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Pollen morphology and taxonomy of *Genista* sects. *Acanthospartum* Spach and *Fasselospartum* P. Gibbs (*Genisteae*, *Fabaceae*)

Abstract

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A pollen analysis was carried out on 72 samples from six taxa of *Genista* sects. *Acanthospartum* and *Fasselospartum*. The taxa were compared in terms of quantitative and qualitative characters, examined by LM and SEM; the joint use of all characters provides the most complete information. The combined analysis of the pollen characters allows noticing some differences among the taxa. The pollen differences confirm the separation of *G. fasselata* (an eastern Mediterranean species) from the sect. *Acanthospartum* and its inclusion in a distinct section, *Fasselospartum*, as suggested by Gibbs (1966). The results of the present pollen analysis support the distinction of the three species included by Greuter & al. (1989) in *G. acanthoclada* aggr.: *G. acanthoclada* (an eastern Mediterranean species), *G. sardoa* (a Sardinian endemic) and *G. valdes-bermejoi* (endemic to Mallorca); *G. sardoa* appears the most distinct species of the aggregate, followed by *G. valdes-bermejo*i. The releved pollen differences support the subdivision of *G. acanthoclada* into two subspecies: *G. acanthoclada* subsp. *acanthoclada* and *G. acanthoclada* subsp. *echinus*, as recognised by Vierhapper (1919). Within *Genista acanthoclada* subsp. *echinus*, some pollen differences were found between the two formae (f. *rhodica* and f. *tenuior*), described by Vierhapper (1919).

Key words: Numerical analysis, Palynology, Systematics.

Introduction

Genista L. (*Genisteae*, *Fabaceae*) includes about 110 species distributed mainly in the Mediterranean region. The genus delimitation is uncertain. For example, not all authorities agree on segregating *Cytisanthus* O. F. Lang, *Chamaespartium* Adans., *Pterospartum* (Spach) K. Koch, *Echinospartum* (Spach) Fourr. and *Teline* Medik. from *Genista* (see Gibbs 1966, 1968; Polhill 1976; Greuter & al. 1989; Talavera 1999; Talavera & Gibbs 1999). Also the attribution of the species to infrageneric taxa (subgenus, section) and the relationships among taxa themselves are questionable and open to discussion. To contribute to the systematics of *Genista*, several studies were carried out using various approaches (for references see Rizzi Longo & al. 2006).

The pollen morphology was investigated as well: fragmentary data were published by Faegri (1956), Polhill (1976) and Pardo & al. (1994). Pollen grains of *Genista* show a relatively uniform morphology as regards the main pollen characters. However, a certain variability in pollen characters was noted, also within the same taxon (Rizzi Longo & Feoli Chiapella 1981); for this reason, it appeared correct to examine several populations per taxon, scattered within the distributional range, in order to assess the infra- and interspecific variability (Rizzi Longo 1986). Morphological and biometric analyses were undertaken by the authors on pollen grains in order to contribute to the systematics of various groups of taxa of different rank belonging to *Genista*. Detailed studies of pollen characters, not only by light (LM), but also by scanning (SEM) and trasmission electron microscope (TEM) were carried out on *Genista* sect. *Spartioides* Spach (Rizzi Longo & Feoli Chiapella 1993), sect. *Voglera* (Gaertn., Mey. & Schreb.) Spach (Feoli Chiapella 1983), sect. *Spartocarpus* Spach (Rizzi Longo & Feoli Chiapella 1994) and sect. *Cephalospartum* Spach emend. P. Gibbs (Rizzi Longo & al. 2006). The ultrastructure of the exine of 15 species of *Genista*, chosen so as to represent all the sections of the genus, was also examined by TEM (Ghirardelli & al. 1994).

In the present study, a detailed pollen analysis of sects. *Acanthospartum* Spach and *Fasselospartum* P. Gibbs is carried out by LM and SEM. The pollen analysis was undertaken to contribute to clarify the systematic correlations among these taxa. The two sections belong to subgenus *Spartocarpus* Spach, the two other sections of which, *Spartocarpus* and *Cephalospartum*, were already examined. With this research the pollen analysis of the whole subgenus will be completed.

Systematics of the sections

Genista sect. *Acanthospartum* comprises only *Genista acanthoclada* aggr., which includes three endemic vicariant species distributed in the Mediterranean region: *G. acanthoclada* DC., *G. sardoa* Valsecchi and *G. valdes-bermejoi* Talavera & L. Sáez (Greuter & al. 1989). The taxa of the section are spiny shrubs with opposite branching, trifoliolate opposite or alternate leaves, flowers with broadly or angular ovate standard petal, longer or shorter than the keel, ovoid-acuminate 1-2 seeded legume. The opposite branching is a rather rare character in *Genista*, being shared only by species of sections *Spartocarpus*, *Acanthospartum* and *Cephalospartum,* all belonging to subgen. *Spartocarpus.*

Genista acanthoclada is distributed in the eastern Mediterranean region: Greece, Turkey, Syria, Lebanon and Libya (Vierhapper 1919; Gibbs 1966; Maire 1987; Greuter & al. 1989; Jafri 1980 and Mouterde 1978-84). Two subspecies were described: ssp. *acanthoclada* and ssp. *echinus* (Spach) Vierh. (Vierhapper 1919; Gibbs 1966; Greuter & al. 1989). *Genista acanthoclada* ssp. *acanthoclada* is distributed in Greece (Ionian Isles, Peloponnese, Attica, Aegean Isles and Crete), western Turkey and Libya, particularly in Cyrenaica, while ssp. *echinus* (= *G. echinus* Spach) occurs in south-western and southern Turkey, Rhodes and Karpathos (Dodecanese), Syria and Lebanon (Vierhapper 1919; Gibbs 1966; Maire 1987; Greuter & al. 1989). Within ssp. *echinus*, Vierhapper (1919) recognized two *formae*: f. *tenuior* Boiss. (Anatolia, Syria and Lebanon) and f. *rhodica* Vierh. (Rhodes and Karpathos).

Genista sardoa Valsecchi (= *G. acanthoclada* ssp. *sardoa* (Bèg. & Landi ex Landi) Valsecchi) is a Sardinian endemic, distributed in the western coastal zone (Valsecchi 1975, 1984).

Genista valdes-bermejoi Talavera & L. Sáez (= *G. balearica* Willk. ex Porta) is endemic to Mallorca (Balearic Islands) and grows in few localities, mostly on the mountain chain facing the north-western coast of the isle (Bonafè Barceló 1978; Talavera 1999).

