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Pollen morphology and taxonomy of the genus *Sideritis* L. (*Labiatae*) in north Africa

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دراسة حبوب اللقاح و دورها في تصنيف جنس سيديريتس (الشفويات) في شمال افريقيا

على عكس باقي أجناس فصيلة الشفويات تعتبر مورفلوجيا حبوب اللقاح من أهم الوسائل التصنيفية لجنس "سيدريتيس" خاصة على مستوى الفصائل تجنفـقضهذزنتج. يعتبر حجم الحبوب وشكلها زينتها (ornementation) من أهم الخصائص التي تمكن و بسهولة من تصنيف هذا الجنس سواء على مستوى الفصائل أو على مستوى الانواع.

الكلمات المفتاحية : التصنيف – حبوب اللقاح – المجهر الالكتروني.

Morphologie pollenique et taxonomie du genre *Sideritis* (*Labiatae*) en Afrique du Nord

À la différence des autres genres des Labieés, la morphologie pollenique s'est avérée très prometteuse pour la systématique du genre *Sideritis* L. en particulier au niveau des sections. La taille des grains de pollen, leur ornementation et leur forme en vue polaire sont autant de caractères très utiles pour la séparation aussi bien des sections que des espèces au sein des sections

Mots clés : Systématique - Palynologie- Microscope électronique à balayage

Pollen morphology and taxonomy of the genus *Sideritis* (*Labiatae*) in north Africa

This palynological investigation has proved taxonomically very useful especially at the sectional level of the taxonomic hierarchy.The size of pollen grains, their shape and their sculpturing are all very determinate characters for delineation both at the sectional or the specific levels.

Key Words : *Sideritis*- Systematics -Palynology- Scanning electron microscope

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INTRODUCTION

Although palynology is a relatively recent discipline, being first used only in the mid-nineteenth century, and progressing rapidly during the last seventy years (Huynh,1972), it still provides new information to the investigator.besides its numerous applications, palynology is of great use in plant taxonomy and still contributes to the elucidation of taxonomic problems both in the higher and lower ranks of the taxonomic hierarchy. Such evidence has served in many instances to place taxa of uncertain affinities. however, when used, the interpretation of palynological results should always be complemented by other lines of evidence.

The characters recognised as of important taxonomic value in pollen grains include the number and position of colpi and the exine sculpturing. The value of such critical details have been confirmed by the scanning elctron microscope.

Many palynological studies have been carried out on the family Labiatae, for example (Brozova, 1962; Emboden, 1964 ; Erdtman Henderson *et al.*, 1962; Huynh, 1968, 1969, 1972) and others.The family is described as stenopalynous in that it has a rather uniform pollen morphology.Pollen grains of this family are tricolpate, tetracolpate or hexacolpate. Brozova (1962) has shown that the hexacolpate condition is derived from tricolpate one. This was supported and emphasized by Huynh (1972) while working on the genus *Sideritis*. He stated that the basic type pollen grain of Labiatae is tricolpate. This then gives rise to either tetracolpate or hexacolpate grains following two trends in evolution:

- (i) Multiplication of meridional apertures resulting in tetracolpate type.
- (ii) "de novo "aperturation of the sporoderm resulting in the hexacolpate type.

According to Leitner (1942), in Labiatae tricolpate grains are shed in the binucleate stage and hexacolpate grains in the trinucleate stage.The correlation between the binucleate condition and tricolpate grains was also reported by Waterman (1960) studying the Labiatae of Michigan. In his study Waterman was able to key out some of the plants that occured in natural populations or were cultivated, to species or to a group of species on the basis of the shape of the pollen, its size and sculpturing.

As for the genus *sideritis* it has been studied previously by El -Gazzar & Watson (1968) and Risch (1956) who was able to identify three pollen types which have been subsequently fully characterized by Huynh (1972) as follows:

•1. Tricolpate pollen type. Characterized by three meridional colpi of the same length, subdividing the pollen into three mesocolpia of about the same size. This type is found exclusively in the species of section *Hesiodia* where it occurs under two distinct forms:

- "montana type" where the central zone of the poles (apocolpia) and also of the mesocolpia show a reticulum of larger mesh size than the surrounding areas. This type was detected in *S. montana* and *S. remota* out of the three species forming this section.
- "balansae type" found in *S. balansae*. In this type the reticulum is uniform and no central zones were observed.

