Caryophyllales:
New Insights into the Phylogeny, Systematics and Morphological Evolution of the Order

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Caryophyllales: New Insights into the Phylogeny, Systematics and Morphological Evolution of the Order. Proceedings of the Symposium held on 24th-27th September 2012 at Moscow M.V. Lomonosov State University

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The articles in this Symposium volume emphasize the most important problems and results of molecular phylogeny, diversity, photosynthetic pathways, ecology, morphology and anatomy within the order Caryophyllales. The work presents recent results from fundamental research in theoretical botany and provides a basis for applying biology to a changing world, especially with regard to the radiation and evolution of taxa with a global distribution such as Chenopodiaceae and Amaranthaceae, with their important role in natural and disturbed plant communities. New versions of the phylogeny of many taxa are included, and some taxa are lectotypified in this publication. We believe that this book will be useful for future investigations of phylogeny and evolutionary trends in the plant sciences.

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Images on the title page (clockwise from top left): *Rivina brasiliensis*, *Atriplex holocarpa*, *Enchylaena tomentosa*, semi-desert landscape at Elton Lake (Volga–Ural Interfluvial)

Photos from A.P. Sukhorukov

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FRUIT ANATOMY OF *ANTHOCHLAMYS* (CHENOPODIACEAE/AMARANTHACEAE)

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Introduction

The genus *Anthochlamys* comprises 5-6 species distributed in the Irano-Turanian floristic region. Almost all the taxa can be found in Iran and West Afghanistan. Only *A. tjanschanica* seems to be isolated from other species (Tien-Shan Mountains: Eastern Kazakhstan, Kyrgyzstan and North Tajikistan: Sidorenko et al., 1968). All species are annuals with ridged or sulcate stems that are glabrous or covered in the upper part with simple or branched hairs. The leaves are short-petiolate or sessile, entire, lanceolate or elliptic, and are mostly basally arranged. The inflorescence is a spike. Each bisexual flower is supported by a small bract and consists of a white or pinkish perianth of 5 free segments, 5 stamens and a superior ovary with two stylodia. The fruit is indehiscent, single-seeded, rounded (2.5–4.5 mm in diameter), flattened, in its seed-containing part convex on both sides, and with a prominent, thin, wing-like pericarp outgrowth on the margin that can form a small sinus at the top of the fruit between two stylar remnants (Fig. 1A). The seed has a vertical embryo and copious perisperm. The diagnostics of the genus are mostly based on prominence of stem ridges, leaf shape and presence of apical sinus in the fruit (Aellen, 1950; Hedge, 1997).

