

## Palynological investigations in Valerianaceae – some elementary aspects and problems<sup>1</sup>

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*Summary:* In a comparative study of pollen wall structure by means of light-microscopy, SEM and TEM investigation of longitudinal and cross sections we could distinguish four different pollen types. In contrast to the data in the literature the occurrence of an endexine in pollen of Valerianaceae could be ascertained. For a comparison of different techniques pollen grains from herbarium material were compared with fresh material and TEM-fixed material.

*Zusammenfassung:* In einer vergleichenden Untersuchung der Pollenwandstruktur mit Hilfe des Lichtmikroskops, des REMs und des TEMs, konnten wir vier charakteristische Pollentypen unterscheiden. Im Gegensatz zu den Literaturangaben gelang es uns, das Vorkommen einer Endexine bei Valerianaceen-Pollen nachzuweisen. Für die Bewertung verschiedener Techniken verglichen wir Pollen von Herbarbögen mit Frischmaterial und für die Untersuchung mittels TEM fixierte Proben.

**Keywords:** Valerianaceae, exine structures as taxonomic character, colpus morphology, endexine, pollen

The elucidation of relationships within the Valerianaceae, a family comprising more than 400 species in 11 genera, is known to be difficult and is still discussed today (LARSEN 1986, ERIKSEN 1989, BARRIE 1990, BACKLUND 1996). The results of palynological investigations of WAGENITZ (1956) and CLARKE & JONES (1977) on European taxa and of BLANKENHORN (1978) and CLARKE (1978) on representatives of all genera agree that the family is stenopalynous and that pollen morphological characters only give scarce clues for systematical conclusions, one of the main problems being the high intraspecific variability of structures.

Hitherto mainly light-microscopical studies were performed. There were only sporadic SEM- and TEM-investigations, as those of SKVARLA et al. (1977) and PATEL & SKVARLA (1979) comparing some Valerianaceae in relation with a systematic investigation of Asteraceae. Results of SEM-investigations were included in monographic treatments of some genera as *Aretiastrum* (WEBERLING & UHLARZ 1976) *Nardostachys* (WEBERLING 1978), and *Belonanthus* (WEBERLING D. & F. 1981) and *Phyllactis* (WEBERLING 1981).

Repeatedly during our investigations the question arose, whether after all there might not be structural characters of some, may be limited, systematical value. This especially applies to the hitherto scarcely investigated taxa from Central and South America, one of the centers of diversity of the family.

Therefore it appeared to be desirable to investigate the ultrastructural features, but at the same time not to neglect intraspecific variability. This paper also deals with some methodical questions in order to find a base for a systematical treatment of different taxa. The most important questions in this context were the existence of an endexine and the morphology of the apertures.

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<sup>1</sup> Dedicated to Prof. Hans A. Froebe on the occasion of his 70<sup>th</sup> birthday.

## Methods

For the application of different methods of preparation fresh material was used in most cases. In case of herbarium material the pollen grains were expanded in a wetting agent (glycerin: water 1:10 and 1 or 2 droplets of a detergent) for several (5) hours and then rinsed with water. The state of the grains was controlled by light-microscopy.

**Light-Microscopy:** For semithin sectioning pollen grains were treated as described for TEM-methods. Sections of 0.3–1.0  $\mu\text{m}$  were stained with azur blue/methylene blue according to Richardson and enclosed in EPON.

**Transmission-Electron-Microscopy:** Pollen samples of fresh material were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) and postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2). The same was applied to pollen grains from herbarium material after expanding the grains by a wetting agent (see above). After dehydration in a graded series and infiltration with EPON using propylenoxid as intermedium the material was polymerized at 60°C for two days.

Most of the ultrathin sections (70 nm) were stained with 2% uranyl acetate and lead citrate (according to Reynolds). Instead of staining the sections with uranyl acetate some sections were alternatively en bloc stained with 2% uranyl acetate in 70% ethanol.

For **Scanning-Electron-Microscopy** pollen samples of fresh material were fixed in a mixture (5:5:90) of formol-acetic-70% alcohol (FAA) or glutaraldehyde/OsO<sub>4</sub> (as described for TEM-preparation). Most of these samples as well as samples from herbarium material were acetolysed, dehydrated with FDA, critical point dried and coated with gold in a sputter coater.

For routine paraffine histology the FAA-fixed specimens were dehydrated in a graded ethanol series and infiltrated with paraffine using tertiary butanol as intermedium. After sectioning (8  $\mu\text{m}$ ) and dissolving the paraffine with xylene the grains were ready for investigation in the SEM.

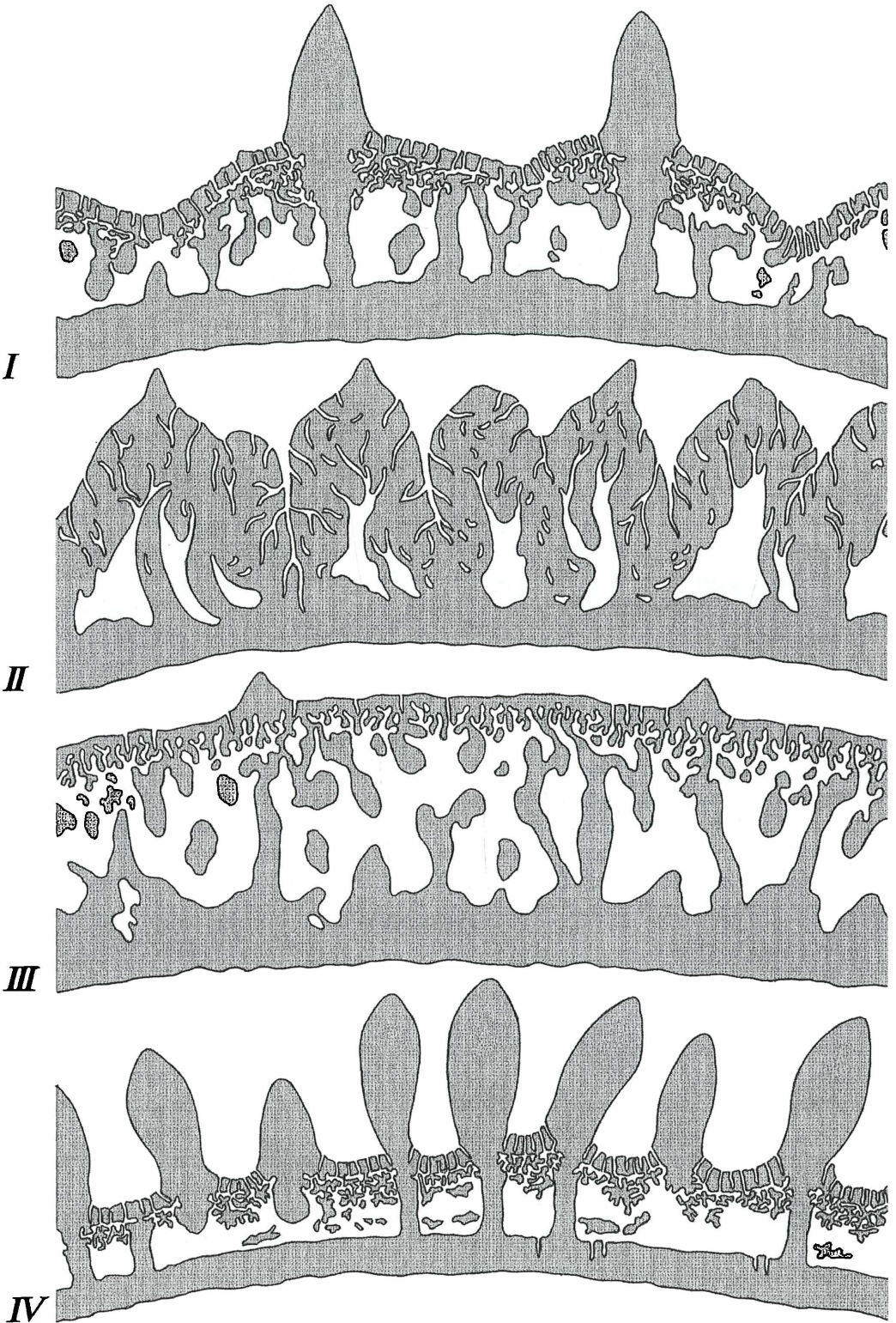
For **preparation of orientated sections** the EPON embedded pollen grains were transferred to Petriperm-dishes (Heraeus) for polymerisation. After that the bottom of the petri-dish consisting of a thin foil of plastic may be easily stripped of when the resin is hardened. Applying this method a thin clearly transparent lamina of resin can be obtained containing the pollen grains in diverse orientations. In the light-microscope these can be selected, marked and cut out. After fixing on a piece of EPON the pollen grains can precisely be oriented for sectioning. It is also possible to obtain nearly complete series of semi- and ultrathin-sections of a pollen grain.

