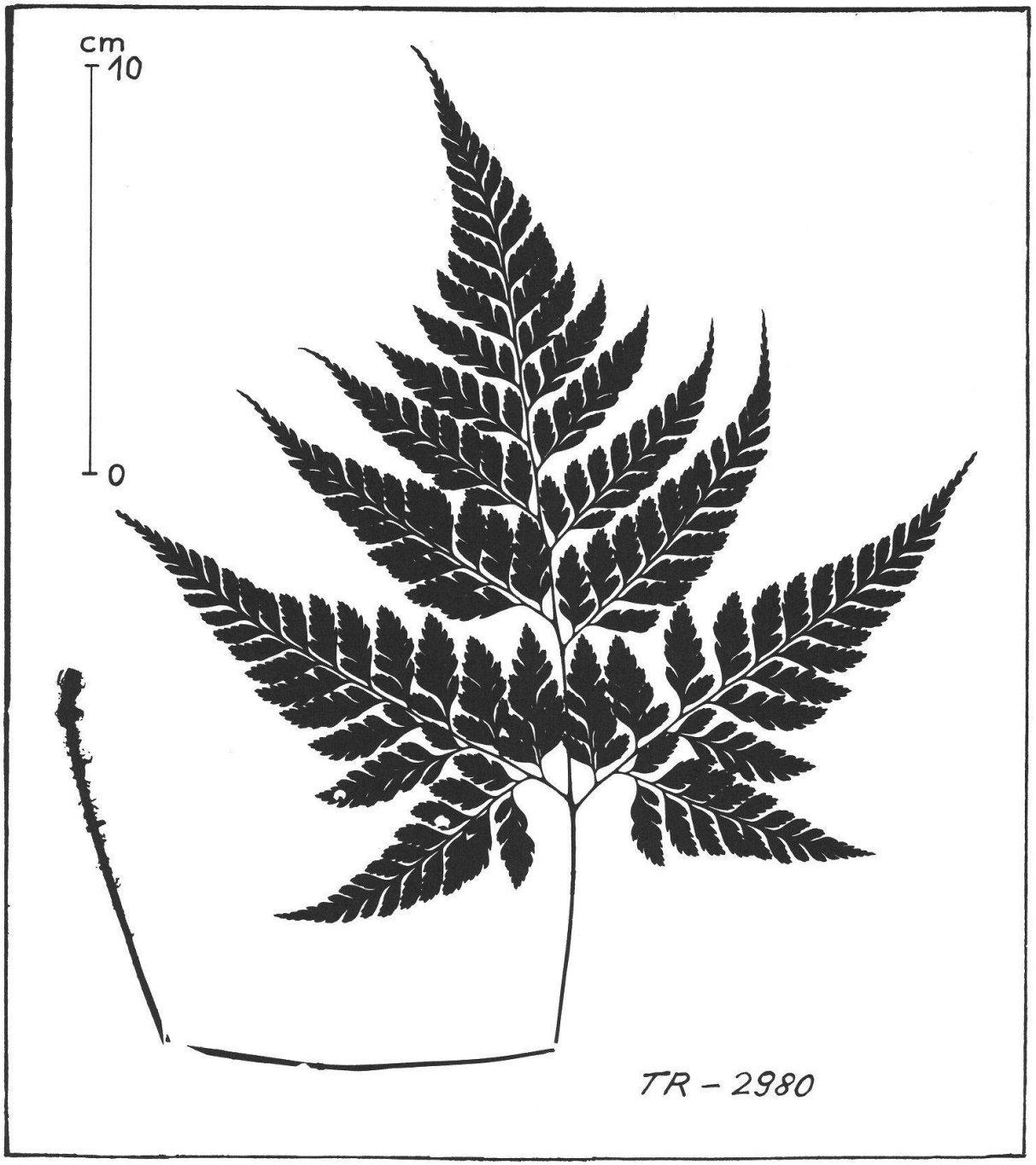

Fig. 1. Silhouette of fertile leaf of *Arachniodes foliosa* (TR-2980 original collection). Bar = 10 cm. Photogr. P. Eglin, Basel.

error) are based on 50 spores and at least 100 guard cells for each sample. Results see Table 1. Scanning electron microscope (SEM) photographs were made by R. V. as described in Rasbach et al. (1990).

2.4. Cytology

Pinnae with immature sporangia were fixed in glacial acetic acid and abs. ethanol mixture 1:3 (vol.:vol.). Staining with acetocarmine, squashing and preparation of perma-

