The Evolution of Entomophily and Diaspore Dispersal in Seed Plants,

the Reproductive Biology of Basal Angiosperms,

and the Development of Beetle Pollination in Gymnosperms and Angiosperms

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ISBN: 978-3-384-57343-8

First published May 2025
(Revision 2, August 2025)

Lektorat: Dr. Dawn Frame

Layout: B. Steinbach

Printing and distribution
on behalf of the authors:

tredition

tredition GmbH, Heinz-Beusen-Stieg 5,
22926 Ahrensburg, Deutschland

Contact address in accordance
with EU Product Safety Regulation:
ilse@cerrado.eu

The German National Library lists this
publication in the German National Bibliography.

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PREFACE

The aim of the present book is to provide an overview of land plant reproductive biology evolution as presently understood. We provide up-to-date information on how plants have changed during the last 400 million years with respect to their reproductive biology (pollination modes, seed dispersal, and partly also breeding systems). Which groups of plants have evolved from what was originally spore or pollen dispersal by wind or water to dispersal by insects (entomophily)? To address this, we explore how extinct groups, such as the Bennettitales, may have reproduced. What were the characteristics of the hypothetical survivors of early angiosperms and were those groups pollination generalists or specialists? These questions are an active area of research and debate today. How were early seeds and fruits constructed and how did they function? How were these diaspores dispersed in gymnosperms and angiosperms? Another phenomenon emphasized in this book is pollination by beetles, which occurred and still occurs in the geologically very old group cycads, and a case is made that it occurred in the extinct Bennettitales. Beetles, along with flies, but also thrips, bees, cockroaches, and even moths, are pollinators of basal and non-basal angiosperms, but in several gymnosperms and basal angiosperms, beetles are especially important pollinators such that cantharophilous characters are deeply imprinted in the flowers of many angiosperm taxa.

The book has three main parts. 1) Development of entomophily and seed dispersal in land plants, 2) Pollination and seed dispersal in certain gymnosperms and basal angiosperms, and 3) Importance of beetles in pollination and discussion of other existing forms of pollination, in certain gymnosperms, and in basal and certain advanced angiosperms.

Together with several of our students and collaborators, we have had a unique opportunity not only to study for many years in Brazil, but by means of expeditions and excursions through several international co-operations, we have been able to carry out plant reproductive biology studies in French Guiana, Peru, Ecuador and Colombia

(South America), Panama and Costa Rica (Central America), Cameroon (Africa), Sabah and Malaya (Malaysia, East Asia), and Queensland (Australia). In addition, working from our base in Germany, we have also had ample opportunities to conduct similar research in Austria and several Mediterranean countries, i.e., Greece, Israel, Croatia, Italy, France, Spain, and Portugal.

Our studies have been the product of collaborations with numerous researchers, technicians, local people and enthusiasts, who among other things have helped identify study plants and animals, and been a source of inspiration and fruitful exchanges. These colleagues who identified plant and animal material were mentioned and thanked in former publications.

We are very grateful to all of them for their support. One person, however, deserves special mention, our colleague and friend, Dawn Frame (Montpellier), who kindly took on the challenge of reading and correcting the book manuscript and she contributed substantially to the chapter on Bennettitales. As Dawn is a well-respected, internationally known tropical botanist in her own right, her knowledge of critical literature and her field experience have contributed significantly to the science of this work. Moreover, our non-native English finds a new voice, color and tone in her phrasing. We are also very grateful to B. Steinbach for his competent layout of this book. Many thanks to the colleagues, who provided several of the photos. Thanks also to the publishers, who gave the license to re-use some of the illustrations, to Annika Lange for literature survey, and Elvira Eberhardt for scanning the slides.

To our knowledge, a book with emphasis on 1) the evolution of reproductive biology in gymno- and angiosperms, 2) pollination modes of basal angiosperms, and 3) the importance of beetle pollination (cantharophily) in gymnosperms and basal and advanced angiosperms, presently does not exist. The facts and discussions presented in this book, based on the authors' broad experience and critical review of the pertinent literature, makes this tome an indispensable tool for biologists interested in the evolution of plant reproductive biology.