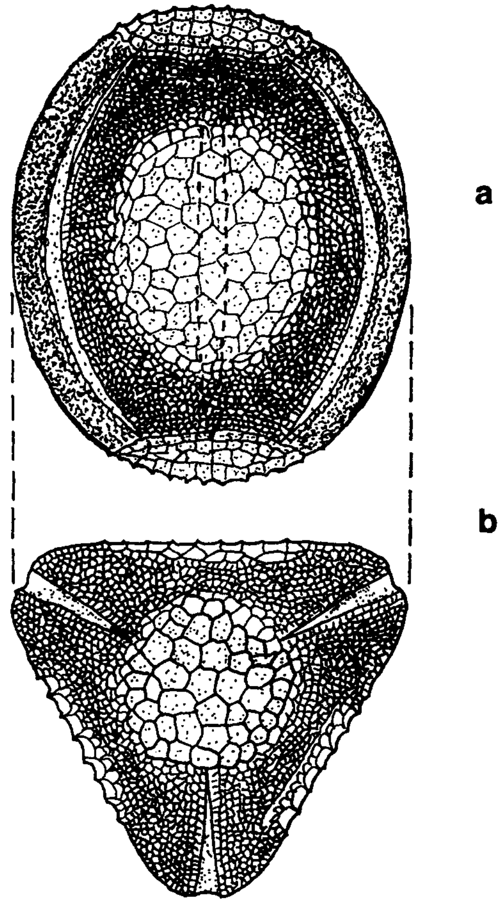

Figure 1. Pollen types of *Sideritis* species . *S. montana*. a : lateral view ; b: polar view

•**2. Tetracolpate pollen type.** Characterized by four meridional colpi of about the same length and dividing the equator into four more or less equal parts. this type is of wide occurence and is encountered in sections *Burgsdorfia*, *Sideritis*, *Empedocleopsis* and *Marrubiastrum*.

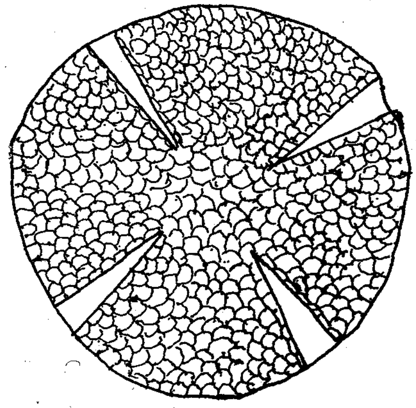

Figure 2. Pollen types of *Sideritis* species. *S. cossoniana* : polar view

•**3. 6- pantocolpate pollen type.** Characterized by six colpi of reduced size. This type is restricted to section *Empedoclea*.

MATERIAL AND METHODS

The source of the material used in this study is given in Appendix. Prior to light and elctron microscope observations, the pollen was prepared using an acetolysis technique modified from Erdtman (1952, 1960) the details of which are given below.

Acetolysis procedure: Anthers from mature flowers were placed in a centrifuge tube with few drops of wetting agent (1% teepol). After 3-5 min. a few drops of lactic acid were added to prevent further expansion of the pollen grains. The tubes were centrifuged at 3000 rpm for 5min. and the supernatant decanted. The pollen was resuspended in glacial acetic acid, centrifuged and the supernatant decanted. The pellet was then resuspended in a freshly prepared acetolysis mixture (9:1 acetic anhydride concentrated sulfuric acid) and heated for 5-10 min. at 100 °C. After centrifugation, the liquid was decanted and the pollen washed three times with distilled water (centrifuging and decanting each time). The samples were then divided into two for light (LM) and scanning (SEM) electron microscopy.

For LM, the material was suspended in 50% glycerol, centrifuged and the supernatant decanted. The tubes were inverted and left to drain in an oven set at 40 °C for 1 hour. Slides were prepared in sets of three. Pollen was mounted in glycerin gelly and sealed with paraffin wax. Measurements of polar and equatorial diameters and of colpus length were taken for a minimum of 10 pollen grains when possible.

For SEM, The samples were passed through 50%, 70%, 90%, 95%,and 100% ethanol before being transferred finally into acetone with centrifugation and decanting at each step. The pollen was then stuck on clean stubs with double-sided sellotape, coated with gold and examined under a JEOL T20 scanning electron microscope. Microphotographs were taken.

