Comparative anatomical study of the fruits of *Angelica pancicii* and *A. sylvestris* (*Apiaceae*) distributed in the Bulgarian flora

Elina Yankova

Institute of Botany, Bulgarian Academy of Sciences, Acad. G. Bonchev St., bl. 23, 1113 Sofia, Bulgaria

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Abstract. Anatomical study of the mature fruits of *Angelica pancicii* and *A. sylvestris* from natural Bulgarian populations was carried out. Some important specific peculiarities were established. The three dorsal ribs of the fruit are more clearly expressed in *A. pancicii* than in *A. sylvestris*. The two lateral ribs of the fruit of *A. sylvestris* are considerably longer and narrower, while in *A. pancicii* they are wider, but shorter.

Key words: anatomy, *Angelica, Apiaceae*, mericarp

Classification of Umbelliferous plants is based on the peculiarities of inflorescence, umbel florets and fruits. These characteristics and especially the structure of fruits are of reliable diagnostic importance. Researchers today tend to use a maximum accessible variety of characteristics relating to the structure of all parts of the plants, although for Umbelliferous plants and especially for the taxonomically rather controversial genus *Angelica* the analysis of anatomical and carpological characteristics holds a priority.

The anatomy of fruits of the different representatives of family *Apiaceae* varies strongly, even between the species of one and the same genus.

So far the anatomy of fruits within genus *Angelica* L. (syn. *Archangelica* Wolf) has been studied by Briquet (1923), Denisova (1961), Tikhomirov & Galakhova (1965, 1967), Pimenov & Kljuykov (2002). No such study has been conducted for *A. pancicii* Vandas. Considering the fact that *A. pancicii* and *A. sylvestris* L. are medicinal plants that concentrate the biologically active substances chiefly in their fruits, the study of their anatomy is quite significant.

This article presents the results of the conducted comparative anatomical study of the fruits of these two species from natural Bulgarian populations.

Material and methods

The studied material, ripe fruits of the species *A. pancicii* and *A. sylvestris* from the central umbel and umbels of the 1st order, was collected from seven natural populations (Table 1) in the period September-October of two consecutive years (2001–2002).

The measurements included 30 seeds per population in three repetitions.

A binocular looking glass and an Amplival light microscope were used for observation of the manual cuts and durable microscopic preparations made after the classical paraffin methods (Nikolov & Daskalov 1966). The staining was made with Heidenhain's hematoxylin.

In the statistical processing of biometric data the Student's criterion was applied (Zaitsev 1984).

Table 1. Origin of the species studied

Species	Population N	Region
A. pancicii	1	Balkan Range (*Western*), around Petrohan, SOM 156911
	2	Mt Vitosha, around Aleko mountain hostel, SOM 156909
	3	Rila Mts, in the region of Borovetz resort, SOM 156910
	4	Mt Sredna Gora (*Western*), Lozenska planina, SOM 159108
A. sylvestris	5	Northeast Bulgaria, between Zhulud and Arkovna villages, SOM 159117
	6	Balkan Range (*Central*), Vezhen summit, SOM 159114
	7	Mt Vitosha, Vladaya village, SOM 159118

Results and discussion

The fruits of *A. pancicii* and *A. sylvestris* consist of two monospermic mericarps linked ventrally by a commissure, which at break-up hang on a common stalk (carpophore). The seed suture widens tangentially and a small bicollateral vascular bundle is observed in it.

In most investigated fruits the two mericarps show an absolutely equal stage of development (Plate I, Fig. 1). In isolated cases, one of the mericarps is smaller in size, and neither an embryo, nor endosperm is observed in it (Plate I, Fig. 2). A similar phenomenon has been also observed in *Archangelica decurrens* Ledeb. by Denisova (1961). Kozo-Poliansky (1914) maintained that the reduction of one carpophyll testified to a greater phylogenetic advancement of these species.

The mericarp is slightly flattened ventrally, with a ventral commissural suture, ovate to broadly oval, or nearly orbicular in shape, dorsally convex. It has five ribs: three dorsal and two lateral ones, which undergo morphological changes in the process of ontogenesis.