Genista acanthoclada aggr. is an example of East-West Mediterranean vicariance, with *G. valdes-bermejoi* and *G. sardoa* distributed in the western, and *G. acanthoclada* in the eastern Mediterranean region. *G. acanthoclada* ssp. *echinus* is by far the easternmost taxon. The aggregate may be included into the palaeogenic and conservative element (after Contandriopoulos & Cardona 1984) of the Mediterranean flora.

Spach (1844) included in sect. *Acanthospartum* also *Genista fasselata* Decaisne (= *G. sphacelata* Spach); furthermore, Gibbs (1966) considered more convenient to include the species in a distinct monospecific section, *Fasselospartum*, because of a series of character differences between *G. fasselata* and *G. acanthoclada*, as the branching (alternate instead of opposite), the spines recurved, axillary and flower-bearing (instead of branches terminated by a spine), a clearly different shape of the pulvinules (black, scale-like instead of swollen and prominent) and the standard (glabrous instead of sericeous). The type of spines observed in *G. fasselata* is rather rare in *Genista*, being absent in all the other taxa of subgen. *Spartocarpus*. *G. fasselata* occurs in isolated localities of the eastern Mediterranean region: Cyprus, Israel (M. Carmel), southern Aegean region (Karpathos, Kasos) (Gibbs 1966; Zohary 1972; Chrtek & Slavik 1981; Greuter & al. 1989).

Materials and methods

Pollen grains from 72 samples of the taxa of *Genista* sect. *Acanthospartum* and from 18 samples of *G. fasselata* (sect. *Fasselospartum*) were examined, using dried material. The geographical origin of the studied populations, scattered within the respective distributional ranges of the taxa, is reported in Fig. 1-3.

The following groups of characters were taken into account:

- *quantitative characters by LM*: length of the polar axis (P), equatorial diameter (E), length of the colpus, equatorial breadth of the colpus, equatorial breadth of the mesocolpium, all in equatorial view; distance between the apices of two ectocolpi in polar view;
- *qualitative characters by LM:* outline in equatorial and polar view; shape of the colpi in equatorial view;
- *qualitative and quantitative characters by SEM:* exine ornamentation at mesocolpium, at apocolpium and at the rim of the apertures; aperture membrane; density of the exine perforations in the interapertural zone at equatorial level.

For light microscopy (LM), the material was acetolysed according to Erdtman (1960). The measurements were made by filar ocular micrometer mounted on a Nikon Optiphot within a standard period after preparation of the slide (4 hours), in order to avoid any alteration in dimensions (Van Campo 1966; Hanks & Fairbrothers 1976; Rizzi Longo 1986). Thirty pollen grains were considered for each sample, since the average values of the quan-

Fig. 1. Geographical origin of the examined populations of the taxa of *Genista* sect. *Acanthospartum* distributed in the western Mediterranean region: ■ *G. valdes-bermejoi*; ▲ *G. sardoa.*

titative characters stabilize after 20-25 measures (Rizzi Longo 1986). Thirty grains were observed for each sample also for the qualitative characters.

For SEM, acetolysed anthers were dehydrated in acetone, dried according to the critical point technique (Anderson 1951). The pollen was coated with gold-palladium and examined with a Philips 500 SEM. Thirty pollen grains were observed for each sample to study the exine pattern; ten counts of the number of the perforations in a standard area (10 μm^2) were carried out at x 5000 for each sample.

The taxa were compared in terms of average value of the quantitative characters and in terms of average frequency of the qualitative character states. The comparison was carried out by analyzing all the characters together, using the Euclidean distance. Single linkage clustering was applied to obtain a hierarchical classification (Sneath & Sokal 1973) and an eigenanalysis was applied to the distance matrix to order the taxa (Lagonegro & Feoli 1981). To link the taxa according to maximum affinity, a minimum spanning tree (Gower & Ross 1969) was constructed. The computer programs used for classification and ordination are described in Podani (1994).

The nomenclature of the taxa follows Vierhapper (1919), Gibbs (1966), Greuter & al. (1989) and Talavera (1999). For pollen terminology see Punt & al. (1994).

Results

Description of pollen grains

The pollen grains of *Genista* sects. *Acanthospartum* and *Fasselospartum* are single, isopolar, radially symmetric, medium-sized, with a perforate suprareticulate tectum. The apertures are three furrows (hardly ever two) located in the equatorial region (3-zonocolpate grains). Sometimes the colpi break in their central part forming a long and rather undefined endoaperture, often resulting in a longitudinal interruption (3-zonocolporate with long

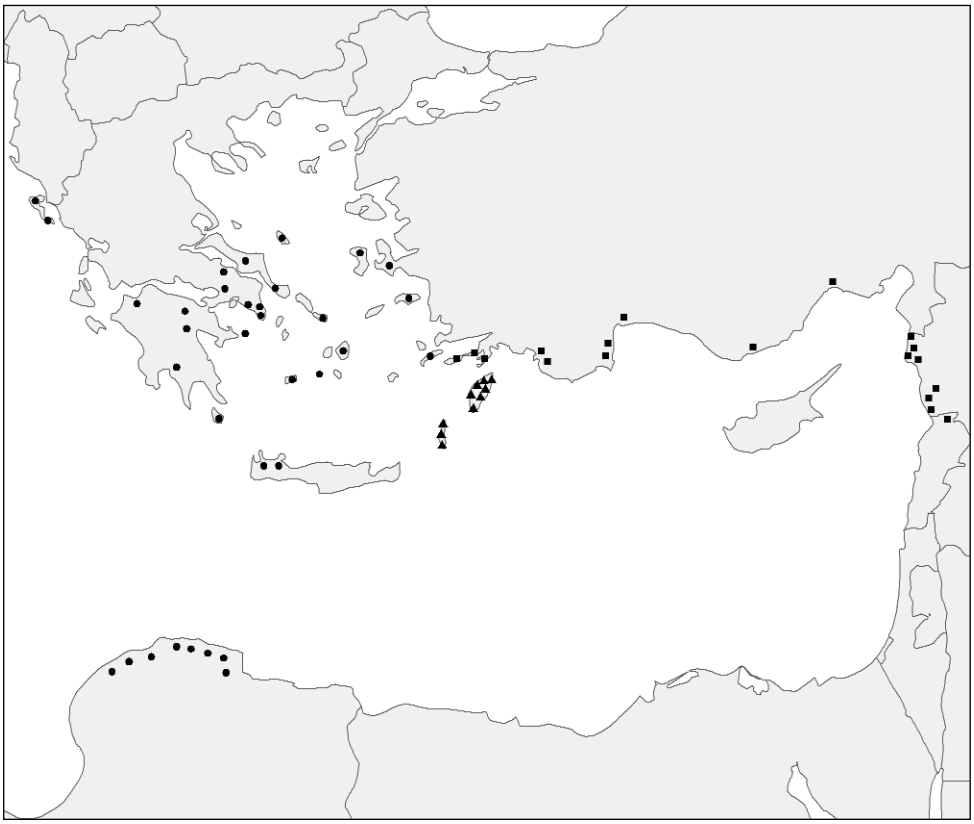

Fig. 2. Geographical origin of the examined populations of the taxa of *Genista* sect. *Acanthospartum* distributed in the eastern Mediterranean region: ● G. *acanthoclada* ssp. *acanthoclada*; ■ *G. acanthoclada* ssp. *echinus* f. *tenuior*; ▲ *G. acanthoclada* ssp. *echinus* f. *rhodica.*