## Investigated specimens

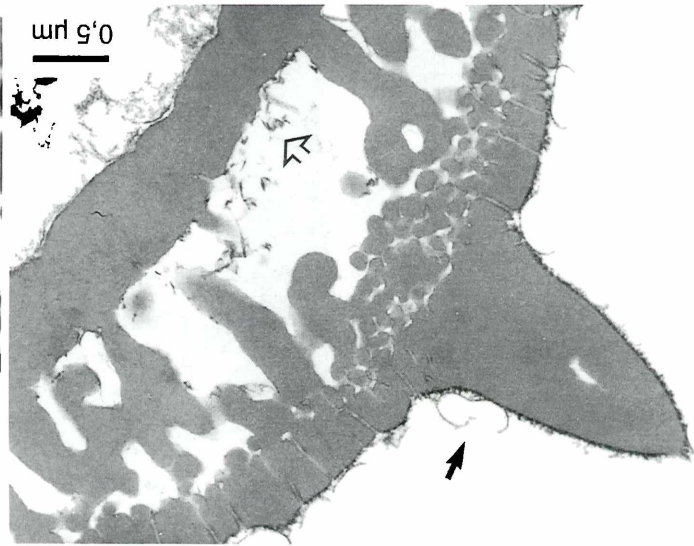
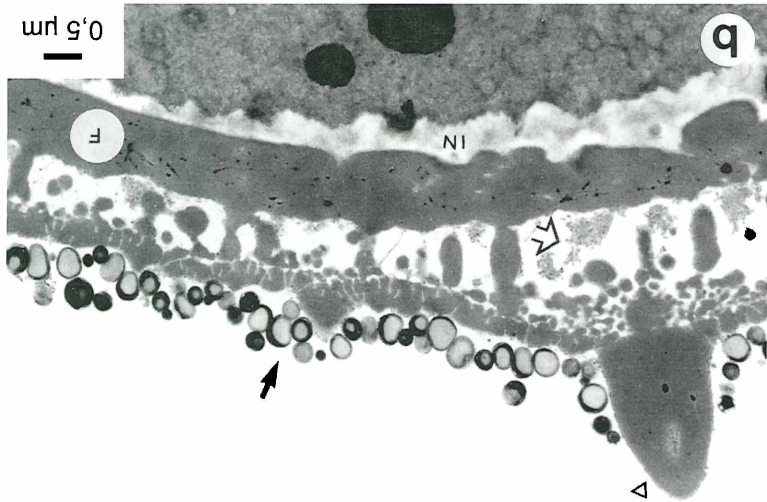
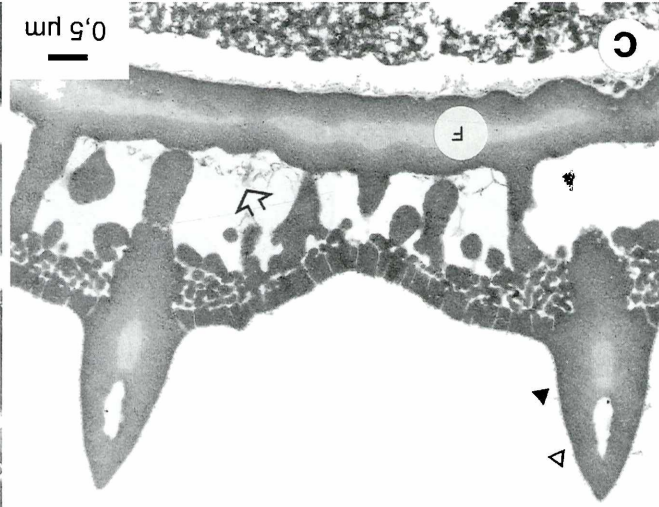
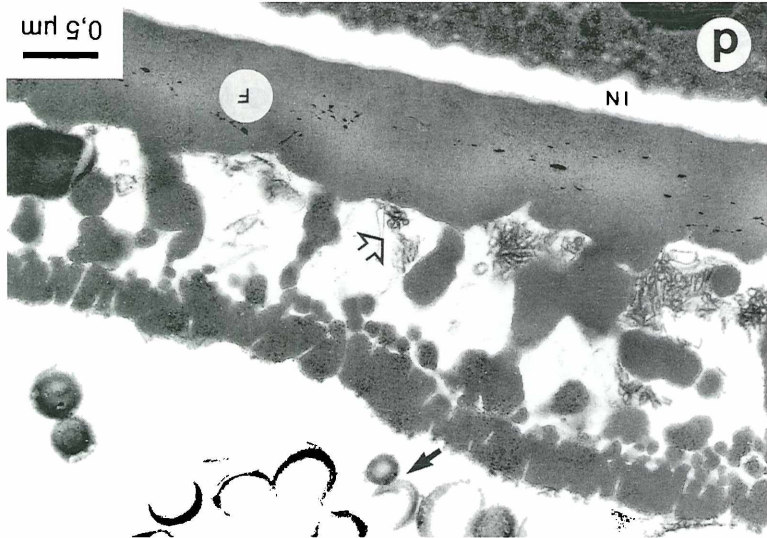
### Type I (*Patrinia*-type)

*Patrinia scabiosaefolia* Fisch.: • Bot. Garden Univ. Ulm. — *Nardostachys jatamansi* (D. Don) DC. (see WEBERLING 1978, BLANKENHORN 1978). — *Valeriana prionophylla* Standl.: • E. Meyer, Costa Rica, Cerro de la Muerte, 3200 m, Nr. 1, 6, 10, 10A, 10B, 10E, 39, 41a, 41b;

Figure 1: Schematic representations of TEM sections through the exine of *Patrinia scabiosaefolia* Fisch., representing exine type I (*Patrinia*-type), *Valeriana palmeri* A. Gray, type II (*V. palmeri*-type), *Valerianella rimosa* Bast., type III (*Valerianella*-type) and *Valeriana pycnantha* A. Gray, representing type IV (*Stangea*-type).







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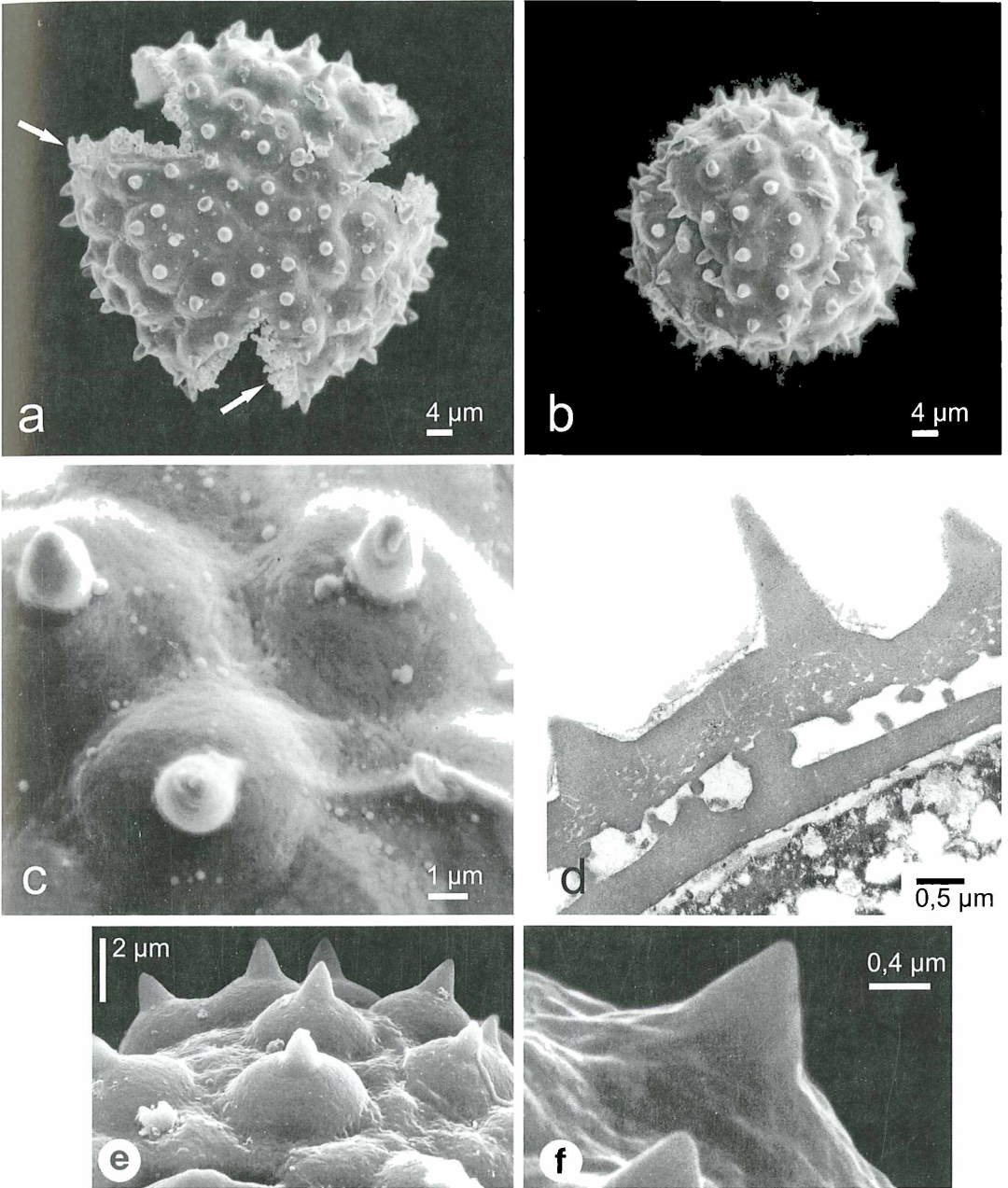


Figure 3: *Valeriana condamoana* Graebn. a) pollen grain in polar view, the arrows point to colpus sculptures, b) equatorial view, c) detail. d) Section through the exine. e) & f) *Nardostachys jatamansi* (D. Don) DC., supratectate structures, spines with socles (e) Smith & Cave 2085 [B]; f) Dr. Prains coll. 35, Sikkim [Z]).

