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Morphology of *Juniperus* Cone and its Implications on Cone Evolution

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Abstract

Background: The basic cone unit in *Pinaceae* is called Bract-Scale-Seed Complex (BSSC), in which the scale is supposed to be equivalent to an axillary shoot bearing ovules in *Cordaitales*. This correlation established by Florin provides a rational foundation on which an interpretation for the origin of cones in at least most *Coniferales* is built, and may be called Florin model for convenience. *Cupressaceae* is a family in *Coniferales*, in which the ovule-scale and its subtending bract are thought fully fused and hard to distinguish by external morphology.

Results: Different from *Pinaceae* and other typical conifers, *Juniperus (Cupressaceae)* appears not following Florin's model closely. For example, the cone of *Juniperus oxycedrus* has only three rather than more BSSCs in a whorl, and its fleshy fructification appears more like a berry rather than a typical coniferalean cone. In this paper morphology and anatomy of *Juniperus oxycedrus* fructifications are documented using Micro CT. New observation demonstrates clearly that three seeds alternate the three surrounding bracts in *Juniperus oxycedrus*.

Conclusion: Such spatial arrangement is quite different from that in typical BSSCs, in which the ovules should be aligned with their subtending bract. Together with other unexpected features in other cupressaceous cones, *Juniperus* may help to expand the avenue through which we can interpret the origin and homology of cones in *Cupressaceae* and other conifers or gymnosperms in general.

Keywords: Conifers • Cone • Evolution • Gymnosperms • *Juniperus* • *Cupressaceae*

Introduction

Cones are reproductive organs characteristic of gymnosperms. They are frequently seen in *Coniferales, Bennettitales, Pentoxyales, Gnetales,* and *Cycadales* [1]. Since 1930s the research of Florin and ensuing botanists appear to have resolved the homology of cones in *Coniferales*, in which a basic unit of a typical cone is called Bract-Scale-Seed Complexes (BSSC) and interpreted as derived from a secondary fertile shoot situated in the axil of its subtending bract [2-6]. The Florin model appears to be very successful and can account for many evolutionary events related with *Coniferales*. However, persisting problems remain unsolved. For example, the spatial relationship between the assumed scale and bract is hard to decipher in some *Cupressaceae*, and the homology between coniferous cones and those in *Cycadales, Pentoxyales,* and *Bennettitales* remains obscure, leaving the evolutionary relationship among these taxa open to debate. At least some of the cones in *Cupressaceae* appear incompatible with the Florin model of cones although the latter appears to have resolved many problems of evolution in other *Coniferales*. It is interesting that, although *Cupressaceous* cones are frequently investigated, the consistent inconsistency between these cones and Florin model is frequently down-played [7,8]. Considering these all, investigating and thus understanding *Cupressaceous* cones is necessary and crucial and has the potential to shed otherwise unavailable light on the evolution of coniferous cones.

Here we document the morphology and anatomy of the cones of *Juniperus* using Micro CT, a new technology available recently. Through the application of this technology, the morphology and organization of *Cupressaceous* cone is more clearly visualized and demonstrated. The goal of this research is to call for decent attention to this non-Florin cone organization in *Cupressaceae,* discuss its potential for complementing the Florin model and prompt an integrated evolutionary map for gymnosperms based on cone morphology.

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Materials and Methods

The material was collected from a tree of *Juniperus oxycedrus macrocarpa* (Silbth. and Sm.) in the Jardí Botànic de Sóller (IPEN ES-0-SOLLE-160019), Mallorca, Spain in June, 2016. The sample was photographed using a Sony ILCE-7 digital camera, scanned using a Bruker SkyScan 1172 at the Institute of Botany, Chinese Academy of Sciences (CAS), Beijing, China. The machine-generated data was used to re-generate 3D image using a VG Studio and final result was output as videos and pictures. All figures were organized as figures using a Photoshop 7.0 for publication.

It is frequently said that the scale and bract are fused each other in the *Cupressaceae*. We cannot confirm or deny this statement. In case of the material studied here, we would not use the word "scale" but only use "bract" in the description because

1) The scales, if present, should be closely related with seeds,

2) We saw nothing other than bracts closely associated with the seeds,

3) We cannot see any trace of scales distinguishable from the bracts anatomically and morphologically,

4) A scale, if present, should be aligned with the seeds rather than a bract.

