Turczaninowia 21 (4): 188–206 (2018)
DOI: 10.14258/turczaninowia.21.4.20
http://turczaninowia.asu.ru

ISSN 1560–7259 (print edition)
TURCZANINOWIA
ISSN 1560–7267 (online edition)

УДК 582.766.5:581.332

Pollen morphology of the genus *Euonymus* (Celastraceae)

O. A. Gavrilova[1], D. A. Britski[1], V. V. Grigorieva[1], V. F. Tarasevich[1], A. E. Pozhidaev[1], V. M. Leunova[2]

[1] Komarov Botanical Institute RAS, Prof. Popov St., 2, St. Petersburg, 197376, Russia. E-mail: olgaangav@gmail.com

[2] Moscow State University, Leninskye gory, 1–12, Moscow, 119234, Russia. E-mail: cedrus@rambler.ru

Keywords: aperture, exine structure, microscopy, sporoderm, systematics.

Summary. Pollen of 62 species from the genus *Euonymus* (Celastraceae) were studied using light (LM), confocal laser scanning (CLSM) and scanning electron (SEM) microscopes. This is approximately half of the species composition of the taxon. The genus, as a whole family, is quite homogeneous by palynomorphological characteristics and represented by 3-colporate pollen type. On the other hand, some species and (or) group of species could be segregated considering the details of structure (aperture structures, exine ornamentation). We have found 3-colporate pollen in all investigated taxa and 19 species also have 2- and/or 4-6-deviated grains with different colpi arrangement. Their structure has been studied in more detail with a CLSM 3-dimentional reconstruction. Grains are isopolar, ellipsoidal or spheroidal medium sized, from 17 to 41 µm in diameter. Colpi are long, spired at the ends. Endoaperture (ora) range from poorly-defined, rounded or slightly elongared along the colpus up to well-defined, with sharp edges, elliptical and elongared along the equator. LM-observing shows H-shaped aperture; CLSM data demonstrate that they are constructed from ora bridge and exine thinning on both sides of parallel colpus. The exine is tectate, columellate, with well-defined columella layer. Exine thickness is from 1.5 to 5.1 µm. The ornamentation varies from reticulate to microperforate. The species groups divided according to their palynomorphological characteristics generally do not coincide with the genus subdivision onto subgenera and sections. The taxonomic significance of various palynological characteristics is discussed.

Морфология пыльцы представителей рода *Euonymus* (Celastraceae)

О. А. Гаврилова[1], Д. А. Брицкий[1], В. В. Григорьева[1], В. Ф. Тарасевич[1], А. Е. Пожидаев[1], В. М. Леунова[2]

[1] Ботанический институт им. В. Л. Комарова РАН, ул. Проф. Попова, д. 2, г. Санкт-Петербург, 197376, Россия

[2] Московский государственный университет, Ленинские горы,1-12, г. Москва, 119234, Россия

Ключевые слова: апертуры, микроскопия, систематика, спородерма, структура экзины.

Аннотация. С помощью светового (СМ), конфокального лазерного сканирующего (КСЛМ) и сканирующего электронного (СЭМ) микроскопов изучена пыльца 62 видов рода *Euonymus* (Celastraceae). Род, как и все семейство, довольно однороден по палиноморфологическим признакам и представлен трехбороздно-оровым типом пыльцевых зерен. Однако некоторые виды и (или) группы видов могут быть выделены с учетом деталей строения (апертур или поверхности экзины). У всех исследованных таксонов обнаружены 3-бороздно-оровые пыльцевые зерна, у 19 представителей наряду с 3-бороздно-оровыми встречаются также 2- и/или 4-6-бороздно-оровые зерна с разным расположением борозд и ор. Структура апертур изучена более подробно с использованием трехмерных реконструкций конфокальной микроскопии. Зерна радиально-симметричные, эллипсоидальные или сфероидальные, среднего размера, от 17 до 41 мкм в диаметре. Борозды длинные, заостренные на концах. Эндоапертуры (оры) встречаются от слабо очерченных, неясных, округлых или чуть вытянутых по борозде до хорошо различимых, с четкими краями, эллипсоидальных, вытянутых по экватору. Наблюдение

Поступило в редакцию 13.02.2018
Принято к публикации 02.09.2018

Submitted 13.02.2018
Accepted 02.09.2018

с помощью СМ показывает Н-образные апертуры, данные КЛСМ демонстрируют, что они состоят из оровой перемычки и утоньшений экзины с двух сторон параллельно бороздам. Экзина столбиковая, тектатная, в большинстве случаев хорошо выражен столбиковый слой. Толщина экзины от 15 до 5,1 мкм. Поверхность варьирует от крупносетчатой до микроперфорированной. Выделенные по палиноморфологическим характеристикам группы видов в основном не совпадают с подразделениями рода на подроды и секции. Обсуждается таксономическое значение отдельных палинологических признаков.

The genus *Euonymus* L. belongs to the family Celastraceae R. Br. of the order Celastrales and contains from 129 (Ma, 2001) to 200 species (Leonova, 1974; Tzvelev, 2004; Savinov, Baikov, 2007), which are deciduous and evergreen shrubs or small trees. Plants of *Euonymus* grow in the undergrowth or along broad-leaved, mixed or coniferous forests in tropical, subtropical and temperate areas of both hemispheres. Because of the beautifully colored seeds, spindle trees are often cultivated as ornamental plants in gardens and parks all around the world.

The most of systematics (Leonova, 1974; Ma, 2001; Tzvelev, 2004; Savinov, Baikov, 2007) accept a genus after R. A. Blakelock (1951): the subgenus *Kalonymus* R. Beck which was allocated by Ya. I. Prokhanov (1949, 1960) to separate genus, and the genera allocated by T. Nakai (1941) as sections are included in *Euonymus*. Now, there are two main worldwide taxonomic systems of the genus: 1) the R. A. Blakelock system (1951) (including with T. Leonova (1974) changes which have made many taxonomical decisions of T. Nakai (1941) and Th. Loesener (1942)), and 2) the J.-S. Ma (2001) system. According to R. A. Blakelock (1951) the genus includes 6 sections in subgenus *Euonymus* and one section in subgenus *Kalonymus*. T. Leonova (1974), and after her, I. Savinov and K. Baikov (2007), I. Savinov (2007) have divided the genus into 12 sections, 10 of them are from subgenus *Euonymus* and 2 of them are from *Kalonymus*. After J.-S. Ma (2001) the genus includes 5 sections, 4 of them are combined into subgenus *Euonymus*, and all sections are allocated on the basis of fruits structure. J.-S. Ma (2001) has united three sections of R. A. Blakelock (1951): *Biloculares* Rouy et Fouc., *Multiovulares* Loes., *Stenocarpus* Blakel. in one section *Euonymus*, while the section *Melanocarya* (Turcz.) Nakai, *Echinococcus* Nakai and *Ilicifolia* Nakai have been left almost invariable, although the number of species has been reduced. The subgenus *Kalonymus* is acknowledged as the youngest group of spindle trees (Shimanovich, 1987; Savinov, Baikov, 2007). N. Tzvelev (2004) doesn't divide a genus into subgenera and has united spindle trees of Eastern Europe into 8 sections, having allocated one new section *Nanevonymus* with one species *E. nanus*. B. Schulz (2006) has described fruits and seeds of 30 widely cultivated species of spindle trees from Central Europe and species' criteria have been discussed. Based on the morphological and molecular data, M. P. Simmons et al. (2012) considered that all infrageneric classifications weren't natural and demanded rather significant revision. Y.-N. Li et al. (2014) concluded that only two sections (*Echinococcus* and *Kalonymus*) of J.-S. Ma (2001) system correspond to molecular groups.

The pollen morphology of the genus has been insufficiently studied, and also pollen data hasn't been used for taxonomy and systematics. There are palynomorphological characteristics of 26 *Euonymus* species, which were obtained mainly with light microscope (LM), rarely scanning electron (SEM) microscope (Erdtman, 1952; Ikuse, 1956; Wang et al., 1960; Nair, 1965; Lobreau, 1969; Heusser, 1971; Huang, 1972; Kupriyanova, 1972; Shimakura, 1973; Poliychuk, 1978; Nakamura, 1980; Artyushenko, Romanova, 1984; Chester, Raine, 2001; Premathilake, Nilsson, 2001; Perveen, Qaiser, 2008). D. Lobreau-Callen and B. Lugardon (1972–1973) have been studied sporoderm ultrastructure of 2 species (*E. europaeus* L., *E. japonicus* Thunb.) using SEM and transmission electron (TEM) microscope.

In our paper, the pollen of 62 species (specimens) from the genus *Euonymus* (Celastraceae) was studied using LM and SEM and partly with confocal laser scanning (CSLM) microscopes. This is about a half of the species' composition of the taxon.

The aim of our research work was the study of pollen morphology of the genus *Euonymu*s by SEM and CLSM and supplement LM data. We tried to specify features of a structure and variety of pollen characteristics, that expand our knowledge of this taxon, and to estimate the value of the pollen morphological characteristics for taxonomy.

Material and methods

Pollen samples were collected in herbarium of the Komarov Botanical Institute, Russian Academy of Sciences (St. Petersburg, Russia) (BIN RAS). The following vouchers were investigated: ***E. acan-***

