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Morphological and anatomical study of the fruits of four species of *Stachyurus* Siebold et Zucc. (Stachyuraceae)

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Abstract
The genus *Stachyurus* Siebold et Zucc. has always been considered as the only genus in the Stachyuraceae, but the systematic position of this family is complicated, since it has been included in various orders of flowering plants by vegetative and generative features. A thorough study of the fruits of representatives of the genus *Stachyurus* has not yet been undertaken. This work presents the results of a morphological and anatomical study of the fruits of four species of the genus *Stachyurus*. Morphological and anatomical studies of the fruits of representatives of the genus *Stachyurus* Siebold et Zucc. from the monotypic family Stachyuraceae are carried out. The fruits are considered as many-seeded 4- false-loculed paracarpous berries. A peculiarity of the fruit anatomy of the studied species *Stachyurus* is the lack of differentiation of the mesocarp. It is composed of layers of similar parenchymatous isodiametric cells with a row of vascular bundles in the center. The exocarp and endocarp are monolayers composed of parenchymatous rectangular cells elongated in a tangential direction. Separate mechanical tissues and various inclusions (resins, gums, etc.) were not found. Stachyurus species differ in the features of fruit surface micromorphology: type of surface ultrastructure, presence of pore-like areas in the cuticle, presence and shape of epicuticular wax. The identified carpological features of *Stachyurus* along with other diagnostic features can be taken into account in further systematic and phylogenetic studies of Stachyuraceae.

Key words
Stachyurus, Stachyuraceae, fruit, morphology, pericarp, anatomy, surface ultractructure, species

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Conflict of interest
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Морфолого-анатомическое исследование плодов четырех видов *Stachyurus* Siebold et Zucc. (Stachyuraceae)

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Аннотация
В семейство Stachyuraceae всегда относили один род – *Stachyurus* Siebold et Zucc., но сложным является систематическое положение этого семейства, поскольку его по вегетативным и генеративным признакам включали

в самые различные порядки цветковых растений. Тщательного изучения плодов представителей рода *Stachyurus* еще не проводилось. В данной работе представлены результаты морфологического и анатомического исследования плодов четырех видов рода *Stachyurus*. Проведены морфолого-анатомические исследования плодов представителей рода *Stachyurus* Siebold et Zucc. из монотипного семейства Stachyuraceae. Плоды представляют собой многосеменные 4-камерные (то есть ложно-гнездные) паракарпные ягоды. Отличительной особенностью анатомии плодов изученных видов *Stachyurus* является недифференцированность мезокарпия. Он представлен слоями однотипных паренхимных изодиаметрических клеток с одним рядом проводящих пучков в центре. Экзокарпий и эндокарпий – однослойные, представленные паренхимными прямоугольными клетками, вытянутыми в тангентальном направлении. Отдельные механические ткани и различные включения (смолы, гумми и т.д.) не обнаружены. Виды *Stachyurus* отличаются по признакам микроморфологии поверхности плодов: типу ультраструктуры поверхности, наличию поровидных участков в кутикуле, наличию и форме эпикутикулярного воска. Выявленные карпологические особенности *Stachyurus* наряду с другими диагностическими признаками могут быть приняты во внимание при дальнейших систематических и филогенетических исследованиях Stachyuraceae.

Ключевые слова
Stachyurus, Stachyuraceae, плод, морфология, перикарпий, анатомия, ультраструктура поверхности, вид

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Introduction
Введение

The monotypic family Stachyuraceae was described by J.C. Agardh in 1858. The genus *Stachyurus* Siebold et Zucc. currently contains 10 species [1, 2]. Only one species grows in Japan (*S. praecox* Siebold et Zucc.). The remaining nine species are widespread-in China, mainly in the southern and eastern regions of this country, and in neighboring countries. The family Stachyuraceae is thought to have originated in the ancient Sikang-Yunnan Plateau, from where it spread westward to Burma, India (Assam), Nepal and Bhutan, and eastward through southwest and southern China to Taiwan and Japan[1].

