THE POLLEN GRAIN OF *POLYGALA FRUTICOSA* BERG. *(POLYGALACEAE)*

by

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Resumen

PAIVA, J. & J. D. SANTOS DIAS (1990). El polen de Polygala fruticosa Berg. (Polygalaceae). *Anales Jard. Bot. Madrid* 47(2): 377-385 (en inglés).

El polen de la familia *Polygalaceae* es polizonocolporado e isopolar. *Polygala fruticosa* Berg., una de las especies sudafricanas, tiene polen 21-23 zonocolporado e isopolar. Las ectoaperturas de tipo colpo son de igual longitud, la endoapertura ecuatorial es completa (endocíngulo). La exina es gruesa, con sexina aproximadamente de igual al doble de gruesa que la nexina. La superficie es psilada y la apocolpia circular. El polen contiene las células vegetativas y generativas antes de salir de las anteras.

Palabras clave: *Polygala,* polen.

Abstract

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The family *Polygalaceae* is polyzonocolporate with isopolar pollen. The pollen of *Polygala fruticosa* Berg., a South-african species, is 21-23-zonocolporate and isopolar. The ectoapertures are colpi of equal length, the endoaperture is a complete equatorial belt (endocingulus). The exine is thick, with the sexine ± twice or as thick as the nexine. The surface is psilate and the apocolpium is circular. The pollen contains the vegetative cell and the generative cell before leaving the anther.

Key words: *Polygala,* pollen.

INTRODUCTION

General features

Polygala L., originally a continental genus, is comprised of about 720 species of which 400 are American, 206 African, 32 European, 70 Asiatic and 12 Australian. The distribution is almost worldwide, naturally occuring representatives of this genus being absent only from the Polynesia, New Zealand and the polar regions. *P. paniculata* L. is an introduced species in Polynesia and *P. serpyllifolia* J. A. C. Hose probably so in the South of Greenland.

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It is the largest genus of the *Polygalaceae* which has 16 to 18 genera and about 1300 species.

Polygalaceae is a remarkable family for the superficial resemblance of the flowers to the well-known papilionaceous flower, but the floral structure is quite different from the *Fabaceae* one. The keel of the latter is formed by two ± fused ventral petals while in the former is formed only by one petal, the posterior, the *Fabaceae* wings are two lower petals and the *Polygalaceae* ones are two lateral and inner sepals, enlarged and often petaloid. The androecium is similar, since in the two families the stamens are usually enclosed by the keel (carina) and the filaments fuse to form a tube around the ovary.

It is divided into three tribes *Xanthopylleae:* one genus extending from India to Yunnan and the New Guinea, North Australia and the Solomon islands. Sepals free, petals free, stamens free, ovary 1-2 locular; *Polygaleae:* 11-13 genera, almost cosmopolitan. Sepals free or two united, petals united, stamens united in one or two groups (1-2 delphous), ovary 2-3 locular; *Moutabeae:* four genera in South America, New Guinea and the Solomon islands. Sepals united, petals united, stamens monodelphous, ovary 2-8 locular.

Polygala range from woody trees up to 15 high, lianes, to small herbs, 1.5 cm high. The flowers are hermaphrodite and strongly zygomorphic. Calyx with five unequal sepals, the three outer are usually sepaloid with the two anterior ones equal and sometimes united; the two inner sepals (wings) are generally petaloid and much larger than the external ones; all are either persistent or caducous. Three petals (rarely five) more or less united, the lowest forming the carina, crested or not; the upper one joined to the carina usually at the base; the two lateral ones usually vestigial or absent. Generally eight stamens, monodelphous, united into a sheath with a long posterior split, usually to the base, adenate at base to petals (exceptional 4-5 to 9-10 stamens). The anthers are obovoid or ellipsoid, 1-2-locular, opening transversly or obliquely, becoming an apical pore. The pistil is bicarpelar with a superior bilocular ovary with an anatropous ovules inserted near the apex on the septum of each locule. The style is simple with a two branched stigma. The fruit is usually a symetrical capsule, bilocular, with two seeds per capsule. Seeds usually have a caruncle which is generally trilobate with the two internal lobes shorter and usually with membranous caruncular appendages, pubescent or rarely glabrous, with a hard testa.