Figure 2: *Patrimia scabiosaefolia* Fisch., exine. Different fixing and staining procedures. a & c) herbarium material, after softening fixed in FAA, a) sections stained with uranyl acetate and lead citrate, c) en bloc staining with uranyl acetate, sections stained with lead citrate; b & d) fresh material fixed in glutaraldehyde and osmium tetroxide, b) see a), d) see c). Arrow (dark): droplets on surface; arrow (light): debris of membranes in the interstices of columellae; arrow-head: particles in the foot layer; triangle (light): hole in a spinula; triangle (dark): centre of spinula; F: basal layer.

Parque Nacional Chirripó, Valle de los Leones, 3100 m, Nr. 13; 3400 m, Nr. 17; 3350 m, Nr. 35 [ULM]; • F. Weberling 8322, Costa Rica, Cerro de la Muerte, 3200 m, 8272, 8272a, b, Volcán Turrialba [ULM/Herb. Weberling].

#### Type I a (*Valeriana coarctata*-type)

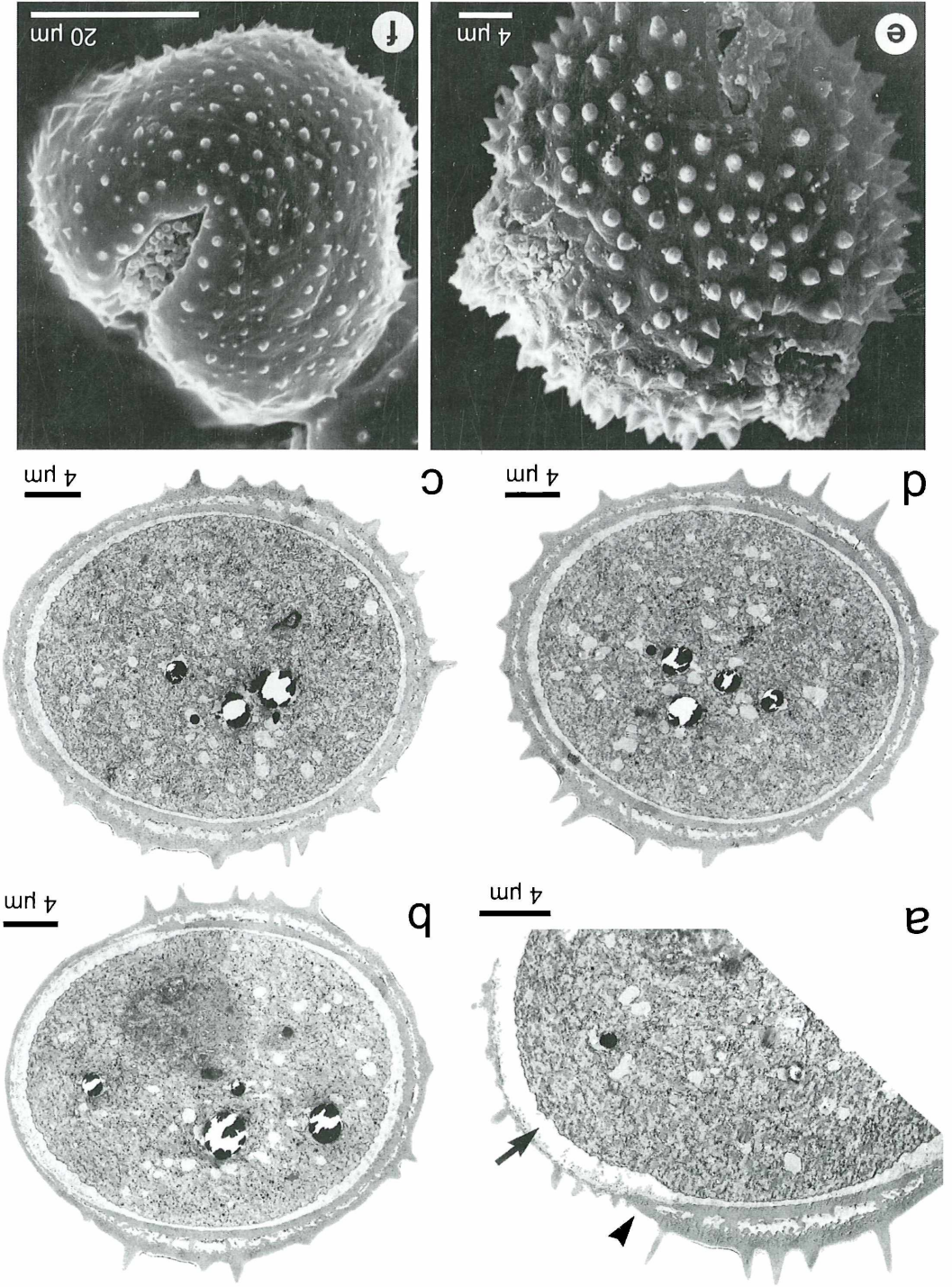
*Valeriana adscendens* Turcz.: • Luteyn et al. 8857 [NY]. — *Valeriana carnososa* Sm.: • Boelcke 15725, Argentina [BAA, ULM/Herb. Weberling]. — *Valeriana clematitis* Kunth: • St. G. Beck 13918, Bolivia [LPB]; • J. C. Solomon 12918, Bolivia [MO]; • J. L. Luteyn 4813, Colombia [NY]; • E. Meyer 2 CR, Costa Rica, [ULM]; • J. L. Luteyn 4813, Colombia [NY]; • A. Molina 16600, Guatemala [F]; • L. O. Williams, A. Molina, T. P. Williams 19609, Guatemala [W]; • J. C. Solomon 12572, Bolivia [MO]; • J. C. Solomon 12918, Bolivia [MO]; • M. H. Grayum 7393 & 7180, Costa Rica [MO]; • A. Molina, W. Burger, B. Wallenta 16407, Guatemala [F]. — *Valeriana coarctata* Ruiz & Pavon: • E. Asplund 5753, Bolivien [US]; • E. Asplund 11773, Peru [US]; • J. Soukop 3615, Peru [US]; • O. Tovar 229, Peru [US]. — *Valeriana condamoana* Graebn.: • Ferreyra 6544 [US]; • D. & F. Weberling 5898, Peru [ULM/Herb. Weberling]. — *Valeriana decussata* Ruiz & Pavon: • St. G. Beck 14721 [LPB]. — *Valeriana eichleriana* Graebn.: • Rambo 45419, Brasil [LIL]. — *Valeriana jasminoides* Briq.: • St. G. Beck 13411, Bolivia [LPB]. — *Valeriana karstenii* (Karsten) Briq.: • A. Schulze 1293, Colombia [B]. — *Valeriana lobata* (Hook. & Arn.) Hoek: • Marticorena et al. 1265 [B]. — *Valeriana microphylla* Kunth: • Luteyn 8396 [NY]. — *Valeriana nivalis* Wedd.: • St. G. Beck 8696 [LPB]. — *Valeriana plantaginea* Kunth: • A. Charpin & F. Jacquemoud 13535 [G]. — *Valeriana polemoniifolia* Phil.: • Marticorena et al. 1599 [B]; • Marticorena et al. 1530, Chile [B]. — *Valeriana thalictroides* Graebn.: • D. & F. Weberling 8119, 9112, Peru [ULM/Herb. Weberling]. — *Valeriana tucumana* Bors.: • E. Budin s.n., 3000 m; n.14543 [SI]. — *Valeriana urbani* Phil.: • S. Venturi 4066, Argentina [SI]. — *Phyllactis bracteata* (Benth.) Wedd.: • B. B. Larsen & B. Eriksen 45320 [ULM]. — *Centranthus macrosiphon* Boiss.: • Bot. Garden Univ. Ulm.

#### Type II (*Valeriana palmeri*-type)

*Valeriana palmeri* Gray: • M. Grayum 8601, Costa Rica, Puriscal, Cerro Bola, 1600 m [CR]; • M. Valerio 591, Costa Rica, Prov. Alajuela, Grecia, 825 m [CR]; • R. L. Willeur 15915, Costa Rica, Prov. Guanacaste, Monteverde, 1200 m [CR]; • Heyde & Lux 7716/2, Guatemala, Dpto. Sta. Rosa, Estanzuela, 830 m [G]; • W. D. Stevens, B. A. Krukoff 9963, Nicaragua, Dpto. de Estelli, 900 m [MO]; • P. Moreno 24612, Nicaragua, Dpto. de Nueva Segovia, Municipio El Jicaro, 700 m [MO]; • P. Moreno 24872, Nicaragua, Dpto. de Matagalpa, 800 m [MO]. — *Valeriana scandens* L. var. *scandens*: • R. Liesner, Judziewiez, Pérez 15316, Costa Rica, Turrialba, Río Reventazón, 550 m [CR]; • C. Wright 21831, Cuba Oriental [W]; • P. Sintenis 302, Puerto Rico [G]; • W. A. Schipp 947, Brit. Honduras, 22 Meilen von Stann Creek River, 3 m [G]; • P. Moreno 19627, Nicaragua, Dpto. de Matagalpa, 580 m [MO]. — *Valeriana scandens* L. var. *candolleana* (Gardn.) Muell.: • E. Meyer 14, Costa Rica, Sta. Elena, 1300 m [ULM]; • E. Meyer 15 CR, Costa Rica, Sta. Elena, 1400 m [ULM]; • E. Meyer 16a [CR], 16b [CR]; • K.

