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Chiave palinologica di identificazione di tre generi appartenenti alla famiglia delle Apiacee

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RIASSUNTO

Sono stati analizzati al microscopio ottico ed al microscopio elettronico a scansione i caratteri morfobiometrici di 10 specie appartenenti alla famiglia delle *Apiaceae*: *Bupleurum fruticosum* L., *B. gerardi* All., *B. lancifolium* Hornem., *B. semicompositum* L., *B. tenuissimum* L., *Echinophora spinosa* L., *E. tenuifolia* L., *Pimpinella anisoides* Briganti, *P. peregrina* L., *P. tragium* Vill. L'analisi ha permesso di caratterizzare 8 morfotipi nei pollini acetolizzati e 3 morfotipi nei granuli non acetolizzati. I caratteri palinologici, confermando l'inquadramento sistematico proposto da Wolff (1959), Tutin *et al.* (1968), Fiori (1969), Snogerup & Snogerup (2001), hanno assunto valore sistematico intergenere ed infraspecifico

Parole chiave: polline, *Bupleurum*, *Echinophora*, *Pimpinella*, melisso-archeo-palinologia

SUMMARY

Palynological key of identification of three genera belonging to family *Apiaceae*

The morphobiometric characters of 10 species belonging to the family *Apiaceae* (*Bupleurum fruticosum* L., *B. gerardi* All., *B. lancifolium* Hornem., *B. semicompositum* L., *B. tenuissimum* L., *Echinophora spinosa* L., *E. tenuifolia* L., *Pimpinella anisoides* Briganti, *P. peregrina* L., *P. tragium* Vill.) have been analysed by light and scanning microscopes. The analysis has allowed us to identify 9 morphotypes in the acetolysed pollen and 3 morphotypes in the not acetolysed grains. The palynological characters, confirming the systematic arrangement suggested by Wolff (1959), Tutin *et al.* (1968), Fiori (1969), Snogerup & Snogerup (2001), have acquired an intergenus and infraspecific systematic value.

Key words: pollen, *Bupleurum*, *Echinophora*, *Pimpinella*, melisso-archeo-palynology

INTRODUCTION

Apiaceae is a family represented by 500 genera in Europe. They are mainly herbaceous plants, more rarely shrubbies, which are largely used for the preparation of medicinal herbs and in medicine. The importance of these plants in melissopalynology for the production of multifloral honeys (De Leonardis *et al.*, 1982, 1984a, 1984b, 1988; Zizza *et al.*, 1985; Simonetti *et al.*, 1989; De Leonardis & Zizza, 1994; Ricciarelli D'Albore, 1998) and the consequent necessity to distinguish the not acetolysed pollen grains during the routine analysis of honey have led Persano Oddo & Ricciarelli Albore (1989) to differentiate: shape *Astrantia* (A) < 25 μm e > 25 μm , shape *Heracleum* (H), *Eryngium*, *Bupleurum*, *Trinia*.

The object of our study was both to analyse thoroughly the diacritic palynological characters of the 3 genera in order to verify the systematic value in relation to the taxonomic arrangement suggested by Wolff (1959), Tutin *et al.* (1968), Snogerup & Snogerup (2001) and both to provide one key for the identification of acetolysed pollen grains (systematic interest) and one for the identification of the not acetolysed grains (melisso-archeopalynological interest).

The pollen grains belonging to the genera *Bupleurum* L., *Echinophora* L. and *Pimpinella* L. with species *B. fruticosum* L., *B. gerardi* All., *B. lancifolium* Hornem., *B. semicompositum* L., *B. tenuissimum* L., *Echinophora spinosa* L., *E. tenuifolia* L. e *Pimpinella anisoides* Briganti, *P. peregrina* L., *P. tragium* Vill. (Pignatti, 1982) have been examined.

MATERIALS AND METHODS

All the pollen specimens have been acetolysed according to the standard method described by Erdtman (1960). They have been observed and measured using a standard Zeiss light microscope, oil immersion 100x (N.A. 1.30) and complex 10x eyepieces.

For SEM studies the acetolysed pollen was washed in distilled water and dehydrated, then put on millipore filters, air-dried and coated with a thin gold layer (100Å) using an Edwards S 150A evaporator. The observations were made using a Jeol Scanning Microscope 35.

