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Xin Wang * and Li-Jun Chen

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Shaolinia: A Fossil Post Between Conifers and Angiosperms

Xin Wang [1,*] and Li-Jun Chen [2]

[1] State Key Laboratory of Palaeobiology and Stratigraphy, Nanjing Institute of Geology and Palaeontology and CAS Center for Excellence in Life and Paleoenvironment, Chinese Academy of Sciences, Nanjing 210008, China
[2] Shenzhen Key Laboratory for Orchid Conservation and Utilization, National Orchid Conservation Center of China and Orchid Conservation and Research Center of Shenzhen, Shenzhen 518114, China; email: chenlj@sinicaorchid.org
* Correspondence: xinwang@nigpas.ac.cn; Tel.: +86-25-8328-2266

Abstract: The greatly diversified flowering plants (angiosperms) are the dominating and defining group in the current earth ecosystem. However, from which group by what way the flowers, especially their gynoecia (key characteristics of angiosperms), are derived have been the questions in botany unanswered over centuries. Such an embarrassing situation can be attributed to the lack of plants with partially enclosed ovules, which are supposed to be a post between gymnosperms and angiosperms. Here we report a fossil plant that has an apparent coniferous vegetative and reproductive morphologies but has a single seed partially wrapped by the subtending bract. Such a morphology suggests that the carpels in angiosperms may be equivalent to bracts enclosing their axillary seed(s). Such a non-traditional interpretation of the homology of angiosperm carpels is compatible with various new progresses made in botany and is in line with Tomlinson's recent hypothesis. Together with other fossil evidence reported recently, it appears that gynoecia in angiosperms are derived in multiple ways.

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1. Introduction

Flowering plants (angiosperms) can be distinguished from gymnosperms by their enclosed ovules before pollination [1–3]. But how such a feature came into existence has been a puzzle for botanists for long time. Formerly, carpels in angiosperms were supposed to be derived from megasporophylls that bear ovules along their margins [4]. But so far there is no fossil evidence favouring this hypothesis. On the contrary, there are increasing evidence rejecting this hypothesis [5–18]. To answer this question, fossil evidence is the only reliable source for morphological information of plants in the past. As angiosperms and gymnosperms are distinguished each other by the status of ovules before pollination, being enclosed or naked [3], therefore seeking an ovule between those two statuses (namely, semi-enclosed) in a fossil plant is apparently more needed than anything else.

2. Materials and Methods

The specimen, including two facing slabs, were collected from the Yixian Formation of Erdaogou, Jianchang, Liaoning (119.7451°E, 40.5288°N). The specimen includes physical connected conifer-like vegetative part and reproductive organs. Specimens were photographed with a Nikon D810 digital camera. Details of the specimens were observed and photographed under a Leica M205A stereomicroscope equipped with a Leica DFC450C digital camera. A seed was removed from the specimen and observed using a Nikon SMZ1500 stereomicroscope equipped with a Nikon DS-Fi1 digital camera and a Leo 1530 scanning electron microscope (SEM). All photographs were saved in TIFF format and organized for publication using a Photoshop 7.0.

3. Results

Shaolinia gen. nov.

Generic diagnosis: Plant woody, including branches, leaves, and connected cone-like organs. Branch rigid, straight, with helically arranged leaves. Leaf *Juniperus*-like, straight or slightly curving. Cone-like reproductive organs alternately arranged along the branch. Reproductive organ including more than 40 lateral appendages helically arranged around a central axis. Each lateral appendage comprising a single axillary seed and subtending bract wrapping the seed from the bottom and laterals. Bract with a pointed tip, gaping adaxially. Seed round-shaped, with isodiametric sculpture.

Type species: *Shaolinia intermedia* gen. et sp. nov.

Etymology: *Shaolinia* dedicated to Dr. Shaolin Zheng, a senior Chinese palaeobotanist.

Stratigraphic horizon: the Yixian Formation.

