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Pollen morphology of the genera *Onobrychis* and *Hedysarum* (Hedysareae, Fabaceae) in Bulgaria

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Pollen morphology of 14 taxa, 12 taxa of the genus *Onobrychis* Adans. and two taxa of the genus *Hedysarum* L., distributed in Bulgaria, was studied with light and electron microscopy. The pollen is 3-colpate, medium or large in size, with reticulate ornamentation. Differences are observed in the dimensions, the aperture shape and the exine stratification. One pollen type with three tentative subtypes are recognized based on size and ornamentation. Pollen morphology confirms a close relationship between the species of both genera.

Key words: Bulgaria, Fabaceae, *Hedysarum*, *Onobrychis*, pollen morphology

INTRODUCTION

The genera *Onobrychis* and *Hedysarum* constitute the main part of the tribe Hedysareae DC. in the sense adopted by Polhill and Raven (1981). Like the remaining five genera in this tribe, they are centred in the area from the Western Himalayas to the Caucasus, and restricted to Eurasia, North America and Africa (Polhill & Raven 1981). In the Bulgarian flora *Hedysarum* is represented with two species. They are distributed in the northeastern part of the country. *Hedysarum tauricum* and *H. grandiflorum* belong to sect. *Multicaulia* and sect. *Subcaulia* respectively. *Onobrychis* comprises twelve taxa, most of them found in all floristic regions (Kozhukharov 1976, 1992). A great part of the investigated *Onobrychis* species are included in sect. *Onobrychis.* The annuals *O. caput-galli* and *O. aequidentata* belong to sect. *Lophonobrychis*.

The pollen morphology of *Onobrychis* and *Hedysarum* is still incompletely investigated. Information is available in some regional pollen morphological studies and in several general surveys of the family (Erdtman 1966, Melhem 1971, Ohashi 1971, Kupriyanova & Aleshina 1972, Pire 1974, Tarnavschi *et al.* 1990). Pollen of both genera is ascribed to one pollen type (*Onobrychis*-type) by Faegri (1956), Faegri and Iversen (1989), and Moore *et al.* (1991). The basis for such a conclusion are the 3 apertures and the reticulate (suprareticulate) ornamentation of the exine. This early description of the pollen morphology was subsequently complemented by Ferguson and

Skvarla (1981), Guinet (1981), Polhill and Raven (1981), Guinet and Ferguson (1989), Moore *et al.* (1991), and Reille (1992).

MATERIAL AND METHODS

Pollen material was obtained from field populations, and from collections in the Herbaria of Sofia University (SO) and Institute of Botany (SOM) (Table 1).

The pollen was acetolysed in the standard way (Erdtman 1960). For light microscopy (LM), slides were prepared by mounting the pollen in glycerol jelly. Fifty measurements of each character were made at a magnification of × 1280. Six characters were measured: *P* (polar diameter), *E* (equatorial diameter), *L* (colpus length), *S* (colpus width), *M* (mesocolpium), *A* (apocolpium), and the *P*:*E* ratio.

For transmission electron microscopy (TEM) acetolysed exines were fixed with 2% osmium tetroxide, pre-stained with uranyl acetate and embedded in epon-araldite (Skvarla 1966). Sections were post-stained by Reinold's (1963) method. The preparation of the material and the microphotographs were done in the Electron Microscopy Laboratory at the Faculty of Biology, Sofia University.

For scanning electron microscopy (SEM),

Table 1. Specimens examined.

Taxon	Locality	Voucher number
Onobrychis alba (W. & K) Desv. ssp. *alba*	Bulgaria, Thracian plain, Besapara hills, near Ognyanovo village	SO 99 678
Onobrychis alba (W. & K) Desv. ssp. *calcarea* (Vand.) Ball.	Bulgaria, Struma Valley, railway station Kresna	SO 98 833
Onobrychis alba (W. & K) Desv. ssp. *laconica* (Orph. ex Boiss.) Hayek	Bulgaria, Slavyanka Mts., Parilski Dol	SO 97 454
Onobrychis aequidentata (Sibth. & Sm.) D'Urv.	Bulgaria, Struma Valley	SOM 90 924
Onobrychis arenaria (Kit.) DC. ssp. *arenaria*	Bulgaria, Thracian plain, Besapara hills, Elenin peak	SOM 19 139
Onobrychis lasiostachya Boiss.	Bulgaria, Thracian plain, Besapara hills, near Ognyanovo village	SO 99 679
Onobrychis caput-galli (L.) Lam.	Bulgaria, Struma Valley near to Kresna	SO 97 457
Onobrychis degenii Dorfl.	Bulgaria, Thracian plain, Besapara hills, near Ognyanovo village	SO 99 680 SOM 147 159
Onobrychis gracilis Bess.	Bulgaria, Strandzha Mts., Lopushna Reserve	SO 96 763
Onobrychis montana DC.	Bulgaria, Pirin Mts., touristic hut Yavorov	SOM 12 943
Onobrychis pindicola Hausskn. ssp. *urumovii* Deg. & Drenowski	Bulgaria, Pirin Mts., Vihren peak	SO 96 788
Onobrychis viciifolia Scop.	Bulgaria, Danube plain, near the town of Montana	SO 97 456
Hedysarum grandiflorum Pall. ssp. *bulgaricum* Koz.	Bulgaria, Danube plain, near the town of Svishchtov; Bulgaria, Hadzhi Dimitrovo village	SO 98 786 SOM 103 624
Hedysarum tauricum Willd.	North-eastern Bulgaria, near the Devnya river; North-eastern Bulgaria, near the town of Preslav	SOM 124 235 SO 44 090

pollen grains were coated as dry specimens with platinum with the aid of an Agar Sputter Coater. The microphotographs were obtained with the Zeiss-DSM-962 SEM of the Institute of Biotechnology, Electron Microscopy Unit, University of Helsinki, Finland.