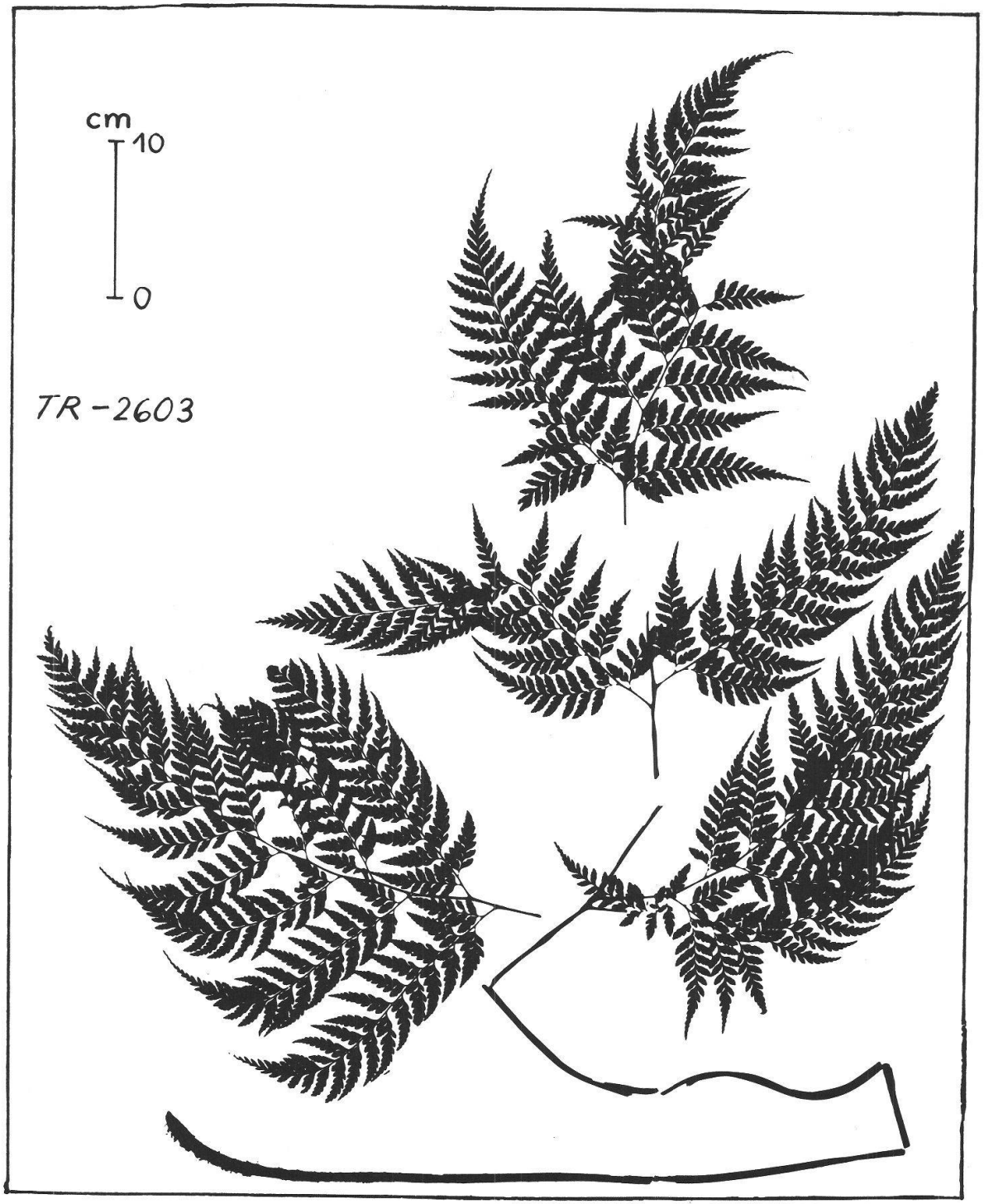

Fig. 2. Silhouette of fertile leaf of *Arachniodes webbiana* (TR-2603 original collection). This very large frond (145 cm long) had to be cut into five parts (including petiole) for pressing. Bar = 10 cm. Photogr. P. Eglin, Basel.