RESULTS AND DISCUSION

The findings of this palynological investigation based on 19 taxa are sumarized in table 1. It is obviously difficult to cover the whole range of variation in the genus. Nevertheless with the material examined and with the aid of previous studies, one can to some extent define the overall similarities and variations within the genus at the sectional and specific levels.

The pollen shape is oblate to prolate. In polar view the cutline of the pollen is either circular as in *S. montana*, *S. cossoniana*, *S. villosa*, *S. gossypina*, *S. maura* and *S. subatlantica* or quadrandular in the remaining species. Of all the samples examined only *S. montana* showed tricolpate pollen grains while all others have tetracolpate ones.

This result is in agreement with the findings of earlier workers such as Huynh (1972) and others who claim that the tricolpate pollen grains are restricted to section *Hesiodia* and that sections *Burgsdorfia* and *Sideritis* have tetracolpate pollen grains.

The position of the colpi is a diagnostic feature especially in quadrangular tetracolpate pollen grains. Thus in most species the colpus is situated in the middle of the square side (pleurotreme Figure 3) except for *S. romana* and and *S. imbricata* where the colpi are in the cormers of the square (goniotreme Figure 4).

Table 1. Palynological data

Taxon	Equatorial diameter			Polar diameter			Colpus length		
	min	mean	max	min	mean	max	min	mean	max
Section *Hesiodia*									
S.montana									
subsp.*ebracteata*		27*			27*			12*	
Section *Burgsdorfia*									
S.romana	24	25.5	30	24	27	30	18	21	24
S.villosa	30	33	36	30	34	36	21	23.2	30
S.gossypina	30	33	36	27	33	39	18	24.2	30
Section *Sideritis*									
S.grandiflora	36	37.2	39	39	40.3	42	24	27.6	33
S.imbricata	30	31	33	33	33	33	21	21.9	24
S. subatlantica									
var. *rifea*	33	35.4	39	30	33	39	18	20.2	24
var. *heterostachya*		27.6	30	34.5	39	21	24.9	30	
S.maireana	24	30.7	36	33	36.6	39	24	25.5	30
S.arborescens									
subsp.*ortanaedae*	30	33	36	30	34.2	36	18	22.2	24
S.antiatlantica	27	29.5	33	30	31.8	36	18	22.5	30
S. jahandiezii	27	29.2	30	27	30.6	36	15	18	21
S. mohamedii	37	28.2	33	36	39	42	24	26.5	30
S. maura	21	28.5	36	27	29	33	15	19	24
S. ochroleuca									
var. *maroccana*	30*	29*	24*						
S. hirsuta	21	27*	18*						
S. incana									
subsp. *virgata*	24	27.8	30	30	31.8	36	15	21	24
var. *albiflora*	30	32	33	33	36.5	39	24	26	27
var. *tomentosa*	24	27	29	30	32	33	15	19.2	21

* = Measurements based only on one pollen grain and thus are less reliable Units=mm

