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Early eudicot reproductive structure: Fruit and flower morphology of *Ranunculaecarpus* Samyl. from the Early Cretaceous of eastern Siberia

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ABSTRACT. Floral and fruit morphology of the early eudicot *Ranunculaecarpus quinquecarpellatus* Samyl. is described based on details from sectioning and microscopy of the permineralized type material from the Albian Buor-Kemyus Formation of the Zyryanka coal basin. Serial sections confirmed most of the originally described characters but revealed additional information, including hypogynous perianth and several stamens with in situ pollen. Each fruit consists of five free follicles inserted on a short receptacle. Follicles are elongate, with a dorsal keel, ventral suture and an attenuate apex, and are thin-walled, with two rows of small seeds in marginal placentation. The seeds are anatropous, ovoid, 1.3–1.7 in length, with an exotesta of cells that are rounded-hexagonal in surface view. The hypogynous perianth is composed of several free tepals. The stamens are short, with tetrasporangiate, dithecal anthers dehiscing by longitudinal slits. Pollen in situ is 18–20 mm long, 13–15 mm in equatorial diameter, with uncertain aperture configuration and a loose reticulum supported by narrow, widely spaced columellae. The combination of macromorphological characters support possible affinity to extant Ranunculaceae. However, *Ranunculaecarpus* is distinguished from modern members of the family by the persistence of the perianth in fruit, a smaller number of stamens (ca 10) than is typical, and pollen that is unlike that of any extant genera. Given that there are also similarities with Saxifragales, the systematic affinities of *Ranunculaecarpus* remain uncertain.

KEYWORDS: Ranunculales, follicles, fossil, permineralized, pollen, seeds, early angiosperms, paleobotany, Albian

INTRODUCTION

The Early Cretaceous angiosperm reproductive structure *Ranunculaecarpus* was described by V.A. Samylina (1960) based on fruits as part of a flora known mainly from leaf fossils from the early-middle Albian deposits of the Buor-Kemyus Formation of the Zyryanka River, a tributary of the Kolyma River in eastern Siberia. Based on this age assignment, the genus is one of the more ancient occurrences of early angiosperms in northeastern Russia, and this apocarpous fruit has been included in assessments of the global diversity of early angiosperm reproductive organs (Takhtajan 1969, Friis & Crepet 1987, Friis et al. 2011).

Ranunculaecarpus quinquecarpellatus Samylina was established on the basis of several specimens of permineralized fruits fractured in various orientations. Samylina (1960) described

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these fruits as ca 1 cm long, consisting of five free carpels, each ca 2 mm wide and containing two rows of small smooth seeds. Details of placentation, the receptacle and other floral parts were not indicated. We reinvestigated the original specimens with the aid of scanning electron microscopy and serial sectioning. These techniques mostly confirm the accuracy of Samylina's morphological description, and provide additional characters of fruit and floral morphology. Here we give visual documentation of the characters of this extinct fruit type and reveal new characters of the flower from which its matured, including features of the perianth, stamens and pollen. These data allow us to consider the taxonomic affinities of *Ranunculaecarpus* more precisely and to discuss some questions of the early evolutionary history of eudicot flowers.

GEOLOGIC OCCURRENCE

We studied the same specimens of *Ranunculaecarpus* that were initially described and named by Samylina (1960), which are housed at the laboratory of palaeobotany of the Komarov Botanical Institute, Russian Academy of Sciences, St. Petersburg. The fossils were collected along with other plant remains from the Buor-Kemyus Formation of the Zyryanka coal basin in 1957 by G.G. Popov and V.A. Samylina. The Zyryanka coal basin extends as a wide strip about 500 km long extending from the Indigirka River to the Kolyma River (Fig. 1).

The first geological investigation of the region was made in 1933–1935 by P.N. Ushakov and V.A. Zimin. The collections of fossil plants from the Cretaceous deposits were described by Prynada (1938) and Kryshtofovich (1938). The stratigraphy of the Zyryanka-Silyap coal field was subsequently studied by Popov (1962). He placed the Early Cretaceous deposits in the Zyryanka Group, which he subdivided into three successive formations: Ozhogina, Silyap and Buor-Kemyus (Fig. 2). The Zyryanka Group conformably overlies the Upper Jurassic Bastak Formation and Ilintas Formation. The more ancient Ilintas Formation is marine and contains mollusks of late Oxford–early Kimmeridge age. In the nonmarine Bastak Formation, only rare and poorly preserved fossil plants of Late Jurassic age were found. The Early Cretaceous deposits of the Zyryanka Group are nonmarine and contain industrial-quality coal seams and abundant fossil plants. The Zyryanka Group is overlain unconformably by Late Cretaceous nonmarine deposits of the Vstrechninskaya Formation which probably is Turonian-Coniacian in age (Kryshtofovich 1938). A systematic treatment of all the floral assemblages of the Zyryanka River region is contained in a series of papers by Samylina (1959, 1960, 1964, 1967), in which

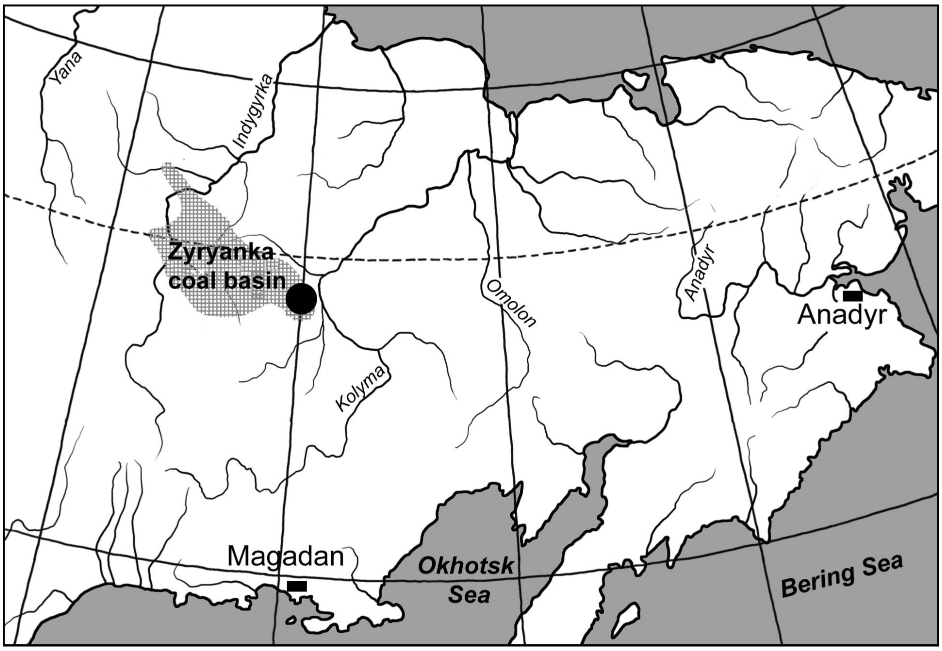

Fig. 1. Map showing the location of the Zyryanka coal basin in eastern Siberia between the Kolyma River and Indigirka River, and the locality of *Ranunculaecarpus quinquecarpellatus* Samyl. (black circle) at the Zyryanka River near Ugolnaya Zyryanka settlement