Works by Erdtman (1969, 1971), De Leonardis *et al.* (1986), Forlani (1986) and Pignatti (1982) have been consulted for the palynological terminology and the *taxa* nomenclature respectively. In particular, the polar perimeter has been defined according to the terminology used by Cerceau-Larrival (1959, 1962a) for *Umbelliferae*.

RESULTS

Bupleurum pollen are characterized by radiosymmetric monads with an internal nexinic subrhomboidal outline. The polar perimeter is subtriangular, whereas the equatorial perimeter is elliptic except in *B. gerardi* (circular, subcircular, elliptic) and in *B. tenuissimum* (oval and elliptic). The equatorial shape is longiassic except in *B. gerardi*, where it is equiassic and longiassic. The 3 longitudinal equatorial colpi (NPC 345) are narrow, with acute apexes and lalongate, lolongate and circular ora. The exine is subtectate and rugulate. The columellar hypertrophy is absent. Size: P 17-32 μm , and E 13-19 μm (Fig.1: a,b,c; Fig.2: a).

In *Echinophora* the bilateral monads have an internal nexinic equatorially-constricted outline. The polar perimeter is suboval, whereas the equatorial perimeter is elliptic. The shape is constantly longiassic. The 2 longitudinal equatorial colpi (NPC 245) are slightly elliptic, constricted at the middle, with lalongate ora in *E. spinosa* and lolongate in *E. tenuifolia*. The exine is subtectate and rugulate. The columellar hypertrophy is absent. Size: P 32-55 μm , and E 15-29 μm (Fig.1: d,e; Fig.2: b).

Pimpinella grains are radiosymmetric monads with an internal nexinic subrectangular outline. The polar perimeter is subtriangular pleurotreme, whereas the equatorial perimeter is elliptic. The shape is longiassic. The 3 longitudinal equatorial colpi (NPC 345) are narrow, with acute apexes and lalongate, lolongate and circular ora. The subtectate exine is rugulate. The columellar hypertrophy appears to be from subpolar to equatorial position. Size: P 26-37 μm , and E 10-17 μm (Fig. 1: f,g,h,i,l; Fig. 2: c)

The internal nexinic (subrhomboidal, equatorially-constricted, subrectangular) outlines, the sizes, the possible presence of columellar hypertrophy, the suboval or subtriangular polar perimeter are useful characters to provide a key for the palynological identification of the acetolysed grains, which has highlighted 9 morphotypes.

Key of acetolysed pollens

- 1 Bilateral monads, di-zonocolporate apertures..... **2**
- 1 Radiosymmetric monads, tri-zonocolporate apertures **3**
 - 2 Polar axis 44(50)55 μm *E. spinosa* type
 - 2 Polar axis 32(34)37 μm *E. tenuifolia* type
 - 3 Equatorial subrhomboidal shape,
 - absent columellar hypertrophy..... **4**
 - 3 Equatorial subrectangular shape,
 - present columellar hypertrophy **6**
 - 4 Polar axis 28-31 μm *B. fruticosum* type
 - 4 Polar axis 23-26 μm *B. lancifolium* type
 - 4 Polar axis 18-22 μm **5**
 - 5 Width mesocolpium 11-13 μm *B. gerardi*
 - 5 Width mesocolpium 6-8 μm *B. tenuissimum* type*
 - 6 Polar axis ≥ 30 μm *P. anisoides* type
 - 6 Polar axis < 30 μm **7**
 - 7 Subpolar columellar hypertrophy..... *P. tragium* type
 - 7 Subpolar and equatorial columellar hypertrophy *P. peregrina* type

B. tenuissimum type* comprises: *B. tenuissimum*, *B. semicompositum*.

The formulation of a palynological key of the not acetolysed grains, which has allowed us to identify 3 morphotypes, has been developed on the basis of all those characters easily usable and visible through a light microscope during routine analysis in the melisso-archeopalynological field.