Fig. 3. Geographical origin of the examined populations of *Genista fasselata* (sect. *Fasselospartum*).

ectoaperture and diffuse lolongate endoaperture, corresponding to tricolporoidate grains after Erdtman 1952). Such apertures was already observed in *Genisteae* by various authors, as Misset & al. (1982) in *Ulex* and *Stauracanthus*, Rizzi Longo & Feoli Chiapella (1994) and Rizzi Longo & al. (2006) in *Genista*, Pardo & al. (2000) in *Cytisus* s.l.

Quantitative pollen characters (LM)

Table 1A presents the means and standard deviations of all the quantitative characters considered by LM and of the ratios in the taxa of the sections.

The pollen grains of *Genista acanthoclada* aggr. and *G. fasselata* are small to medium-sized (sensu Erdtman 1952) and usually spheroidal (oblato-spheroidal to prolato-spheroidal), with a mean P/E ratio of 1.05-1.08 in *G. acanthoclada*, *G. valdes-bermejoi* and *G. fasselata*; only in *G. sardoa* the grains are mostly subprolate with a mean P/E ratio of 1.19. The grains of *G. fasselata* differ from the taxa of *G. acanthoclada* aggr. because of their size, their average values being the highest for the equatorial diameter (25.1 μm) and the

distance between the apices of two ectocolpi (6.9 μm). The mean apocolpium index is the highest (0.28) in *G. fasselata*, while it varies between 0.17 and 0.19 in *G. acanthoclada* aggr. Within *G. acanthoclada* aggr., the most distinguishable species is *G. sardoa*, which has the highest P/E ratio and basically higher P values (27.1 μm).

Qualitative pollen characters (LM)

Table 1B shows the average frequency of qualitative character states by LM in the taxa of the sections.

The pollen grains present generally an ellipsoid form in equatorial view. In *Genista fasselata*, the outline is in most cases broadly elliptic (94 %). In *G. acanthoclada* aggr., the most frequent outline is narrowly elliptic; the frequency of broadly elliptic grains is rather high only in *G. acanthoclada* ssp. *acanthoclada*, where subrhomboidal grains are present too, even if in low percentage. The outline in polar view is more frequently circular in *G. fasselata* (52%), subcircular or more rarely subtriangular in *G. acanthoclada* aggr. (see Plate 1). The taxa show a certain variability in the apertural types: colpate pollen grains prevail in all the taxa (80-100%); colpi with diffuse endoaperture are less frequent. The most frequent shape of the furrows in direct view is equatorially constricted (60%) in *G. fasselata*, rectangular in *G. acanthoclada* aggr. (44-51 %). The rims of the colpi are straigth in *G. fasselata*, most frequently wavy in *G. acanthoclada* aggr. The colpi in profile view may be curved or angular; the latter occur more frequently in *G. valdes-bermejoi* and *G. fasselata*. When viewed in profile, a thickening of the apertural region towards the poles can be observed; it is generally longer to half of the colpus, but it can be also equal or shorter.

Ultrasculptural exine characters (SEM)

Table 1C shows the average frequency of the qualitative character states by SEM of the exine ornamentation (see Plate 1) and the average number of the perforations in a standard area (10 μm^2) of the mesocolpium at equatorial level.

In the examined taxa, the tectum at the interapertural zone is supramicroreticulate perforate, with some small unperforate and more rised areas. Fossulate areas have been rarely observed only in *Genista acanthoclada* ssp. *echinus* f. *tenuior*. The muri are more frequently blunt in all taxa. Small perforations of the tectum, with or without inclusions, are present on the bottom of the lumina. The number of the perforations per 10 μm^2 is 24 in *G. fasselata*, while in *G. acanthoclada* aggr. varies from 22 in *G. acanthoclada* ssp. *acanthoclada* to 37 in ssp. *echinus* f. *tenuior*. Generally, only one circular perforation (or, more rarely, irregular as in *G. fasselata*) can be observed; sometimes, many small perforations are present on the bottom of the lumina, either separated or confluent (especially in *G. valdes-bermejoi*). At the apocolpium, the reticulum usually extends unchanged across the poles, but in some cases lowers, particularly in *G. acanthoclada* ssp. *echinus* f. *tenuior* and in *G. fasselata*. The supratectal reticulum mostly tends to disappear towards the rim of the colpi in the equatorial region, especially near the aperture, and the density of the perforations of the tectum usually lowers. Only in *G. acanthoclada* ssp. *echinus* f. *tenuior* and in

Table 1. Pollen characters in *Genista* sects. *Acanthospartum* and *Fasselospartum*. Means and standard deviations (μm) of quantitative characters (LM) and average frequencies of qualitative characters states (LM, SEM) are given.

