The significance of *Runcaria* (Middle Devonian, Belgium) in the evolution of seed plants.

[L'importance de *Runcaria* (Dévonien moyen, Belgique) pour l'évolution des plantes à graines]

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Introduction

The advent of reproduction by seeds was one of the most essential evolutionary steps in plant history: the vast majority of living plants are seed plants (spermatophytes). The seed habit includes the following set of defining characteristics: (1) heterospory, (2) occurrence of a single megaspore that germinates within an indehiscent megasporangium (nucellus) retained on the sporophyte, (3) enclosure of the megasporangium in an integument, and (4) capture of pollen before seed dispersal (ROTHWELL & SCHECKLER, 1988; HAIG & WESTOBY, 1989). Contrasting hypotheses about the single / multiple, saltational / gradual origin of the seed habit (CHALONER, 1989; GALTIER & ROWE, 1989; DIMICHELE *et alii*, 1989; BATEMAN & DIMICHELE, 1994), and identification of the closest relatives of seed plants (aneurophytalean or archaeopteridalean progymnosperms) (ROTHWELL & ERWIN, 1987; ROTHWELL & SERBET, 1994; MARSHALL & HEMSLEY, 2003) are still matters of considerable debate. Early seeds did not possess the whole set of characters that define modern seeds. They lacked a true micropyle and an entire integument, and none has yet been discovered containing an embryo. Hence they are called preovules. A suite of characters observed in the Late Devonian preovules *Moresnetia* and *Elkinsia* and in a number of younger taxa define

hydrasperman reproduction (ROTHWELL & SCHECKLER, 1988). In hydrasperman preovules, prepollen capture was realized by the modified apex of the nucellus. Prepollen were directed into a hollow lagenostome, then retained in a pollen chamber. A central column attached to the pollen chamber floor sealed the chamber to provide optimal conditions for prepollen germination. The hydrasperman syndrome has been presented as shared by all basal members of the seed plant clade (ROTHWELL & SCHECKLER, 1988). Discovery of the Lower Carboniferous preovule *Coumiasperma* challenged this view. GALTIER and ROWE (1989, 1991) suggested that the possession of a massive nucellar tip and the lack of pollen chamber characterized another primitive seed organization that either preceded and was ancestral to the hydrasperman type, or evolved independently and was adapted to wet habitats.

At least six different types of Late Devonian (around 365 Ma) preovules or preovule-like structures are known (PRESTIANNI, this volume). This diversity strongly suggests an earlier evolution of the seed habit. The Middle Devonian (around 385 Ma) *Runcaria heinzelinii* STOCKMANS 1968 has been recently redescribed on the basis of new specimens, and interpreted as a precursor of seed plants (GERRIENNE *et alii*, 2004). Since then, more new specimens have been isolated from the original fossiliferous slab. Some of them are illustrated here.