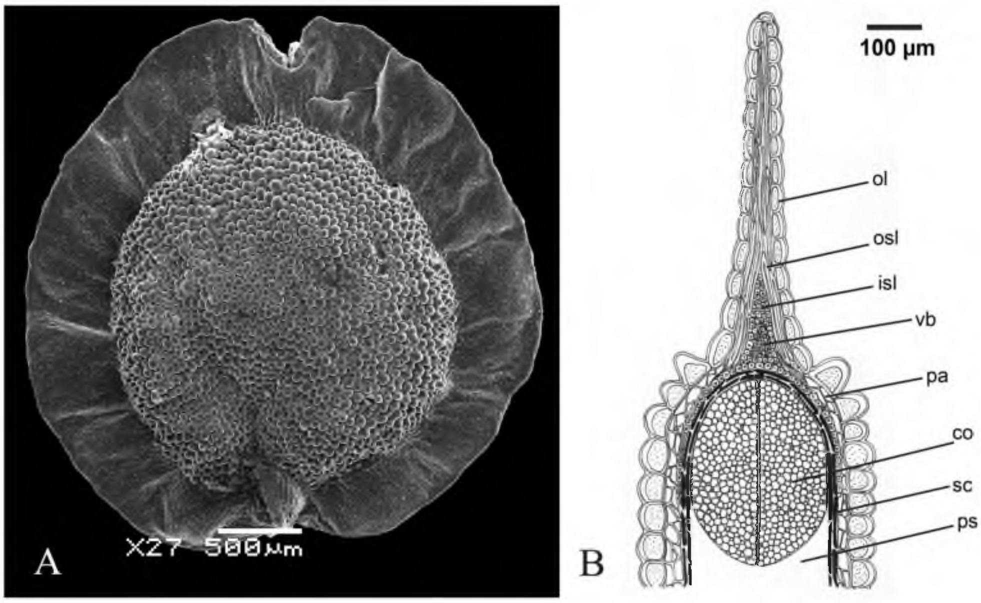

Fig. 1. A. Fruit of *Anthochlamys turcomanica*. Scale bar: 500 µm; B. Cross-section of *A. polygaloides* fruit. – Abbreviations. Pericarp: ol – outer layer, pa – subepidermal parenchyma, osl – outer sclereid layer, vb – vascular bundle (derivate), isl – inner sclereid layer. Seed: sc – seed coat, co – cotyledons, ps – perisperm. Scale bar: 100 µm

Within the Chenopodiaceae/Amaranthaceae group the three genera *Anthochlamys*, *Agriophyllum* and *Corispermum* form their own clade (Kadereit et al., 2003) that can be recognized at the subfamilial level as Corispermoideae Ulbr. (Ulbrich, 1934). This clade splits into two subclades – *Agriophyllum* and *Corispermum+Anthochlamys*. The close relationship between *Corispermum* and *Anthochlamys* is also supported by morphological data. However, the genus *Anthochlamys* still remains one of the least studied groups, especially with regard to fruit/seed structure and its implications for evolution.

The first results describing the fruit anatomy of three *Anthochlamys* species (*A. afghanica*, *A. multinervis* and *A. tianschanica*) were provided by Sukhorukov (2007). The aims of the present study are:

(1) to investigate the fruit anatomy of all species in the genus from a taxonomic point of view;

(2) to reveal evolutionary trends in the carpology of the *Anthochlamys–Corispermum* lineage;

(3) to clarify whether the details of fruit structure in *Anthochlamys* appear to be unusual within the Caryophyllales.

Materials and Methods

Our own observations on the ecology of the *Anthochlamys* species were carried out in Uzbekistan and Kazakhstan during expeditions in 2005-08. 2-3 detached fruits of *Anthochlamys* species were obtained from the collections at MW, LE, and W with the permission of the herbarium staff in each case. The material studied is listed in Appendix 1. In order to compare the fruit/seed characters of *Anthochlamys* with other Caryophyllales and especially with closely related taxa from the subfam. Corispermoideae, ca. 250 members of the order were investigated earlier (Kadereit et al., 2010; Sukhorukov, 2007; 2008 a; 2008 b; 2011), and recent results are still being analyzed (Sukhorukov and Mavrodiev; Sukhorukov et al., in prep.). Cross-sections were cut either by hand or with a microtome. For tissue staining the following solutions were used: 0.2% aqueous toluidine blue to stain living tissues, Sudan IV for revealing fatty substances, and Lugol's iodine for starch. Prior to scanning electron microscopy (SEM) the material was dehydrated in aqueous ethyl alcohol solutions of increasing concentration, then in alcohol-acetone solutions and pure acetone. SEM observations were made with a JSM--6380 (JEOL Ltd., Japan) at 15 kV after critical-point drying and sputter-coating with gold-palladium. The carpological terms used are according to Werker (1997).

Results

Fruit anatomy of *Anthochlamys*

In cross-section the pericarp of the fruit comprises two zones (Fig. 1 B) – an outer zone (I) of 1-3(-5) layers represented by parenchymatous unsclerified cells, and an inner zone (II) of mechanical tissue (sclereids). The number of cell layers in zone I varies depending on the fruit topography. In the wing area and in the central (seed-containing) part of the fruit there are 1-3 layers. In the transition zone between the wing and the seed-containing part, there are usually 3-5 layers.