Almost all Stachyuraceae are evergreen or deciduous shrubs, sometimes climbing, only S. himalaicus is a small tree up to 10 meters in height. Leaves are simple, alternate, serrate to serrulate, with pinnate venation; stipules are small, early caducous. Flowers are small, bisexual or functionally unisexual and then plants dioecious, actinomorphic, shortly pedicellate or sessile, 4-merous [3], collected in pendulous or erect axillary racemes or spikes[2]. Anthers versatile, introrse, opening throughlongitudinal slits. Pollen grains are 3-colporate [4]. Gynoecium with four carpels, they are congenitally united at the base, free only at the top, with short style, stigma large, capitate, the four free carpel tips are postgenitally united and form a single stigmatic surface [4]. The superior, syncarpous ovary is 4-locular and with axile placentation in the lower part (the synascidate zone), but in the middle and upper parts (the symplicate zone) the partitions (the four septa) are incomplete and the ovules are borne on deeply intruded placentas [4, 5]. The ovules are numerous, arranged in two alternating rows in each carpel, bitegmic, crassinucellate [5], anatropous and syntropous[3]. Fruit berry-like[4][5],, with leathery pericarp and deciduous calyx [3]. Fruit anatomy has not been studied. Seeds numerous, arillate, with small, straight embryo[6], with copious, fleshy, oily and proteinaceous endosperm and short [3, 5], fleshy funicle, 2n = 24 [4].

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In the 20th century the taxonomists treated the family Stachyuraceae in different orders of flowering plants. Some researchers[7][8][9],[10],, placed Stachyuraceae near to the Flacourtiaceae family in the order Vilolales. According to the flower and sporoderm morphology, *Stachyurus* shows maximum similarity with Flacourtiaceae, especially with the tribe Scolopieae[11]. According to the characters of wood anatomy[12], Stachyuraceae resembles both Flacourtiaceae and Hamamelidaceae. The family Stachyuraceae was included in the order Hamamelidales by J. Hutchinson[13][14],. A. Cronquist 9 notes that the *Stachyurus* inflorescence resembles *Populus* (Salicaceae) and believes that the ancestors of both orders Violales and Salicales were very similar. However, such characters as placentation type (intrusive parietal, but not parietal one), the fruit morphology (a berry with distinct locules formed by fully fused septas), and particularly the seed structure (spermoderm is derived only from the outer integument) make Stachyuraceae much closer to Theales, and especially to the family Theaceae[15][16][17],[18],,.

A new stage in the study of Stachyuraceae began in 2002. The investigation of pollen (shape, size, aperture type and exine sculpture) of 37 species belonging to 19 genera from seven families (Stachyuraceae, Dilleniaceae, Actinidiaceae, Saurauiaceae, Ochnaceae, Staphyleaceae and Theaceae) with light and scanning electron microscopy[19] showed that Stachyuraceae, Staphyleaceae and Ternstroemoideae (Theaceae, but now often accepted as a separate family of Ternstroemiaceae) are closer to each other than to the rest of the studied families. According to cladistic analysis of Stachyuraceae and related families (Dilleniaceae, Ochnaceae, Theaceae, Actinidaceae and Staphyleaceae) in which 72 characters including of plant growth habits, external morphology, wood anatomy, flower structure, pollen morphology, embryology and chromosome number were analyzed using maximum parsimony method[20]. The family Stachyuraceae is shown to be more closely related to Staphyleaceae than to the families Actinidiaceae, Dilleniaceae, Ochanceae and Theaceae. Representatives of the new order Crossosomatales, first proposed by A.L. Takhtajan [8], are also actively involved in the investigation. Based on the study of the rbcL plastid DNA sequence, it was found that all four genera of the family Crossosomataceae form a well-supported clade with representatives of the families Stachyuraceae and Staphyleaceae [5, 6, 7]. In the APGII [8] and APG IV [9] systems the family Stachyuraceae is placed in the order Crossosomatales close to the family Staphyleaceae [1, 10].

The purpose of this work is to describe the morphological, anatomical structure of the fruits and their surface ultraculture in four Stachyurus species in order to identify their common features and differences that can be used with other diagnostic features for a more thorough consideration from different sides of the question of the systematic position of Stachyuraceae.