1

INTRODUCTION

Spore- or pollen-dispersal by wind or water is an omnipresent phenomenon in the reproduction of plants. It occurs in lower and higher groups, such as algae, lichens, bryophytes, ferns, gymnosperms and angiosperms. A new and usually more efficient method of spore and pollen transfer is entomophily, that is transfer by insects. This system of transfer became dominant in angiosperms and together with pollination by birds and mammals, is one of the characteristics responsible for the success and species richness of this plant group. However, entomophily is not an exclusive phenomenon of angiosperms, but is an 'invention' already developed in certain mosses as well as gymnosperms, predating the development of angiosperms. For example, the moss family Splachnaceae has several entomophilous species. Among gymnosperms there are also several groups that have switched to entomophily. Several extinct Bennettitales, as well as members of the extinct and extant cycads and Gnetales, probably attracted in the past, and still attract, insects as pollen vectors.

Our intention with the present book is to provide an updated overview of the development and evolution of entomophily in plants. We will start with the description of entomophily in the moss family Splachnaceae, continue to gymnospermous groups, and lastly will show the broadness of pollination by insects, water and wind in basal angiosperms. Further, we will provide an overview of beetle pollination, which is the most frequent specialized pollination mode in basal angiosperms. As beetle pollination is not only a phenomenon of basal but also of more advanced angiosperms, the question arises as to what are the common features and differences of cantharophily among different plant groups? Reproduction of plants not only means spore and pollen dispersal and consequent fertilization and embryo development, but also seed and fruit transport and the expansion of plants. Therefore, we will also provide information on, and discuss, these important processes.

Unless otherwise mentioned, photographs are by the authors.

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SPORE DISPERSAL IN BRYOPHYTES (MOSSES)

Entomophily in Splachnaceae

Wind and, to a lesser extent, water dispersal of spores is common in mosses and liverworts (e.g., Melchior and Werdermann 1954). One moss family, Splachnaceae, is distinctive by having entomophilous spore dispersal. About half of the 70 to 80 species of this family have remarkable adaptations for entomophily. There are adaptations to substrates of animal origin (e.g., mammalian dung, skeletal remains), as well as morphological and chemical adaptations (Cameron and Troilo 1982, Pyysalo et al. 1983, Cameron and Wyatt 1986, Koponen 1990, Marino et al. 2009, McCuaig et al. 2015).

Koponen (1990) and Marino et al. (2009) have provided detailed information on the history of studies of Splachnaceae, distribution of species, species biology and scent emissions, adaptations to entomophily, and associated spore dispersing flies; the following section is partly based on their reports. The first mention of entomophily in Splachnaceae is attributed to Bryhn (1897), who observed flies visiting *Splachnum rubrum*. Bryhn noticed that these flies carried *S. rubrum* spores on their body, and later observed the same phenomenon in other *Splachnum* species, as well as in the Splachnaceous genera *Tetraplodon* and *Aplodon*. Although Bryhn was aware of correlations between morphological adaptations of Splachnaceae to specialized substrates, and to spore dispersal, why the flies visited the mosses remained unclear. Later authors, such as Pfaehler (1904), Goebel (1915), Bequaert (1921) and Wettstein (1921) realized that odor emitted from the moss attracts the flies. Wettstein (1921) confirmed that the insect-attracting odors are emissions released by the stomata of the hypophysis of the moss sporophyte. Additionally, scent and other features of animal dung on which the mosses grow is attractive to the flies. Scatophagidae and other fly groups that visit these mosses are well-known dung-visiting flies.

Entomophilous members of Splachnaceae are concentrated in the northern hemisphere, especially in arctic, subarctic, boreal and alpine regions and grow on substrates of animal origin. Several authors studying the mineral composition of entomophilous Splachnaceae report that these mosses tolerate higher concentrations of certain elements, such as nitrogen, calcium and phosphorus, than other common boreal and arctic mosses, and that they accumulate the minerals from the animal feces on which they grow (e.g., Koponen 1990).

Splachnaceae colonies on herbivore dung can withstand competition from other mosses often only for 1-2 years, and each colony can produce sporophytes only

Fig. 1
***Splachnum luteum*.**
The yellow-colored, odor-emitting hypophyses of mosses are attractive to spore-transporting flies.
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once. The reason for this is that longevity in Splachnaceous mosses is dependent on the persistence of the substratum, which, in the case of dung, is of relatively short duration. Bacterial and fungal activity lead to chemical decomposition, and several insects, Annelida and Nematoda mechanically decompose the animal droppings.