thocarpus Franch. (*E. acanthocarpus* Franch. var. *sutchuenensis* Franch. ex Diels): "China 1907–1909, s. n."; ***E. actinocarpus*** Loes. (*E. angustatus* Sprague): "Hong Kong, Shek–O, Y. W. Tsam 1178. 24 V 1940"; ***E. alatus*** (Thunb.) Siebold: "Sakhalin, South-Sakhalin reservation, S. Kravchenko s. n. 22 VI 1950"; ***E. americanus*** L.: "Baltimore, North Carolina, № 422в. 26 V 1894"; ***E. artropurpureus*** Jacq.: "Texas, Houston, A. Travers 323. 24 V 1957"; ***E. benthamii*** Lundell (*E. acuminatus* Benth.): "Mexico, Benth. № 452, 1870"; ***E. bungeanus*** Maxim.: "Manchuria, Dalniy, J. Vasilieva, s. n. 1903"; ***E. cornutus*** Hemsl.: "China, Western Szechuan, E. H. Wilson 1047. 07 VIII 1907–1909"; ***E. czernjaevii*** Klokov: "USSR, Donetsk, E. Mordak, L. Sveschnicova s. n. 02 V 1977"; ***E. dasydictyon*** Loes. et Rehder: "Tibet, highlands between the stations of O-Lun-shi and Makhchuk. 18 V 1893"; ***E. dichotomus*** Heyne ex Wall.: "India orientalis. Herb. Wight. 484"; ***E. echinatus*** Wall.: "Nepal, Bagmati zone, Kathmandu District Sheopuri Ridge, D. N. Nicolson 3345. 13 V 1967"; ***E. elegantissimus*** Loes. et Rehder: "China, Peking, W. Y. Hsia s. n. 15 IV 1926"; ***E. europaeus*** L. "Belorussia, Gomel, N. Kozlovskaya s. n. 23 V 1962"; ***E. fimbriatus*** Wall.: "N. W. Himalaya, Deota Temple, D. Dultne 22545, 09 V 1989"; ***E. frigidus*** Wall.: "Sikkim, Sunducrophro, C. B. Clarke 35672a. 04 VI 1884"; ***E. gibber*** Hance (*E. miyakey* Hayata): "China, Hainan, S. K. Lau s. n. 18 V 1933; ***E. glaber*** Roxb.: "Calcutta s. n."; ***E. grandiflorus*** Wall.: "Chao Cim ssu im Kreise Teng fong, Honan, A. K. Schingler s. n., VIII 1907"; ***E. hamiltanianus*** Wall.: "Cult. in garden, Kew, R. A. Blakelork. 06 X 1977"; ***E. hederacea*** Champ. ex Benth. (*E. radicans* Miq.): "Suchumi, T. Leonova s. n. 05 VI 1955"; ***E. integerrimus*** Prokh.: "China, prov. Gansu, dist. Piplan, M. P. Petrov s. n. 10 VI 1957; ***E. japonicus*** Thunb.: "Crimea, Alupka, K. K. Kossinskiy s. n. 1911"; ***E. juzepczukii*** Leon.: "Forest flora of Siwalik and Jaunsar Deobau divisions. Mohammed Yusuf R. 32. 25 V 1923"; ***E. lanceifolius*** Loes.: "China, Western Szechuan, E. H. Wilson. 06–11 VIII 1907–1909"; ***E. latifolius*** (L.) Mill.: "Crimea, Mangup, N. N. Tzvelev, D. B. Geltman, N. A. Medvedeva, G. A. Mustafina 692. 18 V 1984"; ***E. laxiflorus*** Champ. ex Benth. (*E. paniculatus* Wight ex Lawson): "Imperial Forest college, Dehra Doon, Indu Bhushan Maitra B. Sc. Roll., № 1. IV 1924"; ***E. maackii*** Rupr.: "South-Usury distr., N. V. Schipchinskaya 227. 15 VI 1913"; ***E. macropterus*** Rupr.: "Kaliningrad, E. Pobedimova 52, 09 V 1950"; ***E. maximomowiczianus*** Prokh.: "Kedrovaya pad reservation, V. N. Vasiliev, E. V. Volkova, L. I. Ivanina s. n. 5 VI 1950"; ***E. melananthus*** Franch. et Sav.: "Japan, Oita, Naoiri-gun, Kujumachi, Mt. Kuro-dake, Shinya Masyda 101072, 27 V 1989"; ***E. miniatus*** Tolm.: "Sachalin, Chechov, F. M. Chernyaeva s. n. 13 VI 1963"; ***E. moldavicus*** Klokov: "Russia, Voronezh distr. 30 IV 1930"; ***E. myrianthus*** Hemsl.: "Cult. Royal Bot. Garden Kew, R. A. Blakelork. 11 V 1944"; ***E. nanus*** M. Bieb.: "Ukraine, Vinnica distr., s. n. 03 VI 1928"; ***E. nitidus*** Benth. (*E. chinensis* Lindl.): "Hohg Kong, Shin Ying Hu 5222. 04 V 1968"; ***E. obovatus*** Nutt.: "Michigan (U.S.A.), Frere Rolland – German № 6443. 17 V 1956"; ***E. occidentalis*** Nutt. ex Torr. "Washington, Richard R. Halse 4040. 21 VI 1990"; ***E. oxyphyllus*** Miq.: "Sapporo, S. Galeenobii 2567. VI 1882"; ***E. patens*** Rehder: "Log. Kiang-si, Lu-shan. VIII 1934"; ***E. pauciflorus*** Maxim.: "Russia, Vladivostok, E. Pobedimova, G. Konovalov 70. 05 VI 1959"; ***E. phellomanus*** Loes.: "China, Sothwestern Kansu, J. F. Rock 12822. VII 1925"; ***E. pendulus*** Wall.: "Western Himalaya, prov. Simla, № 486, 20 V 1856"; ***E. planipes*** Koehne: "Sakhalin, South-Sakhalin reservation, S. Kravchenko s. n. 22 VI 1950"; ***E. porphyreus*** Loes.: "China, Western Szechuan, E. H. Wilson 968. 06 VIII 1908"; ***E. przewalskii*** Maxim.: "South-western Kansu, J. F. Rock s. n. VII 1925"; ***E. sachalinensis*** (F. Schmidt) Maxim.: "South Sachalin, M. G. Popov, K. D. Stepanova s. n. 16 VI 1950"; ***E. sacrosanctus*** Koidz: "Primorsky region, Nikolsk, M. Fritz s. n. 1912"; ***E. salicifolius*** Loes.: "China, Yunnan, № 11718a; ***E. sanguineus*** Loes.: "Western Hupeh, E. H. Wilson 3111. 05 VII 1907–1909"; ***E. semenovii*** Regel et Herder: "Almaty, Flora Illiensis, 1886"; ***E. sieboldianus*** Blume: "Sachalin, s. n."; ***E. subtriflorus*** Blume: "Japonia insula Jesso, circa Hakodate, Dr. Albrecht s. n. 1861–1863"; ***E. szechuanensis*** Chen H. Wang: "China, Szechuan, Dr. Aug. Henry 5562, III 1889; ***E. theifolius*** Wall.: "China, Yunnan, A. Henry 10544"; ***E. tingens*** Wall.: "Imperial Forest College, place of collection Deoban, Indu Bhusman Maitra B. Sc., № 19. V 1924"; ***E. velutinus*** Fisch. et C. A. Mey.: "Herb. Bunze, Riga, Albursgebing Talysh geb. 28 VIII 1847"; ***E. verrucosus*** Scop.: "Baschkortostan, Ufa, B. A. Fedchenko, 19. 15 VI 1926"; ***E. vidalii*** Franch. et Sav.: "Japan, Yokohama, Maximowicz 1221. 06–18 V 1862"; ***E. yakushimensis*** Makino: "Japan, Kagoshima, T. Yahara, S. Akiyama s. n. 14 VII 1987"; ***E. yedoensis*** Koehne var. ***koehneana*** Loes.: "China, western Hupeh, E. H. Wilson 353, 06–10 VII 1907–1909"; ***E. yesoensis*** Koidz.: "Kuril isl., Schikotan, E. Pobedimova, G. Konovalov 701. 11 VII 1959".

Pollen was acetolysed according to G. Erdtman (1952). Unstained grains were mounted in glycerin jelly and were sealed with paraffin. LM studies were conducted in the laboratory of palynology in BIN RAS. Pollen grains have been studied with SEM JEOL JSM-6390 (SEM) and with an LSM 780 (CLSM) at the Core Centrum 'Cell and Molecular Technologies in Plant Science' in BIN RAS. CLSM investigation was after O. Gavrilova (2014). The palynological terminology follows W. Punt et al. (2007) and M. Hesse et al. (2009).

Results

Pollen grains of all investigated species are 3-colporate, medium sized, from 17 to 41 µm in diameter, with tectate or semitectate, columellate exine, perforate or reticulate types of ornamentation. Morphological and morphometrical pollen characteristics are presented in Tables 1 and 2. The species are presented according to subgenera and sections of J.-S. Ma (2001) system, the species, recognized in that system, are in bold, the other names are listed as synonyms.

General pollen morphology: the form and sizes. Pollen of all species is **radially symmetric,** from prolate or prolate spheroidal to oblate or oblate spheroidal (Fig. 1, 4). Grains in polar view are from 3-4-lobate (Fig. 1: 1, 6; Fig. 4: 8, 11, 12, 15) to circular (Fig. 1: 2, 5). Only pollen of 4 species *E. gibber, E. japonicus, E. laxiflorus, E. nitidus* are 3-angular (Fig. 1: 3, 4; Fig. 4: 1) in polar view. Pollen equatorial view are round or elliptic. Polar axis and equatorial diameter varies from 17 to 41 µm.

Table 1

Morphological and morphometrical characteristics of pollen grains of species from the subgenus *Euonymus* of genus *Euonymus* (Celastraceae)

Species	Pollen size (polar axis x equatorial axis, µm) and pollen grains form	The number of colpi, their features, % of deviated grains	Endoapertures – Ora, their features and diameter, µm	Ornamentation, their features, lumina, µm, (width of muri, µm)	exine thickness, µm
2. Echinococcus					
E. acanthocarpus	33–36 × 28–30 oblate spheroidal	3–(4)–(6)–(1), syncolpi, 5 %	round, H–shaped, 5–6	reticulate, heterobronchate, 0.2 and 0.5–1.4 (0.4)	3.4–3.6
E. actinocarpus	27–29 × 25–27 oblate spheroidal	3	round or quadrangular, H–shaped, 5	reticulate, heterobronchate, 0.3 and 0.4–0.6 to 1.2 (0.4)	3.0–3.5
E. americanus	30–31 × 27–31 prolate spheroidal to oblate spheroidal	3	not clear, H–shaped, 3	reticulate–perforate, 0.2–1.2 (0.7–1.1)	2,4– 3,2
E. echinatus	27–32 × 25–35(40) oblate spheroidal	3–(4)–(6), 11 %	not clear, 3	reticulate, 0.8 up to 1.7 (0.2–0.3)	2.3–3.6
E. obovatus	25–30 × 25–29 oblate spheroidal	3	round, 4	reticulate, heterobronchate, 0.1–0.5 and 0.7–1.5 (0.2)	3.5–3.8
3. Melanocarya					
E. alatus	31–35 × 26–30 prolate spheroidal to oblate spheroidal	3	not clear, 3–4	reticulate, 1–3 (0.5–0.7)	3.5–4.4
E. pendulus	28–30 × 26–30 oblate spheroidal	3–(2)–(1), 7 %	round, H–shaped, 5–6	reticulate, heterobronchate, 0.2–0.5 and 0.6–1.2 (0.3)	2.7–3.0
E. sacrosanctus	31–35 × 27–30 prolate spheroidal	3–(4)–(6), 20 %	round, not clear, 3–4	reticulate, 0.8 up to 2.0 (0.4)	3.0–4.5
E. subtriflorus	22–30 × 20–25 prolate spheroidal to oblate spheroidal	3	round or oval, not clear, 5–6	reticulate, 1.0 up to 2.5 (0.3)	2.5–3.1
4. Ilicifolia					
E. benthamii	28–33 × (25)28–30 oblate spheroidal	3	round, not clear, 5–6	reticulate, 0.4–1.2 (0.5)	2.7– 3.7

Continuation of table 1

Species	Pollen size (polar axis x equatorial axis, µm) and pollen grains form	The number of colpi, their features, % of deviated grains	Endoapertures – Ora, their features and diameter, µm	Ornamentation, their features, lumina, µm, (width of muri, µm)	exine thickness, µm
E. hederacea	25–28 × 25–27 oblate spheroidal	3	equatorial elon–gated, H–shaped, not clear, small–sized, 1–2	reticulate, heterobronchate, 0.5–0.9 up to 1.3 and 0.1–0.2 (0.4–0.5)	2.3–2.6
E. japonicus	22–25 × 20–24 prolate spheroidal to oblate spheroidal	3–(2), 1 %	round, small–sized, H–shaped, 2	reticulate–perforate, 0,1 up to 1.0 (0,3 up to 1)	2.0–3.5
E. patens	29–30 × 29–32 oblate spheroidal	3	H–shaped, not clear, small–sized, 1	reticulate, heterobronchate, 0.1–0.2 and 0.6–1.0 (0.3)	3.0–3.4
E. theifolius	26–28 × 25–28 prolate spheroidal	3–(4), W–shaped colpi, 10 %	H–shaped, not clear, round, 3	reticulate, heterobronchate, 0.1 and 0.5–1.3 (0,3)	2.0– 2.8
E. yakushimensis	30–35 × 27–33,5 prolate spheroidal to oblate spheroidal	3	H–shaped, round, 5–6	reticulate–perforate, 0.25 up to 1.2 (0.3 up to 1.0)	3.2
			5. Euonymus		
E. artropurpureus	24–25 × 22–25 oblate spheroidal	3	round, H–shaped, 2–5	reticulate, 0.7 up to 3.0 (0.4–0.5)	2.2–2.7
E. bungeanus	29–31 × 27–28 oblate spheroidal	3	round, 4–5, H–shaped	reticulate, heterobronchate, 0.7–2.1 and 0.2–0.3 (0.4)	3.0–3.5
E. czernjaevii	24–27 × 23–26 prolate spheroidal to oblate spheroidal	3	not clear, 3	reticulate, heterobronchate, 0.1–0.2 up to 0.3–1.0 (0.3)	2.2– 2.5
E. dichotomus	24–27 × 23–25 prolate spheroidal to oblate spheroidal	3	round, small–sized, 1.5	(micro)reticulate, 0.1–0.3 (0.5)	2.3– 3.0
E. europaeus	28–30 × 26–30 prolate spheroidal to oblate spheroidal	3	round, not clear, 3	reticulate, hetero–bronchate, 0.8 up to 1.8 and 0.2–0.5 (0.5)	2.3–2.9
E. gibber	24–29 × 25–30 oblate spheroidal	3	equatorial elongated, 5–7	reticulate, hetero–bronchate, 0.2 up to 1.3 (0.4–0.8)	2.3
E. glaber	21–29 × 20–26 prolate spheroidal to oblate spheroidal	3	not clear, small–sized, 1	reticulate–perforate, 0.1 up to 0.8 (0.4)	1.4–2.0
E. grandiflorus	34–38 × 32–35 prolate spheroidal to oblate spheroidal	3–(4)–(6), 50 %	H–shaped, small–sized, not clear, 1–2	reticulate, 0.6–0.7 to 1.7 (0.4)	3.5–3.7
E. hamiltonianus	28–30 × 25–29 prolate spheroidal to oblate spheroidal	3–(2), 2 %	round or equatorial alongated, not clear, 3–4, H–shaped	reticulate, 1.0 up to 2.2 and 0.2–0.5 (0.2)	2.4–3.5
E. integerrimus	30–34 × 30–37 oblate to oblate spheroidal	3–(4)–(6), 1 %	not clear, small–sized, 1–2	reticulate, 0.4–0.8 up to 1.3 (0.5)	3.4–3.5
E. juzepczukii	25–28 × 23–28 prolate spheroidal to oblate spheroidal	3	round, 4	reticulate, 1.0–1.5 (0.2)	2.0