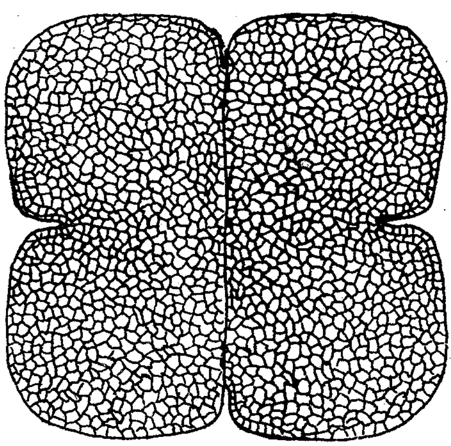

Figure 3. Pollen types of *Sideritis* species . *S. hirsuta* : polar view

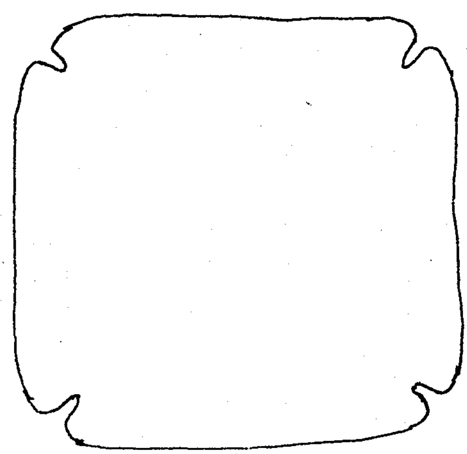

Figure 4. Pollen types of *Sideritis* species . *S. imbricata* : polar view

The average size of pollen grains ranges from 25.5 µm to 37.2 µm in equatorial diameter and from 27 µm to 40.36 µm in polar diameter. *Sideritis romana* has the smallest pollen grains amongst the species studied with a polar diameter of (24)-27- 30 um and an equatorial diameter of (24)-25.5-(30) mm. The biggest pollen grains are found in *S. grandiflora* with a polar diameter of (39) - 40.36 -(42) mm and an equatorial diameter of (36)- 37.2-(39) mm

The pollen sculpturing exhibits a wide range of variability. The pollen of *S. montana* is completely different from that of any other species studied, with the central zone of the poles and mescolpia having a reticulum of larger mesh size than the surrounding areas (Figure 1 & 5).This type was reported by Huynh (1972) to be found only in *S. montana* and *S. remota* in the entire genus. Thus, this characteristic on its own makes these species readily distinguishable from all the others studied. *Sideritis romana* has a more or less smooth reticulum (Figure 6), while the reticulum of *S. cossoniana, S. villosa* and *S. gossypina* is made up of a coarser reticulum (Figures 2, 13 & 14). The other species have a reticulum with an intermediate mesh size.

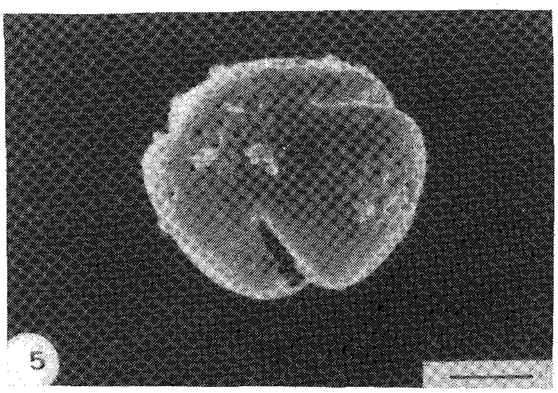

Figure 5. Scanning electronmicrographs of pollen of *Sideritis montatna*

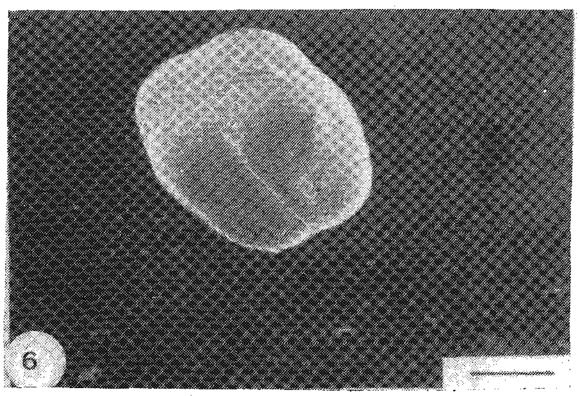

Figure 6. Scanning electronmicrographs of pollen of *Sideritis romana* (sacle bar = 12.5 µm)