The plant nomenclature follows Kozhukharov (1976, 1992). The pollen terminology in general follows Faegri and Iversen (1989) and Punt *et al.* (1994).

RESULTS

The measurements of six characters of the studied taxa are shown in Table 2. The pollen grains of *Hedysarum* and *Onobrychis* are 3-colpate, prolate and perprolate. The ectocolpi are elongated, shallow or deep, narrowing at the poles. The colpus membrane is covered by large granules. The sexine is thicker than the nexine. The infratectum is composed of two elements: unbranched columellae and granules. The tectum is incomplete, thinner than the columellae layer. The ornamentation is reticulate, the lumina different in shape and size in *Onobrychis,* and finely reticulate in *Hedysarum*. In equatorial view the pollen grains are elongated, elliptic to rectangular-obtuse, and in polar view they are circular, triangular-obtuse or triangular.

Based on pollen size, shape in polar and equatorial view, and ornamentation, one pollen type and three tentative subtypes could be recognized.

Subtype I
(Fig. 1A–D)

Polar diameter less than 25 µm, ornamentation finely reticulate.

Pollen grains prolate (*Hedysarum tauricum*, *H. grandiflorum*), P:E = 1.56–1.88, dimensions $P \times E$ = 18.4–24.6 × 10.4–15.6 µm. Pollen grains triangular-obtuse in polar view, elongated, rectangular-obtuse to elliptic in equatorial view. Ectocolpi straight, colpus membrane covered by different in size sculptural elements. $L \times S$ = 15.8–20.8 × 0.8–1.6 µm. Exine 0.75 µm thick in the mesocolpium. Foot layer thin, discontinuous, tectum incomplete.

The pollen grains of *Hedysarum tauricum* and *H. grandiflorum* belong in this subtype.

Subtype II
(Figs. 1E–H, and 2A–H)

Polar diameter above 25 µm, ornamentation reticulate. Pollen grains circular, with shallow colpi

Table 2. Taxa examined for pollen subtypes, with measurements (µm) of the mean and ranges for the polar (*P*) and equatorial (*E*) axes, length (*L*) and width (*S*) of the colpus, mesocolpium (*M*) and apocolpium (*A*) and the shape index (*P*:*E*)

Subtypes and taxa	*P*	*E*	*L*	*S*	*M*	*A*	*P*:*E*
Subtype I							
Hedysarum tauricum	18.4(20.9)22.8	10.4(11.1)15.6	15.8(17.9)19.8	1.3(1.0)1.6	5.4(6.9)7.8	3.2(2.8)3.4	1.77
Hedysarum grandiflorum	20.6(22.3)24.6	12.0(13.7)15.6	11.8(18.4)20.8	0.8(1.0)1.2	7.2(8.8)10.8	3.2(3.9)4.4	1.63
Subtype II							
Onobrychis degenii	36.8(45.9)52.8	17.6(21.2)25.6	32.8(42.7)49.6	1.6(1.6)2.0	12.0(14.6)19.2	4.0(4.8)6.4	2.17
Onobrychis viciifolia	30.4(38.4)41.6	16.0(17.8)20.0	27.2(35.0)38.4	0.8(1.2)1.6	8.8(11.3)17.6	4.0(4.6)5.2	2.15
Onobrychis gracilis	25.6(31.6)36.0	13.6(16.1)19.2	22.4(28.0)32.0	0.8(1.3)2.0	9.6(11.2)14.0	4.8(5.4)6.0	1.97
Onobrychis alba ssp. *laconica*	33.6(36.2)38.4	17.6(18.7)21.6	29.6(33.0)35.2	1.2(1.6)2.0	11.2(12.1)13.6	4.0(4.1)4.8	1.94
Subtype III							
Onobrychis montana	32.0(35.5)40.0	15.2(18.0)20.0	28.8(32.2)36.8	0.8(1.2)1.6	8.8(11.5)13.6	3.6(4.4)4.8	1.97
Onobrychis caput-galli	30.4(36.9)41.6	18.4(21.2)24.8	27.2(33.9)38.4	1.2(1.6)2.0	12.0(14.8)17.6	4.0(5.1)6.4	1.74
Onobrychis aequidentata	29.6(43.3)57.6	16.8(21.4)28.8	27.2(40.7)55.2	0.8(1.7)2.4	9.6(13.8)18.4	2.4(3.4)4.8	2.02
Onobrychis pindicola	31.2(34.8)38.4	16.0(17.3)20.0	28.0(31.6)35.2	0.8(1.0)1.6	9.6(11.0)12.8	4.0(4.6)5.6	2.02
Onobrychis arenaria	30.4(33.7)37.6	12.8(16.9)19.2	27.2(30.6)34.4	1.2(1.9)2.0	7.2(11.3)14.4	4.0(4.8)5.2	2.0
Onobrychis alba ssp. *alba*	29.4(38.4)41.6	14.1(18.3)20.8	26.9(35.1)38.4	0.2(1.3)2.0	9.0(12.1)14.4	3.8(4.8)5.6	2.1
Onobrychis alba ssp. *calcarea*	29.6(32.7)36.0	12.8(15.3)18.4	26.4(29.3)32.0	0.6(0.9)1.2	7.2(9.7)12.0	3.6(4.6)5.6	2.13
Onobrychis lasiostachya	28.8(32.8)36.8	12.8(15.3)17.6	26.4(30.4)34.4	0.8(1.0)1.2	7.2(9.9)11.2	2.0(2.7)4.0	2.14