In dung-specific Splachnaceae, the distally expanded part of the seta, the hypophysis, is green in the juvenile phase, but during maturation takes on a characteristic size, shape and color, becoming white, yellow, pink, purple, olive brown or black (**Fig. 1**). According to several authors, the large, colored hypophyses, which also emit odor (see below), are attractive to flies that transport the clumped sticky spores. The attracted flies belong to Scatophagidae, Sarcophagidae, Muscidae, Fanniidae, Tachinidae, Calliphoridae, and Anthomyidae. Upon insect landing on the sporophyte and sensing the hypophysis, the sticky moss spores adhere to the hairs of the insects.

Those flies that afterwards visit animal droppings, sites where they frequently meet conspecific mates and copulate, transmit spores to these substrates and thereby found new moss colonies. Studies by Cameron and Wyatt (1986) indicate that wind is an ineffective agent of spore dispersal in entomophilous species of Splachnaceae.

In spore-dispersing Splachnaceae, the sporophyte produces attractive odors. Scent compounds, which are produced mainly in the hypophysis, were determined in several entomophilous species to be volatile octane derivatives and organic acids, such as acetic, propionic and butyric acids (Pyysalo et al. 1978); a later study by the same authors revealed additional compounds such as phenylacetylene, valeric, capronic, benzoic, phenylacetic and palmitic acids (Pyysalo et al. 1983). These early chemical scent studies, although highly suggestive, were carried out on cut plant material, and hence, subject to artifacts (Raguso and Pellmyr 1998, Dobson et al. 2005). More recent studies using less invasive methods found that pungent smelling mature sporophytes of *Splachnum ampulaceum* produced over 50 volatile organic compounds, including short-chain oxygenated compounds, unsaturated irregular terpenoids, fatty acid-derived 6- and 8-carbon alcohols and ketones, and the aromatic compounds acetophenone and p-cresol (McCuaig et al. 2015). In many cases, sporophyte color and odor, as well as visiting flies, are species-specific, indicating that entomophilous Splachnaceae co-exist by signal diversification (Marino et al. 2009).

In summary, dung mosses have a very specific ecology, they are entomophilous (have their spores dispersed by insects, in this case flies) and, with respect to substrate, are coprophilous growing mainly on animal droppings, a feature that doubtless helps to attract the flies. Marino et al. (2009) remark that "The sporophytes are like deceptive angiosperm flowers that attract insects by using sensory cues that mimic decaying organic matter used by such flies for oviposition and for larval food."

COMMENTS ON ENTOMOPHILY IN MOSSES

As explained above, entomophilous spore dispersal occurs in the moss family Splachnaceae. In Splachnaceae several species produce a colored hypophysis and emit a pungent smell that attract mainly flies, which serve as spore dispersers.

Thus, several of the so-called 'lower plants' or 'cryptogams' use color and scent to attract insects into becoming spore dispersers. As will become evident further on, mosses' methods of insect attraction are nearly as sophisticated as those of gymnosperms and flowering plants; the only difference is that the number of entomophilous groups in these mosses, and cryptogams in general, is relatively small.

3

POLLINATION AND REPRODUCTIVE BIOLOGY IN SEED FERNS

Pteridosperms (Pteridospermophyta, Seed ferns)

Seed ferns are the earliest seed plants and consist of several groups basal to remaining seed plants (Hilton and Bateman 2006). According to Labandeira et al. (2007), their ovules had a pollination drop system (Owens et al. 1998, Gelbart and Aderkas 2002) and typically bore wind-dispersed prepollen. As far as is known, all extant gymnosperm lineages (cycads, Ginkgoales, Gnetales and conifers) produce pollination drops, which are aqueous secretions that receive pollen and transport it to the ovule interior; among other components such as sugars, pollination drops contain carbohydrate-modifying enzymes and defence proteins (Prior et al. 2019).

The Late Carboniferous medullosacean seed fern *Pachytesta illinoensis* is the earliest seed plant having a well-documented syndrome consistent with insect pollination (Labandeira et al. 2007). This said, most pteridosperm species during the Triassic and Jurassic were generally wind-pollinated, with the likely exception of *Peltaspermum thomasii*, which may also be a candidate for insect pollination (Labandeira et al. 2007).