Continuation of table 1

Species	Pollen size (polar axis x equatorial axis, μm) and pollen grains form	The number of colpi, their features, % of deviated grains	Endoapertures – Ora, their features and diameter, μm	Ornamentation, their features, lumina, μm, (width of muri, μm)	exine thickness, μm
E. lanceifolius	29–32 × 27–28 prolate spheroidal	3	not clear, 2	reticulate, 0.6–1.2 up to 2.0 (0.3)	3.5–4.0
E. laxiflorus	23–26 × 26–29 oblate spheroidal	3–(1)–(2), 11 %	round, clear, 3	reticulate, 0.1–0.7 up to 1.5 (0.6–0.7)	2.0
E. maackii	28–30 × 25–31 oblate spheroidal	3	H–shaped, round, 4–6	reticulate, heterobronchate, 0.2–0.3 and 0.6–2.0 (0.3)	2.7–3.1
E. melananthus	33–35 × 29–33 oblate spheroidal	3	round, not clear, 3–4	reticulate, (0.6) 1.0–2.0 up to 27 (0.3)	3.5–4.0
E. moldavicus	27–29 × 25–27 oblate spheroidal	3	round, 3	reticulate, 0.5 up to 1.0 (0.4–0.5)	2.5–3.1
E. myrianthus	21–23 × 22–25 oblate spheroidal	3	small–sized, not clear, 1–2	perforate, 0.2–0.5 (0.2–0.9)	1.8–2.0
E. nanus	21–25 × 20–23 prolate spheroidal to oblate spheroidal	3	round, oval, 3–4	reticulate, heterobronchate, 0.1–0.2 and 0.3–1.0 (0.5)	3.9–4.8
E. nitidus	17–20 × 18–22 oblate spheroidal	3	round, H–shaped, not clear, 2–3	perforate, 0.1–0.4. (0.3–0.8)	2.3
E. occidentalis	31–35 × 31–40 oblate spheroidal	3	not clear, round, H–shaped, 3	reticulate, 0.5–1.2 (0.2)	3.2–4.0
E. pauciflorus	27–32 × 23–29 prolate spheroidal to oblate spheroidal	3–(4)–(6), W–shaped, 20 %	round, not clear, 3–4	reticulate, 0.5 up to 2.0 (0.3)	2.2–4.0
E. phellomanus	32–35 × 28–32 oblate spheroidal	3	round, not clear, 4–5	reticulate, heterobronchate, 0.5–0.8 up to 2.0 (0.3)	3.0–4.0
E. przewalskii	27–30 × 25–28 oblate spheroidal	3	round, H–shaped, 4–5	reticulate, heterobronchate, 0.2–0.3 and 0.4–1.2 (0.4)	2.9–3.3
E. salicifolius	24–30 × 28–32 oblate to oblate spheroidal	3	round, H–shaped, 2–4	reticulate, heterobronchate, 0.1–0.2 and 0.5–1.5 (0.5)	2.0 – 2.7
E. semenovii	25–28 × 22–27 prolate spheroidal to oblate spheroidal	3	oval, equatorial elongated, 4	reticulate, heterobronchate, 0.2–0.3 and 0.5–1.5 (0.2)	2.0–3.8
E. sieboldianus	30–34 × 29–31 prolate spheroidal to oblate spheroidal	3–4–(6), 2 %	round, equatorial elongated, 5	reticulate, 0.5 up to 2.2. (0.4)	2.2–3.0
E. tingens	27–31 × 28–31 oblate spheroidal	3–(4), 9 %	equatorial elongated, not clear, 4–6	reticulate, hetero–bronchate, 0.2 and 0.5–1.4 (0.6–0.7)	2.3
E. velutinus	25–35 × 22–33 prolate spheroidal to oblate spheroidal	3	round, clear, 3–4	reticulate, 1.0–1.8 (0.2)	2.0–3.8
E. verrucosus	27–29 × 25–30 prolate spheroidal to oblate spheroidal	3	round, H–shaped, 3–4	reticulate, 0.5–1.5 (0.4)	3.0

End of table 1

Species	Pollen size (polar axis x equatorial axis, µm) and pollen grains form	The number of colpi, their features, % of deviated grains	Endoapertures – Ora, their features and diameter, µm	Ornamentation, their features, lumina, µm, (width of muri, µm)	exine thickness, µm
E. vidalii	30–35 × 27–33 oblate spheroidal	3	round, 5	reticulate–perforate, 1.0–1.8 up to 2.5 (0.5–1.3)	3.2
E. yedoensis var. *koehneana*	32–38 × 32–35 oblate spheroidal	3	not clear, equatorial alongated, 4–7	reticulate, heterobronchate, 0.2–0.3 and 0.4–1.6 (0.3–0.7)	4.2

Table 2

Morphological and morphometrical characteristics of pollen grains of species from the subgenus *Kalonymus* of genus *Euonymus* (Celastraceae)

Species	Pollen size (polar axis x equatorial axis, µm) and pollen grains form	The number of colpi, % of deviated grains	Endoapertures – Ora, their features and diameter, µm	Ornamentation, their features, lumina, µm (width of muri, µm)	exine thickness, µm
E. cornutus	29–32 × 27–30 oblate spheroidal	3–(2), 1 %	Round or quadrangular, H–shaped, 2–4	reticulate, heterobronchate, 0.7–1.7 and 0.3–0.4 (0.4)	3.4–3.5
E. dasydictyon	31–37 × 25–31 oblate spheroidal	3	not clear, small–sized, 2	reticulate, 0.7–1.5 up to 1.8. (0.4)	3.4–4.0
E. elegantissimus	35–37 × 33–37 oblate spheroidal	3	not clear, 3	reticulate, 0.5–0.9 up to 2.0 (0.5)	3.5–4.0
E. fimbricatus	25–30 × 23–30 prolate spheroidal to oblate spheroidal	3–(2), ≤ 1 %	not clear, round, small–sized, 2	reticulate, 0.5 up to 2.0. (0.3)	2.0–3,2
E. frigidus	23–24 × 23–25 oblate to oblate spheroidal	3	not clear, small–sized, 1	verrucate–reticulate, non–mesurable	1.8–2.1
E. latifolius	38–41 × 30–41 prolate spheroidal to oblate spheroidal	3–(4)–(6), 39 %:	round, not clear, 5	reticulate, heterobronchate, 0.5 up to 3 and 0.2–0.3. (0.5)	4.3–5.7
E. macropterus	32–34 × 30–35 prolate spheroidal to oblate spheroidal	3	round not clear, 2–3, H–shaped	reticulate, hetero–bronchate, 1.2 up to 2.7 and 0.3 (0.5–0.7)	2.0–3.1
E. maximomo-wiczianus	30–32 × 25–30 prolate spheroidal to oblate spheroidal	3–(4), ≤ 1 %	not clear, small–sized, 1	reticulate, 0.5 up to 2.0 (0.2)	3.9–4.8
E. miniatus	30–37 × 31–38 oblate spheroidal	3	round, not clear, 3	reticulate, 1.0 up to 3.0 (0.4–0.5)	3.0–4.0
E. oxyphyllus	31–38 × 29–37 oblate spheroidal	3	not clear, round, 5	reticulate, 1.0–2.5. (0.2)	3.9–5.1.
E. planipes	31–35 × 29–32 prolate spheroidal to oblate spheroidal	3–(2)–(1), 4 %	not clear, oval or round, 3	reticulate, heterobronchate, 1.0–2.0. (0.2)	2.5–4.8
E. porphyreus	31–34 × 30–33 prolate spheroidal to oblate spheroidal	3	not clear, 5–6	reticulate, heterobronchate, 0.6–1.2 up to 2.3	4.0–4.3
E. sachalinensis	31–35 × 31–33 oblate spheroidal	3	round, not clear, 3	reticulate, 1.0 up to 3.0 (0.6)	3.0–4.3
E. sanguineus	31–33 × 27–35 prolate spheroidal to oblate spheroidal	3–(2), 4 %	round or equatorial elongated, not clear, 4	reticulate, 0.5 up to 2.0 (0.3–0.4)	2.0–2.7
E. szechuanensis	30–32 × 25–31 oblate spheroidal	3	round or equatorial elongated, 5	reticulate, 0.8 up to 2.0 (0.4–0.5)	3.0–4.5
E. yesoensis	30–32 × 29–34 prolate spheroidal to oblate spheroidal	3–(2), 1.5 %	H–shaped, round, 5	reticulate–perforate, 0.2–1.5 (0.5–0.9)	1.5–1.8

Apertures. All examined species have compound pollen apertures and its grains belong to 3-colporate apertural type. The colpi are long, often wide, with arrow-headed, pointed (Fig. 1, 4), and colpi membranes are granulate, with small, round granules. The ends of colpi sometimes merge in apocolpium and syncolporate grains have been found (Fig. 1: 5).

The number of colpi varies, grains with different number and positions of ecto- and endoapertures have been revealed in some specimens. This phenomenon has been called pollen heteromorphism (Nadot el al., 2000). We found 2-colporate or 1-syncolporate (Fig. 3: 7, 8; Fig.4: 4, 5, 10) pollen, with W-like colpi band in pairs (Fig. 4: 13, 14), 4-colporate (Fig. 4: 13, 14, 15), 3-colporate-3-rugate and 4-colporate-4-rugate (Fig. 3: 1, 5, 6, 9; Fig. 4: 11, 12) pollen.