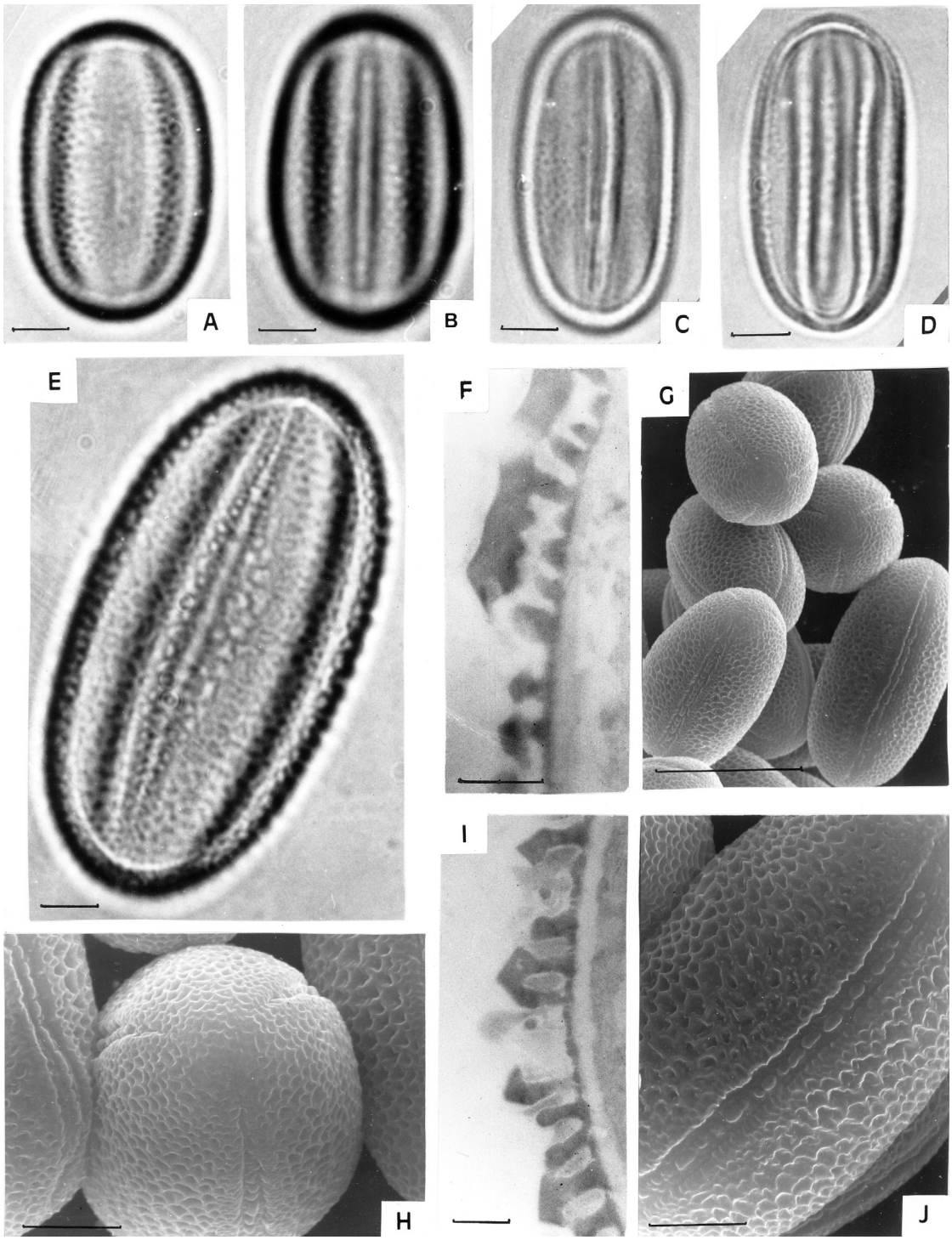

Fig. 1. — A–B: *Hedysarum grandiflorum.* LM equatorial view and colpus. Scale bar = 4 μm. — C–D: *H. tauricum.* LM equatorial view and colpus. Scale bar = 4 μm. — E–F: *Onobrychis alba* ssp. *laconica.* — E: LO colpus and ornamentation. Scale bar = 4 μm. — F: TEM section of exine. All scale bars of TEM = 1 μm. — G–J: *O. degenii.* — G: SEM polar and equatorial view. Scale bar = 20 μm. — H: SEM polar view and apocolpium. Scale bar = 5 μm. — I: TEM section of exine. — J: SEM colpus and ornamentation. Scale bar = 5 μm.