Figure 4: a–d) *Valeriana condamoana* Graebn., series of median longitudinal sections in the colpus region. On the right side of a) the colpus shows the margo, the colpus sculptures and the thickened intine, in b) the margo exclusively formed by the tectum can be seen, in c) the transition from the margo to the normal exine, but still without supratracteal sculptures, in d) the exine near the colpus, the intine still thickened (material: D. & F. Weberling 5898). e) *V. laurifolia* Kunth (= *V. dematitidis* Kunth s. l.), pollen grain in polar view (J. L. Luteyn 4813, Colombia [NY]); f) *V. robertianifolia* Briq., pollen grain in oblique equatorial view, (O. Jiménez 956, Costa Rica, Cord. de Talamanca [CR]).





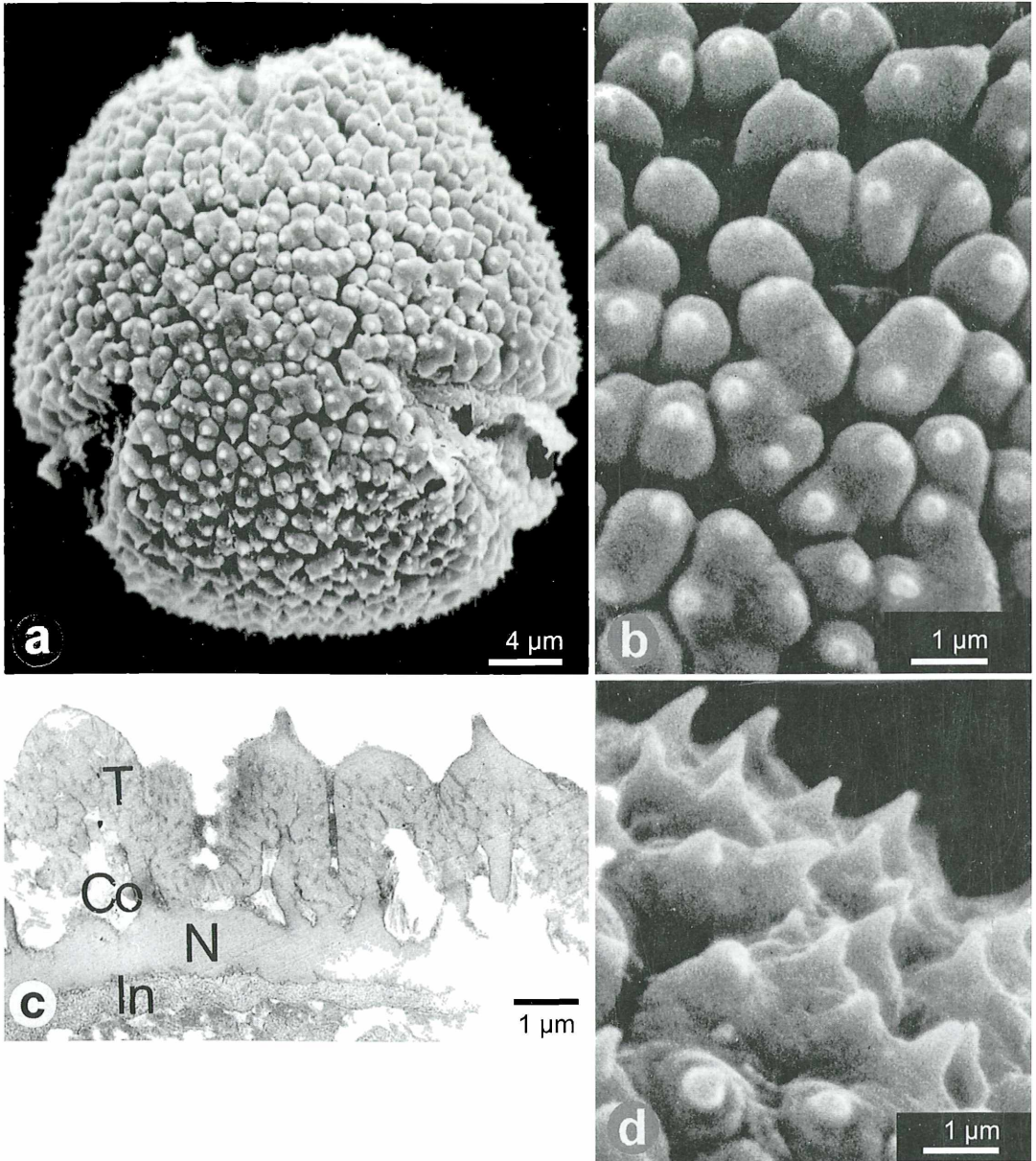
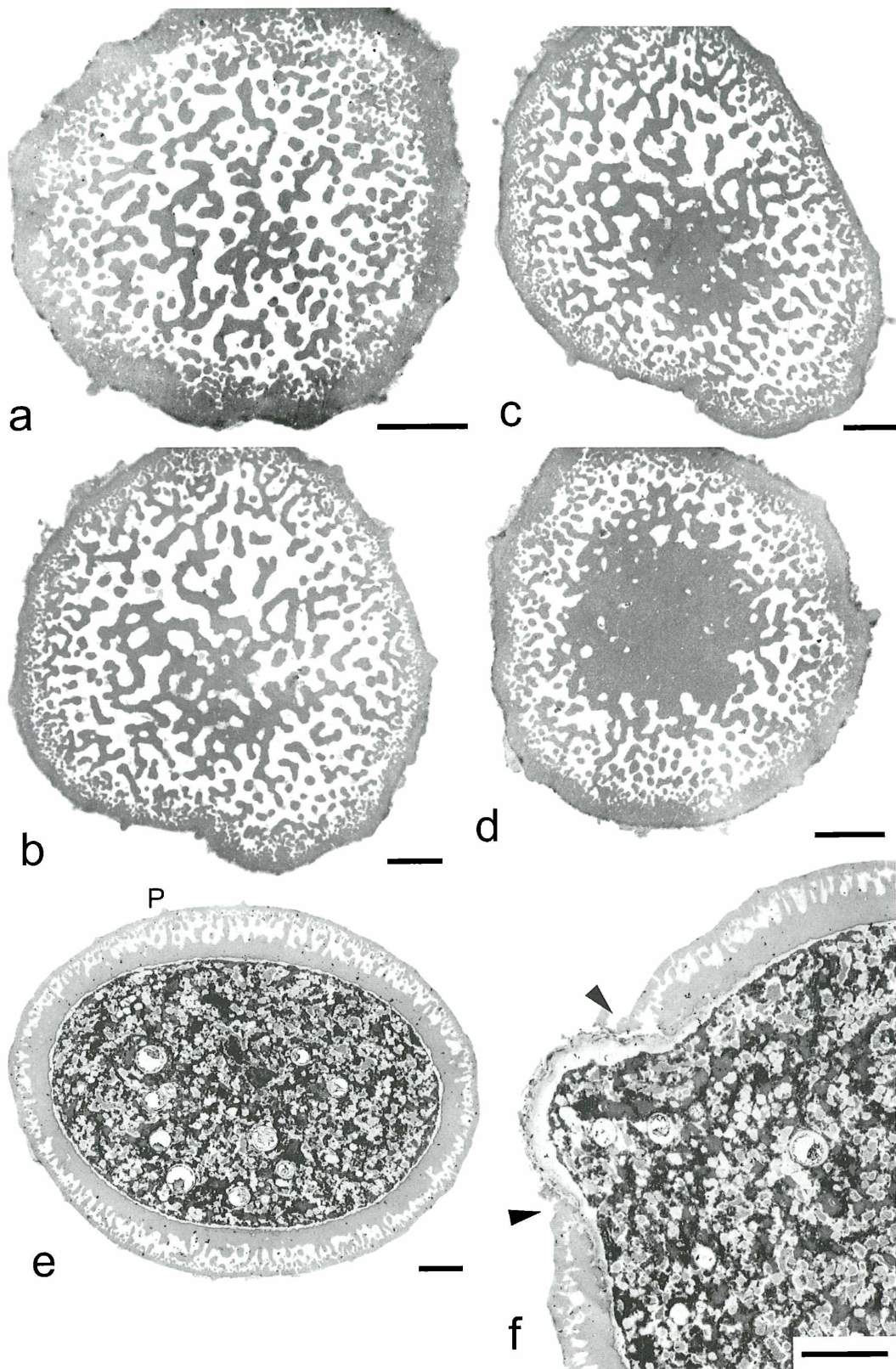


Figure 5: Pollen grains with wavy tectum. a–c) *Valeriana palmeri* Gray, b) detail, c) TEM section through exine, the foldings are partly filled with pollen cement (material: W. D. Stevens & B. A. Krukoff 9963, Nicaragua [MO]); d) *V. scandens* L. var. *scandens* (material: C. Wright 221 831, Cuba [W]).

Figure 6: a–d) *Valerianella rimosa* Bast., Series of tangential sections through the exine, from the periphery towards the center. e) median longitudinal section, showing the mesocolpium on both sides, the exine thicker in the polar regions p, due to the increased length of the columellae, foot layer thicker in the equatorial region, f) equatorial section through the colpus, the arrowheads point to the margo (material: Botan. Garden Ulm). a–d) bar = 2 µm, e & f) bar = 1 µm.





