MORPHOLOGICAL CLADISTIC ANALYSIS OF THE CORDAITEAN CONES AND IMPLICATIONS FOR PLANT LINEAGES

A Thesis

by

JULIAN EDUARDO CORREA

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Chair of Committee,	Anne Raymond
Committee Members,	Thomas Olszewski
	James Woolley
Head of Department,	Rick Giardino

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ABSTRACT

The cordaiteans were a group of Carboniferous and Permian woody plants, which thrived in wetlands and *terra firma* habitats. Cordaitean cones from wetland habitats are exquisitely well preserved and contain a suit of characters that make their fossils prime candidates for cladistic analysis; historically, there are more male cones than female cone. Cordaitean morphological characteristics make this clade a possible precursor lineage of modern conifer and gnetalean lineages. However, the exact relation between fossil seed-plant clades and modern gymnosperm groups remains enigmatic. The resent discovery of a bisexual cordaitean cone calls into question the relationship between cordaiteans, gnetaleans and angiosperms. This study performs three cladistic analyses in order to find the relations of taxa within the cordaitean clade and other early plant lineages using primarily PAUP* and TNT. An analysis of male only cones provides a phylogram for the relations between the cordaitean taxa; the results also suggest a bisexual ancestry for the clade. A second analysis attempted to introduce the morphologically distinct female cones; the results produce a similar strict consensus tree. However, many of the female cones do not pair with their associated male cones identified in 'whole-plant' reconstructions of cordaitean lineages. Instead, female cones formed separate lineages. A final analysis incorporated other plant lineages and assigned traits associated with seeds and pollen to both male and female cones. The results support an early bisexual ancestry to the cordaiteans, and suggests that the cordaiteans are a polyphyletic clade, which gave rise to the voltzialean-conifer lineage. Including more fossil taxa lineages may prove useful, as well as establishing other criteria to compare characters to taxa in cladistic analysis.

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Another round of thanks to all my colleagues in Geology and Geophysics who made sure I never stopped working, including (but not limited to) Jenn Hendricks, Robert Grajeda and Jonathan Sneed. Thank you all so much.

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INTRODUCTION

Cordaiteans were Late Paleozoic woody trees and shrubs with large strap-shaped leaves that produced seeds and pollen in compound cones (Florin 1938-1954; Taylor et al., 2009: Fig. 1, Fig. 2). They flourished in wetland and terra firma environments, in the tropics and temperate zones of the paleo-northern and paleo-southern hemispheres (Ignatiev and Meyen, 1989; Raymond et al., 2010; Césari and Hüniken, 2013). Cordaiteans have been proposed as the ancestors of conifers (Florin, 1951), the sister group of conifers (Taylor et al., 2009), and as the ancestors of gnetaleans (Eames, 1952; Rothwell and Stockey, 2013). Morphological cladistic analyses identified cordaiteans as the sister group of coniferophyta, ginkgoes and conifers (occasionally gnetaleans, and accepted for the purposes of this study as such), or of conifers only (Hilton and Bateman, 2006; Doyle, 2006; Rothwell, Crepet and Stockey, 2009: Fig 3). The discovery of a permineralized, bisexual cordaitean cone from the Kalo Formation of Iowa (Pennsylvanian, mid-Moscovian, and its implications for seed plant evolution have sparked a new interest in cordaitean phylogeny (Raymond et al., 2013, 2014). Both Florin (1938-1945, 1951) and Harris (1947) noted the similarity between angiosperm flowers and the secondary fertile shoots of cordaitean cones. Rudall and Bateman (2010) explored the possible homology of cordaitean cones, conifer seed cones, gnetalean cones and angiosperm inflorescences. In their exploration of teratoid bisporangiate cones in conifers, Flores-Rentiería et al. (2011) speculated that the ancestral angiosperm had bisexual reproductive organs. In this study, I explore the phylogeny of cordaiteans and their relationship to callistophytalean seed ferns, voltzialean conifers, gnetaleans and angiosperms using reproductive organs and their associated seeds and pollen.

Most cladistics analyses of seed plant phylogeny relied on whole-plant reconstructions and incorporated only a few cordaitean species, and relatively few fossil taxa overall. For example, Hilton and Bateman (2006) incorporated 48 species in their phylogenetic analysis of land plant diversity, 25 fossil species, among them three cordaiteans; Doyle (2006) incorporated 34 species, 13 fossil species, among them one cordaitean. Because relatively few cordaiteans have been reconstructed as whole plants, these studies capture only a small fraction of the diversity of fossil cordaiteans.

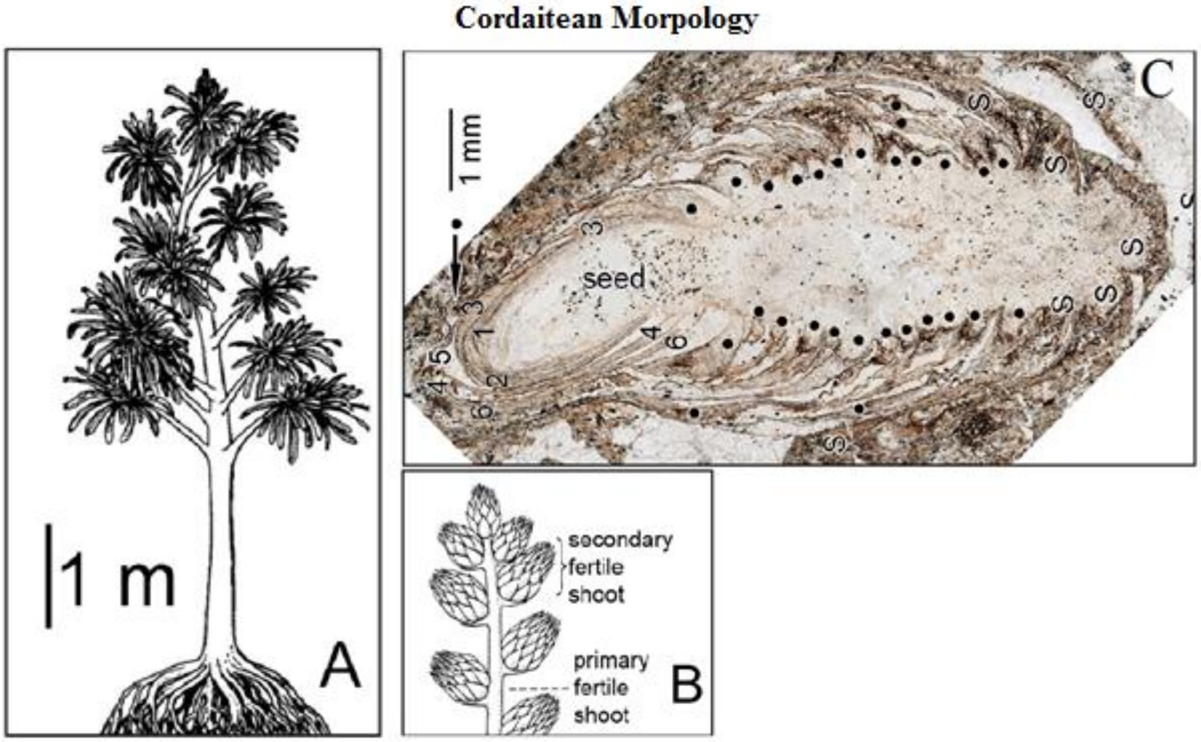


Figure 1. A. Reconstruction of cordaitean plant from an Iowa wetland (Cridland, 1964). B. Diagram of the cordaitean compound cone adapted from Fry (1956). C. Longitudinal section of the secondary fertile shoot of the new bisexual cordaitean cone showing (S) basal sclerenchymatous scales, (□) male scales, upper sterile scales numbered 2 – 6, fertile female scale (1), and seed.

Because permineralized species often reveal more characters than adpressed species (facilitating both whole-plant reconstructions and cladistic analysis) preservation type influences the choice of taxa for cladistics analysis. Not surprisingly, the six cordaitean species reconstructed as whole plants (Table 1) belong to permineralized specimens that grew in paleotropical mires. Cladistic analysis of Carboniferous and Permian cordaiteans has focused on wetland taxa, excluding species that flourished in temperate, terra firma habitats, which are generally preserved as adpressions.

Table 1 Cordaitean known from whole plant reconstructions.

Whole Plant Taxa

Species (stem, pollen cone)	Age, Location	Source
Mesoxylon priapi, *Gothania priapi*	Late Pennsylvanian (Kasimovian) U.S.A.	Trivett and Rothwell, 1985
Pennsylvanioxylon/Cordaixylon birame, *Cordaianthus concinnus*	Mid-to-Late Pennsylvanian (mid to Costanza, 1985 late Moscovian) U.S.A.	
Pennsylvanioxylon/Cordaixylon dumusum, *Cordaianthus dumusum*	Late Pennsylvanian, (late Moscovian, Kasimovian) U.S.A.	Rothwell, 1993
Pennsylvanioxylon/Cordaixylon tianii, *Cordaianthus tianii*	Early Permian, China	Hilton et al., 2009a
Shanxioxylon taiyuanensis, *Cathayanthus ximinensis*	Early Permian, China	Hilton et al., 2009b
Shanxioxylon sinense, *Cordaianthus ramentrarus*	Early Permian, China	Wang et al., 2009

Restricting cladistics analysis to whole-plant reconstructions further limits and biases the choice of species. Due to the difficulty of reconstructing whole plants based on disarticulated organs, most whole-plant reconstructions of cordaiteans come from low diversity assemblages. For example, the *Pennsylvanixylon/Cordaixylon birame* plant reconstructed by

Costanza (1983; 1985) is the only cordaitean known from the Carrier Mills Mine near Murphysboro, Indiana. The other two whole plant reconstructions of North American cordaiteans (*Pennsylvanixylon/Cordaixylon dumusum* and *Mesoxylon priapi)* are the only two cordaiteans known from North American Kasimovian mires (Rothwell, 1982; Trivett and Rothwell, 1993). The Early Permian Taiyuan Formation of China is exceptional in this regard: the remaining three cordaiteans known from whole-plant reconstructions come from this formation, which appears to have had a diverse assemblage of cordaiteans (Hilton et al., 2009a, b; Wang et al., 2003). In addition to the three species placed in whole-plant reconstructions, Hilton et al. (2001) reported two other cordaitean seed genera from the Taiyuan Formation (*Parataxaspermum* and *Diplotesta*). Nonetheless, none of the cordaitean species unique to the mixed cordaitean assemblages from the Pennsylvanian of Iowa or France have been reconstructed as whole-plant taxa (four of five species from Iowa; eight or more species from France). There are 20 permineralized seed species assigned to cordaiteans (Hilton et al., 2003) and more than 16 permineralized cone species derived from 10 or 11 species in contrast to the six cordaitean species known from whole-plant reconstructions (Table 1).

Hilton et al. (2003) proposed a cladistic phylogeny of Chinese, European and North American cordaiteans based on seeds (Fig. 4), which Johnson et al. (2007) extended to include the Siberian seed, *Cardiocarpus angarensis*. Both phylogenetic studies incorporated a large number of species due to the abundance and diversity of cordaitean seeds (Hilton et al. 2003; Johnson et al., 2007). In the Hilton et al. (2003) study, North American *Mitrospermum* species formed a separate clade, however French species with mitrospermid vascular organization did not join this clade. With the addition of the Siberian cordaitean seed, the

North American *Mitrospermum* clade disappeared (Johnson et al., 2007). Though some of the results showed promise, the bootstrap values and decay indices in both analyses were low, which Hilton et al. (2003) attributed to the low number of synapomorphic conditions for *Cardiocarpus*, one of the most diverse genera of cordaitean seeds.

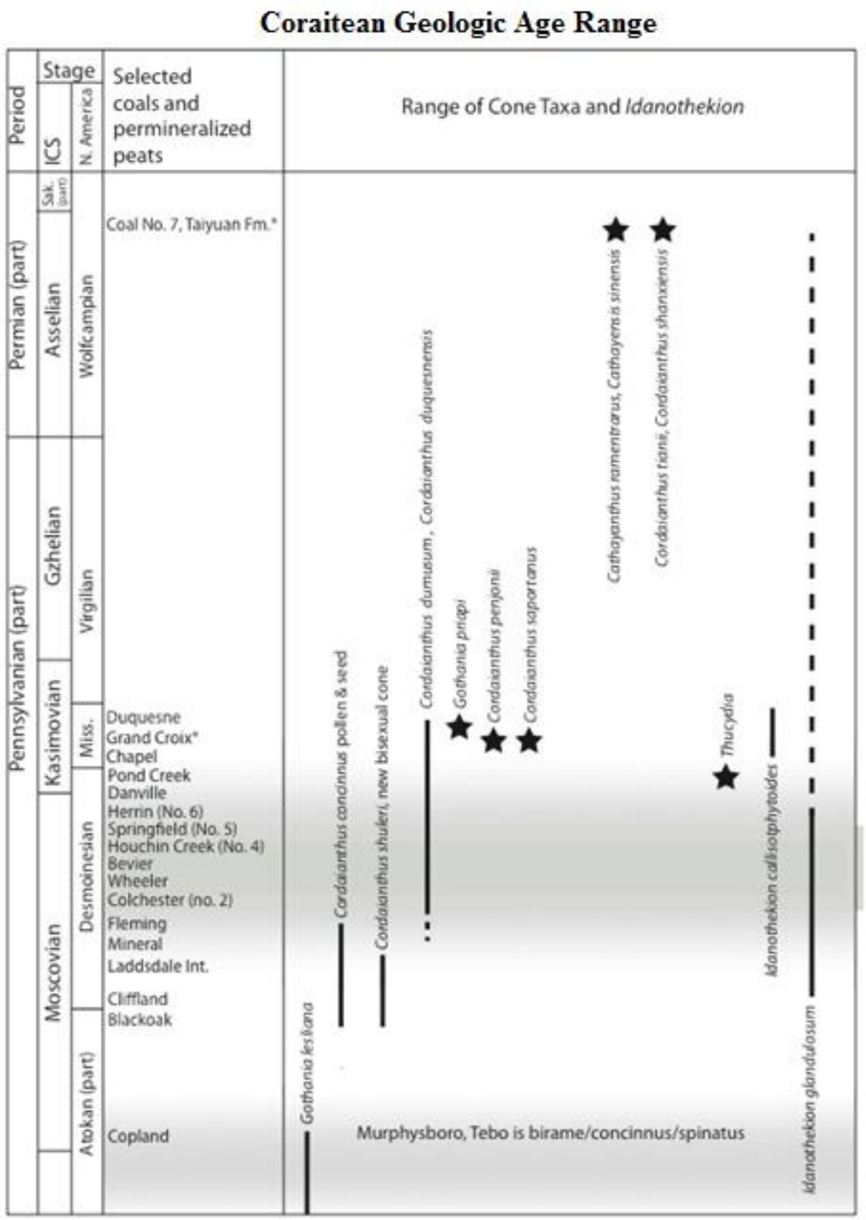
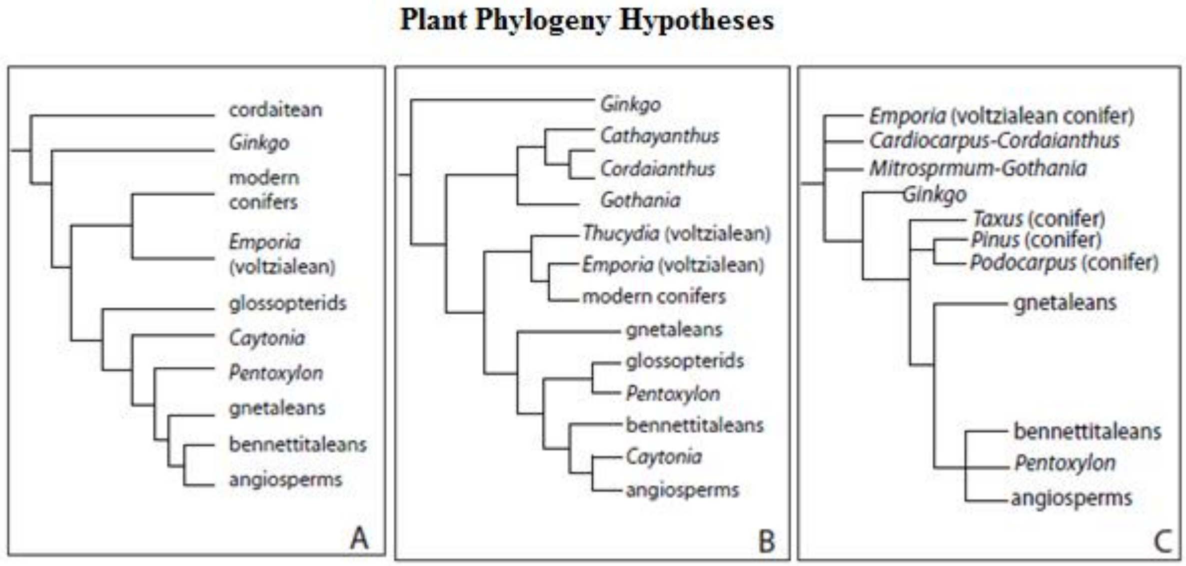


Figure 2. Range of permineralized cordaiteans, the early conifer, *Thucydia*, and *Idanothekion*, the pollen organ of *Callistophyton.* All coals and permineralized peats are North American except Grand Croix (France) and Coal No. 7 (North China). The range of Idanothekion glandulosum has been extended to the Early Permian (dashed line) based on the occurrence of its seed in coal balls from Coal No. 7 in China (Hilton et al., 2002).

Although the species diversity of cordaitean cones is lower than that of cordaitean seeds, cones have a broader suite of functions in plant reproduction than seeds (Table 2). Cordaitean cones provide nutrition and protection to male and female gametophytes, the embryo and the seed, and they have vegetative organs (e.g. bracts and basal sterile sheathing scales) as well as reproductive organs (e.g. fertile scales, pollen sacs, the nucellus). In this thesis, I use reproductive organ systems (compound cones and their associated ovules and pollen) to explore cordaitean phylogeny (Fig. 1B, C).

Figure 3. Modified tree topologies from phylogenetic analyses from – A) Doyle (2006), B) Hilton and Bateman (2006)**,** C) Rothwell, Crepet and Stockey, 2009.



Cladistic analyses based on single organs or organ systems remain controversial (Bateman et al., 1992; Hilton et al. 2006). Using organs rather than whole plants in cladistic analysis may seem disadvantageous; however I expect reproductive axis (cones, fronds or flowers) to yield insight into cordaitean phylogeny for the following reasons:

1) The organ system chosen, the reproductive axis, has a complex suit of characters that have proven useful for understanding plant development and evolution using both

traditional morphology (Chamberlain, 1935; Florin, 1951), genetic-DNA (Coen, Meyerowitz, 1991; Bowman, et al, 2012) and mixed (Doyle, Endress, 2000; Hilton and Bateman, 2006)analysis;

2) Selective pressures involved in the growth, protection and dispersal of both male gametophytes (pollen) and embryos (seeds) shape the reproductive axis. Thus, traits associated with the reproductive axis and its associated pollen and seed may provide more insight into phylogeny than analyses based on seeds alone;
3) Even with selective pressures, plant reproductive organs are conservative in evolution, as mutations to reproductive organs can prevent reproduction (Wu, 2010).
4) Reproductive organs have been used to place fossil plants in modern groups (e.g. *Psaronius* in marratialean ferns: Taylor et al., 2009), and to trace the diversification of plant lineages (e.g. conifers – Chamberlain, 1935; Florin 1938-1945; angiosperms – Doyle, 2008; seed plants – Rudall and Bateman, 2010).

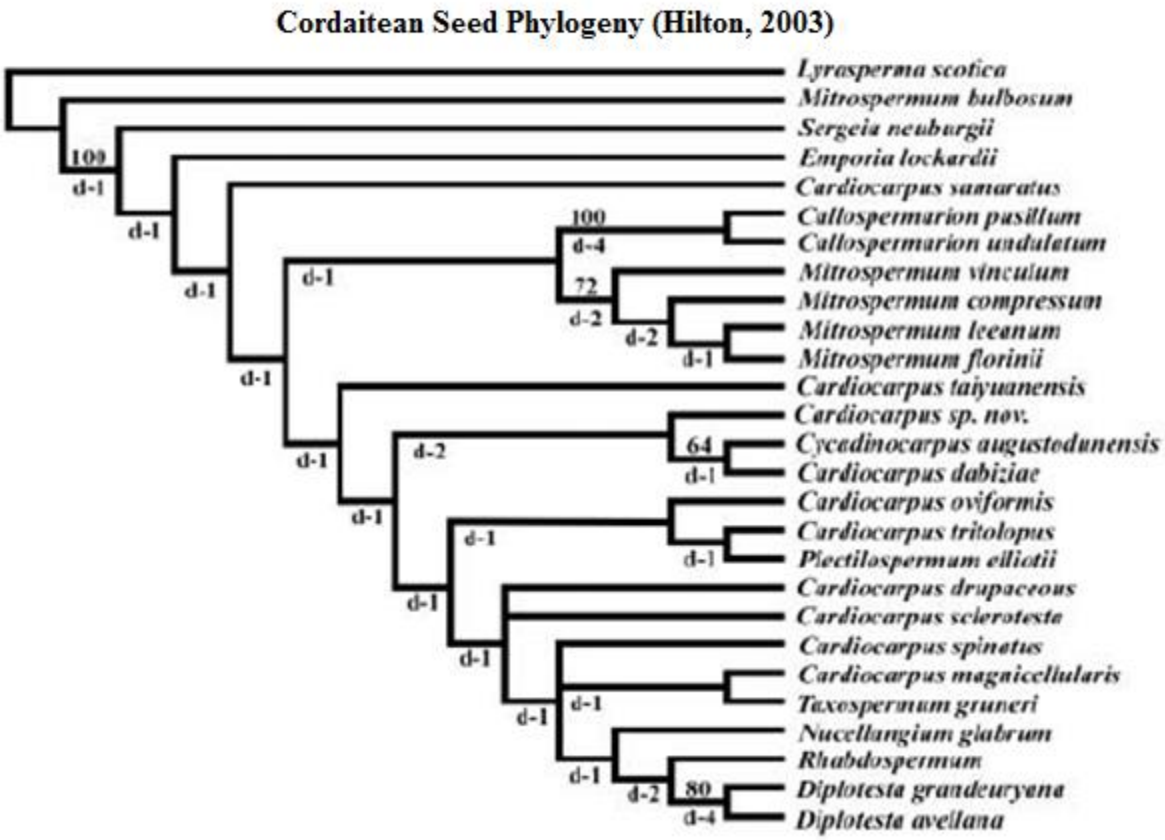


Figure 4. Analysis of Cordaitean seeds, from Hilton, 2003, with bootstrap values above the branch and Bremer support the branches

5) As discussed above, relatively few of the cordaitean taxa known from seeds or cones have been reconstructed as whole-plants. Using reproductive organ systems for cladistics analysis increases the total number of species in the analysis, enables me to include endemic species from France, and to expand the stratigraphic range of the analysis to include the oldest known permineralized as well as the youngest known permineralized species of China.

Table 2. Reproductive functions of cordaitean cones and seeds.

Function of Seed vs Cones

Function	Seeds	Cones
Formation and nurture of male and female gametophytes	No	Yes
Pollen dispersal	No	Yes
Pollen capture and ovule sealing	Yes	Yes
Embryo protection	Yes	Yes
Embryo dispersal	Yes	Yes

MATERIALS AND METHODS

Taxa

Cordaites

Cordaiteans appear during the Lower Carboniferous (Sze, 1936; Wang, 1998; Taylor et al., 2009) and disappear during the Permian. The traits that distinguish cordaiteans from other seed plants, suggesting that they are a natural group, include woody (pycnoxylic) stems and branches with large primary bodies; strap-shaped leaves with parallel venation; compound cones bearing secondary fertile shoots subtended by bracts; secondary fertile shoots with vegetative scales at the base and fertile scales at the apex; and in nearly all cordaiteans, seeds and pollen sacs are borne at the tips of the fertile scales (Taylor et al., 2009; Rothwell review article; Costanza, pers. com.) All cordaiteans probably had compound cones, although some cone species occur only as secondary fertile shoots (Florin, 1951; Hilton et al., 2009a). In most, the primary cone axis bore two rows of secondary fertile shoots, each subtended by a sterile bract (Fig. 1B). The development of secondary fertile shoots in the axils of the sterile bracts echoes the development of compound cones in the axils of the leaves. The secondary fertile shoots of cordaitean cones are flower-like organs with spirally-arranged vegetative and fertile scales (Florin, 1938-1945, 1951; Harris, 1947). In all completely preserved cordaitean cones, the basal scales are sterile. In most, the upper scales are fertile; however some have a mix of fertile and sterile scales at the cone tip. Most cordaitean cones are unisexual, with the exception of a new bisexual cone from the Kalo Formation (Pennsylvanian, mid-Moscovian, or latest Atokan, earliest Desmoinesian) of Iowa. The reproductive propagules of nearly all cordaiteans develop at the tip of the fertile scales.

The male gametes were monosaccate true pollen (*Florinites*) or pre-pollen (*Felixipollenites, Sullisaccites*) that developed in pollen sacs attached to the fertile scales. The female gametes were ovules that grew from the tips of fertile scales. Adpressed cordaitean seed cones have branched fertile scales with multiple seeds attached to each fertile scale (or in the case of the new bisexual cone, adaxially from the base of the apical fertile scale); no permineralized seed cone has more than one attached seed per fertile scale (Ignatiev and Meyen, 1989).

For simplicity, I refer to cordaiteans known from whole plant reconstructions by the name of their pollen cone. Table 3 contains a list of the cordaitean cones used in this study and their associated organs and stratigraphic ages. The full traits matrix used in these analyses appears in APPENDIX 1. Documentation of the traits appears in APPENDIX 2, with documentation of the new bisexual cordaitean cone in APPENDIX 3.

Gothania

The cordaiteans that bore *Gothania* cones had *Mesoxylon* stems, monosaccate prepollen (i.e. pollen with a true suture that germinated from the proximal surface) and *Mitrospermum* seeds (Daghlian and Taylor, 1979; Costanza, 1983; Rothwell and Trivett, 1991). The seed cones of the *Gothania* plant remain unknown. Despite this, paleobotanists have confidence that *Gothania* pollen cones grew on plants that bore *Mitrospermum* seeds because these are the only cordaitean cone and seed in Early Pennsylvanian permineralized assemblages from Europe (Phillips, 1980). Male cones had relatively few scales compared to other cordaiteans (25 – 30), and bore a row of pollen sacs at the tip of the fertile scale. Rothwell (1988) suggested that *Gothania* pollen cones developed simultaneously (i.e. all the pollen matured at a single time). The following traits may be associated with simultaneous development in cordaiteans: a large primary body having a low width/length (W/L) ratio in the primary fertile shoot; a reduced number of cone scales and absence of sclerenchyma in the basal sterile scales. I include two *Gothania* species in this study: 1) The earliest known permineralized cordaitean cone, *Gothania lesliana* (Daghlian and Taylor, 1979), which bore *Felixipollenites* prepollen and is associated with *Mitrospermum compressum* seeds (Daghlian and Taylor, 1979; Costanza, 1983; Trivett and Rothwell, 1991); 2) *Gothania priapi,* which bore *Sullisaccites* prepollen and is associated with *Mitrospermum vinculum* seeds (Trivett and Rothwell, 1985, 1991).

Cordaianthus

A diverse group of cordaiteans bore *Cordaitanthus* cones and species currently assigned to *Cordaianthus* almost certainly belong to more than one cordaitean clade (Ignatiev and Meyen, 1989). *Cordaianthus* cones produced on *Pennsylvanioxylon/Cordaixylon* stems that bore *Florinites* pollen, (i.e. pollen with a functional suture, which germinated from the distal surface), associated with *Cordaianthus* seed cones that bore *Cardiocarpus* seeds may form a natural clade (Costanza, 1983, 1985). Male *Cordaianthus* cones have a large number of scales (40 or more). Florin (1951) described the development of pollen sacs in *C. penjonii*. As they matured, fertile scales developed five to six ridges and bifurcated at the tip. Pollen sacs formed at the edge and ridges of the bifurcating tip, resulting in a ring of four to six pollen sacs. Each fertile scale produced a ring of pollen sacs; however, the configuration of ridges at the tip shows bilateral, rather than radial symmetry. Although tip bifurcation not been documented in all *Cordaianthus* pollen cones, fertile scales of both male and female *Cordaianthus* cones developed similar ridges in a bilaterally symmetric pattern. Rothwell (1988) suggested that *Cordaianthus* cones developed sequentially (i.e. more basal fertile scales matured before fertile scales at the tip of the cone, so that cones produced pollen and

Table 3 Male and female cones and reproductive fronds proposed for use in this study.