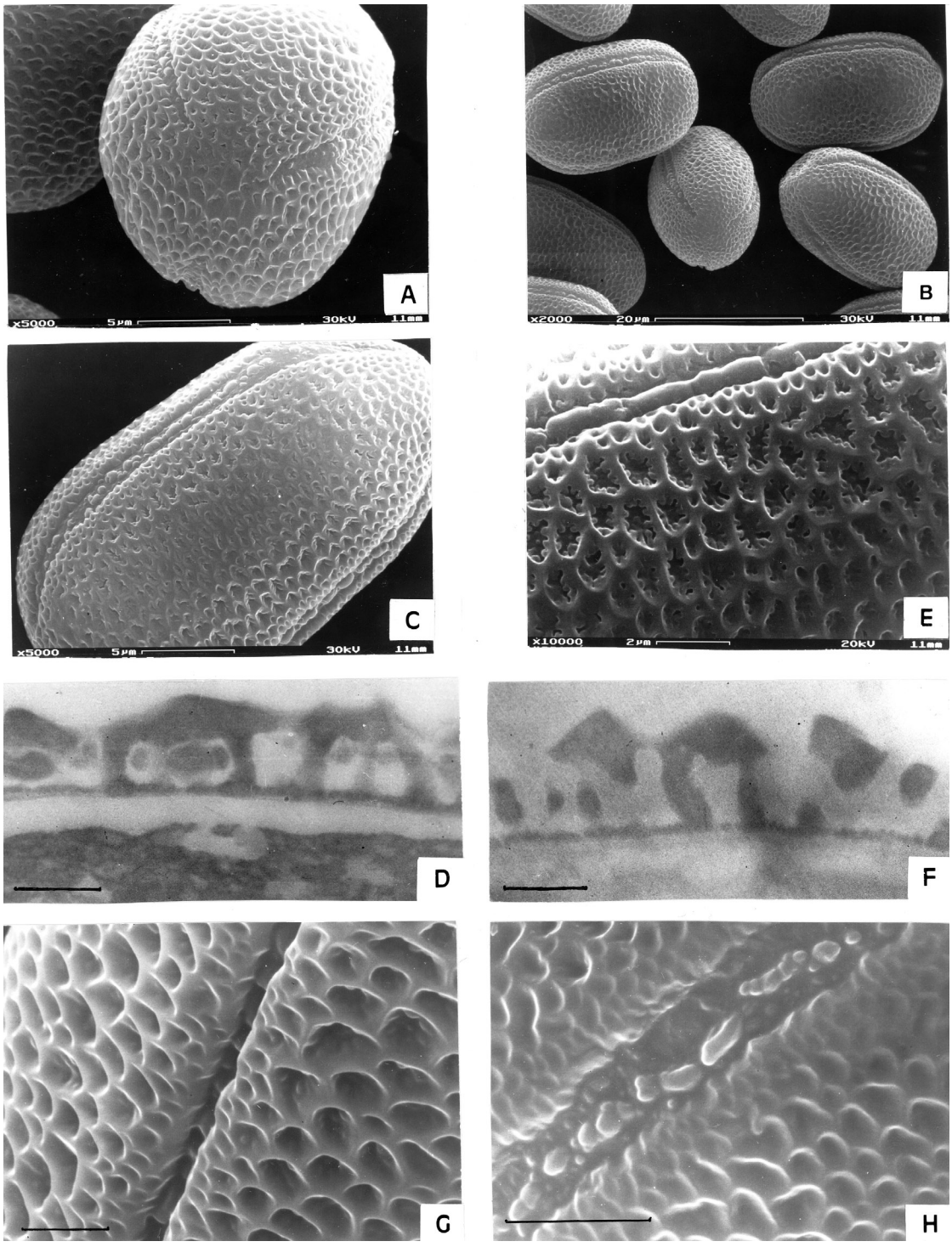

Fig. 2. — A–D: *Onobrychis gracilis*. — A: SEM polar view and ornamentation in apocolpium. Scale bar = 5 µm. — B: SEM polar view and equatorial view. Scale bar = 20 µm. — C: SEM colpus and ornamentation. Scale bar = 5µm. — D: TEM section of exine. — E–F: *O. viciifolia*. — E: SEM ornamentation. Scale bar = 2 µm. — F: TEM section of exine. — G–H: SEM colpus and ornamentation. Scale bar = 2 µm.

in polar view. Ornamentation reticulate, lumina of the reticulum sharply decreasing in size at the extreme margin of the colpi.