Shaolinia intermedia gen. et sp. nov.

Specific diagnosis: the same as the genus.

Description: The specimen includes two facing parts of the distal portion of a woody branch, 68 mm long, 17 mm wide, preserved as a partially coalified compression and impressions on yellowish tuffaceous siltstone (Figures 1a-b). The branch is about 1.1 mm wide in the proximal portion, tapering distally (Figures 1a-b). There are several small axillary lateral branches up to 4.9 mm long, with a few leaves (Figures 1a-b). The leaves are *Juniperus*-like, 0.9-4.4 mm long and 0.2-0.5 mm wide, straight or slightly curving to the distal of the branch (Figures 1a-b, 2a). Four cone-like reproductive organs are inserted alternately along the branch (Figures 1a-b, 2b-d). The cone-like organ is 7.4-10.9 mm in length and 4.2-4.7 mm in diameter, with over fifteen lateral appendages helically arranged around a central axis (Figures 1a-b, 2b-e). Each lateral appendage is round-triangular in shape in adaxial view, about 2.3 mm long, 0.8 mm high, and 1.5 mm wide, including an axillary seed and a subtending bract, and the latter wraps the former from the bottom and laterals (Figures 2b-e, 3a-b). The seed is round in shape, 0.63-0.78 mm x 0.81-1.06 mm, more or less flattened during the fossilization, the seed coat has isodiametric sculpture (Figures 2d-f, 3a-b, 4a-d). The bract has a pointed tip, folding longitudinally and adaxially, gaping adaxially (Figures 2d-e, 3a-b).

Etymology: *intermedia*, Latin for the morphology of the lateral appendages that falls between carpels in some angiosperms and lateral appendages of conifers.

Holotype: JCEDG0001.

Depository: National Orchid Conservation Center of China and Orchid Conservation and Research Center of Shenzhen, Shenzhen, China.

Figure 1. General view of *Shaolinia intermedia*, showing several axillary branches (arrows), leaves and four cone-like reproductive organs (1-4) arranged along the branch, on two facing slabs. Scale bar = 1 cm.