Key of not acetolysed pollens

- 1 Bilateral monads, di-zonocolporate apertures
 - polar suboval perimeter *E. spinosa* type*
- 1 Radiosymmetric monads, tri-zonocolporate apertures
 - polar subtriangular perimeter **3**
 - 3 Equatorial subrhomboidal shape
 - absent columellar hypertrophy *B. tenuissimum* type**
 - 3 Equatorial subrectangular shape, present columellar hypertrophy *P. anisoides* type***

E. spinosa type* comprises: *E. spinosa*, *E. tenuifolia*.

B. tenuissimum type** comprises: *B. fruticosum*, *B. gerardi*, *B. lancifolium*, *B. tenuissimum*, *B. semicompositum*.

P. peregrina type *** comprises: *P. anisoides*, *P. peregrina*, *P. tragium*.

DISCUSSION

Palynological contributions on the family *Apiaceae* are numerous but often fragmentary and concern few species (Anefrod, 1960; Faegri & Iversen, 1964, Cauwet, 1970; Jacques-Felin, 1970; Aytug *et al.*, 1971, Erdtman 1971, Moore & Webb 1978; Punt 1984; etc.). The most complete and interdisciplinary studies are by Cerceau-Larrival (1959, 1962a, 1962b, 1963, 1965, 1967, 1968, 1971, etc.) and Cerceau-Larrival & Deroquet (1975) which not only allowed us to correlate the shape of fruits with the symmetry of radiosymmetric pollen, the pollen shape with the size of cotyledonous leaves, the value of the P/E ratio with the phenotype stability of the belonging tribe, but also to further deepen the knowledge of the shape of the pollen grains, the variability of the sporodermic wall and the presence of columellar hypertrophy as adaptation to environmental conditions.

In the genus *Bupleurum* the peculiar and discriminating character of the pollen grains is the subrhomboidal shape (primitive character) except for *B. sibiricum* Vest ex Roem. & Schult. and *B. chinense* DC. (Merrgen, 1994) which have a suboval or subrectangular shape (more advanced characters). The subrhomboidal shape can only be found in five or six genera of *Apiaceae* of which some, like *Bupleurum* and *Azorella* Lam. are certainly very ancient. The ancestors of these last two genera could have been connected with the Tertiary arctic and antarctic paleoflora (Cerceau-Larrival, 1968, 1974). De Leonardis *et al.* (1997) have highlighted a close palynological affinity between *taxa* from genus *Bupleurum* with those from genus *Smyrniium* L. for the presence in both genera of “subrhomboidal-shaped” grains. On the basis of biometric and morphological data these authors included *Smyrniium perfoliatum* L. and *S. rotundifolium* Miller in the morphotype *B. lancifolium*, characterized by subtriangular polar perimeter and “butterfly-shaped” ora.

With regard to the species studied by us, Candau (1987) has included *B. fruticosum* in the morphotype *Bupleurum lancifolium*, characterized by subrhomboidal equatorial shape, polar axis (16-28 μm) and equatorial axis (9-18 μm). In relation to the data by Candau (1987), the values observed by us have highlighted differences in the length of polar axes in *B. fruticosum* e *B. lancifolium*, whereas they have confirmed the data observed in *B. tenuissimum* e *B. semicompositum*.

Our palynological study on species belonging to *Bupleurum* genus has shown that they can be clearly differentiated on the basis of the length of the polar axis and the mesocolpium.

The positioning of the 5 species in the palynological key is in accordance with the systematic arrangement suggested by several authors. As a matter of fact, Wolff (1959) has included *B. subovatum* Link (syn. *B. lancifolium* Hornem.) in the sect. *Perfoliata* Godr. subsect. *Rugosa* Briq., *B. fruticosum* in the sect. *Coriacea* Godr., *B. gerardi*, *B. tenuissimum* and *B. semicompositum* in the sect. *Eubupleura* Briq. and, precisely, the first in subsect. *Juncea* Briq. the second and the third in subsect. *Trachycarpa* (Lange) Briq. ser. *Tuberculata* Wolff. Tutin *et al.* (1968) have included *B. lancifolium* in the sect. *Bupleurum* L. (syn. sect. *Perfoliata* Godron in Fiori, 1969), *B. fruticosum* in the sect. *Coriacea* Godron, *B. gerardi*, *B. tenuissimum* L. ssp. *tenuissimum* and *B. semicompositum* in the sect. *Isophyllum* (Hoffm.) Dumort, the first in subsect. *Juncea* Briq. and the second and the third in subsect. *Trachycarpa* (Lange) Briq. Snogerup & Snogerup (2001) have subdivided *Bupleurum* genus in 2 subgenera, namely subgenus *Tenoria* including *B. fruticosum* and subgenus *Bupleurum* including *B. lancifolium* in

the sect. *Bupleurum* and *B. tenuissimum*, *B. semicompositum*, *B. gerardi* in the sect. *Aristata* Godron subsect. *Juncea* Briq. The close palynological affinity between *B. tenuissimum*, *B. semicompositum* and *B. gerardi* has therefore been confirmed. Differently from Neves & Watson (2004) whose molecular study has found a greater affinity of *B. gerardi* with *B. lancifolium* than with *B. tenuissimum* and *B. semicompositum*.