POLLINATION AND REPRODUCTIVE BIOLOGY IN GYMNOSPERMS 1

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Bennettitales (Cycadeoidophyta, Bennettitaleans)

The Bennettitales, an extinct Gymnosperm group, were widespread in both hemispheres from the Middle Triassic to the Late Cretaceous, and there is evidence that certain taxa persisted into the Oligocene in high-latitude refugia of Tasmania and eastern Australia (McLoughlin et al. 2011). The Bennettitales early attracted interest because of their ‘flowers’, and Newell Arber and Parkin (1907) proposed that angiosperms and Bennettitales were derived from a common ancestor. The earliest undisputed bennettitalean leaf dates from the lower Permian, and as indicated from the findings of leaves from the upper Permian, that although never common, the Bennettitales were a wide-spread element of the paleoequatorial flora by the end of the Permian (Blomenkemper et al. 2021). It is thought that Bennettitales and Cycadales evolved at the same time “...and first appeared in seasonally dry environments in paleoequatorial regions” (Blomenkemper et al. 2021). During the Mesozoic, Bennettitales along with cycads, conifers and Ginkgoales were common elements of the landscape. Bennettitales were originally thought to be closely related to cycads based on their superficial vegetative resemblance, but it has long been established that these two lineages bear little in common (Thomas and Bancroft 1913). Fossils of Bennettitales are readily distinguishable by their brachyparacytic (syndetocheilic) stomata. Modern-day cladistic analyses based on morphological data, including seed structure, place Bennettitales close to Gnetales and angiosperms (Friis et al. 2009). Hilton and Bateman (2006) and Rothwell et al. (2009) argue against this view and, based on their analyses, find that

Table 1 - Summary of sexual systems in selected Bennettitales

Family	Genus	Sexual system
Williamsoniaceae	*Williamsonia* (female flowers) *Weltrichia* (male flowers)	Diclinous; dioecious and/or monoecious
	Williamsoniella	Bisexual; dichogamous
Cycadeoideaceae	*Cycadeoidea*	Bisexual; strongly dichogamous
	Monanthesia	Bisexual; strongly dichogamous, monocarpic