The outer cells of the outer layer are mamillate (Fig. 2 A) or (mostly near the wing) papillose (Fig. 2 B), 30-75 µm thick, except for the wing, which has smaller, flat cells (Fig. 2 C).

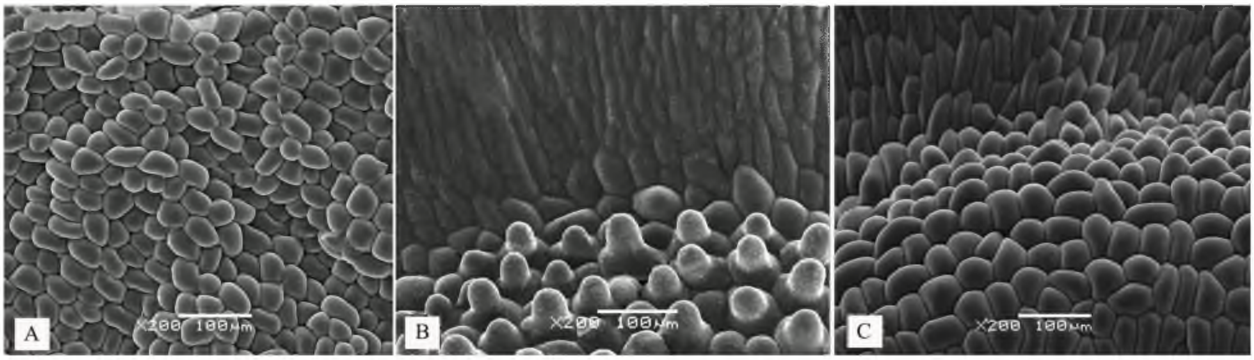

Fig. 2. A. Ultrasculpture of pericarp surface in the median part of *A. afghanica* fruit. Scale bar: 100 µm; B. Papillae in the seed-containing part of *A. polygaloides* fruit (near the wing). Scale bar: 100 µm. C. Pericarp surface at the transition between the central part and the wing of *A. multinervis* fruit. Lower half: mamillate ultrasculpture of the seed-containing fruit part; upper part: cells in the wing area. Scale bar: 100 µm.

Some cells are impregnated with brown pigment (turning blue when stained with toluidine). The cells of the inner layers of zone I are usually thinner. The mechanical tissue (zone II) is discontinuous and present only in the wing area. In the fruit of some species one thin layer (up to 5 µm) of sclerenchymatous parenchyma can sometimes be seen in the central part instead of sclereids. In the wing area the sclereids are oriented in different directions. The innermost layers (adjoining the seed coat) are oriented parallel to the fruit axis (in cross-section they appear round). The outer layers comprising the greater part of the wing are arranged perpendicularly to the fruit axis. The wing is always easily discernible, (0.55)0.8–1.6 mm long and appearing narrowly triangular in cross-section. Air cavities between zone II of the pericarp and the seed coat are either not visible or are up to 50 µm in diameter.

The seed coat is only (3)5–8 µm thick, closely attached to the pericarp and consisting of two strongly compressed cell layers impregnated with tannins. There is abundant perisperm. The embryo is well developed, with two cotyledons located mostly parallel or obliquely to the seed (and fruit) surface. Rarely (in some fruit of *A. polygaloides* and *A. tjanschanica*) the cotyledons are oriented perpendicularly to the seed/fruit axis.

The carpological characters of each species

Despite the uniformity of the topography, some pericarp characteristics can be useful for taxonomy. The most important ones are given in Table 1. Surface topography cannot be regarded as taxonomically important.