If the diacritic characters of pollen of *B. fruticosum* (perennial) in comparison with those of the annual species examined by us are taken into consideration, this condition could reflect a greater sporophyte vigour in species with a perennial *habitus* than in species with an annual *habitus*, as it has already been shown by studies by Mulcahy (1971, 1974), Johnson & Mulcahy (1978) and De Leonardis *et al.* (1995).

In the *Echinophoreae* pollen grains are characterized by bilateral symmetry with a nexinic equatorially-constricted outline, suboval polar perimeter, presence of di-colporate apertures and absent hypertrophy. The peculiarity of these characters correlated to the presence of particular morphological characteristics, such as the inflorescence, the fruit, etc... have contributed to consider this tribe an extremely homogeneous group, quite distinct from the other tribes of the family (Cerceau-Larrival, 1962*b*). In the species examined by us, the apertures consist of 2 longitudinal colpi with ora, confirming the observations carried out by Erdtman (1971) on *Anisosciadium isosciadium* Bornm. and on *Echinophora sibthorpiana* Guss. and by Cerceau-Larrival (1959) on *E. spinosa*, but differing from those of Pla Dalmau (1961) who, in *E. spinosa*, observed 3-colporate grains.

Finally, as to the species belonging to the *Pimpinella* genus, our morphological observations have confirmed those by Cerceau-Larrival (1959) on *P. tragium* and by Erdtman *et al.* (1961) on *P. major*.

Candau (1987) has included *P. villosa* Schousboe (polar axis 28-36 μm , equatorial axis 10-18 μm) in the type *Scandix pecten-veneris*, characterized by a subrectangular nexinic outline. The parameters measured by Candau (l.c.) in *P. villosa* have proved to be more similar to the values measured by us in *P. anisoides* rather than in *P. tragium* and *P. peregrina*. Moreover, the palynological key elaborated by us has highlighted a greater affinity between *P. peregrina* and *P. tragium* than with *P. anisoides*, condition which is in accordance with the arrangement suggested by Fiori (1969) and Tutin *et al.* (1968).

CONCLUSIONS

Only to the species examined by us, it has been pointed out how the three above mentioned genera can be distinguished on a palynological level on the basis of several morphological characters (gene expression) such as symmetry, shape and columellar hypertrophy. The diacritic characters of the three genera could therefore represent a useful diagnostic element in all the palynological routine analysis in the melisso-archeo-palynological field. On the contrary, on an intragenus level, the acetolysed pollen grains of the different species have been distinguished only as a result of an in-depth biometric investigation which has allowed us to characterize 9 morphotypes and has confirmed the systematic arrangements suggested by several authors. It can therefore be concluded that, similarly to the carpological studies by Özcan (2004), which have pointed out different sculpture patterns in the *taxa* of distinct sections and subsections suggested by Snogerup & Snogerup (2001), the palynological characters found by us could also be further diacritic taxonomic parameters on the intergenera and infraspecific levels.

Specimina selecta

- Bupleurum fruticosum* L.: Fiumedinisi (ME), CAT 03052010 G. Ferrauto
Bupleurum gerardi All.: Tortorici (Me), CAT 01050525 G. Ferrauto
Bupleurum lancifolium Hornem.: Monte Capodarso (CL), CAT 00051506 G. Ferrauto
Bupleurum semicompositum L.: Licata (AG), CAT 02062015 G. Ferrauto
Bupleurum tenuissimum L.: Ficuzza (PA), CAT 01061015 G. Ferrauto
Echinophora spinosa L.: Donnalucata (RG), CAT 02063001 G. Ferrauto
Echinophora tenuifolia L.: Leonforte (EN), CAT 01091510 G. Ferrauto
Pimpinella anisoides Briganti: Etna (CT), CAT 03072808 G. Ferrauto
Pimpinella peregrina L.: Floresta (ME), CAT 01050616 G. Ferrauto
Pimpinella tragium Vill.: Quacella – Madonie (PA), CAT 01063012 A. Zizza & G. Ferrauto