Pollen grains

One of the most notable element of the *Polygalaceae* is the typical polyzonocolporate pollen grain. CHODAT (1896), the great monographer of the family, had already considered the morphology of the pollen grains one of the most important features of the *Polygalaceae:* "Es ist das sicherste kemzeichen dieser Familien", and wrote a short paper about the pollen grains of *Polygalaceae* (1889).

Also, when SPRAGUE (1940) delt with the systematic position of the genus *Diclidanthera* had emphasized the pollen morphology of that family: "the case was clinched by examination of the pollen, which was of the type peculiar to Polygalaceae". The shape and features of the pollen grains of *Diclidanthera* were one of the most important used by ERTMANN (1944) to include that genus in *Polygalaceae.* The fist one who have used the pollen grains features justifying the inclusion

of *Diclidanthera* in *Polygalaceae* was O'DONELL (1941), although who has made the first attempt to include that genus in the *Polygalaceae* was MARTIUS (1856). Also BRETELER (1969, 1970) used the help of a palinologist (PUNT) when proved that *Falya,* a monotip genus from Madagascar, is a synonymous of *Carpolobia,* a genus of *Polygalaceae,* instead of being included in the *Dichapetalaceae:* "the pollen of *Falya leandriana* Desc. has been investigated by PUNT and he confirms that, without any doubt, *Falya* belongs in *Polygalaceae*". Anyway, HALLÉ & HEINE (1967) had already doubt the inclusion of *Falya* in the *Dichapetalaceae,* because of the pollen grains: "Son gros pollen sphérique (36 μm) et 10-colpé est très different de celui des *Tapura* et des autres Dichapétalacées".

The former drawings of *Polygalaceae* pollen grains we found are the excellent drawings of *Polygala* pollen grains by BAUER (F. A.) made while this Austrian artist was living in England (1788-1840). Those drawings are kept in the Library of the Department of Botany of the British Museum (Natural History) in London.

The first one who used pollen grains features to help classifying *Polygalaceae* was PURKINJE (1830) with short descriptions and drawings of pollen grains of *Polygala chamaebuxus* L. and *P. speciosa* Sims [= *P. virgata* Thunb. var. *speciosa* (Sims) Harv.].

Very few authors have used the pollen grains features in taxonomical studies of *Polygalaceae,* but lastly some papers were published using pollen grains in this purpose, such as NAUMAN (1981), MERXMÜLLER & HEUBL (1983), HEUBL (1984) and VILLANUEVA & RAMOS (1986).

MATERIAL AND METHODS

Material

Fresh anthers were collected from *Polygala fruticosa* Berg. cultivated in the Botanical Garden of Coimbra. As they are very small, the anthers were fixed intact.

Pollen-bearing parts were also removed by means of forceps from herbarium material (BM; COI; K).

Methods

Light microscopic (LM) observations with measurements were made on pollen from mature anthers which had been prepared according to the acetolysis method as described by ERDTMAN (1969) or HIDEUX (1972), with small changes, when necessary. The mounting for microscope slides was in glycerine jelly (REITSMA, 1969), as well as in silicone oil (ANDERSEN, 1960). The slides were examined with a Leitz Laborlux microscope.

For scanning electron microscopy (SEM) studies the open anthers have also been prepared by acetolysis and the pollen grains coated with a film of about 300 Å thickness, of evaporated gold/palladium, using stub plattens with "silver dag" and a "Mac-leod, MPS-1" sputter coater. An Hitachi S 800 stereoscan electron microscope was used for examination and for taking the micrographs.