Taxa Organ Association

Male Organ	Female Organ	Seed	Stem	Formation, Age, Continent	Authors
Gothania priapi	undiscovered.	*Mitrospermum vinculum*	*Mesoxylon priapi*	Duquesne Coal, Late Penn. North America	Trivett & Rothwell, 1991
Gothania lesliana	undiscovered	*Mitrospermum compressum*	stem similar to *Mesoxylon multirame*	Hamlin Coal, Hyden Fm., Middle Penn., North America	Daghlian & Taylor, 1979; Costanza, 1983
Cordaitanthus tianii	*Cordaitanthus shanxiensis*	*Cardiocarpus tuberculatus*	*Cordaixylon - Pennsylvanioxylon tianii*	Taiyuan Fm., Early Permian, China	Hilton et al., 2009
Cordaianthus concinnus pollen	*Cordaianthus concinnus* seed (both varieties)	*Cardiocarpus spinatus*	*Cordaixylon - Pennsylvanioxylon birame*	Kalo Fm. to Houtchin Creek (No. 4) Coal, Carbondale Fm., Mid to Late Penn., North America	Costanza, 1984; Raymond et al., 2010
Cordaianthus dumusum	*Cordaianthus duquenensis*	*Cardiocarpus oviformis*	*Cordaixylon - Pennsylvanioxylon dumusum*	Calhoun Coal, Matoon Fm., Duquesne Coal, Conemaugh Fm., Late Penn., North America	Rothwell & Warner, 1984
Cordaianthus penjonii	unknown cordaitean seed cone	unknown cordaitean seed	unknown cordaitean stem	Grand Croix, Rive De Gier Fm., Late Penn., France	Florin, 1951

Table 3 - Continued

Male Organ	Female Organ	Seed	Stem	Formation, Age, Continent	Authors
Cordaianthus saportanus	unknown cordaitean seed cone	unknown cordaitean seed	unknown cordaitean stem	Grand Croix, Rive De Gier Fm., Late Penn., France	Florin, 1951; Doubinger et al., 1995
Cordaianthus shuleri	*Kaloconus sp. nov.*	*Nucellangium glabrum*	Alpha paratype of *Mesoxylon thompsonii*	Kalo Formation, Mid. Penn., North America	Raymond et al., 2013, 2014; Fry, 1956; Raymond & Costanza, unpublished data
Cathayanthus ramentrarus	*Cathayanthus sinensis*	*Cardiocarpus samaratus*	*Shanxioxylon sinense*	Taiyuan Fm., Early Permian,China	Wang et al., 2003 Hilton et al., 2009a
Cordaitanthus ximinensis	*Cordaitanthus xishanensis*	*Cardiocarpus taiyuanensis*	*Shanxioxylon taiyuanense*	Taiyuan Fm., Early Permian,China	Wang et al. 2009
Idanothekion callistophytoides	*Dicksoniites pluckenetii*	*Callospermarion pusillum*	*Callistophyton poroxyloides*	Parker Coal, Patoka Fm. to Calhoun Coal, Matoon Fm. and Duqesne Coal, Conemaugh Fm., Late Penn.,North America	Stidd & Hall, 1970; Rothwell, 1975, 1980; Phillips, 1980; Taylor et. al., 2009
Idanothekion glandulosum	*Dicksoniites pluckenetii*	*Callospermarion undulatum*	*Callistophyton boyssetti*	Kalo Fm., Mid. Penn., North America to Taiyuan Fm., Early Permian, China	Stidd & Hall, 1970; Rothwell, 1975; Phillips, 1980; Hilton et al., 2002; Taylor et. al., 2009; Raymond & Costanza, unpublished data

Table 3 - Continued

Male Organ	Female Organ	Seed	Stem	Formation, Age, Continent	Authors
Thucydia mahoningensis pollen cone	*Thucydia mahoningensis* seed cone	*Thucydia mahoningensis* seed	*Thucydia mahoningensis*	Black shale above Mahoning coal, Conemaugh Group, Late Penn., Ohio	Hernandez-Castillo et al., 2001a,b
Archaefructus liaoningensis (Bisexual – flower)	*Archaefructus liaoningensis (Bisexual – flower)*	*Archaefructus liaoningensis* (seed)	*Archaefructus liaoningensis* (herbaceous)	Yixian formation, early Cretaceous, Liaoning, China	Sun, et. al., 2002; Friis, et. al., 2003
Archaeopteris halliana	*Archaeopteris halliana* (heterosporous)	heterosporous	*Callixylon*	Frasnian and Famennian, late Devonian, Euramerica and China	Beck, 1960; Fairon-Demaret et al., 2001
Welwitschia mirabilis	*Welwitschia mirabilis*	*Welwitschia mirabilis*	*Welwitschia mirabilis*	Modern, Namibia, Africa	Martens, 1977; Chamberlain, 1935

seeds over an extended period of time).

The following traits may be associated with sequential development in cordaitean cones: small primary body having a large W/L ratio in the primary fertile shoot; a large number of cone scales; and sclerenchyma in the basal sterile scales.

I include three species of *Cordaianthus* pollen cones and three species of *Cordaianthus* seed cones, found in organic connection to *Pennsylvanioxylon-Cordaixylon* stems in this study. These six cones belong to three species and may form a natural clade: 1, *Cordaianthus concinnus,* which bore *Florinities* pollen and is associated with *Cordaianthus concinnus* seed cones bearing *Cardiocarpus spinatus* seeds (Delevoryas, 1953; Costanza, 1983, 1985); 2, *Cordaianthus dumusum,* which bore *Florinites* pollen and is associated with *Cordaianthus duquesnensis* seed cones bearing *Cardiocarpus oviformis* seeds (Rothwell and Warner, 1993, Rothwell, 1988); 3. *Cordaianthus tianii,* which bore unidentified pollen, and is associated with *Cordaianthus shanxiensis* cones bearing *Cardiocarpus tuberculatus* seeds (Hilton et al., 2009a). Both these cones occur only as secondary fertile shoots, although the secondary fertile shoot of *C. tianii* have been found in organic attachment to *Pennsylvanioxylon/Cordaixylon tianii* stems (Hilton et al. 2009a).

Lastly, *C. ximinensis* which bore *Florinites* pollen and a *Cardiocarpus taiyuanensis* seed when associated to the *C. xishanensi* (Wang, et al., 2009). The sample described in the for the holotype (Wang, 1991; Hilton, et al, 2009) was sample which had poor preservation of its fertile scales, or at least its apical scales. The taxa is excluded for this reason.

I also include two *Cordaianthu*s pollen cone species described from secondary fertile shoots, both of which bore *Florinites* pollen: *C. penjonii*, and *C. saportanus* (Florin, 1951;

Daighlian and Taylor, 1979). These cones differ from other *Cordaianthus* cones in having fertile scales inserted among the basal, sclerenchymatous scales (*C. penjonii*: Florin, 1951); and in having bilaterally symmetric secondary fertile shoots *(C. saportanus*: Florin, 1951). Finally, I include *C. shuleri* (Fry, 1956?), which bore *Florinites* pollen. Like *C. penjonii, C. shuleri* has fertile scales inserted among the basal sclerenchymatous scales. This cone differs from other *Cordaianthus* pollen cones in having rod-shaped fertile scales with four to five radially, symmetrically arranged ridges at the tip, each giving rise to a pollen sac. A ring of sterile scales surrounds the apical fertile scales.

Cathayanthus

The cordaiteans that bore *Cathayanthus* cones had *Shanxioxylon sinense* stems, *Florinites* pollen and *Cardiocarpus* seeds (Wang et al., 2003). In both the seed and pollen cones of *Cathayanthus*, the primary fertile shoot bore secondary fertile shoots in helical attachment, causing Wang et al. (2003) to assign these cones to a new genus. I include both *Cathayanthus* cones in this study. Specimens of the seed cone, *Cathayanthus sinensis*, having a large number of *Cardiocarpus samaratus* seeds at the same developmental stage in organic attachment (Wang et al., 2003), suggest that seed cones in this species experienced simultaneous development. Like *Gothania* cones, *Cathayanthus sinensis* seed cones have a large primary body; unlike *Gothania* cones, they have a large W/L ratio in the primary fertile shoot, sclerenchymatous basal scales and a large quantity of cone scales. *Cathayanthus ramentrarus*, the pollen cones of the *Cathayanthus* plant, had relatively few cone scales (14 – 18: Wang et al., 2003), a character which may be correlated with simultaneous development.

The undescribed bisexual cone

I also include the recently discovered bisexual cordaitean cone, which comes from the informally named Cliffland coal of the Kalo Formation (mid-Moscovian, Pennsylvanian: Fig. 2), in this analysis. This cone bore two rows of secondary fertile shoots, each subtended by a sterile bract. From base to tip, the secondary fertile shoots of the new bisexual cone bore: 1, sterile, sclerenchymatous scales which sometimes had fertile male scales inserted among them; 2 parenchymatous rod-shaped scales with four to six pollen sacs at the tip; 3, a ring of six or seven parenchymatous, sterile scales that were crescent-shaped in cross section; and 4, an apical fertile scale which closely surrounds a basally and adaxially attached *Nucellangium* seed (Fig. 1C). The pollen sacs contain *Florinities* pollen. Three characters link the new bisexual cordaitean cone with *C. shuleri* pollen cones: rod-shaped, parenchymatous male scales; male scales inserted among the basal sclerenchymatous scales; and an upper ring of sterile scales separating the apical fertile scale or scales from lower fertile scales. This association between *C. shuleri* pollen cones and the new bisexual cordaitean suggests that the *C. shuleri* plant had bisexual seed cones and unisexual pollen cones. The presence of fertile male scales inserted among the basal sclerenchymatous scales of the new bisexual cordaitean cones suggests sequential development. In the best preserved specimen with an attached seed, most of the fertile male scales have pollen sacs, consistent with sequential development.

Callistophytaleans

The Callistophytales were shrubby or vining seed ferns with manoxylic wood (i.e. wood with large tracheids and abundant wood parenchyma) belonging to the Pteridospermophyta (seed ferns) that grew in Pennsylvanian and Permian wetlands (Rothwell 1975, 1980). Callistophytaleans had compound, pinnate fronds that bore either pollen organs or seeds attached to the abaxial (lower) surface of the frond (Rothwell, 1975, 1980; Meyen and Lemoigne, 1986; Galtier and Bethoux, 2002). The pollen organ, *Idanothekion*, is a synangium consisting of a ring of 5-9 elongated sacs fused at the base, dehiscence (release of pollen from the synangium) occurred through an inward facing longitudinal slit. *Idanothekion* bore monosaccate true pollen (*Vesicaspora*) that produced a pollen tube from the distal surface (Rothwell, 1980).

Callistophytaleans bore *Callospermarion* seeds. Like cordaitean ovules, *Callospermarion* seeds had 180 degree bilateral rotational symmetry, causing Rothwell (1980) and Hilton et al. (2003) to link the callistophytaleans to cordaiteans. In addition, *Callospermarion* seeds have the same seed integument vascular pattern as the cordaitean seed genus, *Mitrospermum* (Rothwell, 1981; Hilton et al., 2003). However, these similarities could result from convergence: glossopterids, a group of Gondwanan seed ferns also have bilaterally symmetric seeds with 180 degree rotational symmetry. In cladistic analyses of seed plants based on morphological data, the callistophytaleans do not join conifers, cordaiteans and ginkgoes (Hilton and Bateman, 2006; Doyle, 2006: Fig. 3). I include two species of *Idanothekion* in this analysis as a possible outgroup for the cordaiteans: *Idanothekion callistophytoides* (Stidd & Hall, 1970; Rothwell 1980), the pollen organ of *Callistophyton poroxyloides*, which bore *Callospermarion pusillum* seeds; and *Idanothekion glandulosum* (Millay & Eggert, 1970; Rothwell 1980), the pollen organ of *Callistophyton boyssetti*, which bore *Callo. undulatum* seeds.

Thucydialeans

Thucydia mahoningensis is the only species in the conifer family, *Thucydiaceae*, from the Late Pennsylvanian of North America, and also the only conifer known to have had compound pollen cones, viewed as a primitive trait for conifers (Hernandez-Castillo, 2001).

Thucydia trees consisted of an orthtropic (upright) trunk and two orders of plagiotropic (parallel to the ground) branches, with pycnoxylic wood and scale leaves (Hernandez-Castillo et al, 2001a, 2001b). Known from a single, detached cone, the compound pollen cone of *Thucydia* bore spirally-attached, secondary fertile shoots in the axils of bracts. The short secondary fertile shoots had a five to eight basal sterile scales followed by three to four fertile scales, consisting of a short stalk with a single pollen sac at the tip (Hernandez-Castillo et al., 2001a,b). All of the fertile scales developed on the adaxial side of the secondary fertile shoot (Hernandez-Castillo et al., 2001a,b). The pollen *Potonieisporites* occurs within the pollen sacs; *Potonieisporites* has a monolete suture and probably germinated from the proximal surface (Gomremanant ankov, 2009).

The ovulate secondary fertile shoots of *Thucydia mahoningensis* grew in fertile zones of leafy (vegetative) shoots (Hernandez-Castillo et al. 2001b). These shoots had a basal sterile zone, an intermediate fertile zone, and an upper sterile zone and grew directly on the orthotropic stem (i.e. the trunk) of the tree Hernandez-Castillo et al. (2003). Within the fertile zone, fan-shaped (flattened and bilaterally symmetric) secondary fertile shoots grew in the axils of bracts. These shoots had 10 – 15 basal sterile scales and three to four apical fertile scales. The fertile scales, which curved back toward the ‘primary fertile axis’ bore seeds on the adaxial surface at the tip of the scale. The micropyles of these seeds would have pointed towards the branch that bore the secondary fertile shoots (Hernandez-Castillo et al., 2001b).

If the seed-bearing branches of *Thucydia mahoningensis* grew directly on the orthotropic stem, the entire fertile branch might constitute the primary fertile shoot, with spirally attached secondary fertile shoots borne in the axils of bracts in the middle, and closely spaced bracts or leaves above and below the fertile zone. Costanza (1983, 1985) described bracts on the primary fertile shoot of *C. concinnus* cones that did not subtend secondary fertile shoots (i.e. that did not bear a secondary fertile shoot in the axil). Using this interpretation, the sterile – fertile – sterile pattern of Thucydia seed cones echoes the sterile – fertile – sterile pattern seen in the fertile branch complex of progymnosperms: Archaeopteris fissilus, A. haliiana, A. hibernica, A. macilenta, A. obstusa, Svalbardia, and Taen. However, Thucydia seed cones lack vegetative secondary fertile shoots in the axils of upper and lower bracts. The distribution of bracts without axillary secondary fertile shoots at the base and in the middle of cordaiteans cones is poorly known; however North American cordaiteans terminate in secondary fertile shoots (*Cordaianthus concinnus, C. shuleri*, new bisexual, *C. dumusum*).

Gnetaleasns – *Welwitschia mirabilis*

Welwitschia mirabilis belongs to a living monospecific gnetalean genus. Viewed by Martens (1977) as a plant that has lost the capacity for axial vegetative growth, modern *Welwitschia* plants consist of two continuously growing leaves attached to a short trunk (called the crown) with a well-developed tap root; branches bearing compound cones grow in axils of the leaves (Chamberlain, 1935; Martens, 1977: Fig. 5). *Welwitschia* is monoecious (plants are all male or all female: Chamberlain, 1935). Male cones consist of a primary cone axis bearing four ranks of sterile bracts. Small, bilaterally-symmetric secondary fertile shoots develop in the axils of most bracts. However, a few of the most basal bracts are sterile, and the secondary shoots in the axil of the uppermost bracts do not mature (Chamberlain, 1935, Mundry and Stützel, 2004). The secondary fertile shoots have two sets of basal sterile scales, followed by a whorl of six fertile male scales, with an apical female scale bearing a sterile ovule (Chamberlain, 1935, Mundry and Stützel, 2004).

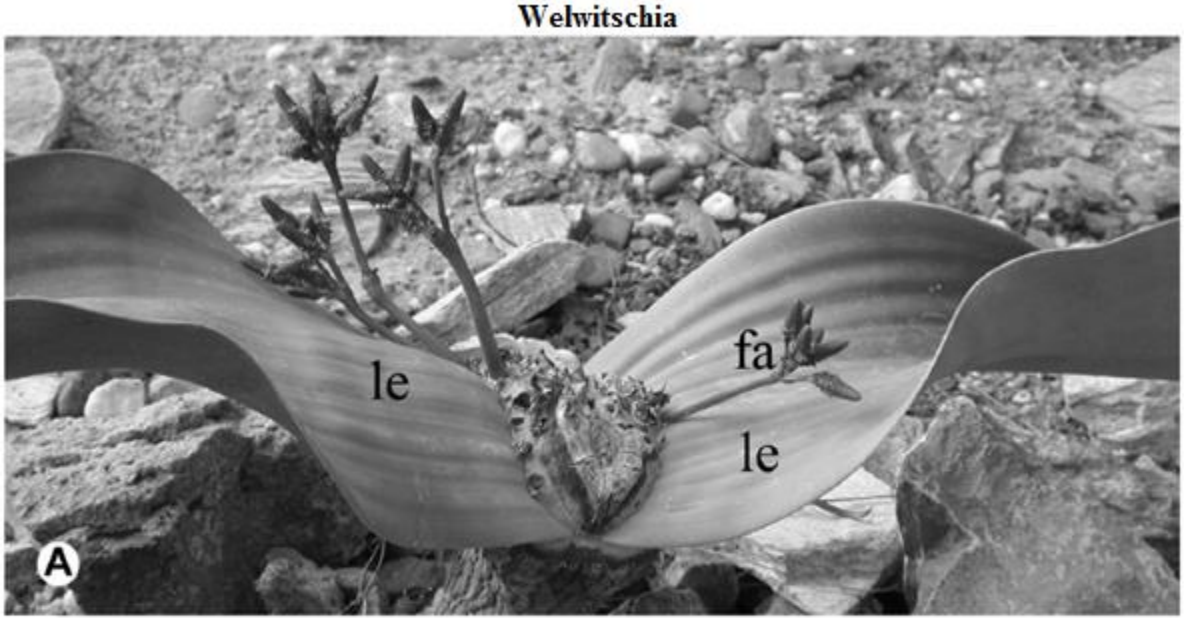


Figure 5 *Welwitschia* plant showing two leaves (le) emerging from the crown, with branches bearing compound male cones (fa), growing out of the crown in the axils of the leaves (from Mundry and Stützel, 2004).

Welwitschia has unisexual compound seed cones that bore four rows of secondary fertile shoots, each in the axil of a sterile bract. As in the male cones, the lowermost bracts do not have secondary fertile shoots in their axils and the secondary fertile shoots of the uppermost bracts do not mature (Chamberlain, 1935). In the female cones of *Welwitschia,* secondary fertile shoots have only one set of sterile scales, which fuse and grow around the apical seed to form a second integument, which disperses with the seed (Chamberlain, 1935).

Angiosperms – Archaeofructaceans

Some of the earliest documented flowering plants belong to *Archaefructaceae* family from the Early Cretaceous of China (Sun, et. al., 2002; Friis, et. al., 2003). Originally considered Late Jurassic in age, the plant has since been dated as Early Cretaceous (Sun, et. al., 2002; Friis, et. al., 2003). In coding *Archaefructus*, I follow Sun et al. (2002) who interpreted *Archaefructus* as a flower (i.e. a simple cone) with leaf-like tepals at the base, intermediate stamens consisting of a short filament with four pollen sacs, and apical carpels (see also Taylor, Taylor and Krings, 2009). Friis et al. (2003) viewed *Archaefructus* as an inflorescence (compound cone) with reduced male flowers, consisting of stalks bearing two stamens at the base, and reduced female flowers consisting of a carpel on a stalk at the top. *Archaefructus* appears to have been an aquatic plant, which could account for the loss of the perianth (sepals and petals: Friis et al., 2003).

Archaeopteridaceans

Including a distant outgroup in the analysis may help to reveal the relationship between cordaiteans, callistophytaleans, conifers (*Thucydia),* gnetaleans (*Welwitschia*), and angiosperms (*Archaefructus)*. Thus we include the progymnosperm *Archaeopteris halliana*, (family Archaeopteridaceae*)*, (Beck, 1960; Fairon-Demaret et al., 2001). Kendrick and Crane (1997) viewed progymnosperms as sister of the Spermatophyta (seed plants). Archaeopterideans first appeared in the Devonian of North America (Arnold 1939). They were large, heterosporous trees with picnoxylic wood and three types of buds, producing three types of lateral organs: 1, buds that produced short-lived branches at the growing tip of the trunk. These may have been the plagiotrophic branch systems with two orders of branching that bore leaves or leaves and sporophylls. 2, lateral, adventitious buds that produced large, long-lived branches. and 3, adventitious buds that could produce either shoots or roots as needed (Meyer-Berthaud et al., 2000; Fairon-Demaret et al., 2001).

The short-lived plageotrophic branch systems of *Archaeopteris* grew in the axils of stipules (leaf-like organs) and consisted of two orders of branches (Beck, 1962).

Archaeopteris may have shed these branches as a unit (Beck, 1962). The primary branch bore secondary branches and leaves in the same ontogenetic spiral (Beck, 1971). Large leaves formed on the abaxial side of the primary branch; smaller leaves formed on the adaxial side (Beck, 1962; Fairon-Demaret and LePonce, 2000).

Fertile branch systems bore sterile secondary branches at the base, fertile secondary branches in the middle and sterile secondary branches at the top of the primary branch. Both fertile and secondary branches bore helically attached pinnules. Fertile secondary branches had sterile pinnules at the base, fertile pinnules in the middle and sterile pinnules at the tip. In some species, some of the fertile branches may have been entirely fertile; however in *Archaeopteris halliana* , fertile branches always have a few basal sterile pinnules and all of the unbroken fertile branches have sterile pinnules at the tip.

Archaeoptteris halliana had fan sterile pinnules and deeply dissected, fertile pinnules. On the fertile pinnules, the basal lobes are sterile; the middle lobes have adaxially-attached sporangia bearing microspores or megasores, intermixed on the pinnule; and the upper lobes are sterile. Thus the fertile branch system of *Archaeopteris halliana* is self-similar, with the primary fertile branch, the secondary fertile branch and the the pinnules having a basal sterile, intermediate fertile and upper sterile pattern. Among the archeoterids, *A. fissilis, A. hibernica, A. macilenta,* and *A. obtuse* and have a sterile-fertile pattern similar to that of *A. halliana* (*A. roemeriana*: Fairon-Demaret and LePonce, 2001; Fairon-Demaret et al., 2001; Beck, 1962; Andrews et al., 1965; Andrews, 1970 in Boureau, 1970). *Svalbardia* and *Tanaitis,* other progymnosperm genera, have the same fertile, sterile, fertile pattern as *Archaeopteri*s (Høeg, 1942; Krassilov et al., 1987). In *Tanaitis*, secondary branches attached to the primary branch in a spiral pattern (Krassilov et al., 1987). *Svalbardia* may have had spirally attached secondary branches as well (Høeg, 1942, see also Banks, 1976).

Beck (1962) suggested that a single fertile scale of a cordaitean cone could be homologous to the entire fertile branch system of *Archaeopteris*. Given the similarity of organ attachment in the two, it seems more appropriate to regard the fertile branch system of *Archaeopteris* as homologies to a compound cordaitean cone. Both have two rows of secondary branches (fertile shoots) attached to the primary branch (fertile shoot), and both have spirally attached pinnules (scales). Further, both attached to the stem in the axil of a stipule (leaf). Accordingly, I treat the fertile branch system of *Archaeopteris haliana* as a compound cone, borne in the axil of a stipule/leaf (Table 4).

Table 4. Proposed homologues for cordaitean and *Archaeopteris* fertile branch systems.

Archaeopteris Homologues

Cordaitean	Archaeopteris
Leaf subtending primary fertile shoot	Stipule (not used in analysis)
Primary fertile shoot	Primary Branch
Sterile Bract	No homolog
No homologue	Proximal sterile secondary branch
Secondary fertile shoot	Fertile Secondary Branch
No homologue	Distal sterile secondary branch
Basal sterile scale	Basal sterile pinnules of fertile secondary branch
Fertile scales	Fertile pinnules
Pollen sac	Microsporangium

Programs and Parameters

I use TNT (Goloboff, 2000) and PAUP* (Swofford, 1990) to analyze cordaitean cones. Both these programs use parsimonious methods for analysis and both have systematic enumeration-state space search tecniques to run matrices. The size of the data (14 – 25 taxa with 39 characters) set is small enough to use exact parsimony methods. In PAUP*, branch and bound searches were used to produce cladograms. I run bootstrap analyses with 1000 branch and bound replicates and about 10,000 saved repetitions of mostparsimonious tree topologies in memory to produce values for the male-cone analyses. I run the analyses incorporating male-and-female cones and the analysis incorporating additional lineages with only 100 branch and bound replicates and 500 saved repetitions to accommodate time constraints due to the increased number of taxa. I extrapolate Bremer decay values by searching all trees one length value longer than the branch and bound trees and checking for collapsing nodes in the strict consensus - repeating with continued searches of increasing tree lengths. When the nodes would search for too many trees, I perfom a converse constraint branch search to obtain the Bremer decay values. It is important to note that bootstrap value in morphological analysis often get criticized due to the few characters included, and favor Bremer values (Forey, 2014). TNT's implicit enumeration was used to construct tree topologies to compare the programs different results (Goloboff 1999) for runs of male-only, male-female and plant lineages, and to map synapomorphies (APPENDIX 4) for runs of male-only analysis. The male-cone only analysis underwent a Bayesian analysis for further comparison of results. MrBayes (Hulsenbeck and Ronquist, 2001) was used with a standard datatype for morphological characters and run for 3 million generations, four parallel runs and four chains sampling every 1000 trees with a gamma rate prior. All trees were produced in FigTree (2014) for observations.

I use PAUP* to identify the traits that were important for determining tree topology in the male cone analysis and mapped those traits onto the strict concensus tree produced in PAUP* for that analysis. Using Mesquite (Maddison and Maddison, 2008), I trace the characters used in the male-cone analysis (listed in APPENDIX 2) and mapped them onto the same tree using parsimony ancestral state reconstruction (APPENDIX 5). The number of steps, C. I., R.I., Bias, Asymmetry and MK1 rates for the characters were also obtained with respect to the PAUP* male-only strict consensus (APPENDIX 6). The traits were mapped using parsimony ancestral state reconstruction.

I explore the data by running different sets of cordaiteans All analyses included the new bisexual cone, which has both male fertile scales with attached pollen sacs and female fertile scales with attached seeds. In all analyses, I use the progymnosperms, *Archaeoperis* and *Tanaitis*, and the callistophytaleans as outgroups. In all cases, I run analyses with and without *Cordaianthus ximinensis* and *C. xixhanensis*, which have much missing data.

1) A male-cone only analysis. This analysis included characters of pollen associated with male cones where known. Although its scope is relatively narrow, the resulting phylogenetic tree is not complicated by mixing female and male reproductive organs.

2) A male and female cone analysis. This analysis included characters of pollen associated with male cones where known and of seeds associated with female cones. However I did not score male cones for the characters of their associated seeds, nor did I score seed cones for the characters of their associated pollen. Coding male and female cones as separate taxa in the analysis allows me to test the whole-plant reconstructions of cordaiteans. Nonetheless, if

male and female cones have different morphologies, they may join different clades in the results of the analysis.

3) A male and female cone analysis using the same outgroups as before, adding the voltzialean conifer, *Thucydia*; the modern gnetalean, *Welwitschia,* and the ancient angiosperm, *Archaefructus*. In this analysis, I treated pollen and seed characters as in the first male and female cone analysis. As in that analysis, treating male and female cones as separate taxa in the analysis enables me to test whole-plant reconstructions of cordaiteans, although male and female cones of the same species that have very different morphologies may join different clades.

4) A male cone analysis in which I scored male cones for the characters of their associated seed species. This analysis included the voltzialean conifer, *Thucydia*; the modern gnetalean, *Welwitschia,* and the ancient angiosperm, *Archaefructus,* as well as the new bisexual cone. Scoring male cones with the characters of their associated seeds, where known, increases the number of characters assigned to each cone.

5) A male and female cone analysis in which I scored male cones for the characters of their associated seed (if known), and seed cones for the characters of their associated pollen. Once again, this analysis included the voltzialean conifer, *Thucydia*, the modern gnetalean, *Welwitschia*, and the ancient angiosperm, *Archaefructus*. Scoring both male and female cones with the characters of their associated pollen and seeds, where known, increases the number of characters assigned to each cone, and mitigates the problem of morphologically dissimilar male and female cones assigned to the same species joining different clades in the resulting phylogenetic tree.

A final analysis was done to compare the traits to the taxa. I use a clustering approach with R (R Core Team, 2014) to aggregate taxa and characters simultaneously using Ward's criterions, an optimization function for heuristic cluster searches. A modified dataset of all the taxa and characters, by making all values one number higher and all none-coding values a zero, is used for this purpose. The searches aggregate both taxa and traits.

RESULTS

Analyses with and without *Cordaianthus ximinensis* and *C. xixhanensis,* which have much missing data, yielded similar results. However analyses including these taxa produced a greater number of most parsimonious trees (an average of 15 more trees depending on the analysis), a strict consensus with more polytomies and lower bootstrap and Bremer support values. The large portion of missing data is due to the poor preservation of the specimens (Hilton, et. al, 2006b). Since the missing data appear to destabilize the analyses, I present the analytic results excluding *C. ximinensis* and *C. xixhanensis*.. Analyses results including *C. ximinensis* and/or *C. xixhanensis* appear in APPENDIX 4.

Male only results

An analysis with PAUP* using branch-and-bound searches on a character matrix consisting of pollen cones plus the new bisexual cone (14 species), using the 39 traits produced 3 equally parsimonious trees (Fig. 6), with a value of 47 for the best tree score. Of the 39 characters, 30 were parsimoniously informative; 3 were parsimoniously uninformative and 6 were constant characters (traits that were informative for other analyses). A TNT analysis with implicit enumeration produced one similar, equally parsimonious tree (APP6. 1), with different branch lengths. The *Idanothekion* outgroup-sister clade emerge with a bootstrap of 99.4 and Bremer decay value of 3.