Table 1. Fruit characteristics of the *Anthochlamys* species

Species	**Fruit length/width ratio (mm)**	**Thickness of seed-containing part of fruit (mm)**	**Number of pericarp layers in seed-containing part of fruit**	**Pericarp thickness in seed-containing part of fruit (µm)**	**Thickness of outer pericarp layer (µm)**	**Cell-wall outlines of outer pericarp layer**	**Wing length in cross-section (mm)**	**Wing thickness (µm)**
A. afghanica	2.5-3 x 2.7	0.4-0.5	2-3	55-60	30-40	+/- round	0.6-0.8	70-100
A. multinervis	3-3.5 x 3	0.5-0.65	1-2(3)	45-55	(35-) 40-50	+/- round	0.8-1.2	90-130
A. polygaloides	3.5-4.5 x 3.7-4.5	0.7	2(3)	40-60 (-75)	30-45 (-50)	+/- round	0.8-1.6	75-100
A. rechingeri	3-3.2 x 3-3.2	0.65-0.8	2(3-4)	50-75	(35-) 40-60 (-65)	+/- round	0.55-0.7	60-75
A. tianschanica	3.3-5 x 3-4	0.7-1	2(3)	40-50	30-40 (-45)	convex or +/- round	0.7-1.1	60-75
A. turcomanica	3.3-4 x 3.2-3.7	0.7-0.9	2(3)	55-90	50-75	+/- round	0.8-1	60-100

Small fruit diameter and small wing length are additional parameters supporting the species level of *A. rechingeri*. The geographical isolation of *A. tjanschanica* has not led to the evolution of fruit-structure peculiarities.

Discussion

The relationship between Corispermum and Anthochlamys

The first known *Anthochlamys* species was described as *Corispermum polygaloides* (Fischer et al., 1835). However, since 1837, when Fenzl established *Anthochlamys*, its generic status has been supported in all monographs, e.g. Volkens (1893), Ulbrich (1934), Iljin (1936), Aellen (1950), Hedge (1997) and Kadereit et al. (2003). Even the thesis that *Corispermum patelliforme* might represent a morphological link between the genera (Mosyakin, 2003) is not supported by carpological characters (Sukhorukov, 2007).

The diversification of *Anthochlamys* is clearly associated with the areas (Central Iran and Afghanistan) located to the south of all the diversity centres of *Corispermum*, including in particular the 'Nitidum' group. In this area are found only *C. lehmannianum*, at the southern edge of the area, and the endemic species *C. rechingeri* (compare Hedge, 1997; Sukhorukov, 2008). Moreover it can be postulated that the evolution of the genera is linked to different substrates: the ecotopes of *Corispermum* are sands while *Anthochlamys* prefers gypsum soils or variegated rocks. The morphological differences belong mostly to the number of perianth segments: there are 5 prominent perianth segments in *Anthochlamys* and (0)1(2-3) in *Corispermum*. Another character is the convexity of the fruit (excluding the wing area): bilaterally convex in *Anthochlamys* and unilaterally convex in *Corispermum*.

The present study shows that there are several genus-specific characters in *Corispermum* and *Anthoclamys* (Tab. 2). There are also many characters common to them, although in some cases they have arisen indepedently in the two genera (Tab. 3).

Table 2. New genus-specific characters in the fruit anatomy of *Corispermum* and *Anthochlamys*.

Corispermum	*Anthochlamys*
Papillae if present located over all the seed-containing part of the fruit. Wing thickness decreases gradually towards the top of the fruit.	Papillae if present located at the edge of the seed-containing part of the fruit. Wing thickness almost equal in all parts (except the base and top of the fruit

Table 3. Pericarp characters common to *Corispermum* and *Anthochlamys* (but with some features derived independently in the two genera)

Corispermum	*Anthochlamys*
Outer cell walls of outer pericarp layer (if not papillated) usually flat or slightly convex (except in the 'Declinatum' group). Air cavity often present in the base of the wing. Mechanical tissue present in wing and mostly in seed-containing part.	Outer cell walls of the outer pericarp layer convex or semi-round. Air cavity often not present in the base of the wing. Mechanical tissue present in wing area and at edge of seed-containing part.