Mehlreter, Guatemala, Volcán Suchitán, 1900 m [CR]; • C. Whiteford 1609, Belize, Toledo, 40 m [CR]; • W. A. Haber 1392, Costa Rica, Parque Nacional Monteverde, 1350 m [CR]; • A. Grijalva, S. M. Araquistain 24, Nicaragua, Cerro Mombachito, 800 m [MO]; • W. D. Stevens 11851, Nicaragua, Dpto. de Matagalpa, Esquipulas, 980 m [MO]; • W. D. Stevens 64579, Mexico, Chiapas, 900 m [MO]. — *Valeriana sorbifolia* Kunth: • Heyde & Lux 7716/1 Guatemala, Dpto. Sta. Rosa, Estanzuela, 830 m [G]; • v. Türckheim 7715/14, Guatemala, Depto. de Alta Vera Paz, 4300 m [G]; • v. Türckheim 7716/15, Guatemala, Dpto. Alta Vera Paz, 1350 m [G]; • W. A. Haber 3561, Costa Rica, Prov. Guanacaste, Monteverde, 800 m [MO]. — *Valeriana tenella* Killip: • M. L. & R. Schnetter, Colombia, Straße von Bogotá nach Coachí, 2500 m [ULM/Herb. Weberling]; • F. J. Breteler 3394, Venezuela, Merida, 2450 m [Instituto Forestal Latino/ Americano].

### Type III (*Valerianella*-type)

*Valerianella rimosa* Bast., *Centranthus ruber* DC., *Valeriana bracteosa* Phil.: • O. Zöllner 3586, [ULM/Herb. Weberling]. — *Valeriana fonckii* Phil.: • C. Joseph 5853 [US]; • De Barba 2189 [LIL] this sample corresponds with type Ia; • J. Böhnert s.n., Chile, Prov. Talca [B]; • Smith & Sparre 224 [B]. — *Valeriana macrorrhiza* DC. (= *V. gilliesii* [Hook. & Arn.] Stuck. & Briq.): • Darwinion 14532, Argentina [SI]; • O. Boelcke et al. 10349, Chile [SI]; • Marticorena et al. 10 [B]. — *Valeriana pilosa* Ruiz & Pavon: • R. Schnetter [ULM/Herb. Weberling]. — *Valeriana pulchella* Mart. & Gal.: • J. Bernasconi 15091, Argentina [SI]; • E. Meyer 2b, 3 and 4, Costa Rica, Cerro de la Muerte, 3300 m and 3400 m, 3300 m [ULM]; • E. Meyer 18 and 36, Costa Rica, Parque Nacional Chirripó, Valle de Crestones, 3400 m and 3350 m [ULM]; • E. Meyer 40, 42 and 44, Costa Rica, Cerro de la Muerte, 3400 m and 3350 m [ULM]; • C. Bazer s.n., Costa Rica, Cerro de la Muerte, 3300 m [ULM/Herb. Weberling]. — *Valeriana radicalis* Clos: • Grandjot 14525, Chile [SI]. — *Valeriana robertianifolia* Briq.: • O. Jimenez 956 [CR]; • J. G. Laurito 5970 [CR]; • J. Linden (Typus) 7716/3 [G]. — *Valeriana urticaefolia* Kunth: • E. Meyer, 2CR [ULM]; • H. Hilger s.n., Guatemala [ULM]; • F. Ventura 4246 [CR]; • W. D. Stevens, B. A. Krukoff & A. Grijalva 15037 [MO]; • W. D. Stevens & B. A. Krukoff 14828 [MO]; • W. D. Stevens, B. A. Krukoff & O. M. Mantiel, s.n., Nicaragua, Dpto. de Estelli [MO]; • W. D. Stevens & B. A. Krukoff 10252 [MO]; • P. Moreno 21947 [HNMN]; • W. D. Stevens & B. A. Krukoff 18058 [MO]. — *Belonanthus hispidus* (Wedd.) Graebn.: • D. & F. Weberling 6523, Peru, [ULM/Herb. Weberling]. — *Belonanthus spathulatus* (Ruiz & Pavon) Schmale: • J. Krach 7591, 7690 [M].

### Type IV (*Stangea*-type)

*Stangea henrici* Graebn.: • B. D. & F. Weberling 8159, Peru [ULM/Herb. Weberling]; — *Stangea wandae* Graebn.: • D. & F. Weberling 5895, Peru [ULM/Herb. Weberling]; • D. & F. Weberling 6584, Peru [ULM/Herb. Weberling]. — *Stangea paulae* Graebn. & Tessend.: • A. Weberbauer 1036, Peru [HBG]. — *Valeriana pycnantha* A. Gray: • D. & F. Weberling 6583, Peru [ULM/Herb. Weberling]; • J. C. Solomon 13216 [MO]. — *Valeriana globularis* A. Gray, *Valeriana petersenii* Weberling & Reese-Krug (see REESE-KRUG & WEBERLING 1996).

Explanation of abbreviations in the legends of the figures:

C	columellae	In	intine	T	tectum
Co	colpus	N	nexine	Ta	tapetum
E	exine	Pr	protoplast		



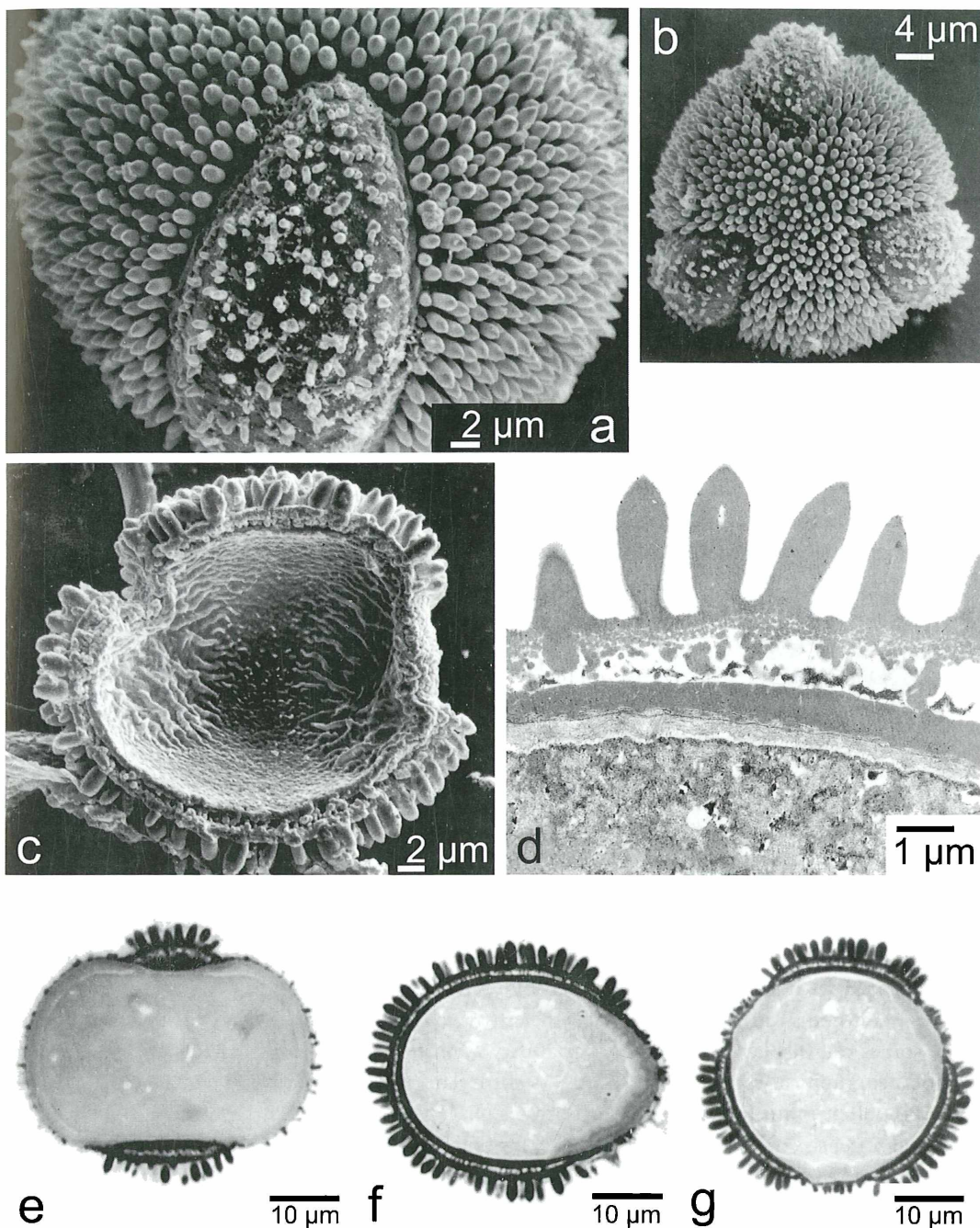


Figure 7: *Valeriana pycnantha* A. Gray, pollen grains, in which the surface is densely covered with bacula. a–c) SEM, a) equatorial view on the colpus, b) polar view on the entire grain, c) section through the pollen grain, d) TEM section through the exine, e–g) semi-thin sections through pollen grains, e) longitudinal section, colporae left and right, f) longitudinal section, colpus on the right side, g) equatorial section, all three showing supratectal bacula in the colpus (material: D. & F. Weberling 6583).