	G. acanthoclada ssp.acanthoclada	G. acanthoclada ssp.echinus f. tenuior	G. acanthoclada ssp.echinus f. rhodica	G. valdes-bermejoi	G. sardoa	G. fasselata
A) QUANTITATIVE POLLEN CHARACTERS (LM)						
in equatorial view						
polar axis length (P)	25,1 ± 1,94	24,4 ± 1,26	24,6 ± 1,47	25,1 ± 1,49	27,1 ± 2,50	27,2 ± 2,15
equatorial diameter (E)	23,7 ± 1,30	23,3 ± 2,56	23,3 ± 0,80	23,4 ± 1,04	23,0 ± 2,27	25,1 ± 1,82
colpus length	19,2 ± 1,59	19,3 ± 1,24	19,2 ± 1,04	19,7 ± 1,82	20,4 ± 3,14	18,4 ± 1,82
equatorial colpus breadth	2,1 ± 0,33	2,1 ± 0,28	2,3 ± 0,24	2,0 ± 0,24	2,5 ± 0,12	2,4 ± 0,19
equatorial mesocolpium breadth	16,4 ± 1,54	16,5 ± 1,98	16,0 ± 0,72	16,2 ± 1,24	16,5 ± 3,35	16,9 ± 1,73
in polar view						
distance between the apices of two ectocolpi	4,2 ± 0,75	4,3 ± 0,54	4,70 ± 0,70	4,3 ± 1,42	4,2 ± 0,36	6,9 ± 1,48
P/E ratio	1,06 ± 0,09	1,06 ± 0,15	1,05 ± 0,05	1,07 ± 0,06	1,19 ± 0,19	1,08 ± 0,07
apocolpium index	0,17 ± 0,03	0,18 ± 0,04	0,19 ± 0,03	0,17 ± 0,04	0,19 ± 0,03	0,28 ± 0,07
B) QUALITATIVE POLLEN CHARACTERS (LM)						
outline in equatorial view						
narrowly elliptic	44,1	88,9	100,0	66,7	75,0	5,6
broadly elliptic	47,1	11,1	0,0	33,3	25,0	94,4
subrhomboidal	8,8	0,0	0,0	0,0	0,0	0,0
outline in polar view						
circular	12,4	6,1	10,4	7,3	4,8	51,7
subcircular	55,7	39,1	52,6	60,1	58,5	1,8
3-lobate	1,8	12,9	3,8	9,9	6,8	8,4
subtriangular	29,4	41,7	33,1	22,1	27,5	35,9
triangular	0,5	0,2	0,0	0,5	2,5	2,1
apertures						
fused apertures	8,4	2,1	4,9	3,2	1,2	0,4
two furrows not fused	0,9	0,4	0,9	4,7	8,3	0,0
three furrows not fused	90,7	97,5	94,1	92,2	90,5	99,6
apertures in equatorial view						
colpi	91,0	81,1	84,6	100,0	100,0	79,6
colpi with diffuse endoaperture	9,0	18,8	15,4	0,0	0,0	20,4
in direct view						
rectangular	50,1	49,9	45,7	43,7	50,8	16,9
boat-shaped	28,7	25,3	34,2	29,8	27,5	22,7
equatorially constricted	21,2	24,8	20,1	26,5	21,7	60,3
straigth rim	27,7	37,3	40,8	26,5	23,0	100,0
wavy rim	72,4	62,7	59,2	73,5	77,0	0,0
in profile view						
curved	51,3	63,2	55,9	34,8	81,0	45,5
angular	48,7	36,8	44,1	65,2	19,0	54,5
long thickening towards the poles	59,9	54,9	69,3	40,5	55,0	69,7
medium length thickening towards the poles	30,5	27,0	16,4	44,0	28,2	9,2
short thickening towards the poles	9,6	18,1	14,3	15,5	16,8	21,1

Table 1. (continued.)

	G. acanthoclada ssp.acanthoclada	G. acanthoclada ssp.echinus f. tenuior	G. acanthoclada ssp.echinus f. rhodica	G. valdes-bermejoi	G. sardoa	G. fasselata
C) ULTRASCULPTURAL EXINE CHARACTERS (SEM)						
tectum ornamentation at mesocolpium						
homogeneously supramicroreticulate perforate	86,8	90,4	93,9	86,7	88,7	95,7
percentage of unperforate areas	13,2	6,3	6,1	13,3	11,3	4,3
percentage of fossulate areas	0,0	3,3	0,0	0,0	0,0	0,0
muri						
angular	37,8	40,8	38,4	42,5	45,0	46,0
blunt	62,2	59,2	61,6	57,5	55,0	54,0
lumina						
with one circular perforation	73,6	69,2	73,8	68,3	77,5	66,0
with one irregular-shaped perforation	16,4	20,4	18,7	18,3	15,0	26,3
with several separated perforations	0,8	2,9	1,3	0,0	3,7	2,3
with several joined perforations	9,2	7,5	6,3	13,3	3,7	5,3
density of perforations (n°/10 μm^2)	22	37	28	29	23	24
tectum ornamentation at apocolpium						
pattern similar to mesocolpium	84,2	65,9	88,8	90,0	92,5	75,3
with lower reticulum	13,6	34,2	11,2	10,0	7,5	24,7
with higher reticulum	2,2	0,0	0,0	0,0	0,0	0,0
tectum ornamentation at furrow rim						
pattern similar to mesocolpium	31,1	70,0	41,1	87,5	37,5	35,7
with lower reticulum	10,5	0,0	6,7	0,0	20,0	13,6
with trend to disappearing reticulum	58,3	30,0	52,2	12,5	42,5	50,7
less densely perforate	96,9	100,0	100,0	97,5	100,0	88,7
more densely perforate	3,1	0,0	0,0	2,5	0,0	11,3
colpus membrane						
smooth	44,1	55,4	36,1	47,5	20,0	60,7
granulate	20,6	23,8	23,4	25,0	37,5	26,0
microverrucate	33,4	10,9	40,5	20,0	42,5	13,3
verrucate	1,9	10,0	0,0	7,5	0,0	0,0

G. valdes-bermejoi, the tectum ornamentation at the furrow rim displays a pattern mostly similar to that of the mesocolpium. The aperture membrane is often smooth, but may be also granulate or micro-verrucate (as in *G. sardoa*), less frequently verrucate, especially in *G. acanthoclada* ssp. *echinus* f. *tenuior* and in *G. valdes-bermejoi*.

The qualitative characters, particularly by SEM, are seldom discriminant among taxa; only the frequency of the character states may result useful in order to distinguish the taxa.

Ultrastructural exine characters (TEM)

In a former study (Ghirardelli & al. 1994), the ultrastructure of the exine of *Genista acanthoclada* ssp. *acanthoclada* (sect. *Acanthospartum*) and *G. fasselata* (sect. *Fasselospartum*) was examined by TEM in the frame of an analysis of 15 species representative of all the sections of *Genista.*

In both species, the exine shows four layers: tectum, columellar layer, foot layer and endexine (see Plate 1). The tectum, interrupted by perforations, has a supratectal reticulum. In comparison with other taxa of *Genista*, the exine in *G. acanthoclada* ssp. *acanthoclada* and *G. fasselata* is not very thick (0.73 and 0.74 μm, respectively). The ectexine (tectum, columellar layer and foot-layer) is thicker in *G. fasselata* (0.67 μm) than in *G. acanthoclada* ssp. *acanthoclada* (0.59 μm); by contrast, the endexine is thicker in the latter species (0.13 μm).