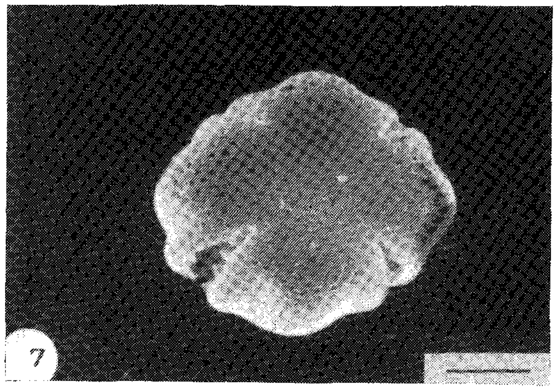

Figure 7. Scanning electronmicrographs of pollen of *Sideritis imbricata*

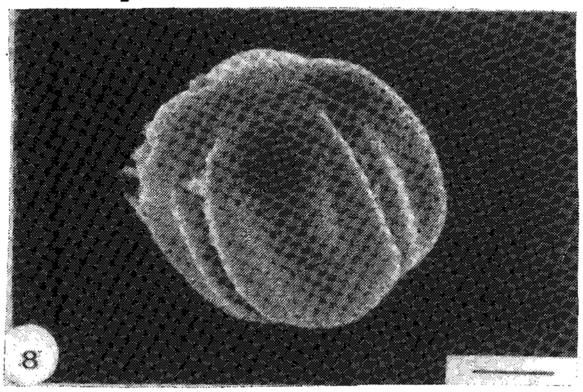

Figure 8. Scanning electronmicrographs of pollen of *Sideritis antiatlantica*

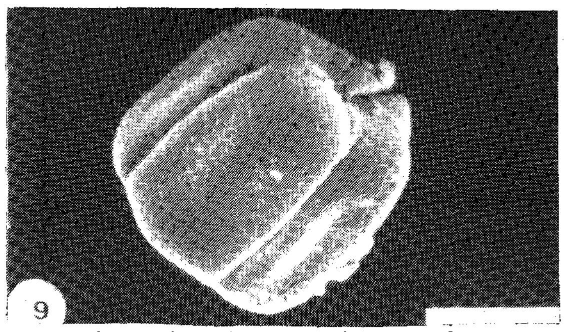

Figure 9. Scanning electronmicrographs of pollen of *Sideritis incana* var. *tomentosa*

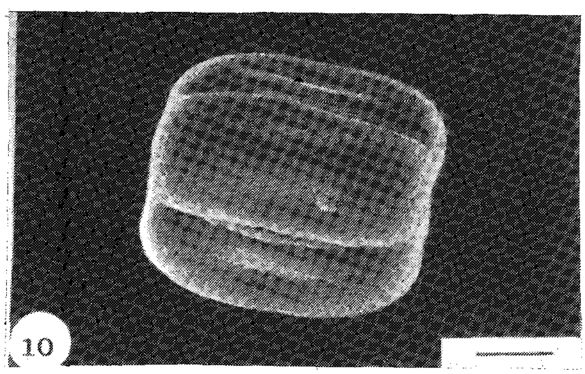

Figure 10. Scanning electronmicrographs of pollen of *Sideritis mohamedii*

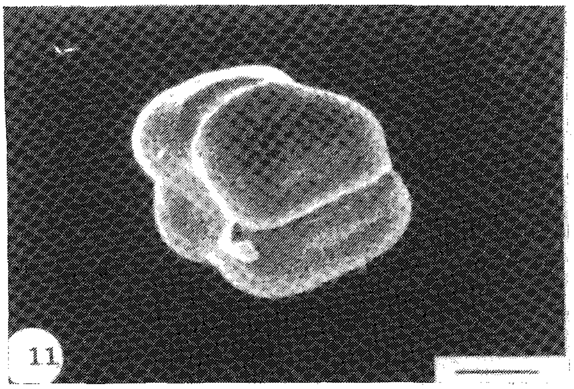

Figure 11. Scanning electronmicrographs showing pollen types of *Sideritis* species and their magnifications *S. jahandiezii*

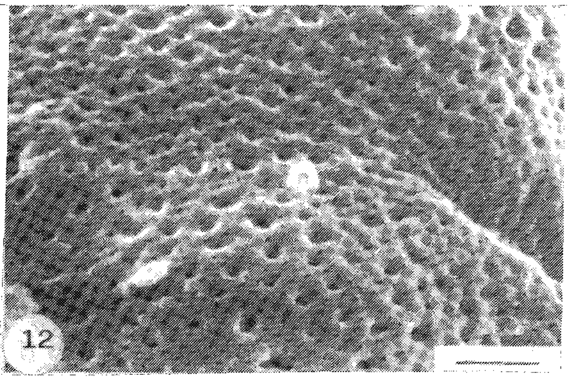

Figure 12. Scanning electronmicrographs showing pollen types of *Sideritis* species and their magnifications *S. jahandiezii*