The application of CLSM and 3D-reconstruction of separate grains allows us to specify the disposition of ecto-and endoapertures in deviate pollen forms. The last two types of the deviations (3-colporate-3-rugate and 4-colporate-4-rugate) have three or four meridonal colpi (or parts of colpi), starting in one grain pole, but do not reach the other; colpi join

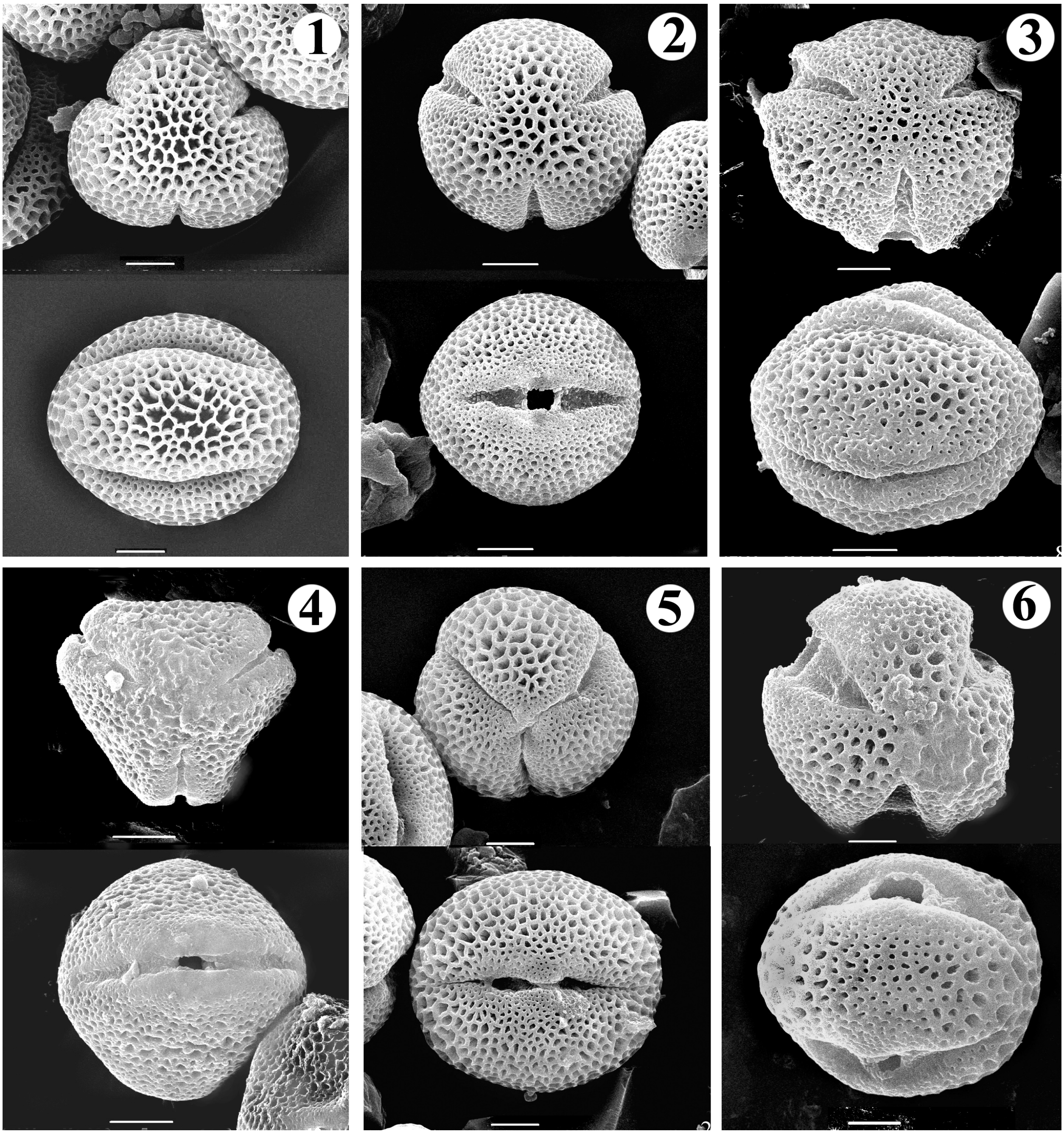

Fig. 1. Polar view and equatorial view of the pollen of *Euonymus* species (SEM): 1 – *E. melananthus*; 2 – *E. hederaceus*; 3 – *E. gibber*; 4 – *E. japonicas*; 5 – *E. acanthocarpus*; 6 – *E. vidalii.* Scale bar – 5 µm.

together near the other pole by three or four rugas, which are perpendicular to colpi. The ora (3 or 4 respectively) are placed in the center of colpi. The percentage of deviant forms can be in amounts of up to 50 % of grains in various *Euonymus* species.

Nearly one half of species from the subgenus *Kalonymus* (7 from 16) and near a fourth (quarter) of species from the subgenus *Euonymus* (13 from 47) show the occurrence of atypical pollen forms. The sections *Echinococcus, Melanocarya, Ilicifolia* demonstrate many species with pollen heteromorphism, but, in the section *Euonymus,* it has been found that only 6 specimens have deviated pollen grains from 31 investigated species.

Among the representatives of the subgenus *Kalonymus* 1–2-colporate deviated pollen forms are more common (5 species), and 4–6-apertural grains appear rarely (2 species). The representatives of subgenus *Euonymus* often have 4-colporate or 3-colporate-3-rugate and 4-colporate-4-rugate atypical grains (7 species), and rarely have 1–2-colporate (5 species), *E. acanthocarpus* has all types of deviant forms. The percentage of deviant pollen grains among the representatives of the genus

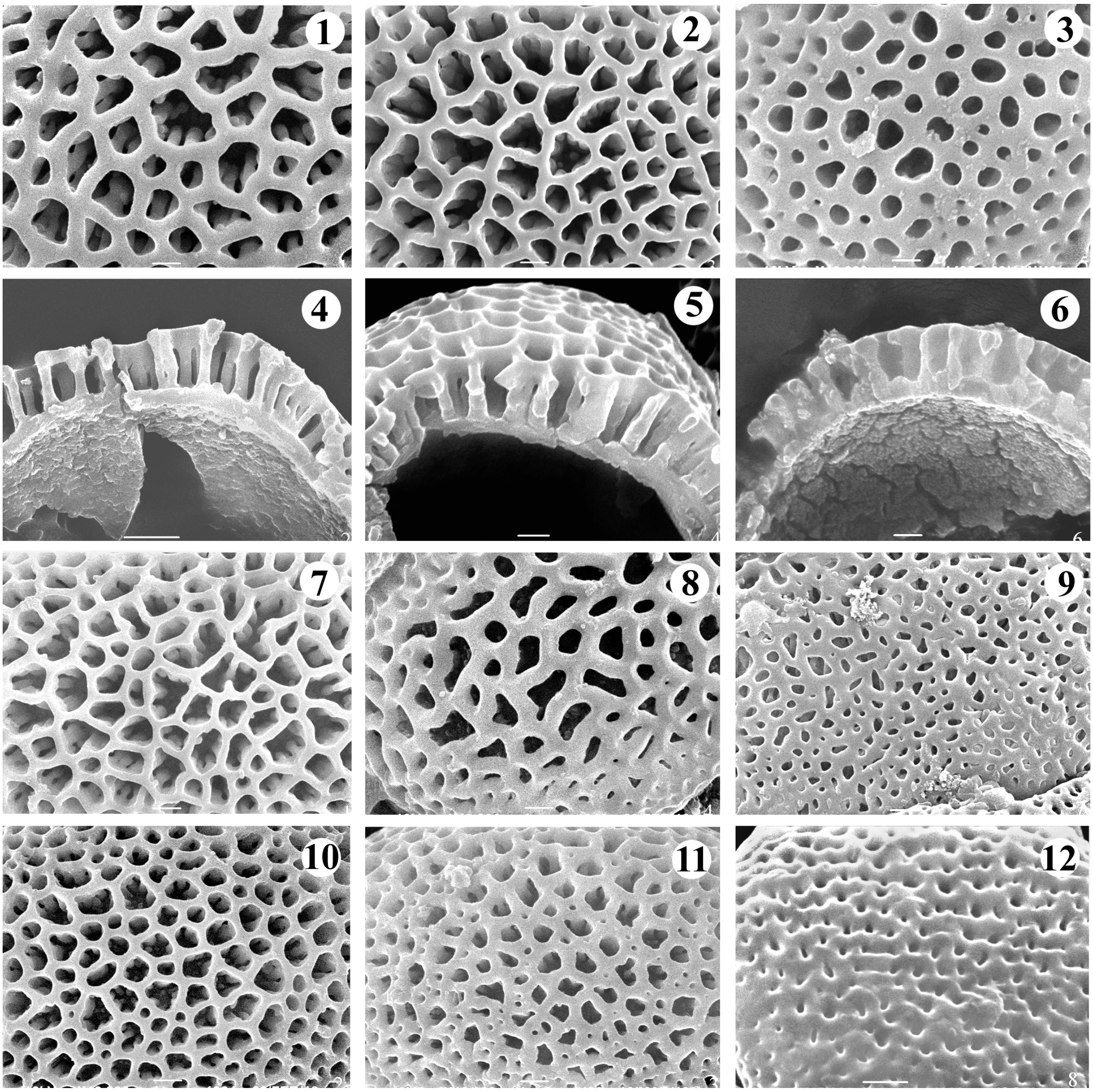

Fig. 2. Pollen ornamentation (1–3, 7–12) and sporoderm cleavage (4–6) of *Euonymus* species (SEM): 1, 4 – *E. porphyreus*; 2, 5 – *E. acanthocarpus*; 3, 6 – *E. americanus*; 7 – *E. phellomanus*; 8 – *E. laxiflorus*; 9 – *E. glaber*; 10 – *E. pendulus*; 11 – *E. nanus*; 12 – *E. nitidus.* Scale bar – 1 µm.

Euonymus varies from individual pollen grains up to 11 % into specimen, but the percentage of atypical grains in E. *sacrosanctus* and *E. pauciflorus* reaches 20 %, *E. latifolius* has 39 % and *E. grandiflorus* possess 50 % of deviated pollen forms.

Endoapertures have been revealed and are seen as obscure, not clear circumscribed, slightly outlined (Fig. 5: 11, 12), with the diameter no more than colpi wideness or up to clear, round, oval, oblong or quadrangular (Fig. 5: 5, 6), extending along the equator, which are sometimes significantly exceed the colpi width and pointed at the ends (Fig. 5: 8, 9).

H-shaped apertures formed by ora and exine thinning side by side to colpi (Fig. 4: 6, 9; Fig. 5) appear in pollen of many *Euonymus* species. CLSM optical sections confirmed the exine thinning are on two sides of the colpi (Fig. 5: 4, 7, 10), which are visible in transmitted light (Fig. 4: 6, 9; Fig. 5: 5, 8, 11). This thinning sometimes is a little longer than the colpi length, but in some cases, they are side by side to the entire colpus and connect to each other on the pole, which are shown at SEM micrographs (Fig. 5: 1 3). The most distinct H-shaped ora are detected in the pollen of 1/3 part of the species from the subgenus *Euonymus* (16 of 47) and only of 2 species from the subgenus *Kalonymus*.

Ornamentation. Under a light microscope the pollen sculpture is usually reticulate, lumina specific by different sizes and shapes. Texture varies from pointed to reticulate (Fig. 4).

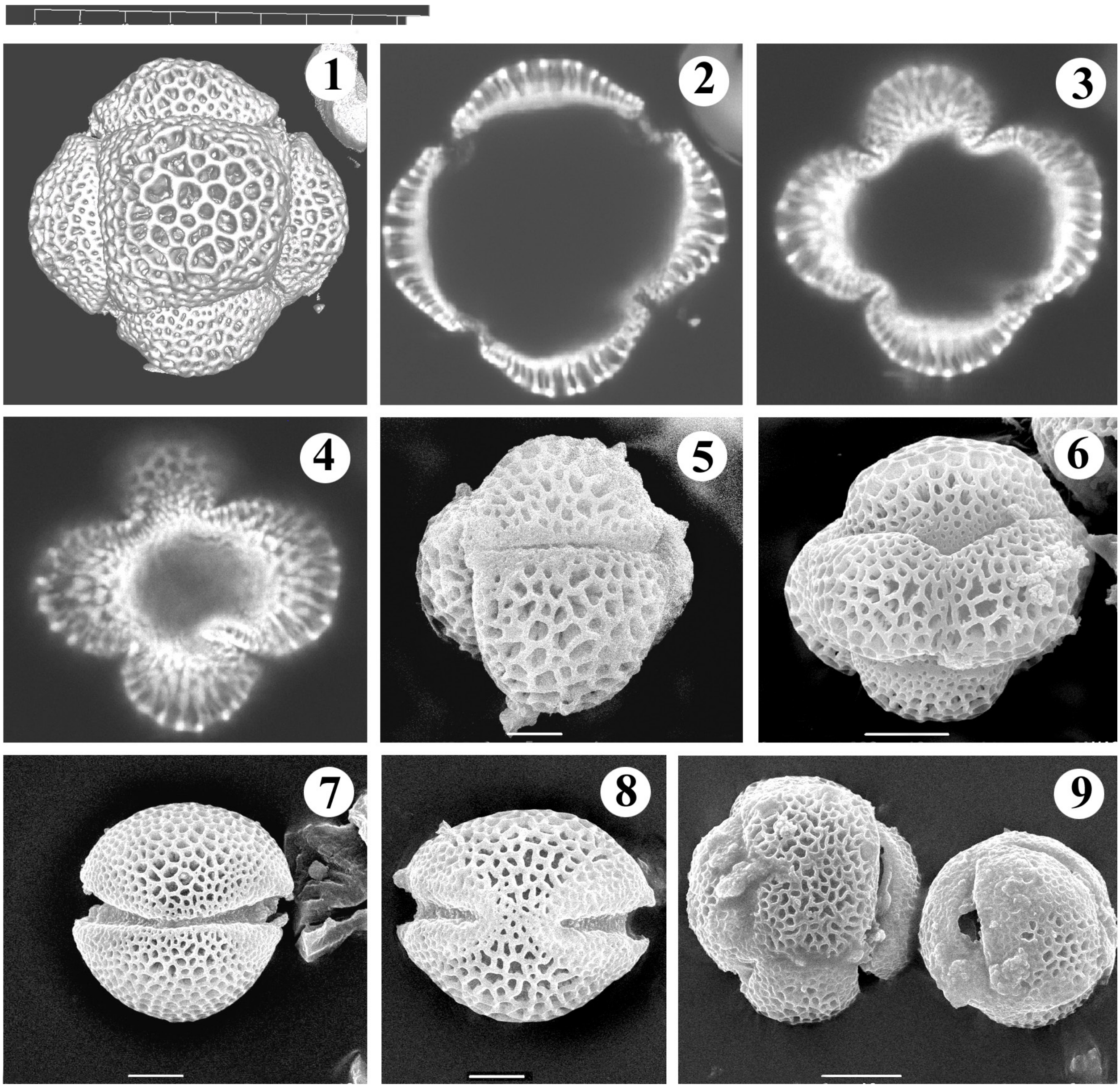

Fig. 3. General view and virtual sections through sporoderm of the pollen of *Euonymus* species (1–4 – CLSM, 5–9 – SEM): 1–5 – *E. latifolius*; 6 – *E. pauciflorus*; 7 – *E. pendulus*; 8 – *E. laxiflorus*; 9 – *E. echinatus*. Scale bar – 5 µm.