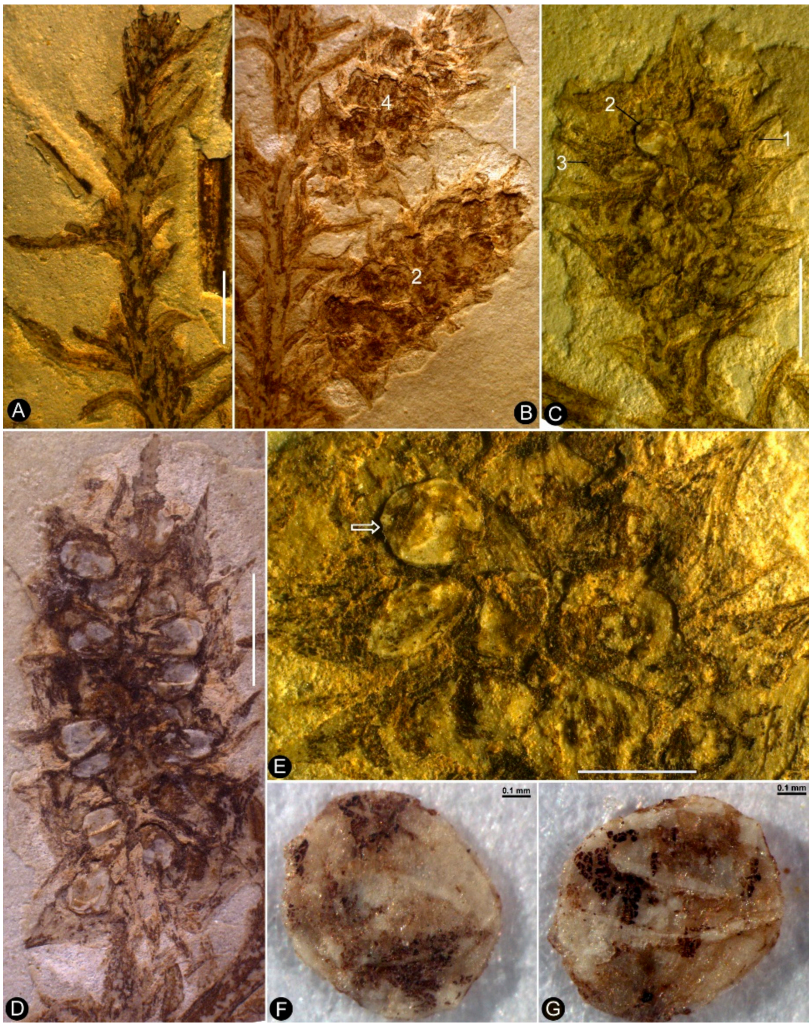

Figure 2. Leaves and reproductive organs of *Shaolinia intermedia*. **A**. The distal portion of the branch with helically arranged leaves. Scale bar = 2 mm. **B**. Detailed view of the reproductive organs (No. 2 and 4). Note the helically arrange lateral appendages. Scale bar = 2 mm. **C**. Detailed view of reproductive organ No. 3, with helically arranged lateral appendages. Scale bar = 2 mm. **D**. Detailed view of reproductive organ No. 2, showing seeds inside the lateral appendages. Scale bar = 2 mm. **E**. Detailed view of the distal portion of the reproductive organ shown in Figure 2c. The arrowed seed is removed and shown in Figures 2f-g and 4a-d. Scale bar = 1 mm. **F-G**. Detailed views of the seed removed from the organ shown in Figure 2e. Scale bar = 0.1 mm.

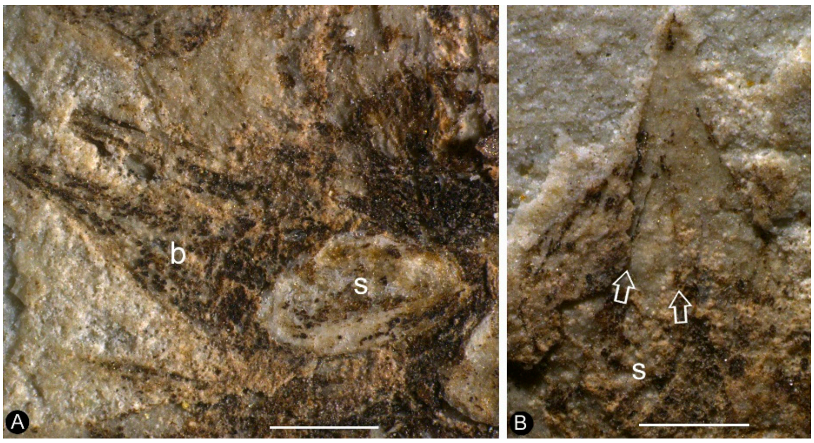

Figure 3. Lateral appendages with *in situ* seed inside and an adaxial gap. **A**. Detailed side view of lateral appendage No. 3 in Figures 2c, showing the seed (s) wrapped by the bract (b). Scale bar = 0.5

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mm. **B**. Detailed side view of top lateral appendage in Figures 2c, showing the adaxial gap with two margins (arrows) of the bract. Scale bar = 0.5 mm.