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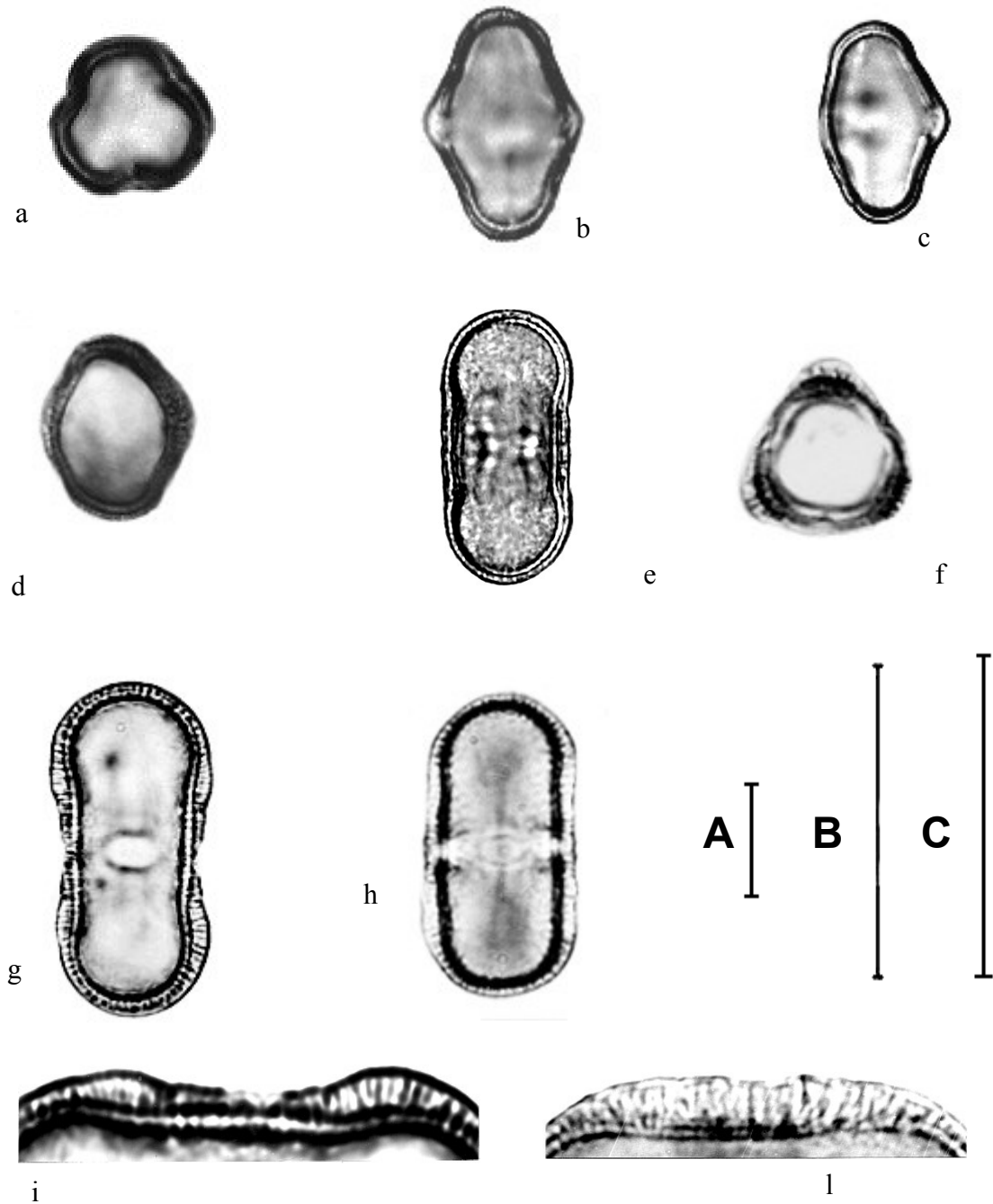


Fig. 1. Palynological micrographs with O.M. Of *Bupleurum*, *Echinophora* and *Pimpinella* morphotypes. *Bupleurum fruticosum* L.: a, polar view; b, equatorial view, optical section; c, equatorial view, profile of os. *Echinophora spinosa* L.: d, polar view; e, equatorial view, optical section. *Pimpinella anisoides* Briganti: f, polar view; g, equatorial view, optical section; h, equatorial view, mesocolpium; i, subpolar columellar hypertrophy. *P. peregrina* L.: l, subpolar and equatorial columellar hypertrophy. (Scale 10µm A figs.a,b,c,d,e,f,g,h; B fig.i; C fig.l).