By SEM (Fig. 2) we found reticulate (the lumina wider than 1 μm, sometimes with big-sized lumina wider than 2.0 μm) or microreticulate (the lumina and muri smaller than 1 μm), perforate (holes less than muri and less than 1μm in diameter – Praglowski, Punt, (1973), Punt et al. (2007), Hesse et al., 2009) ornamentation. The brochus (mesh of a reticulum consisting of one lumen and the adjoining half of the muri – Erdtman (1952), Punt et al. (2007), Hesse et al. (2009)) vary in size and contour, and

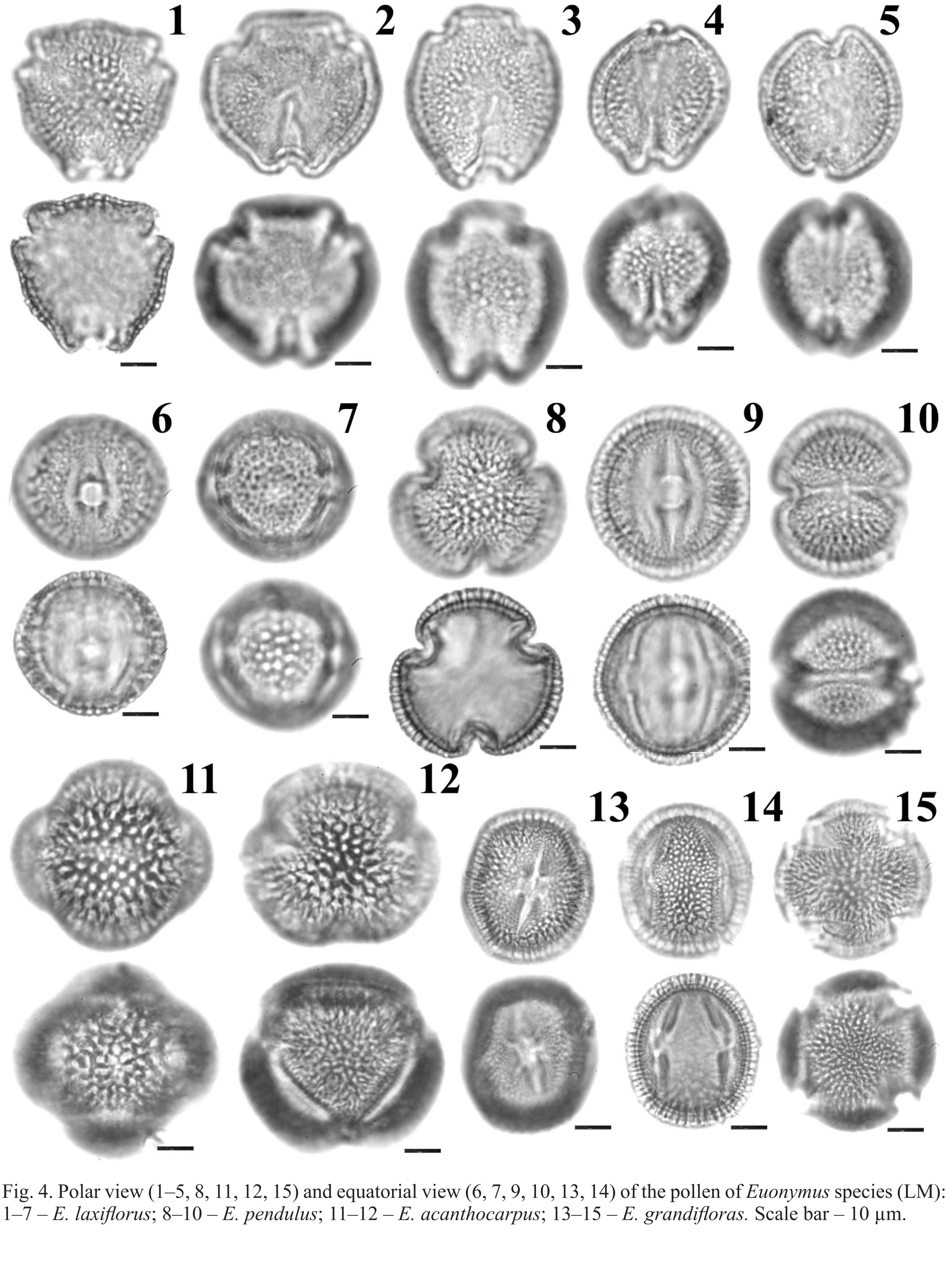

Fig. 4. Polar view (1–5, 8, 11, 12, 15) and equatorial view (6, 7, 9, 10, 13, 14) of the pollen of *Euonymus* species (LM): 1–7 – *E. laxiflorus*; 8–10 – *E. pendulus*; 11–12 – *E. acanthocarpus*; 13–15 – *E. grandifloras.* Scale bar – 10 μm.

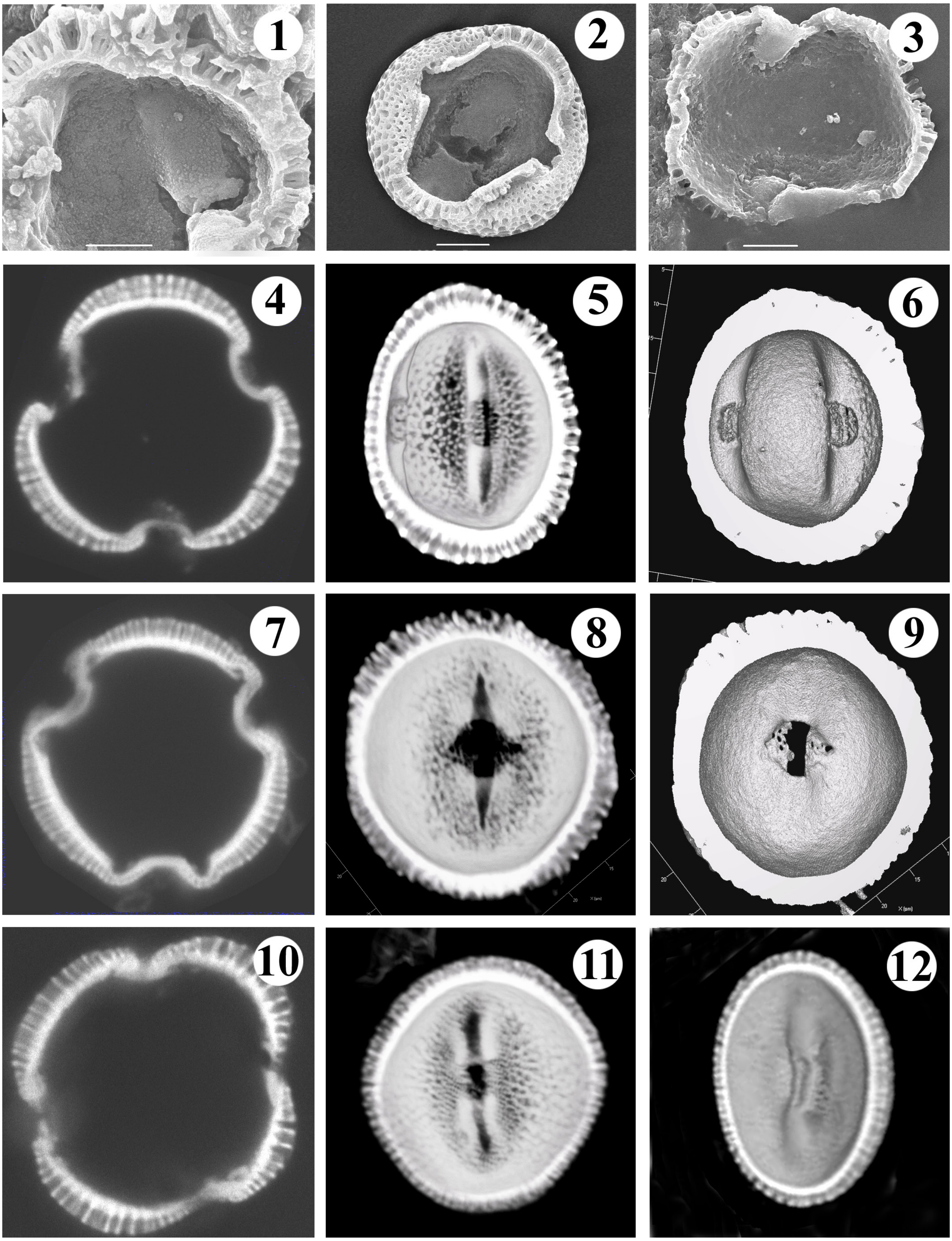

Fig. 5. Sporoderm cleavage (1–3), virtual sections through sporoderm (4, 7, 10) and reconstructed grains (5, 6, 8, 9, 11, 12) of the pollen of *Euonymus* species (1–3 – SEM, 4–12 – CLSM: 4, 7, 10 – optical sections; 5, 8, 11, 12 – transmissional mode; 6, 9 – surface mode): 1 – *E. macropterus*; 2 – *E. cornutus*; 3, 11 – *E. theifolius*; 4 – *E. europaeus*; 5, 6 – *E. latifolius*; 7 – *E. patens*; 8, 9 – *E. szechuanensis*; 10 – *E. echinatus*; 12 – *E. glaber.* Scale bar – 5 µm.

there are heterobronchate surfaces with lumina at different sizes (Fig. 2: 3, 11). The lumina has round, angular, elongated or oblong, rarely oval or irregular outline, its sizes range from smaller than 0.1 µm up to 3.5 µm in diameter. The width of muri is 0.2–0.8 µm, and rarely riches up to 1.3 µm. The distance between the perforations is usually 0.2–0.9 µm. The muri are smooth, direct, rare flexuose or ridge like. The columellae and the bottom of the mesh are visible sometimes, free standing columellae are found sometimes (Fig. 2: 1, 2, 7, 10). Along the colpi the size of lumina decreases, sometimes near the colpi surface it becomes perforated, only in some species (*E. benthamii, E. dichotomus, E. frigidus, E. myrianthus, E. nitidus, E. obovatus, E. occidentalis, E. planipes, E. salicifoliaus, E. yakushimensis*) the mesh reduction is not revealed. On apocolpium the lumina is usually less or equal to mesocolpium lumina size; on the other hand, only the species *E. glaber*, *E. yedoensis* var. *koehneana* and *E. vidalii* (Fig. 1: 6) showed an increase in the size of lumina on apocolpium compared with mesosolpium.