Pollen grains prolate (*Onobrychis gracilis*, *O. alba* ssp. *laconica*) and perprolate (*O. degenii*, *O. viciifolia*). *P*:*E* = 1.94–2.17, dimensions $P \times E$ = 25.6–52.8 × 13.6–25.6 µm. Pollen grains elliptical elongated in equatorial view. Ectocolpi long, shallow, colpus membrane covered by large and small sculptural elements. $L \times S$ = 22.4–49.6 × 0.8–2 µm. Exine 1–1.25 µm thick in the mesocolpium. Foot layer thin, continuous in *O. gracilis* and *O. degenii*, discontinuous and equal in thickness to the nexine in *O. viciifolia*. Columellar layer 0.6–1 µm thick, composed either of columellae and granules (*O. viciifolia*) or only of long, straight, and different in height columellae (*O. degenii*, *O. alba* ssp. *laconica*). Tectum 0.6 µm thick, about two times thinner than the columellar layer. Ornamentation reticulate the largest in size lumina in the intercolpia (1.5–1.75 µm). Lumina around the apertures less than 1 µm in diameter, resembling perforations.

The pollen grains of *Onobrychis gracilis*, *O. alba* ssp. *laconica*, *O. degenii* and *O. viciifolia* belong in this subtype, but they show some differences. The smallest are the pollen grains of *O. gracilis*, while the largest belong to *O. degenii*. The ornamentation of *O. alba* ssp. *laconica* differs from the other subspecies by the presence of perforations around the apertures. This subspecies shows close similarity with *O. viciifolia*.

Subtype III
(Figs. 3A–H, 4A–H and 5A–H)

Polar diameter above 25µm. Pollen grains triangular-obtuse or triangular in polar view, with deep colpi, ornamentation reticulate. Lumina of the reticulum of the same size at the extreme margin of the colpi.

Pollen grains prolate (*Onobrychis montana, O. caput-galli*) and perprolate (*O. alba* ssp. *alba*, *O. pindicola*, *O. arenaria*, *O. alba* ssp. *calcarea*, *O. aequidentata, O. lasiostachya*). *P*:*E* = 1.74–2.14. Large or medium in size pollen grains $P \times E$ = 28.8–57.6 × 12.8–28.8 µm. Pollen grains triangular-obtuse or triangular (*O. lasiostachya*) in polar view, and elliptical elongated to rectangular-obtuse in equatorial view. Ectocolpi long, deep, the colpus membrane covered by large and small sculptural elements. $L \times S$ = 26.4–55.2 × 0.64–2.4 µm. Exine 0.75–1.5 µm thick in the mesocolpium. Exine thinnest in *O. lasiostachya* (0.75 µm) and thickest in *O. caput-galli* (1.5 µm). Foot layer thin, continuous in *O. caput-galli*, *O. montana, O. alba* ssp. *alba*, and *O. lasiostachya*. Tectum 2–3 times thinner than the nexine in *O. arenaria* and equal in thickness to the nexine in *O. pindicola*. Columellar layer 1.0–1.25 µm thick. Columellae straight, of different heights. Columella and groups of columellae visible in the lumina of the reticulum. Tectum uneven, 0.7 µm thick, approximately one half as thick the columellar layer. Ornamentation reticulate, lumina equal in size and shape in the intercolpium and at the extreme margin of the colpi. Diameter of the largest lumina about 1.5–3 µm.

The pollen grains of *Onobrychis montana*, *O. caput-galli*, *O. alba* ssp. *alba*, *O. pindicola*, *O. arenaria*, *O. alba* ssp. *calcarea*, *O. aequidentata* and *O. lasiostachya* belong in this subtype. Here are included the evolutionarily advanced species of section *Lophonobrychis* (*O. aequidentata*, *O. caput-galli*) and more primitive representatives of section *Onobrychis* (*O. pindicola* and *O. montana*).

Onobrychis lasiostachya is closely related to *O. arenaria* in macromorphology and some authors consider *O. lasiostachya* as subspecies (Tutin *et al*. 1968, Greuter *et al*. 1989). In pollen morphology, namely outline in polar view, ornamentation and exine structure, *O. lasiostachya* differs considerably from the other species. The pollen grains have the longest, narrow and almost closed colpi. The lowest metric values for the apocolpium and mesocolpium are encountered in *O. lasiostachya*.

DISCUSSION

The results of our investigation show that pollen morphology of the bulgarian representatives of *Onobrychis* and *Hedysarum* is comparatively homogenous and confirm the general description presented by Ohashi (1971), Ferguson and Skvarla (1981), Faegri and Iversen (1989), and Moore *et al.* (1992).