All male cordaitean cones form a clade with 55.9% bootstrap support and a Bremer value of 1. A 'bisexual' clade, consisting of *C. shuleri*, the new bisexual cone and *C. penjonii* form a clade at the base of another cordaitean clade (56.3%bootstrap Bremer-1). This clade unites *C. shuleri* with the new bisexual cone (93.6% bootstrap, Bremer-2). These two cones probably represent the pollen-only and bisexual seed cone of a single species, mirroring the growth of Gnetalean cones. *C. penjonii* shares the trait of having fertile scales inserted among the basal sclerenchymatous scales with both *C. shuleri* and the new bisexual cone. *C. penjonii* is known only as a detached secondary fertile axis. These three cones share the unique trait of having fertile scales inserted among the basal sclerenchymatous scales.

The remaining unisexual cones form a clade (55.6% bootstrap, Bremer-1), which contains a polytomy of three different cordaitean clades and *C. concinnus*. The *Gothania* clade is well supported clade (96.8% bootstrap, Bremer-3) and the cones share large primary axis with an equally large aspect ratio and a unique arrangement of their pollen sacs on the few thin crescent fertile scales. The two remaining clades join Chinese cordaiteans with Euramerica cordaiteans- *C. ramentrarus* with *C. saportanus* (bootstrap: 67.1%, Bremer: 1) and *C. tianii* with *C. dumusum* (bootstrap: 60.1%, Bremer 1). This would suggest a wide distribution of Euramerica and Chinese

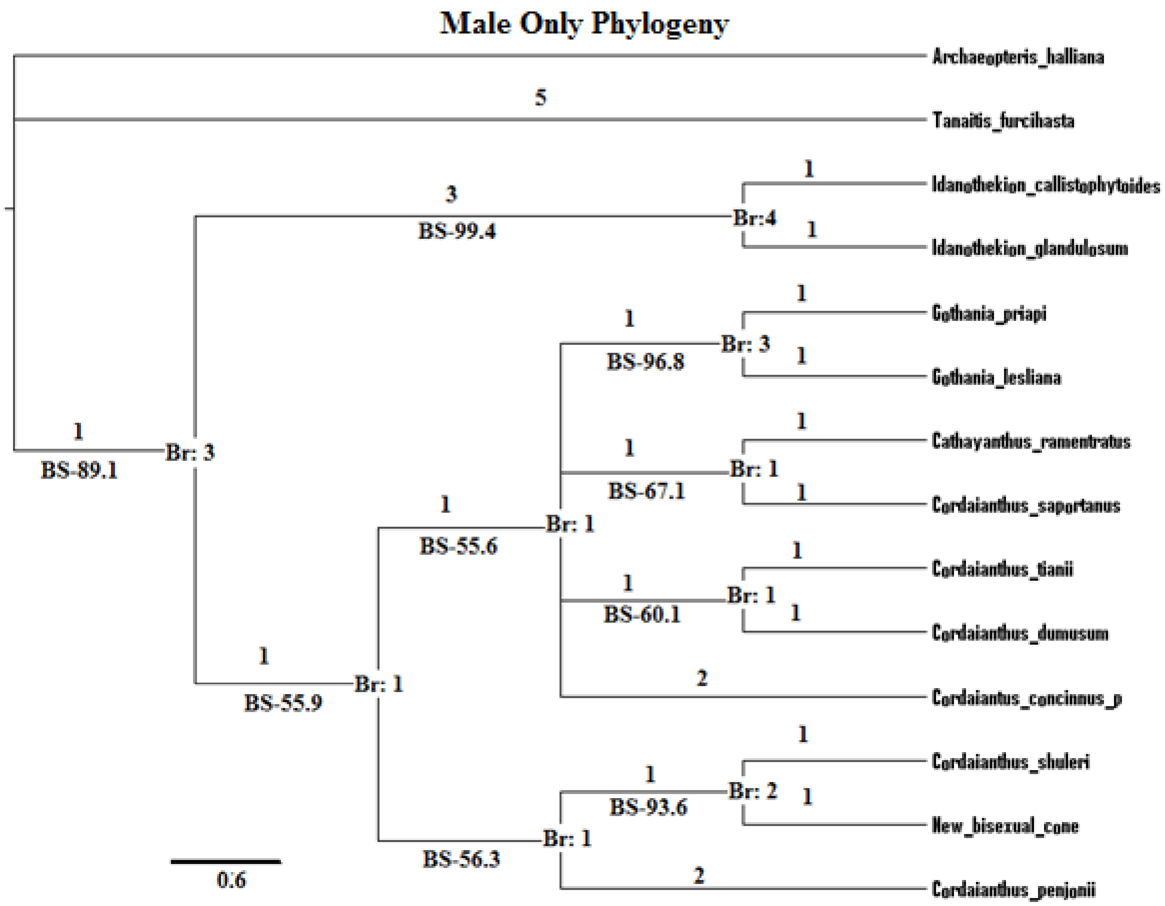


Figure 6. a strict consensus tree of 3 most parsimonious trees of length 47 of male-only cones. The branch lengths measures are above the branches, while the bootstrap values are below them; Bremer values are within the lines. The C.I. was 0.9149 the R.I. was 0.9149 for the tree - all calculated with autopomorphies. All PAUP* trees were optimized with accelerated transformation optimization.

cordaiteans. *C. rememtrarus* and *C. saportanus* share the unique trait of bilaterally symmetrically arranged secondary fertile shoots (Florin, 1951; Wang et al., 2003). This clade jumps position between a *Gothania* lineage or a *C. tianii-dumusum* lineage, each on a separate equallparsimonious tree. *C. tianii* and *C. dumusum* share the unique trait of adventitious or epicormic production of pollen cones on older branches, below the growing tip (Rothwell, 1993; Wang et al., 2009).

Male-female analysis

Runs that include seed cones (16 species, 39 traits) yielded 3 equally parsimonious trees with a best tree score of 50 (Fig. 7). Of the 39 characters, 30 were parsimoniously informative; 3 were parsimoniously uninformative and 5 were constant characters (traits that were informative for other lineages). The *Idanothekion* clade reemerges with high support: 98.9bootstrap & Breme of 4.

In the analysis of male and female cones, all cordaitean cones form a clade with 53.9% bootstrap support and Bremer support of 1. The new bisexual seed cone, *C. shuleri*, and *C. penjonii* emerge at the base of the cordaite clade (56.3% bootstrap, 1 Bremer). This clade forms a polytomy with a clade of two female cones and the greater cordaitean clade. The two female cones, *C. shanxiensis* and *C. concinnus*, clade (53.2% bootstrap, 2 Bremer) share no unique traits; they share a few common cordaitean traits - parenchymatous sterile scales intermixed with fertile scales on the upper part of the secondary fertile shoot (Costanza, 1983, 1985; Hilton et al., 2009a) - though *C. shanxiensis* has quite a bit of missing data. Why this clade forms, rather than the female cones joining their corresponding male cones seems particularly strange; it may form in part due to both coding similarly from their female characteristics, *C. shanxiensis*' missing characters and/or a lack of characteristics which would group them with their respective male cones.

Within the greater cordaitean clade consisting of unisexual cordaitean cones, the *Catha. ramentrarus and Cordai saportanus* clade re-emerges and the seed cone associated with *Cathay. ramentrarus, Catha. Sinensis,* appropriately groups with its corresponding male cone; the whole clade has weak support (bootstrap <50%; Bremer 1). *Cathayanthus* cones share the unique trait of helical attachment of the secondary fertile shoots on the primary cone axis (Wang et al., 2003).

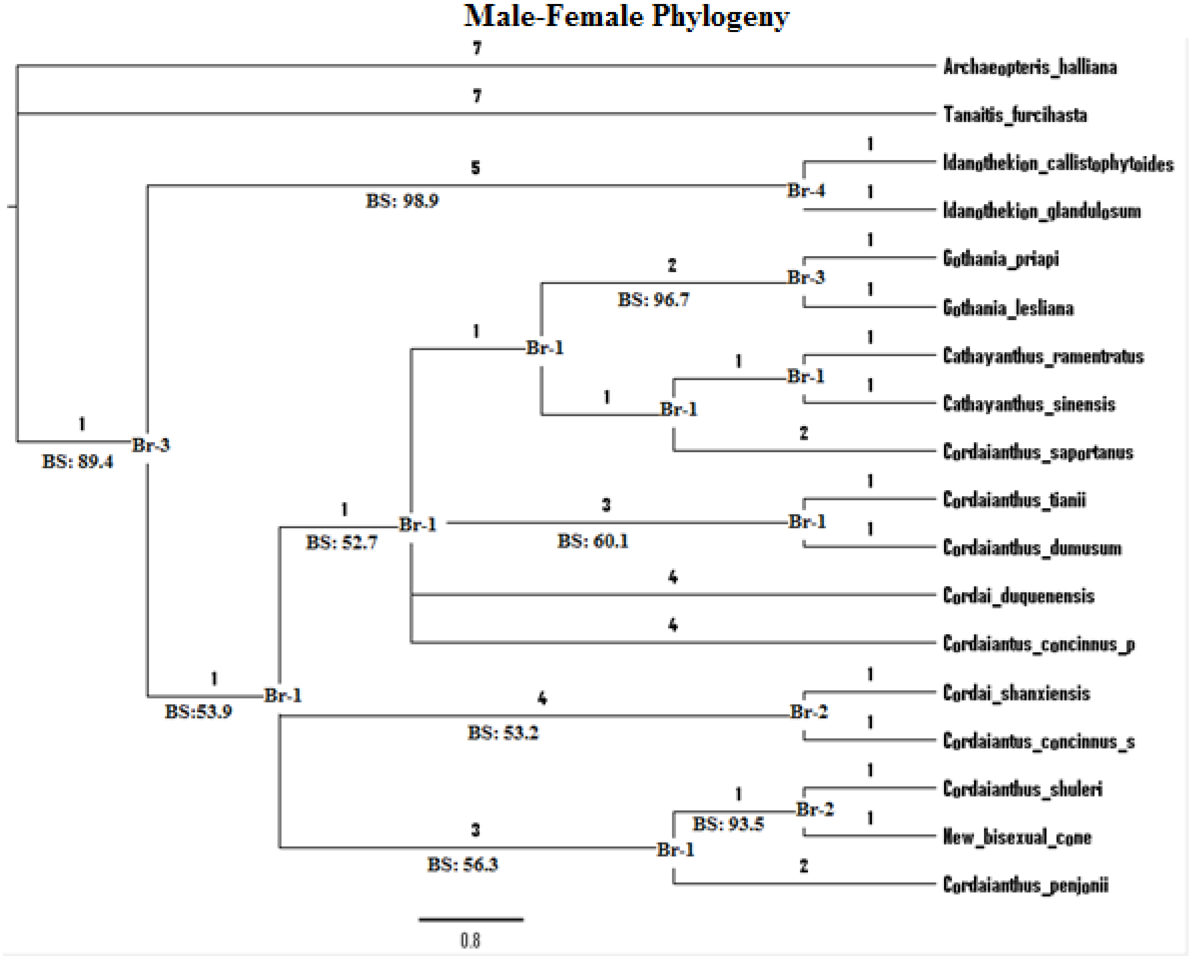


Figure 7. Strict consensus tree of 3 most parsimonious trees of length 50 of male-female cones. The branch lengths measures are above the branches, while the bootstrap values are below them; Bremer values are within the lines. The C.I. was 0.8800 the R.I. was 0.8909 for the tree. The Rescaled consistency index (R.C.) = 0.8370.

The clade has forms firmly in this part of the analysis as a sister clade to the *Gothania* clade with a bootstrap support of <50% and a Bremer support of 1. The *Gothania* clade again has high support values, BS 96.7% and Br 3. The clade which combines *Gothania* cones bearing *Mitrospermum* seeds and *Sullisaccites* or *Felixipollenites* pre-pollen with *Cathayanthus* cones bearing *Cardiocarpus* seeds and *Florinities* pollen shares many reproductive ecology and developmental traits. Cones assigned to *Cordaitanthus* form a clade within the greater cordaitean clade of unisexual cones or fall outside in a polytomy with the aforementioned clades within the greater cordaitean clade. *C. tianii* and *C. dumusum* form a weakly supported clade (<50% BS ; Br 1). The two other cones, *C. concinnus* (male) *and C. duquesnensis* from a polytomy with all the other 'greater cordaiteans,' failing to united *C. duquesnensis* with the corresponding *C. dumusum*.

An observation that became more and more pronounced as the study continued is the difficulty in trying to code for female cones separately from the male cones. The problem comes from coding a species as though its male and female cones were separate organisms. The idea could help solidify the whole-plant reconstructions, since most reconstructions remain in 'hypothesis' realm age have little external support. However, when executed, the idea proved difficult as many of the corresponding cones had traits that didn't match well. This might be due in part with how the cones are produced: male cones produced a material for swift dispersal and the morphology of cones may reflect this selective pressure. For example, some modern conifers even produce female woody cones and herbaceous male cones (Chamberlain, 1935). Regardless, these subtle morphological differences made the search for the evolution between male and female cones difficult.

A side note on support values (both bootstrap and Bremer) for the trees. Most of the resulting trees produced low values for most of the branches and lineages (BS <50% and Bremer: 1) in all of the analysis. Originally, this may seem easily attributed to the small size of the data matrices, however increasing the size of the matrices (Doyle, 2006; Hilton et. al, 2003; Hilton and Bateman, 2006) didn't show a great increase in support values (bootstrap <50% and Bremer: 1; Fig. 3, Fig. 4) on most branches. I have not been able to identify a reason for these low values, but the lack of support may just be an artifact of the nature of characters in plant descriptions. Since other studies have analyzed and interpreted the evolutionary relations of their corresponding taxa with low support values, result interpretations made here seem appropriate as well.

Plant lineage analysis

An analysis containing all the taxa available for the study yielded 104 equally parsimonious trees with a length of 73. The resulting topology of the strict consensus formed a polytomy of most of all the cordaiteans, gnetaleans and *Archaeofructus* plants. Though these results may reveal the true nature of the relations between these taxa, the results are not very falsifiable, and suggest the data is insufficient to resolve this number of taxa. I choose to look at the 50% majority consensus to interpret the possible lineages the data yielded (Fig. 8).

Idanothekion appears as sister clade to all the other ingroups and the genus has a 99.2% bootstrap and Bremer of 4. The sister clade has 55.4% bootstrap and Bremer 1 values and contains *Archaefructus*, the cordaites, conifers and gnetaleans. *Archaefructus* emerges at the periphery of the other clades, with the *C. shuleri* and new bisexual cone clade (97.8% bootstrap, Bremer-2) following in the next clade. This is particularly interesting as these early lineages, albeit paraphyletic, share bisexuality. The next clade in the lineage is the *C. shanxiasis* and *C. concinnus* (female) with 54.8% bootstrap and 1 Bremer. The following node forms a polytomy between some of the cordaites and a cordaitean-voltzialean conifer-gnetalean clade (referred to as the ‘voltzialean’ lineage henceforth). Among the cordaitean cones within the polytomy, the *C. tianii* - *C. dumusum* clade re-emerges (56.2% bootstrap, Bremer-1). These cones share the trait of forming from adventitious buds, on older stems and branches below the growing tip. However, other *Cordaianthus* cones do not join this clade, and the two *Cathayanthus* cones do not form a clade in this analysis. *C. duquenensis* and *C. concinnus* (pollen cone) split independently at the polytomy here as well. The ‘voltzialean’ lineage starts off with cordaitean cones (*C. saportanus*, *Cathay. sinensis* and *Cathay. ramentrarus*) that bore Florinites pollen or Cardiocarpus seeds. The pollen and seed cones of the living gnetalean, *Welwitschia*, form a weakly supported clade (BS, <50%, Br, 1) which joins the *Gothania* – *Thucydia* clade with weak support (BS, <50%, Br, 1). The gnetalean and *Thucydia* appearing in sister clades give the ‘voltzialean’ lineage its name. The gnetalean shares the trait of having very few cone scales with *Thucydia*, and all three (gnetalean, *Thucydia* and *Gothania*) taxa share simultaneous development of cones. The seed and pollen cones of *Thucydia* form a clade with 60% bootstrap support and Bremer support of 1, which joins with the *Gothania* clade (BS; 91 % Br: 1), suggesting that the voltizalean conifers are sister to the *Gothania* lineage, which would make cordaiteans a polyphyletic group. Like *Gothania*, *Thucydia* has pre-pollen, in this case *Potoniesporites* (Gomankov, 2009). In addition, Thucydia and Gothania both have basal sterile scales that lack sclerenchyma.

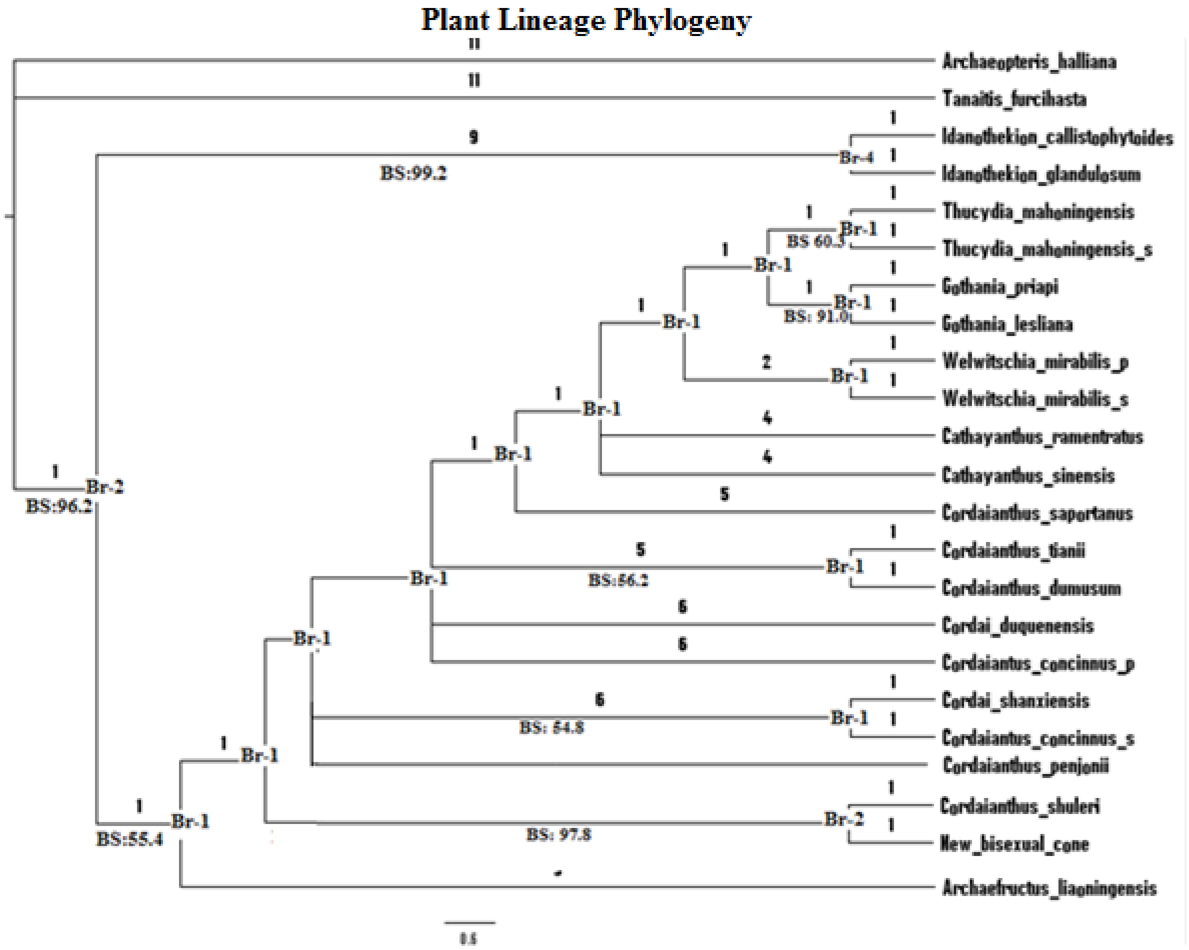


Figure 8 – 50% majority consensus tree of 104 trees, with a 73 value best score found, of male-female cones and the other lineages. The branch lengths measures are above the branches, while the bootstrap values are below them; Bremer values are within the lines. The C.I. was 0.7397 the R.I. was 0.8000 for the tree.

I ran two additional analyses to better understand links between taxa. The first of these analyses consisted of all the male cones and all the possible lineages: *Archaeofructus*, *Thucydia* and the gnetaleans (Fig. 9a). This analysis yielded six most parsimonious trees, with a score of 66 for the best tree. In the strict consensus clade, the callistophytalean pollen organ, *Idanothekion*, formed the closest outgroup-sister clade consisting of all other seed plants, including the angiosperm, *Archaeofructus*. Within this clade, *Archeofructus* is the sister clade of all cordaitean cones plus the voltzialean conifer, Thucydia, and the gnetalean, *Welwitschia*. The cordaitean – *Thucydia* – *Welwitschia* clade has very little structure, with most taxa splitting straight from a polytomy. However a few of the clades revealed in previous analyses reappear within the polytomy: *Thucydia* with the *Gothenia* cones, *C. tianii* with *C. dumusum*, and *C. shuleri* with the new bisexual cone.

For the next analysis, I honored the available whole-plant reconstructions of *Thucydia* and cordaitean taxa, assigning pollen traits to the appropriate seed cone, and seed traits to the appropriate pollen cone where known (Fig. 9b). For example, I coded both *Gothania* pollen cones as having the vascular pattern of their associated seed, *Mitrospermum*. Likewise, I coded the *Cordaianthus concinnus* seed cone as having true pollen, because its associated pollen cone bears *Florinities* pollen. This analysis included all possible lineage taxa and yielded 26 most parsimonious trees, with a best tree score of 75.

Exploratory Phylogeny of Plant lineages

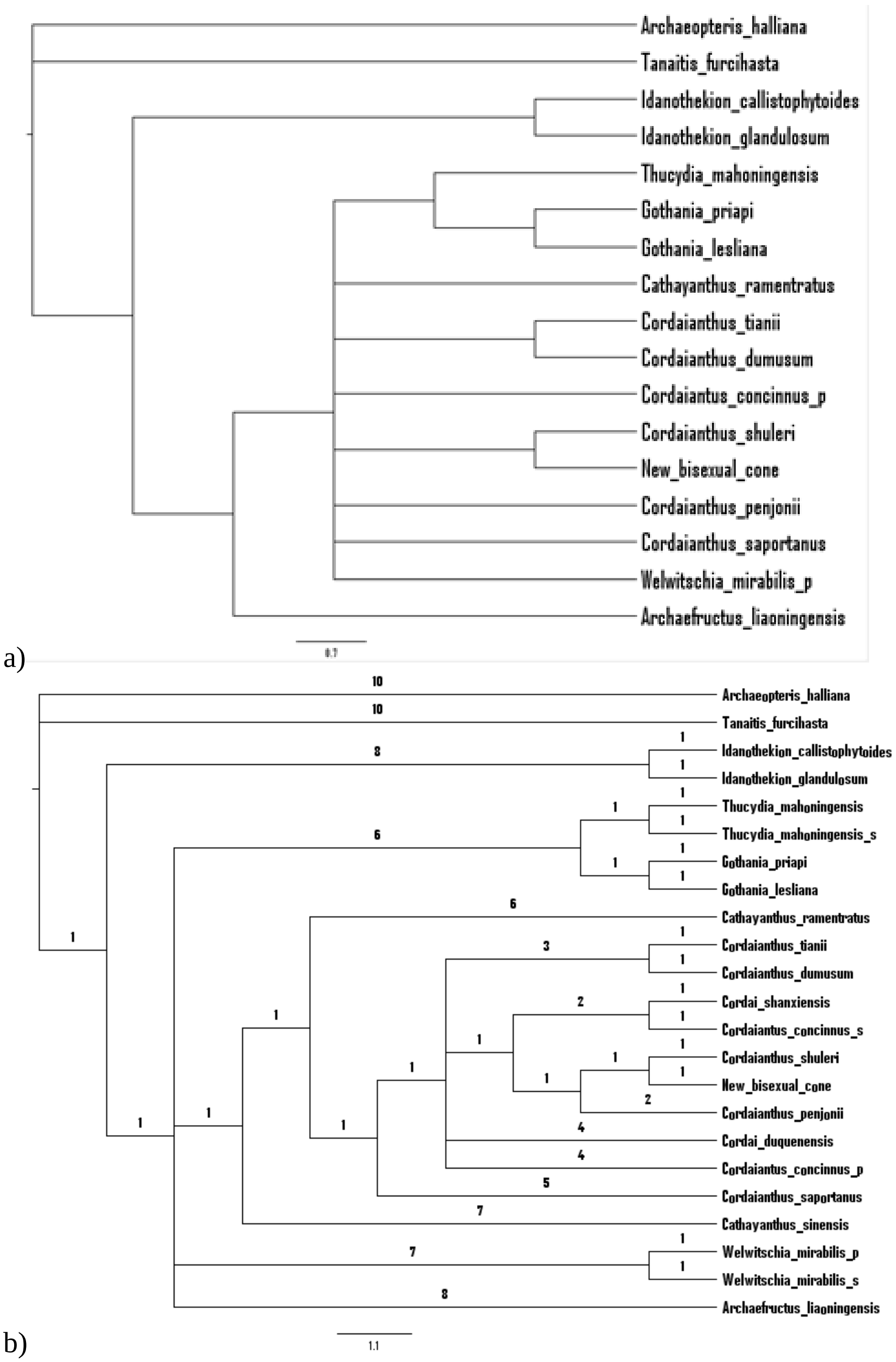


Figure 9 a) Strict consensus of 6 (score 66) trees using male-only cordaitean cones and the plant lineages. b) Strict consensus of 26 trees (Score 75) of all taxa with taxa coded with pollen characters assigned to female cones and vice-versa.

The strict consensus resolves with a strange relation among the previewsly observed lineages. Within the non-callistophytalean seed plant clade, four lineages form a polytomy: the angiosperm, *Archaeofructus*; pollen and seed cones of *Welwitschia*; a cordaitean clade consisting of *Cordaianthus, Cathayanthus,* and the new bisexual cone; and a *Gothania – Thucydia* clade. Within the *Cordaianthus – Cathayanthus* clade, the result reverses the pattern seen in previous analyses, in which a clade containing *C. shuleri*, the new bisexual cone and *C. penjonii* was sister to a large 'unisexual' cordaitean clade; *C. shuleri*, the new bisexual cone and *C. penjonii* and a sister *C. shanxiensis* and *C. concinnus* (seed) clade now form the tip of the *Cordaianthus – Cathayanthus* clade. The previous top, *C. sinensis* and *C. ramentrarus*, now are the basal most members of the *Cordaianthus – Cathayanthus* clade.

The characters that are important for determining the tree topology of the male cone analysis appear in Fig. 10. The following traits separate all seed plants from the progymnosperm outgroups (*Archaeopteris* and *Tanaitis*): trait 1) heterosporous or seed-bearing; trait 2) bisexual or unisexual reproductive organs; trait 26) sterile – fertile patterning of the reproductive axis; trait 38) pollen or spore germination surface. Within the seed-plant clade, the following traits determine the separation of callistophytalean pollen organs (*Idanothekion*) into a clade: trait 4) presence of a cone; trait 11) pollen sac location with respect to axis bearing the fertile organ; trait 19) surface of attachment of sporangium, synangium pollen sac, or seed on the fertile scale or sporophyll; and trait 32) organization of pollen sacs. Two traits determine the separation of cordaiteans from callistophytaleans: trait 16) venation of the organ bearing seeds or pollen sacs; and trait 29) shape of the organ bearing seeds or pollen sacs.

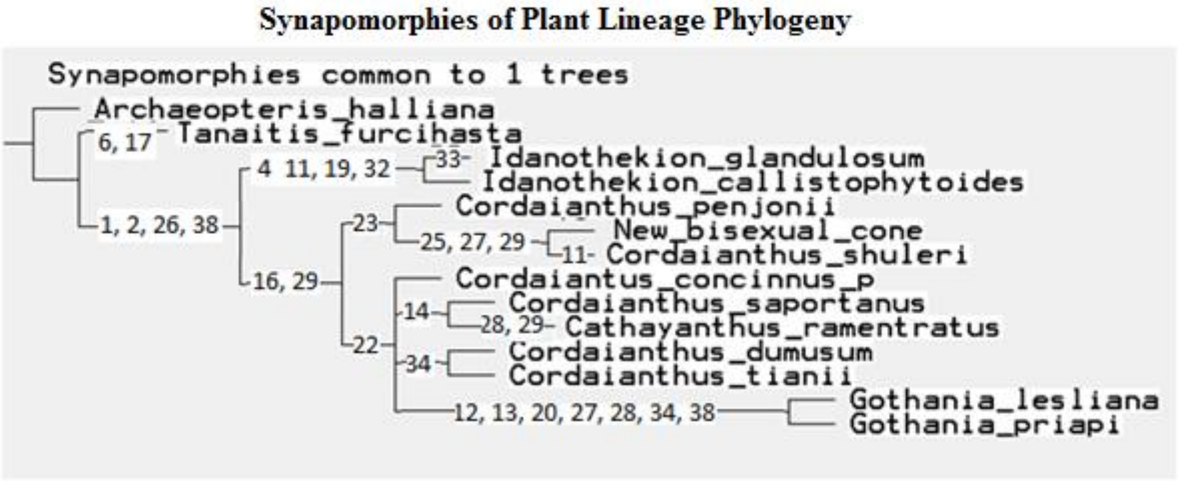


Figure 10. TNT cladogram with synapomorphies mapped into the branches (Modified from APP4. 1 so that trait numbers match Appendix 2).