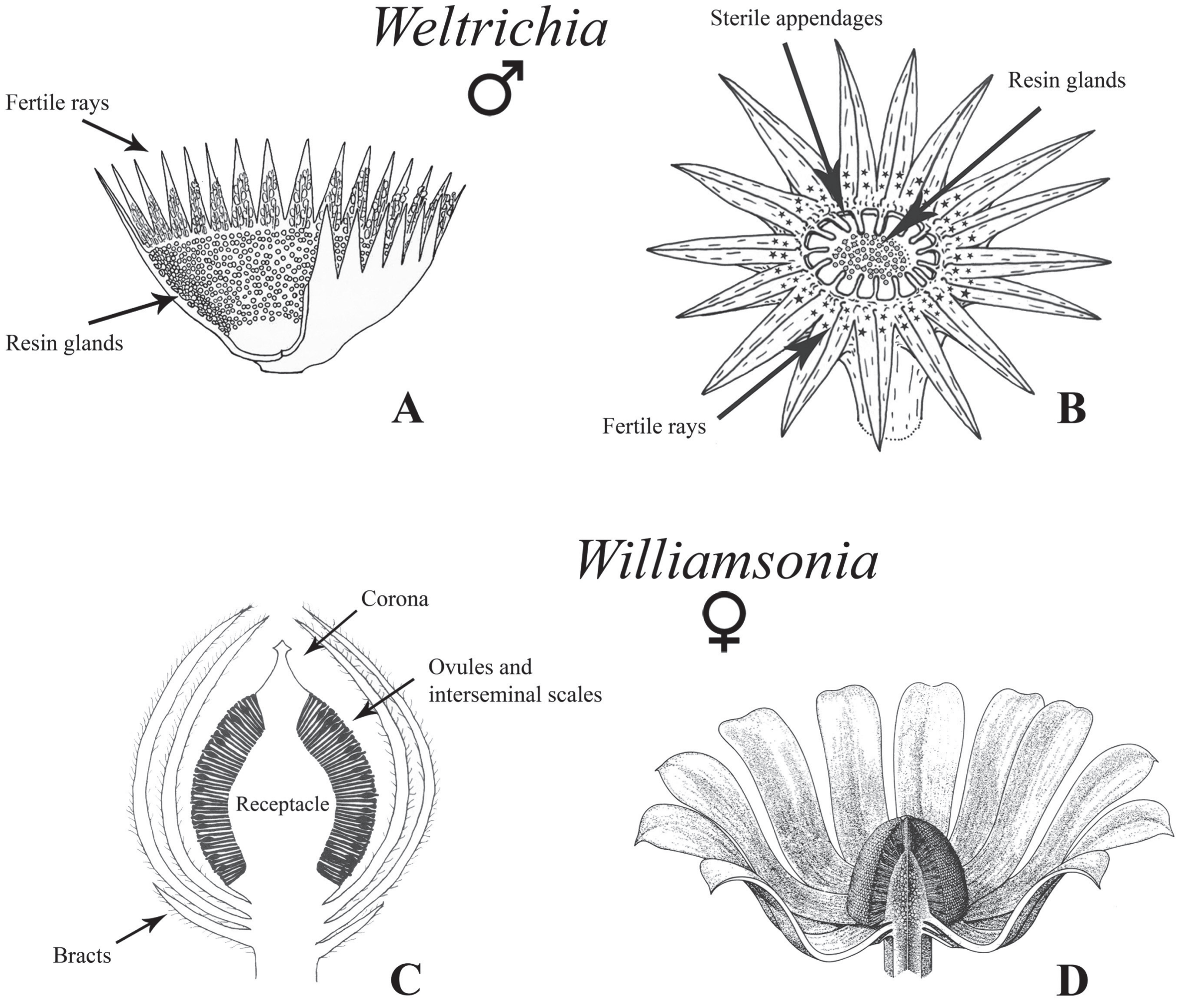

Fig. 2 - Reconstructions of *Weltrichia* and *Williamsonia* flowers.

(A) *Weltrichia sol*, cut-away view of whole flower, ca. one-third natural size; modified from Harris (1969). (B) *Weltrichia givulescui*, illustrating features of *Weltrichia* flowers; depending on species resin glands and/or sterile appendages may be lacking; *ca.* natural size; modified from Popa (2019).

(C) Diagrammatic representation of *Williamsonia* flower; modified from Watson and Sincock (1992).

(D) *Williamsonia margotiana*, almost fully opened flower; cut-away to show inferred gynoecium form, ca. one-half natural size.