The readers are welcome to interpret otherwise, but we do not think that this would influence the validity of our following discussion and conclusion.

Results

The cones of *Juniperus oxycedrus macrocarpa* were collected when they were mature. The fructifications appeared fleshy and baccate due to the presence of three seeds inside the fructification. The fructifications were about 19 mm long and 15 mm in diameter. Some longitudinal veins were seen in the fleshy bracts that surround the seeds inside. The apices of the fructifications were triangular in shape suggestive of the tripartite organization of the cone. Three radiating sutures were seen on the cone apex implying that positioning of former bracts (Figure 1).

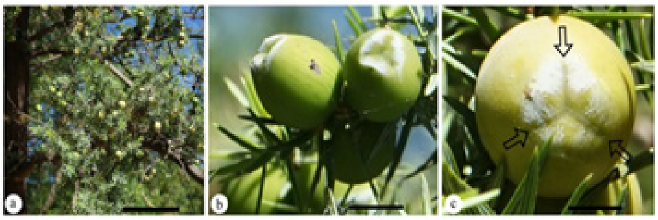

Figure 1. Shoot and fructifications of *Juniperus oxycedrus macrocarpa* (Silbth. and Sm.). A. Part of the shoot of a tree of *Juniperus oxycedrus macrocarpa* in the Jardí Botànic de Sóller, Mallorca, Spain. Bar=10 cm; B. A couple of fructifications on a branch. Note the longitudinal veins within the fleshy bract. Bar=1 cm; C. Top view of a fructification, showing its triangular apex with three radiating sutures (arrows) between three adjacent bracts. Bar=5 mm.

The three sutures were also seen in Micro CT slices. The spatial relationship between the fleshy bracts and inside seeds could be revealed thanks to Micro CT technology. Through the observation of video and pictures, it became clear that the seeds alternate, rather than opposite, the bracts (Figure 2).

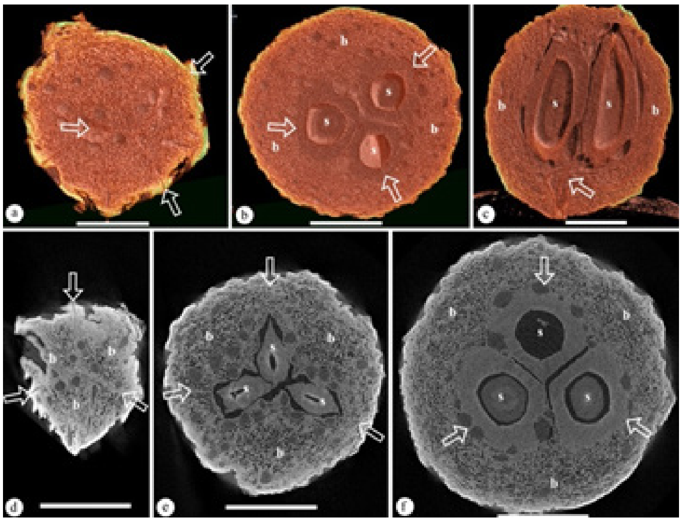

Figure 2. Micro CT observation of a fructification *Juniperus oxycedrus macrocarpa* (Silbth and Sm) Regenerated in VG Studio. A. Transverse section across the tip of a fructification, showing three radiating sutures (arrows) between adjacent bracts; B. Transverse section across the middle of the same fructification in the same orientation showing three seeds (arrows) corresponding the three radiating sutures; C. Longitudinal section across the middle of the same fructification, showing two of the three seeds supplied by vascular bundles (arrow) and surrounded by bracts; D-F. Three transverse sections at different levels of the upper portion of the fructification in the same orientation, showing the correspondence in position between the sutures between bracts and seeds (arrows).

Note: All bars are 5 mm long, b-bract; s- seed.