Within the cordaitean clade, trait 23, insertion of fertile male scales among the basal sclerenchymatous scales, determines the separation of *Cordaianthus penjonii*, *C. shuleri*, and the new bisexual cone from all other cordaiteans. Within this clade, the new bisexual cone and C. shuleri form a clade determined by three characters: (character 25) presence of a ring of sterile scales separating the apical fertile scales from the intermediate fertile scales; (character 27) number of sterile scale morphologies; and (character 29) the shape of the fertile scale of sporophyll, which is rod-shaped in these two cones.

Character 22, the presence of sterile scales above fertile scales on the primary cone axis, determines the large clade consisting of cordaiteans with unisexual cones. Within this large clade, a number of traits determine the *Gothania* clade, including: trait 12) the aspect ratio of the primary body (the xylem and phloem) of the primary fertile shoot, which is long and narrow in Gothania cones; trait 13) the size of the primary body of the primary fertile shoot; trait 20) the configuration of the pollen sacs at the tip of the fertile scale; trait 27) the number of sterile scale morphologies; trait 28) the number of cone scales; trait 34) simultaneous development of pollen or seeds; and trait 38) germination surface of the pollen or microspore. Within the large clade of cordaiteans with unisexual cones, trait 14, symmetry of the secondary fertile shoot determines the *Cathayanthus – Cordaianthus saportanus* clade. Likewise, trait 34, epicormic or adventitious production of pollen cones, determines the *C. dumusum – C. tianii* clade. However, no character brings all *Cordaianthus* cones together, or all Cordaianthus plus *Cathayanthus* cones together.

Multivariate analysis

I used two-way cluster analysis to investigate linkages between characters in the traits matrix (APPENDIX 1). To do this, I adapted the traits matrix to fit in the uninformative and the missing traits in order to run under a multivariate statistical analysis by making them a zero value; since zeros are informative data in cladistic analyses in a different way than just absence in ecological analyses, so all the values received an additional value (+1) which should maintain the relations between all traits. I used the vegdist to compute dissimilarity indices and standardized the data using the Wisconsin shortcut to determine the overall similarity in taxa based on shared traits, and in traits based on their distribution among taxa. Two-way cluster analysis produces a phonetic classification of taxa, based on overall similarity rather than homology or shared ancestry (Mishler, 2009). The resulting heat map can reveal characters with the same distribution among taxa, due to shared ancestry, shared ecology or a combination of both (Fig. 11).

Most of the characters did not arrange with a lot of particular support. A red branch of characters appears to heat-up most of taxa. Though none of the characters seem to have some particular pattern (type of symmetry, extend of bisexuality, shape of female fertile scales, etc.) they may be highly influential to the analysis based on how they cluster and heat-up. The dark green branch has characters which may coordinate with reproductive ecology (developmental): xylem pith size and aspect ratio, tip of primary shoot fertility and continuous or simultaneous growth. Most of the outgroups and the Chinese cordaites heat-up to these characters. An interesting thing to note comes at the bottom with the *Idanothekions* and the *Shanxioxylon taijuanense* cones – the branch divisions seem to match the heat blocks of the two lineages taxa groupings. How this influences this analysis or the cladistic analyses remains uncertain, but this may be the reason that the *Shanxioxylon taijuanense* cones caused noise in the analyses. Furthermore, since this analysis treats missing and incongruent data in the same way, the behavior may just seem to show this.

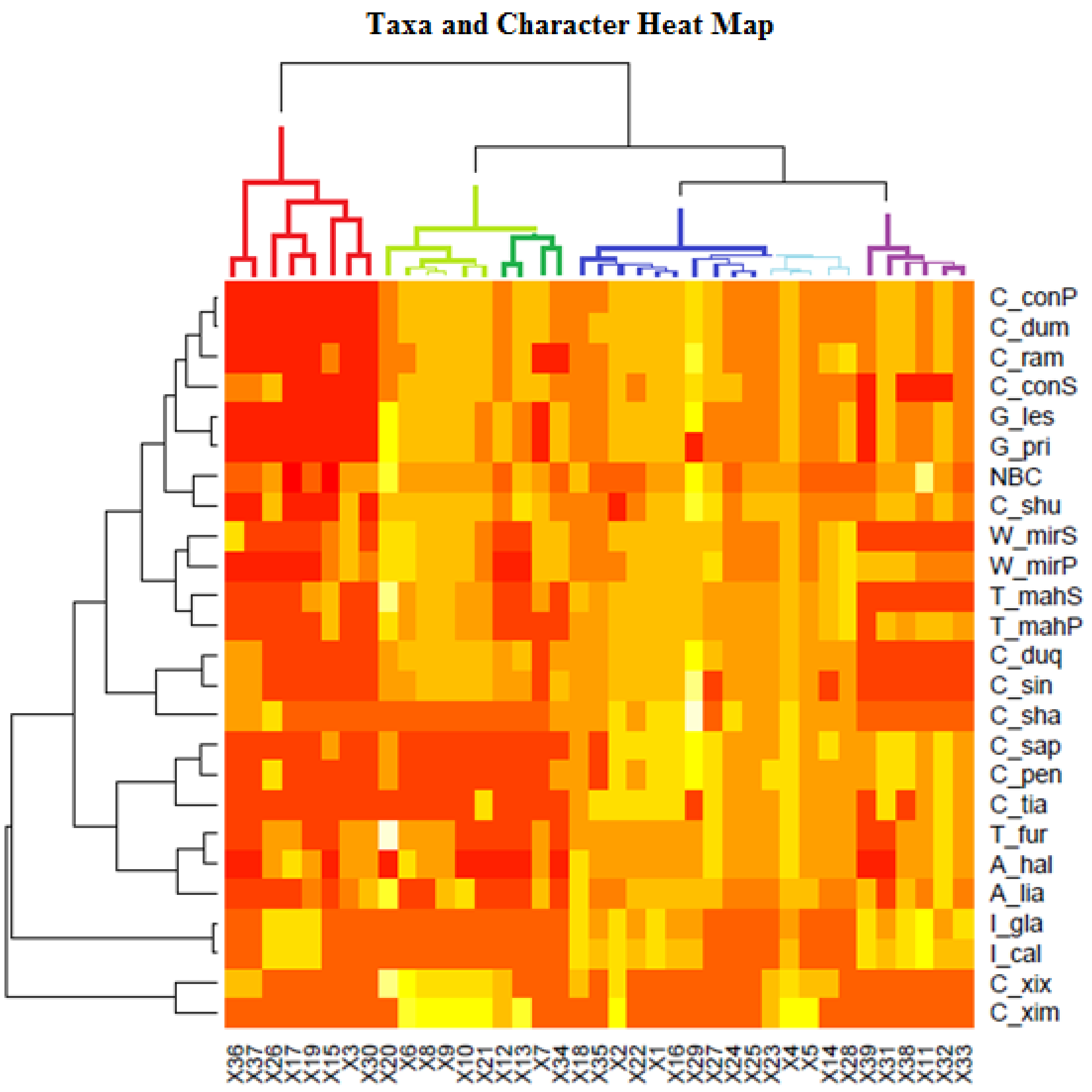


Figure 11 Heat map of characters (top dendrogram – bottom values) and taxa (side dendrogram – right values).

DISCUSSION

Bootstrap and Bremer support values in phylogenetic studies of ancient land plants

In this study, most of the resulting trees had low bootstrap support values and low Bremer support values for most branches and lineages (BS <50% and Bremer: 1). This might result from the small size of the data matrices, however moderately sized matrices (Doyle, 2006; Hilton et. al, 2003; Hilton and Bateman, 2006) showed similarly low support values (BS <50% and Bremer: 1; Fig. 3, Fig. 4) on most branches. I have not been able to identify a reason for these low values, but the lack of support may be intrinsic in the nature of morphological clados distic analysis; Hilton (2003) attributed low support values to a low number of synapomorphies within the cordaitean clade. Since other studies have analyzed and interpreted the evolutionary relations of their corresponding taxa despite low support values, the results and interpretations made here are valid.

Phylogenetic implications of cordaitean lineages

Most of the analyses suggest two cordaitean lineages: a *Gothania* lineage, which has *Mesoxylon* stems, *Mitrospermum* seeds and monosaccate pre-pollen, and a new lineage with bisexual seed cones, *Nucellangium* seeds, and monosaccate pollen (Fig. 6 – 8). The pollen cone, *C. shuleri*, and perhaps *C. penjonii* belong to the new lineage with bisexual seed cones. The characters that determine the *Gothania* lineage fall into two categories, characters related to pollen and characters related to the size and complexity of the cones (Fig. 10). *Gothania* cones have prepollen that germinated from the proximal rather than the distal surface, considered a primitive trait (Poort et al., 1996). A row of pollen sacs formed at the tip of the fertile scale, a derived character for cordaiteans (Fig. 10). Rothwell (1988) suggested that the pollen in *Gothania* cones developed directly. Characters related to the size and complexity of *Gothania* may correlate with direct development (Fig. 10), including the large size of the primary body, which might function to deliver nutrients and water quickly to the developing cone, the low number of cone scales and having simple cones with only one sterile scale type, the basal sterile scales.

The new bisexual cone, which bore *Nucellangium glabrum* seeds, forms a clade with its probable pollen cone, *Cordaianthus shuleri* (Appendix 3). These two taxa share a number of derived traits consistent with belonging to the same species, including the upper sterile scales, a ring of sterile scales separating the apical fertile scales from the intermediate fertile scales, and rod-shaped fertile male scales that develop a radially symmetric array of four to five ridges, each of which give rise to a pollen sac. Further, although cordaitean seed cones, including the new bisexual seed cone are rare, the seed associated with the new bisexual cone, *Nucellangium,* co-occurs with the pollen cone *C. shuleri*, throughout its range, except in the Laddsdale coal interval/Seccor Coal, where *Nucellangium* occurs without *C. shuleri* (Raymond et al, 2010; Costanza, 1983).

The relationship of *Cordaianthus penjonii* to the cordaitean lineage with bisexual seed cones remains uncertain. *C. penjonii* comes from the silicified Grand Croix flora of France, and no bisexual cones have been described from this assemblage (Doubinger et al., 1995). In addition, *C. penjonii*, the new bisexual cone and *C. shuleri* develop pollen sacs in different ways. The fertile male scales of *C. penjonii* develop 4 to 5 bilaterally, but not radially, symmetric ridges at the tip, resulting in a bilaterally symmetric array of pollen sacs at the tip of the scale, a pattern also seen in *C. saportanus, C. dumusum* and *C. concinnus* pollen cones (Florin, 1951; Rothwell, 198?; Costanza, 1983, 1985).

The new lineage with bisexual cones is sister to all lineages with unisexual cones in the male only and male-female cone analyses (Figs. 6 - 8). In contrast, the analyses assigning characters of the associated seed and pollen to all cones regardless of gender which incorporated all outgroups (Fig. 9), nests the new lineage with bisexual cones groups within *Cordaianthus* and *Cathayanthus* clade. I discuss the implications of this dichotomy below in the Plant Bisexuality section.

Traditional systematic investigations would suggest one or two additional lineages, a 'true' *Cordaianthus* lineage with *Pennsylvanioxylon/Cordaixylon* stems, *Cardiocarpus* seeds, and monosaccate pollen, and a *Shanxioxylon* lineage with *Cathayanthus* or *Cordaianthus* cones, *Cardiocarpus* seeds and monosaccate pollen (Trivett and Rothwell, 1991; Costanza, 1983, 1985; Wang et al., 2003, 2009; Hilton et al., 2009b). My analyses provide no support for a *Cordaianthus – Pennsylvanioxylon/Cordaixylon* lineage including *C. concinnus* seed and pollen cones, *C. dumusm, C. duquesnensis, C. tianii*, and *C. shanxixylon*, although parts of this lineage persistently form small clades (i.e. the *C. tianii – C. dumusum* pollen cone clade, determined by the occurrence of epicormic or adventitious cones in both species, and the *C. shanxiensis – C. concinnus* seed cone clade, determined by the presence of sterile intermixed with fertile scales on the secondary fertile shoot above the basal sclerenchymatous scales : Figs. 6 - 8).

The results of this phylogenetic study offer equivocal support for the *Cathayanthus* lineage, which emerges in the male cone analysis and the male-female analysis (Figs. 6, 7). In the both analyses, *C. saportanus*, a French cordaitean, joins *C. ramentrarus* to form a clade. The secondary fertile shoots of both cones have bilateral symmetry (Florin, 1951; Wang et al., 2003). However, *C. saportanus* is a detached secondary fertile shoot and thus lacks the other definitive character of *C. ramemtrarus*, helical attachment of the secondary fertile shoots to the primary fertile shoot (Wang et al., 2003). Renault (1879) described a compound cordaitean cone, *C. subglomeratus*, from Grand Croix, which had secondary fertile shoots spirally attached to the primary cone axis. In both taxa all the fertile scales occur at the apex of the secondary fertile shoot. However Renault's specimen revealed the secondary fertile shoots of *C. subglomeratus* in oblique longitudinal section, and their symmetry in transverse section remains unknown. Nonetheless, *Cathayanthus* may also occur in the Grand Croix assemblage.

Neither of the characters that unite the *Cathayanthus* lineage in the male cone and the male-female analyses (spirally-arranged secondary fertile shoots on the primary fertile shoot, and bilaterally symmetric secondary fertile shoots) appear in all cones associated with *Shanxioxylon* stems (Fig. 10). *C. saportanus* and *C. ramemtrarus* (both pollen cones) have bilaterally symmetric secondary fertile shoots. As previously discussed, *C. saportanus*, known only from detached secondary fertile shoots, may have had spirally attached secondary fertile shoots, and may belong to the *Cathayanthus* lineage. However, Wang et al., (2003) described the seed cone associated with *Cathay. ramentrarus, Cathay. sinense,* as radially.. Further, although both cones of the *Shanxioxylon sinense* plant have spirally arranged secondary fertile shoots and its pollen cone has bilaterally symmetric secondary fertile shoots, the cones of the *Shanxioxylon taiyuanense* plant have tetrastichous (on four sides of the axis) attachment of secondary fertile shoots and radial symmetry, leading Hilton et al. (2009b) to place them in *Cordaianthus*.

As illustrated by the discussion of *Cathayanthus* cones, a number of factors contribute to the failure of phylogenetic analysis to consistently identify lineages associated with *Cardiocarpus* seeds. For the most part, the cones and seeds of this lineage have generalized morphology with few synapomorphies (Wang et al., 2003). The *Cathayanthus* lineage has some potential synapomorphies; however no character appears unique to *Cordaianthus* pollen or seed cones (Fig. 10). Many members of the *Cordaianthus* and *Cathayanthus* lineages occur as detached secondary fertile shoots, which presents a challenge in identifying synapomorphies. Of the ten cones associated with these two potential lineages, five are known only from detached secondary fertile shoots: *C. saportanus, C. tianii, C. shanxiensis, Cathay. ximinensis*, and *Cathay. sinensis*. Discovery of complete cones belonging to these taxa might enable identification of synapomorphies for these two potential lineages. Or, identification of these potential lineages may require a whole plant analysis that incorporates vegetative anatomy.

In the analysis of male cones with limited outgroups (progymnosperms and callistophytaleans), *Gothania* formed a separate clade (Fig. 7). In the analysis of male and female cones, the *Gothania* clade joins with the *Cathayanthus* clade to form a *Gothania* – *Cathayanthus* clade, which brings together cordaitean cones that had *Mitrospermum* seeds and monosaccate prepollen (*Gothania*), and with cones that had *Cardiocarpus* seeds and monosaccate pollen (*Cathayanthus*). Poort et al. (1996) considered monosaccate prepollen primitive with respect to true pollen. Thus this grouping implies that true pollen evolved more than once within the cordaitean lineage, or that the appearance of prepollen in *Gothania* is a reversal. Other characters that link *Gothania* and *Cathayanthus* may relate to their reproductive ecology (Fig. 10). Rothwell (1988) suggested that both *Gothania* cones had simultaneous development of pollen; *Cathayanthus sinensis* appears to have had simultaneous development of seeds based on the presence of multiple, mature ovules or seeds in one of the specimens. Character states that might correlate with simultaneous development include: a large primary body in the primary fertile shoot to supply simultaneous development of pollen and seeds; photosynthetic basal sheathing scales lacking sclerenchyma, a tissue requiring high metabolic investment that may serve primarily to protect long-lived, continually developing cones; relatively few cone scales and few sterile scales relative to the number of fertile scales. Two-way cluster analysis of the character matrix revealed correlation between direct development and the size of the primary body, but not traits associated with cone scales.

Coding of male and female cones

Ideally, inclusion of male and female cones in the same phylogenetic analysis should yield a phylogenetic analysis in which male cones and female cones belonging to the same plant cluster together. The new bisexual cone and its probable pollen cone, *C. shuleri*, consistently formed a clade, as did *Thucydia mahoningensis* seed and pollen cones (Figs. 6 - 9). However, pollen and seed cones of *Shanxioxylon sinense* formed a clade in only one analysis, and seed and pollen cones assigned to different species of *Pennsylvanioxylon/Cordaixylon* never formed a distinct clade, even in the analysis in which I coded pollen cones for their associated seed characters and seed cones for their associated pollen characters (Fig. 9B). This result suggests that *Cathayanthus* and *Cordaianthus* pollen and seed cones experienced different selective pressures, with male cones selected for swift dispersal of pollen and seed cones selected for nurture and protection of seeds. Leslie (2011) documented divergence in the size and morphology of conifer pollen and seed cones after the Jurassic, attributed to conifer seed predation by birds and mammals. In modern conifers woody seed cones generally persist longer than herbaceous pollen cones (seed cones 2 – 20 yrs, pollen cones 1 - 2 yrs: Chamberlain, 1935, Keeley and Zedler, 1998). Leslie's (2011) study suggests that, within the cordaitean-conifer-gnetalean clade, similarity between male and female cones is a primitive character. Regardless of its cause, variation in the anatomy and morphology of pollen and seed cones complicates the use of reproductive organs to trace the evolution of seed plants using cladistics techniques.

Pollen evolution and implications for voltzialean conifer phylogeny

Seed plants evolved from a heterosporous ancestor with spores having a proximal germination surface, perhaps through an intermediate prepollen stage in which pollen germinated from the proximal rather than the distal surface (Taylor et al., 2009). In my phylogenetic analyses, the fossil group with the most advanced pollen, the callistophytealean seed ferns, which had distal germination and pollen tubes (Rothwell 1972), diverged early from cordaiteans, voltzialean conifers, gnetaleans and angiosperms (Figs. 8, 9). In most analyses, *Gothania*, a cordaitean with prepollen occurred nested within a clade of other cordaitean lineages that had true pollen (Figs. 6-8, 9a). Poort et al. (1996) considered prepollen as primitive with respect to true pollen. Results of Doyle's (2006) phylogenetic study also suggested that true pollen and distal pollination appeared early, leading Doyle to speculate that the presence of prepollen in the voltzialean conifers was a reversal, rather than a primitive character. Generally, reproductive organs are viewed as evolutionarily conservative. However, just as heterospory evolved iteratively in plants that reproduced with spores (DiMichele and Bateman, 1994), pollen and pollen tubes may have evolved iteratively from prepollen in more than one plant lineage.

Because both have prepollen, the voltzialean conifer, *Thucydites*, consistently formed a clade with *Gothania*, in analyses that include all of the plant lineages considered in this study (Figs. 8, 9). This result implies that cordaiteans as traditionally defined (Taylor et al., 2009) are paraphyletic. All voltzialean conifers apparently have prepollen (Taylor et al., 2009), and could be monophyletic. Based on the early appearance of *Gothania* and *Felixipollenites* in the permineralized record (Fig. 2), Gomankov (2009) proposed the evolution of both *Potoniesporites* and *Florinities* from *Felixipollenites,* supporting the link between *Thucydia,* which has *Potoniesporites* prepollen, and the *Gothania* clade with *Felixipollenities* and *Sullisaccites* prepollen. Whether or not the occurrence of prepollen in voltzialean conifers and *Gothania* is primitive or a reversal, a phylogenetic link between the *Gothania* lineage and voltzialean conifers would minimize the number of reversals from pollen to prepollen, or independent evolutionary events leading to the appearance of pollen in the cordaitean-conifer-gnetalean clade. Future analyses will test this working hypothesis by including more voltzialean conifer species.

Results of this study suggest that angiosperms are more closely related to conifers than to the callistophytalean seed ferns, consistent with Doyle (2006) and Hilton and Bateman (2006), but not with Doyle (2012: Fig. 12). Incorporating additional taxa into the analysis could test this hypothesis, and Magallon’s (2010) hypothesis that cycads, not conifers are the closest living relatives of angiosperms. Other candidates for addition include conifers with true pollen; cycads, a Late Paleozoic group with unisexual cones that persists today; benettitaleans, a Mesozoic group, possibly related to cycads (Taylor et al., 2009) with simple bisexual cones; caytonialeans, a Mesozoic group with unisexual reproductive organs having adaxially attached seeds; and other, more conventional angiosperms.

Flowers and cone bisexuality

Bisexual reproductive organs (i.e. flowers) distinguish modern angiosperms from living seed plants and may play an important role in the evolution of angiosperm diversity. Yet, the role of bisexual reproductive organs in plant evolution remains unclear, although a variety of hypotheses have been advanced to explain the origin of this condition (Chamberlain, 1935; Frolich and Parker, 2009; Flores-Rentería et al., 2011; Glover et al., 2015). Hypotheses based on the genetics and developmental biology of angiosperms generally consider bisexual reproductive organs as derived with respect to unisexual reproductive organs (Frolich and Parker, 2009; Rudall and Bateman, 2010; Theissen and Melzer, 2007. Conversely, based on the fossil record and the developmental biology of non-angiosperm seed plants, Chamberlain (1935) hypothesized that early seed plants had bisexual reproductive organs, which were lost in most seed-plant lineages and retained in angiosperms (see also Land, 1904, Flores-Rentería et al., 2011). Part of the problem of resolving the history of bisexual reproductive organs in land plants comes from the prominence of unisexuality in the lineages leading to coniferophyta (Doyle, 2012; Magallon and Sanderson, 2005). However, given the presence of sterile ovules in some gnetalean pollen cones, bisexuality probably emerged in the coniferophyta, at least within the gnetaleans (Chamberlain, 1935). Likewise, Flores-Renteria et al. (2011) invoked the widespread occurrence of teratoid female top, male bottom bisexual cones in living conifers to infer a bisexual ancestor for conifers. Magallon and co-workers proposed an early divergence between angiosperms and coniferophytes, in the Late Paleozoic or Early Mesozoic, and from a very old lineage, cycads or bennettitaleans **(**Magallon and Sanderson, 2005; Magallon, 2010; Doyle, 2012: Fig. 12**).** The fossil record has very few specimens that would definitively resolve any of the hypotheses to explain the origin of bisexuality as few non-angiosperm fossils exhibit this condition (Bateman and DiMichele, 2002; Raymond et al., 2013, 2014; Wang, 2004).

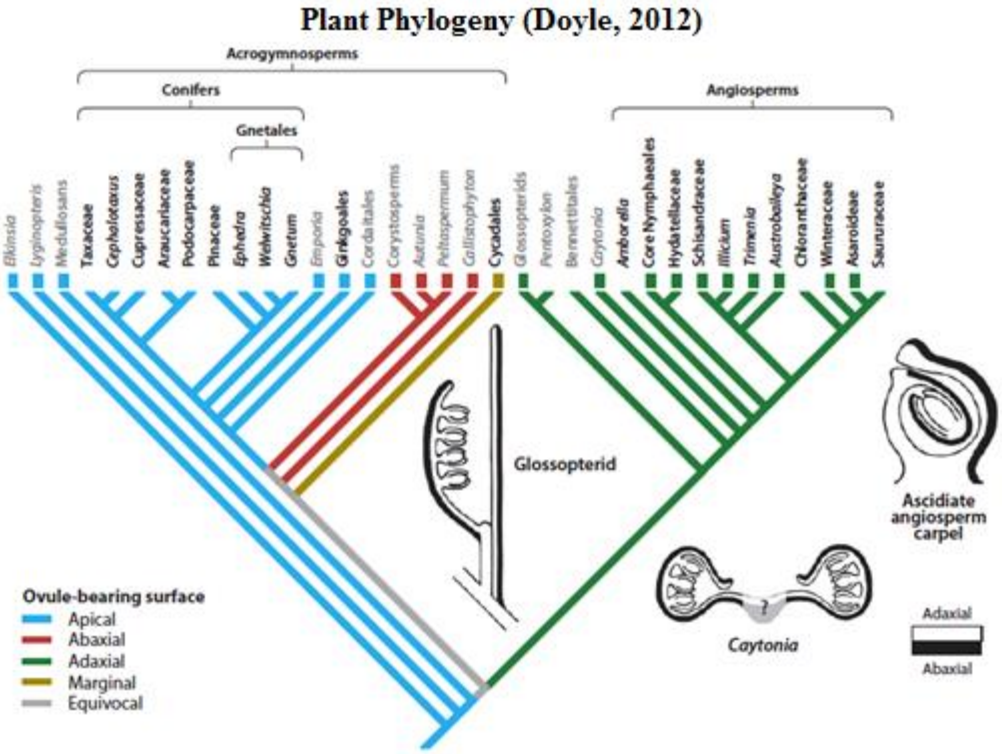


Figure 12 Phylogenetic tree from Doyle, 2012 showing the ancestry of angiopsperms. One of the few trees incorporating morphological data that links conifers and gnetaleans. The new cordaitean lineage with bisexual cones bears seeds on the adaxial surface of the fertile scale as do angiosperms, caytonialeans and glossopterids, according to the interpretation adopted by Doyle (2012).

The discovery of the new bisexual cone and its phylogenetic position in these analyses appears to support Chamberlain's (1935) hypothesis. Chamberlain proposed that the ancestor of modern seed plants possessed bisexual reproductive organs. Three lines of evidence support the existence of a bisexual ancestor for coniferphyta:

1. Teratoid bisexual conifer cones occur commonly in most conifer lineages except Sciadopityaceae, Cupressaceae and Taxaceae, and such cones almost always have the

angiosperm gender pattern: male bottom, female top (Chamberlain, 1935; Flores-Rentería et al., 2011).

2. The gnetaleans, which have been linked to conifers in recent molecular phylogeny studies (Chaw et al., 2000; Sanderson et al., 2000; Magallon and Sanderson, 2005; Qui et al., 2006), probably had an ancestor with bisexual cones (Land, 1904). The secondary fertile shoots of *Welwitschia* pollen cones have an apical sterile ovule, and in the compound pollen cones of *Gnetum*, the ultimate secondary fertile shoot is female and bears a sterile ovule. In addition, teratoid bisexual pollen cones similar to *Welwitschia* pollen cones occur in *Ephedra* (Land, 1904; Mehra, 1950).

3. Wang (2004) described a bisexual cone in adpression from the Permian of China, *Palaeognetaleana auspicia*, which he identified as a gnetalean.

The analysis of male only cones (Fig. 6), and of male and female cones including the plant lineages (Fig. 8) provide cautious support for Chamberlain's hypothesis of the early appearance of bisexual reproductive organs. In both, the cordaitean lineage with bisexual cones is sister to all other cordaiteans. In the analysis of male and female cones (Fig. 7), the cordaitean lineage with bisexual cones is sister to two clades, one composed of *Cordaianthus* seed cones and another composed of the remaining cordaitean cones. Based on these trees, I interpret the cordaitean lineage with bisexual cones as primitive relative to other cordaiteans, and *Cordaianthus penjonii*, as a closely related, possibly unisexual lineage. This interpretation carries three implications. One, angiosperms inherited the basal sterile, intermediate male, apical female patterning of their flowers from a distant (Paleozoic) ancestor as suggested in Flores-Rentería, et al. (2011)**.** Two, the seeds of ancestral cordaiteans may have been attached adaxially to the base of the apical fertile scale. Adaxially attached megasporangia also occur in the fertile branch systems of archaeopterideans (Fairon-Demaret et al., 2001), interpreted here as large compound cones. However, Doyle (2012) proposed adaxially attached seeds as a key innovation in the evolution of angiosperms (Fig. 12). Three, as previously discussed, the prepollen-pollen transition happened independently in callistophytaleans and coniferophyta. The presence of prepollen in the *Gothania – Mitrospermum* and voltzialean conifer lineages could be either primitive or a reversal.

In the analyses that support Chamberlain's hypothesis of early bisexual reproductive organs, the *Gothania - Mitrospermum* lineage appears highly derived with respect to other cordaiteans. The seeds and cones of this lineage share a number of unique characters, suggesting that they are derived with respect to other cordaiteans (Wang et al., 2003). However, the *Gothania – Mitrospermum* lineage may not be derived:

1. It appears earlier in the fossil record than either the *Cordaianthus – Cardiocarpus* lineage or the lineage with bisexual cones;
2. It has primitive male gametophytes (prepollen with as proximal germination surface as opposed to pollen with a distal germination surface);
3. The primary body of its associated stem, *Mesoxylon*, is relatively primitive compared to *Shanxioxylon* and *Pennsylvanioxylon/Cordaixylon* (Wang, 1997). Citation is Wang, Shi-jun, 1997. A study on origin and evolution of Cordaitaceae in the Late Paleozoic. Acta Phytotaxonomica Sinica 35(4): 303-310)

The analysis of whole-plant male and female cones including the plant lineages (Fig.

9B) identified the *Gothania* - *Thucyida* lineage as sister to other cordaiteans. In this analysis, the cordaitean lineage with bisexual cones forms a clade with members of the *Cordaianthus* lineage, consistent with the position of a derived lineage. The results of this analysis appear to support the hypothesis that early seed plants had unisexual reproductive organs, and imply that bisexual reproductive organs evolved independently in the new cordaitean lineage, the gnetaleans (*Welwitschia*) and angiosperms (*Archaefructus*). Incorporating additional groups into the analysis might enable me to resolve the position of the *Gothania* lineage and the new cordaitean lineage with bisexual cones, and to identify the most primitive lineage. For example, *Gothania* remains the only cordaitean lineage for which no seed cone has been described. Discovery and inclusion of *Gothania* seed cones might help to resolve its position in these analyses.