Research method
Методика исследований

The fruits of four species of Stachyurus – *S. chinensis* Franch, *S. himalaicus* Hook.f. e*t Thomson, S. praecox* Siebold et Zucc., *S. yunnanensis* Franch were studied. The fruits were obtained from the carpological collection and herbarium of the Komarov Botanical Institute the Russian Academy of Sciences (St. Petersburg, Russia).

Since the material from the herbarium is dry, to restore the normal cellular organization, the fruits were softened for 10 days in a thermostat in a mixture of Strasburger: 90% ethyl alcohol, glycerin and water in the same proportions. The study of the morphology of fruits was carried out using binoculars of different resolution, since the external and sometimes internal structure of the fruits was studied [11]. The terminology used to describe the morphology of fruits is given here according to the source[21].

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To study the anatomy of the fruits, specimens were made according to the generally accepted methodology[22]. The fruits were poured into paraffin blocks. Fruit sectioning was performed in two ways – manually with a razor blade and for thinner and clearer sections a sliding microtome was used. For further work, only cross sections of mature fruits in their middle part (10-25 μm thick) were taken. To determine the degree of lignification of the cell walls in different areas of the pericarp, a lignin detection reaction was carried out by adding phloroglucin and hydrochloric acid. For the possible identification of different cellular structures and inclusions, as well as their possible chemical composition, staining reactions with I-KI solution and Sudan III were performed according to the accepted rules [22]. Fully prepared and stained specimens were placed in a drop of glycerin on a microscope slide and covered with a coverslip. Excess glycerin around the edges of the slide was removed with a napkin. The edges of the coverslips were fixed with varnish for reliable preservation of more successful specimens.

The prepared specimens were examined at various magnifications of the "Biolam" light microscope. In order to get a more detailed picture of the anatomical structure of the fruit, the "RA-4" plotter was used. The study of the micromorphology of the fruit surface was carried out using a HITACHI S-405A electron scanning microscope [11]. The main ultrastructural features of the fruit surface were noted and micrographs were taken.

The carpological descriptions of *Stachyurus* species are given in a previously accepted specific order [11].

Results and discussion

Результаты и их обсуждение

***Stachyurus chinensis* Franch.** The fruit is a berry, glabrous, globose, 6-7 mm in diameter, with the remaining long curved column up to 2.5 mm long (Fig. 1a). The fruit surface is leathery, thin. On the surface of ripe fruits, protrusions are noticeable due to the increase in size of fully developed numerous seeds. The fruit is 4-loculed, with numerous seeds on intrusive placentas. The seeds are small, curved, up to 1.5 mm in diameter, with fleshy funicular aril.

The pericarp consists of 10-16 layers of cells (Fig. 2a). The exocarp is represented by a single-layered epidermis of rectangular thin-walled cells, elongated in the tangential direction. The mesocarp is unspecialized and consists of 8-14 layers of parenchymatous cells of isodiametric shape. A single circle of vascular bundles is located in the middle layers of the mesocarp. The endocarp consists of a single-layered epidermis of thin-walled, tangentially elongated cells.

The ultrasculpture (or primary sculpture) is wavy. The cuticle is continuous; its ridges are visible. Epicuticular wax is in the form of granules (Fig. 3a).

***Stachyurus himalaicus* Hook.f. et Thomson.** The fruit is a berry, subglobose, 7-8 mm in diameter, with the remaining long column up to 2.5 mm long (Fig. 1b). The pericarp is leathery and it is so thin that numerous protruding seeds can be seen on the surface of the ripe fruit. The fruit is 4-locular, with numerous seeds on intrusive fused placentas (Fig. 1e). The seeds are small, curved, up to one mm in diameter, with a fleshy aryl.

The pericarp consists of 20-26 layers of cells (Fig. 2b). The exocarp is represented by a single-layered epidermis of rectangular thin-walled cells that are elongated in the tangential direction. The mesocarp is unspecialized and consists of 18-24 layers of isodiametric parenchymatous cells. A single circle of vascular bundles is localized in the middle layers of cells. The endocarp is formed by a single-layered epidermis of thin-walled cells, strongly elongated in the tangential direction.

The surface of the fruit is wavy. The cuticle is continuous; its thickenings are visible as ridges. The epicuticular wax is in the form of small granules (Fig. 3b).