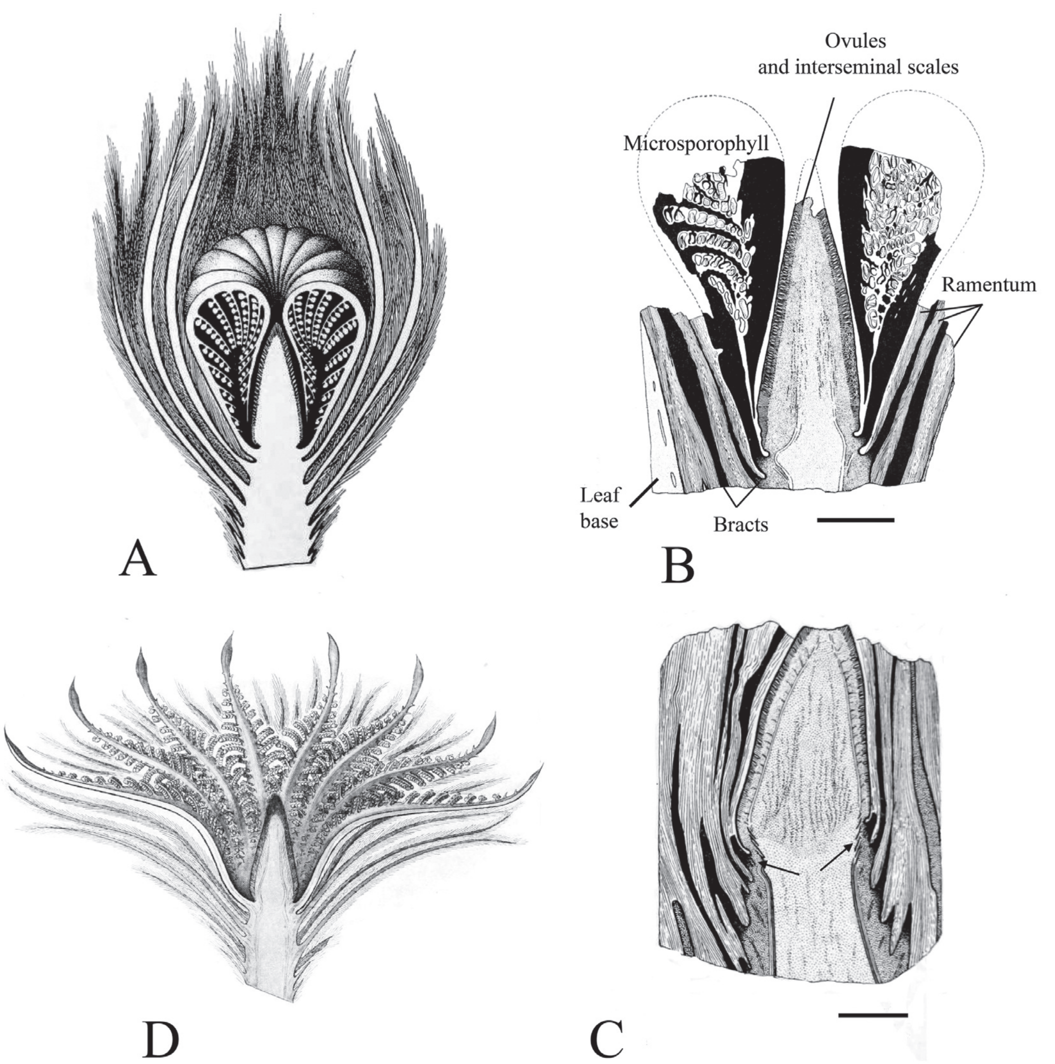

Fig. 3 - *Cycadeoidea* flowers.

(A) Restoration of unexpanded bisexual flower with part of enveloping bracts removed; slightly reduced from natural size, from Wieland (1906: 165; fig. 88).

(B) *C. dacotensis*. Composite drawing based on two parallel longitudinal sections of bisexual flower, upper part of nearly mature microsporophylls and top of immature ovulate cone missing, but general shape indicated by light tracing.
Scale bar = 10 mm. Modified from Wieland (1906: 145; fig. 72. Plate XXVI, S360 & S361).
(https://collections.peabody.yale.edu/search/Record/YPM-PB-005061; image of S361 unavailable).

(C) *C. dacotensis*. Longitudinal section of immature female-stage flower (ovulate cone) showing remains of abscised staminate disk indicated by arrows. Scale bar = 10 mm. Modified from Wieland (1906: 181; fig. 97. Plate XLII, S540) (https://collections. peabody.yale.edu/search/Record/YPM-PB-005153).

(D) Idealized restoration of *C. ingens* flower in nearly longitudinal section showing hypothetical expansion of mature microsporophylls; surrounding armor of old leaf bases arbitrarily removed; ca. half natural size, from Wieland (1906: 106; fig. 54).

WITH KIND PERMISSION OF PHYTON (HORN, AUSTRIA).

the Bennettitales are most closely related to basal pteridosperm groups (see below). In a pilot study using infra-red spectroscopy, Vajda et al. (2017) examined extant and fossil gymnosperm cuticles and found that Bennettitales were close to the extinct Nilssoniales, and as long recognized, but distantly related to cycads.

Two families (**Table 1**) are generally recognized on the basis of growth habit: Williamsoniaceae (stout to slender branched shoots bearing relatively exposed reproductive structures) and Cycadeoideaceae (massive, tall or short in stature, sparsely branched trunks bearing flowers on short stalks embedded among persistent leaf bases) (Watson and Sincock 1992). This said, many researchers think that Williamsoniaceae may be paraphyletic (Pott and Axsmith 2015, McLoughlin et al. 2018, and see Anderson et al. 2007). The oldest recognized bennettitalean reproductive structure, *Williamsonia eskensis*, is from the Middle Triassic of Australia (McLoughlin et al. 2018), and the earliest *Cycadeoidea* reproductive structures date from the Upper Jurassic. Williamsoniaceae has long been considered the older of the two families (Stewart 1983, Popa 2019) and was widespread whereas Cycadeoideaceae is thought to have been more restricted in distribution, essentially to western Laurasia and into the Tethyan margin of northern Gondwana (McLoughlin et al. 2018). Although a common and emblematic floristic element for much of the Mesozoic, the Bennettitales petered out towards the end of the Cretaceous (Watson and Sincock 1992).