CONCLUSIONS

In this study, I purposely adopted ‘cordaitean-tinted’ glasses in identifying traits and coding character states, perhaps most radically in interpreting the fertile branch systems of archaeopteridaleans as compound cones. This largely affected the results.

The results of this study suggest two primary lineages within the cordaiteans: a *Cordaianthus shuleri, C. penjonii* - new bisexual cone clade and a *Gothania* clade. In the male cone only analysis, a *Cathayanthus* clade emerged; however, this clade joins the *Gothania* clade in subsequent analyses. No analysis yielded a clade containing all members of the *Cordaianthus – Cardiocarpus – Pennsylvanioxylon/Cordaixylon* lineage.

The clade containing the cordaitean with bisexual cones tends to emerge earlier than the other cordaitean clade, offering cautious support for Chamberlain’s hypothesis of the early appearance of bisexual reproductive organs.

The *Cathayanthus* clade (Fig. 6) incorporates Euramerican (*C. saportanus*) and Chinese (*Cathayanthus*) taxa, as do the *C. dumusum– C. tianii* and the lone *C. concinnus* seed and C. shanxiensis clades. The emergence of clades containing Chinese and Euramerican species suggests cordaitean clades had broad geographic ranges.

Inconsistent linkages between the *Cathayanthus* and *Gothania* clades may be due to shared traits correlated with reproductive ecology, in this case direct development of pollen and seeds. Two-way cluster analysis may be helpful in identifying character states that reflect common ecology rather than phylogeny (we hope).

Both *Gothania* and the voltzialean conifer, *Thucydia,* have monosaccate prepollen, and form a clade in all analyses that include both groups. However, pollen may have evolved from prepollen independently in cordaitean and conifer lineages. The inclusion of more conifer cones may help to resolve this question.

Through the analysis, the phrasing and understanding of how the cones are described changed the results to a large extent. The analyses performed here often emphasized the first tests executed (the male-cone only analysis) and this influenced the results. Trying to tease out the particular relations between characters and taxa using R revealed some level of relations, however the implications of this analysis remain unclear. A criterion to evaluate these relations could help in future analyses using purely morphological traits of small clades.

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APPENDIX 1

Character Matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Archaeopteris_halliana	0	0	0	1	0	1	0	0	0	-	0	?	?	0	-	0	1	1	0	-	?	0	0	0	0	0	1	0	0	0	?	1	0	?	0	-	-	0	-
Tanaitis_furcihasta	0	0	0	1	0	0	0	0	0	-	0	?	?	0	-	0	0	0	-	6	?	0	0	0	0	0	1	0	0	0	?	1	0	?	0	-	-	0	-
Idanothekion_callistophytoides	1	1	-	0	-	-	-	-	-	-	2	-	-	-	-	0	1	1	1	-	-	0	-	-	-	1	-	-	0	-	0	0	0	?	0	-	-	1	1
Idanothekion_glandulosum	1	1	-	0	-	-	-	-	-	-	2	-	-	-	-	0	1	1	1	-	-	0	-	-	-	1	-	-	0	-	0	0	1	?	0	-	-	1	1
Thucydia_mahoningensis	1	1	-	1	0	0	?	1	1	0	1	?	?	1	1	1	-	0	-	2	0	1	0	0	0	-	0	2	1	-	1	1	0	?	0	-	-	0	-
Thucydia seed	1	1	-	1	0	0	0	1	1	0	-	?	?	1	1	1	-	1	0	5	0	1	0	0	0	-	0	2	1	-	-	-	-	?	0	?	?	-	-
Gothania_priapi	1	1	-	1	0	1	?	1	1	1	0	1	0	0	-	1	-	0	-	3	0	1	0	0	0	-	0	1	?	-	1	1	0	1	0	-	-	0	-
Gothania_leslia	1	1	-	1	0	1	?	1	1	1	0	1	0	0	-	1	-	0	-	3	0	1	0	0	0	-	0	1	3	-	1	1	0	1	0	-	-	0	-

na																																							
Cathayanthus_ramentratus	1	1	-	1	0	0	?	1	1	1	0	0	1	1	0	1	-	0	-	0	1	1	0	0	0	-	1	2	5	-	1	1	0	?	0	-	-	1	0
Cathayanthus_sinensis	*1*	*1*	-	1	0	0	?	1	1	1	-	0	0	?	?	1	-	0	-	0	1	1	0	0	0	-	?	0	5	-	-	-	-	1	0	0	0	-	-
Cordaianthus_tianii	*1*	*1*	-	1	0	?	?	?	?	?	0	?	?	0	-	1	-	0	-	?	1	1	0	0	0	-	1	0	?	-	1	1	0	?	1	-	-	?	?
Cordai_shanxiensis	1	1	-	1	0	?	?	?	?	?	-	?	?	0	-	1	-	0	-	?	?	0	0	1	0	1	?	0	5	-	-	-	-	0	0	0	0	-	-
Cordaianthus_dumusum	*1*	*1*	-	1	0	1	1	1	1	1	0	0	1	0	-	1	-	0	-	0	1	1	0	0	0	-	1	0	2	-	1	1	0	0	1	-	-	1	0
Cordai_duquenensis	1	1	-	1	0	1	?	1	1	1	-	0	1	0	-	1	-	0	-	0	1	1	0	0	0	-	1	0	2	-	-	-	-	0	0	0	0	-	-
Cordai_concinnus_p	*1*	*1*	-	1	0	1	1	1	1	1	0	0	1	0	-	1	-	0	-	0	1	1	0	0	0	-	1	0	2	-	1	1	0	0	0	-	-	1	0
Cordaiantus_concinnus_s	*1*	*1*	-	1	0	1	1	1	1	1	-	0	1	0	-	1	-	0	-	0	1	0	0	1	0	1	1	0	2	-	1	-	0	0	0	0	0	-	-
Cordaianthus_shuleri	*1*	*?*	1	1	0	1	1	1	1	1	0	0	2	0	-	1	-	0	-	1	1	0	1	0	1	1	2	0	4	-	1	1	0	0	0	-	-	1	0
Cordaiant	*1*	*1*	-	1	0	?	*?*	?	?	?	0	?	?	0	-	1	-	0	-	0	?	0	1	0	0	1	1	0	2	-	1	1	0	0	?	-	-	1	0

hus_penjonii																																							
Cordaianthus_saportanus	*1*	*1*	-	1	0	?	?	?	?	?	0	?	?	1	0	1	-	0	-	0	?	1	0	0	0	-	1	0	2	-	1	1	0	?	?	-	-	1	0
New_bisexual_cone	*1*	*0*	1	1	0	1	1	1	1	1	6	0	2	0	-	1	-	(0,1)	0	5	1	0	1	0	1	1	2	0	4	1	1	1	0	0	0	0	0	1	0
Cordai_ximinensis	?	1	-	1	1	2	?	1	1	1	?	0	2	?	?	?	-	?	?	?	1	?	0	?	?	-	?	?	?	-	?	?	?	?	?	-	-	?	?
Cordai_Xixhanensis	?	1	-	1	0	2	?	1	1	1	-	0	?	0	-	?	-	0	?	4	1	?	0	?	?	-	?	?	?	-	-	-	-	?	?	0	0	-	-
*Welwitschia_mirabilis_p**	1	0	1	1	0	2	1	1	1	1	0	?	?	1	0	1	-	0	-	2	0	1	0	0	0	-	2	2	1	0	1	0	0	1	0	-	-	1	1
*Welwitschia_mirabilis_s**	1	1	1	1	0	2	1	1	1	1	-	?	?	1	0	1	-	0	-	2	0	1	0	0	0	-	1	2	1	-	-	-	-	1	0	2	-	-	-
Archaefructus_liaoningensis	1	0	0	1	1	-	1	-	1	2	0	-	-	0	-	1	-	(0,1)	0	5	?	1	0	0	0	-	1	0	1	1	?	2	0	?	0	?	?	1	1

*Seed (s) and pollen (p) cones divided for the different aspects of the analysis.

The '?' codes for data that could not be identified in the fossil, usually due to poor preservation. The '-' codes for a character which does not apply to the taxon.

APPENDIX 2

Characters used in the cladistic analysis

Character 1 – Heterosprous (0) Pollen & seed (1)

Character 2 – Bisexual (0) unisexual (1)

Character 3 – Complete functional bisexuality (0) vestigial bisexuality (1). Trait refers to Gnetalean state where the female ovule is considered vestigial.

Character 4 – Secondary fertile shoot absent (0) Present (1)

Character 5 – Compound (0), simple (1) cone(s)

Character 6 – Secondary (II°) axis arrangement on the primary (I°) axis: Spiral (0), 2-ranked (1), 4-ranked (2)

Character 7 – I° axis tip sterile (0), fertile (1)

Character 8 – Sections of II° axis sterile, present (0), absent (1)

Character 9 – Bract subtending fertile appendage absent (0), present (1)

Character 10 – Bract arrangement from the axis bearing it helical (0), 4-ranked (1) single at branch tip

Character 11 – Pollen sac(s) location relative to fertile scale or frond bearing it: helical (0) adaxial (1), abaxial (2)

Character 12 – I° axis pith aspect ratio large [≤0.5] (0), small [>50] (1)

Character 13 – I° axis pith body size big (0), small (1)

Character 14 – II° axis symmetry radial (0), bilateral (1)

Character 15 – II° axis bilateral symmetry type, two mirror planes (0), single mirror plane (1)

Character 16 – Sporophyll bearing propagule shape lobed multivained (0), scale (1)

Character 17 – Lobed sporophyll three dimensional (0), planar (1)

Character 18 – Fertile structure to gamete/propagule attachment Edge (0), surface (1)

Character 19 – adaxial (0), abaxial (1)

Character 20 – Fertile structure attachment configuration; 4-6 bifurcating asymmetrical ridges (0) 4-6 bifurcating symmetrical ridges (1), single sac/ovule (2), Row at primordia at tip (3) fractile triangle (4), Male apical/female adaxial (5) , sac at each lobe/tip (6)

Character 21 – Sclerenchyma basal sterile scales absent (0), present (1)

Character 22 – I° axis sterile scale above fertile present (0), absent (1)

Character 23 – Fertile scales distributed among basal sterile scales absent (0), present (1)

Character 24 – Sterile fertile scale apically intermingled absent (0), present (1)

Character 25 – Unique sterile scales ring absent (0), present (1)

Character 26 – Sterile/fertile/Sterile II° shoot present (0), absent (1)

Character 27 – Number of sterile scale morphologies: 1 type (0), 2 types (1), 3 types (2)

Character 28 –Quantity of cone scale:s many (0), few (1), very few (2)

Character 29 –Fertile organ shape: pinnule (0), short stalk scale (1), crescent ‘croissant’ scale (2), crescent thin ridgey (3), parenchymatous narrow scale (4), helmet semi-circular scale (5)

Character 30 – Difference between male/female scales in same cone absent (0), present (1)

Character 31 –Vascularized pollen bearing organ present (0), absent (1)

Character 32 –Synangium (0), single pollen sac (1), paired pollen sac (2)

Character 33 – Dehiscence parenchymatous (0), schleranchymatous (1)

Character 34 – continuous I° axis growth (0), simultaneous I° axis growth (1)

Character 35 –Epicormic/adventitious absent (0), present (1)

Character 36 – Ovule vascular bundles distribute outside the sclerotesta (0), pass through and return from the sclerotesta (1), unvascularized integument (2)

Character 37 – Ovule vascular bundles equant (0), plate-like ellipsoidal (1)

Character 38 – Pollen/microspore germinate proximal (0), distally (1)

Character 39 –Pollen tube absent (0), present (1)

APPENDIX 3.

DESCRIPTIONS OF THE NEW BISEXUAL CORDAITAN CONE FROM THE PENNSYLVANIAN KALO FORMATION OF IOWA

Anne Raymond[1], Suzanne Costanza[2] and Julian Correa[1]

[1] Dept. of Geology & Geophysics, Texas A&M University, College Station TX 77843
[2] Harvard University Herbaria, 26 Oxford Street, Cambridge, MA 02138, USA

Summary

Discovery of a permineralized bisexual cordaitean cone from the Kalo Formation (Moscovian) of Iowa indicates that two Paleozoic members of the cordaitean – conifer - gnetalean lineage were bisexual, *Palaeognetaleana* auspicia (Wang, 2004) and the new cordaitean cone. Morphological and anatomical investigations reveal the new cone as a compound cone with bisexual secondary fertile shoots, reminiscent of angiosperm flowers in their overall organization. Its secondary fertile shoots have a single seed attached to the base of the apical cone scale, which is closely surrounded by sterile scales. Below the sterile scales are male scales, with apical pollen sacs attached radially. Sterile, sclerenchymatous scales occur at the base of the cone. We identify the apically attached organ as a seed based on the presence of four nested cuticles: 1, the external epidermal cuticle of the seed's outer integument, 2, the inner epidermal cuticle of the seed endotesta, 3, the exterior cuticle of the nucellus, and 4, the membrane of the megagametophyte. Tracheids and parenchyma cells occur inside the megagametophytic membrane, indicating the presence of an embryo. Three cordaitean seed genera occur in the Kalo Formation, *Cardiocarpus*, *Mitrospermum* and *Nucellangium*; however, the venation and attachment of the developing seed suggest that the new cone bore *Nucellangium* seeds. In the new cone, the primary cone axis and organization

of the secondary fertile shoots match that of *Cordaianthus shuleri* (Darrah) emend. Fry, except that *Cordai. shuleri* had fertile male scales at the apex of the secondary fertile shoot, rather than a single fertile female scale. Thus, the *Nucellangium* plant apparently had bisexual female cones and pollen-only male cones, described as *Cordai. shuleri*.

Specimens

The new bisexual cone comes from a concretion of permineralized peat from the coal that was mined at the Williamson No. 3 Mine in Lucas Co., Iowa (Raymond et al., 2010). This locality yields a diverse cordaitean assemblage, including *Nucellangium glabrum, Mitrospermum florini, M. leeanum* (rare), *Cardiocarpus magnicellular*is and the small variety of *Cardio. spinatus*, which Brotzman (1974) informally named 'var. *macilentus*'. In Iowa, this diverse cordaitean assemblage occurs only in the Kalo Formation, which is mid-Moscovian (Pennsylvanian) in age (Raymond et al., 2010). The Kalo Fm. has two informally-named coals: the lower, the Blackoak coal, is mid-Moscovian (latest Atokan) in age; the upper, the Cliffland coal, is mid-Moscovian (earliest Desmoinesian) in age. The diverse cordaitean assemblage from the Williamson No. 3 Mine could have come from either coal.

Table 3-1 lists the specimens of the new bisexual cone, most of which come from the Thompson-Darrah coal ball collection of the Harvard Botanical Museum. The type specimen was discovered in a previously uncut concretion (HU64666),

Table 3-1 **Specimens of the New Bisexual Cone**

Specimen, Location	Locality	Description
HU64666, TAMU on loan from Harvard University	Williamson No. 3 Mine, Lucas Co., Iowa	Primary fertile shoot with the ultimate secondary fertile shoot, and lateral fertile shoots with an attached seed. Male

		scales bearing anthers.
HU40696, TAMU on loan from Harvard University	Shuler Mine, Dallas Co., Iowa	Detached secondary fertile shoot with attached seed, many male scales missing.
HU67676, TAMU on loan from Harvard University	Williamson No. 3 Mine, Lucas Co., Iowa	Secondary fertile shoot with upper sterile scales, intermediate male scales, basal sclerenchymatous scales. Seed has dispersed
HU slide 463, TAMU on loan from Harvard University	One of three Iowa mines: Williamson No. 3, Shuler, or Urbandale Mine, Polk Co., Iowa	Secondary fertile shoot with attached ovule, male scales bearing anthers.
HU slide 526, TAMU on loan from Harvard University	One of three Iowa mines: Williamson No. 3, Shuler, or Urbandale Mine, Polk Co., Iowa	Secondary fertile shot with attached seed/ovule. Male scales with remnant pollen sacs.
HU slides Bx8-1, 80-85, 2 of 6 slides at TAMU on loan from Harvard University	One of three Iowa mines: Williamson No. 3, Shuler, or Urbandale Mine, Polk Co., Iowa	Primary fertile shoot with two lateral secondary fertile shoots, one with attached seed/ovule, fertile male scales with attached pollen sacs.
UIUC8809-3, Slide 18825, U. Il Urbana-Champaign	Oskaloosa, Mahaska Co., IA, probably the Star Mine in the Kalo Formation	Primary fertile shoot with two attached lateral secondary fertile shoots, one bearing a seed and fertile male scale with pollen sacs.

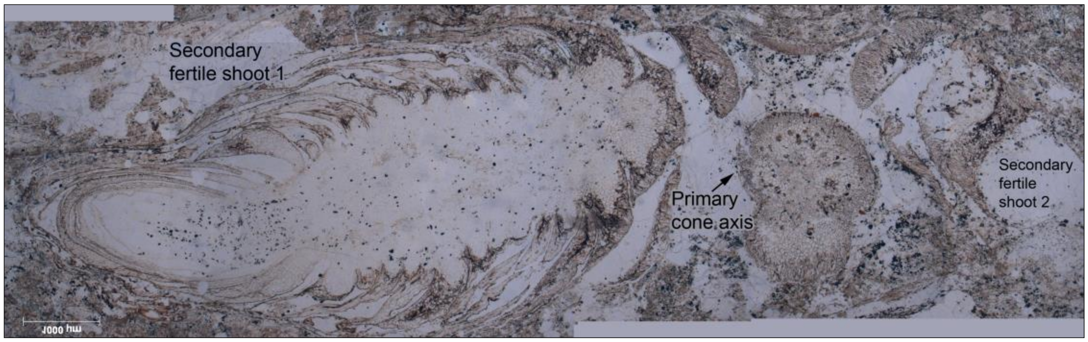


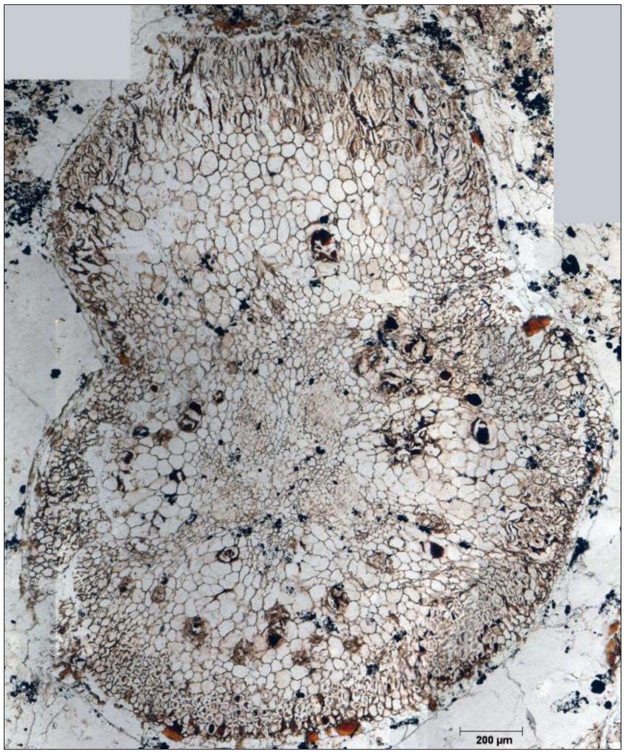
Fig. 3-1. Most informative specimen of the new bisexual cone showing two secondary fertile shoots and the tip of the primary cone axis. Secondary fertile shoot 1, on the left side of the image, has an attached apical seed. Subsequent peels show that this shoot is attached to the primary cone axis. Secondary fertile shoot 2, at the right of the image, is incomplete. HU64666, Slide 1.2

selected for a paleoecological study of the Williamson No. 3 assemblage. Part of this specimen was lost to the saw cut; however the remaining material includes the primary fertile shoot, the terminal secondary fertile shoot and one lateral secondary fertile shoot in organic attachment (Fig. 3-1). An additional specimen, consisting of a secondary fertile shoot with an apically attached seed and badly degraded male scales occurs in a previously-cut coal ball from the Shuler Mine, Dallas County, Iowa (HU 40696). Three additional specimens with attached ovules or seeds occur in slides from the Thompson-Darrah collection of the Harvard Botanical Museum. These specimens came from one of three Kalo Formation mines: the Williamson No. 3 Mine in Lucas Co., IA (either the Blackoak or Cliffland coal); the Shuler Mine (Cliffland coal) in Dallas Co., IA; or the Urbandale Mine, in Polk Co., IA (Cliffland coal). Costanza (1983, Fig. 138) pictured a specimen from the University of Illinois Urbana-Champaign (Specimen 8809-3, UIUC slide 18825, from the Star Mine, Oksaloosa, IA) in her dissertation, showing the primary fertile shoot in organic connection with two lateral secondary fertile shoots, one of which has an attached ovule. Additional specimens with dispersed ovules occur in the coal ball containing the type specimen (HU 64666) and another Williamson No. 3 coal ball (HU 67676), and a coal ball from the Shuler Mine.

Description

Cone Structure

The most informative specimen is a fragment of a compound cone that includes the primary fertile shoot, one lateral secondary fertile shoot and the terminal secondary fertile shoot. The original cut through the specimen exposed the lateral secondary fertile shoot and its attached seed in oblique longitudinal section, as well as the tip of the primary fertile shoot in transverse section and part of another, sub-opposite secondary fertile shoot (Fig. 3-1). The seed-bearing lateral secondary fertile shoot and the terminal secondary fertile shoot are in organic connection to the primary fertile shoot, revealed by repeated peeling of the original surface. The top of the terminal secondary fertile shoot lies in the upper slice of the coal ball; the upper portion of the seed-bearing lateral secondary fertile shoot was lost to the saw kerf.



The tip of the primary fertile, shown in Fig. 3-2, is about 1.7 mm in diameter, and appears to be giving off a bract toward the top. The vascular system (stele) has a distorted U-shape with a central pith, open toward the departing bract. The xylem is poorly preserved. The inner cortex is parenchymatous with abundant large, filled cells that may have been secretory. The cells of the outer cortex are smaller in diameter than those of the inner cortex, and have thick walls, suggestive of fibers. The primary fertile shoot has a thick, waxy cuticle.

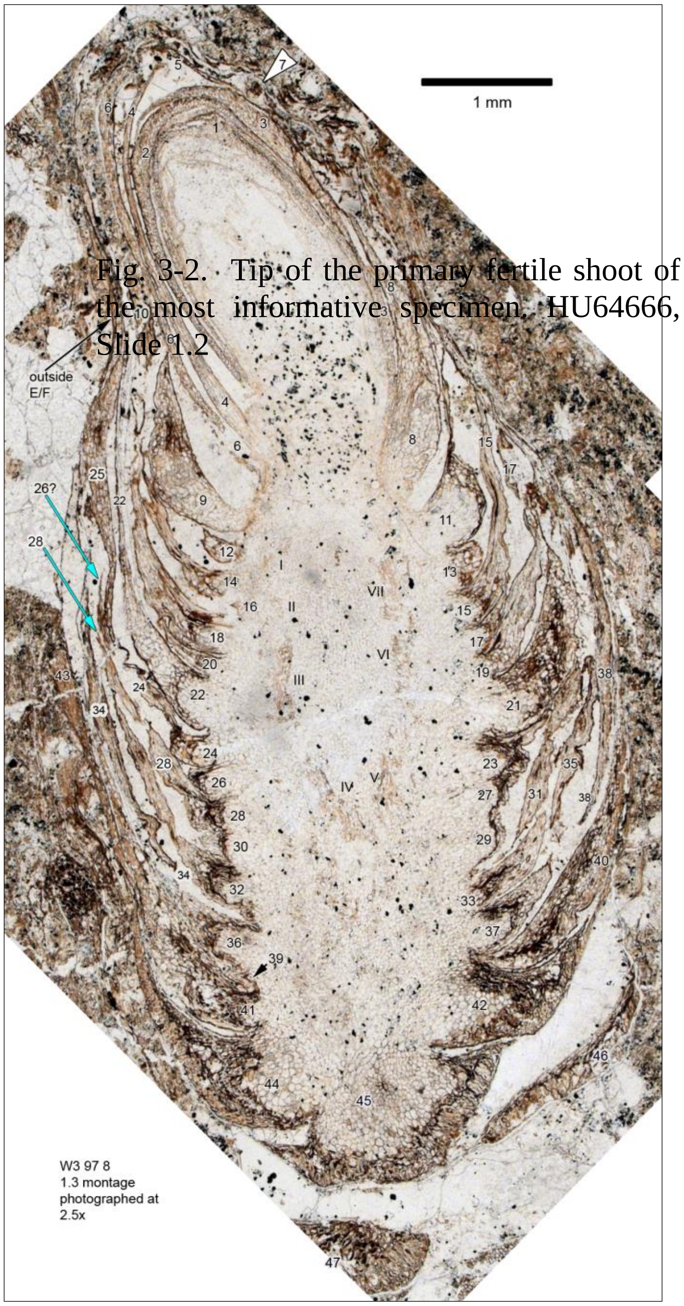


Fig. 3-2. Tip of the primary fertile shoot of the most informative specimen. HU64666, Slide 1.2

The lateral secondary fertile shoot is approximately 10 mm long and 4 mm wide. Its axis is approximately 2 mm wide and has a broad pith surrounded by seven dispersed axial bundles (Fig. 3-3). The pith is about 0.7 μm in diameter, measured at its widest point,

Fig. 3-3. Secondary fertile shoot of the new bisexual cone with attached seed. HU 64666, slide 1.3

approximately 4 mm above the most basal attached scale. The stelar tracheids range from 20 to 40 μm in diameter. In axial bundle III of Fig. 3-3, tracheids towards the axis center may have helical or scalariform wall features, while tracheids towards the axis periphery may have reticulate secondary walls (Fig. 3-4). The axial bundles do not appear to have ray parenchyma cells. Based on the presence of secondary wall thickening on radial and tangential walls and the absence of ray parenchyma, we interpret these as metaxylem following Rothwell (1982).

The new bisexual cone has approximately 47 visible, closely spaced, spirally arranged cone scales (Fig. 3-3). Based on *Cordaitanthus concinnus* ovuliferous cones, which had 25 or fewer scales in longitudinal section, and up to 92 scales in serial transverse sections (Costanza, 1985), the new ovulate cone may have 170 or more cone scales. All of the cone scales are rhombic in cross section at their attachment point and have a single vascular bundle. There are five different types of scales.

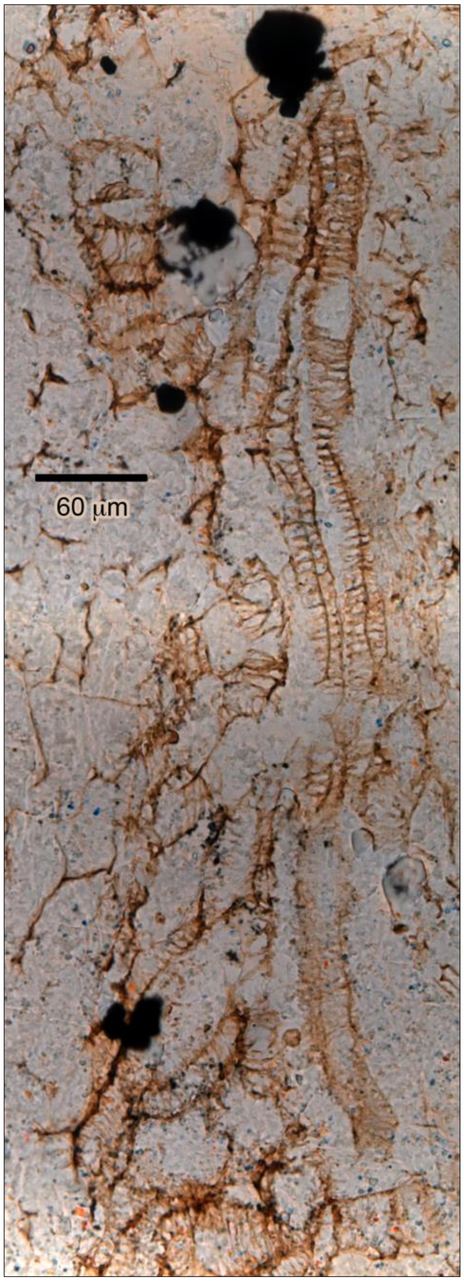


Fig. 3-4. Vascular bundle III of secondary fertile shoot, enlarged from Fig.3-3. HU64666, slide 1.4

The sterile scales occur at the base of the secondary fertile shoot, crescent shaped in transverse section and recurved in longitudinal section without a pronounced keel (Fig. 3-3, scale nos. 47 – 38). These scales, approximately 3.5 mm long and almost 1 mm thick at the base, have a thick zone of abaxial sclerenchyma (up to 400 µm thick). In distal basal scales, this abaxial sclerenchymatous zone becomes progressively thinner. Adaxially, these scales consist of thin-walled parenchyma cells and have a single vascular strand (e.g. scale 45 in Fig. 3-3). The transverse section of a mature bisexual cone that has dispersed its seed has approximately 28 basal sclerenchymatous scales (Fig. 3-5).