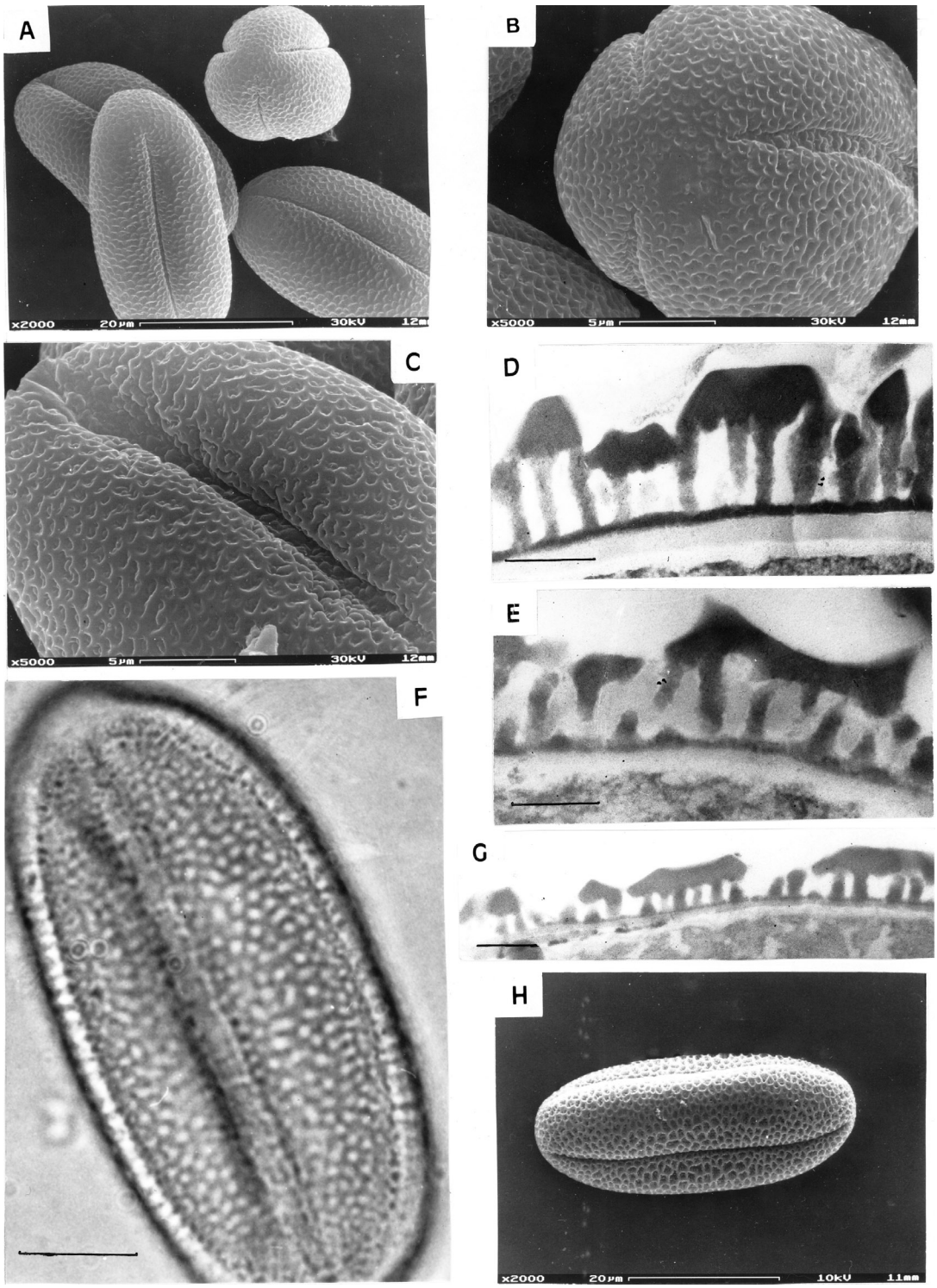

Fig. 3. — A–E: *Onobrychis caput-galli*. — A: SEM polar view and outline. Scale bar = 20 µm. — B: SEM apocolpium and ornamentation. Scale bar = 5 µm. — C: SEM colpus and ornamentation. Scale bar = 5 µm. — D, E: TEM sections of exine. — F: *O. aequidentata.* LM colpus and ornamentation. Scale bar = 8 µm. — G–H: *O. alba* ssp. *alba*. — G: TEM section of exine. — H: SEM equatorial view and ornamentation. Scale bar = 20 µm.