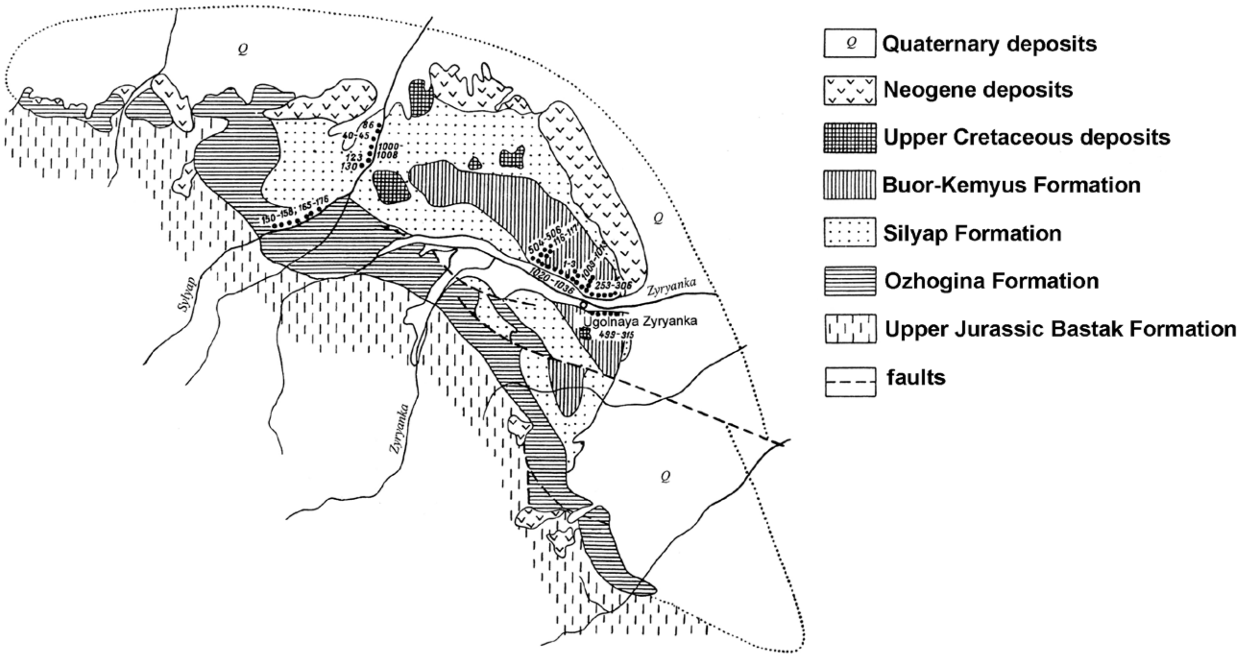

Fig. 2. Geological map of the Zyryanka River basin, indicating the position of the Buor-Kemyus Formation in relation to the underlying Silyap, Ozhogina, and Bastak formations and overlying Upper Cretaceous, Neogene and Quaternary deposits

she assessed their ages based on comparisons with floras from other areas. The Ozhogina Formation was dated as Neocomian, possibly also including Upper Jurassic sediments. The age of the Silyap Formation was determined as Aptian, and that of the Buor-Kemyus Formation as early-middle Albian (Samylina 1974).

The Buor-Kemyus Formation is about 3000 m thick and consists of sandstones, siltstones and mudstones, with numerous coal layers. In the middle part of the formation there are lenses and layers of conglomerates up to 400 m thick. Floras of the Buor-Kemyus type are widely distributed in northeastern Russia. Similar assemblages were found in the Omsukchan Formation in the upper reaches of the Kolyma River, in the Bol'shoy Anuy, Eropol and Penzhina river basins, and in several other localities (Samylina 1976). In the Aynakhkurgen and Umkuveem depressions and in the Penzhina River basin, plant-bearing deposits with floristic assemblages of the Buor-Kemyus type overlie marine deposits with mollusks of late Aptian age. The development of the younger Grebenka flora with predominance of angiosperms began in the late Albian-Cenomanian, as indicated by marine mollusks found together with the floral remains (Herman 1999). These data confirm the early-middle Albian age of the Buor-Kemyus flora.

The Buor-Kemyus flora includes about 80 species. The most characteristic taxa are *Osmunda cretacea* Samyl., *O. denticulata* Samyl., *Arctopteris kolymensis* Samyl., *A. rarinervis* Samyl., *Onychiopsis psilotoides* (Stokes et Webb) Ward, *Coniopteris onychioides* Vassilevsk. et Kara-Murza, *C. bicrenata* Samyl., *Asplenium dicksonianum* Heer, *A. rigidum* Vassilevsk., *Acrostichopteris* sp., *Nilssonia magnifolia* Samyl., *N. comtula* Heer, *Nilssoniopteris prynadae* Samyl., *Anomozamites* sp., *Ginkgo pluripartita* (Schimp.) Heer, *G. paradiantoides* Samyl., *Sphenobaiera biloba* Pryn., *S. flabellata* Vassilevsk., *Phoenicopsis magnum* Samyl., *Pagiophyllum triangulare* Pryn., *Podozamites* spp., *Schizolepis cretaceus* Samyl., *Sciadopitys* sp., *Pityophyllum* sp., *Cyparissidium gracile* (Heer) Heer, *Parataxodium* sp., *Sequoia* sp. and small-leaved angiosperms. Ferns, ginkgophytes, cycadophytes, Czekanowskiales and conifers prevail in this flora. Angiosperms are rare in numerical occurrence but their diversity is rather high, including about 20 species: *Araliaephyllum kolymense* (Krysht.) Golovn., *Ranunculaecarpus quinquecarpellatus* Samyl., *Trochodendroides potomacensis* (Ward) Bell, *T. buorensis* Golovn., *Crataegites borealis* Samyl., *Celastrophyllum kolymense* Samyl., *Araliaecarpum kolymensis* Samyl., *Caricopsis compacta* Samyl., *C. laxa* Samyl., "*Rogersia*" *denticulata* Samyl., *Dicotylophyllum obliquum* Samyl., *D. zyrjankense* Samyl. and *Dicotylophyllum* spp. (Samylina 1960, Golovneva & Alekseev 2010, Golovneva 2018).