Discussion

According to the well-accepted Florin model of *Cordaitales-Coniferales* evolution [2-4], BSSC in a typical cone should comprise a subtending bract and a secondary fertile shoot in its axil. This spatial relationship is easy to understand and accept considering axillary branching is almost ubiquitous in seed plants, in which a branch is always in the axil of a subtending leaf [1,9,10]. Such compatibility between hypothesis and observation in most seed plants at least partially contributes to the success of the Florin model for cone evolution. Not surprising, such a model is also frequently applied to account for the organization of cones in *Cupressaceae*, a family in Conifers. According to Farjon [11], as far as back to 1893 Jack has started paying attention to the positioning of ovules in *Cupressaceous* cones. For example, ovules have been seen between bracts in *Callitris, Diselma, Fitzroya Widdringtonia* Plate 16.1-4,6, *Juniperus oxycedrus*, *Juniperus communis*, *Juniperus brevifolia, Juniperus phoenica* Plate 11.5-6, *Libocedrus plumosa*, *Libocedrus bidwillii, Tetraclinis, Cupressus macnabiana, Cupressus guadalupensis, Cupressus arizonica, Cupressus goveniana* Plate 9.2-3 [11]. In the meantime, ovules have been seen on the cone apices in *Juniperus tibetica, Juniperus squamata, Juniperus satuaria, Juniperus recurva, Juniperus przewalskii, Juniperus pingii, Juniperus indica, and Juniperus angosturana* Farjon 2005. According to Farjon 2005, Jack 1893, Kubart 1905, Noren 1907, Renner 1907, Gaussen 1967, Jagel 2001, Farjon and Garcia 2002 have addressed the morphology of ovules and bracts in *Cupressaceous cones*. Unfortunately, such a consistent inconsistency between *Cupressaceous cones* and the Florin model is largely ignored, probably due to the success and dominance of the Florin model, which appears valid for many *coniferous cone*. Beyond *Coniferales*, there are still other cones in gymnosperms including *Pentoxyales* and *Cycadales* that appear beyond the coverage of the Florin model. The homology among typical cones of *Coniferales* and these gymnosperms is still mysterious, hindering a comprehensive appreciation of the evolution of gymnosperms.

Part of the reason of the current academic situation is due to the shortcoming of traditional documenting technique, which cannot demonstrate the morphology and anatomy of cones to botanists and general public. Although paraffin section has contributed much to our understanding of plant anatomy in the past decades, its two dimensional presentation of three dimensional morphology and anatomy requires extra effort and education to correctly conceive the three dimensional relationship among different parts in an organ of interest. Newly developed technologies enable us to visualize and demonstrate the anatomy of plant organs in an easy-conceive way. Micro CT is one of such new technologies. Its application leaves no space for anyone to ignore the anatomical fact in *Cupressaceous* cones. This is the reason we try to apply Micro CT to call for attention to the long-ignored fact about *Cupressaceous* cones.

According to the Florin model for *coniferous cones*, each basic unit of coniferous cone comprises a subtending bract and a secondary fertile shoot in its axil. This interpretation implies that a secondary shoot and its subtending bract are aligned in the same radius, namely, the secondary fertile shoot opposite the corresponding bract. This implication is well confirmed in most *Coniferales*, including *Pinaceae*, *Araucariaceae*, and Taxodiaceae. This explains the success and wide acceptance of the Florin model. If this implication were not confirmed in the reality, the fate of the Florin model would be quite different. It is interesting that the alignment between ovules and bracts expected by Florin model is not seen in at least above mentioned taxa in *Cupressaceae*. The existence of such consistent discrepancy between the Florin model and botanical observation undermines the validity of Florin model, or at least reduces the applicable scope of the model.

As documented here, the ovules consistently alternating the bracts in Juniperus are of importance in that they cast doubt over the validity of the Florin model in the *Cupressaceae*, and, more importantly, prompt new more widely applicable interpretations, at least including the *Cupressaceae*. Thus a new interpretation for cones valid in wider scope is needed in botany. It becomes more interesting, as you see below, that all cones in gymnosperms may be derived from a single common ancestor, and the Florin model may well be a specialization of a more general model applicable for more gymnosperms.