The ribs are heteromorphic. The dorsal ribs that are shaped like cones with rounded apexes are equal in size and comparatively low. The two lateral ribs are higher and wider, lengthened parallel to the commissural axis and thus acquiring a wing-like shape. Owing to this, some authors determine them as wings (Denisova 1961).

In *A. pancicii* the three dorsal ribs are wider and more convex than those in *A. sylvestris* (Plate II, Figs 1, 2). The two lateral ribs in *A. sylvestris* are considerably more lengthened and narrower, almost filiform at the ends in some mericarps (Plate II, Fig. 4), and in *A. pancicii* they are wider but shorter (Plate II, Fig. 3).

A biometric analysis of these data has shown the following:

Of the morphometriacally measured characteristics most strongly varied the width of the dorsal and lateral rib and the size of epidermal cell (V = 69.52 % for the width of the dorsal rib in the population N 5 of *A. sylvestris*; V = 65 % for the size of the epidermal cell in the same population, and V = 61.70 % for the width of the lateral rib in a population N 1 of *A. pancicii.* The length and width of the mericarp showed moderate variability (V = 39.7 % for the mericarp width in a population N 7 of *A. sylvestris*; V = 25.53 % for the mericarp length in the same population), while the length of lateral rib and thickness of the cuticle showed low intrapopulation variability (V = 15.92 % for the length of lateral rib in a population N 5 of *A. sylvestris* and V = 15.10 % for the cuticle thickness in a population N 4 of *A. pancicii.*.

On an interspecies level, the length and width of the mericarp, the length of lateral rib and the width of dorsal rib showed higher variability in *A. sylvestris*; while the width of lateral rib and the size of the epidermal cell were more variable in *A. pancicii*.

On an interpopulation level among the populations of *A. sylvestris,* the length and width of the mericarp, the width of lateral rib and the thickness of cuticle varied the strongest in the population N 7, while the width of dorsal rib, the length of lateral rib and the size of the epidermal cell varied most strongly in the population N 5. Among the populations of *A. pancicii,* the length and width of the mericarp varied the strongest in the population N 4; the length of lateral rib, the width of dorsal rib and the size of the epidermal cell varied most in the population N 2; the width of lateral rib in the population N 1; and the thickness of cuticle in the population N 3.

Of the analysed morphological characteristics, we have considered the width of lateral and of dorsal rib, and the length of lateral rib as taxonomically important for differentiation of the two species. Judging by these parameters, the proven differences between the two species according to Student's criterion amounted to 100 % in relation to the width of lateral and dorsal ribs, and to 91.66 % in relation to the length of lateral rib.

Table 2. Morphological variability of the fruits of *A. pancicii* and *A. sylvestris* (statistical variation analysis)

Species	Populat. N	Mericarp length		Mericarp width		Lateral rib length		Lateral rib width		Dorsal rib width		Epidermal cell size		Cuticle thickness	
		M±m, cm	V, %	M±m,cm	V, %	M±m,c m	V,%	M±m, cm	V, %	M±m, cm	V,%	M±m, cm	V, %	M±m ,cm	V, %
A. pancicii	1	0.50±0.01	10.92	*** 0.43±0.01	14.55	0.091±0.001	9.23	*** 0.047±0.003	61.70	*** 0.038±0.002	14.92	0.002±0.00004	20.00	0.00026±0.003	11.53
	2	0.51±0.004	7.88	* 0.43±0.02	9.84	* 0.094±0.001	13.72	*** 0.048±0.004	59.80	*** 0.038±0.0001	48.29	0.0025±0.0001	60.24	0.00026±0.003	12.69
	3	0.52±0.002	13.00	*** 0.41±0.002	19.70	0.093±0.003	10.75	*** 0.048±0.002	45.83	*** 0.038±0.001	47.82	0.0019±0.004	21.05	0.00027±0.003	11.85
	4	0.50±0.1	13.00	*** 0.47±0.01	15.29	0.094±0.01	6.91	*** 0.052±0.001	25.00	*** 0.04±0.001	18.50	0.0019±0.0002	10.10	0.0003±0.00004	15.10
A. sylvestris	5	*** 0.62±0.01	9.34	*** 0.45±0.01	10.44	*** 0.103±0.002	15.92	*** 0.04±0.0004	10.50	*** 0.021±0.002	69.52	0.0016±0.001	65.00	0.00032±0.003	10.12
	6	0.5±0.01	12.00	*** 0.37±0.01	12.63	*** 0.111±0.002	12.61	*** 0.039±0.001	17.18	*** 0.023±0.002	46.20	* 0.0022±0.0001	22.94	0.0003±0.003	10.94
	7	*** 0.47±0.01	25.53	*** 0.38±0.02	39.70	*** 0.115±0.002	13.04	*** 0.037±0.001	45.83	*** 0.024±0.003	45.83	0.0024±0.0003	12.65	0.00025±0.003	12.75