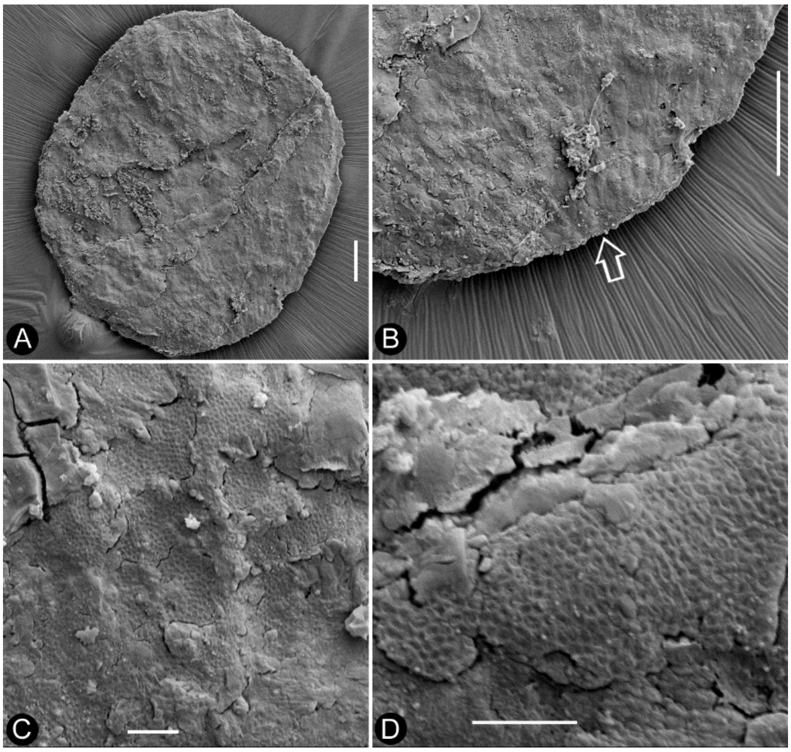

Figure 4. SEM view of the *in situ* seed found in *Shaolinia intermedia*, from the reproductive organ No. 2 shown in Figures 2c, e-g. A. Seed in whole. Scale bar = 0.1 mm. B. Converging cellular arrangement suggestive of possible micropyle (arrow) of the seed. Scale bar = 0.1 mm. C. Seed coat sculpture. Scale bar = 10 μm. D. Detailed view the seed coat sculpture. Scale bar = 10 μm.

Remarks: We refrain us from using the terms like "carpels" and "flowers" in our description as they would make as if we preferred to treat *Shaolinia* as an angiosperms, although we cannot rule out the possibility that later evolution *Shaolinia* may turn into a true angiosperm bearing flowers and carpels.

The term "bract" is equivalent to that in conifers, and the "axillary seed" is equivalent to and comparable to the ovuliferous scale more reduced (reduced into a single seed) than in conifers.

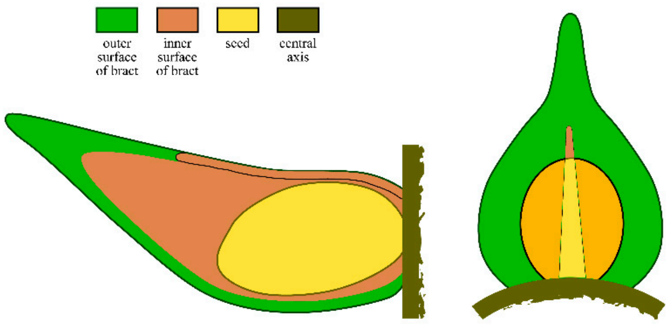

Figure 5. Sketches of lateral appendages of *Shaolinia intermedia*. A. Longitudinal radial profile of a lateral appendage showing a bract and an adaxial seed attached to the central axis and partially wrapped. **B**. Adaxial view of a lateral appendage showing a bract and a partially wrapped adaxial seed inside. Note the bract gapes adaxially.