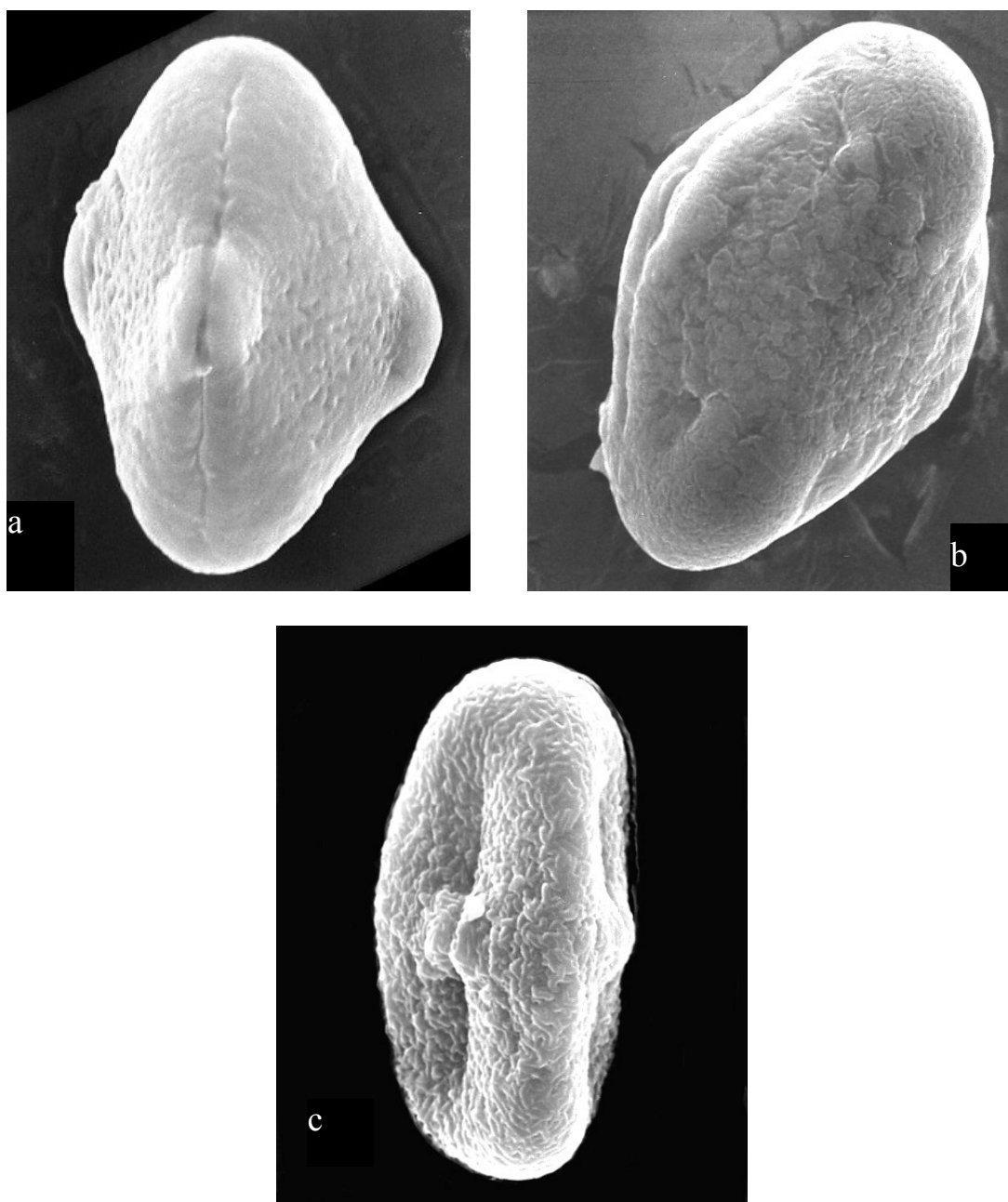


Fig. 2. Palynological micrographs morphotypes with S.E.M. *Bupleurum fruticosum* L.: a, equatorial view; (3000x). *Echinophora spinosa* L.: b, equatorial view (3000x). *Pimpinella peregrina* L.: c, equatorial view (3000x).