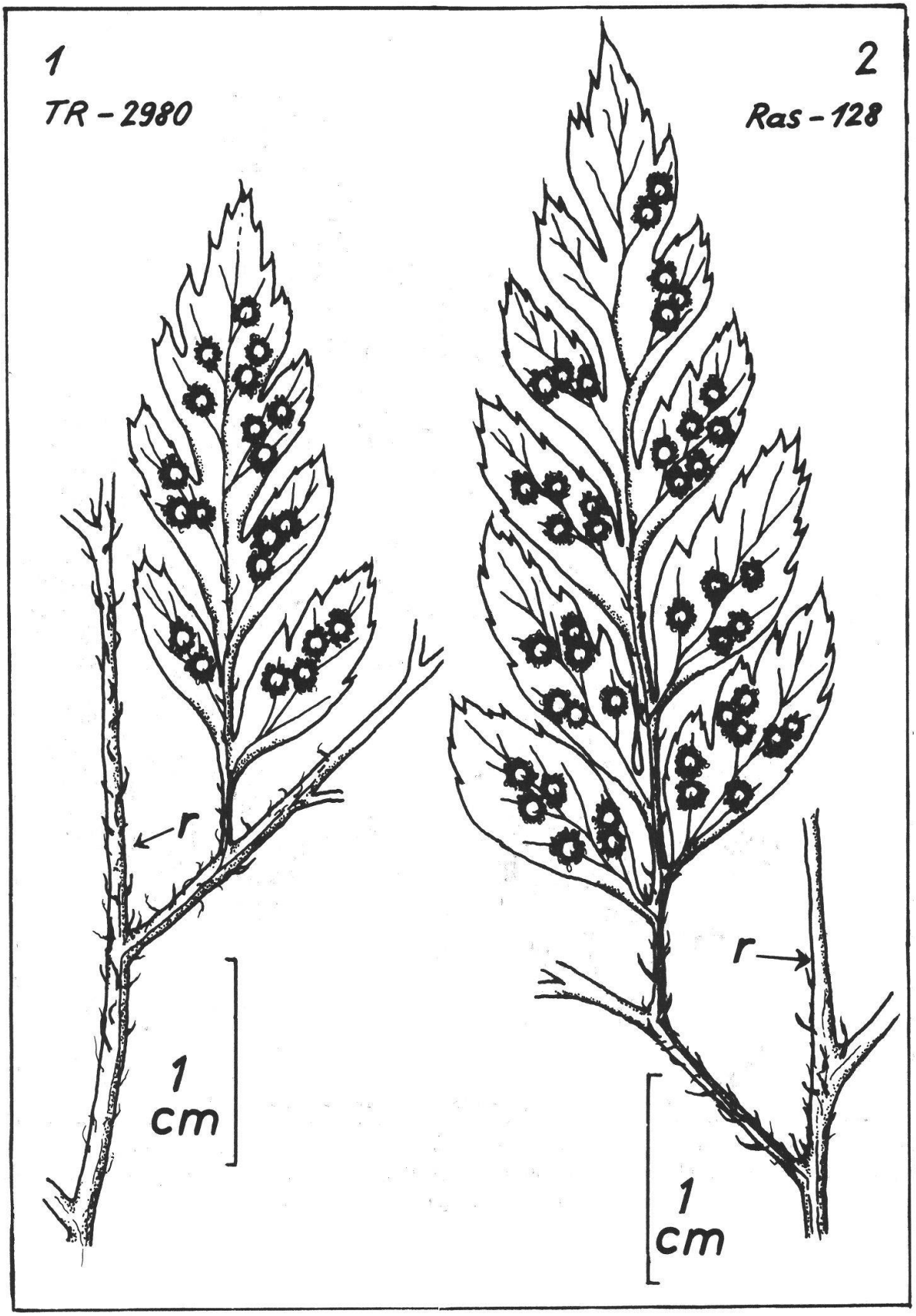

Fig. 3. Pinnules with sori. 3.1 = *Arachniodes foliosa* (TR-2980). 3.2 = *A. webbiana* (Ras-128). r = rachis. Drawings HR.

Tab. 1. Size of spores, guard cells and scales of petiole bases

Name of plant and collectors number	Ploidy level [1]	Lexo [2]	g-cells [3]	Scales of petiole bases [4]
A. foliosa TR-2980	4×	HR (32) 35-38 (42.5) RV (28) 32-34-36 (39)	n.p.	n.p. [44]
TR-2980			(33) 42-46-49 (58)	8.7 (12.3) [63]
TR-2980, RV 287+ RV 395				
A. webbiana TR-2603	2x	HR (29) 31-33 (35)		n.p. [145]
RV 3140		RV (25) 28-30-33 (36)	(33) 37-40-43 (52)	15.1 (25.0) [104]
A. webbiana TR-5225 =Ras 96		HR (29 32-34 (35)	(35) 38-43 (47)	8.0 (12.0) [69]

[1] 2x = diploid, 4× = tetraploid.
[2] Lexo = length of exospore in µm.
[3] g-cells = length of guard cells in µm.
[4] Mean length of largest scales of petiole bases in mm (in round brackets the maximum) [in angular brackets the length of the fronds in cm from which the scales were taken], the scales are somewhat smaller on juvenile fronds, n.p. = not prepared.

nent slides were performed according to Manton 1950: 293–299. Meristematic root tips were treated with 0.1% colchicin in water for 12–16 hours at c. 4 °C, excess of liquid quickly removed on blotting paper and root-tips fixed in acetic ethanol. For softening the roots were warmed in 1N aqueous hydrochloric acid at 60 °C for 15–20 minutes, stained with Feulgen (*Schiff's* reagent) for 2 hours, squashed in 45% acetic acid, examined and photographed using phase contrast.

2.5 Chemical analysis

Extraction of rhizome powder was performed as described by v. Euw et al. (1980: 301), see also Widén et al. (1991), using the "standard" method. Both species yielded only small amounts of crude ether soluble material. No attempts were made to isolate pure compounds on a preparative scale. In *A. foliosa* the crude ether extract was used directly for thin-layer chromatography (TLC) on buffered SiO_2 (v. Euw et al. 1980: 298-299) with at least two different solvent systems and direct comparison with authentic reference compounds. In *A. webbiana* the crude ether extract was first processed by the "MgO"-procedure and the so concentrated "MgO-filicins" analysed in TLC. Results are given in table 2.

3. Results

3.1 Gross morphology

A. foliosa and *A. webbiana* turned out to be indistinguishable by gross morphology (as visible with the naked eye).

3.2 Microcharacters

In both taxa small reduced scales (paleasters) terminating in a long uniseriate hair are common on the dorsal side of the lamina. Stomata (polocytic) and epidermal patterns

Tab. 2. Semiquantitative results for composition[1] of phloroglucinols in *Arachniodes foliosa* and *A. webbiana*. + + + = more than 20% of crude ether extract for crude "phenolics" ("MgO-filicins"); + + = 10–20%; + = 5–10%; ± = 1–5%; − = less than 1%

Name of plant collectors number	Ploidy [2]	Origin	Amount dry rhizome in g	Amount crude ether extr. [3]	Amount cation free [4]	Amount "MgO-filicin"	2-B [5]	5-BB [6]	6-BB [7]	7-BB [8]	7-AB [9]	8-BB [10]	10-BB [11]	10-AB [12]	20-BBB [13]
A. foliosa TR-2980	4×	Ken[14]	12.7	0.054 (0.42%)	n.p.[15]	n.p.	(+)	(+)	−	+ +	−	+	(+)	−	−
A. webbiana	2×	Mad[14]	97.3	0.37 (0.38%)	n.p.	0.0437 (11.8%)	(+)	(+)	−	+ +	−	(+)	(+)	−	+ +

[1] Estimated from intensity of spots in TLC
[2] 2x = diploid, 4x = tetraploid
[3] In g (in % of dry rhizome)
[4] In g (in % of crude ether extract)
[5] 2-B = Aspidinol-B, chemical structure (5-B) and JUPAC name of this and the following phloroglucinols see table 4 and Appendix (8.1) in Widén et al. 1991
[6] 5-BB = Flavaspidic acid-BB
[7] 6-BB = Aspidin-BB
[8] 7-BB = Para-aspidin-BB
[9] 7-AB = Para-aspidin-AB
[10] 8-BB = Desaspidin-BB
[11] 10-BB = Albaspidin-BB
[12] 10-AB = Albaspidin-AB
[13] 20-BBB = Trispara-aspidin-BBB
[14] Ken = Kenya, Mad = Madeira
[15] n.p. = not prepared