MATERIAL AND METHODS

One of the permineralized fruits initially observed in transverse section (Pl. 2, fig. 1; Takhtajan 1969: Pl. XIII, fig. 3) was opened along a natural crack for examination of oblique longitudinal surfaces (Pl. 1, fig. 2) by reflected light microscopy and by scanning electron microscopy (SEM). One of the two fragments, exposing surface details of seeds, was cleaned in a sonicator, temporarily mounted on an aluminum stub, sputter-coated with palladium, and examined with a Hitachi S-4000 FE-SEM (Pl. 2, fig. 7). Upon completion of the SEM observations, the palladium was removed by soaking the specimen in a solution of sodium cyanide in water, and the specimen was reassembled with cyanoacrylate glue.

Another specimen, which had originally been broken into two pieces by an oblique longitudinal fracture, was photographed to record the features revealed on the broken surfaces (Pl. I, fig. 3), then reassembled with cyanoacrylate glue and sectioned serially with a Microslice II annular diamond saw using a very thin blade (thickness 0.04 mm) in order to minimize loss of material in the cutting process. Six transverse cuts were made: three at the apical end, each ca 1 mm apart, and three at the base ca 0.8 mm apart (Pl. I, fig. 4), to provide serial transverse views (Pl. I, figs 5–10). The central wafer, ca 3.3 mm in length (D in Pl. I, fig. 4), was subsequently sectioned into longitudinal wafers to illustrate features not obvious from the transverse sections (Pl. 1, figs 12, 13, Pl. 2, fig. 6). Sections intercepting anthers were ground thinner (ca 40 microns) to facilitate transmitted light microscopy. To document in-situ pollen, anthers on the surface of these sections were viewed without coverslips by epifluorescence microscopy, with a green (530–585 nm) excitation filter fitted on a Zeiss Axiophot microscope. The surface of one section intercepting anthers was etched with weak HCl in an attempt to expose the pollen grains for SEM, but this failed and the section was instead remounted on a glass slide for light microscopy and archiving.

SYSTEMATIC PALEOBOTANY

Genus: ***Ranunculaecarpus*** Samyl., 1960

Ranunculaecarpus quinquecarpellatus Samyl.

Pl. 1, figs 1–13, Pl. 2, figs 1–7, Pl. 3, 1–13

1960 *Ranunculaecarpus quinquecarpellatus* Samylina 1960: 336; pl. 1, fig. 3–5, text-fig. 1.

Holotype. Coll. BIN 509, spec. N 791 (=396-4 of Samylina 1960), Northeast Russia, Kolyma River basin, Zyryanka River, locality 396, Buor-Kemyus Formation, early-middle Albian, designated by Samylina (1960: pl. 1, fig. 5), stored at Komarov Botanical Institute, St. Petersburg.

Additional specimens. Coll. BIN 509-792a (=396-12), 509-792b (=396-2a), same locality and repository as holotype.

Description and remarks. Fruits apocarpous, 8–9 mm long and 3–4 mm in diameter, consisting of five free follicles inserted on the same level of a short receptacle. Follicles elongate, ± cylindrical, ca 1.5–2.0 mm wide with dorsal keel, apparently possessing a dorsal vascular bundle, ventral suture and attenuate apex. Sections near apex revealed tapered ends of follicles (Pl. 1, fig. 5) but did not intercept styles or stigma. However, the natural longitudinal fracture of the specimens shows narrowed tips of carpels that probably were stigmatic (Pl. 1, fig. 3). Successively lower sections reveal that the carpels touch each other ventrally but are not fused (Pl. 1, fig. 6–11). Longitudinal keel on dorsal side of each carpel (Pl. 2, figs 4, 5). No lateral veins observed. Follicles thin-walled, 0.1 mm thick along lateral walls, abruptly thickened to 0.2 mm at dorsal keel (Pl. 2, figs 4, 5) and composed of rounded thin-walled cells 0.05–0.8 mm in diameter. Inside this cell layer is a homogenous dark substance that likely represents one or more layers of obliterated thin-walled cells.

Each follicle contains two rows of small seeds with ca 9–11 seeds in each row. Placentation is at the ventral edge of each carpel (marginal) (Pl. 2, figs 4–6). Seeds ovoid, 1.3–1.7 mm in length and 0.6–0.8 mm in diameter. Seed coat composed of two layers. Outer layer (exotesta) consists of large cells with thickened anticlinal walls, readily visible in transverse (Pl. 2, figs 4, 5) and longitudinal sections (Pl. 2, fig. 6). Outer periclinal walls apparently thinner and often collapsed, resulting in strongly concave surface of each cell. Exotestal cells rounded-hexagonal in surface view, 75–150 μm wide, forming honeycomb pattern on seed surface (Pl. 2, fig. 7). Inner layer of seed coat (seen best by SEM, Pl. 2, fig. 7) consists of 1–2 rows of cells that are ± isodiametrical at apex (15–18 μm diam.), and elongate (15–18 μm wide, at least 60 μm long), longitudinally aligned over the majority of seed length. Raphe within testa visible in sections of seeds, indicating anatropous condition (Pl. 2, fig. 6). Surface of raphe composed of cells similar to ordinary exotesta cells but much smaller. No cellular structure preserved inside raphe. Raphes of the ovules in different rows face each other, indicating that ovules were syntropous (sensu Endress 1994). Inside