*** P = 5 %; *** P = 0.1 %**

Concerning the length and width of the mericarp, the proven differences amounted to 66.66 % in relation to the mericarp width and to 50 % in relation to its length. This means that these two characteristics are not so important as the above-mentioned three for distinct differentiation of *A. pancicii* from *A. sylvestris*.

The size of the epidermal cell and cuticle thickness are almost equal in both species. This was supported by the statistical data processed according to Student's criterion, with proven differences only in 8.33 % of the cases for the size of the epidermal cell and in no case for the cuticle thickness.

The morphological characteristics analysed on interpopulation level differ stronger in *A. sylvestris*, where the cases with mathematically proven differences in the characteristics between the different populations are more numerous as compared to *A. pancicii*: in 66.66 % for *A. sylvestris* against 50 % for *A. pancicii* in relation to mericarp length and width of the dorsal rib; in 33.33 % for *A. sylvestris* against 16.66 % for *A. pancicii* in relation to mericarp width; in 66.66 % for *A. sylvestris* against 16.66 % for *A. pancicii* in relation to the lateral rib; in 33.33 % for *A. sylvestris* against 0 % in *A. pancicii* in relation to width of the lateral rib and cuticle thickness. Only the size of the epidermal cell showed greater difference between the populations of *A. pancicii* as compared to *A. sylvestris*: the differences are mathematically proven only for *A. pancicii* (Table 2).

The difference in measured width of the lateral and dorsal ribs and length of the lateral rib between the two species has been proven almost to 100 %, while on interpopulation level this difference was not so clearly expressed.

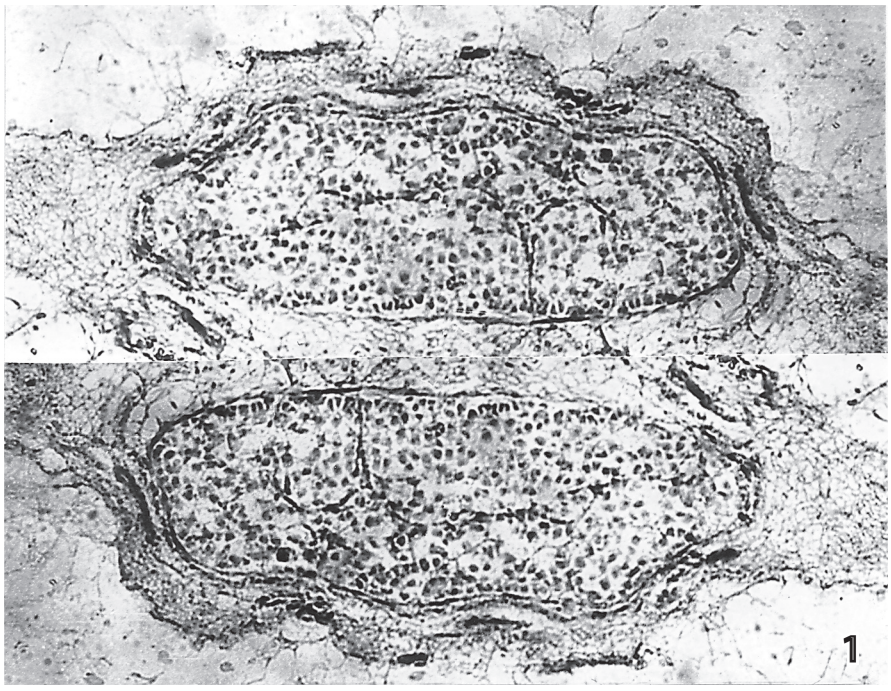
Mericarp structure in the two investigated species is typical for the representatives of family *Apiaceae*. It is built of three main parts: exocarp, mesocarp and endocarp.