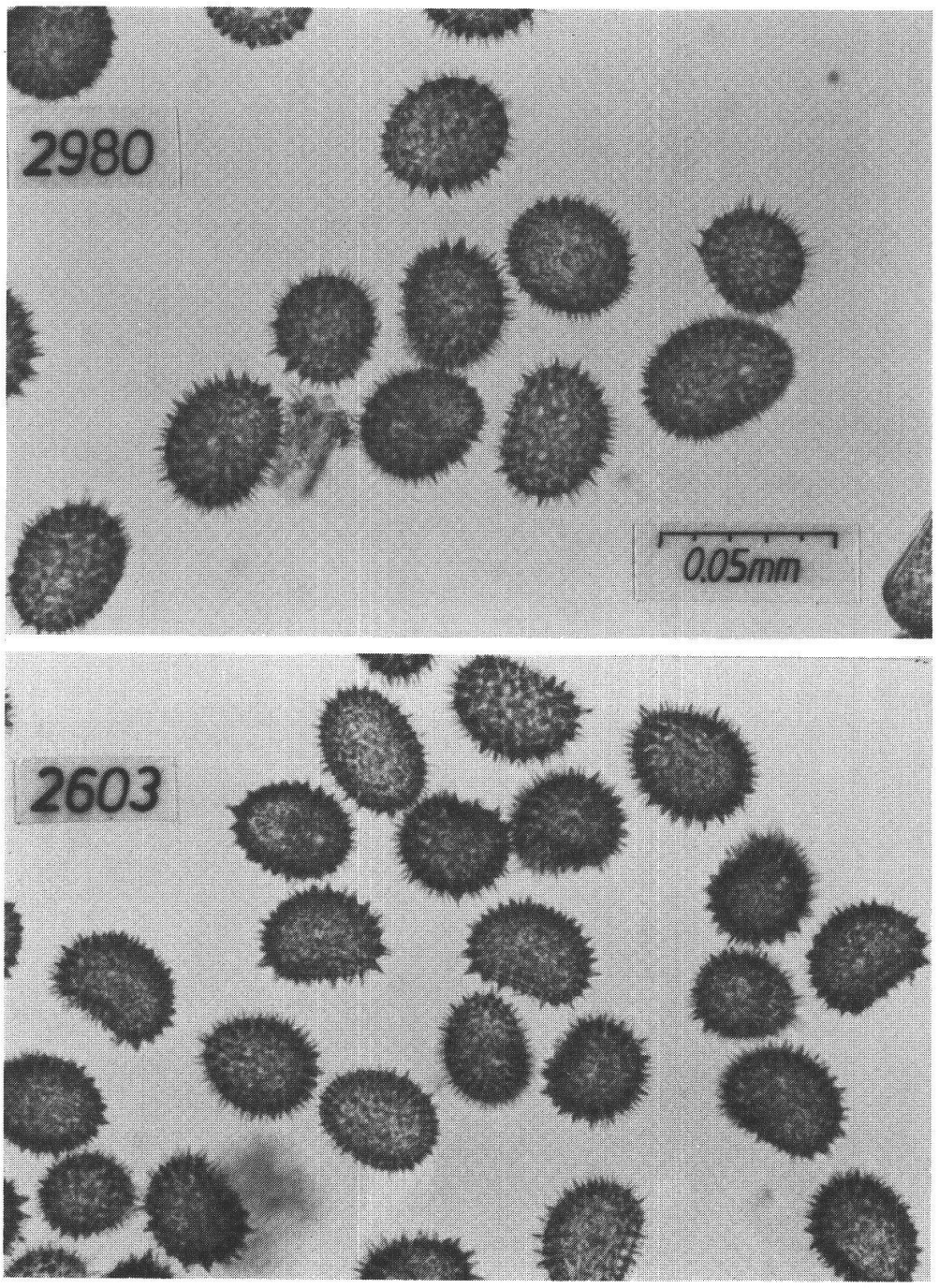

Fig. 4. Photographs of spores under the light microscope. 4.1 = *Arachniodes foliosa* (TR-2980). 4.2 = *A. webbiana* (TR-2603). Prep. and photogr. HR.

are similar in both taxa, but in *A. foliosa* the anticlinal epidermal cell walls are more distinctly 1- to 2-sinuate (Fig. 6.1), whereas in *A. webbiana* these anticlinal walls are mainly 1-sinuate (Fig. 6.2). Epidermal cells are smaller in the diploid taxon; though ranges for the guard cell length overlap, their means are significantly different (see Table 1). Stomatal density is higher in *A. webbiana* (due to its smaller cells).

Both taxa have similar reniform (not peltate, as wrongly stated in Burrows & Burrows 1990), dorsally and marginally glabrous (eglandular) indusia (Fig. 6C, D). In both