***Stachyurus praecox* Siebold et Zucc.** The fruit is a berry, globose or broadly elliptic, 7-12 mm long (including a small residual column) and up to 9 mm in diameter (Fig. 1c). The pericarp is leathery; seed protrusions are visible on the surface of the fruit. The fruit is 4-locular, with numerous seeds on intrusive fused placentas. The seeds are small, curved, up to 1.5 mm in diameter, with a fleshy aryl.

The pericarp consists of 22-27 layers of cells (Fig. 2c). The exocarp is represented by a single-layered epidermis, consisting of thin-walled rectangular cells that are slightly elongated in the tangential direction. The mesocarp is unspecialized and consists of 20-25 layers of isodiametric parenchymatous cells. The vascular bundles are found in the middle layers of the mesocarp cells and form a single circle. The endocarp is represented by a single-layered epidermis of thin-walled cells, elongated in tangential direction.

The surface of the fruit is wavy. The cuticle is continuous. Granules of epicuticular wax form separate clusters (Fig. 3c, Fig. 3d).

***Stachyurus yunnanensis* Franch.** The fruit morphology of *S. yunnanensis* is similar to *S. himalaicus* – it is almost globose up to 7 mm in diameter, with a long column remaining up to 2.5 mm in length (Fig. 1d). The main difference is that *S. yunnnanensis* has larger protrusions of seeds on the surface of the fruit.

The pericarp consists of 22-30 layers of cells (Fig. 2d.). The exocarp is formed by a single-layered epidermis of thin-walled, rectangular, tangentially elongated cells. The mesocarp is unspecialized and consists

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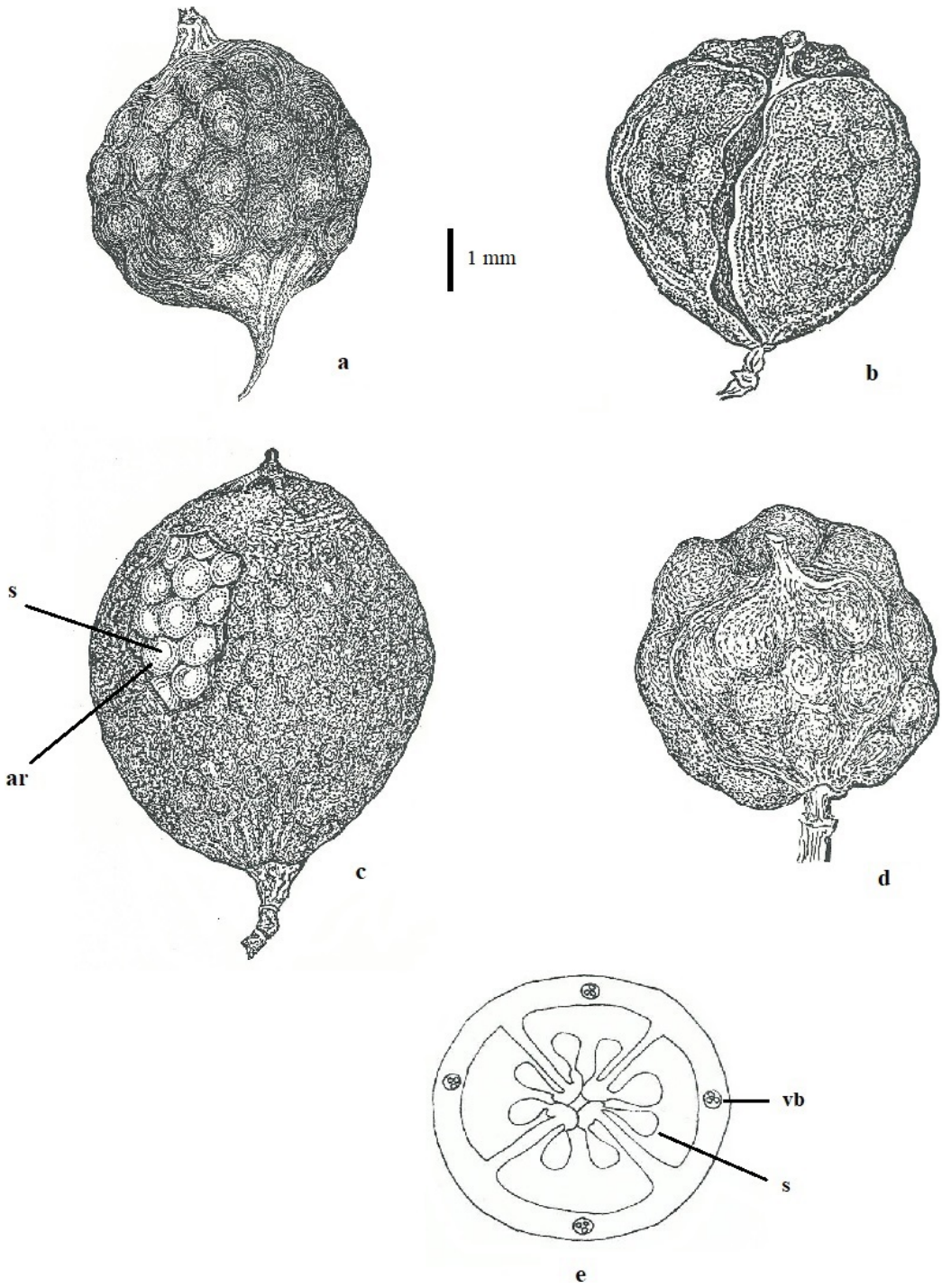