## Results

The pollen grains of Valerianaceae are known as trizonocolporate to tricolporoidate, more or less spheroidal (suboblate to subprolate), the exine surface usually being echinate or microechinate and the sexine exhibiting (often only slightly) branched columellae. In all the species investigated the tectum was provided with microperforations (fig. 1, 2, 3d, 6f, 7d, 8f, 9c). As mentioned already by BLANKENHORN (1978), the colpi usually are covered with sculptures.

According to the structure of the exine at least 4 different "types" of pollen grains can be distinguished (figs. 1, 2/3, 6, 5, 8), which in reality, however, represent slightly distinct reference points (!) within a more or less continuous spectrum of forms. At least the form designated as "type II" has not been reported before.

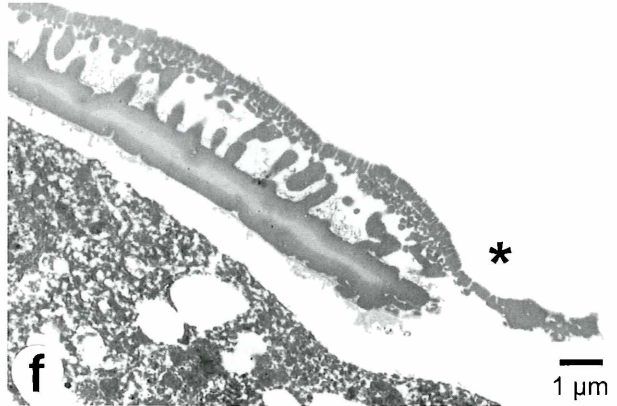
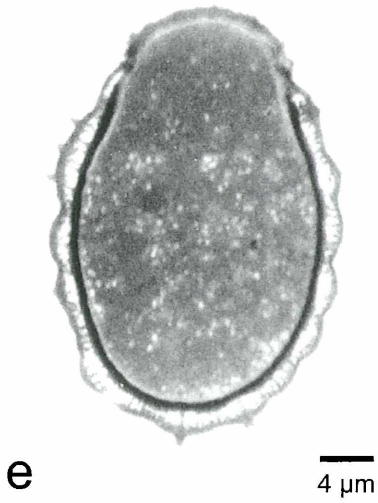
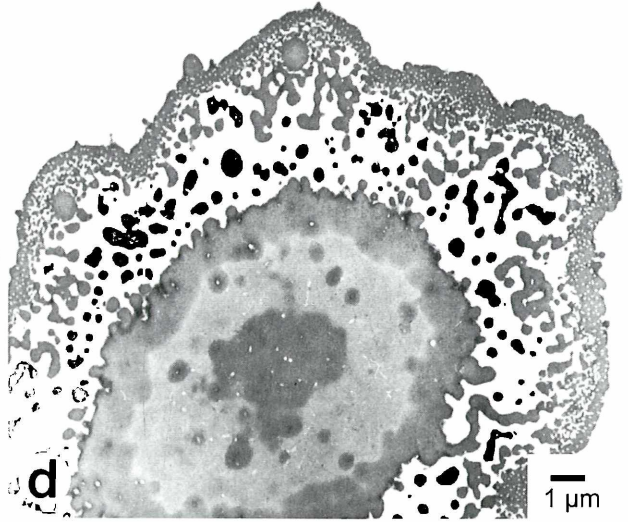
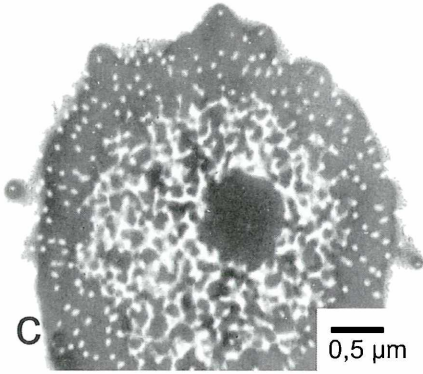
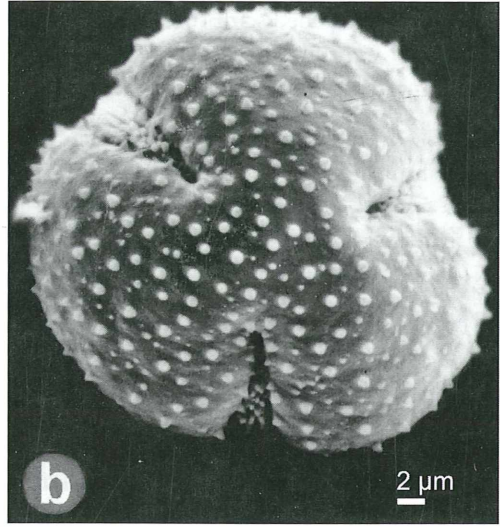
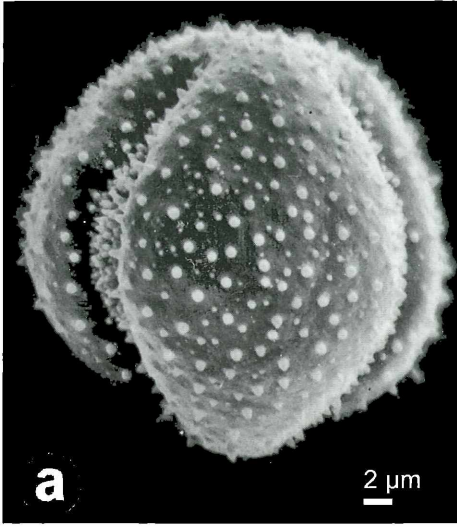
### Type I (*Patrinia*-type, fig. 1 I)

Because of its occurrence in the genera *Patrinia* and *Nardostachys*, which commonly are regarded as primitive, this "*Patrinia*-type" (BLANKENHORN 1978) is considered to be plesiomorphic. Its exine is characterized by the suprategate spines and microechinae ( $< 1 \mu\text{m}$ ), the spines being underlain by dome shaped "socles" or cushions (figs. 2a, c, 3e, f). The latter term was coined already by BLANKENHORN (1978), who investigated 8 species of this genus. The term "verrucae", used in the English literature (not by PATEL & SKVARLA 1979), does not appear appropriate in these cases. In TEM sections (figs. 2, 8c) they appear as "localized out-pockenings supported by highly branched networks of the distal portions of the columellae" (PATEL & SKVARLA 1979: 86). Usually a strong columella is seen extending from the foot layer into the socle and continuing into the terminal spine. Frequently a central lumen can be seen in the tip of the spine (figs. 2a, c), which, however, may be filled with unknown substances (fig. 2b). Due to the increase in columellar ramification towards the tectum and the occurrence of microperforations the distal zone of the infrategate can appear somewhat granular (fig. 2a). The thickness of the socles and the length of the supra-tergate structures varies between species (and even within a species). The biggest cushions are found in the genus *Patrinia*. Those of *Nardostachys jatamansi* (D. Don) DC. (fig. 3e, f) exhibit a decreasing size from the western (Nepal) to the eastern part of the area (Tibet, Szechuan, Sikkim), which corresponding with the variability of further characters (WEBERLING 1978, BLANKENHORN 1978: fig. 14).

### Type Ia (*Valeriana coarctata*-type)

To some extent the pollen grains of this type display a transition to type III. They may be supplemented with spines being underlain by socles, as is often seen in *V. condamoana* Graebn. (fig. 3a-c), although in general they lack socles (fig. 3d). On the other hand the size and density of the spines can display a remarkable variability as observed in *V. clematitis* Kunth s. l., a species of wide geographical range with a high variability in other characters as e.g. leaves. Different from the pollen grain in fig. 4e, other samples (J. C. Solomon 12918 [MO]) the pollen grains displayed a dense covering with spines of  $3 \mu\text{m}$  height. Within the material of *V. fonckii* Phil. some samples (e.g. C. Joseph 5853 [US]) were fitting better in type III.

Figure 8: Pollen grains with thick exine, distinct layer of long columellae and scant sculptures. a & b) *Centranthus ruber* DC., a) equatorial view, b) polar view (material: Botan. Garden Ulm, 7/1991, fix. FAA); c-f) *Patrinia scabiosaefolia* Fisch., c) base of a dome shaped socle, d) tangential section through a pollen grain, showing the exine with tectum (outermost ring), columellae and foot layer (innermost ring, bright zone, and its innermost layer - gray central part); e) pollen grain in longitudinal median semi-thin section TEM, f) colpus region in longitudinal section, showing the margo \* and below the intine (material: Botan. Garden Ulm, 8/1991, fix. FAA).



## Type II (*Valeriana palmeri*-type, fig. 1 II)

In cross-sections the tectum of this type appears to be waved or folded and partially extremely thickened, forming arched sculptures which bear a single spine in their culmination. In a revision of Mexican and Central American species of *Valeriana*, presenting a TEM study BARRIE (1990) erroneously interpreted this type as intectate (in contrast to CLARKE 1978).

In *Valeriana palmeri* Gray (fig. 5a–c) this character is especially strong pronounced. It is probably a distinguishing feature of *Valeriana* Sect. *Sorbifoliae*, as *V. palmeri*, *V. scandens* f. *scandens* (fig. 5d) and f. *candolleana*, *V. sorbifolia*, *V. tenella*. However, *V. robertianifolia* (fig. 4f), sometimes included in the Sect. *Sorbifoliae*, although differing from this group by its more or less exerted stamina (see F. G. MEYER 1951: 461), fits better into type III.