Exine. The exine is semitectate or tectate, columellate, from 1.5 to 5.1 µm in thickness (Fig. 2:

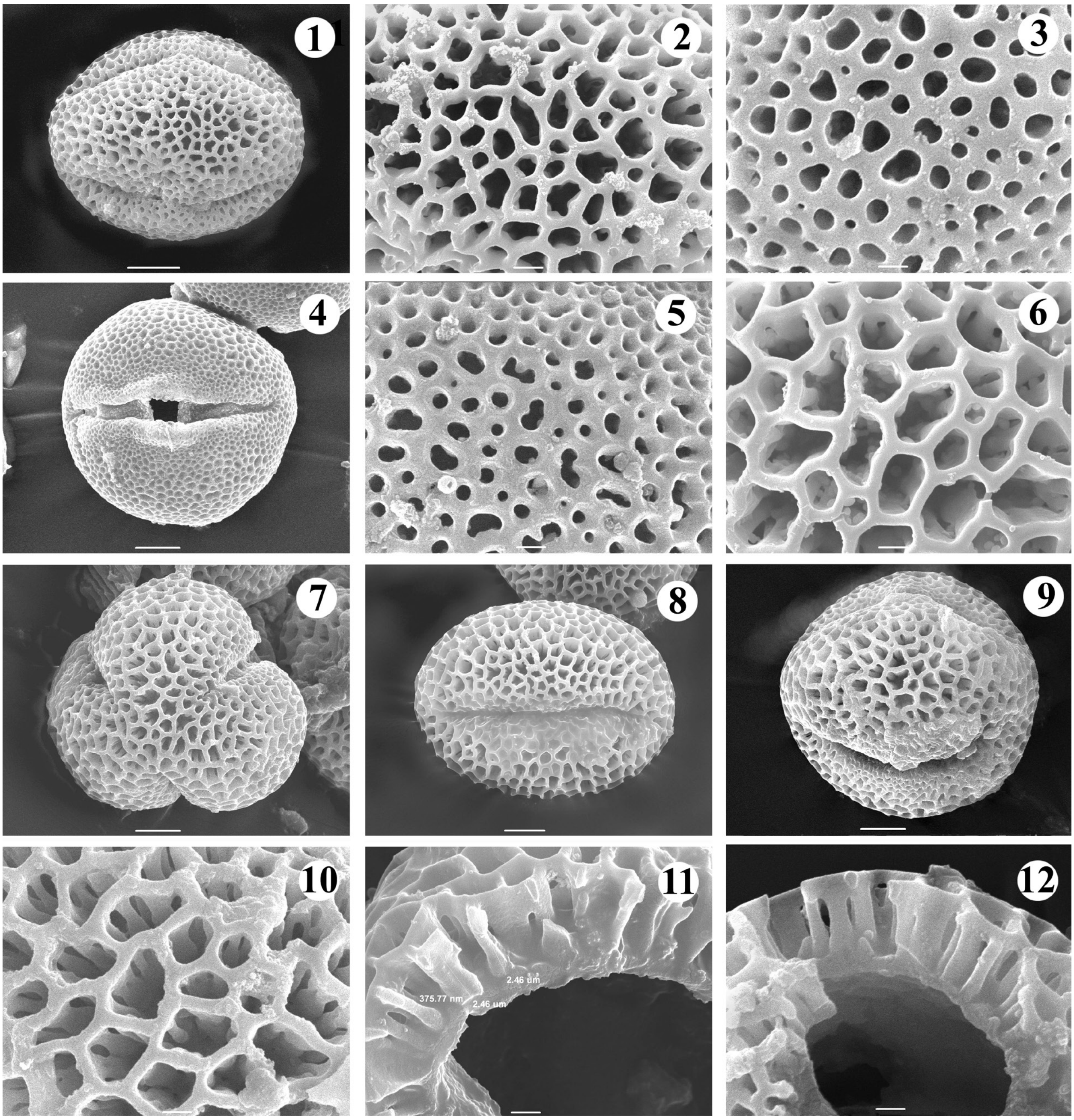

Fig. 6. Pollen general view (1, 4, 7–9), pollen ornamentation (2, 3, 5, 6, 10) sporoderm cleavage (11, 12) of *Euonymus* species (SEM) 1, 2 – *E. obovatus*; 3 – *E. americanus*; 4, 5 – *E. yesoensis*; 6 – *E. oxyphyllus*; 7, 10 – *E. miniatus*; 8, 11 – *E. planipes*; 9, 12 – *E. sachalinensis.* Scale bar – 5 µm (1, 4, 7–9), 1 µm (2, 3, 5, 6, 10. 11, 12).

4–6; Fig. 3: 2, 3; Fig. 5: 1–5, 7, 8, 10–12; Fig. 6: 11, 12). The exine in the center of mesocolpium or/and apocolpium is usually thickened by columellae elongation. The columellae are clear, smooth, and often long, the distance between them (each to the other) is equal or slightly smaller than their thickness. The tectum and the foot layer (with endexine) are usually not thick, but approximately equal each other in thickness. The thickest exine layer is columellaete infratectum, as it is often more than twice (rarely the same thickness) of the tectum or foot layer wideness. CLSM data show the same distribution of the extexine layers, whereas the border of the endexine and the foot layer is not visible with SEM and CLSM.

Discussion

Palynomorphological features

According to palynomorphological data the genus *Euonymus* as the other genera of the family Celastraceae, is quite homogeneous. The pollen is represented by 3-colporate type with reticulate variations of surface ornamentation (Lobreau, 1969; Perveen, Qaiser, 2008). The pollen morphology of all studied species is very uniform, all the characteristics studied have a small range of variability. However, morphological features of some species have been revealed both in the structure of apertures and in exine ornamentation.

We observed parallel to the colpi exine thinning by LM. Similar thinnings have been found in other angiosperm families, such as Crassulaceae, Araliaceae, Linaceae, by our unpublished data. CLSM studies confirmed both sides have exine thinnings parallel to the colpus, which in the combination with ora, looks like H-shaped endoaperture (Fig. 4: 6, 9; Fig. 5). The TEM investigation of Celastraceae sporoderm was conducted by D. Lobreau-Callen and B. Lugardon (1972–1973) on an example of the exine of both acetolized and non-acetolized grains of *E. europaeus*, *E. japonicus* and *Celastrus orbiculatus*. The endexine structure has been illustrated, and the authors showed that thinning parallel to the colpi is formed in endexine.

Variations in the number and arrangement of apertures were found in several specimens. This article provides a quantitative content of atypical pollen grains in the studied specimens. The deviant pollen forms are widely distributed in flowering plant taxa. It has been found that, as in many flowering plant taxa, pollen forms with different aperture arrangement may be arranged in continuous regular series (Pozhidaev, 2009), in which, from form to form, the aperture position changes smoothly and continuously to follow a regular geometric pattern, that demonstrates non-specificity at a high taxonomic level (families, orders and subclass of dicotyledons and above; compare Fig. 4 of this article and figures in the paper of A. Pozhidaev (2009)). I. Dajoz et al. (1991, 1993) showed that 3-aperturate and 4-aperturate pollen grain of heteromorphic species *Viola diversifolia* differ by germination and longevity. In *Viola calcarata* the ratio of pollen with four and five apertures is variable according to altitude (Till-Bottraud et al., 1999). The authors (Till-Bottraud et al., 2001) supposed pollen heteromorphism to be an evolutionarily stable strategy. C. Prieu et al. (2016) have investigated the connection between aperture patterns and harmomegathy in the pollen of wild type and three *Arabidopsis thaliana* mutants with zero, four, or a mix of four to eight apertures, and have suggested that "pollen grains with few apertures survive better than pollen with many apertures". A. Mignot et al. (1994), S. Nadot et al. (2000), R. Humphrey (2016) and our unpublish data show that some angiosperm taxa have deviated pollen forms, but the others have only typical pollen grains.

The most common type of ornamentation of the *Euonymus* pollen is reticulate, a few parts of the species have microreticulate or perforate pollen surface or sometimes we found heterobrochate surfaces (Fig. 2).

We can't group species by pollen characteristics, because we found many intermediate types such as in pollen size or in endoaperture or surface structure. Endoapertures differ by size from gross, clear up to small, unclear; exine thinning side by side to colpi are found from observable to poor distinguishable; the ornamentation varies by lumina and muri sizes.

The most characteristic features of different species which help us to tell the difference between species by pollen grains are size and form of endoapertures and pollen ornamentation. These provide an opportunity to use palynomorphological data in pollen-spore analysis and clarify some taxonomical questions.

Pollen morphology for systematics

The genus is divided into two subgenera (*Euonymus* and *Kalonymus*) and from 4 to 12 sections. Subgenus *Kalonymus* is recognized the most recent group of *Euonymus*. There is no pollen with H-like ora (except 2 species) and there are significantly more common species with deviated pollen forms in this subgenus.

J.-S. Ma (2001) has realized the last world revision of the genus and has reduced the number of sections up to 4 in the subgenus *Euonymus* and has not find divisions within the subgenus *Kalonymus*. The number of species was reduced to 129, as more than 50 species were considered as synonymous.

After J.-S. Ma (2001) the subgenus *Kalonymus* includes 16 species. These species are classified into *Kalonymus* by other authors (Blakelock, 1951; Leonova, 1974; Tzvelev, 2004; Savinov, Baikov, 2007) too. We have studied the pollen of 16 species from the subgenus, but J. S. Ma (2001) names some of them as synonymous of these investigated specimens (*E. dasydictyon, E. miniatus, E. planipes, E. porphyreus, E. yesoensis*). *E. maximowiczianus* is not noted in this system (only *Kalonymus maximowicziana* Prokh. as synonym of *E. sachalinensis)*.

J.-S. Ma (2001) considers *E. dasydictyon* и *E. porphyreus* as synonymous of *E. frigidus*, as the first two taxa belong to different series of T. Leonova systems (1974), and the third taxon refers to the other section by T. Leonova (1974) and R. A. Blakelock (1951). Pollen of *E. dasydictyon* and *E. porphyreus* is very similar, but grains of *E. porphyreus* have larger ora and smaller lumina in the surface ornamentation. Grains of *E. frigidus* are smaller in size, mainly oblate, verrucate-reticulate ornamentation, which is a unique type in the genus, and the exine is more than twice thin. Palynomorprological data confirms the separation of this species. Molecular data (Li et al., 2014) also contradicts the association of *E. porphyreus* and *E. frigidus* in one species.

E. yesoensis is referred to synonyms for *E. oxyphyllus,* which is consistent with R. A. Blakelock's (1951) and T. Leonova's (1974) systems, and is not comport with palynological data. Pollen of these species has different ornamentation – reticulate with lumina from 3.0 μm in *E. oxyphyllus* and reticulate-perforate in *E. yesoensis* (Fig. 6: 4–6).

E. miniatus and *E. planipes* are synonymous of *E. sachalinensis* (Fig. 6: 7–12). Pollen grains of these taxa do not differ. Pollen morphology has allowed these taxa to combine in one species.

R. A. Blakelock (1951) and T. Leonova (1974) classified *E. yakushimensis* in the subgenus *Kalonymus,* but J.-S. Ma (2001) included this species in the subgenus *Euonymus* (section 4 *Ilicifolia).* According to the palynological characteristics this species is like to representative of section *Ilicifolia*: *E. yakushimensis* grains have distinctive H-shaped aperture and reticulate-perforate surface; but the species of the subgenus *Kalonymus* have a very rare H-shaped ora, and the surface is mainly reticulate with the lumina more than 2 μm.

In general, the pollen of most studied species of the subgenus *Kalonymus* can be attributed to one group of pollen grains with reticulate often with a big size of lumina sculpture and a clearer ora. Pollen of *E. frigidus* and *E. yesoensis*, discussed above, as well as pollen of *E. cornutus*, with distinctive H-shaped aperture (Fig. 5: 2), are segregated from this group. It is impossible to separate the subgenus species by pollen features series, which were established in T. Leonova (1974) and I. Savinov, K. Baikov (2007) systems. Although recent studies (Simmons et al., 2012; Li et al., 2014; Sidorov, Trusov, 2015; Sidorov et al., 2014) confirmed the isolation of the subgenus, the main group of *Kalonymus* pollen may also include pollen of a large number of species of another subgenus.