Numerical analysis

Fig. 4 represents the dendrogram of the taxa of *Genista* sects. *Acanthospartum* and *Fasselospartum*, based on all the considered pollen characters, both qualitative and quantitative, examined by LM and SEM. *G. fasselata* (sect. *Fasselospartum*) is linked to the taxa of sect. *Acanthospartum* at high level of distance. Within *G. acanthoclada* aggr., the most distinct taxon is *G. sardoa*. *G. acanthoclada* ssp. *acanthoclada* and *G. acanthoclada* ssp. *echinus* f. *rhodica* have the highest affinity, followed by *G. acanthoclada* ssp. *echinus* f. *tenuior* and *G. valdes-bermejoi*.

Fig. 5 represents the ordination of the studied taxa, based on the same set of characters, and the overimposed minimum spanning tree. Also here, *Genista fasselata*, which is settled on the upper left side of the graphic, is isolated from the taxa of *G. acanthoclada* aggr., on the right side of the graphic. A pattern similar to that of the dendrogram may be observed. The link between *G. fasselata* and *G. acanthoclada* aggr. occurs through *G. acanthoclada* ssp. *echinus* f. *tenuior*.

Different groups of populations of *Genista acanthoclada* ssp. *acanthoclada* and ssp. *echinus* were studied separately. For ssp. *acanthoclada*, the populations of the Greek mainland and islands, of Turkey and of Cyrenaica (Libya) were taken into account; for ssp. *echinus*, the populations of Turkey, Syria-Lebanon, Rhodes and Karpathos were examined separately. Fig. 6 represents the ordination of the various groups of populations, based on all pollen characters, and the overimposed minimum spanning tree. The groups of populations of ssp. *acanthoclada* is placed on the left side of the graphic, those of ssp. *echinus* on

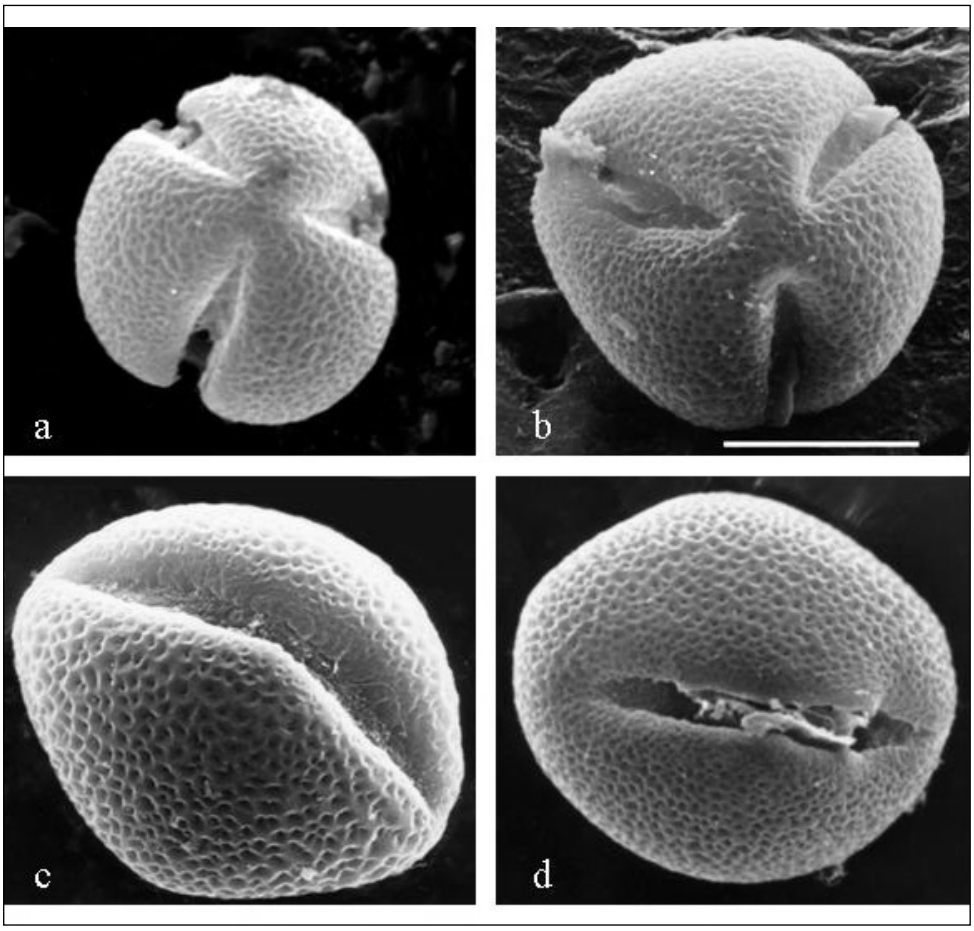

Plate 1. SEM micrographs of *Genista* sects. *Acanthospartum* and *Fasselospartum*: **a**, **b**: polar view; **c, d**: equatorial view. (a) Subcircular outline, *G. acanthoclada* ssp. *acanthoclada*. (b) Subtriangular outline, *G. acanthoclada* ssp. *echinus* f. *rhodica*. (c) Subrhomboidal outline, colpus with granulate to microverrucate membrane, *G. sardoa*. (d) Broadly elliptic outline, colpus with diffuse endoaperture, tectum ornamentation at furrow rim with disappearing reticulum, *G. fasselata*. Scale bar – 10 μm.

the right side. The group of Cyrenaica of ssp. *acanthoclada* is placed on the second axis, in the lower part of the graphic. Within ssp. *acanthoclada*, the populations of Greece result rather contiguous; the only population of Turkey examined results isolated. The populations of Cyrenaica are more distant, and present the greatest affinity with ssp. *echinus*, group of Rhodes. Within ssp. *echinus*, the populations of Turkey, Rhodes and Karpathos result very similar, the group of Syria – Lebanon rather isolated. The link between the two subspecies occurs through the populations of ssp. *acanthoclada* of the Greek islands and those of ssp. *echinus* of Rhodes.