## Type III (*Valerianella*-type, fig. 1 III)

Pollen grains of the *Valerianella*-type, a term also coined by BLANKENHORN (1978) already can be distinguished from the others by its vigorously thickened exine, whereas the microechinate supratectate structures are slightly pronounced (fig. 6e, f, fig. 9e, f), often inconspicuous, according to BLANKENHORN (1978: 123) measuring no more than 0.5  $\mu\text{m}$ . However, in *Centranthus ruber* (fig. 8a, b) the sculptures on the colpus extend up to 6  $\mu\text{m}$  (fig. 9b, d). In relation to the tectum, the columellae are longer in comparison to the other types described here. Frequently they are repeatedly branched towards the tectum (fig. 9c). This also can be seen in *Centranthus ruber* as well as in *Valerianella rimosa* in tangential sections through the exine (fig. 6a–d, fig. 9a). The exine usually is relatively thick (fig. 6e, f, fig. 9f), especially in the polar region, due to an increase in columella-length, whereas the foot layer is evidently thicker within the equatorial region. The margin of the colpus is encircled by a thickened tectum (fig. 6f arrowheads, fig. 9b, d), which is curled in turgescence stage of the pollen grain. The sculptures covering the colpus may be ramified (fig. 9b, d). This form occurs in the genera *Fedia*, *Valerianella*, *Centranthus* p. p., *Belonanthus* (D. & F. WEBERLING 1981: 31, fig. 17), *Aretiastrum* (WEBERLING & UHLARZ 1977: 236, fig. 9), *Phyllactis* (WEBERLING 1981: 304, fig. 4) and in some species of *Valeriana*, especially the most argentine-chilenean-andean endemics (*V. radicalis* Clos, *V. macrorrhiza* DC. [= *V. gilliesii* (Hook. & Arn.) Stuck. & Briq.], *V. fonckii* Phil. etc., within the material of latter species, De Barba 2189 [LIL], corresponding better with type Ia), but beside this also in some annual groups of *Valeriana* as *V. robertianifolia* Kunth (fig. 4f) and *V. urticaefolia* Kunth.

## Type IV (*Stangea*-type, fig. 1 IV)

In the fourth type the surface of the pollen wall is densely covered with bacula (fig. 7). Apart from their higher linear dimension the supratectate sculptures are essentially similar to that of the other types, which also usually contain a central cavity (fig. 7d). The exine is much thinner compared to the other types, which is mainly due to the little height of the columellae (fig. 7f, g). Especially, when observed by light-microscopy, they prove to be very short (fig. 7f, g).

This type is limited to the genus *Stangea* (from which BLANKENHORN, 1978, derived the term) and a group of *Valeriana* species (*V. pycnantha* A.Gray a. o.) of low semi-rosette habit and entire leaves growing in the high Andes of Peru, Bolivia, N. Chile and N. Argentina. In contrast to the current opinion here also some relations become apparent in the systematical dispersion of this type.



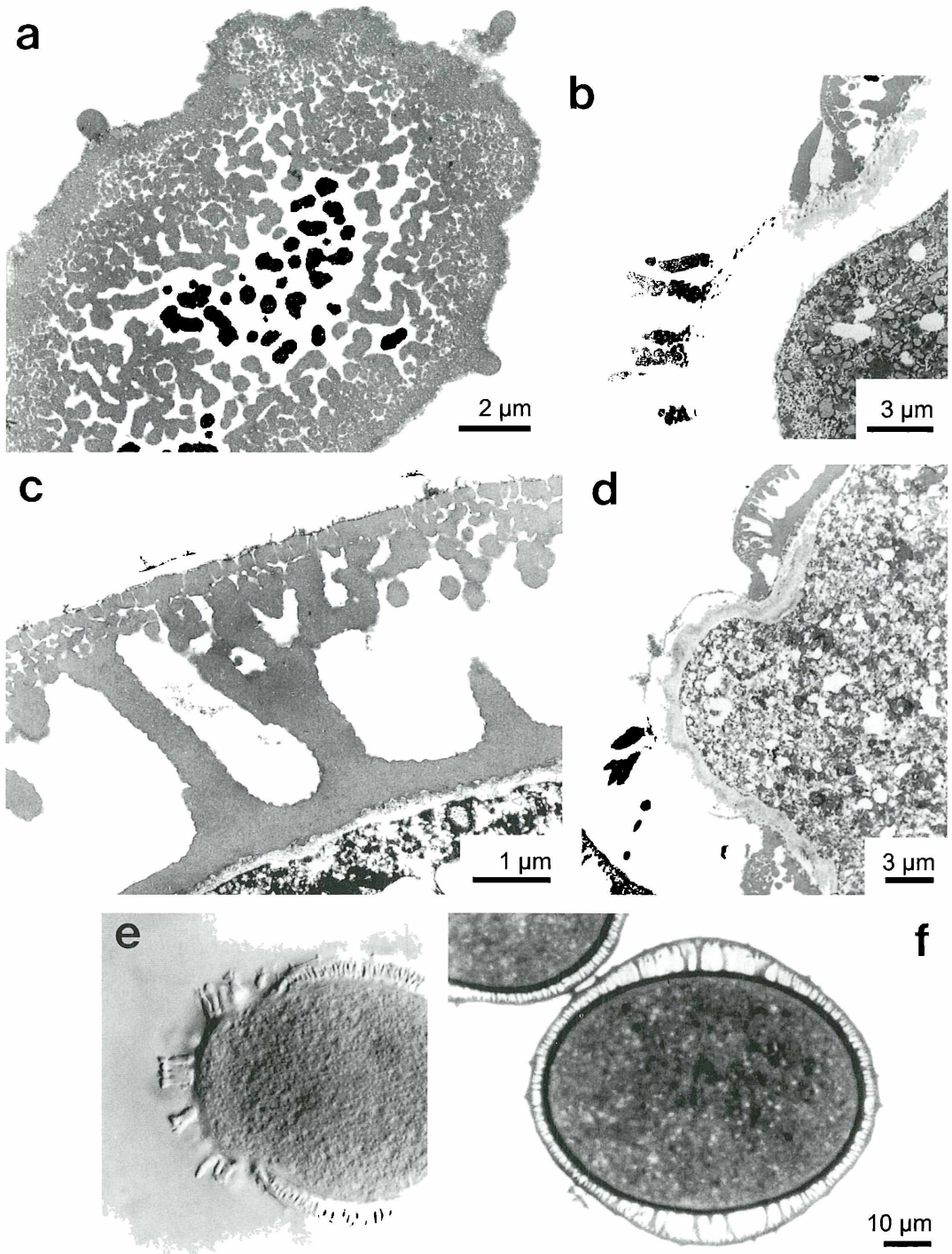


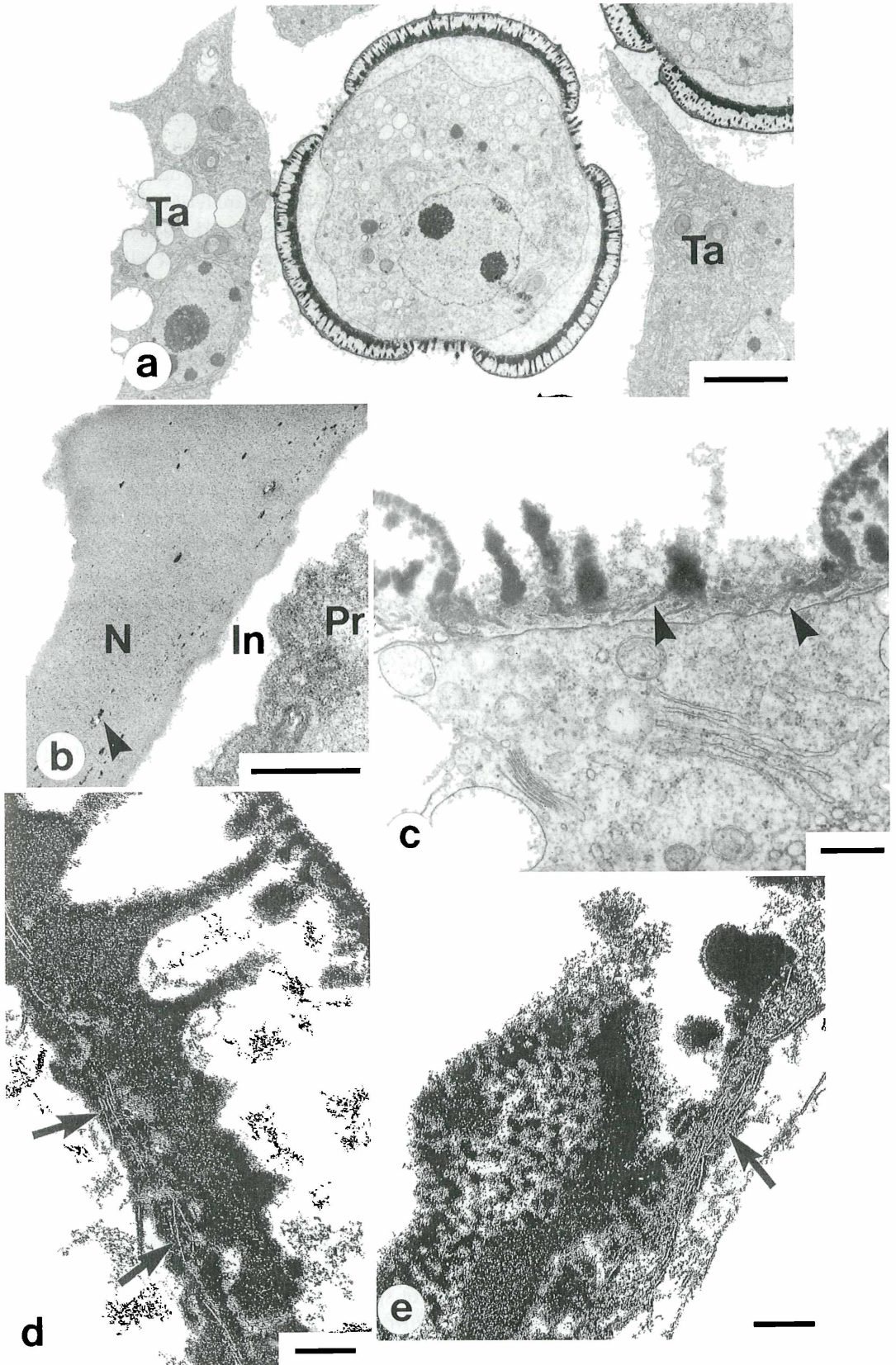
Figure 9: *Centranthus ruber* L. a) tangential section through the exine, b & d) transversal section through the colpus region (d), b) detail, c) section through the exine, pronounced layer of long and ramified columellae, micro-perforations; e) optical section through the entire pollen grain (Differential Interference Contrast), f) longitudinal semi-thin-section through the mesocolpium (material: Botan. Garden Ulm).