Fig. 1. Fruit morphology:
a – *Stachyurus chinensis* Franch.; b – *S. himalaicus* Hook.f. et Thomson; c – *S. praecox* Siebold et Zucc.; d – *S. yunnanensis* Franch.;. e – schematic transverse section in the middle of an unripe fruit *S. himalaicus* Hook.f. et Thomson (scheme). Scale bar is one mm. Legend: vb – vascular bundles, s – seeds on intrusive on intrusive fused placentas (in the lower and middle parts of the fruit) – 1e, and mature seeds – 1c, ar – fleshy funicular aril

of 20-28 layers of isodiametric parenchymatous cells. A circle of vascular bundles is localized in the middle layers of cells. The endocarp consists of a single-layered epidermis of thin-walled cells, highly elongated in the tangential direction.

The surface of the fruit is striated. The cuticle is continuous and its ridges are visible. Epicuticular wax in the form of granules (Fig. 3d, Fig. 3e).

The fruits of the Stachyuraceae are shown to be polyspermous berries with four locules. Seed protrusions can be seen on the surface of the fruit, indicating that the pericarp is thin, not composed of numerous layers of cells, and it does not have mechanical tissues. In total, the pericarp consists of 10-30 cell layers. The exocarp of all *Stachyurus* species studied is single-layered and consists