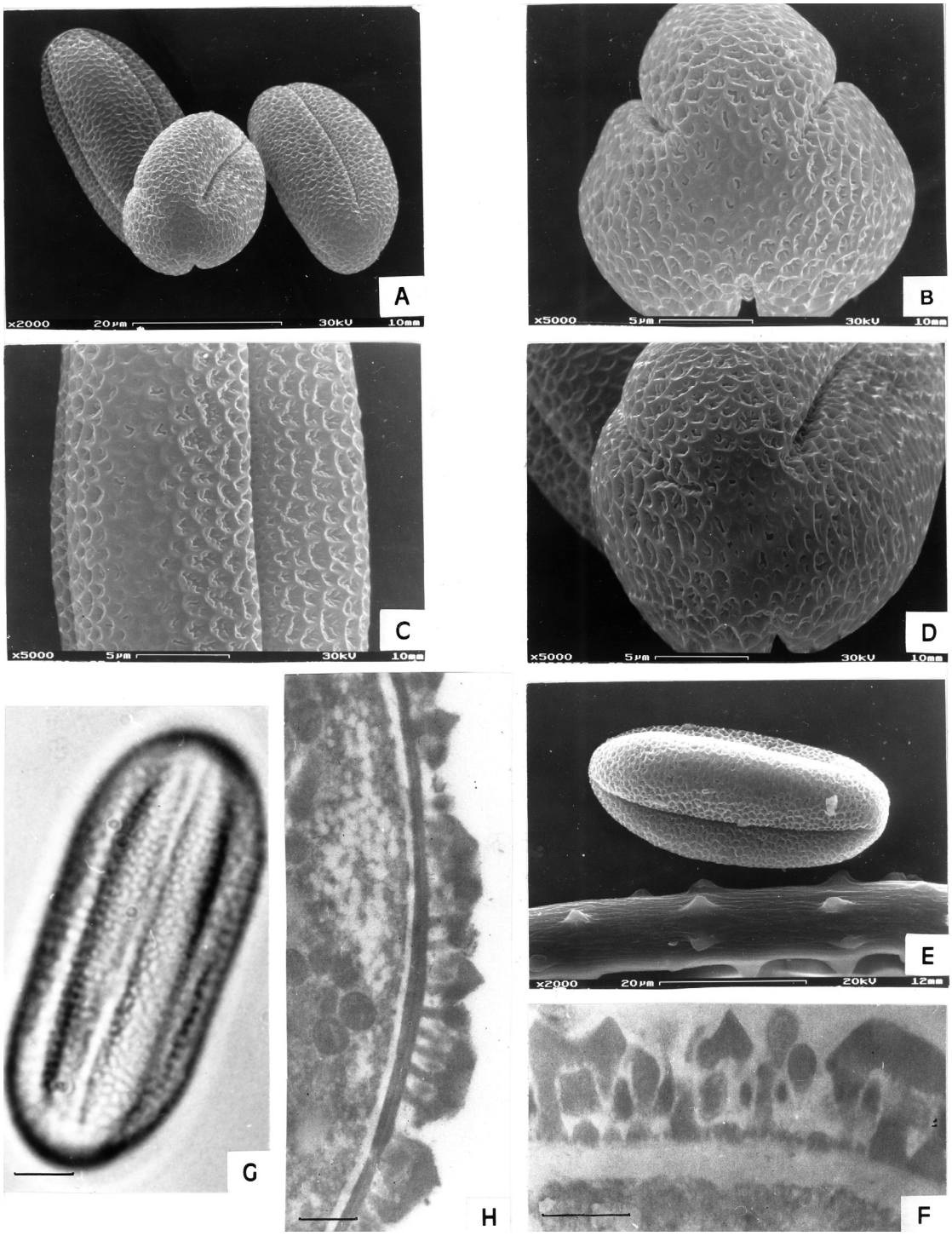

Fig. 4. — A–D: *Onobrychis montana*. — A: SEM polar view. Scale bar = 20 µm. — B–D: SEM apocolpium, colpus and ornamentation. Scale bars = 5 µm. — E–F: *O. pindicola*. — E: SEM equatorial view. Scale bar = 20 µm. — F: TEM section of exine. — G: *O. alba* ssp. *calcarea*. LM colpus and ornamentation. Scale bar = 4 µm. — H: *O. montana*. TEM section of exine.