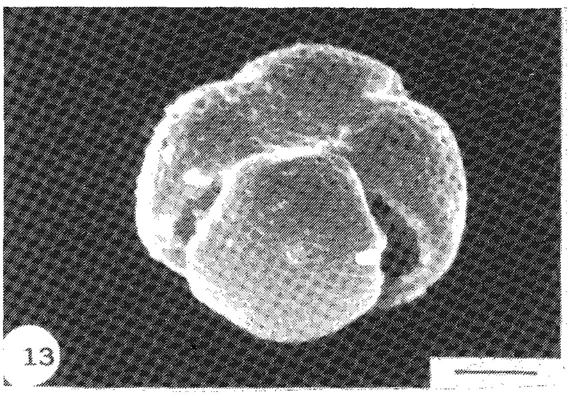

Figure 13. Scanning electronmicrographs showing pollen types of *Sideritis* species and their magnifications *S. villosa*

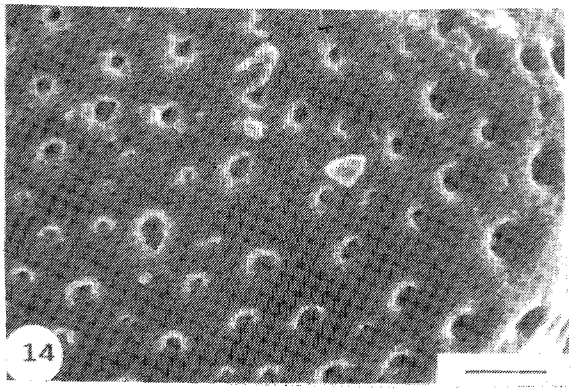

Figure 14. Scanning electronmicrographs showing pollen types of *Sideritis* species and their magnifications *S. villosa*

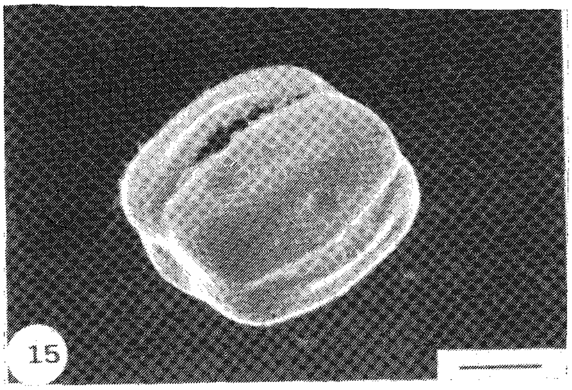

Figure 15. Scanning electronmicrographs showing pollen types of *Sideritis* species and their magnifications *S. incan* subsp. *virgata*

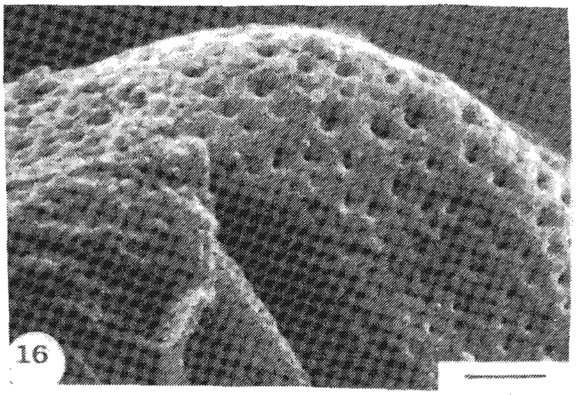

Figure 16. Scanning electronmicrographs showing pollen types of *Sideritis* species and their magnifications *S. incan* subsp. *virgata*

It follows from these observations that the study of the pollen provides useful taxonomic information in *Sideritis* unlike the situation in many of the other genera of the family.

Section *Hesiodia*, represented by *S. montana*, can be sorted out at first sight on the basis of pollen morphology.

The section *Burgsdorfia* can be also easily distinguished from section *Sideritis* by examining both pollen size and ornamentation.

However, within section *Burgsdorfia* a clear division can be made between *S. romana* and the other species of the section (viz. *S.cossoniana, S. villosa* and *S. gossypina*) on the basis of the shape in polar view (square in the former and circular in the latter) and the size of the reticulum.

Within section *Sideritis*, both the shape of the grains (circular in *S. maura* and *S. subatlantica* and square in the others) and the position of the colpi can be used with adventage in discriminating species or species groups.

CONCLUSION

To sum up, it is at once apparent that we are dealing with a relatively stenopalynous group. Nevertheless the pollen grain morphology varies within a limited range, in size, sculpturing and aperture number.