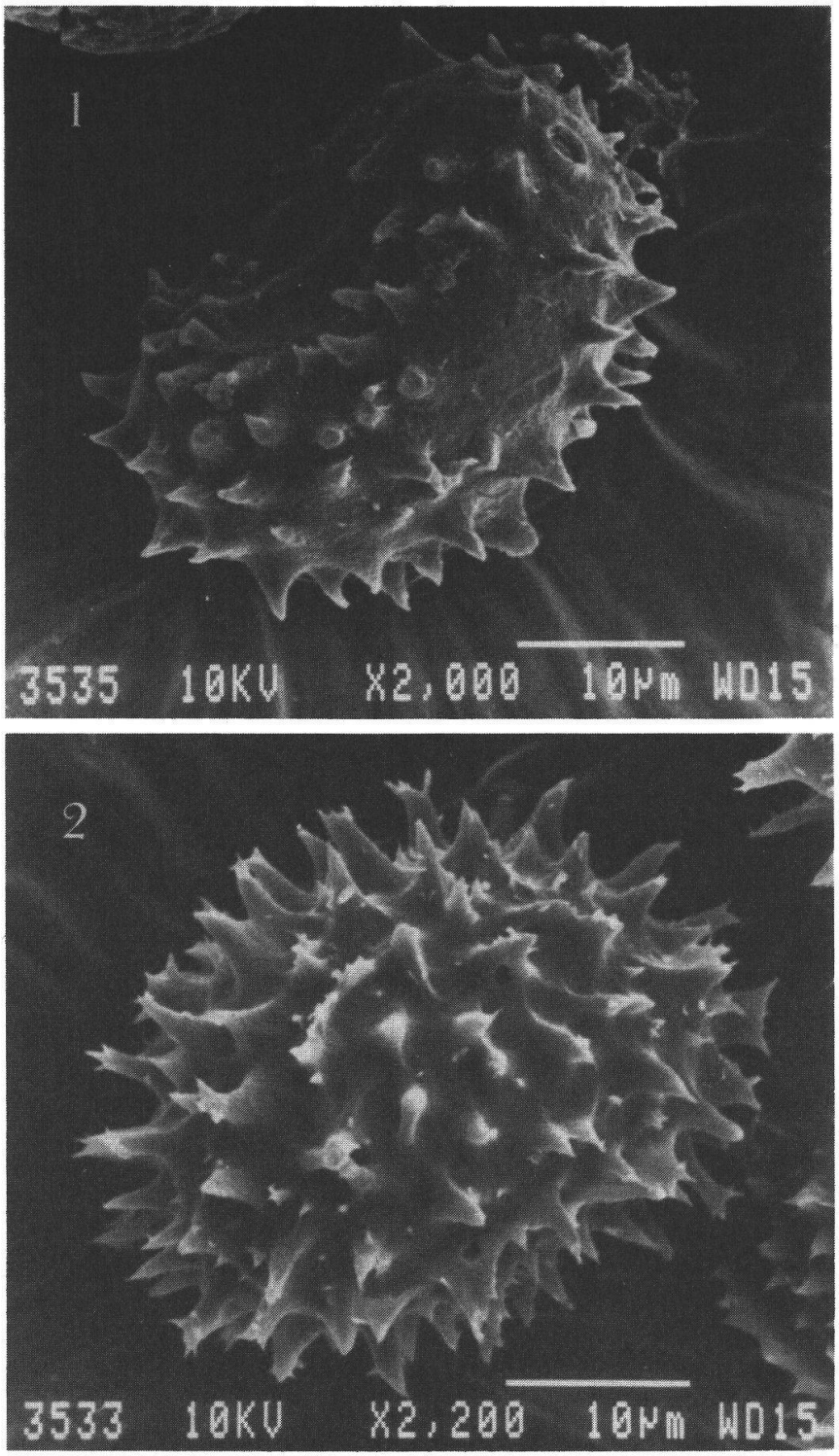

Fig. 5. Scanning electron microscope (SEM) photographs of spores. 5.1 = *Arachniodes foliosa* (TR-2980, not quite mature). 5.2 = *A. webbiana* (TR-2603, mature spore). Bar = 10 µm. Prep. and photogr. RV.