For transmission electron microscopy (TEM) studies, the anthers were dissected from individual flowers and fixed in 2.5 % glutaraldehyde in 0.1 M phosphate

buffer (SÖRENSEN in MILLONIG, 1976) at pH = 7.2, at room temperature for 4 hours. Anthers were then washed several times in buffer, treated with 1% osmium tetroxide, dehydrated in a graded alcohol series, and embedded in SPURR (1969) resin. Sectioning was carried out on an III-LKB Ultratome ultramicrotome with a Dehmer diamond knife and collected on uncoated copper 200-300 mesh grids, covered in some instances by formvar film. Sections were stained with uranyl acetate (VALENTINE & WRIGLEY, 1964) and lead citrate (REYNOLDS, 1963; VENABLE & COGGE SHALL, 1964). Observations and electron micrographs have been made with a Siemens Elmiskop 101 transmission electron microscope.

RESULTS

The terminology is according the suggestions towards simplification and unification proposed by WITTMAN & WALKER (1965) and REITSMA (1970) and NILSSON & MULLER (1978), with some very common ERDTMAN (1969) and FAEGRI & IVERSEN (1964) terms between brackets.

Description

The pollen grain of *Polygala fruticosa* Berg. is of the type peculiar to *Polygalaceae*. It is 21-23-zonocolporate, rarely 19 or 24, isopolar, with radial symmetry (fig. 1 A-E); equatorial view elliptic; polar view circular with inset colpi (fig. 1 A); from subtransverse (oblate-sphferoidal) to suberect (prolate-sphferoidal), with *P/E* ratio = 0.90-1.14 ($\bar{X} = 1.05 \pm 0.06$). Big size; $P = 48.17$-65.45 ($\bar{X} = 58.86 \pm 3.45$) µm; $E = 44.16$-57.60 ($\bar{X} = 51.97 \pm 2.73$) µm. Ectoapertures, colpi of equal length 33.5-45.5 µm, fairly wide (2.5-5.5 µm) (fig. 1 D-E), ends obtuse, membrane psilate-punctate, margins thickened, slightly irregular; endoapertures, pores situaded along the equator of the grain (fig. 1 E-F), describing a bond-like structure, which is a complete equatorial belt (endocingulus) (fig. 1 E, H), 6.75-11.5 µm high, margins thickened, costae 3-3.5 µm wide. Exine rather thick. Nexine 4.2-5.3 µm thick, ± similar thickness throughout (fig. 1 G, I; fig. 2 C), but 2-3 times thicker in the endocingulus margins (fig. 2 A); nexine 2 (endonexine) twice as thick as the nexine 1 (ectonexine), 2.92-3.70 µm thick, granular nexine 1 (ectonexine) fibrous, 1.28-1.6 µm thick (fig. 1 G, I; fig. 2 C). Sexine ± twice as thick as the nexine in the apocolpium (fig. 2 G) and of equal thickness in the mesocolpium (fig. 1 G, I; fig. 2 C), except in the endocingulus margins; sexine 1 (basosexine HESLOP-HARRINSON, 1963) consisting of a thin layer (fig. 1 I, K); sexine 2 a closely packed pillon-like elements (columellae) (fig. 1 G-K; fig. 2 C), 1.6-1.8 µm thick in the mesocolpium; sexine 3 is a tectate layer, 2.10-2.19 µm thick (fig. 1 G-K; fig. 2 B, C, G). Surface psilate in the mesocolpium (fig. 1 D, E); apocolpium circular (fig. 1 C), psilate, rarely with a few ± aperturoid depressions.

Ultrastructural aspects

Sporoderm

TEM micrographs show a collumellate tectate sexine with a very thin basic layer (sexine 1) (fig. 1 I). At the ectoaperture margins the sexine 1 becomes thinner