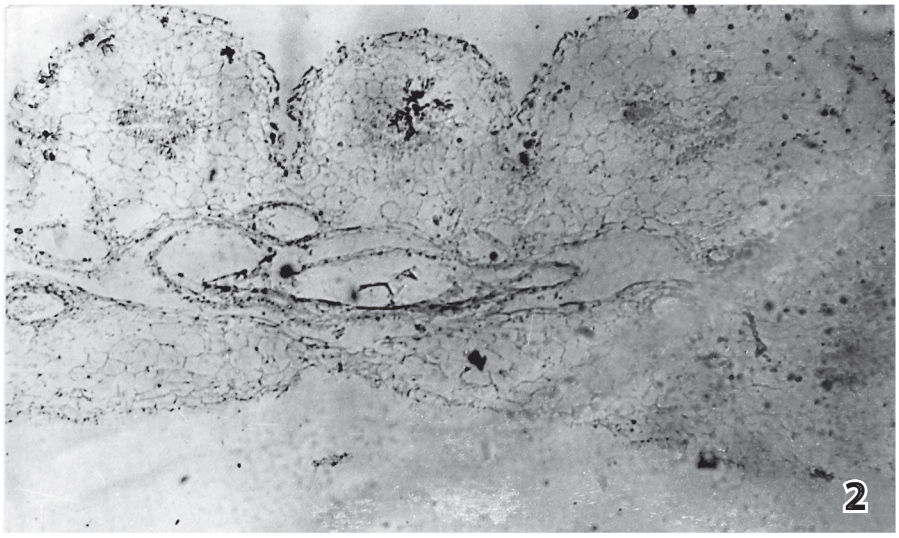
Exocarp: it is one-layered, built of rectangular epidermal cells. It encloses the mericarp, breaking out only at the commissure. The exocarp cells are slightly widened tangentially. Their outer walls are faintly thickened, with small cuticular ribs. In some epidermal cells papillae occasionally formed, similarly to *A. decurrens* (Denisova 1961). The exocarp is followed by the powerful ring of the mesocarp which constitutes the main mass of the mericarp.

Mesocarp: its development was described in detail by Briquet (1923) for *A. officinalis* Hoffm., who differentiated four layers of the mesocarp: the chlorenchyma, epicarp, sculptural parenchyma, and in-depth parenchyma.

In the ripe fruits of *A. pancicii* and *A. sylvestris*, of the four layers, the sculptural and the in-depth parenchyma have been observed. The sculptural parenchyma constitutes the main layer of the mesocarp and is built of sclerified cells with thickened walls and porous fissures. According to Pervukhina (1950), such already dead and filled in with air cells serve to supply moisture to the developing seed and reduce the fruit weight. Furthermore, they also ensure good thermal isolation of the seed. The other layer of the mesocarp, the so-called in-depth parenchyma, is built of large thin-walled cells. In the fossae between the ribs and at their base, stretches of unsclerified cells remain,