Based on the fossil record it appears that most well-characterized members of Williamsoniaceae have unisexual flowers (Pott 2014): *Williamsonia* is a form genus for female flowers and *Weltrichia* for male ones (**Table 1**). There is one notable exception, *Williamsoniella*, wherein the flower is bisexual, but dichogamous. All known members of Cycadeoideaceae have bisexual flowers that are strongly dichogamous in such a fashion that the male reproductive structures and pollen are not present when female reproductive structures reach sexual maturity.

It has long been thought that the Bennettitales were insect pollinated (e.g., Hughes 1976, Crowson 1981, Pott 2014, Popa 2019, van der Kooi and Ollerton 2020), however, there are few detailed explanations about how this may have occurred. A new hypothesis treating pollination biology in Bennettitales was recently proposed by Frame and Gottsberger (2023) and as it is pertinent to our discussion of the evolution of beetle pollination in gymnosperms and angiosperms, we will reiterate it here. For a more detailed discussion of this theory, we refer readers to the aforementioned publication.

Bennettitalean female reproductive structures are similar and consist of a conical or dome-shaped receptacle bearing ovules and interseminal scales; there are often sterile appendages distally and this structure is called the ‘corona’, and sometimes proximally (**Fig. 2**). Gynoecia are subtended by a whorl of free or basally laterally fused male reproductive organs, i.e., androecium (Cycadeoideaceae **Fig. 3**; *Williamsoniella*, **Fig. 4**) or not (*Williamsonia*, **Fig. 2**), followed by helically arranged sterile bracts inserted on a peduncle.

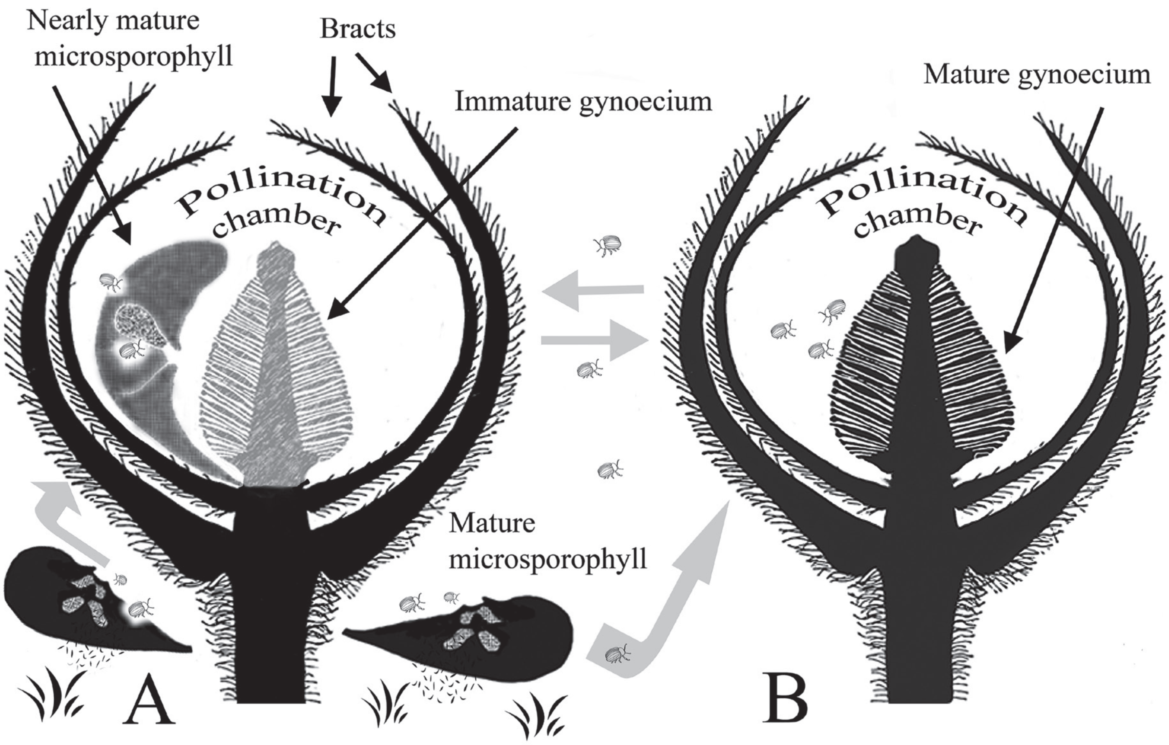

Fig. 4 - Hypothetical schema of pollination by insects (beetles) in bisexual Bennettitales, flower based on diagrammatic restoration of *Williamsoniella* in longitudinal section.