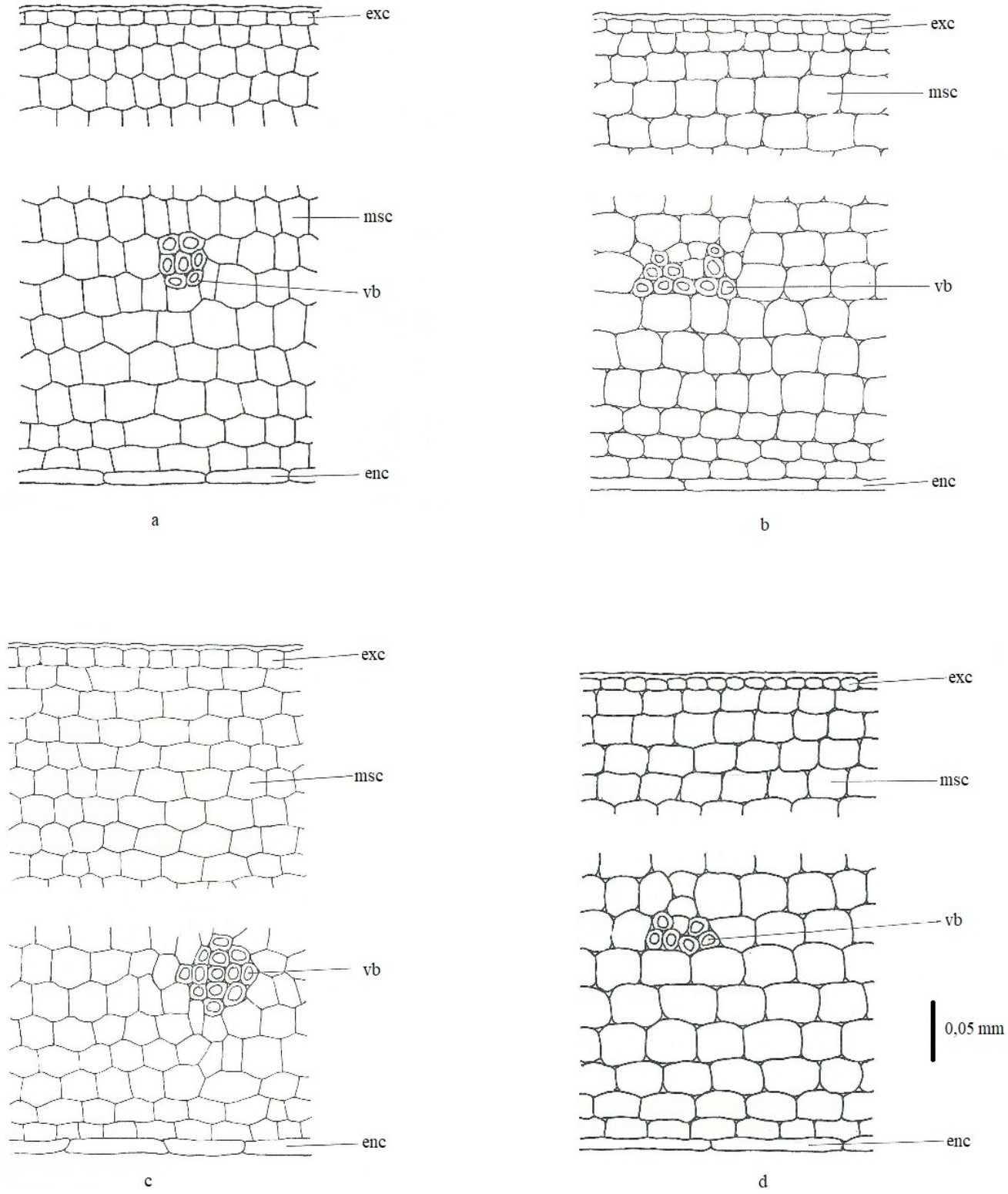

Fig. 2. Fruit anatomy:
a – *Stachyurus chinensis* Franch.; b – *S. himalaicus* Hook.f. et Thomson; c – *S. praecox* Siebold et Zucc.; g – *S. yunnanensis* Franch. Scale bar is 0.05 mm.
Legend: exc – exocarp, msc – mesocarp, enc – endocarp, vb – vascular bundles

of thin-walled rectangular cells. The mesocarp is unspecialized and undifferentiated, and in fact it is a zone composed of layers of thin-walled parenchymatous cells of isodiametric shape. The endocarp is single-layered and is represented by thin-walled cells elongated in the tangential direction. Representatives of Stachyuraceae are characterized by wavy, rarely- striate (S. yunnanensis) ultrasculpture of the pericarp surface. The cuticle is continuous. Epicuticular wax is deposited in the form of granules (*S. chinensis*, *S. yunnanensis*), small granules (*S. himalaicus*) or separate clusters (*S. praecox*).