Plate I

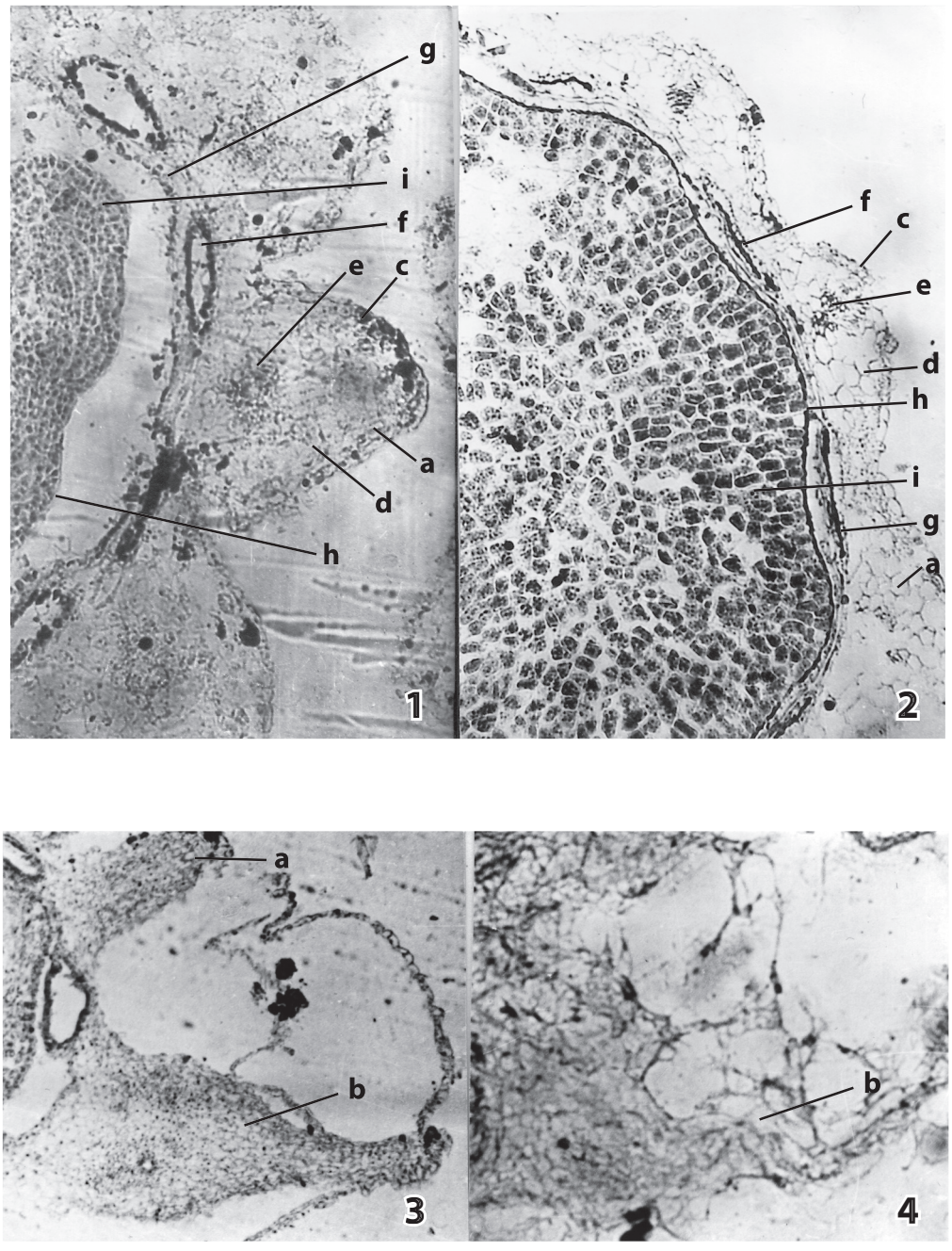




Figs 1-2. Transversal cut of the fruits of *A. pancicii* and *A. sylvestris*.

1, a fruit with equally developed mericarps of *A. sylvestris*; **2**, a mericarp with missing embryo and endosperm of *A. pancicii*.

Plate II



Figs 1-4. Transversal cut of a mericarp of *A. pancicii* and *A. sylvestris.*
1, 3, *A. pancicii*; **2, 4**, *A. sylvestris*: ***a*** – dorsal rib; ***b*** – lateral rib; ***c*** – exocarp; ***d*** – mesocarp; ***e*** – vascular bundles in the ribs; ***f*** – essential oil ducts; ***g*** – endocarp; ***h*** – episperm; ***i*** – endosperm.

and one or two layers of such cells usually reach up to the top of the ribs.