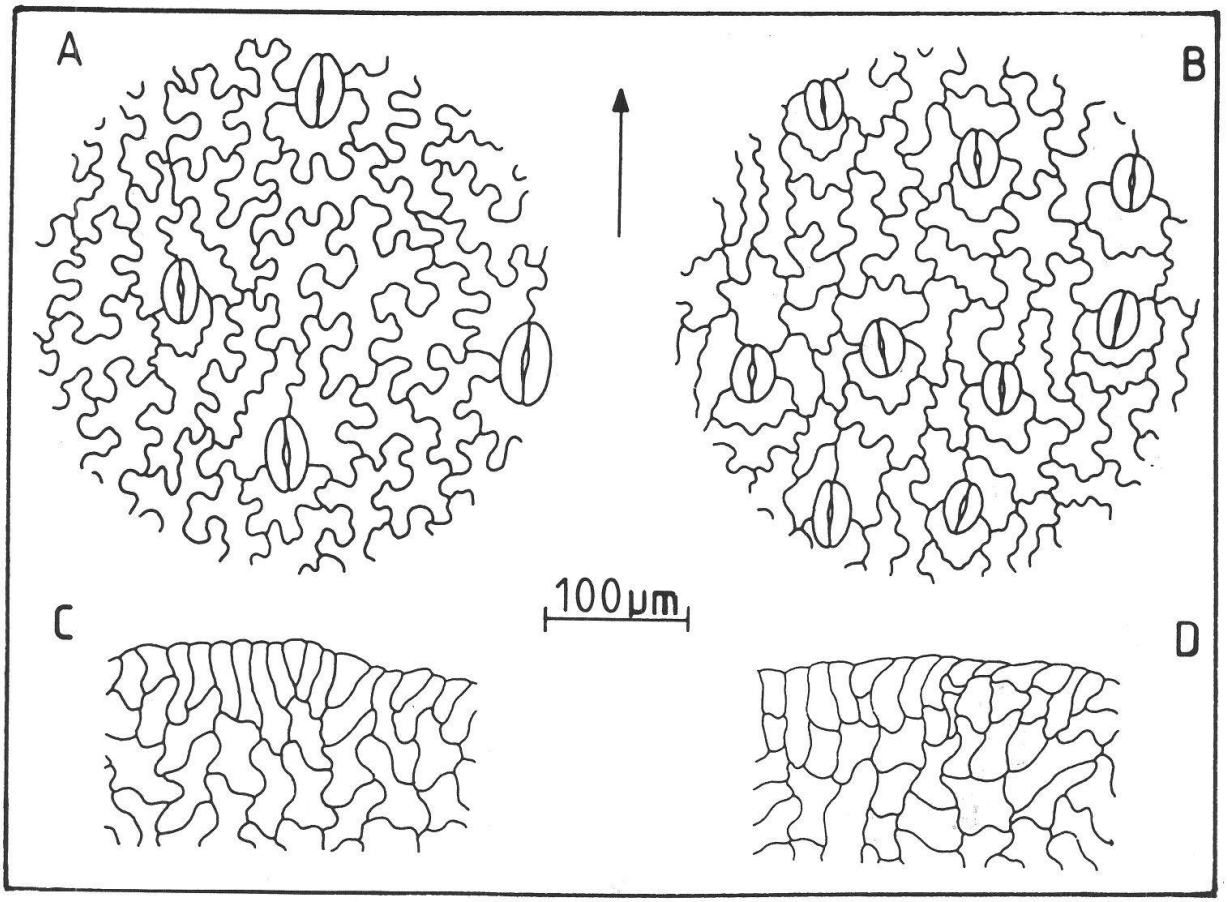

Fig. 6. Epidermis preparations (A and B) and edges of indusia (C and D). A and C = *Arachniodes foliosa* from Kenya, fertile frond of original collection, TR-2980. B and D = *A. webbiana*, fertile frond of plant collected in Madeira and cult. in Gent, RV 3141. The arrow points to the course of the adjacent vein towards the margin. Prep. and drawings. RV.

species sporangia are entirely glabrous [without (unicellular) glands or hairs], stalks are 3-seriate up to the capsule.

The perispore structure (Figs. 4 and 5) of both taxa is typical Dryopterioid, consisting of only two layers without connecting columellae (pillars). The minor differences in SEM-morphology (Fig. 5) are probably due to the immature state of the *A. foliosa* spores. The echinate morphology is aberrant within the genus (see Huang 1981: 77, Pl. 82; Mitui 1982: 163; Tryon & Lugardon 1991: 430–432), but is reminiscent of similar cases known within *Dryopteris*, e.g., *D. formosana* (Christ) C. Chr. and *D. ruwenzoriensis* C. Chr. ex Fraser-Jenkins. Perisporal spines in *A. webbiana* are up to c. 6 µm long, those of (cultivated) Kenyan *A. foliosa* (slightly immature) up to 4 µm [in South African material up to 5 µm according to Welman (1970: 82)].

Apart from the size differences (Table 1 and Fig. 7), the paleae are similar, non clathrate (= isotoechus) and uniformly pale to dark (rare in *A. foliosa*) brown, with smooth to undulate or sparsely and minutely dentate margins (toothlets often set with a glandular cell). The apex is long-filiform and ends in a long uniseriate hair terminated by a glandular cell. It is remarkable that diploid *A. webbiana* is sometimes a larger plant (occasionally also with larger scales) than the tetraploid.