One of the important studies on plant organ evolution was performed about twenty years by Crane and Kenrick. After careful study of living and fossil plants, Crane and Kenrick came to a hypothesis that the variety of organs seen in living and fossil plants is a result of long time diverted development of reproductive organs throughout the geological history [12]. In their paper, their hypothesis is exemplified by the provenance of microphylls in lycopsids and interseminal scales in Bennettitalean cones. Actually, the derivation of integument can also be taken as a result of diverted development, as suggested by Benson and favored by laters [13-16]. The earliest reproductive organs in land plants are aggregates of sporangia, micro- or mega-, borne on shoot terminals, as seen in the earliest land plants [16]. It is conceivable that each of the ancestral female cones may comprise an axis and clusters of ovules (megasporoclads) helically arranged along its sides. This situation may be exemplified by the lax cone of *Cycas*, in which clusters of ovules are helically arranged around the shoot apex. If each of the megasporoclads in *Cycas* is reduced into an ovule, a cone with helically arranged ovules/seeds around its axis, just as seen in *Pentoxyales*, may come into existence. The diverted development (sterilization) of these lateral appendages (ovules), as seen in *Pentoxylalean cones*, may produce interseminal scales, which surround and protect their fertile peers (ovules) in *Bennettitales*. The hybridization between axillary branching and the cones of *Pentoxyales*, turning sterilized ovules into subtending bracts, may give rise to cones seen in Cordaitales and typical *Coniferales*. Further modification of the secondary fertile shoot may make it a shoot terminating in an ovule with a micropylar tube, as seen in Gnetales. Lacking involvement of axillary branching, sterilization of some ovules into bracts in *Pentoxylalean cones* might produce the cone configuration documented here for *Cupressaceae*, namely, some of the former ovules may be sterilized and function as protecting bracts. When clusters of ovules (rather than single ovule) are retained, clusters of ovules dispersed between bracts as seen in Widdringtonia, *Juniperus oxycedrus, Juniperus communis, Juniperus brevifolia, Libocedrus plumosa, Libocedrus bidwillii, Tetraclinis, Cupressus macnabiana, Cupressus guadalupensis*, and *Cupressus arizonica* become something easy to conceive. When all except the terminal ovule(s) is retained and all others are sterilized into protecting surrounding and subtending bracts, the situation in Callitris rhomboidea [17,18]. *Juniperus tibetica, Juniperus squamata, Juniperus satuaria, Juniperus recurva, Juniperus przewalskii, Juniperus pingii, Juniperus indica, Juniperus angosturana,* and *Platycladus orientalis* may come into existence [8,11]. The significance of this interpretation lies in that it removes the former implicit requirement on spatial relationship between ovules and bracts, namely, the aligned arrangement of ovules and bracts in gymnospermous cones required by the Florin model becomes surplus and unnecessary. Ovules and bracts become independent each other and they have the freedom to combine and coalescence anyway in the new interpretation. Such a great freedom of combination among plant parts makes the great variety of gymnospermous cones easy to appreciate. Thus this lift of unnecessary restriction on spatial relationship between ovule and bract makes a common Bau-plan for gymnospermous cones within reach. If this is the case, botanists will not have to give ad hoc interpretations for various cones and will not have to play ostrich ignoring botanical facts any more. It may well be that the Florin model is a specialization of universal model that is applicable for all gymnospermous cones. If this is true, drawing a conceivable and rational evolutionary roadmap for gymnosperms will be a mission possible for botanists in the near future.

Conclusion

The most intriguing feature of *Juniperus oxycedrus macrocarpa* is the presence of angiospermy in it. The Micro CT observation demonstrates clearly that three seeds are all fully enclosed by the fleshy bracts. For general public, angiospermy may appear idiosyncratic of angiosperms, therefore *Juniperus oxycedrus macrocarpa* documented should be logically placed in angiosperms, apparently, an absurd conclusion. Then an interesting question pops up: What is the real difference between angiosperms and gymnosperms, then? Tomlinson and Takaso gave a clear answer which was accepted and endorsed by me namely, the real difference between angiosperms and gymnosperms is whether the ovules are enclosed or not at the time of pollination. If the enclosure is before the pollination, the plant is an angiosperm. Now it seems that the timing of two events, pollination and seed-enclosing, in the life cycle of plants matters in plant classification.

Declarations

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Competing interests

The authors declare that they have no competing interests. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Availability of data and materials

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Consent for publication

Not applicable.

Ethics approval and consent to participate

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