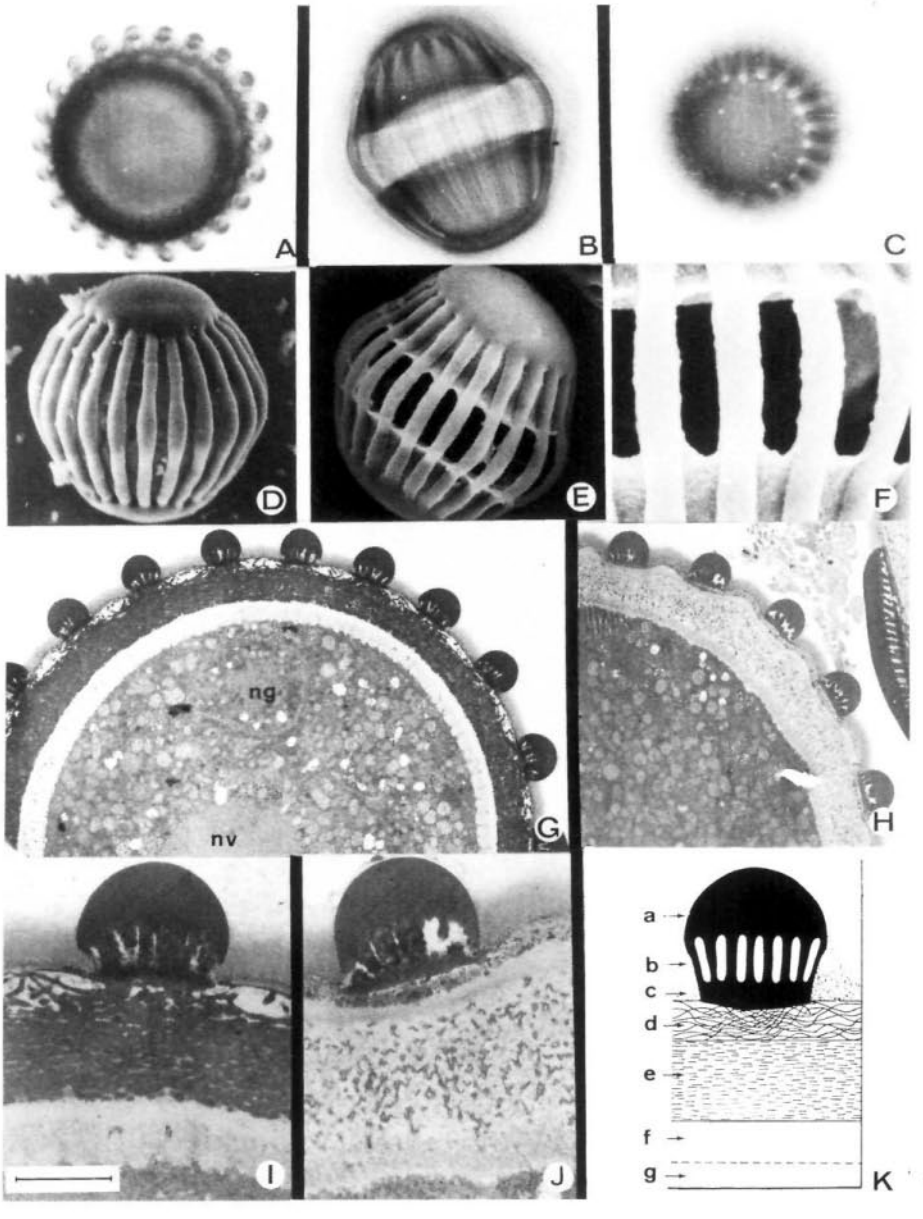

Fig. 1.—Pollen of *Polygala fruticosa* Berg.: A-C, LM views (A, polar view; B, equatorial view; C, apocolpium); D-E, SEM views (D, emature grain; E, mature grain; F, endocingulus section); G-J, TEM sections (G & I, non-equatorial sections; H & J, equatorial sections); K, sporoderm stratification sheme. a, sexine 3 (tectum); b, sexine 2 (collumellae); c, sexine 1 (basosexine); d, nexine 1 (ectonexine); e, nexine 2 (endonexine); f, ectointine; g, endointine; ng, nucleus of generative cell; nv, nucleus of vegetative cell. Scales: A-E, 20 μm; G & H, 5.2 μm; I & J, 1.7 μm.