Endocarp: it is one-layered, built of large tangentially elongated cells and outlines the internal boundary of the pericarp.

Above the endocarp, in the in-depth parenchyma of the mesocarp, six large schyzogenous essential oil ducts of the groove type are situated in both species: two on the commisural and four on the dorsal side of the fruit. They all are tangentially widened and covered inside with one layer of epithelial cells, over 10 in number in both investigated species. Denisova (1961) identified four to six epithelial cells in *A. decurrens*. In single fruits, extremely seldom, five essential-oil ducts have been observed, which could be explained by obliteration and destruction of one of the ducts (Tamamshian-Denisova 1961). The same number (6) of essential-oil ducts were established by Tikhomirov & Galakhova (1965) in *A. sylvestris.* This gives us grounds to assume that the number of essential-oil ducts in both investigated species is permanent, contrary to *A. decurrens*, which has a greater number of essential-oil ducts (Denisova 1961). Similar variation in the number of essential-oil ducts within the framework of one genus was established in genus *Lilaeopsis*: from six in some species to a greater number in others (Affolter 1985).

Vascular bundles are located in the ribs, named "mestomes" by Kozo-Poliansky (1914). They are single, collateral, almost orbicular, slightly widening radially in the dorsal ribs, and tangentially in the lateral ones, where vascular bundles are separated from the endocarp by 3–5 layers of cells, and in the dorsal ribs with 2–4 layers. During the bundle development its phloem divides into two parts, with a xylem between them.

The epidermis is built of what has remained of the integument, represented by 3–4 layers of cells on the ventral side of fruit and one layer on the dorsal side. It fits tightly to the endocarp. On the commissural side it gets detached from the seed and one can observe there that the outer layer, which is best preserved and represents the integumental epidermis, is formed of one layer of thin-walled cells. Such a structure of epuidermis was established in other representatives of genus *Angelica* (Denisova 1961), as well as in representatives of other genera of *Apiaceae* (Gupta & Gupta 1964; Affolter 1985).

In the mature fruits of *A. pancici i*and *A. sylvestris* the endosperm occupies the entire space under the seed cover. It is built mosaic-like of large rectangular cells, many of which have polyploidised and formed single spots in the endosperm tissue. A transverse cut of the endosperm is kidney-shaped, convex in the centre and slightly flattened towards the endocarp. Considering this characteristic, we can refer the fruits of the two investigated species to the *Orthospermae*-type, according to the classification of Koch (Denisova 1961). To the same type are also referred the fruits of *Aegopodium podagraria* L., *Pastinaca sativa* L. and *Heracleum* sp. studied by Alexandrov & Klimochkina (1947).

The embryo in the two investigated species is small: 0.5–0.6 mm in length and 0.2–0.3 mm in width. Considering the fact that the mature mericarp measures 0.5–0.6 cm in length and 0.4–0.5 cm in width, we could maintain that the embryo in *A. pancicii* and *A. sylvestris* is 10 times smaller than the seed. It is situated in an elongated ovate cavity in the frontal part of the endosperm. The mature embryo of the two investigated species could be referred to the third (axial) division of linear type, according to the classification of Martin (1946).

Conclusion

A comparative anatomical analysis of the ripe fruits of the species *A. pancicii* and *A. sylvestris* has shown the following:

The fruit conforms to the main differentiation lines characteristic of the fruits of family *Apiaceae*.

The two investigated species differ in some morphometric characteristics of the ribs. The dorsal ribs are wider and more convex in *A. pancicii*, while the lateral ribs are longer in *A. sylvestris*.

The morphological characteristics of lateral and dorsal rib width and lateral rib length differed distinctly in the two investigated species and this was proven by the statistical data processing. The same characteristics are not so distinct on interpopulation level, being somewhat more pronounced between the populations of *A. sylvestris*. This gives us grounds to assume that these characteristics are taxonomically important for differentiation of the two species.

Intrapopulation variability of the morphological characteristics of fruits in the two investigated species of genus *Angelica* was expressed stronger in *A. sylvestris*. Differences in these characteristics between the populations of the various species are also more distinct between the populations of *A. sylvestris*.

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