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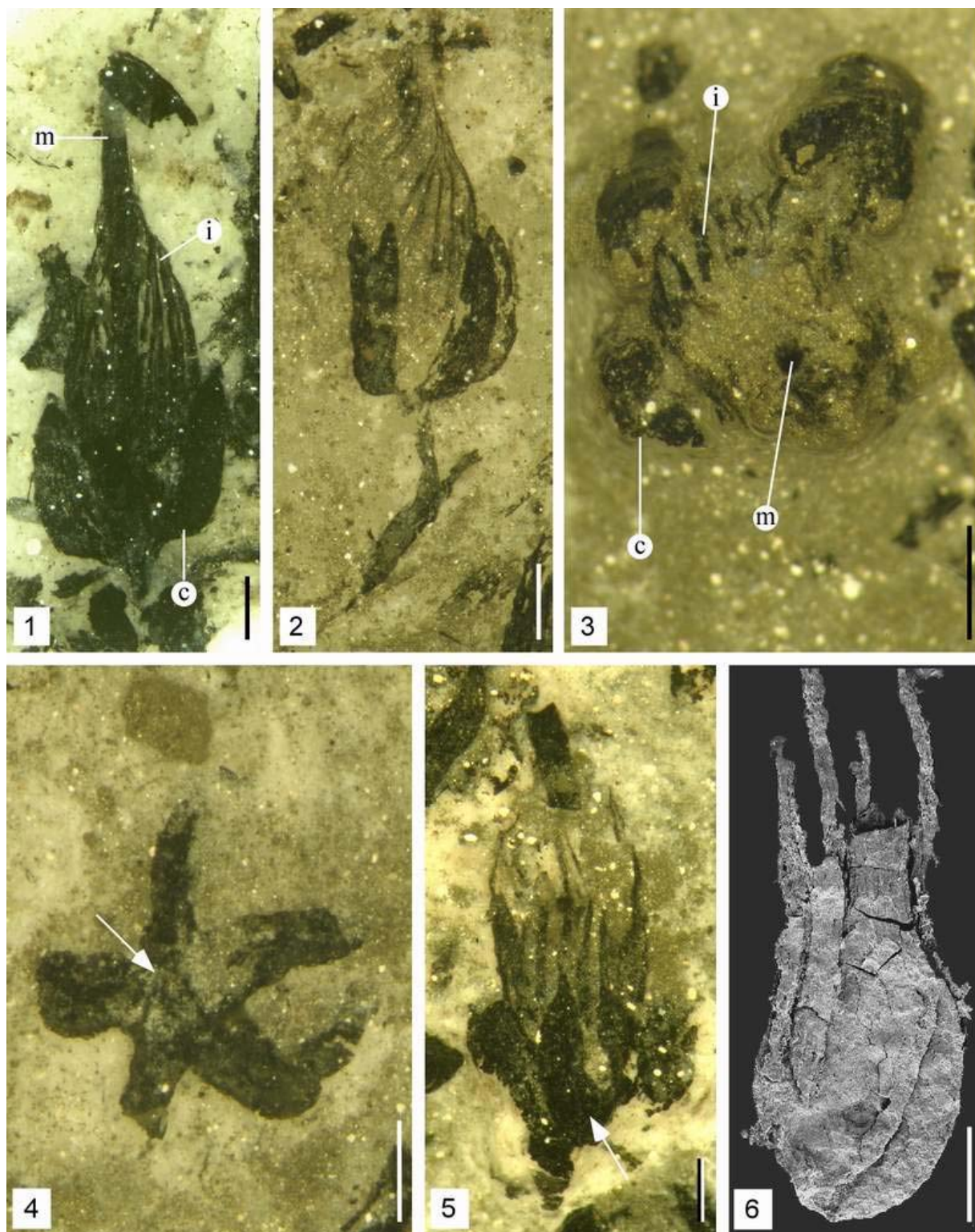M.Fairon@ulg.ac.be

Plate 1: *Runcaria heinzelinii* STOCKMANS 1968
Figure 1. Specimen IRSnB 104866/Slide 2. Nearly complete preovule. c: cupule segment; i = integumentary lobe; m = megasporangium. Scale bar = 1 mm.
Figure 2. Specimen IRSnB 104866/Transfer2. Preovule at the tip of a dichotomizing axis. Scale bar = 1 mm.
Figure 3. Specimen IRSnB 104866/Transfer2, specimen destroyed. Preovule as seen from above, showing the radial symmetry. Three cupule segments are visible. c: cupule segment; i = integumentary lobe; m = megasporangium (transverse section of the columnar extension). Scale bar = 1 mm.
Figure 4. Specimen IRSnB 104866/Transfer2, specimen destroyed. Cupule with 5 segments (seen from below). Arrow indicates broken stalk. Scale bar = 1 mm.
Figure 5. Specimen IRSnB 104866/Transfer2. Preovule with cupule segment divided in two halves (arrow). Scale bar = 1 mm.
Figure 6. Specimen IRSnB 104866/Stub2. Basal part of the megasporangium. Note the faint hexagonal pattern on its surface. Scale bar = 0.5 mm.