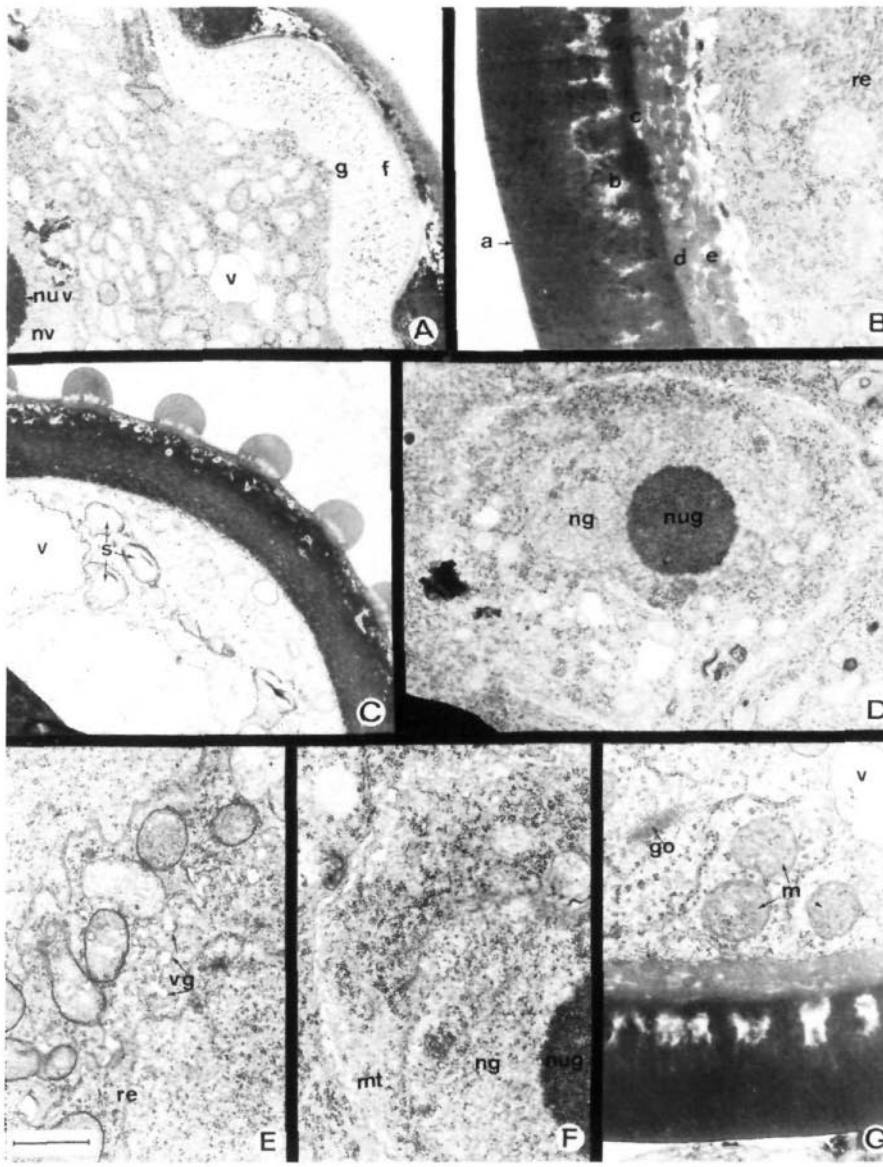

Fig. 2.—TEM sections of pollen of *Polygala fruticosa* Berg.: A, endocingulus intine; B, apocolpium section; C, non-equatorial section; D, generative cell; E, generative cell wall plate; F, generative cell section; G, apocolpium section. a, sexine 3 (tectum); b, sexine 2 (collumellae); c, sexine 1 (basosexine); d, nexine 1 (ectonexine); e, nexine 2 (endonexine); f, ectointine; g, endointine; go, Golgi apparatus; m, mitochondria; mt, microtubules; ng, nucleus of generative cell; nug, nucleole of generative cell; nuv, nucleole of vegetative cell; nv, nucleus of vegetative cell; re, endoplasmic reticulum; s, starch; v, vacuoles; vg, generative cell wall plate vesicles. Scales: A, 2.50 μm; B & G, 1 μm; C, 2.77 μm; D, 1.66 μm; E & F, 1.11 μm.