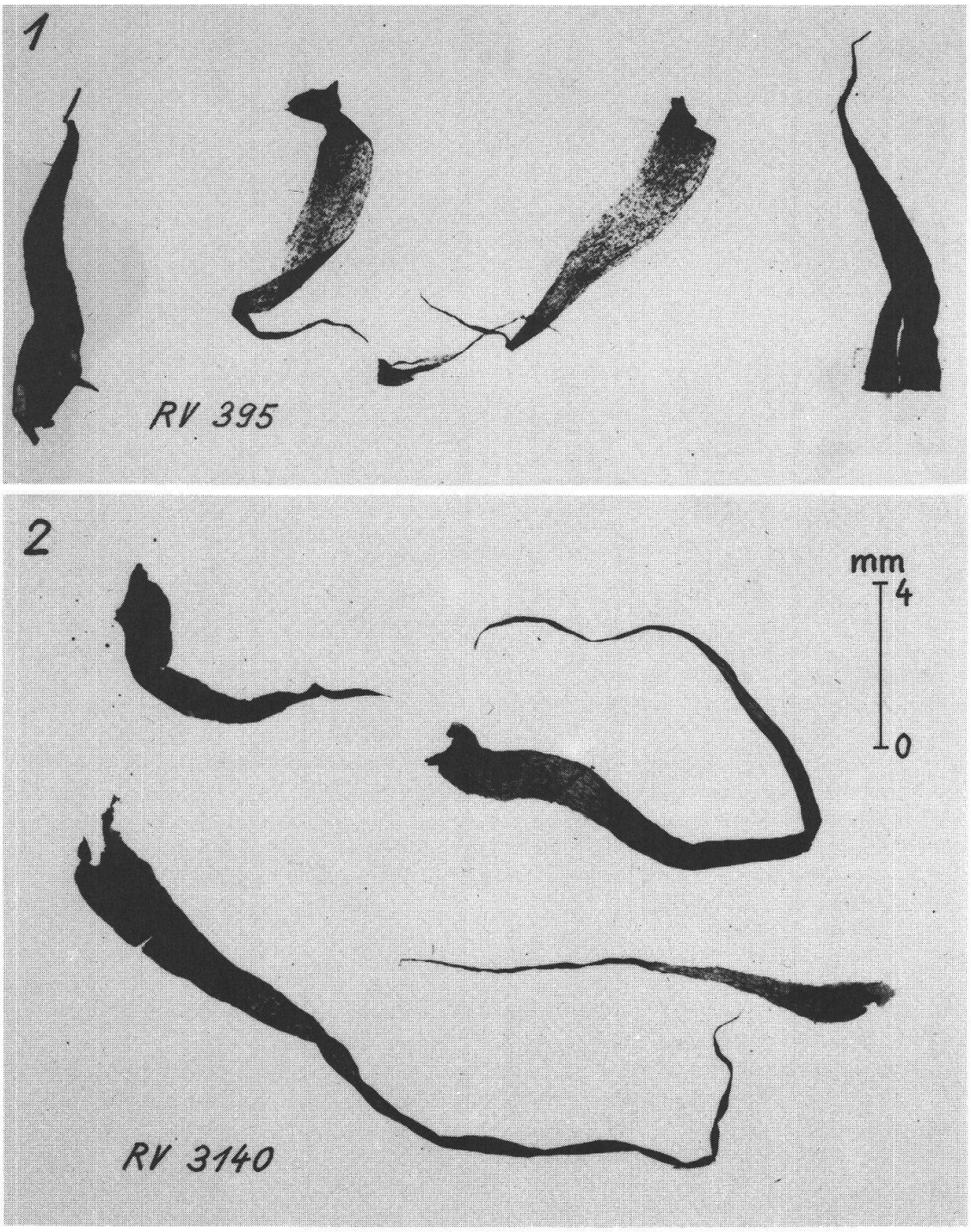

Fig. 7. Photographs of scales from petiole bases. 7.1 = *Arachniodes foliosa* from Kenya, RV 395, frond 63 cm long. 7.2 = *A. webbiana* from Madeira, RV 3840, frond 104 cm long. Prep. by RV and photogr. by P. Eglin, Basel.

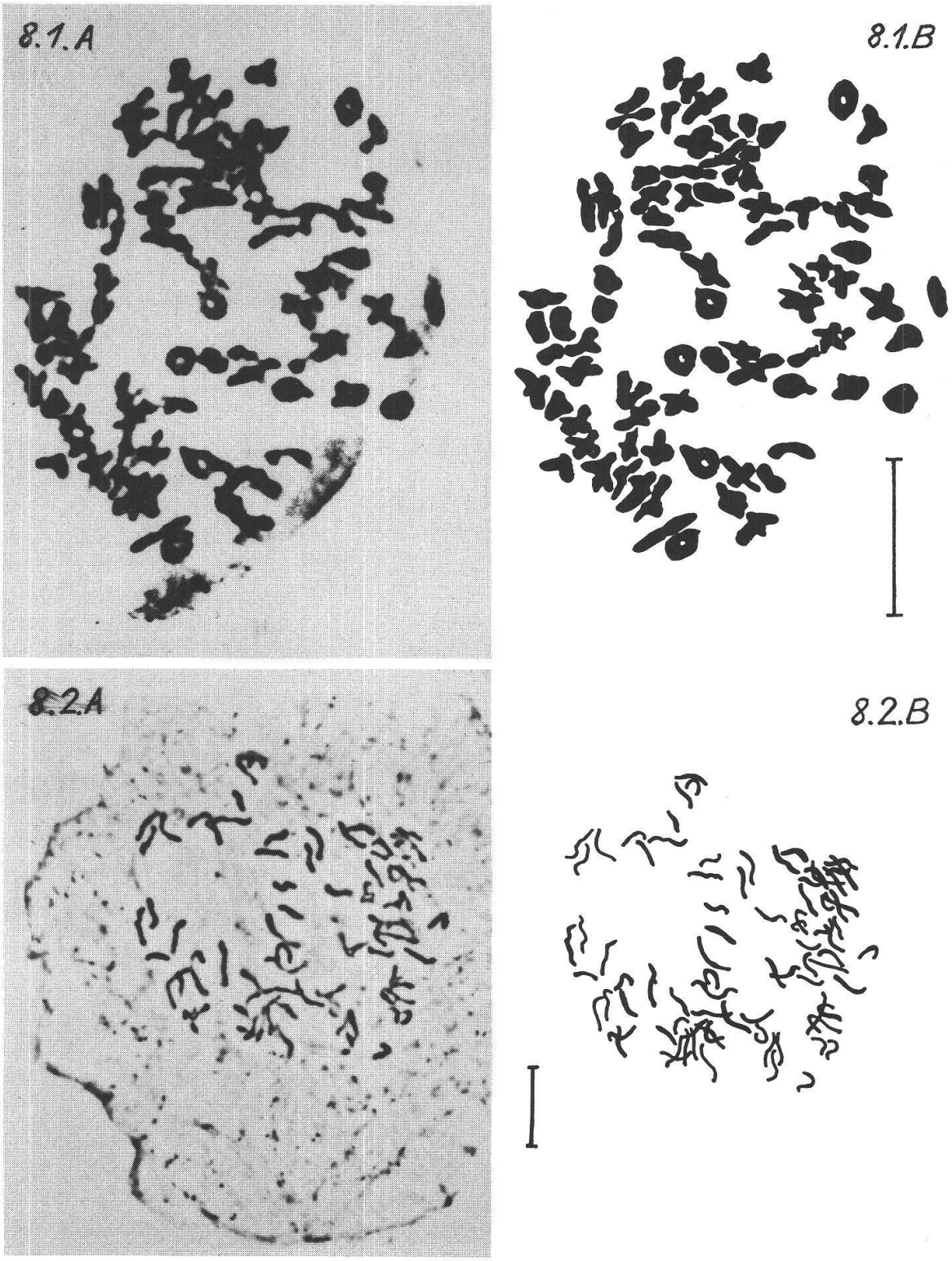

Fig. 8. Cytology: 8.1.A and 8.2.A = photographs, 8.1.B and 8.2.B = explantory diagrams. 8.1.A and 8.1.B = *Arachniodes foliosa* TR-2980, spore mother cell at meiosis (metaphase 1) showing 82 pairs of chromosomes. Bar = 10 µm (magnification ca. 2000 ×). 8.2.A and 8.2.B = *A. webbiana* TR-5223, cell of root-tip at mitosis showing c. 82 single chromosomes. Bar = 10 µm (magnification = 1000 ×). All det. and photogr. by MG.

In spite of some overlapping values the two taxa can usually be differentiated by the size of their spores and guard cells (see Table 1).

Confirming chemical and cytological results, micromorphology shows that both species seem to be more closely related to each other than to any other species of the genus (so far examined). Apart from their geographical distribution and frond size, *A. foliosa* and *A. webbiana* can be positively identified by their different scale and stomata size.