About the age of *Runcaria*

Hundreds of *Runcaria* preovules occur in a single large slab of sandstone from the Bois de Bordeaux Formation collected at the "Plan incliné de Ronquières" locality (Belgium) (Stockmans, 1968). Data regarding the age of the locality are numerous. All plant horizons at Plan incliné de Ronquières belong to the upper (but not the uppermost) part of the Bois de Bordeaux Formation, which from conodont evidence is regarded as Givetian or earliest Frasnian (Bultynck, 1991). Other plant remains collected from the same locality include the progymnosperm genera *Protopteridium* (= *Rellimia*) and *Svalbardia* and the cladoxylopsids *Pseudosporochnus* and *Calamophyton* (Stockmans, 1968). The concurrent range of those four genera is restricted to the Givetian (Edwards *et alii*, 2000). *Runcaria* is found in association with axes and dispersed leaves of the lycopsid genus *Leclercqia*, that ranges from late Emsian to late Givetian (Meyer-Berthaud *et alii*, 2003). Finally, palynological samples yielded more than 30 species of trilete micro- and megaspores that constrain a middle to late Givetian age (TA Oppel Zone; 385 Ma) (Gerrienne *et alii*, 2004).

Description

Specimens of *Runcaria* consist of 6.5 to 8 mm long cupulate preovules (Pl. 1, fig. 1) borne singly at the tip of small axes bifurcating at 40° to 70° (Pl. 1, fig. 2). These stalks do not exceed 3 mm in length and 0.4 mm in diameter. Each preovule is radially symmetrical (Pl. 1, fig. 3). Cupules form a short cup supporting a single preovule. They comprise four to five segments (Pl. 1, fig. 4) free from each other except at the base, each segment ranging from 1.6 to 3 mm in length and 1.1 to 1.7 mm in width. Cupule segments divide in two halves; each half separates distally in two acute tips (Pl. 1, fig. 5). Lateral edges of the cupule segments are decurrent and form rims along the stalk. Preovules consist of a radially symmetrical megasporangium surrounded by a 4.7 to 5.2 mm long laciniate integument. The megasporangium comprises a sessile oval body bearing a distal extension that emerges above the integument and ends up in an enlarged head. The columnar extension is 4 to 5.2 mm long and 0.2 to 0.4 mm wide; the proximal oval body, 1.4 to 2 mm long and 0.9 to 1.3 mm wide. A faint oval mark in the oval body of some specimens may represent the imprint of a megaspore or a megagametophyte, two structures that cannot confidently be observed in ovules preserved as adpressions. The junction between the oval body and the distal extension does not show any bulge that would suggest the occurrence of a pollen chamber. The columnar extension is probably hollow and may be locally enlarged. Its surface is covered by longitudinal ribs separated by deep furrows and twisted counterclockwise. In contrast, the oval body shows a faint polygonal pattern on its surface (Pl. 1, fig. 6). The distal head ending the columnar extension is up to 470 μm wide and 780 μm long. It is made up of cells with thinner walls than those of other cells of the megasporangium. Its external surface is smooth and may show a distal projection. There is no evidence of an opening or dehiscence mark on any part of the megasporangium. The integument separates into a minimum of 16 lobes free from each other and from the megasporangium down to the base. The lobes converge distally, their tips appressed or wrapping around the columnar extension at about 2/3 of its length. Lobes either lie vertically along the megasporangium or separate from it in a counterclockwise spiral, to form the loose walls of a chamber surrounding the basal part of the megasporangium. Individual lobes are up to 100 μm wide and are comprised of elongate thickened cells. In the chamber, they are separated from each other by spaces exceeding 100 μm in width. Many microspores occur around the cupulate preovules and among the integumentary lobes, but none adhere to the distal head of the megasporangium or to the column.