and is eventually lacking in colpi (fig. 1 G-I). The sexine is ± of equal thickness to the nexine in the mesocolpium (fig. 1 G), but much thicker in the apocolpium (fig. 2 G). At the endoaperture borders the nexine becomes ± twice as thick as in the mesocolpium (fig. 2 A). Nexine 2 (endonexine) is considerably more compact, granular and ± twice as thick as the nexine 1 (ectonexine) (fig. 1 G, J; fig. 2 C). The nexine 1 (ectonexine) is a fibrous layer, thicker at the colpi (fig. 1 G; fig. 2 C). The intine is much thinner in the apocolpium (fig. 2 G). In the mesocolpium it shows two distinct layers (fig. 1 G, I). The inner layer is twice as thick as the outer one. In the endocingulus (endoaperture) region there is an accumulation of amorphorous cellulosic material and the intine becomes much thicker, showing several layers (fig. 2 A). The plasmalemma which is the inner intine layer is followed by a thin layer obviously thicker than the former. After which there is a very thick layer, 3-4 times as thick as the previous layer. There are 3-4 very thin outer layers which are much thinner than the inner ones (excluding the plasmalemma). The thick middle layer and one of the thin external ones are granular while the others appear more homogeneous.

Measurements

The following averages of measurements were taken from different sites of a few TEM micrographs of one pollen grain: extra-apertural intine 2.47 µm thick (inner layer 1.64 µm thick, outer layer 0.83 µm thick); apertural intine in the colpi 5.46 µm thick, apertural intine extra colpi 4.62 µm thick; nexine 4.58 µm thick [nexine 2 (endonexine) 3.28 µm thick, nexine 1 (ectonexine) 1.30 µm thick]; endocingulus border nexine 12.60 µm thick [nexine 2 (endonexine) 8.44 µm thick, nexine 1 (ectonexine) 4.16 µm thick]; sexine 3.78 µm thick [sexine 1 (basosexine) 0.4 µm thick, sexine 2 (collumelae) 1.64 µm thick, sexine 3 (tectum) 2.14 µm thick]; endoaperture height 9.13 µm.

Vegetative and generative cells

The first formed layer of the generative cell wall has a rather electron transparent composition and may well comprise of callose (fig. 2 D-F). In the cytoplasm of the generative cell starch grains are found as well as in the vegetative cell while no vacuoles were found (fig. 2 D). Starch grains are a general feature in the cytoplasm of the vegetative cell of mature pollen grain as well as some vacuoles (fig. 2 A, G). Plastids are found in the cytoplasm as well as Golgi bodies with adjacent vesicles (fig. 2 B, G). Almost no lipid droplets are present. Near anthesis the generative cell appears to lie free in the vegetative cytoplasm. In the cytoplasm of the generative cell the nucleus occupies most of the space (fig. 2 D). Mitochondria, Golgi bodies and the rough endoplasmic reticulum are frequently present in the vegetative cell (fig. 2 A-C, G).

Discussion

As Erdtman (1952) suggested, the pollen grains in *Polygalaceae* seem to be reminiscent of those in *Malpighiaceae* and *Tremandraceae* of the same order *(Polygalales)*, and the pollen morphology suggests some affinity of the *Malpighiaceae*

to the *Trigoniaceae* and *Tremandraceae* and also to 2 families in allied orders such as the *Humiriaceae (Linales)* and *Zygophyllaceae (Sapindales)*. The *Malpighiaceae* are in some respects the most archaic family of the *Polygalales* and form a sort of link between the *Polygalales* and *Linales*. The *Malpighiaceae* cannot be considered as directly ancestral to the rest of the *Polygalales* (CRONQUIST, 1981). Pollen thought to represent the *Polygalaceae* occurs in Paleocene and more recent deposits (CRONQUIST, 1981).

With zonocolporate pollen grains, exine with a well defined stratification and entirely endexinous, *Polygala* is not a primitive genus. Included in the subclass *Rosidae* the *Polygalaceae* are not the most primitive family of that subclass as the pollen grains are zonocolporate.

BIBLIOGRAPHIC REFERENCES

ANDERSEN, S. T. (1960). Silicone oil as mounting medium for pollen grains. *Dan. Geol. Unders.* 4(1): 1-24.