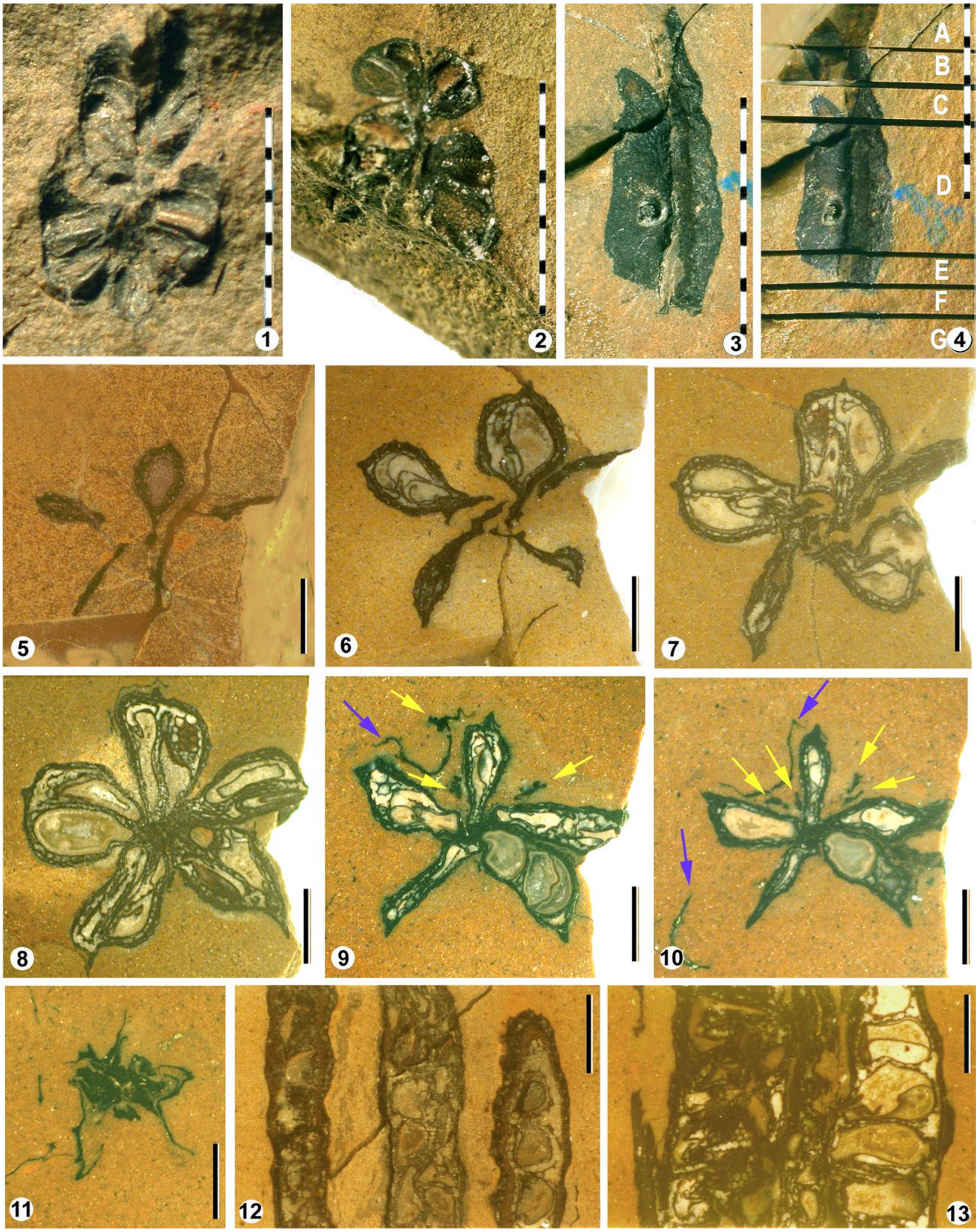

Plate 1. *Ranunculaecarpus quinquecarpellatus* Samyl. fruits. **1**. Obliquely fractured fruit, exposing several seeds, holotype, BIN 509-791; **2**. Obliquely fractured fruit, BIN 509-792b; **3–13**. Specimen imaged before and after sectioning, BIN 509-792a; **3**. Fruit exposed in longitudinal fracture, showing two of the follicles, including a narrow apical protrusion at right; **4**. Same specimen after sawing, showing the position of transverse sections viewed in figs 5–10; **5**. Transverse section in upper part (B top), showing free tips of five follicles; **6**. Transverse section of specimen in upper part of fruit (C top), showing dorsal keel and ventral suture of follicles; **7**. Transverse section in middle part of fruit (C bottom); **8**. Transverse section in middle part of fruit (E top), showing two rows of seeds; **9**. Transverse section in lower part of fruit (E bottom), showing tepal (blue arrow) and three anthers (yellow arrows); **10**. Transverse section near base of fruit (F top) with persistent perianth (blue arrows) and two pairs of anthers (yellow arrows); **11**. Transverse section near base of receptacle, showing at least three tepals diverging (F bottom); **12**. Tangential section of fruit from fig. 4, increment D, near periphery, revealing three free carpels and serial seeds; **13**. Tangential section of fruit from fig 4, increment D, near central axis. Scale bars: 1–4 = 5 mm, 5–13 = 1 mm