J.-S. Ma (2001) divided the subgenus *Euonymus* into 4 sections. The section *Echinococcus* is represented by 18 species. We studied the pollen of 5 specimens of this section, including *E. obovatus* that J.-S. Ma (2001) recognized as synonyms of *E. americanus*. Pollen of these species differs both in structure/size of ora and thickness of muri, so *E. obovatus* has a reticulate exine surface with narrow muri, but *E. americanus* has a reticulate-perforate ornamentation with rather thick muri (on the verge of a perforated) (Fig. 6: 1–3). R. A. Blakelock (1951), T. Leonova (1974) and I. Savinov, K. Baikov (2007) splitted this section into two series, what have not agreed with the research of Y-Ch. Zheng et al. (2012). Neither the complexity of palynological features, none any one of the regular features (size, structure apertures, exine structure, surface type) have not confirmed the division of the section *Echinococcus* into the series, as well as the isolation of this section within the genus and the affinity to the subgenus *Kalonymus*, that has been affirmed by recent molecular studies (Li et al., 2014).

The *Melanocarya* section consists of 11 species. Four of the studied pollen samples belong to 2 species by J.-S. Ma (2001), *E. sacrosanctus* and *E. subtriflorus* referred to synonyms of *E. alatus.* T. Leonova (1974) believed that *E. subtriflorus* is synonymous with *E. alatus*. Although pollen of *E. subtriflorus* is a little smaller sized, the differences of pollen grains among these three taxa are inconsiderable, it is permissible to recognize their association, the similarity of *E. sacrosanctus* and *E. alatus* as confirmed by R. Sidorov, N. Trusov (2015). The fourth our specimen of the section – *E. pendulus* (Fig. 2: 10; Fig. 3: 7; Fig. 4: 8–10) is described by J.-S. Ma (2001) as synonym of *E. lucidum* D. Don; we have not studied *E. lucidum* and have not made a comparison with synonym. *E. salicifolius* (= *E. lawsonii* var. *salicilolius*) is included in *Melanocarya* section by R. A. Blakelock (1951) and in *Euonymus* section by J.-S. Ma (2001). A distinctive set of features of pollen of this species was not detected, the pollen grains are medium-sized 24–32 µm in diameter, with heterobronchate surface and well-expressed H-liked apertures. The above-described pollen grains also are found in *E. pendulus* (section *Melanocarya)* and in part of the representatives of the *Euonymus* section.

The *Ilicifolia* section includes 20 species. We investigated 6 pollen samples. *E. patens* (Fig. 5: 7) is synonymous with *E. hederaceus*, and it is confirmed by palynomorphological data. Pollen of *Ilicifolia* species has well-expressed, H-shaped apertures (except *E. benthamii*), perforate or reticulate-perforate (diameter of lumina less than 1.3 µm), and often heterobronchate exine surfaces. The section is considered close to the primitive form. I. Savinov, K. Baikov (2007) discussed the possibility of *Ilicifolia* location as a separate subgenus. R. Sidorov, N. Trusov (2015) believed that this allocation is acceptable, as they found that the ratio of the LCD-composition lipid seeds and aril of *E. japonicus* differs from those characteristics of the other species of the genus. However, this study comprises of only one species from the section and the pollen of *E. japonicus,* which has the rare feature – 3-angular outline in polar view and is distinct in the complex of palynomorphological characteristics. The pollen of the other species of the section is more typical for the genus.

Euonymus is the largest section of the subgenus *Euonymus*; it includes 64 species, about a half of the genera species according to J.-S. Ma (2001). We have investigated 31 pollen specimens from this section, 12 samples (*E. czernjaevii, E. moldavicus, E. velutinus, E. bungeanus, E. integerrimus, E. lanceifolius, E. pauciflorus, E. juzepczukii, E. sieboldianus, E. vidalii, E. yedoensis* var. *koehneana, E. przewalskii)* are reduced to synonyms by J.-S. Ma (2001).

All these investigated species, except *E. salicifolius* and *E. grandiflorus*, belong to 4 series of the section *Biloculares* according to R. A. Blakelock (1951). Application of pollen data to taxonomy of *E. salicifolius* is discussed in a paragraph of the section *Melanocarya. E. grandiflorus* is a species of section *Multiovulatus* by R. A. Blakelock (1951), T. Leonova (1974), I. Savinov, K. Baikov (2007). Pollen of *E. grandiflorus* (Fig. 4: 13–15) is characterized by quite large sizes, 32–37 µm in diameter.), reticulate surface, small-sized ora. In *E. grandiflorus* we have found the most number of deviated pollen grains (50 %) in the genus. The division on section and series of systems of R. A. Blakelock (1951), T. Leonov (1974), and I. Savinov, K. Baikov (2007) on the basis of pollen data is not confirmed.

We noted that the most diverse and distinctive pollen features were found in the representatives series *Myrianthi* (section *Biloculares*). These characteristics are the next: polar view of *E. gibber*, *E. laxiflorus*, *E. nitidus* grains is 3-angular (Fig. 1: 4, Fig. 4: 1, 2), pollen of *E. nitidus* have minimal sizes 17–22 µm in diameter, pollen of *E. dichotomus* and *E. myriantus* is small too, no more than 25 µm in diameter; three representatives (*E. dichotomus, E. myriantus, E. nitidus*) from all subgenus *Euonymus* have perforate exine ornamentation (Fig. 2: 12); (exine ornamentation of *E. glaber* (Fig. 2: 9) is reticulate-perforate and reticulate in *E. gibber*, *E. laxiflorus* (Fig. 2: 8), *E. tingens*); on apocolpium lumina in *E. glaber* is more than mesocolpium lumina size. I. Savinov, K. Baikov's (2007) brings three sections *Ilicifolia, Multiovulatus* and *Myrianthus* of primitive spindle trees together in the group; this group is the most diverse from a palynomorphological point of view. Exine surface in this group vary from perforate and microreticulate up to reticulate with small-sized lumina, but large-sized lumina is not a commonly found.

As for synonyms of the species mentioned in the section *Euonymus* J.-S. Ma (2001) they refer to the section *Biloculares* series *Lophocarpi* and *Pseudovyenomi* by R. A. Blakelock (1951). According to J.-S. Ma (2001) *E. europaeus* (Fig.5: 4) includes *E czernjaevii, E moldavicus* and *E. velutinus*. T. Leonova (1974) did not recognize *E. moldavicus* as separate species, while the other three taxa put together in the *Lophocarpae* series of the section *Euonymus*.

J.-S. Ma (2001) classified *E. juzepczukii, E. sieboldianus, E. lanceifolius, E. vidalii, E. yedoensis* var. *koehneana* into *E. hamiltonianus.* After R. A. Blakelock (1951) *E. sieboldianus, E. lanceifolius, E. yedoensis* var. *koehneana* also are synonyms of *E. hamiltonianus.* Pollen grains of *E. juzepczukii, E. lanceifolius, E. sieboldianus, E. hamiltonianus* are from 23 to 34 µm in diameter, ora are more or less expressed from 2 to 5 µm in diameter, the surface is mainly reticulate with large lumina sizes, columellas and the bottom of lumina are often visible. Near colpa, the size of lumina decreases, on the apocolpium lumina usually are equal or less than on the mesocolpium, exine from 2.2 to 4.0 µm thick, columellas are clear and long. Grains of this group are similar among themselves, so association is allowed. R. A. Blakelock (1951) put *E. maackii* to *E. hamiltonianus* var. *maackii* and J.-S. Ma (2001) represents *E. bungeanus* as *E. maackii* synonym. *E. maackii* grains belong to the above described pollen group, they are very similar to those at *E. bungeanus.* Distinctive features of pollen of two samples are quite substantial. Grains are larger usually from 30 µm in the diameter, ora large, more than 4–7 µm in the diameter, a surface is reticulate perforate or reticulate, and there is an increase of the lumina sizes on an apocolpium in comparison with mesocolpium.

E. semenovii and *E. przewalskii* were united by J.-S. Ma (2001) in one species, while T. Leonova (1974) and I. Savinov, K. Baikov (2007) believed these two species can be treated in one series. Its pollen is also poorly distinguishable, within morphological fluctuations into one species.

E. integerrimus and *E. pauciflorus* were reduced by J.-S. Ma (2001) to *E. verrucosus* as synonyms. T. Leonova (1974) and I. Savinov, K. Baikov (2007) relegated these to one series. Their pollen is very similar, its association is admissible.

N. Tzvelev (2004) has allocated *E. nanus* into one new section *Nanevonymus*. N. Tzvelev (2004) didn't divide the genus into subgenera and considered *Kalonymus* as one section. All spindle trees of Eastern Europe have been united in 8 sections. Pollen of *E. nanus* is typical for the genus.

The species which pollen is similar by some characteristics as well as on their complex, often belong to different sections and subgenera. Palynological data do not confirm modern taxonomical systems.

The reduction of some taxa (*E. obovatus = E. americanus; E. dasydictyon = E. frigidus; E. porphyreus = E. frigidus; E. yesoensis = E. oxyphyllus; E. czernjaevii = E. europaeus; E. velutinus = E. europaeus; E. vidalii = E. hamiltonianus*; *E. yedoensis* var. *koehneana = E. hamiltonianus*) to synonyms is not confirmed palynologically.

But the next species (*E. planipes = E. sachalinensis; E. miniatus = E. sachalinensis; E. moldavicus = E. europaeus; E. sacrosanctus = E. alatus; E. subtriflorus = E. alatus; E. patens = E. hederaceus; E. bungeanus = E. maackii; E. przewalskii = E. semenovii; E. juzepczukii = E. hamiltonianus*; *E. sieboldianus = E. hamiltonianus*) can be synonyms by pollen data.

Conclusion

3-colporate, reticulate pollen of representatives of the genus *Euonymus* is typical for the family Celastraceae as a whole.

No correspondence of palynomorphological data to the accepted sectional subdivision of subgenus *Euonymus* (Ma, 2001) has been found. In addition, no corresponding data have been discovered on taxonomic groups isolated within the genus according to R. A. Blakelock (1951), T. Leonova (1974), N. Tsvelev (2004) and I. Savinov and K. Baikov (2007), neither by complex of pollen characters nor by one of them separately (the shape of the pollen grain, the structure of the apertures, the structure of the exine, surface sculpture, dimensions).

The species which pollen is similar by some characteristics as well as on their complex often belong to different sections and subgenera. The palynological data do not confirm modern taxonomical systems.

The palynomorphological data do not confirm the status of *Kalonymus* neither as a subgenus, nor a separate genus.

The reduction of half species into synonyms is not proved by pollen morphology.

Nevertheless, the pollen peculiarities of certain species are allowed in order to use the obtained data in the pollen-spore analysis.

Acknowledgements

The study was carried out within the framework of institutional research project No. 01201255609 of the Komarov Botanical Institute of the Russian Academy of Sciences. The research was done using equipment of The Core Facilities Center “Cell and Molecular Technologies in Plant Science” at the Komarov Botanical Institute RAS (St. Petersburg, Russia).

REFERENCES / ЛИТЕРАТУРА

Artyushenko A. T., Romanova L. S. 1984. *Morphologiya pyltsi reliktovykh, endemichnykh i redkich vidov flori Ukrainy* [*Pollen morphology of relic, endemic and rare species of Ukraine flora*]. Naukova dumka, Kiev, 48 pp. [In Russian]. (***Артюшенко А. Т., Романова Л. С.*** Морфология пыльцы реликтовых, эндемичных и редких видов флоры Украины. Киев: Наукова думка, 1984. 48 с.).

Blakelock R. A. 1951. A synopsis of the genus *Euonymus* L. *Kew Bull*. 2: 210–290.

Chester P. I., Raine J. L. 2001. Pollen and spore keys for Quaternary deposits in the northern Pindos Mountains, Greece. *Grana* 40: 299–387. DOI: 10.1080/00173130152987535

Dajoz I., Till-Bottraud I., Gouyon P. H. 1991. Evolution of pollen morphology. *Science* 253: 66–68. DOI: 10.1126/science.253.5015.66

Dajoz I., Till-Bottraud I., Gouyon P. H. 1993. Pollen aperture polymorphism and gametophyte performance in *Viola diversifolia*. *Evolution* 47: 1080–1093. DOI: 10.1111/j.1558-5646.1993.tb02137.x

Erdtman G. 1952. *Pollen morphology and plant taxonomy: Angiosperms.* Stokholm, 539 pp.