BRETELER, F. J. (1969). The African Dichapetalaceae, I. *Acta Bot. Neerl.* 18(2): 375-386.

CHODAT, R. (1889). Le pollen des Polygalacées. *Arch Sc. Phys. Nat. Guive*, Sér. 3, 21(3): 269-270.

CHODAT, R. (1896). Polygalaceae. *Engl. & Prantl, Pflanzenfam.* 3(4): 323-345.

CRONQUIST, A. (1981). *An integrated system of classification of flowering plants.* Columbia University Press. New York.

ERDTMAN, G. (1944). The systematic position of the genus Diclidanthera Mart. *Bot. Not.* 38: 80-84.

ERDTMAN, G. (1969). *Handbook of Palynology, Morphology, Taxonomy, Ecology. An introduction to the study of pollen grains and spores.* Munksgaard.

ERDTMAN, G. (1971). *Pollen morphology and plant taxonomy-Angiosperms (An Introduction to Palynology, I).* Ed. 3. New York.

FAEGRI, K. & H. IVERSEN (1975). *Textbook of pollen analysis.* Ed. 3. Copenhagen.

HALLÉ, N. & H. HEINE (1967). Deux nouvelles espéces africaines du genre Tapura Aubl. (Dichapetalaceae). *Adansonia*, sér. 2, 7(1): 43-51.

HESLOP-HARRINSON, J. (1963). An ultrastructural study of pollen wall ontogeny in Silene pendula. *Grana Palynol.* 4(1): 7-25.

HEUBL (1984). Systematische untersuchungen an mitteleuropäischen Polygala-Arten. *Mitt. Bot. München* 20: 205-428.

HIDEUX, M. (1972). Techniques d'étude du pollen au MEB: effects comparés des différents traitements physico-chimiques. *Micron* 3: 1-31.

MARTIUS, C. F. P. (1856). *Flora Brasiliensis* 7(17): 1-36, t. 1-14.

MERXMÜLLER, H. & G. HEUBL, G. (1983). Karyologische und palynologische Studien zur Verwandtschaft Polygala chamaebuxus L. *Bot. Helv.* 93(2): 133-144.

MILLONIG, G. (1976). *Laboratory Manual of Biological Electron Microscopy.* M. Saviolo edit. Vercelli: I-VII: 1-67.

NAUMAN, C. E. (1981). Polygala grandiflora Walter (Polygalaceae) re-examined. *Sida* 9: 1-18.

NILLSON, S. & J. MULLER (1978). Recommended palynological terms and definitions. *Grana* 17: 55-58.

O'DONELL, C. A. (1941). La position sistematic de "Diclidanthera" Mart. *Lilloa* 6(1): 207-212.

REITSMA, T. J. (1969). Size modification of recent pollen grains under different treatments. *Rev. Palaeobot. Palynol.* 9: 175-202.

REITSMA, T. J. (1970). Suggestions towards unification of descriptive terminology of Angiosperm pollen grains. *Rev. Palaeobot. Palynol.* 10: 39-60.

REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *Journ. Cell. Biol.* 17: 208-213.

SPRAGUE, T. A. (1940). Taxonomic Botany, with special reference to the Angiospermes. *Huxley, The New Systematics:* 435-454.

SPURR, A. R. (1969). A low viscosity epoxy resin embedding medium electronic microscopy. *Journ. Ultr. Res.* 26(1-2): 31-43.

VALENTINE, R. C. & N. G. WRIGLEY (1964). Graininess in the photographic recording of electron microscope images. *Nature* 203(4946): 713-715.

VENABLE, J. H. & R. COGGESHALL (1964). A simplified lead citrate stain for use electron microscopy. *Journ. Cell. Biol.* 52: 407-408.

VILLANUEVA, E. & A. RAMOS (1986). Contribución al estudio polínico de Polygala L. (Polygalaceae) en la Península Ibérica. *Anales Jard. Bot. Madrid* 42(2): 377-388.

WITTMANN, G. & D. WALKER (1965). Towards simplification in sporoderm description. *Pollen & Spores* 7(3): 443-456.

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