The flattened wing-like outgrowth in *Anthochlamys* is a rare trait within the core Caryophyllales

Many taxa of the order Caryophyllales (in its recent circumscription: Brockington et al., 2009; Schäferhoff et al., 2009) growing in arid regions are characterized by diverse adaptations for wind dispersal (e.g. Ulbrich, 1928). The dissemination of indehiscent (single-seeded) fruit is often linked to wing-like or tuberculate appendages of the perianth, for instance in many Chenopodiaceae (Kühn et al., 1993), especially representatives of the subfamily Salsoloideae, and part of Polygonaceae (Brandbyge, 1993). Sometimes the seed coat of dehiscent fruit can develop flattened wing-like projections (some species of Caryophyllaceae). However, in most cases dissemination is promoted by two or several modes, termed heterodiaspory (Sukhorukov, 2010).

Anemochory seems to be the preferred mode of dissemination in *Anthochlamys*, but the fruit (without the perianth) is the only dispersal unit. In contrast to the closely related *Corispermum*, the fruit wing is always prominent in *Anthochlamys*. This obligate presence of the flattened pericarp outgrowth is a unique character within the entire Chenopodiaceae clade. The study of fruit evolution in Caryophyllales *sensu novo*, including Polygonaceae and Limoniaceae, shows a rare convergent tendency to develop pericarp outgrowths (Sukhorukov et al., in prep.). It is found only in a few orders, namely in the fruit of *Sarcobatus* (Sarcobataceae), in some Polygonaceae (Ronse Decraene et al., 2000), and in the nutlets of *Gisekia* (Gisekiaeae), but without any similarities in anatomical structure.

Conclusions: Fruit structure in six *Anthochlamys* species is quite uniform, differing only in details. Fruit diameter, length and thickness of the wing are additional parameters supporting the recognition of *A. rechingeri* at the species level. The geographic isolation of *A. tianschanica* has not affected the evolution of its carpological traits. The genus-specific features of *Anthochlamys* have been identified. The closely related genera *Corispermum* and *Anthochlamys* are similar in fruit anatomy, but the diversification of both genera proceeded under different ecological and geographical conditions. Although many members of the Caryophyllales appear to be anemochorous, it is postulated that the 'winged' pericarp arises only rarely in the order. The prominent flattened wing of the pericarp is characteristic of *Anthochlamys* and seems to be unique within the Caryophyllales.

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Appendix 1

List of specimens investigated

Anthochlamys afghanica Podlech: Afghanistan, prov. Kandahar, Keshkenakhud, X.1978, D. Podlech *32681* (LE);

A. multinervis Rech. f.: 1) Iran, prov. Semnan, road from Semnan to Firuzkuh, alt. 1400 m, IX.1974, I. Hedge & al. *14736* (LE); 2) Iran, Kavir Kashan, XI.1974, Termé, Moussari *30276-E* (W-02617);

A. polygaloides (Fisch. & C.A. Mey.) Fenzl: 1) Iran, Tehran prov., W of Mardabad, IX.1974, I. Hedge & al. *14685* (W-00441); 2) Iran, Azerbaijan prov., Mianeh, X.1974, P. Wendelbo & Shir-delpur *14925* (W-8430);

A. rechingeri Aellen: Iran, Bahram, VIII.1948, P. Aellen *s.n.* (W-5425; K). Several fruits from both specimens are used for the carpology.

A. tianschanica Iljin ex Aellen: 1) [Uzbekistan], Namangan, VII.1922, N. Androsov *66* (MW); 2) Uzbekistan, Fergana valley, Tuzul-say, VIII.1950, A.D. Pyatayeva *258* (LE); 3) Kyrghyzstan, Dzhalalabad prov., valley of Naryn river, VI.2000, Pimenov & al. *50* (MW);

A. turcomanica Iljin: 1) Turkmenistan, Chuli, IX.1914, I. Ivanov *s.n.* (LE); 2) Turkmenistan, the same place, VI.1971, A.V. Mescheryakov *s.n.* (LE).