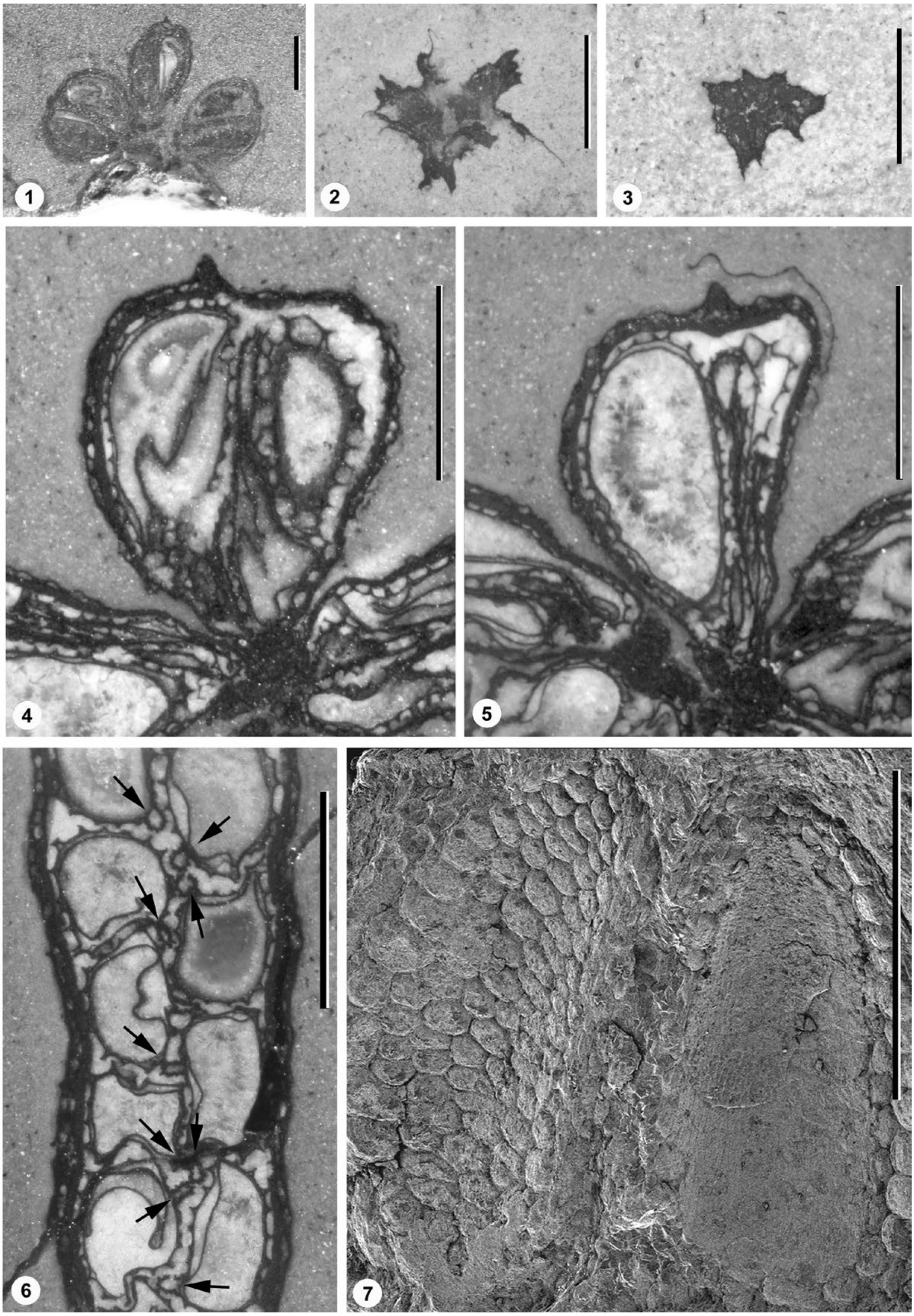

Plate 2. *Ranunculaecarpus quinquecarpellatus* Samyl. **1**. Transverse section, showing detail of same specimen as Pl. 1, fig. 2. Note five free carpels, BIN 509-792b; **2**. Transverse section detail at base of receptacle, BIN 509-792a; **3**. Transverse section detail of pedicel, BIN 509-792a; **4**. Detail of carpel, showing dorsal keel and thin wall, containing seeds with seed coat of large cells, transverse section, BIN 509-792a; **5**. Fruit with thin tepal draped around carpels, transverse section, BIN 509-792a; **6**. Carpel with two rows of seeds, tangential section external to that of Pl. I, fig. 12. Note protrusion of seed coat on each seed inferred to contain raphe (arrows), BIN 509-792a; **7**. Seed coat exterior of large rounded-hexagonal cells with thickened cell walls, inner layer (exposed on seed at right) consisting of 1–2 rows of small flattened and elongated cells with thin walls. Same specimen as Pl. 1, fig. 2; BIN 509-792b, SEM. Scale bars: 1–7 = 1 mm

seed coat, clear dark line without recognizable cellular structure can be seen in sections; this line is similar in color and thickness to inner walls of exotesta cells; it often follows the seed coat outline but in some cases has contracted away from the seed coat. Although no cellular content can be seen inside the line, it is likely that this is the outer border of the nucellus. No embryo can be seen.

Transverse sections near the base of the fruit reveal a hypogynous perianth of free tepals (Pl. 1, figs 9–11, Pl. 2, fig. 2), and stamens with anthers having longitudinal dehiscence (Pl. 1, figs 9, 10, Pl. 3, figs 1–4). The precise number and arrangement of perianth parts and the number of stamens could not be determined, but the anthers appear to be arranged with two between each of the adjacent carpels (Pl. 1, figs 9, 10, Pl. 3, fig. 1). Although only two of these pairs were observed in the available sections, we infer that this pattern is repeated around the periphery of the gynoecium for an estimated total of 10 stamens, but it is not clear whether the androecium was spiral or whorled. Tepals 1–1.3 mm wide and very thin. Stamens short (estimated less than 500 μm based on disappearance in successive sections). Anthers 300–360 μm wide, tetrasporangiate and dithecal (Pl. 3, figs 2–4); each theca dehiscent by longitudinal slit. Connective nearly as wide as thecae. Pollen within anthers was studied by epifluorescence (Pl. 3, figs 5–7) and transmitted light (Pl. 3, figs 8–13). Grains prolate, 18–20 μm long, 13–15 μm in equatorial diameter, with loose reticulum supported by narrow, widely spaced columellae (Pl. 3, figs 5–13). Lumina of reticulum irregular, varying in size, 1–3.5 μm in diameter (e.g. grain “a” in Pl. 3, figs 9, 10, grain “b” in Pl. 3, fig. 10, and in the upper part of grain illustrated in Pl. III, figs 11–13). Middle focus on individual grains shows columellae extending from nexine to tectum (e.g. grain “a” in Pl. 3, fig. 8). No apertures were identified with certainty in the fossil material. One grain (central grain in Pl. 3, figs 11, 12) has the appearance of being colpate but this may be an artifact of natural folding combined with damage of the surface during preparation of the thin section. Different focus levels through other grains appear to exclude a tricolpate morphology. The possibility that the grains are polyporate with very indistinct apertures cannot be ruled out, although the smooth, uninterrupted nexine surface (grains “a” and “b” in Pl. 3, figs 6, 7) suggests that the grains are inaperturate.