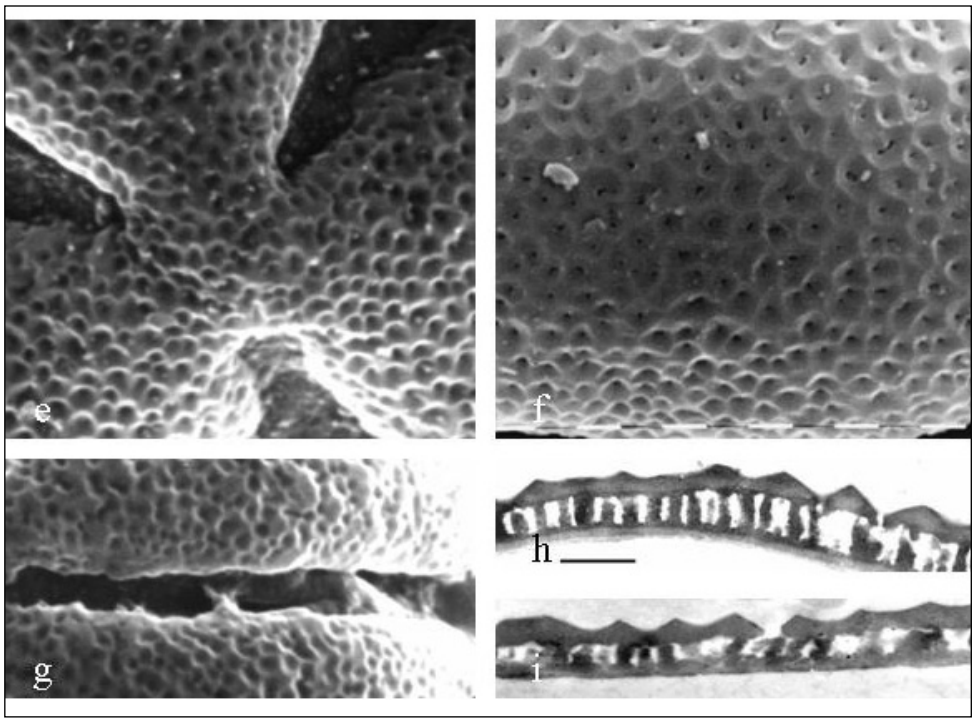

Plate 1. (continued). SEM micrographs of *Genista* sects. *Acanthospartum* and *Fasselospartum*: **e**: polar view; **f, g**: equatorial view. TEM micrographs of the exine in the interapertural zone at equatorial level: **h, i**. (e) Tectum ornamentation at apocolpium with pattern similar to mesocolpium, *G. sardoa*. (f) Tectum at mesocolpium homogeneously supramicroreticulate perforate, blunt muri, lumina with one circular or irregular-shaped perforation, rarely with several perforations, *G. fasselata*. (g) Tectum ornamentation at furrow rim with pattern rather similar to mesocolpium, presence of some small unperforate areas, *G. valdes-bermejoi*. (h) Exine with thin foot layer and thick endexine, *G. acanthoclada* ssp. *acanthoclada*. (i) Exine with thick tectum and foot layer, *G. fasselata*. Scale bar – 1 μm.

Discussion

Pollen analysis

The morphological and biometrical analysis of the pollen grains of *Genista* sects. *Acanthospartum* and *Fasselospartum* confirms the main characters already described in various taxa of *Genista* and of other genera of *Genisteae*. The comprehensive analysis of the pollen characters allows to point out some differences among the taxa of the two sections, as underlined by the numerical analysis.

Genista fasselata (sect. *Fasselospartum*) results clearly distinguishable from all the taxa of *G. acanthoclada* aggr. (sect. *Acanthospartum*) for several pollen characters: quantitative by LM (in particular, the highest values of the equatorial diameter and of the distance between the apices of two ectocolpi) and qualitative by LM (in particular, the high fre-

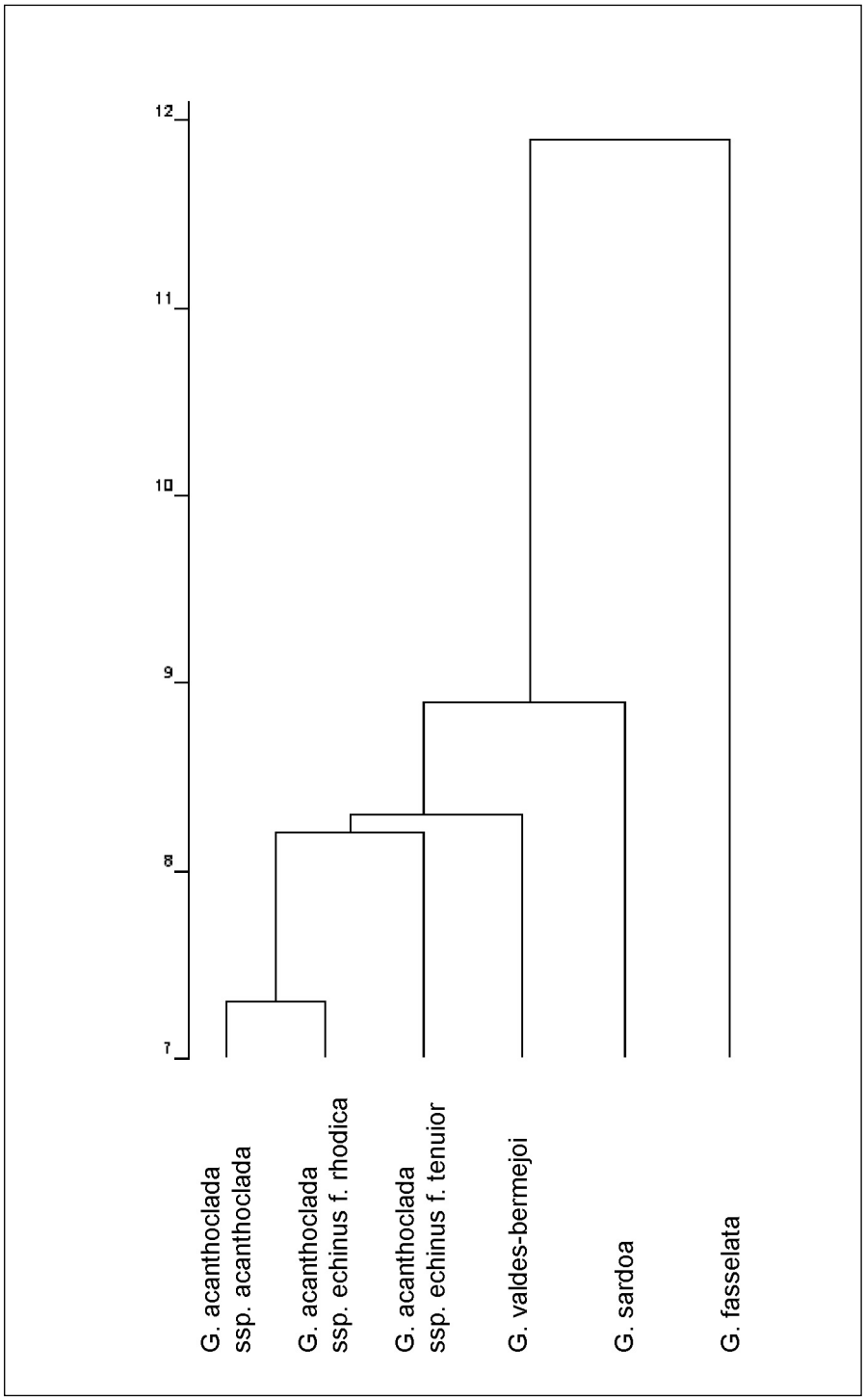

Fig. 4. Dendrogram of the taxa of *Genista* sects. *Acanthospartum* and *Fasselospartum* based on all the considered pollen characters, both quantitative and qualitative, examined by LM and SEM.