Differences in size are often striking and provide a useful taxonomic character, especially in conjunction with the other morphological features of the grains. these observations are in accordance with those of Faegri and Deuse who stated :

"It is commonplace among palynologists that the size of pollen grains is a rather unreliable character, varying with the previous treatment of preservation. On the other hand, there are important pollen types within which a further taxonomic differentiation cannot be made by means of qualitative morphology, and where more or less successeful attempts have been made to differentiate by means of size statistics."

The most relevant pollen characters of this investigations have been shown to be those of fine structure. These characters, combined with those previously mentioned, may be of great importance in differentiating sections or other taxa of whatever level.

As a result, support for macroscopic morphological characters which can lead to the splitting of the genus might be found.

ACKNOWLEDGEMENTS

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APPENDIX : SOURCE OF MATERIAL

S. montana

Rejdali R25 (IAV); Rejdali R95 (IAV); Hammoumi R1 (IAV); RNG Uni.BM exp.(1974) 254 (RNG,E); Jahandiez 790 (E,LD); Lindberg 2587 (LD); Davis D58733 (E); cosson 9/7/1880 Costantine s.n. (LD); Facture 15/5/1921 Oran s.n. (LD); faure 18/6 1934 oran s.n.(E); Murbeck 18/4/1896 Bir saad s.n (L.D).

S. romana

Rejdali R188 (IAv); Jury, Rejdali and Watson 8363 (IAV,RNG);Murbeck 11/5/1896 Sousse (Tunisia) s.n. (LD); kralik 296 (BM, LD); davis & Lamond D57767 (E,RNG), font- Ouer 334 (BM); Karlsen et al.M87-46 (LD); Dahlygreen & Lassen 56-15(L); Dahlgreen et la. M9-53 (LD).

S. cossoniana

Rejdali R 140 (IAV); rejdali R141 (IAV); Rejdali R35 (IAV); Rejdali R75 (IAV); Rejdali R 162 (IAV); Ball B2917 (E); jahandiez 204 (LD); Jahandiez 180 bis(E); Davis D48542 (E), Samuelson 6427 (LD); Maire 13/431 Agadir s.n. (LD); Lindberg 2497 (LD); Hammoumi R7 (IAV); Erik Way 236 (LD); Bramell, Richardson & Murray 211 (RNG); Bramwell, Richardson & Murray 276 (RNG); Miller, Russel & Sutton 271 (RNG); Bramwell, Richardson & Murray 276 (RNG), Miller 271 (RNG); Bramwell, Richardson & Murray 276 (RNG) .

S. villosa

Balls B2463 (BM); Balls B2967 (E); Lindberg 2607 (LD); Jahandiez 625 (BM, LD); Jahandiez 230 (LD); Lewalle 9975 (IAV), Rejdali R6 (IAV), Rejdali R163 (IAV); Jury; Rejdali and Watson 8848 (IAV, RNG); RNG Univ.BM exp.1974 732 (BM, RNG), RNG Univ. BM exp.1974 544 (RNG, BM, E) Murbeck 5/5/1921 amizmiz s.n. (LD); Murbeck 6/5/1921 Imi-n- Tala s.n. (LD), Davis D48948 (E); Bannerman 22/3/1952 Tizi n-Test s.n. (BM).

S. gossypina

Maire 295 (MAD); Hammoumi R2 (IAV); Rejdali R17 (IAV)

S. Subatlantica var.*rifea*

font -Quer 379 (RAB), Font-Quer 531 (BM);Font-Quer 380 (RAB); Sennen 7685 (RAB); Sennen 7686 (BM); Sennen 8889 (RAB; Sennen 9530 (RAB), Sennen 7980 (BM).

S. subatlantica Var. *heterostachya*

Sennen 7686 (BM,RAB).

S. briquetiana

Rejdali R150; Jury, Rejdali and Watson 8489 (IAV, RNG), Sennen 8897 (BM); Sennen 9736 (BM); Sennen8898 (RAB; Sennen 8485 (LD), Sennen 8486 (E), Sennen & Mauricio 1932 Djbel Kerber s.n. (BM)

S. imbricata

Dahlgreen & Lassen 19-57 (LD); 19-27 (LD), Maire 20/7/1930 Djbel Dersa Tetouan s.n. (RAB); Font-Quer 556(BM), Font-Quer 534 (BM)/

S. maura

Balansa 564 (BM, E); Cosson 13/5/1875 Ouilis Dohras s.n. (LD), 16/5/1875 Dohra s.n. (E); Warion 79 (E).