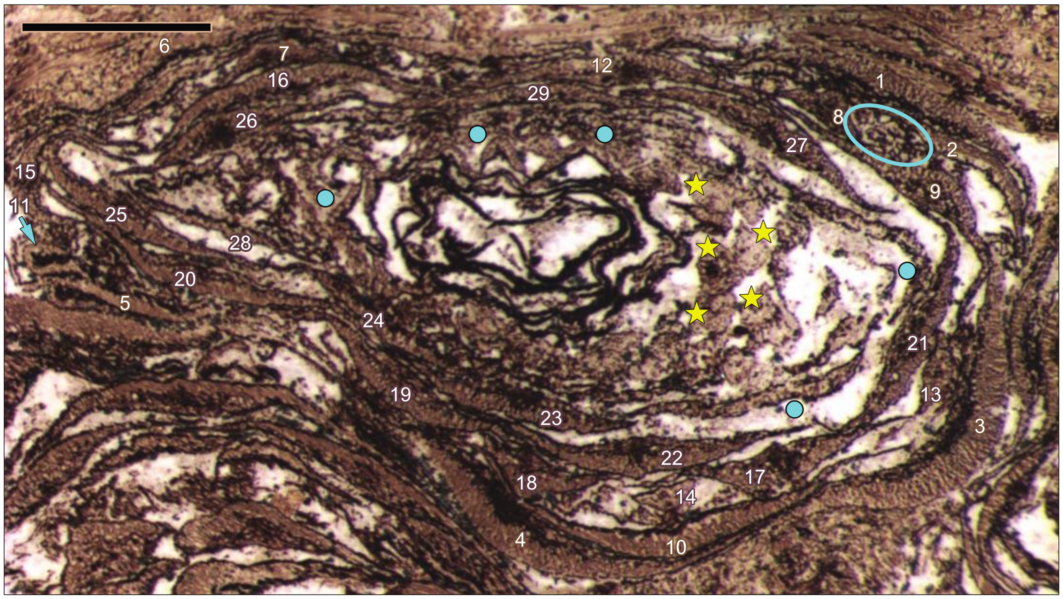


Fig. 3-5. Transverse section of mature bisexual cone that has dispersed its seed. Approximately 27 basal sclerenchymatous scales, numbered in white, surround the inner fertile scales. Blue dots mark the transitional scales between the male fertile scales and upper and lower sterile scales. Yellow stars mark the fertile male scales. In this specimen, many of these appear to have rotted. Approximately 8 or 9 upper sterile and one fertile scale? at the center of the cone would have surrounded the seed, which has probably dispersed. Scale is 1 mm. HU67676 (W3-97-19), peel.

A variable number of transitional scales occur distal to the basal sterile scales. Transitional scales are largly parenchymatous, lozenge-shaped in transverse section above the base, and about half as wide as basal sterile scales, becoming v-shaped in cross section at the tip, and are easiest to discern in transverse sections of the secondary fertile shoot (Fig. 3-4, scales 37 – 25; Fig. 3-5, turquoise dots). These scales form between zones of sterile scales and male scales; the cone in Fig. 3.5 preserves only a few of the outer set of transitional scales. Distal to the transitional scales are approximately 18 proximal male scales, parenchymatous, rhombic in transverse section, and recurved with swollen bases in longitudinal section with (Fig. 3-3, scales 24 – 7; Fig. 3-5, yellow stars). All appear fertile, bearing remnants of apically-attached pollen sacs, discussed below. Although most proximal male scales are distal to the basal sheathing scales, transverse sections of the secondary fertile shoot reveal that proximal male scales, often associated with transitional sterile scales, can occur interspersed with sterile basal sheathing scales. In Figure 3-5, the turquoise circled area between scales 1, 2, 8, and 9 contains pollen sac remnants, indicating the presence of fertile scales, and scale 14 is a transitional scale.

Approximately 14 upper sterile scales, largely parenchymatous, erect in longitudinal section, crescent shaped and possibly keeled in transverse section, occur near the tip of the cone, closely surrounding the developing seed (Fig. 3-3, scales 2 – 6). The transverse section of a senescent cone has approximately 14 blackened and shriveled upper sterile scales at the center of the cone (Fig. 3-5). The missing seed has probably dispersed. This specimen indicates that the apical scales would have been crescent shaped, and possibly keeled in transverse section. In longitudinal section, upper sterile scales have thinner bases than male scales and are erect rather than recurved (Fig. 3-3, scales 2 – 6).

Upper sterile scales have both longitudinally tabular (Fig. 3-6A, scale 4), and isodiamteric cells (Fig. 3-6A, scales 4, 6). The tabular cells occur at the center base of the scale associated with the vascular strand. The proximal male and upper sterile scales of the best specimen have scattered multicellular trichomes (hairs: Fig. 3-6A, scale 9, arrow). However, one specimen of the new bisexual cone from the Thompson-Darrah Collection has abundant multicellular trichomes on the margin and abaxial surface of the upper sterile scales (Fig. 3-6B,C). The ovule/seed attached to this specimen is small, suggesting that these trichomes on the distal sterile scales form early in cone growth and ovule development and suggesting that the trichomes on the upper sterile scales formed early in cone development

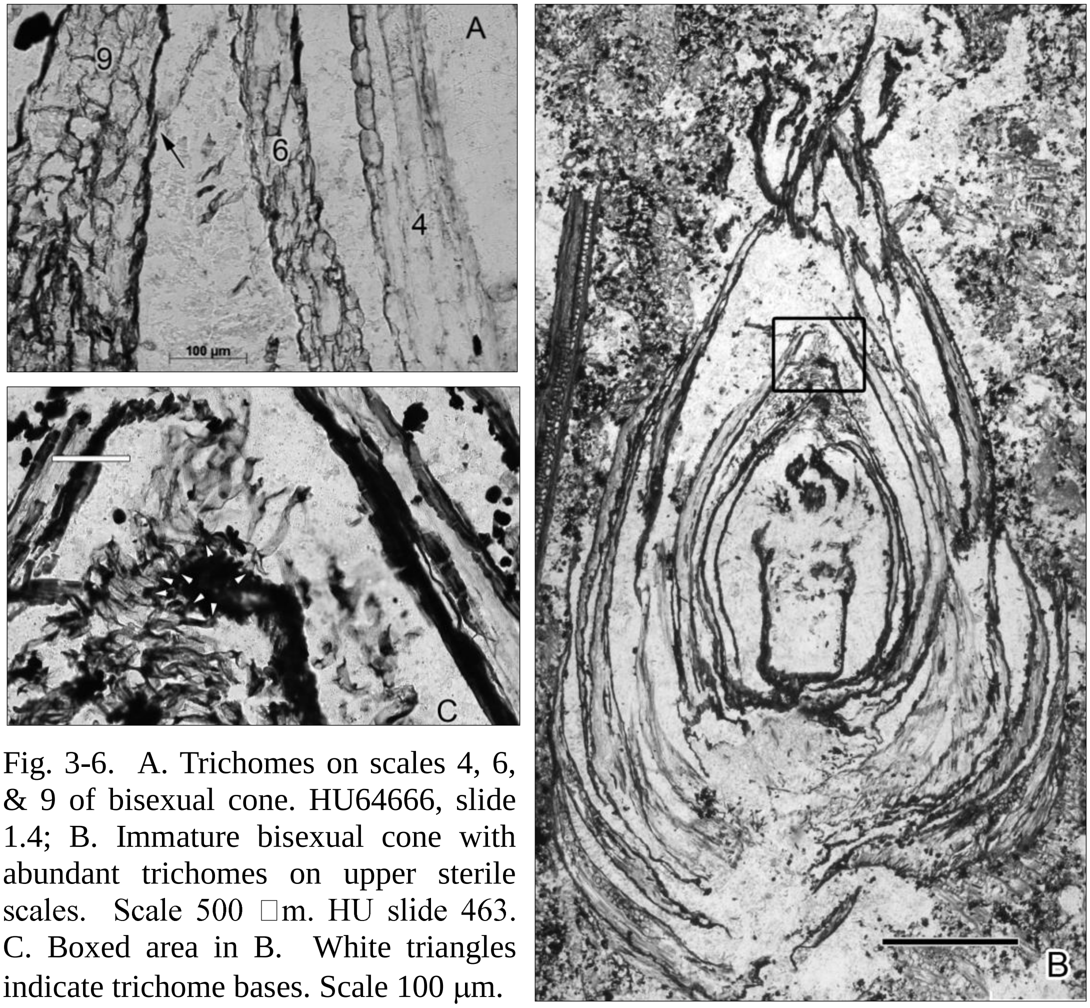


Fig. 3-6. A. Trichomes on scales 4, 6, & 9 of bisexual cone. HU64666, slide 1.4; B. Immature bisexual cone with abundant trichomes on upper sterile scales. Scale 500 □m. HU slide 463. C. Boxed area in B. White triangles indicate trichome bases. Scale 100 µm.

and disappeared when the seed became mature.

The apical, seed-bearing scale is similar in shape to the upper sterile scales and closely surrounds the basally attached, developing seed (Fig. 3-7). Although the cell walls at the junction of the fertile scale and seed are thin and difficult to trace, the developing seed, the fertile scale, and the surrounding upper sterile scales all have external epidermis and cuticle (Fig. 3-8). The pattern of nested cuticles indicates that the developing seed is in organic connection with the secondary fertile axis and the apical fertile scale. In both specimens with attached seeds, the longitudinal axis of the seed lies at an angle to the longitudinal axis of the secondary fertile shoot, which suggests that the seed is basally attached to the cone scale rather than apically attached to the tip of the secondary fertile shoot (Fig. 3-3, 3-7). Because the upper sterile scales closely surround the developing seed, we interpret the new bisexual cone as uni-ovular (Fig. 3-3, 3-7).

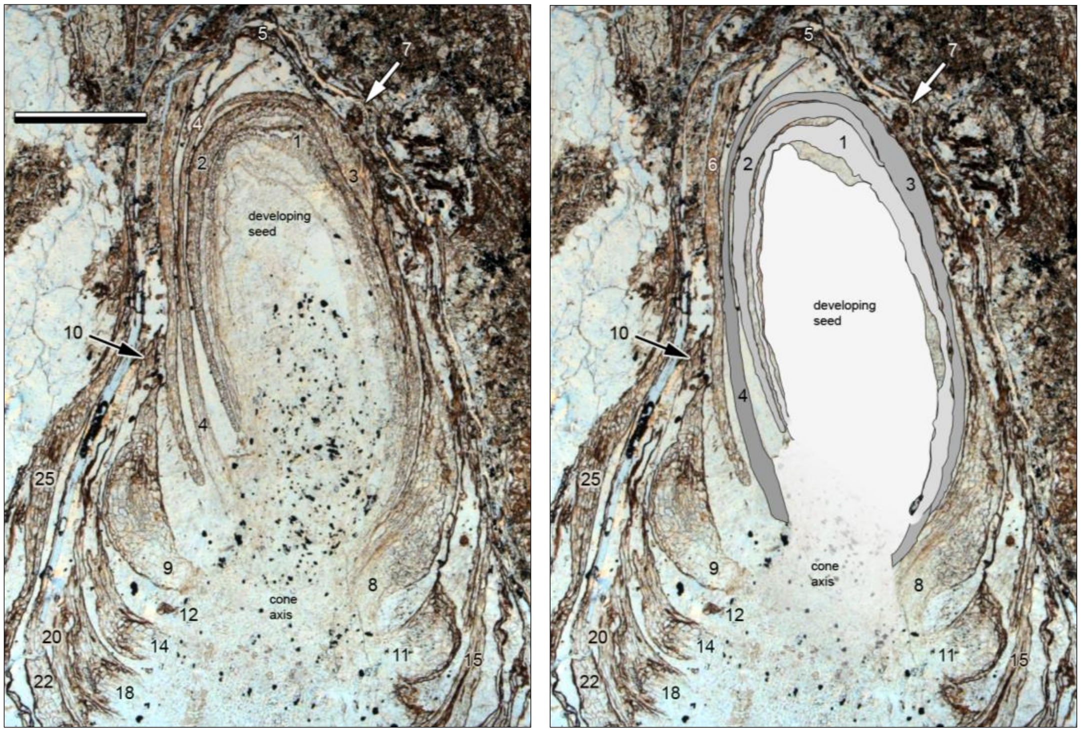


Fig. 3-7. Upper sterile scales, apical female scale and developing seed of the most informative specimen of the new bisexual cone. Scale 1 mm. HU64666. Slide 1.5.

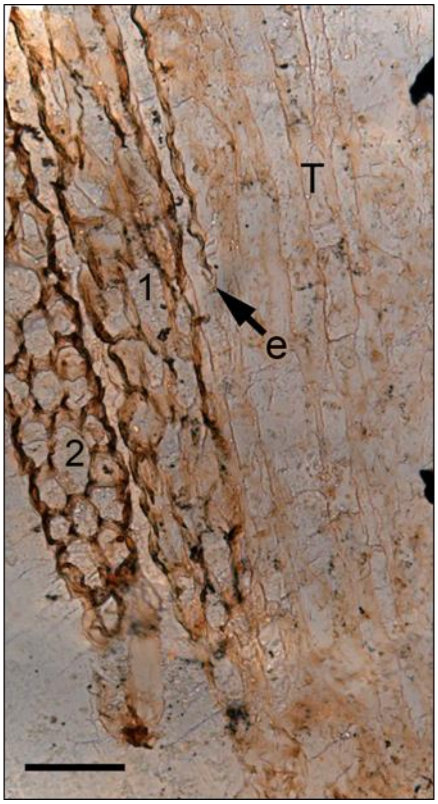


Fig. 3-8. Left side of attached seed of new bisexual cone. 1, fertile scale; 2, innermost upper sterile scale. e, outer epidermis of seed, T, tabular cells of seed integument. Scale 100 □m. HU64666, slide 1.4.

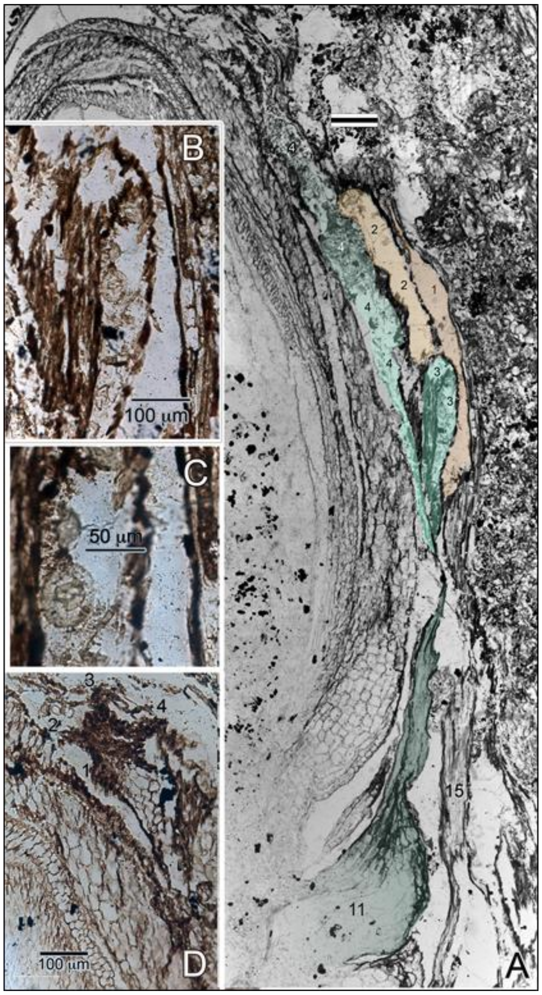


Fig. 3-9. A. Pollen sacs attached to scales 11 (sac 3 and 4, tinted green) and 15 (sacs 1 and 2, tinted orange) of the most informative specimen of the new bisexual cone. Scale 200 □m. B. Tip of sac 3 enlarged. C. *Florinites* grain in sac 3. D. Cone Scale 7 showing four radially symmetric ridges (numbered 1 -4) that will give rise to pollen sacs. HU64666, slide 1.1.

Pollen and Pollen Sacs

In the most informative specimen, most of the pollen sacs are empty and fragmented; however cone scales 11, 18 and 24 retain remnants of pollen sacs, visible in Fig. 3-3 as membranes at the tip of the scale. Scales 11 and 15 have attached pollen sacs, one of which contains *Florinites* pollen (Fig. 3-9A-C).

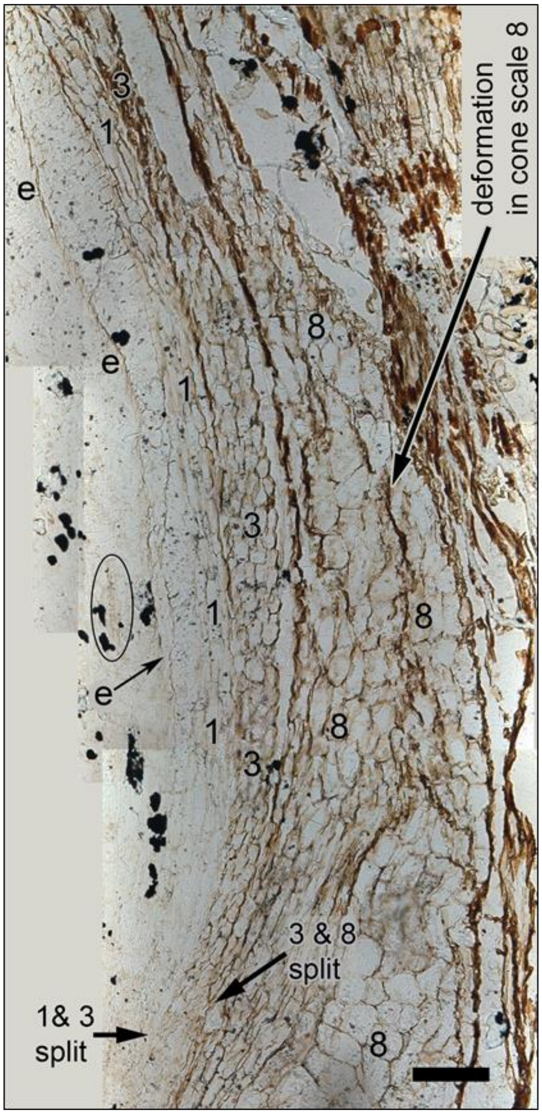


Fig. 3-10. Right side of attached seed of the new bisexual cone. Scales are numbered; 1, fertile scale; e, outer epidermis of seed. Ellipse indicates position of tracheids in the seed integument, shown enlarged in Fig. 3-11. Scale 100 μm. HU64666, slide 1.2.

The tip of cone scale 7 has four pollen sacs in organic attachment (Fig. 3-9D). In addition to empty, fragmented pollen sacs, masses of *Florinites* pollen, some of which may be enclosed in fragmented pollen sacs, occur between the rhombic fertile male scales. The most informative specimen of the new bisexual cone preserves an extraordinary amount of pollen between the cone scales, even for a cordaitean pollen cone. Despite the fragmentary condition of the pollen sacs, the amount of pollen between the cone scales suggests undispersed pollen produced *in situ*.

Seed and seed attachment.

We identify the organ attached to the apical (fertile) scale as a developing seed (approximately 2 mm long and 1.2 mm wide) based on the presence of three nested membranes and portions of a fourth, and faint cell walls and tracheids enclosed by all of these membranes (Fig. 3-10). Beginning at the attachment level of cone scales 7 and 8 and continuing into the middle of the developing seed, the cone axis and the lower half of the developing seed consist of scattered pyritized cells and occasional faint cells and portions of cell walls (Fig. 3-3, 3-7). However, the specimen shows the exterior epidermis and cuticle, tabular cells with faint cell walls, and tracheids along the sides of the lower half of the developing seed (Fig. 3-10).

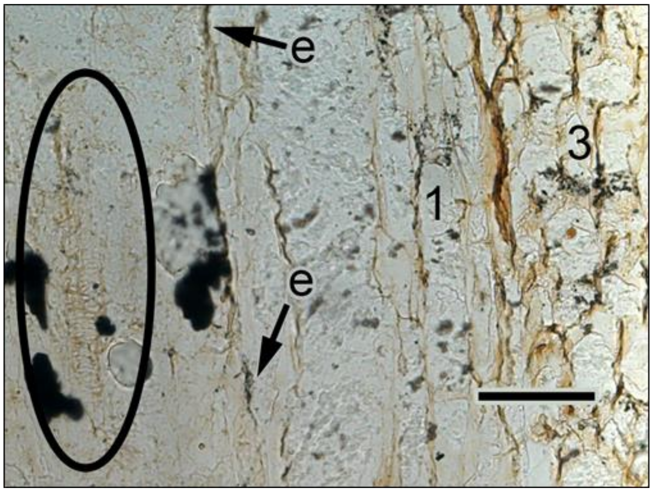


Fig. 3-11. Enlargement of region with tracheids from Fig. 3-10. 1, fertile scale; 3, cone scale 3; e, outer epidermis of developing seed. Ellipse indicates region with tracheids. Black spots are pyrite. Scale 50 m. HU64666, slide 1.3.

The exterior membrane (e in Fig. 3-8, 3-10), which we interpret as the outer epidermis of the developing seed, is first visible 100 μm above the attachment level of the fertile scale on the left and 450 μm above the attachment of scale 3 to the fertile scale on the right. At this level on the left side of the developing seed, extremely thin-walled tabular cells, similar in size and shape to the tabular parenchyma cells of the distal sterile scales, occur inside the outer epidermis of the seed, extending beyond it in the proximal direction (Fig 3-8, T).

On the right side of the developing seed, tracheids, 20 – 25 μm wide with reticulate secondary wall structure, occur inside the outer epidermis on both sides of the developing seed near the base (Fig. 3-10, 3-11). These appear to belong to the integumentary vascular bundles of the seed. On the left side, the tracheids appear above the level of the well-defined tabular cells. On the right, the tracheids lie between the outer epidermis of the seed and the middle membrane, in a region with very faint cell walls (Fig. 3-11). The presence of the integumentary vascular system on both sides of the developing seed suggests that the plane of section is close to the primary plane.

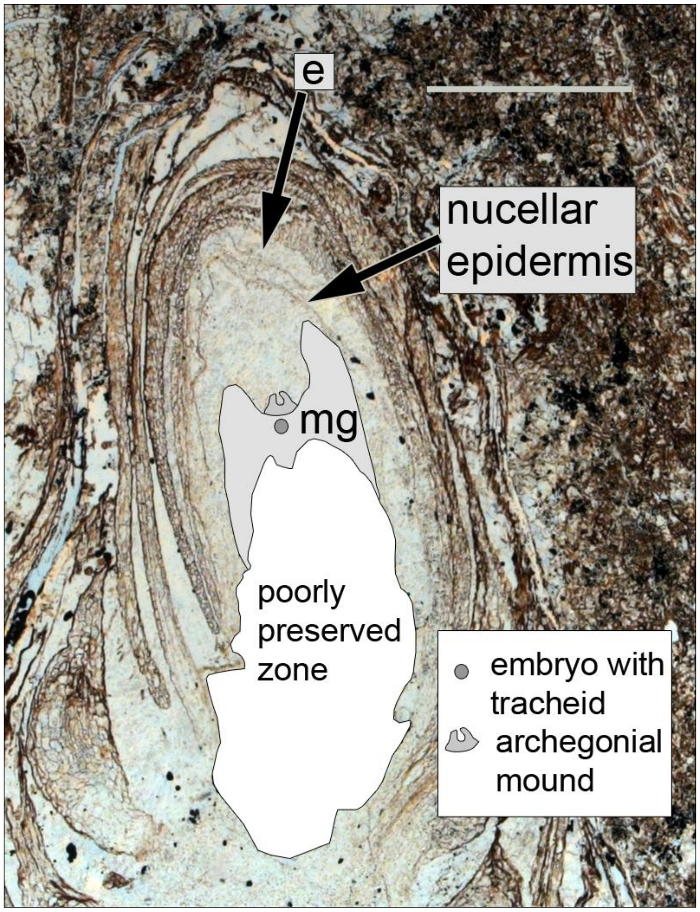


Fig. 3-12. Nested membranes of the attached seed of the new bisexual cone. e, outer epidermis of attached seed, mg, megagametophyte. Embryo and megagametophyte shown enlarged in Figs. 3-13 3-14 Scale 1 mm HU64666 slide 1.6

The seed's outer epidermis lies close to cone scale 1, especially on the right side of the specimen (Fig. 3-12). At the apex of the developing seed, it separates from cone scale 1 and narrows to form a blunt tip, which we interpret as the base of the micropylar beak (i.e. the integument surrounding the micropyle: Figs. 3-12, 3-13, 3-14). In this region, inward pointing cell walls indicate that this exterior membrane is not part of the fertile scale (cone scale 1: Fig. 3-13). Inside the outer epidermis, near the apex of the developing seed, lies a partial membrane with outward pointing cell walls, which we interpret as the inner epidermis of the endotesta of the developing seed (Fig. 3-13). The third membrane begins in the middle of the developing seed, above the attachment level of cone scale 1 (Fig. 3-12, 3-13, 3-14). We interpret this membrane, which has inward pointing cell walls throughout, as the epidermis of the nucellus. At the apex of the developing seed, it forms a blunt tip, which we interpret as the base of the nucellar beak.

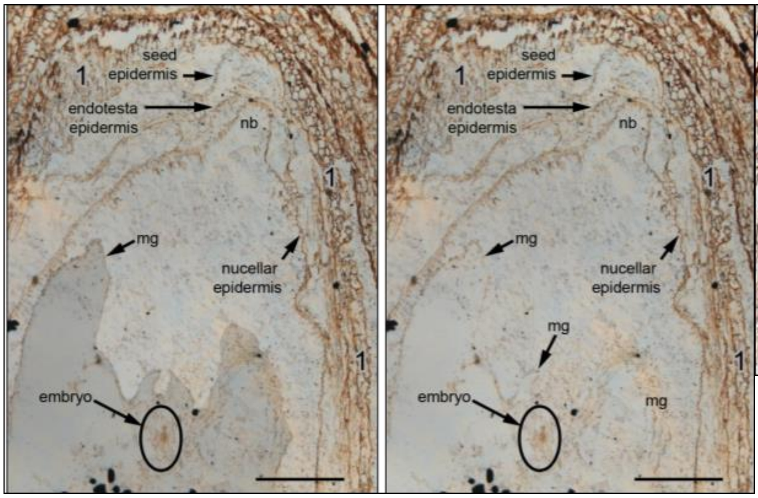


Fig. 3-13. Nested membranes in attached seed of the new bisexual cone, enlarged from Fig. 3-12. The megagametophyte is shaded in the image to the left. 1, fertile scale; mg, megagametophyte; nb, nucellar beak. Scale 200 μm. The embryo is shown enlarged in Fig. 3-14. HU64666, slide 1.6.

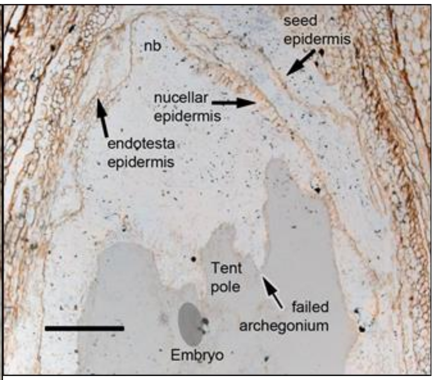


Fig. 3-14. Location of embryo, tent pole, and failed archegonial mound in the attached seed of the most informative specimen of the new bisexual cone. nb, nucellar beak. Scale 200 μm. HU64666, slide 1.4.

The interior membrane, which we interpret as the megagametophye membrane, is extremely thin and has pulled away from the middle membrane, interpreted here as the epidermis of the endotesta (Fig. 3-12 - 3-14). The megagametophyte membrane has two central lobes surrounding a semi-circular chamber. The right central lobe has the shape of a parrot's beak; the left central lobe is less distinct. Lateral to each central lobe is a larger lobe, which we consider as part of the megagametophyte. A group of cells with faint walls and narrow tracheids with reticulate secondary walls, interpreted as the embryo, occurs proximal to (below) the central lobes (Fig. 3-14). These tracheids may belong to the hypocotyl or radicle.

The configuration of the central lobes is similar to the configuration of the archegonial cavity and mound of a dispersed *Nucellangium glabrum* seed described by Stidd and Cosentino (1976). The most informative specimen (HU64666) preserves only the nucellar epidermis of the nucellus (Figs. 3-12 – 3-14). The remnant archegonial mounds (and cavities) of the megagametophytic membrane have shrunken, into the megagametophyte, leaving the central tent pole and the megagametophyte on each side to form three lobes.

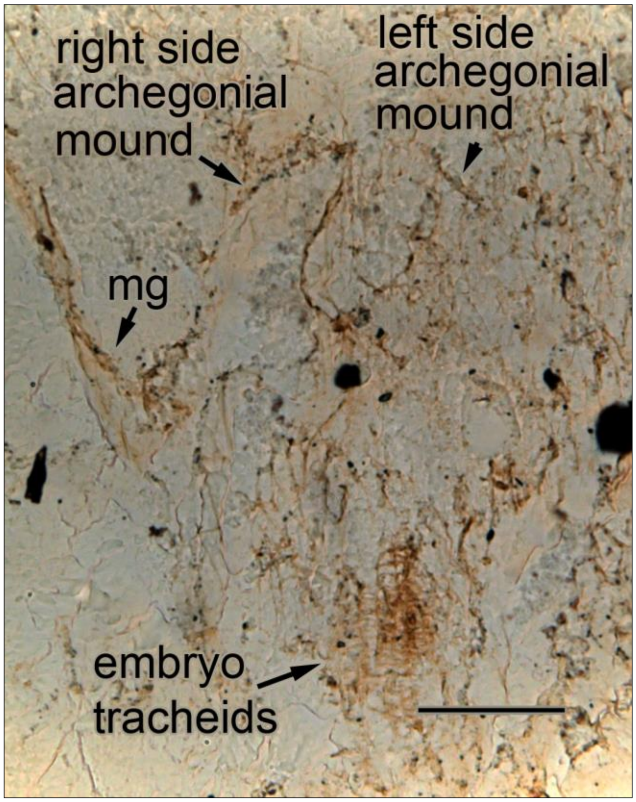


Fig. 3-15. Embryo in the attached seed of the new bisexual cone. Enlarged from Fig. 3-13. mg, megagametophytic membrane. Scale 50 μm. HU64666, slide 1.6.