(A) Pollination chamber formed by bracts enclosing immature gynoecium and nearly mature androecium (shading denotes degree of maturity). Nearly mature androecium attracts beetles; beetles meet, mate, feed on pollen and oviposit as microsporophylls mature (male-stage flower). Soon after, mature microsporophylls abscise and fall to the ground along with beetles and developing larvae (shown at bottom, not to scale). Adult beetles carrying pollen can return to male-stage flowers or enter female-stage flowers, thereby effectuating pollination. Larvae in fallen microsporophylls mature into adults in decaying microsporophylls and adjacent ground litter.

(B) Pollination chamber formed by bracts enclosing mature gynoecium (female-stage flowers) shown with some attracted beetles. Bract movement not shown but implied. Restored flower ca. 3.5 times natural size; modified from Harris 1969).

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Williamsoniella coronata is particularly instructive. It had a central axil bearing ovules terminated apically by a ‘corona’ of sterile appendages and bore a perianth of sterile bracts below the androecium (**Fig. 4**). Flowers were dichogamous and the nearly mature to mature androecium abscised prior to ovule maturity (Harris 1944), thus flowers were protandrous and obligatory out-crossers. Although *Williamsoniella coronata* differed considerably from *Cycadeoidea* vegetatively, with respect to its ‘flower’, it was rather similar (Harris 1944). We refer to bennettitalean reproductive structures as flowers as is traditional, by doing so there is no implied direct homology of bennettitalean floral appendages to those of angiospermous flowers (Sporne 1965, Frame & Gottsberger 2023). All members of Cycadeoideaceae were bisexual and like *Williamsoniella*, sexually mature female and male reproductive structures are never found in a flower at the same time. Here, too, flowers were protandrous and dichogamous, and male reproductive structures disintegrated possibly months before ovules became mature. No longer exposed openly on slender branches or axils as in *Williamsonia*, in *Cycadeoidea*, the flower was retained, embedding in the robust trunks that distinguish the family.

Frame and Gottsberger (2023) interpret the particular morphology and temporal integrity of bisexual bennettitalean flowers to be indicative of a pollination chamber, i.e., a disposition of perianth members to form a more or less closed flower where pollinators, especially beetles, meet and carry out activities such as feeding or mating (**Fig. 4**), similar to what is found in many present-day basal angiosperms (e.g., Gottsberger 1970, 2016a). What is striking in the Bennettitales, is that the flower was *protandrous*, whereas in cantharophilous angiosperms, the flower is almost always *protogynous.* Perianth movement, that is, creation and dissolution of the pollination chamber, is coordinated with maturation of sexual structures, scent, nutritional tissues and retention and release of pollinators. Frame and Gottsberger (2023) hypothesize that in *Cycadeoidea* flowers the chamber is formed twice, in the male-stage, and in the female stage, which occurs much later. Pollen is doubtless a tangible reward in the first stage, and specialized interseminal scales apically and basally likely play a role in attraction and/or reward in female-stage flowers.

With respect to the form genus *Weltrichia* (unisexual male-flowers), Popa (2019) has presented a dazzling array of reconstructions of different species. These radial symmetrical, frequently robust, coriaceous flowers were distinguished by a central cup and radiating rays that bore numerous pollen sacs (fertile rays); pollen sacs might be sessile or attached to appendages (**Fig. 2**). Various sterile appendages were sometimes present decorating the adaxial rim of the cup (**Fig. 2**), and in certain species (e.g., *Weltrichia sol*) resin glands have been found covering the adaxial face of the cup (Harris 1969, Popa 2019). Frame and Gottsberger (2023) interpret the central cup in *Weltrichia*, which is often protected by hairs or ornamented by appendages to function as part of the pollination chamber. The distal portions of *Weltrichia* are thought to have been variously upright to distally spreading and these authors suggest that the rays may have undergone movement to form pollination chambers. It is of note that in *Weltrichia* there are frequently resin bodies; when present they are always found proximal to the pollen sacs on floral rays (Popa 2019). Several authors (e.g., Pott 2014, Popa 2019) consider the resin as pollinator attractants, their attraction may be as food bodies, sites of odor and/or color, and/or they may act as guides to the central cup possibly by light reflection (Frame and Gottsberger 2023). It is of note that in certain Araceae blossoms, see chapter on Araceae, resin is produced, which among other things, serves