4. Discussion

Angiosperms can be distinguished from their gymnosperm peers by their enclosed ovules (angio-ovuly) before pollination [1–3], a character that can ensure the pollination to be carried out in

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an angiospermous (not gymnospermous) way. However, how such a feature is derived from former gymnosperms with naked ovules is a crucial question of interest in botanical studies on the origin of angiosperms, because the answer to this question will found the relationship between angiosperms and gymnosperms and unite both into an integral system of seed plants. To answer this question, a fossil plant with semi-enclosed ovule is of key importance. Although conifer-like in vegetative morphologies, *Shaolinia* has its seed wrapped by its subtending bract. Despite the seed/ovule is not fully enclosed as in typical angiosperms, the seed-wrapping tendency of *Shaolinia* is intriguing because it is not hard to extrapolate further evolution from conifers and *Shaolinia* to fully-enclosed ovules/seeds in angiosperms. *Shaolinia* is not alone in term of such an implication, as the recently reported *Combina triassica* has given exactly the same implication [19]. Such a congruous ovule-enclosing tendency in conifer-like *Shaolinia* and *Combina* makes it intriguing to relate conifers with angiosperms, an evolutionary scenario anticipated by some botanists [3].

Axillary branching is a pattern frequently seen in angiosperms and many gymnosperms. Its history can be dated at least back to the Middle Pennsylvanian [20], and is the Bau-plan underlying the lateral appendages of conifer cones. As proven by Florin's works [21–24], the lateral appendages in the reproductive organs of Cordaitales and Coniferales are compound organs comprising an axillary ovule-bearing branch and a subtending bract. Various metamorphisms of these two parts have given rise to diverse cones/gynoecia in these two groups. This information has been well known for long time. However, its implication for the homology of carpels has never been explored before, most likely due to the dominance of Arber and Parkin's hypothesis [4], in which a carpel was assumed derived from a so-called "megasporophyll", which, however, has been proven non-existent [9-10]. Most botanists have been misled by such a groundless speculation. The first obvious light elucidating the homology of carpels emerged more than 20 years ago when Roe et al. [5] demonstrated that the ovules in *Arabidopsis* are independent of the carpel wall and borne on a branch. Morphologically, the most significant progress was made recently when Zhang et al. [6] revealed that the carpels in *Michelia figo* (Magnoliaceae) are composite organs derived from a former axillary ovule-bearing branch and its subtending foliar part. The varying morphology of carpels in a single tree of *Michelia figo* [6] demonstrates a great resemblance to the lateral appendages of *Shaolinia* in term of axillary seeds and subtending foliar parts. Such observations make it obvious that axillary branching frequently seen in many seed plants is a feature shared by conifers (besides others) and angiosperms, and a carpel may well be derived from an axillary ovule-bearing branch (equivalent to a placenta) and its subtending foliar part (equivalent to ovarian wall). Configuration and organization of lateral appendages in *Shaolinia* is comparable the carpels in *Illicium lanceolatum* (Schisandraceae) [25], and the only difference between these two lies in the extent the carpel wall (= bract) is closed. This speculation has been favoured by a Triassic conifer-like reproductive organ, *Combina triassica* [19]. Now the occurrence of *Shaolinia* seems to reinforce and strengthen the evidence chain for the derivation of carpels in angiosperms from bract-scale complexes in conifers, as suggested by Tomlinson [3], and solves the recalcitrant problem of origin of angiosperms and their carpels.

Based on comparison of gynoecium organization in early angiosperms [13–18,26–33], it is obvious that the gynoecia of these early angiosperms are diversified in morphology and organization. Although gynoecia in Magnoliales and Amborellales cannot be excluded from the list of ancestral taxa, there are other types of gynoecia that may be plesiomorphic and derived independently in the geological history. For example, the Early Jurassic *Nanjinganthus* is hard to accept for many because of its "noncarpellate" inferior ovary [30–31,34], a feature that used to be thought much derived in angiosperm systematics. It appears more plausible that the gynoecia in angiosperms were derived from different taxa independently (*33-35*), an evolutionary scenario unexpected previously.

5. Conclusions

The new fossil sheds a new light on the evolution of female reproductive organs, suggesting potential homology between some conifers and angiosperms. In line with previous fossil reproductive morphology, there appear to be various independent ways to derive the reproductive organs (flowers) in angiosperms.

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