Stidd and Cosentino (1976) described a 'membrane-like' substance lining the archegonial cavity of the detached seed. The archegonial cavity of the attached seed is lined with a membrane, which may account for its persistence after the formation of the embryo (Fig. 3-15). However, the archegonial cavity of the attached seed (~ 40 μm in a seed 1.2 mm wide) is considerably smaller than the cavity in the dispersed seeds of Segal (1969: ~200 μm in a seed 5.6 mm wide) or Stidd and Cosentio (1976: ~350 - 450 μm in a seed 8 mm wide).

Discussion

The new bisexual cone is cordaitean and most similar to Cordaianthus shuleri Fry

The attachment of secondary fertile shoots to the primary fertile shoot in two rows, spiral insertion of cone scales on the secondary fertile shoot, cone scale morphology, and size identify the new bisporangiate cone as cordaitean. The anatomy of its primary and secondary fertile shoots, and the organization, morphology and anatomy of its scales with the exception of the apical seed-bearing scale match that of *Cordaianthus shuleri* Fry, which was first found in a coal ball from the Shuler Mine, which exploited the Cliffland coal of the Kalo Formation (Fry, 1956; Raymond et al., 2010). *Cordaianthus shuleri* Fry also occurs in coal balls from the Urbandale Mine, which exploited the Cliffland coal of the Kalo Formation and from the Williamson No. 3 Mine in Lucas County, Iowa, which exploited one of the two Kalo Formation coals.

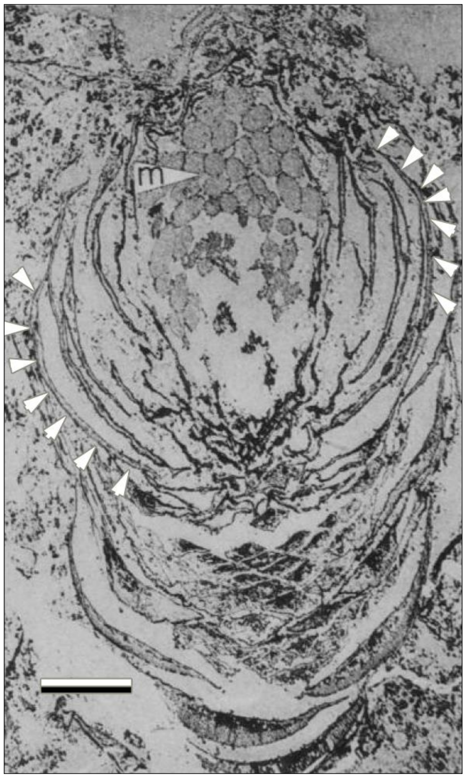


Fig. 3-16. Oblique longitudinal section of Cordai. shuleri Fry. Small white arrow heads point to upper sterile scales. Grey arrow with m points to pollen sacs. Scale 1 mm. After Fry, 1956.

Darrah (1940) described *Cordaianthus shuleri* typified by material from the Shuler Mine in Iowa. However, the type slides of *Cordai. shuleri* Darrah 1940 and of Fry's 1953 and 1956 revised *Cordai. shuleri* can not be located at the Botanical Museum of Harvard University despite efforts to locate this material, or at Cornell University (W. Crepet pers. comm.). Accordingly, in our discussion, we rely on Darrah's publications and Fry's thesis and subsequent publications, supplemented by observations of *Cordai. shuleri* material from the Thompson-Darrah collection at the Harvard Botanical Museum.

Because basal cross-sections of both cones are

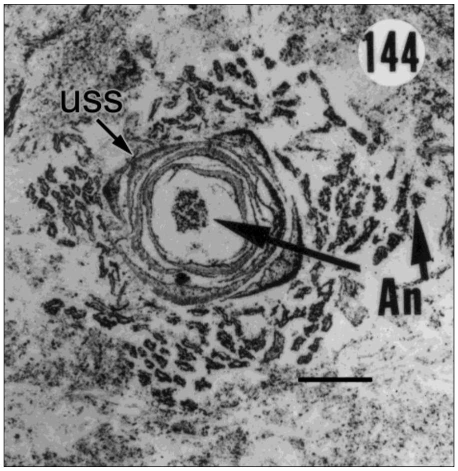


Fig. 3-16. *Cordaianthus shuleri* cone showing pollen sacs (anthers) of the proximal fertile scales, the upper sterile scales and anthers from the apical fertile scales. An, pollen sacs, uss, upper sterile scales. Scale 100 μm. UIUC18630BBot, A series, slide 18097, Star Mine, Mahaska Co., IA, after Costanza (1983).

very similar, particularly in immature cones, we originally considered that *Cordai. shuleri* might be immature bisexual cones, or bisexual cones sectioned below the tip of the secondary fertile shoot. However an oblique section of *Cordai, schuler*i (Darrah) emend. Fry through the center of the cone, originally published by Fry (1956, Plate 8, Fig. 9,) clearly shows transitional and distal sterile scales surrounding the apical male scales (Fig. 3-15, small white arrows). Fry (1956) did not recognize the distinct shape or significance of these scales. Similarly, Costanza (1983) figured a secondary fertile shoot of *Cordai. shuleri* that clearly shows anthers from the proximal fertile scales and a ring of upper sterile scales surrounding the apical fertile male scales. These crescent-shaped upper sterile scales have not been described in other cordaitean cones and strongly support the hypothesis that *Cordai. shuleri* cones are the pollen cones of the plant that produced the new bisexual cone. The new cordiatean with bisexual cones probably had compound pollen-only male cones and compound bisexual female cones, with bisexual secondary fertile shoots.

The new bisexual cone differs from other ovulate cordaitean cones

The female cone of *Mitrospermum* has not been described. All *Cardiocarpus* seed cones bore multiple ovules or seeds apically attached to parenchymatous cone scales, which were rhombic or helmet-shaped in transverse section (Rothwell, 1982; Trivett and Rothwell, 1985; Rothwell, 1993; Costanza, 1985, Hilton et al. 2009a). In contrast, the new bisexual cone bore a single seed basally attached to the apical cone scale, closely surrounded by crescent shaped, parenchymatous upper sterile scales. Further, the ovules of most *Cardiocarpus* cones were probably exerted (i.e. borne on long cone scales that extended beyond the tips of the sterile scales) at maturity, with the exception of *Cardio. samaratus – Cathayanthus sinensis* (Wang et al., 2003). In contrast, the seed of the new bisexual cone was basally attached to the apical cone scale and closely surrounded by the upper sterile scales throughout its development.

Ignatiev and Meyen (1989) placed *Cordaitanthus duquesnensis*, the ovulate cone associated with the *Cardiocarpus oviformis* plant, in *Rothwelliconus*, but excluded the ovulate cone of *Cardio. spinatus* because one had epicormic pollen cones (the *Cardio. oviformis* plant) and the other had axillary pollen and seed cones (the *Cardio. spinatus* plant). Nonetheless, these two North American *Cardiocarpus* appear to form a natural group that includes *Cordai. shanxiensis – Cardio. tuberculatus*. All three have similar fertile scales: parenchymatous, helmet-shaped at the base, with four wings at the tip (Rothwell, 1982; Costanza, 1985; Hilton et al., 2009a). *Cordai. shanxiensis* and *Cordai. concinnus* seed cones share the trait of having sterile scales intermixed with fertile scales along the axis of the secondary fertile shoot (Costanza, 1983, 1985; Hilton et al., 2009a). All three plants have similar male cones, leaves, and stems. The stems of *Cordai. concinnus* and *Cordai. tianii/Cordai. shanxiensis* have been placed in *Cordaixylon*, whereas the stem of *C. spinatus* has been placed in *Pennsylvanioxylon* (Costanza, 1985, Rothwell, 1993). However, these two stem genera are very similar and probably represent the same taxon.

The seed and pollen cone of the *Cardiocarpus magnicellularis* plant have not been described; yet Brotzman (1974) noted the strong similarity of *Cardio. magnicellularis* and

Cardio. spinatus seeds. For example, the outer sarcotesta of both species consists of thin-walled, mucilaginous or resinous cells (Brotzman, 1974). *Cardio. magnicellularis* generally co-occurs with *Cardio. spinatus* (except in the Upper Grassy Creek Coal of Cross Mountain, TN: Raymond et al., 2010), and cones of the two species may be difficult to distinguish.

Based on their cladistic analysis of cordaitean ovules, Hilton et al. (2003) suggested that *Cardiocarpus* was not a natural group. Two different genera of ovulate cones from the Permian of China bore *Cardiocarpus* seeds. As discussed above, the ovulate cones of the *Cardio. tuberculatus* plant (*Cordaianthus shanxiensis*) are similar to those of *Cardio spinatus (Cordai. concinnus* seed cones: Costanza, 1983, 1985; Hilton et al., 2009a). Two other *Cardiocarpus* species from the Permian of China, *Cardio. samaratus* and *Cardio. taiyuanensis,* appear to belong to *Shanxioxylon* stems (Wang et al., 2009; Hilton et al., 2009b). The *Cardio. samaratus* plant had *Cathayanthus sinensis* seed cones with spiral attachment of secondary fertile shoots to the primary fertile shoot (Wang et al., 2009). The *Cardio. taiyuanensis* plant bore *Cordaianthus xishanensis* seed cones, with tetrastichous (in four rows) attachment of the secondary fertile shoots to the primary fertile shoot (Hilton et al., 2009b). In both cones, the secondary fertile shoots had multiple fertile scales, which produced a single, seed attached to the tip of the fertile scale, similar to *Cordaianthus* seed cones (Wang et al., 2009; Hilton et al., 2009b). This seed attachment differs from that of the new bisexual cone, which had only one female fertile scale and produced a single seed, adaxially attached to the base of the fertile scale.

Cordaitean seed cones appear to be quite rare in the permineralized record. Rothwell (1982) based his description of *Cordaianthus dumsum* on four detached secondary fertile shoots; subsequently, he discovered two additional specimens, one of which preserved the primary cone axis (Rothwell, 1993). Costanza (1983, 1985) based her description of *Cordaianthus concinnus* seed cones on two compound seed cones. Hilton et al. (2009a) described *Cordai. shanxiensis* seed cones based on two isolated secondary fertile shoots, neither of which shows the base of the specimen. Wang et al. (2003) reported 15 secondary fertile shoots of *Cathayanthus sinensis*, and only one primary cone axis. Hilton et al. (2009b) apparently based their description of *Cordai. xishanensis* on one cone consisting of a primary cone axis and at least three attached secondary fertile shoots. We base our description of the new bisexual cordaitean cone on three cones that include the primary cone axis, each with two attached secondary fertile shoots, three additional detached secondary fertile shoots with attached ovules/seeds, and two secondary fertile shoots that have dispersed their seeds. The scarcity of cordaitean seed cones in coal balls, especially cones that preserve secondary fertile shoots in organic attachment to the primary cone axis (eight total specimens, spread over five species, with one species known only from secondary fertile shoots), contributes to the difficulty of discovering synapomorphic characters for *Cordaianthus* seed cones.

The new bisexual cone differs from other cordaitean pollen cones

The new bisexual cone differs from all other cordaitean pollen cones in having both fertile male and a single fertile seed scale (Table 3-2). As discussed above, it is most similar to *Cordainthus shuleri*, which is the only other cordaitean cone to have a zone of upper sterile scales, and which may be its pollen cone.

Aside from its bisexuality and having a zone of upper sterile scales, the new cone differs from the remaining *Cordaianthus* cones (*Cordai. concinnus,* Cordai. dumusum, *Cordai. penjonii*, *Cordai. saportanus* and *Cordai. tianii*) in having a radially symmetric ring of four to six pollen sacs, attached to the tip of the fertile male scales. In *Cordaianthus* species other than *Cordai. shuleri*, the pollen sacs form a bilaterally symmetric array (Table 3-2), although we note that the configuration of pollen sacs in *Cordai. tianii* remains unknown (Hilton et al., 2009a).

The new bisexual cone differs from all *Cathayanthus* cones in the attachment of the secondary fertile shoots to the primary fertile shoot: spiral or tetrastichous in *Cathayanthus*; opposite or sub-opposite in the new bisexual cone (Wang et al., 2003; Hilton et al., 2009b). The configuration of pollen sacs in *Cathayanthus* species is poorly known (Wang et al., 2003; Hilton et al., 2009b). The tip of fertile scales belonging to *Cathay. xishanensis*, the seed cone of *Cathay. ximinensis*, have a distinctive triangular shape with well-developed ridges, which seems distinct from the bilaterally symmetric ridges that form at the tip of *Cordaianthus* pollen and seed cones (Florin, 1951; Rothwell, 1982, 1993; Costanza, 1983, 1985). However, the fertile scale tips of *Cathay. ramentrarus* pollen and *Cathay. sinensis* seed cones are less distinctive, and may be similar to the bilaterally symmetric ridged scales seen in most *Cordaianthus* cones (with the exception of *Cordai. shuleri*).

Table 3-2. Comparison of the new bisexual cone to other cordaitean pollen cones.

Cone (source)	Pollen sac configuration	Shape fertile scale tip	Number cone scales*	Upper sterile scales	Bisexual
New bisexual cone (this thesis)	Radially symmetric array of 4-5 sacs	Rhombic	~47[2], 18 male 1 seed	Yes ~14[1]	Yes
Cordaianthus shuleri (Fry, 1956; this thesis)	Radially symmetric array of 4-5 sacs	Rhombic	95[4], many fertile	Yes ~12[1]	No

Cordai. concinnus pollen (Costanza 1983, 1985)	Bilaterally symmetric array of 4-6 sacs	Bilaterally symmetric, 4-5 ridges	~52[3], 20 fertile	No	No
Cordai. dumusum (Rothwell, 1993)	Bilaterally symmetric array of 4-6 sacs	Bilaterally symmetric, 4-5 ridges	55-65[4], 8-12 fertile	No	No

Table 2 cont.

Cone (source)	Pollen sac configuration	Shape fertile scale tip	Number cone scales*	Upper sterile scales	Bisexual
Cordai. tianii (Hilton et al., 2009a)	?	?	>40[4] >25-30 sterile 10-15 fertile	No	No
Cordai. penjonii (Florin, 1951)	Bilaterally symmetric array of 4-6 sacs	Bilaterally symmetric, 4-5 ridges	~80[1], ~31 fertile	No	No
Cordai. saportanus (Florin, 1951)	Bilaterally symmetric array of 4-6 sacs	Bilaterally symmetric, 4-5 ridges	~44[1], 10 fertile	No	No
Cathayanthus ramentrarus (Wang et al., 2003)	?	Bilaterally symmetric, triangular with abaxial ridge	14-18[4], 3-5 fertile	No	No
Cathay. ximinensis (Hilton et al., 2009b)	?	?	20-30 sterile scales[4], ? fertile	No	No
Gothania lesliana (Daghlian and Taylor, 1979)	Row of 4	Thin crescent	~25[4], 10 fertile	No	No

G. priapi (Trivett and Rothwell, 1985)	Row of 4	‘Narrow’	~28[4], 5 fertile	No	No

* visible in transverse[1], longitudinal section[2], serial transverse section[3], cited in source[4]

Finally, the attachment of pollen sacs in the new bisexual cone differs from that seen in *Gothania* pollen cones, produced on the plant that bore *Mitrospermum* seeds. *Gothania* cones have a row of pollen sacs at the tip of the fertile scale; the new bisexual cone has a ring of pollen sacs on the tip of the fertile scale (Table 3-2). .

The seed attached to the new bisexual cordaitean cone

The new bisexual cone comes from the diverse cordaitean assemblage of the Kalo Formation of Iowa (middle Moscovian, latest Atokan – earliest Desmoinesian, which includes five cordaitean ovule species: *Cardiocarpus spinatus* (informal variety ‘*macilentus*’ of Brotzman, 1974), *C. magnicellularis*, *Mitrospermum florini*, *Mitrospermum leanum* and *Nucellangium glabrum* (Raymond et al., 2010). We associate the new ovulate cone with *Nucellangium glabrum* based on the following criteria: 1) The inner epidermis of the endotesta is strongly developed considering that the specimen is a developing seed. Stidd and Cosentino (1976) noted that the endotesta of immature *Nucellangium* ovules and seeds has a well-developed inner epidermis, which Brotzman (1974, her Fig. 198) figured, but did not discuss. In other cordaitean ovules from the diverse cordaitean assemblage (*Mitrospermum florini, M. leeanum, Cardiocarpus spinatu*s informal variety macilentus, and *C. magnicellularis*), the inner epidermis of the endotesta is relatively thin (Brotzman, 1974). 2)

The shape of the central lobes is consistent with the shape of the archegonial cavity and mound of *Nucellangium glabrum*. Stidd and Cosentino (1976) and Brotzman (1974) noted a 'membrane-like substance' lining the archegonial cavity in *Nucelluangium glabrum*, which may account for the persistence of the archegonial cavity and mound in this developing seed. 3) Although not fully developed, the inward pointing cell walls of the exterior cuticle are consistent with the radial palisade found on the exterior of *Nucellangium glabrum*. Brotzman (1974) suggested that this radial palisade developed relatively late in the ontogeny of *Nucellangium*, as the ovules/seeds came close to reaching their final size.

The presence of two integumentary vascular bundles in the developing seed is consistent with *Nucellangium glabrum, Cardiocarpus spinatus* var. macilentus, or *C. magnicellularis*, all of which have two integumentary vascular bundles. *Mitrospermum leeanum* and *M. florini* have tracheal plates that extend into the integumentary wings of the ovule (Brotzman, 1974), although early in development *Mitrospermum* may have had lateral vascular bundles instead of the tracheal plates.

As discussed above, the development of pollen sacs from radially symmetric ridges, at the tip of the male cone scale is unlike that of both *Cardiocarpus – Cordaianthus* cones and *Mitrospermum – Gothania* cones. In Cardiocarpus – Cordaianthus cones, the pollen sacs developed from the bifid tip of the fertile scale, or from bilaterally symmetric ridges of the unbranched fertile scale, such that they formed a bilaterally symmetric array of pollen sacs. In *Mitrospermum* - *Gothania* cones, a row of pollen sacs developed on tip or the upper margin of the fertile cone scale.

Implications for the reproductive biology of cordaiteans

Modern conifer embryos do not have tracheids and a key conifer innovation may be delayed development of seeds in cones (Mapes et al., 1989). Rothwell (1982) suggested that cordaitean ovules developed directly, consistent with the presence of tracheids in the embryo of the developing *Nucellangium* seed attached to the new bisexual cone. Stidd and Cosentino (1976) pictured a ‘proliferated’ (fungally attacked) *Nucellangium* ovule with a v-shaped vascular stand of radially aligned tracheids, indicating that infected *Nucellangium glabrum* ovules could form trachieds.

Mature *Nucellangium glabrum* ovules and seeds are noted for having a wide range of sizes, from 8 - 15 mm long, from 7.2 – 12.7 mm wide in the primary plane, and from 5.5 – 7.9 mm wide in the secondary plane, with an average size of approximately 12 mm x 10 mm x 5 mm (Brotzman, 1974; Segal, 1969). The developing seed described here is one fourth the length of the smallest mature *Nucellangium* and one sixth the length of the average mature *Nucellangium.* This seed is smaller than the other, dispersed *Nucellangium* known to have archegonia, which are 9-10 mm in length (Segal, 1969; Stidd and Cosentino, 1976). Nonetheless, it is considerably larger than the largest known attached *Cardiocarpus* ovules: the largest ovule attached to a *C. duquenensis* cone is approximately 320 μm long; the largest ovule attached to a *C. concinnus* cone is approximately 145 μm; (Rothwell, 1982; Costanza, 1985). Rothwell (1982) noted a large size range in mature *Callospermarion* (callistophytalean) ovules.

Because it has tracheids and yet lacks the semi-sclerotic layer, we assume that this developing seed died before lignification of the semi-sclerotic layer. Similarly, although the radial palisade of the exterior epidermis has begun to develop, the radial palisade cells did not develop completely before the seed became permineralized. The specimen also lacks the secretory cells scattered throughout the parenchymatous outer integument found in dispersed, immature *Nucellangium*. Had these cells been present prior to permineralization, we would expect to see them.

Evolution of bisexual reproductive organs in land plants

The organization of the new bisexual cordaitan cone is similar to the organization of angiosperm flowers in having sterile basal scales (tepals, sepals, petals), intermediate fertile male scales (stamens), and an apical fertile female scale (carpel), which closely surrounds an adaxially attached seed. However, angiosperm flowers have no sterile organs separating the fertile male from the fertile female scales in the position of the upper sterile scales in the new bisexual cordaitean cone. Except for the ring of upper sterile scales, the new bisexual cordaitean cone shares similar sterile-fertile patterning (i.e. sterile base, intermediate male, upper female) with angiosperm flowers and bisexual bennettitalean cones (Coen and Meyerowitz, 1991; Crane and Herendeen, 2009; Rothwell et al., 2009; Rudall and Bateman, 2010). It shares similar fertile patterning (i.e. male bottom, female top) with nearly all teratoid conifer cones (Chamberlain, 1935; Flores-Rentería et al., 2011, Rudall et al. 2011) and with the compound pollen cones of *Welwitschia* and *Ephedra* pollen as interpreted by (Hufford et al., 1996; Mundry and Stützel, 2004).

In angiosperms, ABCE floral patterning, controlled by the A-, B-, C- and E-gene families, determines the sterile-fertile patterning of flowers (Coen and Meyerowitz, 1991; Theissen and Melzer, 2007). Similarities in the sterile-fertile patterning of bisexual reproductive organs from widely separated plant lineages (i.e. the cordaitean-conifer-gnetalean lineage, the bennettitaleans and the angiosperms) suggest that angiosperms inherited ABCE floral patterning from a distant ancestor.

Within the conifer-gnetalean-cordaitean lineage, bisexual cones occur in both cordaiteans (this thesis) and gnetaleans (*Palaeognetaleana auspicia* from the Late Permian of North China (Wang, 2004). Coupled with the widespread occurrence of teratoid bisexual cones in many families of conifers (Chamberlain, 1935; Flores-Rentería et al., 2011; Rudall et al., 2011), these fossils support the phylogenetic connection between conifers, cordaiteans and gnetaleans (Bowe et al., 2000; Chamberlain, 1935; Chaw et al., 2000; Florin, 1951; Eames, 1952; Rothwell and Stockey, 2013).

Bisexual reproductive organs occur in three land-plant lineages: the cordaitean-conifer-gnetalean lineage; bennettitaleans; and angiosperms. Following molecular phylogenies that link gnetaleans with conifers, not cycads or angiosperms, Doyle (2012) placed angiosperms with bennettitaleans and glossopterids, in a separate lineage from cordaiteans and conifers. If early seed plants had unisexual reproductive organs, this tree suggests that bisexual reproductive organs arose independently within the two lineages. A number of hypotheses have been proposed to explain the evolution of bisexual reproductive organs from unisexual organs: out of the female, out of the male, mostly male and shifting boundaries (Theissen et al., 2002; Theissen and Melzer, 2007; Frohlich and Parker, 2009).

Conversely, Chamberlain (1935) suggested that early seed plants had bisexual reproductive organs. He attributed the great diversity of unisexual reproductive organs in fossil and living gymnosperm groups to the independent evolution of unisexual reproductive organs in different wind pollinated lineages, hypothesizing that bisexual wind-pollinated plants would have experienced strong selectional pressure favoring unisexual reproductive organs to foster out-crossing and limit self-pollination (Chamberlain, 1935).

The new cordaitean lineage with bisexual seed cones appears to have had unisexual pollen cones. If the ancestor of this lineage had unisexual cones, the combination of bisexual seed cones and unisexual pollen cones appears to support the ‘out of the female’ hypothesis for the evolution of bisexual reproductive organs within the cordaiteans (Theissen et al., 2002). In this evolutionary scenario, the pollen cones of the new bisexual cordaitean would be primitive relative the derived, bisexual seed cones. If the ancestor of this lineage had bisexual cones, the combination of unisexual pollen cones and bisexual seed cones would support Chamberlain’s (1935) hypothesis that gymnosperms derived from ancestors with bisexual reproductive organs. In this evolutionary scenario, the pollen cones of the new bisexual cone would be derived related to its primitive bisexual seed cone.

Gnetaleans, the other ‘bisexual’ member of the conifer-cordaitean-gnetalean lineage, includes some species that have unisexual seed cones and pollen cones with sterile seeds, interpreted as secondary fertile shoots by Chamberlain, 1935; Hufford, 1996) and as compound cones by Mundry and Stützel (2004). This condition might support the ‘mostly male’ (Frolich and Parker, 2000) or ‘out of the male’ (Theissen et al., 2002) hypotheses for the evolution of bisexual reproductive organs in land plants. However, if this group had an ancestor with fully bisexual cones, as suggested by Wang (2004), unisexual reproductive organs are derived within the group. The evolutionary trajectory hypothesized by Wang (2004) for gnetaleans, is consistent with Chamberlain’s (1935) hypothesis that wind pollinated lineages with bisexual cones tend to become unisexual.

Conclusions

The Pennsylvanian cordaitean that bore *Nucellangium glabrum* seeds and *Cordaianthus shuleri* pollen cones has bisexual seed cones. The presence of tracheids within the attached seed suggests that *Nucellangium* seeds developed directly. The reproductive patterning (sterile-fertile and male-female) of the new bisexual cordaitean cone is very similar to that of angiosperm flowers, and bennettitalean bisexual cones. Its male-female patterning is similar to that of most teratoid bisexual conifer cones and the bisexual pollen cones of some gnetaleans. The presence of similar reproductive patterning in these widely separated land-plant lineages suggests that angiosperms inherited the genes that control their floral patterning from a distant ancestor. The occurrence of bisexual reproductive organs in the conifer-gnetalean-cordaitean lineage, in bennettitaleans, and in angiosperms, suggests that bisexual reproductive organs arose independently in the conifer-gnetalean-cordaitean lineage and in angiosperms and bennettitaleans following Doyle (2012), if not in all three lineages. Conversely, the occurrence of bisexual reproductive organs in these three lineages may support the hypothesis of Chamberlain (1935) and Flores-Renterìa et al., (2011) that early gymnosperms had bisexual reproductive organs.

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APPENDIX 4

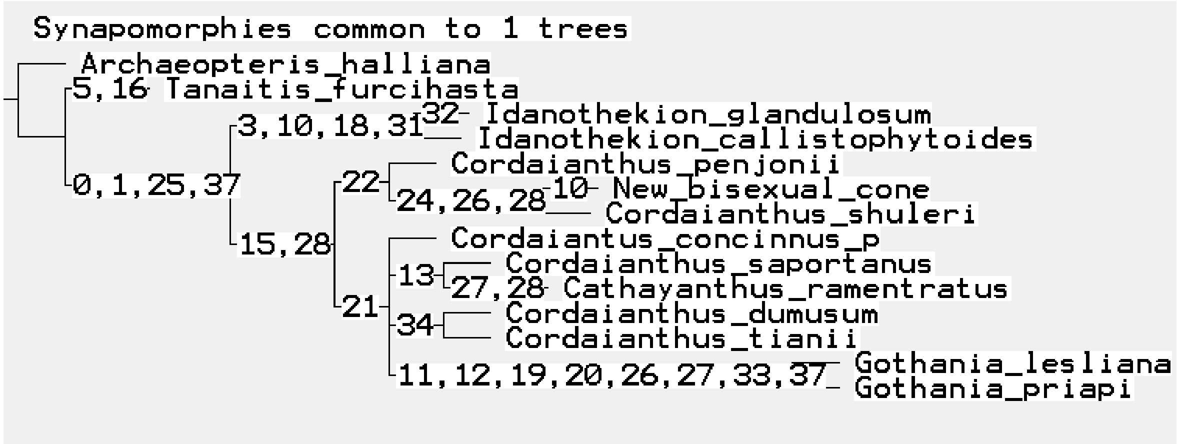
Of the 33 non-constant traits for the male-only cone analysis, I chose 3 groups of traits using both TNT, which lists several synapomorphies (APP4. 1), and knowledge of paleobotany. The first groups concerns the patterning observed in the secondary axis (traits 23-26, APPENDIX 2). These traits appear early in the cordaitean node and follow through into the *C. shuleri* lineage. They are particularly interesting as the patterning resembles that of flowers – some form of sterile material (scales – sepal/tepals) that lead of into fertile (male) material. This similarity did not go unnoticed in early observations of cordaiteans (Harris, 1947) and the pattern appears more obvious when compared to the new bisexual cone which adds the final step to the flower, an apical female gamete. The cordaites themselves have multiple arrangements further divided by the different types of sterile scale morphologies observed (27). They show up as a few synapomorphies in the analysis (APP4. 1), but failed to resolve the greater cordaitean clade from the analysis (Fig. 6, App. 5 20-23). The traits are highly consistent (APPENDIX 6).

The second group of traits tries to describe the organization of cones (secondary axis) on compound cones (primary axis -traits 6, 14 & 15). The description is partially based on how the vascularization stele plates. The shape of the stele gives of shoots that produce both the bract and the axis that will become the secondary shoot axis. The traits tend to show up on the more derived plants (*C. saportanus* and *C. ramentrarus* - APP4. 1). The traits could prove useful in analysis of more complex vascularization systems in plants, but also failed to resolve the greater cordaitean clade (App. 5 5, 12 & 13).