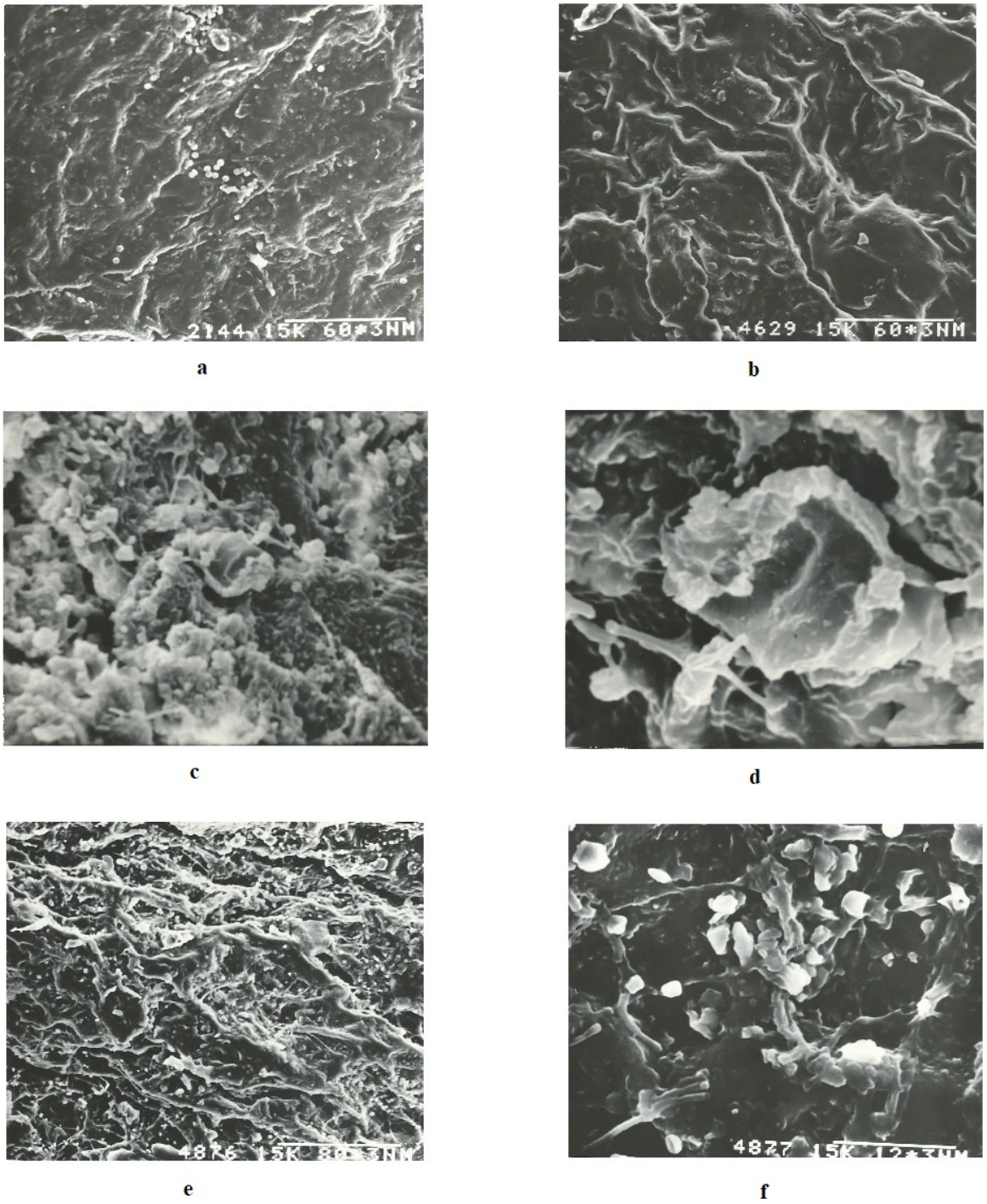

Fig. 3. Fruit surface micromorphology:
a – *Stachyurus chinensis* Franch. (increase × 500); b – *S. himalaicus* Hook.f. et Thomson (× 500);
c – *S. praecox* Siebold et Zucc. (× 500); d – *S. praecox* Siebold et Zucc. (× 1000);
e – *S. yunnanensis* Franch. (× 380); f – *S. yunnanensis* Franch. (× 1000)

Conclusions

Выводы

According to the revealed carpological features (polyspermous fruit, unspecialized mesocarp, lack of mechanical tissues and absence of secretory substances (in cells, in cavities) in the pericarp) and morphological characters (scalariform perforation plates with many bars, anomocytic stomata), the family Stachyuraceae is an unspecialized group of plants. The different species of *Stachyurus* can be distinguished by the characters of fruit surface micromorphology – the type of the pericarp surface ultrasculpture and the presence and shape of the epicuticular wax. An interesting preliminary conclusion is that all the studied fruit samples of the four Stachyurus species are quite archaic in terms of their anatomical features. The revealed new features of the fruit structure supplemented the data on the reproductive biology of the Stachyuraceae.

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