3.3 Chemistry

Both species possess external but no internal glands (see Harada 1951 a, b; 1952 a–c, Harada & Saiki 1956 a–c, Mehra & Mittal 1961, Huurre et al. 1979, with further literature) in agreement with all other typical *Arachniodes* species. The epidermal glands in *A. foliosa* are rather few, small (c. 30 µm), club-shaped or stalked with round heads. *A. webbiana* is densely covered with longer (47–75 µm) club-shaped secretary glands. Also on the scales, especially on the margin and surface of the base, similarly shaped but smaller (33–47 µm) glands were observed. All glands contained much secretion between the cuticle and the outer layer of the cell wall. In this lipophilic secretion the phloroglucinols are located (Huurre et al. 1979), but as in other species of the genus *Arachniodes* (Widén et al. 1976; 1978; 1981; 1983 and other literature cited there), the amount was significantly smaller than in most species of *Dryopteris*. The chemical composition (see Table 2) is very similar in both species, the only difference is the presence of relatively large amounts of trispara-aspidin (20-BBB) in *A. webbiana*, absent in *A. foliosa*.

3.4. Cytology

A. foliosa proved to be tetraploid. Figures 1 and 1 A show a spore mother cell at meiosis with c. 82 pairs. In root-tips $2n = c.\ 164^{I}$ could be counted, as expected for a sexual species.

A. webbiana was earlier examined by Manton (1986) and found to be diploid, with $n = c.\ 41^{II}$ and $2n = 82$. The root count was done on material from Ribeira Funda, squashed and photographed by G. Vida in 1969 (as reported by I. Manton in litt. 9. Oct. 1978). Our count in figures 2 and 2 a is a confirmation.

4. Discussion

Comparison of gross morphology (Figs. 1–3) shows that *A. foliosa* and *A. webbiana* are closely related taxa. This becomes still more evident from examination of their perispore structure (Figs. 4–5). The perispore is conspicuously echinate in both species, i.e., virtually undistinguishable, and the spores are quite different from those of other species of *Arachniodes* so far published. Tryon and Lugardon (1991: 430) examined 26 species and illustrated and described Asiatic representatives as "tuberculate" and American species as having spores with a more "pillared" surface; but none of them shows the echinate surface of *A. foliosa* and *A. webbiana*. The close agreement of gross morphology, micromorphology, perispore morphology, and phloroglucinol composition may be enough reason to assume that *A. foliosa* is an auto-tetraploid taxon evolved from *A. webbiana* by chromosome doubling. This hypothesis might be checked by hybridisation experiments, and we intended to do such crossing experiments. Lack of time did not permit this, so we decided to publish the present preliminary results.

5. Nomenclature

Considering the results given above we hereby reduce *A. foliosa* to a subspecies of *A. webbiana* (oldest name).

A. webbiana (R. Br.) Schelpe ssp. *foliosa* (C. Chr.) Gibby, Rasbach, Reichstein, Widén & Viane subsp. et stat. nov. [Basionym: *Dryopteris foliosa* C. Chr., Dansk Bot. Arkiv 9: 63 (1933)].

6. Final Remark

The echinate spores of both *A. foliosa* and *A. webbiana* which some authors (not Holttum 1991: 26, Fig. 6) assume to be characteristic for the genus *Ctenitis* made us feel a little uncertain. In order to obtain an independent expert judgement we also sent a frond of each species to the late R. C. Ching in Peking, already in poor state of health in 1982 but still very clear in mind. He had most of the known American and Asiatic *Arachniodes* species at his disposal and stated that he accepted both *A. foliosa* and *A. webbiana* as typical representatives of *Arachniodes*, quite distinct from *Ctenitis*. This case is an additional example of the well-known fact that SEM photographs of spores are often most helpful for identification of a species (sometimes even a subspecies or a variety) but do not always provide valid characters for a whole genus.

Zusammenfassung

Arachniodes foliosa aus Kenya, der einzige Vertreter der Gattung im kontinentalen Afrika, erwies sich als tetraploide und sexuelle Sippe. Die nahe verwandte *A. webbiana*, ein Endemit der Insel Madeira, ist diploid und sexuell. Chemische Analyse mittels Dünnschicht-Chromatographie zeigte, daß beide Arten Phloroglucide enthalten. Die vorhandene Menge ist aber, wie bei anderen *Arachniodes*-Arten, merklich kleiner als bei Vertretern der Gattung *Dryopteris*. Beide Arten enthalten fast dieselben Stoffe, nur Trispara-Aspidin ist bei *A. foliosa* abwesend. Dieser geringe chemische Unterschied genügt nicht, um eine spezifische Trennung der zwei Sippen zu begründen, besonders weil deutliche morphologische Unterschiede fehlen. Beide Sippen zeigen sogar genau gleiche Struktur des Perispors (stachelig, ganz verschieden von allen bisher untersuchten amerikanischen und asiatischen Arten), was stark für nahe Verwandtschaft spricht. Der wichtige Unterschied in der Ploideistufe zeigt aber eindeutig, daß zwei verschiedene Sippen vorliegen. Auch die damit gekoppelte Größe der Sporen sowie der Schließzellen in der Epidermis ist zur Unterscheidung brauchbar. Die große Ähnlichkeit der zwei Sippen macht es aber wahrscheinlich, daß *A. foliosa* eine auto-tetraploide Art sein dürfte, die durch direkte Chromosomenverdopplung aus *A. webbiana* hervorgegangen ist. Wir behandeln sie daher als Unterart von *A. webbiana*: als *Arachniodes webbiana* subsp. *foliosa* (C. Chr.) Gibby et al. stat. nov. Wir hatten die Absicht, durch Kreuzungsversuche und zytogenetische Analyse der Hybride einen Beweis für oder gegen diese Theorie zu erbringen. Dies wurde durch Zeit- und Materialmangel verunmöglicht.

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