Up to now, no endexine is described in literature for the Valerianaceae. Indeed, samples of pollen grains treated in the usual way by acetolysis present the nexine as homogeneous layer. However, as reported by REESE-KRUG, MEYER & WEBERLING (1993) and observing the development of the pollen wall by means of TEM, could perceive lamellar structures which develop at the inner side of the exine, while most of the structural elements and sculptures are differentiated already. The appearance of these so called "white lines" is considered as the decisive criterion for the existence of an endexine (ROWLEY 1981, GUÉDÈS 1982). Fig. 10a shows a late microspore stage, when the pollen grains are released, but not yet fully developed. At this stage the tapetum has protruded close to the centre of the locule, its cell walls are dissolved, the cells multinucleate and rather vacuolate, but fused only peripherally. In a higher magnification the "white lines" are clearly discernible (fig. 10d, arrows), as are the lamellate deposits, which are especially developed in the colpus region (fig. 10c, arrowheads, fig. 10e, arrow). In the mature pollen wall these lamellate structures can only be traced by the corresponding inclusions (fig. 10b, arrowhead). In summary it appears to be justified, to assume the occurrence of an endexine in Valerianaceae.

### Colpus morphology

In addition to the question of the occurrence of an endexine the morphology of the colpus can be focussed. Fig. 6e shows a median longitudinal section of the colpus region of *Valerianella rimosa* (type III), showing the mesocolpium on both sides, the exine thicker in the polar regions p, due to the increased length of the columellae, the foot layer being thicker in the equatorial region. In the left part of an equatorial section a colpus is shown in transversal section (fig. 6f). The margin (arrowheads) of the colpus is almost exclusively formed by the tectum, which, however, is absent on the surface of the colpus itself. The distinctly encircled margo can be distinguished by the lack of supratectate sculptures and a thin discontinuous nexine. The tectum can be vigorously thickened (type III) or can be thin (the other types). In contrast to the data in the literature the species investigated by us appeared to have a colpus margo usually free of sculptures. Fig. 4a shows a longitudinal section of the colpus of *Valeriana condamoana* (type Ia), in which the margo (arrow-head), a very thin nexine and below the thickened intine (arrow) is discernible. The latter can also be seen in fig. 4b, and still in 4c and 4d. In fig. 4d the transition from the colpus margin to the mesocolpium with short columellae is visible. The continuation of the tectum forming the margo and covering the margin of the colpus is demonstrated by a longitudinal section of a pollen grain of *Patrinia scabiosaefolia* in fig. 8f. Whereas the margo always remains free of any supratectate sculptures, the surface of the colpus is always covered with such sculptures (fig. 4a, 6f, 7e–h, 9d, b, e, 10a, c), which even could be ramified (fig. 10d, b). In the section through the colpus in fig. 9d, b (*Centranthus ruber*) and fig. 10c, e (*Centranthus macrosiphon*) the thin layer forming the base of the colpus sculptures rather appears as a continuation of the tectum.

Figure 10: Endexine and colpus morphology. *Centranthus macrosiphon* Boiss., a–d) pollen grains, a), c), d) not completely mature within the locule of the anther, tapetum protruded almost into the centre of the pollen sac, cell walls dissolved, cells multinucleate, extremely vacuolate, but fused peripherally only; a) general view; b) detail from mature exine, showing nexine (N), intine (In) and protoplast (Pr), the arrowhead points towards electron dense inclusion; c) equatorial cross section through the colpus region, d) median longitudinal section through the pollen wall in the mesocolpium, the arrows point to the "white lines" e) detail, endexine near the margo, the arrow points to the "white lines" (material: Botan. Garden Ulm). Bar in a) = 5 µm; b & c) = 0.5 µ; d & e) = 0.2 µm.





Concerning the detection of an endexine it should be mentioned that the technique of fixation and staining can be a decisive factor for contrasting the different layers of the pollen wall. Fresh pollen samples fixed with glutaraldehyde followed by osmium tetroxide and sections stained with uranyl acetate and lead citrate as usual for routine procedures in electron microscopy result in rather homogeneous appearing exine layers (fig. 2b) as compared to micrographs after treatment with en bloc staining with uranyl acetate (fig. 2c, d). In fresh material, the foot layer is homogeneous, whereas in FAA fixed material the foot layer seems to be three-layered (fig. 2c). The larger spinulae that otherwise present a homogeneous structure show similar aspects resulting in a two-layered structure. These effects are artifacts (Hesse, pers. comm.). FAA-fixation without en bloc staining doesn't cause these effects (fig. 2a).

The preservation of variety of substances is different as well. Of course FAA-fixation produced most extensive dissolution of substances not to mention the preceding treatment by the wetting agent itself.

In any case, the interstices of columellae present lots of fragmented membranes (fig. 2d), whereas other substances are preserved merely in glutaraldehyde and osmium tetroxide fixed samples. The material in the top of the larger spinulae is in most cases completely extracted by FAA. The same applies to the small opaque electron dense particles in the foot layer (fig. 2d). In addition, fixation in FAA conventional for routine sampling for morphological or anatomical studies in botany, results in complete extraction of the masses of droplets on the surface of the pollen wall (fig. 2a, b). In some cases this is avoidable to a certain extent by postfixation with osmium tetroxide (not illustrated). Contacting aqueous solutions as glutaraldehyde, but even in FAA, some of the fresh pollen samples start almost immediately secreting droplets through the micropores of the tectum (e.g. *Patrinia scabiosaefolia* to a striking extent). It's a matter of nearly seconds and causes severe trouble during fixation because of the extreme hydrophobe character of the pollen wall surfaces.

The pectic substances of the intine are more or less adversely affected by en bloc staining (fig. 2d), thus lacking any contrast and lamellated structure.

## Discussion

As stated by WAGENITZ (1951) the infra-specific variability of pollen structures in Valerianaceae can be highly variable. This applies to the large and polymorphous genus *Valeriana*. The pollen grains classified by BLANKENHORN (1978) as belonging to the *Patrinia*-type were even found in *Valeriana dioica* from Germany, whereas the pollen grains of the North American *V. dioica* subsp. *sylvatica* followed the *Valerianella*-type. Among the results of our investigations the infraspecific variability was exemplified here by *Valeriana clematitis*, a species of wide geographical range with a high variability in other characters. As another example *Valeriana fonckii* (mostly type Ia) may be mentioned here. The dome shaped socles with supratectate spines being characteristic for the *Patrinia*-type not only proved to be variable in size within the genus *Patrinia* and *Nardostachys*, but sometimes can be found in *Valeriana condamoana* also, or even rarely and only slightly pronounced in *V. urticaefolia*. This, however, does not mean that within the Valerianaceae pollen characters of are no taxonomical value. Carefully used they are helpful in characterizing taxa – species or groups of species, as the Sect. *Sorbifoliae* within the genus *Valeriana*, the pollen grains of which altogether follow the *V. palmeri*-type. As mentioned before the pollen-"types" described here may be used as reference-points, useful as

descriptive terms. Even genera, such as *Belonanthus*, *Aretiastrum*, *Valerianella* or *Fedia* can be restricted to one of the "types". In *Valeriana prionophylla*, a widespread species in Costa Rica, no correlation could be found between the size of the pollen grains of plants and the conditions of their habitat. This refers to plants attaining more than 2 m length in favoured conditions, and those of 1–2 cm collected from poor soils of high elevations (3400 m) of the Cordillera de Talamanca.

An additional question investigated, was the occurrence of an endexine. This could be confirmed by the existence of the so called "white lines" (fig. 10c, d, e) in immature pollen walls. As often, in mature pollen stage an endexine can hardly be discriminated any more.

In contrast to the surface of the colpus itself the margo usually was found to be free of supratectate sculptures. WAGENITZ (1956) assumed the sculptures covering the surface of the apertures to be homologous to columellae. This would mean that the layer covering the colpus is identical with the foot layer or the endexine. Indeed in higher magnifications of sections as shown in fig. 4b, a dark line on the inner side of the nexine continued covering the colpus. This interpretation may also be given for the sections through the colpus in fig. 9d, b (*Centranthus ruber*).

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