The *Ranunculaecarpus* fruits co-occurred with lobed leaves described by Samylina (1960) as *Crataegites borealis* Samyl. These leaves are simple, petiolate, with lamina ovate in outline, pinnately dissected into 3–7 lobes, 10–35 mm in length. The lobes have additional secondary lobes or lobe-like teeth which are rounded or triangular. Venation is pinnate or palmately-pinnate, craspedodromous. Although the fruits and leaves were not found in organic connection, the possibility that they might be organs from the same extinct species is supported by similarity to some extant members of the Ranunculaceae. However, the taxonomic affinities of Cretaceous plants with this and similar leaf morphotypes remain controversial (Puebla 2009). Interestingly, a recent study used *Crataegites borealis* as a fossil constraint for a molecular chronogram of Rosaceae (Töpel et al. 2012). The authors stated that “the similarity to modern-day leaves of *Crataegus* is striking and there is no obvious reason to dispute the taxonomic position of the fossils in the crown group of Rosaceae” (Töpel et al. 2012, p. 6). More detailed comparative work is needed to confirm or exclude this assignment.

DISCUSSION

SYSTEMATIC AFFINITIES

Apocarpous organization and fruitlets developing into follicles characterize several extant eudicots, including members of the Paeoniaceae and Crassulaceae (Saxifragales), Rosaceae (Rosales), and Lardizabalaceae and Ranunculaceae (Ranunculales). Most extant eudicots are readily distinguished from *Ranunculaecarpus*, however. Members of Crassulaceae are particularly similar to *Ranunculaecarpus* in their regularly pentamerous and isomerous organisation and in having an apocarpous or nearly apocarpous gynoecium. The seeds are typically very small, 0.5–1 mm long and arranged in two or several rows along the carpel margins (Danilova 1996, Thiede & Eggli 2007), and are similar to seeds of *Ranunculaecarpus* in having a reticulate surface pattern formed from the thickened and concave outer periclinal walls and raised anticlinal walls of the exotesta cells. In the Crassulaceae, however, nectariferous

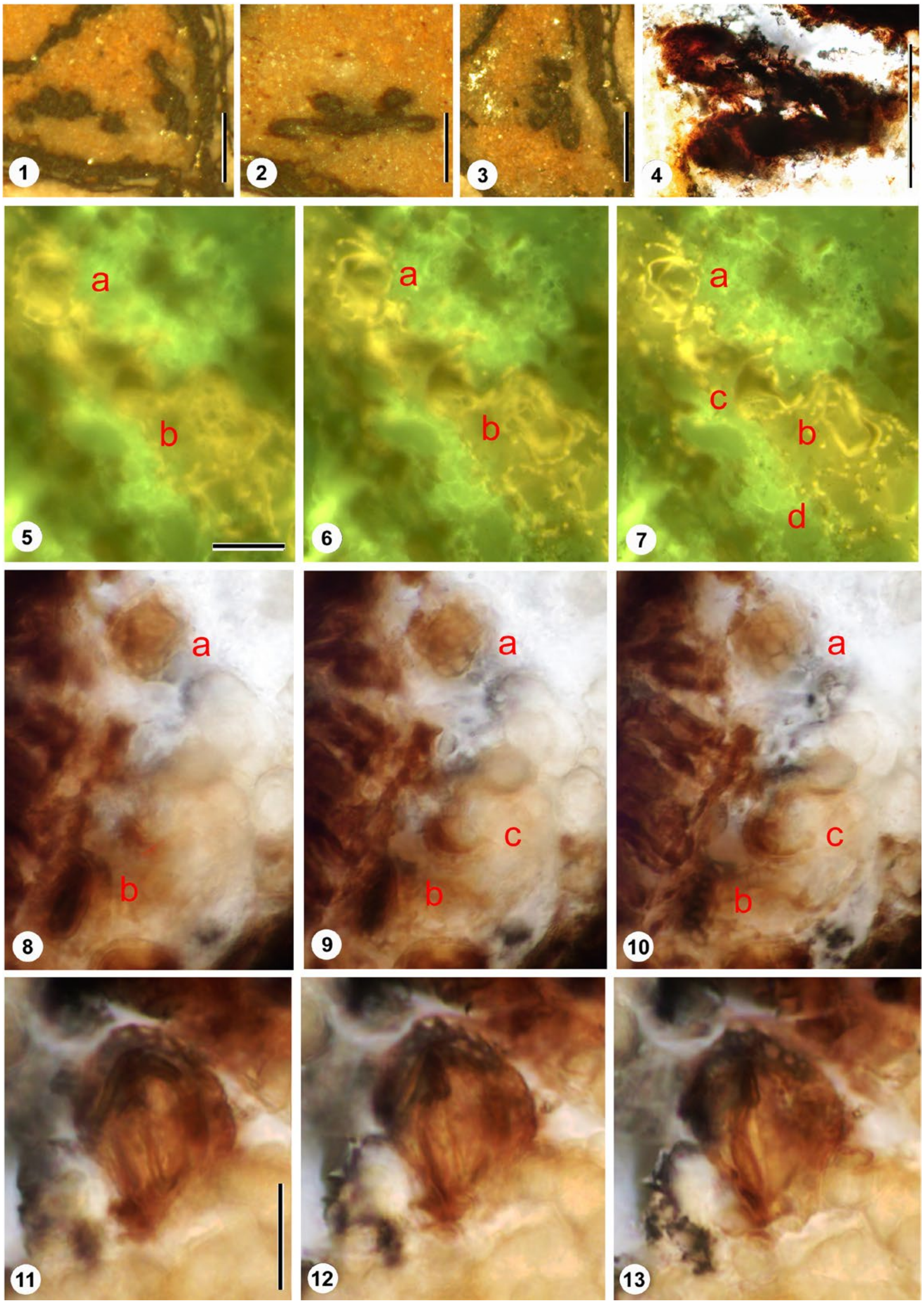

Plate 3. Stamens and pollen of *Ranunculaecarpus quinquecarpellatus* Samyl. **1**. Two anthers in transverse section from Pl. 1, fig. 10; **2, 3**. Anthers enlarged from Pl. 1, fig. 9; **4**. Enlargement of stamen with pollen grains; **5–7**. Successive focus levels of slide containing several pollen grains from anther of fig. 4, viewed by epifluorescence microscopy; **5**. Note pollen in optical section (a) and surface view showing reticulate tectum (grain b); **6**. Note smooth intine with surrounding columellate tectum (grains a and b); **7**. Optical sections showing shape of grain in polar (grain a) and equatorial (grain b) views. Additional grains coming into focus (e.g. c, d); **8–10**. Successive focus levels of same slide, showing several pollen grains from anther of fig. 4, transmitted light microscopy. Three of the grains are labeled a–c, for viewing in successive focus levels. Note that different focus levels show reticulate exine, columellae in profile view, and smooth inner surface of intine; **11–13**. Successive focus levels of pollen grain, showing elongate fold and reticulate tectum with large lumina (visible at upper end of images). Surface exine in lower part of grain was partially ground away during thin-sectioning. Transmitted light microscopy; Scale bars: 1–4 = 0.2 mm, 5 = 25 μm (also applies to 6–10), 11 = 10 μm (also applies to 12, 13)