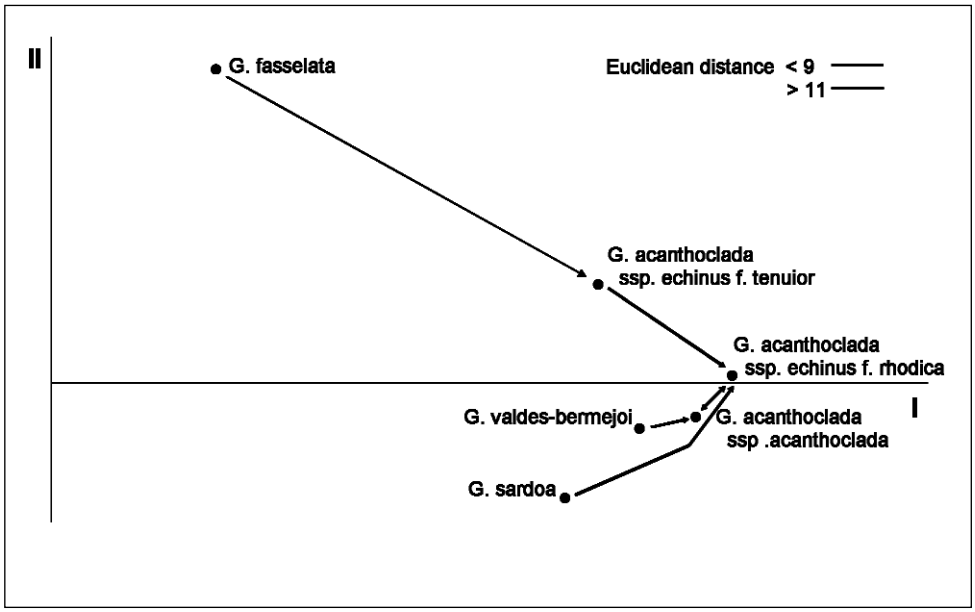

Fig. 5. Ordination of the taxa of *Genista* sects. *Acanthospartum* and *Fasselospartum* on the basis of all the considered pollen characters, both quantitative and qualitative, examined by LM and SEM; the minimum spanning tree, which links the taxa according to maximum affinity, is overimposed.

quency of broadly elliptic grains with circular outline in polar view and of furrows equatorially constricted with straigth rim). Moreover, *G. fasselata* differs from the only taxon of the sect. *Acanthospartum* examined by TEM (*G. acanthoclada* ssp. *acanthoclada*) also for some ultrastructural exine characters, as the thicker ectexine and the thinner endexine. The pollen differences thus confirm the separation of *G. fasselata* from the sect. *Acanthospartum* and its inclusion in a distinct section, *Fasselospartum*, as suggested by Gibbs (1966). *G. fasselata* differs from *G. acanthoclada* on the basis of several morphological characters: in particular, the branching is alternate with axillary spines in *G. fasselata*, opposite with branches terminated by a small spine in *G. acanthoclada*; the pulvinules are black and scale-like in *G. fasselata*, swollen and prominent in *G. acanthoclada*; the standard petal is glabrous in *G. fasselata*, with sericeous hairs in *G. acanthoclada*.

Within *Genista acanthoclada* aggr., *G. sardoa* is the most distinguishable taxon, mainly for the prolate pollen grains and the average frequency of some qualitative characters by LM and by SEM. The species differs from *G. acanthoclada* for some morphological characters: upper branches alternate and rigid in *G. sardoa*, opposite in *G. acanthoclada*; leaflets ovate-lanceolate in *G. sardoa*, smaller and linear-lanceolate in *G. acanthoclada*; flowers borne in terminal racemes in *G. sardoa,* alternate or opposite towards the ends of spinescent branches in *G. acanthoclada*; bracteoles ovate adnate up to half of the calyx in *G. sardoa*, smaller and borne just subtending the calyx in *G. acanthoclada*.

Genista valdes-bermejoi distinguishes itself less from the other taxa of the aggregate for

the complex of pollen characters, mainly for the colpi (mostly angular in profile view) and for the tectum ornamentation at the furrow rim mostly similar to that of the mesocolpium. Morphologically, the species is rather similar to *G. sardoa* in the habitus (short, rigid, alternate or subverticillate branches) and to *G. acanthoclada* in some flower characters, as the size of the petals and the position of the bracteoles; it differs from both species for the leaves (lower trifoliolate and upper unifoliolate) and for the singly borne flowers.

Genista acanthoclada ssp. *acanthoclada* differs from ssp. *echinus* for the complex of pollen characters, mainly for the outline in equatorial view (mostly broadly elliptic grains in ssp. *acanthoclada*, narrowly elliptic grains in ssp. *echinus*). The two subspecies differ for some morphological characters, such as the standard petal length (particularly in relation to the keel) and the pedicel length.

The two *formae* described by Vierhapper (1919) for *Genista acanthoclada* ssp. *echinus* on the basis of some flower characters (standard shape and length, pedicel length) present pollen grains differing for the outline in polar view by LM (mostly subtriangular and trilobate in f. *tenuior*, subcircular in f. *rhodica*) and for some ultrasculptural characters by SEM, like the tectum ornamentation at apocolpium and at furrow rim, and the colpus membrane (mainly smooth in f. *tenuior*, microverrucate in f. *rhodica*). The populations of ssp. *echinus* of Syria-Lebanon differ from those of southern Anatolia, Rhodes and Karpathos