S. antiatlantica

Rejdali R200; (IAV) Rejdali 211 (IAV), Hammoumi R70 (IAV); Hammoumi R1 (IAV). Hamoumi R11 (IAV), Hamoumi R13 (IAV); Hammoumi R12 (IAv), Hammoumi R14 (IAV), Balls B2681 (BM), Maire entre Ighrem et Issaften s.n. (RAB); amire & Wilczek 478 (L).

S. maireana

Font-Quer 533 (BM), Sennen 8890 (BM); Dahlygreen et al . M36 -35 (LD).

S. arborescens subsp. *arborescens*

Sennen 8490 (RAB); Sennen 9726 (RAB) Sennen 8902 (RAB).

S.arborescens subsp. *ortanaedae*

Font-Ouer 373 (RAB); Font-Quer 374 (RAB); Sennen 9734 (RAB), Faune 10/6/1931 D.J. Gerbouz s.n. (E, LD).

S. ochroleuca var.*denticulata*

Ibrahim Avril 1873) Dj. Ounsa s.n. Ibrahim Aout 1873) Dj. Ounsa s.n. (LD);L. Hubuy 6/8/1933 Seksaoua s.n. (RAB), Maire 1924 Glaoua (Tizi-n- Telouat) s.n. (RAB).

S. ochroleuca var. *getula*

Rejdali R 190 (IAV), Jury, Rejdali & Watson 8511 (IAv, RNG), kralik 53 (RAB), Rrik way 298 (LD), Samuelson 7597 (LD), Merxmuller & Obrwinkler Merx. 22230 (LD),Bourgeau 53 (LD).

S.mohamedii

M. Hammoumi, A. M'Hamedi & M. Ait Lafkih 1985 Agard -n- ousatad s.n. (IAV).

S. jahandiezii

Nain 339 (RAB); L. Huby 3/7/1934 Dj. Ayachi s.n. (RAB), Jahandiez 695 (RAB); Humbert Mai 1927 Ait Ouallou s.n. (LD); Rejdali 20 (IAV); Rejdali 115(IAV),Rejdali &Watson 9245 (IAV, RNG).

S. hirsuta

jahandiez 522 (BM, E); Davis D555098 (E); lewalle9606 (IAV); Lewalle 10459 (IAV), RNG Univ./BM exp. (1974) 699 (RNG, BM); RNG Univ./BM exp.1974 929 (BM, RNG), 1056 (BM, RNG); Rejdali R198 (IAV); R180 (IAV); Rejdali R 217 (IAV); Jury, Rejdali and Watson 9256 (IAV, RNG), Font-Quer 333 (BM); Ball Tassaremout 9/5/1871 (B.M).

S. incana var. *albiflora*

Davis D58804(E, BM): Davis D58819 (BM, E); Faure 17/5/1930 Gerbouz s.n. (LD).

S. incana subsp. *incana*

Miller & Harely 722 (BM), RNG Univ. exp. (1979) s.n. (RNG); Segura zubizarreta 16944 (RNG).

S. incana subsp. *tomentosa*

Davis D55033 (BM); RNG Univ./BM Exp. (1974) 1033 (RNG), Stennis 19247 (BM); Jahandiez 428 (BM, LD, E); Font-Quer 557 (BM), Rejdali 216 (IAV), Rejdali & Heywood R162 (IAV.

S. incana subsp. *virgata*

I.B.K. & F.M Richardson 34 (RNG), Hammoumi R12 (IAV); Sennen 8488 (BM); 8903 (BM);Sennen 8905 (BM); Sennen 9740 (RAB); Sennen 9741 (BM);Sennen et Mauricio 3/7/1930 Kebdana s.n. (BM), Debeaux 4643 (LD);Faure 11/7/1911 Oran s.n.(E) ; Faure 17/5/1930 Gerbouz (BM, E): Bourgeau 40 (E); Dahlygreen *et al.*M51-02 (LD) ; RNG Univ./BM exp. (1974) 948 (RNG, BM).

Abreviation of herbaria

BM	: British museum Herbarium
E	: Royal botanic Gardens, Edinburgh Herbarium
RAB	: Institut Scientifique Herbarium
LD	: Lund Herbarium
RNG	: Reading University Herbarium