scales are constantly associated with the dorsal part of the carpel bases (e.g., Takhtajan 2009). In Paeoniaceae, both pericarp and seed coat are composed of numerous cell layers, and in contrast to the fossil, the pericarp surface is pubescent. Most importantly, in Paeoniaceae the mechanical layer of the seed coat is formed by subepidermal cells of the outer integument, which differentiate as macrosclereids (Fedotova 1988). Fruits of Rosaceae formed from apocarpous gynoecia typically have a hypanthium and only a few seeds per carpel. Despite the differences emphasized here, affinity of *Ranunculaecarpus* to Saxifragales sensu APG cannot be completely ruled out.

In Lardizabalaceae the fruit wall is typically fleshy and thick (Chernykh 1998), seeds are typically with a smooth, shiny surface, and the exotesta is composed of narrow palisade-shaped cells with minute polygonal facets (Corner 1976). Features shared between *Ranunculaecarpus* and Ranunculaceae (Tamura 1993) include bisexual flowers, a short receptacle, distinct tepals that are not connate, stamens with dithecal anthers, superior apocarpous ovaries, and apparently conduplicate carpels (not the case for all Ranunculaceae) containing numerous anatropous and syntropous ovules usually with marginal placentation. Fruits are variable in the family, not always follicular, but follicles similar to those of *Ranunculaecarpus* occur in several genera.

The main feature of fruit wall anatomy that can be recognized in *Ranunculaecarpus* is the occurrence of thick-walled cells in the exocarp. Mesocarp and endocarp cells are apparently obliterated and were most likely thin-walled and unlignified. Follicular fruits of extant Ranunculaceae mostly possess thickened and lignified endocarp cells often differentiated as fibers (Chernykh 1998, Cheng et al. 2015), while follicles with thin-walled, unlignified endocarp cells are less common among extant Ranunculaceae. Among taxa with a thin inner fruit wall, *Cimicifuga foetida* L. has only slightly thickened endocarp cells, but cell wall thickenings are present in the exocarp as well as mesocarp, and two types of hairs are present on the fruit surface (Chernykh 1998). In some species of *Caltha* L. all the layers of the fruit wall have thin-walled cells (Chernykh 1998, Cheng et al. 2015). Other species of Ranunculaceae, such as *Leptopypum fumarioides* (L.) Reichenbach, have fruit wall anatomy more similar to that of *Ranunculaecarpus*, with thick-walled exocarp cells that are slightly elongated tangentially, a mesocarp of 2–3 layers of thin-walled cells that are isodiametric or tangentially elongate, and an endocarp of a single layer of thin-walled, tangentially elongate cells (Chernykh 1998). In his classification of Ranunculaceae, Tamura (1993) paid special attention to the presence/absence of prominent lateral veins (or reticulation) in follicles. No prominent lateral veins are visible in *Ranunculaecarpus* follicles. According to Tamura (1993), such follicles are characteristic for the extant genera *Asteropyrum* and *Coptis* (see also Dezhi & Robinson 2001).

The seed anatomy of *Ranunculaecarpus* is similar to that seen in some extant Ranunculaceae. As summarized by Trifonova (1988), seed coat anatomy in Ranunculaceae correlates with fruit morphology. In single-seeded indehiscent fruits, the pericarp serves a protective function, and the seed coat usually lacks specialized layers of cells with thickened walls. In plants with follicular fruits, the outer cells of the outer integument (exotesta) form a protective layer. The exotesta cells are relatively large and thick-walled, as is the case in *Ranuculaecarpus*. The patterns of cell wall thickening vary among extant Ranunculaceae (Trifonova 1988). For example, in *Consolida* only the outer walls are thickened, in *Caltha cornuta* Schott (syn. *Caltha palustris* L.) all the walls are thickened to the same extent, and in *Trollius asiaticus* L. only the inner and radial walls are thickened. In some extant Ranunculaceae the outermost endosperm cells possess thickened walls (Trifonova 1988). Such a thickened endosperm layer might have been transformed during fossilization into the membrane-like structure observed inside the seed coat of *Ranunculaecarpus*, but alternatively the structure seen in the fossil may represent the outer surface of the nucellus. No embryo is preserved in seeds of Ranunculaecarpus despite the good preservation of other tissues.

There are some significant differences between *Ranunculaecarpus* and extant members of Ranunculaceae. The relatively small number of stamens in the fossil contrasts with the typically numerous stamens of extant Ranunculaceae. The possibility that some of the stamens were lost after anthesis cannot be ruled out, but the receptacle appears too small to support a large number of stamens. Although an androecium with many stamens in a spiral arrangement is common among extant Ranunculaceae, and was previously thought to be the primitive condition in the group, more recent phylogeny-based reconstructions of androecium evolution suggest that whorled androecia are ancestral in the Ranunculales and in eudicots in general (Soltis et al. 2018, Sauquet et al. 2017) and that polyandry might be derived (e.g., Drinnan et al. 1994, Soltis et al. 2018, Damerval & Nadot 2007). It is worth noting in this context that Ranunculaceae also includes taxa with few stamens (e.g. *Xanthorhiza* Marshall), although their low stamen number is considered as a reduction (Ronse De Craene & Smets 1995, Damerval & Nadot 2007). Interestingly, androecia with only ten stamens are also found in some *Aquilegia* L. (Tucker & Hodges 2005), a genus which also has five carpels.

Persisting tepals at the fruiting stage as seen in *Ranunculaecarpus* are unusual for extant Ranunculaceae, where the perianth members typically abscise after anthesis (a notable exception is *Helleborus* with sepals persisting in fruit). In contrast, sepals typically do not abscise after anthesis in some other families, for example in some Rosaceae.