Gavrilova O. A. 2014. Application of confocal laser scanning microscope for pollen wall morphology study. *Bot. Zhurn.* (*Moscow & St. Petersburg*) 99(10): 1139–1147 [In Russian]. (***Гаврилова О. А.*** Применение конфокальной лазерной сканирующей микроскопии (КЛСМ) для исследования морфологии оболочки пыльцевых зерен // Бот. журн., 2014. Т. 99, № 10. С. 1139–1147).

Hesse M., Halbritter H., Weber M., Buchner R., Frosch-Radivo A., Ulrich S., Zetter R. 2009. *Pollen terminology*. Springer-Verlag, Wien, 266 pp. DOI: 10.1007/978-3-211-79894-2

Heusser C. J. 1971. *Pollen and spores of Chili. Modern types of Pteridophyta, Gymnospermae and Angiospermae.* Tuczon, 167 pp.

Huang T.-C. 1972. *Pollen flora of Taiwan.* Taipei, 297 pp.

Humphrey R. P. 2016. Pollen heteromorphism is pervasive in *Thalictrum* (Ranunculaceae). *Plant Systematics and Evolution* 302(8): 1171–1177. DOI: 10.1007/s00606-016-1312-8

Ikuse M. 1956. *Pollen grains of Japan.* Tokyo, 279 pp.

Kupriyanova L. A. 1972. Celastraceae Batsch. In: ***Kupriyanova L. A., Aleshina L. A.*** *Pyltsa dvudolnykh rasteniy flory yevropeyskoy chasti SSSR* [*Pollen of dycotiledon plants of flora of European part of URSS*]. Vol. 1. Leningrad, 86–89 pp. [In Russian]. (***Куприянова Л. А.*** Сем. Celastraceae Batsch // ***Куприянова Л. А., Алёшина Л. А.*** Пыльца двудольных растений флоры европейской части СССР. Т. 1. Л., 1972. С. 86–89).

Leonova T. G. 1974. *Beresklety SSSR i sopredelnykh stran* [*Spindle trees of USSR and neighboring countries*]. Nauka, Leningrad, 132 pp. [In Russian]. (***Леонова Т. Г.*** Бересклеты СССР и сопредельных стран. Л.: Наука, 1974. 132 с.).

Li Y.-N., Xie L., Li J.-Y., Zhang Z.-X. 2014. Phylogeny of *Euonymus* inferred from molecular and morphological data. *J. Syst. Evol*. 52(2): 149–160. DOI: 10.1111/jse.12068

Lobreau D. 1969. Les limites de l'"ordre" des Célastrales d'après le pollen. *Pollen et spores* 11(3): 499–555.

Lobreau-Callen D., Lugardon B. 1972–1973. L' aperture a repli du pollen des Celastraceae. *Nat. Monspel. Ser. Bot. Fasc.* 23–24, I–II: 205–210.

Loesener Th. 1942. Celastraceae. In: *Die Natürlishen Pflanzenfamilien*. Vol. 20B. Eds. A. Engler, K. Prantl. Wilhelm Engelmann, Leipzig, Berlin, 87–197 pp.

Ma J.-S. 2001. A revision of *Euonymus* (Celastraceae). *Thaiszia – J. Bot., Košice* 11: 1–264. URL: https://www.upjs.sk/public/media/5743/thaiszia-11-1-264-2001-ma.pdf

Ma J.-S., Funston A. M. 2008. Celastraceae, *Euonymus*. In: *Flora of China.* Vol. 11 (Oxalidaceae through Aceraceae). Eds. Z. Y. Wu, P. H. Raven, D. Y. Hong. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, 440–463 pp.

Mignot A., Hoss C., Dajoz I., Leuret C., Henry J. P., Dreuillaux J. M., Heberle-Bors E. et al. 1994. Pollen aperture polymorphism in the angiosperms: importance, possible causes and consequences. *Acta Bot. Gallica* 141: 109–122. DOI: 10.1080/12538078.1994.10515144

Nadot S., Ballard H. E., Creach J. B., Dajoz I. 2000. The evolution of pollen heteromorphism in *Viola*: a phylogenetic approach. *Pl. Syst. Evol*. 223: 155–171. DOI: 10.1007/bf00985276

Nair P. K. K. 1965. *Pollen grains of Western Himalayan plants.* Asia Publ. H., London, 102 pp.

Nakai T. 1941. Subdivisions of the genus *Euonymus. J. Jap. Bot*. 17: 615–619.

Nakamura J. 1980. Diagnostic characters of pollen grains of Japan. *Spec. Publ. Osaka Mus. Nat. Hist*. 12: 1–157.

Perveen A., Qaiser M. 2008. Pollen flora of Pakistan. LVIII. Celastraceae. *Pak. J. Bot*. 40(3): 957–962.

Poliychuk Yu. S. 1978. About pollen of Far East spindle trees. In: *Redkiye i ischezayushchiye rasteniya yuga Dalnego Vostoka (biologiya, ekologiya, kariologiya)* [*Rare and disappearing plants of the south of the Far East (biology, ecology, karyology)*]. Vladivostok, 142–144 pp. [In Russian]. (***Полийчук Ю. С.*** О пыльце дальневосточных бересклетов // Редкие и исчезающие растения юга Дальнего Востока (биология, экология, кариология). Владивосток, 1978. С. 142–144).

Pozhidaev A. E. 2009. The structure of the diversity of the morphological character on the example of the location of pollen apertures in flowering plants and the natural ordering of biological diversity, or that such a variety (ways of describing and interpreting). In: *Trudy Zoologicheskogo instituta RAN. Prilozheniye 1*. [Перевод на англ.] 150–182 pp. [In Russian]. (***Пожидаев А. Е.*** Структура многообразия морфологического признака на примере расположения апертур пыльцы цветковых и естественная упорядоченность биологического многообразия, или что такое многообразие (способы описания и интерпретации) // Труды Зоол. института РАН. Приложение № 1, 2009. С. 150–182).

Praglowski J., Punt W. 1973. An elucidation of the microreticulate structure of the exine. *Grana Palynol*. 13: 45–50.

Premathilake R., Nilsson S. 2001. Pollen morphology of endemic species of the Horton Plains National Park, Sri Lanka. *Grana* 40: 256–279. DOI: 10.1080/00173130152987508

Prieu C., Matamoro-Vidal A., Raquin C., Dobritsa A., Mercier R., Goyon P.-H., Albert B. 2016. Aperture number influences pollen survival in *Arabidopsis* mutants. *Am. J. Bot. (Special Issue on the Ecology and Evolution of Pollen Performance)* 103: 452–459. DOI: 10.3732/ajb.1500301

Prokhanov Ya. I. 1949. Celastraceae Lindl. In: *Flora SSSR* [*Flora of USSR*]. Vol. 14. Publishers of Academy of Sciences of USSR, Moscow, Leningrad, 552–577 pp. [In Russian]. (***Проханов Я. И.*** Celastraceae Lindl. // Флора СССР. Т. 14. М.-Л.: АН СССР, 1949. С. 552–577).

Prokhanov Ya. I. 1960. Synopsis of Celastraceae system of USSR. Additions and changes. *Botanicheskiye materialy Gerbariya Botanicheskogo instituta AN SSSR* [*Botan. mater. Herb. Bot. In-ta of Academy of Sciences of USSR*] 20: 409–412 [In Russian]. (***Проханов Я. И.*** Конспект системы бересклетовых СССР. Добавления и изменения // Ботан. матер. Герб. Бот. ин-та АН СССР, 1960. Т. 20. С. 409–412).

Punt W., Hoen P. P., Blackmore S., Nilsson S., Le Thomas A. 2007. Glossary of pollen and spore terminology. *Rev. Palaeobot. Palyn.* 143(1–2): 1–81. DOI: 10.1016/j.revpalbo.2006.06.008

Savinov I. 2007. Some morphological basics for a revision of the tribe *Euonymeae* Loes. (Celastraceae R. Br.). *Wulfenia* 14: 97–104.

Savinov I. A., Baikov K. S. 2007. The analysis of phylogenetic relations in the genus *Euonymus* (Celastraceae R. Br.) using SYNAP method. *Turczaninowia* 10, 3–4: 36–50 [In Russian]. (***Савинов И. А., Байков К. С***. Анализ филогенетических связей в роде *Euonymus* L. (Celastraceae R. Br.) с помощью метода SYNAP // Turczaninowia, 2007. Т. 10, вып. 3–4. С. 36–50).

Schulz B. 2006. Studien zu den Früchten und Semen ausgewählter *Euonymus* – Arten. *Mitt. Dtsch. Dendrol. Ges*. 91: 127–145.

Shimanovich E. I. 1987. *Beresklet* [*Spindle tree*]. Moscow, 64 pp. [In Russian]. (***Шиманович Е. И***. Бересклет. М., 1987. 64 с.).

Shimakura M. 1973. Palinomorfs of Japanese plants. *Spec. Publ. Osaka Mus. Nat. Hist*. 5: 1–60.

Sidorov R. A., Zhukov A. V., Pchelkin V. P., Vereshchagin A. G., Tsydendambaev V. D. 2014. Content and Fatty Acid Composition of Neutral Acylglycerols in *Euonymus* Fruits. *J Am Oil Chem Soc.* 91(5): 805–814. DOI: 10.1007/s11746-014-2425-2

Sidorov R. A., Trusov N. A. 2015. On the possibility of phylogenetic relationship in the genus *Euonymus* in comparison with the data on the LC composition of mature fruit triacylglycerols. In: *"Semicentenary after Konstantin Meyer": XIII Moscow Symposium on Plant Phylogeny: Proceedings of the International Conference* (February 2–6, 2015, Moscow). Moscow, 280–284 pp. [In Russian]. (***Сидоров Р. А., Трусов Н. А.*** О возможности привлечении к решению вопроса филогенетических отношений в роде *Euonymus* данных ЖК-состава триацилглицеринов ариллусов зрелых плодов // Материалы междунар. конф. XIII Московского совещания по филогении растений «50 лет без К. И. Мейера». М., 2015. С. 280–284.)

Simmons M. P., McKenna M. J., Bacon C. D., Yakobson K., Cappa J. J., Archer R. H., Ford A. J. 2012. Phylogeny of Celastraceae tribe *Euonymeae* inferred from morphological characters and nuclear and plastid genes. *Mol. Phylogenet. Evol*. 62, 1: 9–20. DOI: 10.1016/j.ympev.2011.08.022

Till-Bottraud I., Gouyon P. H., Venable D. L., Godelle B. 2001. The number of competitors providing pollen on a stigma strongly influences intraspecific variation in number of pollen apertures. *Evolutionary Ecology Research* 3: 231–253.

Till-Bottraud I., Vincent M., Dajoz I., Mignot A. 1999. Pollen aperture heteromorphism. Variation in pollen-type proportions along altitudinal transects in *Viola calcarata*. *Comptes Rendus de l'Académie des Sciences de Paris, Sciences de la Vie* 322: 579–589. DOI: 10.1016/S0764-4469(00)88528-5

Tzvelev N. N. 2004. Celastraceae. In: *Flora Vostochnoy Yevropy* [*Flora of Eastern Europe*]. Vol. 11. Moscow & St. Petersburg, 437–449 pp. [In Russian]. (***Цвелев Н. Н.*** Celastraceae // Флора Восточной Европы. Т. XI. М.-СПб., 2004. С. 437–449).

Wang F. N., Chein N. F., Chzang T. T. 1960. *Morphology of pollen of Chinese plants*. Beijing, 276 pp. [In Chinese].

Zheng Yan-Chao, Mu Xian-Yun, Li Yan-Nan, Tu Qiang, Zhang Zhi-Xiang. 2012. A Numerical taxonomic study of the *Euonymus* section *Echinococcus* (Celastraceae). *Acta Bot. Yunnanica* 3: 271–286. DOI: 10.3724/SP.J.1143.2012.11183