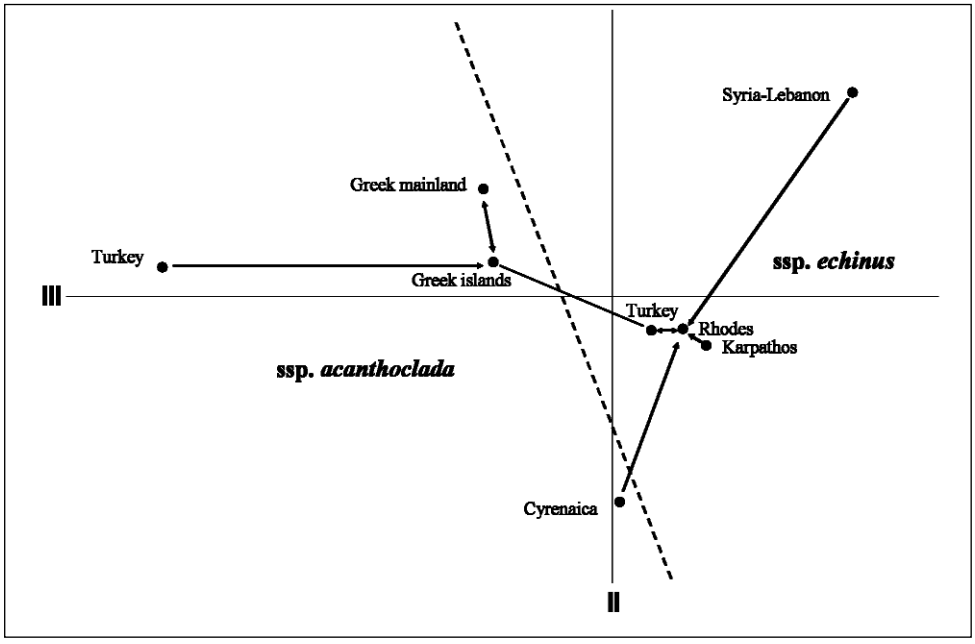

Fig. 6. Ordination of the considered groups of populations of *Genista acanthoclada* ssp. *acanthoclada* and ssp. *echinus* on the basis of all the examined pollen characters, both quantitative and qualitative, by LM and SEM; the minimum spanning tree, which links the groups according to maximum affinity, is overimposed.

for some pollen characters; on the basis of personal preliminary observations, plants of Syria-Lebanon appear to have flowers with a smaller standard. A detailed analysis of the morphology of these populations at the border of the species range seems appropriate.

Within *Genista acanthoclada* ssp. *acanthoclada*, the Greek populations (both from mainland and islands) result rather homogeneous from a palynological point of view, while those from Cyrenaica and the one from western Turkey examined (Izmir region) are better distinguishable. It appears necessary to analyze the morphology of Libyan populations, in order to verify a possible diversification, also considering the remarkable range disjunction between Libyan and Greek populations.

A certain level of correlation among the pollen characters and the macromorphological ones is apparently noticeable.

Comparison with other biotaxonomical data

The karyological data concerning the studied sections are not numerous. Cusma Velari & al. (1997) have reported the chromosome number $2n = 48 + 0\text{-}2B$ for Greek and Turkish populations of *Genista acanthoclada* ssp. *acanthoclada*, Villa (1988) $2n = 52$ for *G. sardoa*. For *G. valdes-bermejoi*, Cardona & Contandriopulos (1983, sub *G. balearica*) and Cusma Velari & al. (2001) have respectively counted $2n = 72$ and $2n = 48 + 0\text{-}2B$ (hardly ever 96). These numbers are traced back to the basic number x = 12 by most authors (among others, Sañudo 1979; Goldblatt 1981; Cusma Velari & al. 2003). Therefore, in sect. *Acanthospartum* both cases of euploidy ($2n = 48$) and ascending aneuploidy ($2n = 52$) were found. The taxa are mostly tetraploid (2n = 48), but a trend towards an increasing level of ploidy may be noticed ($2n = 72$, $2n = 96$). No karyological data relative to *G. fasselata* (sect. *Fasselospartum*) are present in literature.

From a serological survey on the four sections of subgen. *Spartocarpus* (Feoli Chiapella & Kosovel in press), *Genista acanthoclada* ssp. *acanthoclada* (sect. *Acanthospartum*) results very similar to the taxa of sect. *Spartocarpus*. *G. fasselata* (sect. *Fasselospartum*) is slightly more distant. By contrast, the taxa of sect. *Cephalospartum* are clearly different, being even more similar to some species of another subgenus, *Genista*.

Studies relative to phylogenetic analyses on taxa of sects. *Acanthospartum* and *Fasselospartum* are scarce. Only *Genista valdes-bermejoi* has been examined by Pardo & al. (2004) on the basis of nucleotide sequences of nrDNA (ITS region) and cpDNA (*trn*L-*trn*F intergenic spacer): the species results included in the clade of sect. *Spartocarpus*. It is worth noting that, among the sections of the subgen. *Spartocarpus,* the species of sects. *Acanthospartum* and *Spartocarpus* form a monophyletic group, while the taxa of sect. *Cephalospartum* are separated.

Conclusions

The combined analysis of all pollen characters allows noticing some differences among the taxa of *Genista* sects. *Acanthospartum* and *Fasselospartum*. Sometimes, the taxa are more distinguishable on the basis of quantitative characters by LM (as *G. fasselata*), other

times on the basis of qualitative characters by LM (as *G. acanthoclada* ssp. *acanthoclada*, *G. fasselata*) and by SEM (as *G. acanthoclada* ssp. *echinus* f. *tenuior* and *G. valdes-bermejoi*). The joint use of the three groups of characters provides the most complete information.

The pollen differences clearly confirm the separation of *Genista fasselata* (an eastern Mediterranean species) from the sect. *Acanthospartum* suggested by Gibbs (1966), who established for this taxon the new sect. *Fasselospartum*.

The results of the present pollen analysis support the distinction of the three species included by Greuter & al. (1989) in *Genista acanthoclada* aggr.: *G. acanthoclada* (an eastern Mediterranean species), *G. sardoa* (a Sardinian endemic) and *G. valdes-bermejoi* (endemic to Mallorca). *G. sardoa* appears the most distinct species of the aggregate on the basis of pollen characters, followed by *G. valdes-bermejoi*.

The releved pollen differences support the subdivision of *Genista acanthoclada* into two subspecies, *acanthoclada* and *echinus*, as recognised by Vierhapper (1919), Gibbs (1966) and Greuter & al. (1989), but not by Gibbs (1970), Jafri (1980) and Mouterde (1978-84).

Given the found pollen differences, within *Genista acanthoclada* ssp. *echinus* it is possible to advance the re-evaluation of the two *formae* (*rhodica* and *tenuior*) described by Vierhapper (1919).

A morphological study of Libyan populations of *Genista acanthoclada* ssp. *acanthoclada,* presenting pollen grains slightly different from the others, appears necessary in order to verify a possible differentiation in this portion of the distributional range of the species.

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