In many extant Ranunculaceae the carpel insertion is spiral and carpel number is not fixed. The apparent stable carpel number in *Ranunculaecarpus* may indicate a whorled arrangement and a pentamerous and isomerous organization, and would support the idea of the plesiomorphic nature of whorled gynoecium in Ranunculaceae (e.g., Soltis et al. 2018). The evolution of pentamery is of special interest because it characterizes most core eudicots (Endress 2010, Soltis et al. 2003, Ronse De Craene 2010, Sauquet et al. 2017). Among extant Ranunculaceae, whorled pentamerous flowers with five carpels are typical for *Aquilegia* (Tucker & Hodges 2005, Endress 2006, Jabbour et al. 2009). It should be noted that stable occurrence of five carpels can be also seen in spiral flowers with five parastichies, for example in *Nigella damascena* L. (Jabbour et al. 2009).

The pollen of *Ranuculaecarpus* is rather distinctive and apparently differs from that of extant Ranunculaceae. Pollen is variable in extant Ranunculaceae (Erdtman 1986), varying from di- to tricolpate, 6-polyrugate, polyforate, to inaperturate, but the basic types are tricolpate or tricolporate and (micro) reticulate. None are known to have a loose reticulum with large polygonal lumina as seen in *Ranunculaecarpus*. Most Ranunculaceae have pollen with a continuous tectum (not reticulate) that is regularly spinulate, unlike *Ranunculaecarpus* pollen.

COMPARISON WITH OTHER FOSSILS AND THE CRETACEOUS RECORD OF RANUNCULACEAE

The fossil record of reproductive structures of the Ranunculaceae is relatively poor (Pigg & DeVore 2005). The oldest potential example is of small ribbed fruits from Early Cretaceous deposits of Portugal that resemble *Thalictrum* (Friis et al. 1994), but this fossil has not been studied in detail and its systematic affinity was not fully documented. The seeds of *Eocaltha zoophilia* Rodriques-de la Rosa, Cevallos-Ferriz & Silva-Pineda from Campanian deposits of Mexico (Rodriquez-de la Rosa et al. 1998) have some similarities with those of the modern genus *Caltha*. There are, however, floral structures with three-dimensional preservation from the Early Cretaceous that show close similarity to members of the Ranunculales. These include the flowers of *Teixeiraea lusitanica* von Balthazar, K.R. Pedersen & E.M. Friis (von Balthazar et al. 2005), *Kajanthus lusitanicus* M.M. Mendes, G.W. Grimm, Pais & E.M. Friis (Mendes et al. 2014) and *Paisia pantoporata* E.M. Friis, M.M. Mendes & K.R. Pedersen (Friis et al. 2018) from Portugal, and *Kenilanthus marylandensis* E.M. Friis, P.R. Crane & K.R. Pedersen from Maryland, USA (Friis et al. 2017).

The *Kajanthus* flower is distinct from *Ranunculaecarpus* in its trimerous organization, and the *Teixeiraea* flower probably is unisexual. *Paisia* and *Kenilanthus* are comparable to *Ranunculaecarpus* in their apocarpous and pentamerous gynoecium, and *Kenilanthus* have an androecium of about 10 stamens, but *Kenilanthus* differs from *Ranunculaecarpus* in its very long stamens with short filaments and elongated tetrasporangiate, dithecate anthers, and tricolpate, reticulate pollen grains. The perianth and androecium of *Paisia* are distinct from those of *Ranunculaecarpus* with five massive tepals enclosing five stamens. Pollen of *Paisia* is also distinct in its spiny pantoporate configuration (Friis et al. 2018).

In addition to the three-dimensionally preserved floral structures of presumed ranunculalean affinity, there are several impression/compression fossils that may be related to Ranunculales and comparable to

Ranunculaecarpus. *Hyrcantha karatscheensis* Krassilov & Vachrameev from the middle Albian of Kazakhstan comprises a paniculate inflorescence similar to some Ranunculaceae and Paeoniaceae (Krassilov et al. 1983), with terminal bisexual flowers having a small persistent calyx. Each flower consists of three (rarely five) free follicles with sessile stigmas. The details of stamens, pollen and seeds are not preserved. Associated leaves are compound, biternate, with entire-margined leaflets. This plant differs significantly from *Ranunculaecarpus* in floral morphology. *Sinocarpus decussatus* Leng & E.M. Friis from the Barremian-Aptian Yixian Formation of Liaoning, China, was described based on infructescences with decussate arrangement of the fruits (Leng & Friis 2003, 2006). It is distinguished from *Ranunculaecarpus* and from *Hyrcantha* in having a syncarpous gynoecium, in contrast to the apocarpous gynoecium of *Ranunculaecarpus* and *Hyrcantha* (Friis et al. 2011).

Two herbaceous plants from early-middle Albian deposits of the Far East – *Ternaricarpites floribundus* Krassilov & Volynets and *Achaenocarpites capitellatus* Krassilov & Volynets – were assigned to the Ranunculaceae by Krassilov & Volynets (2008). *Achaenocarpites* is characterized by a solitary terminal flower developing into a head of numerous achenes, with tepals partly persistent at fruiting stage. Its leaves are basically ternate, pinnatisect or three-lobed. These lobed leaves are similar in dissection pattern to the leaves of *Crataegites* that co-occur with *Ranunculaecarpus*, but they differ by having entire margins. The fruits of *Ternaricarpites floribundus* consist of three free follicles. The leaves of *Ternaricarpites* are also pinnatisect, but with opposite decurrent lobes.

Leefructus, described as an impression fossil from the Early Cretaceous (Barremian-Aptian) Yixian Formation of the Jehol biota, northeastern China (Sun et al. 2011), bears some resemblance to *Ranunculaecarpus*, but details of its floral organization and the internal structure of fruits and seeds are not preserved.

CONCLUSION

Our investigation, using reflected light and scanning electron microscopy of fractured specimens, supplemented by epifluorescence and both reflected and transmitted light microscopy of physical thin sections, revealed many previously unknown features of the extinct *Ranunculaecarpus* fruit and flower. We also made a preliminary x-ray synchrotron study which indicated that most of the morphological and anatomical details, with the exception of pollen morphology, may now be obtained by non-destructive methods. It may be possible to discover additional *Ranunculaecarpus* specimens hidden within the sedimentary matrix by CT-scanning other leaf-bearing pieces in the collection. Although the similarities to Ranunculaceae first recognized by Samylina (1960) remain striking, the differences that we have enumerated preclude direct assignment to the extant family.

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