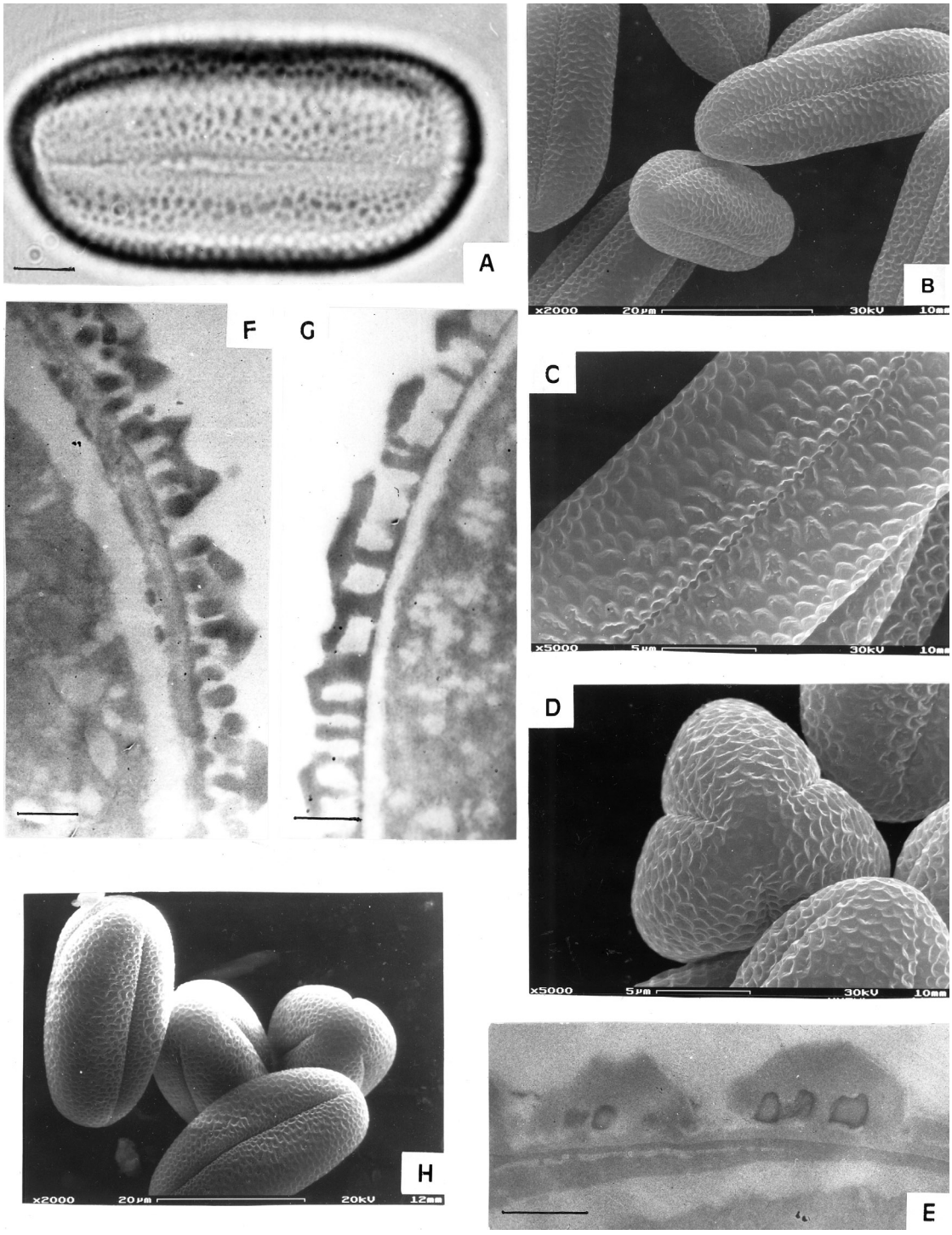

Fig. 5. — A–E: *Onobrychis lasiostachya*. — A: LM colpus and ornamentation. Scale bar = 4µm. — B: SEM apocolpium. Scale bar = 20 µm. — C–D: SEM colpus and ornamentation. Scale bars = 5 µm. — E: TEM section of exine near colpus. — F–G: *O. arenaria*. TEM sections of exine. — H: *O. montana*. SEM polar view, colpus and ornamentation. Scale bar = 20 µm.

Nevertheless, based on pollen size, shape in polar and equatorial view, and ornamentation, three pollen subtypes can be distinguished. The observed differences in the pollen morphology of these groups do not suggest changes in the adopted taxonomical scheme of the genera.

In some groups of the tribes Phaseoleae, Psoraleae, and Loteae, the changes in the exine stratification from a simpler, granular-columellar to a more complicated columellar is linked with changes in the aperture number or type (Stainer & Horvat, 1978, Ferguson 1984, 1990, Guinet & Ferguson, 1989, Diez & Ferguson, 1994). In the present study, such a tendency is not observed probably due to the limited number of the investigated species and similarity of the exine stratification. The pollen grains do not demonstrate differences in the aperture system like Pire (1974) found in *Hedysarum coronarium* and Reille (1992) found in *Onobrychis supina* and *Hedysarum spinosissum*.

The pollen of the species from the group *Onobrychis arenaria* (Kozhukharov 1976, 1992) shows distinct interspecific differences. The macromorphologically similar species *O. arenaria* and *O. viciifolia* can be distinguished by their pollen, mainly by the outline in polar view and the ornamentation around the colpi.

Onobrychis alba is widely distributed in Bulgaria and is highly variable. It also shows variation in pollen morphology. The investigated population of *O. alba* ssp. *laconica* shows differences in ornamentation, dimensions and shape of the grains in equatorial and polar view. This subspecies is thus regarded as representing a distinct subtype of its own.

The similarity of pollen morphology of *Onobrychis pindicola* and *O. montana* reinforces their close relationship based on other features. Differences are observed in exine stratification. The nexine in *O. pindicola* is nearly twice thinner than that of *O. montana*.

Pollen size shows no correlation with the level of ploidy (Kuzmanov 1993) in *Onobrychis aequidentata*, *O. gracilis*, *O. viciifolia*, *O. lasiostachya. Onobrychis gracilis* (2n = 28) has small pollen similar to that of *O. lasiostachya* (2n = 14) while *O. aequidentata* (2n = 14) and *O. alba* ssp. *alba* (2n = 14) have larger pollen grains than the tetraploids *O. pindicola* (2n = 28) and *O. montana* (2n = 28). Slight differences in pollen dimensions were observed among the diploids *O. arenaria*, *O. alba* ssp. *alba*, *O. lasiostachya* and the tetraploids *O. pindicola* and *O. montana.*

In this study, we found no differences in pollen morphology between the annual and perennial taxa of *Onobrychis*.

The thickening of the endexine and the reduction or loss of the foot layer, a tendency established for Fabaceae by Guinet and Ferguson (1989), was found only in *Onobrychis arenaria* and *O. pindicola*.

In the case of the exine stratification, a tendency is observed towards a decrease in the thickness of the endexine and presence of a foot layer, usually linked with the presence of long columellae. The thickest exine is observed in *Onobrychis caput-galli*, *O. montana*, *O. pindicola* and *O. lasiostachya.* These species have high and straight columellae. The thinnest exine is observed in *O. degenii*. The foot layer is thickest (1.5–2 μm) in *O. montana*, *O. caput-galli* and *O. lasiostachya*.

The evolution of the pollen morphological characters has proceeded from a fine reticulum (*Hedysarum tauricum*, *H. grandiflorum*), to a reticulum with lumina sharply decreasing in size at the extreme margin of the colpi (*Onobrychis viciifolia*, *O. gracilis*, *O. degenii*), and reticulum with lumina of the same size at the margin of the colpi (*O. aequidentata*, *O. alba*).

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