Discussion

Despite the lack of evidence concerning its megaspore/megagametophyte content, *Runcaria* displays three characters (modified megasporangial apex, dissected integument, and a cupule) specific to early seed plants, that indicate its spermatophytic affinities (Rothwell & Scheckler, 1988). Salient features of the nucellar apex of *Runcaria* are its extensive length, probably hollow structure, lack of opening and an enlarged cellular tip. Based on that morphology, several scenarios are proposed for the pollination of this ancient seed. All assume that the distal extension of the megasporangium played a significant role because of the selective advantage that its length may have provided in the passive capture of airborne prepollen. If the Ronquières specimens were in a pre-pollination state of development, we are faced with the problem encountered in *Coumiasperma* where antherozoids must penetrate the wall of a closed megasporangium to reach the female gametophyte (Galtier & Rowe, 1989, 1991). The first scenario involves the lysigeneous dissolution of some nucellar cells, better realized at a site where they are thin-walled. This is the case at the enlarged extremity of the columnar extension, but this site occurs at some distance from the megagametophyte within the oval body. We cannot exclude, therefore, that microspores/ antherozoids

caught on the nucellar extension reached the oval body and dissolved cells closer to the presumed site of archegonial development. An alternative mechanism to induce cell wall dissolution, also presented for *Coumiasperma*, involves siphonogamy. However, the earliest evidence for pollen tubes is much later, within the Late Carboniferous callistophytalean pteridosperms (ROTHWELL, 1972). If the Ronquières preovules were fossilized at a post-pollination stage, the distal head may represent a mass of cells produced to seal the columnar extension, open before pollination occurred. Available specimens of *Runcaria* do not provide any information relative to the occurrence of what could be interpreted as a hydrasperman pollen chamber. Furthermore, the mechanism proposed for sealing the preovule after pollination in this third scenario differs significantly from that involving a central column in hydrasperman structures (ROTHWELL & SCHECKLER, 1988). The nucellar extension of *Runcaria* thus differs from that of *Coumiasperma* and that of the hydrasperman preovules. It is currently premature to assume that the runcarian morphology, and the pollination mechanism attached to it, either is ancestral to all younger ovular morphologies and supports the monophyly of the Spermatophytes, or is divergent and represents an extinct group unrelated to *Coumiasperma* and to the hydrasperman seed plants. However, as has been already realized from younger evidence, the morphology of *Runcaria* confirms that pollination was an essential factor driving the evolution of early seed plants (ROTHWELL & SCHECKLER, 1988; HAIG & WESTOBY, 1989; BATEMAN & DIMICHELE, 1994).

An unexpected trait of *Runcaria* is related to the length of the integumentary lobes that leave the distal extremity of the megasporangium free and unprotected. Aerodynamic experiments (NIKLAS, 1981) showed that integument lobes projecting above the nucellar tip, a feature shared by most early preovules, created as many zones of turbulence around the lagenostome and decreased the probability that prepollen were specifically directed to this structure. Hypotheses predicting a similar morphology in the most primitive preovules were thus faced with the paradox that the development of such an integument appeared counter-selective for pollination, a naked nucellus being more efficient for that function (HAIG & WESTOBY, 1989). With its integumentary lobes leaving the nucellar tip free and thus providing direct access for airborne prepollen, *Runcaria* sheds some light on the adaptive significance of the integument in earliest seeds. If adaptive, evolution of the integument was not driven by selective forces related to pollination but to other necessities yet to be identified, presumably related to protection, nutrition and/or dispersal.

Conclusion

The late Middle Devonian *Runcaria* is a complex structure that already possessed the full set of attributes, including a cupule, that characterize younger seeds. It shows that the evolution of the integument was potentially involved in protection, nutrition and/or dispersal. The morphology of the nucellar apex of *Runcaria* differs from that of the hydrasperman preovules which therefore can no longer be presented as the single primitive type in the seed plant stem group. *Runcaria* evolved in the Givetian when progymnosperms were represented by the Aneurophytales and the earliest representatives of the Archaeopteridales (BECK & WIGHT, 1988). The Middle Devonian age of *Runcaria* closes the stratigraphical and evolutionary gap between the Aneurophytales and the oldest seed plants that, prior to this discovery, was presented as a major argument against a direct relationship between these two groups (HILTON, 1998; MARSHALL & HEMSLEY, 2003). Moreover, if seed characters were acquired sequentially, it implies an earlier, possibly early Middle Devonian, origin for the seed habit, when the only known representatives of the progymnosperms were aneurophytaleans.

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