The last group of traits concerns traits with reproductive ecology (traits – 7, 12, 13, 28 & 34). The traits showcase possible developmental characteristics of the primary axis and describe the investment made into the cones; the ‘smaller the trait’ (pith or number of scales) the smaller the overall investment. The cones that produced gametes for multiple seasons probably invested more heavily into the fertile axes. Out of these traits, four form a clade in the heat map (see the dark green clade, Figure 11). They are highly influential of the *Gothania* clade that forms, in all analysis APP4. 1, 3 and 5); these traits seem to have some effect grouping the *Gothania* and the *C. ramentrarus* clade (APP4. 3). Though the traits fail to resolve the greater cordaitean clade (Fig. 6, App. 5 6, 10, 11, 24 and 30) they seem to have a more elaborate pattern that could give structure to the tree. The nature of development makes it difficult to discern as the ecological aspects do not preserve in the fossil record.

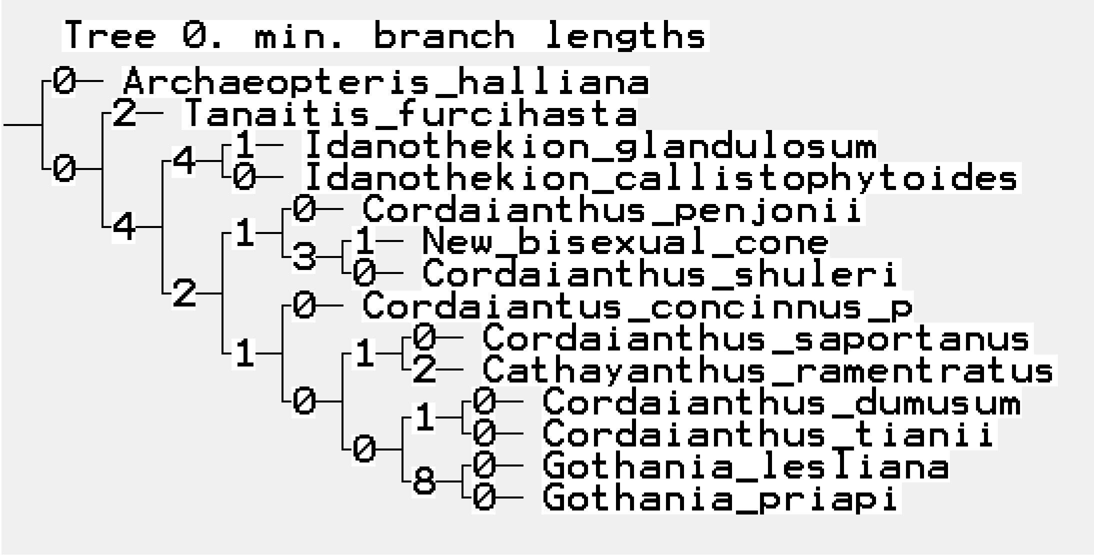
One last set of characters to mention are the ovule vascularization traits (traits 36 and 37) that resemble the Hilton et al, 2003, traits. They seem to create a paradox between derived and primitive traits, specially coupled with pollen traits (traits 31, 38 and 39). The advanced nature of the traits makes *Callistophytalean* plants derived and primitive in nature. The ovule traits would also link them *Gothania* (not mapped, but observed in whole-plant analysis - Figure 9b).

TNT – Analyses conducted using TNT. Traits start counting at 0 and correlate to the characte rs listed in APPENDIX 2 by adding one to the value that appears in the trees.

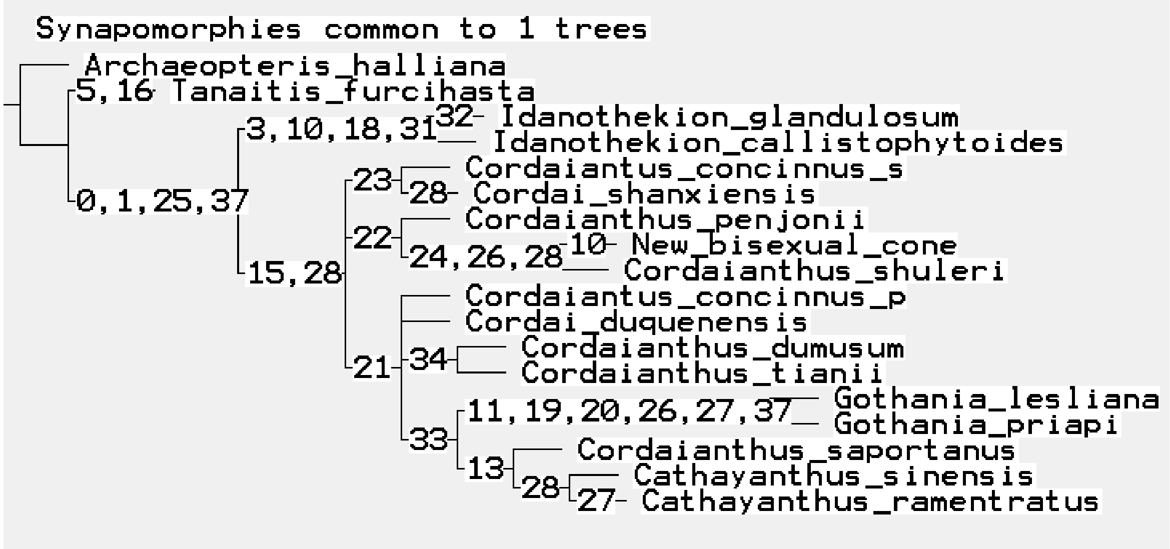


```
Synapomorphies common to 1 trees
 ┌── Archaeopteris_halliana
─┤ ┌5,16 Tanaitis_furcihasta
 └─┤          ┌3,10,18,31┬32 Idanothekion_glandulosum
   │          │          └── Idanothekion_callistophytoides
   │          │            ┌22┬── Cordaianthus_penjonii
   └0,1,25,37─┤            │  └24,26,28┬10 New_bisexual_cone
              │            │           └── Cordaianthus_shuleri
              └15,28───────┤    ┌── Cordaiantus_concinnus_p
                           │    ├13┬── Cordaianthus_saportanus
                           │    │  └27,28 Cathayanthus_ramentratus
                           └21──┼34┬── Cordaianthus_dumusum
                                │  └── Cordaianthus_tianii
                                └11,12,19,20,26,27,33,37┬ Gothania_lesliana
                                                        └ Gothania_priapi
```

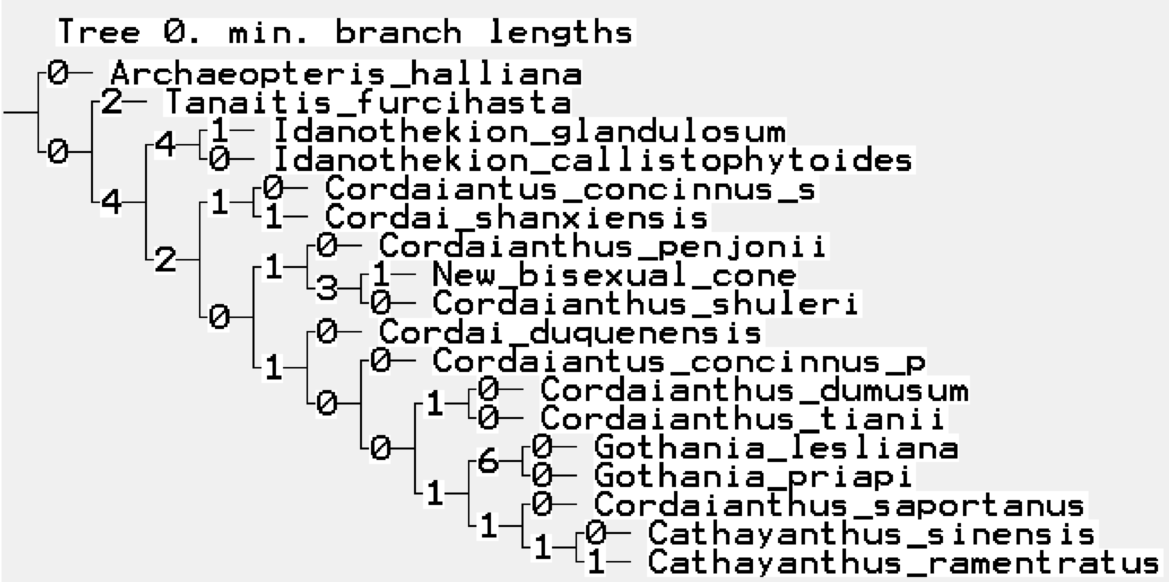
APP4. 1 – TNT of male-only cone analysis; single tree with score of 47. Traits mapped into lineages.



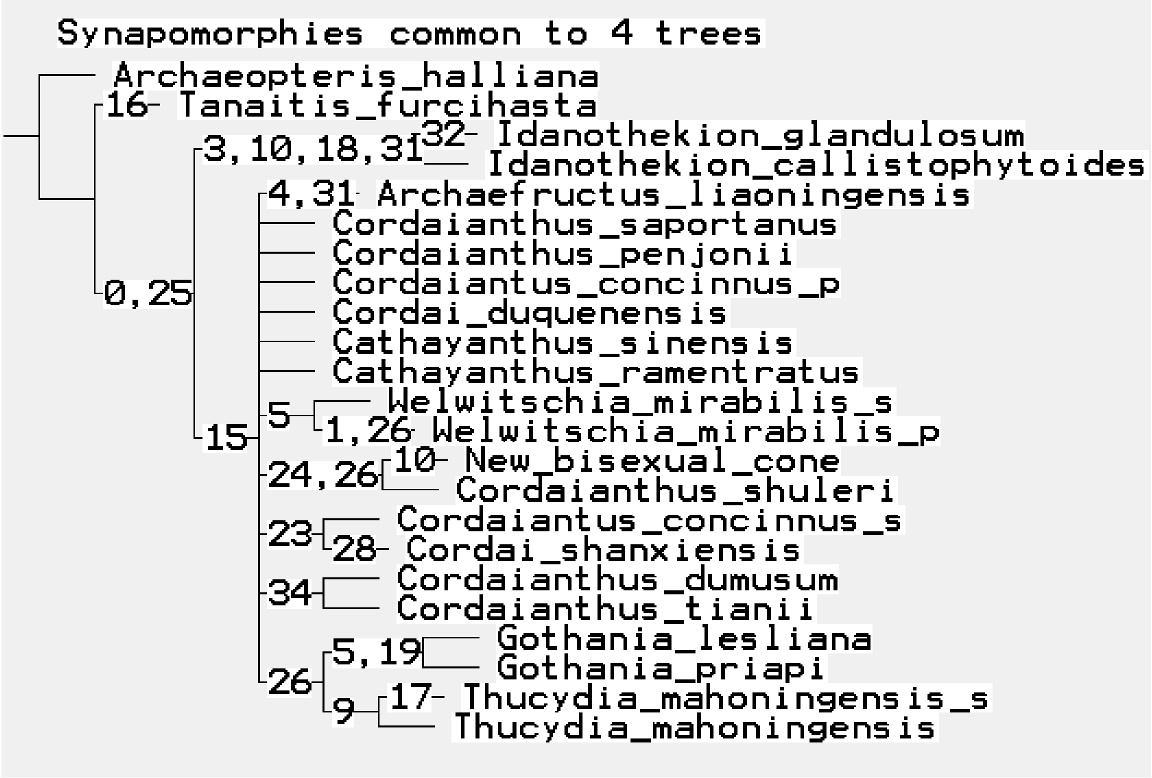
APP4. 2 – TNT of male-only cone analysis with branch lengths mapped.



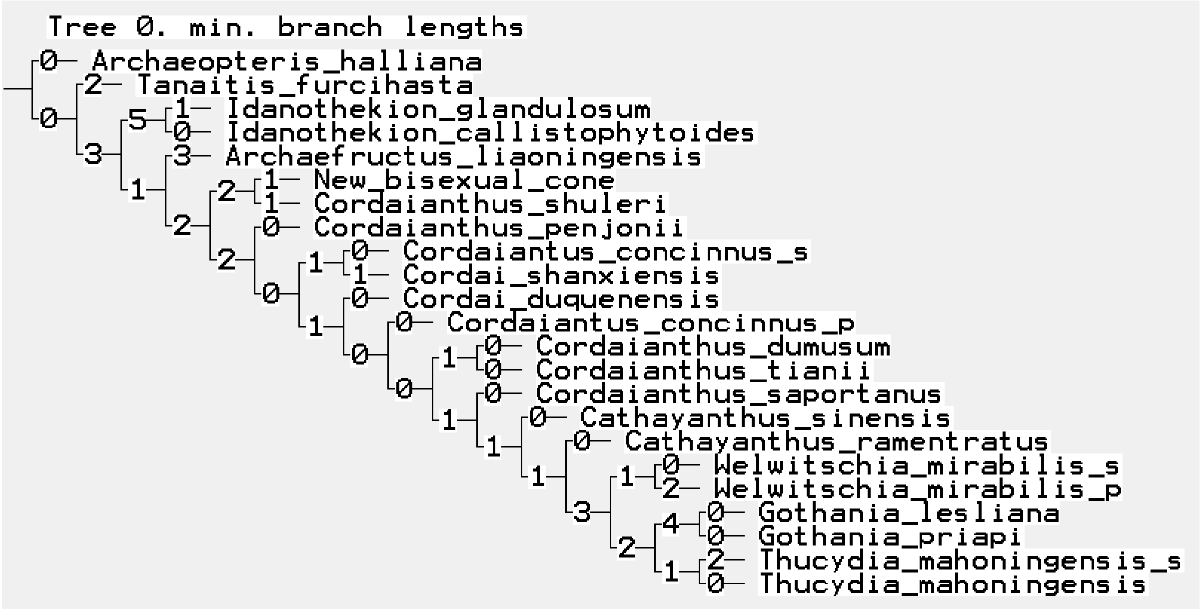
APP4. 3 – TNT of male-female cone analysis; single tree with score of 50. Traits mapped into lineages.



APP4. 4 – TNT of male-female cone analysis with branch lengths mapped.



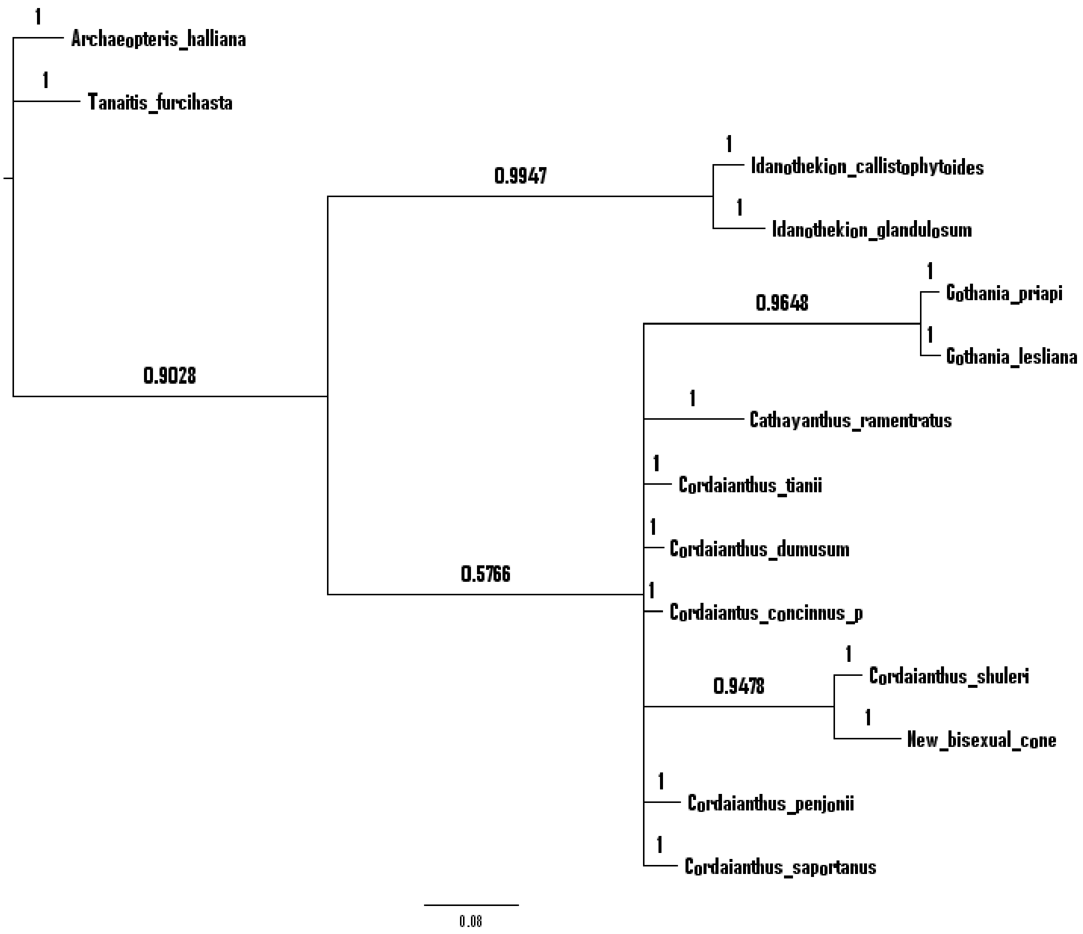
APP4. 5 – TNT of male-female cones & plant lineages analysis; four trees with score of 73. Traits mapped into lineages.



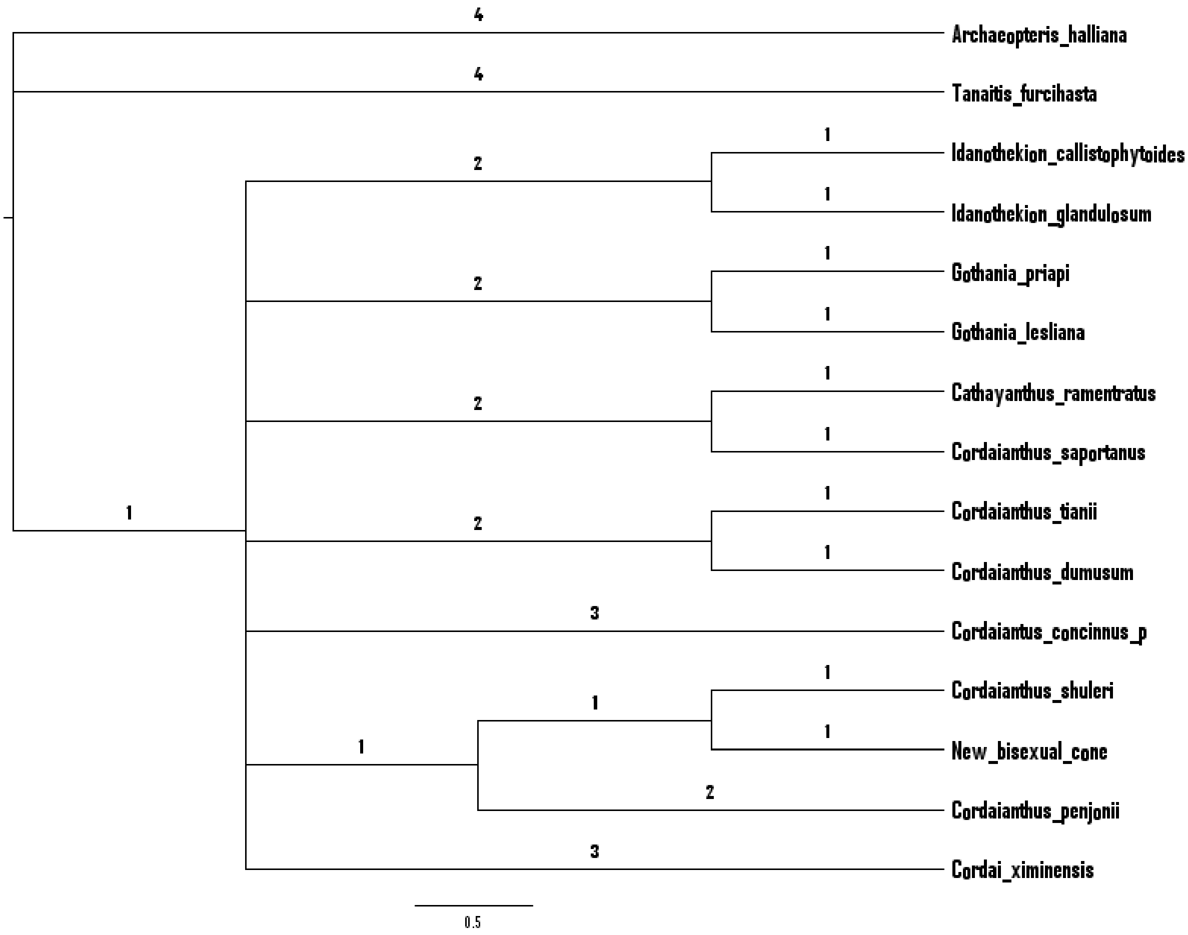
```
Tree 0. min. branch lengths
─┬0─ Archaeopteris_halliana
 └0─┬2─ Tanaitis_furcihasta
    └3─┬5─┬1─ Idanothekion_glandulosum
       │  └0─ Idanothekion_callistophytoides
       └1─┬3─ Archaefructus_liaoningensis
          └2─┬2─┬1─ New_bisexual_cone
             │  └1─ Cordaianthus_shuleri
             └2─┬0─ Cordaianthus_penjonii
                └0─┬1─┬0─ Cordaiantus_concinnus_s
                   │  └1─ Cordai_shanxiensis
                   └1─┬0─ Cordai_duquenensis
                      └0─┬0─ Cordaiantus_concinnus_p
                         └0─┬1─┬0─ Cordaianthus_dumusum
                            │  └0─ Cordaianthus_tianii
                            └1─┬0─ Cordaianthus_saportanus
                               └1─┬0─ Cathayanthus_sinensis
                                  └1─┬0─ Cathayanthus_ramentratus
                                     └3─┬1─┬0─ Welwitschia_mirabilis_s
                                        │  └2─ Welwitschia_mirabilis_p
                                        └2─┬4─┬0─ Gothania_lesliana
                                           │  └0─ Gothania_priapi
                                           └1─┬2─ Thucydia_mahoningensis_s
                                              └0─ Thucydia_mahoningensis
```

APP4. 6 – TNT of male-female cones & plant lineages analysis with branch lengths mapped.

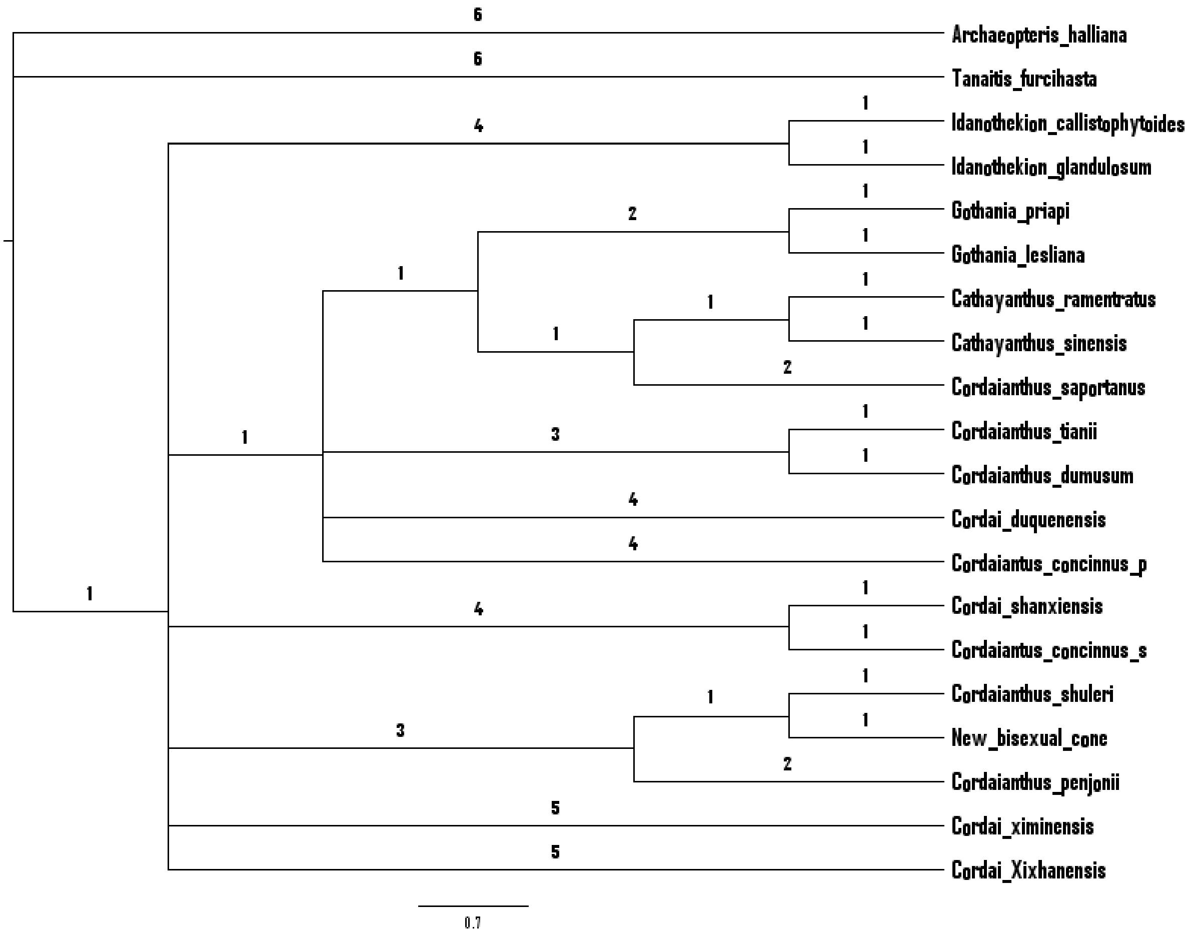
mrBayes – Analysis for male-only cones using mrBayes.



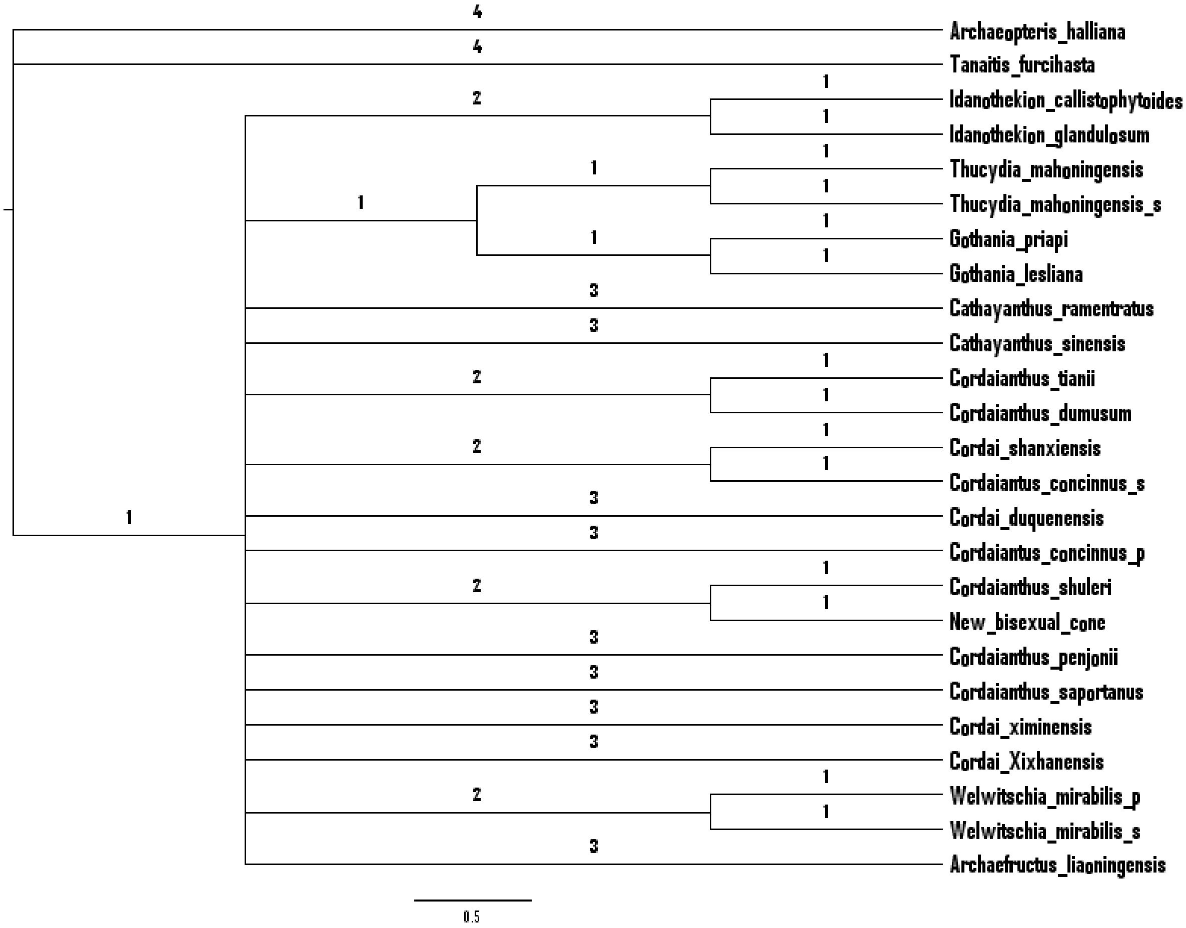
APP4. 7 – Bayesian analysis including credibility values. Burnin set at 100.



APP4. 8 – Analysis including Cordaianthus Ximinensis, male-only cones – PAUP*.



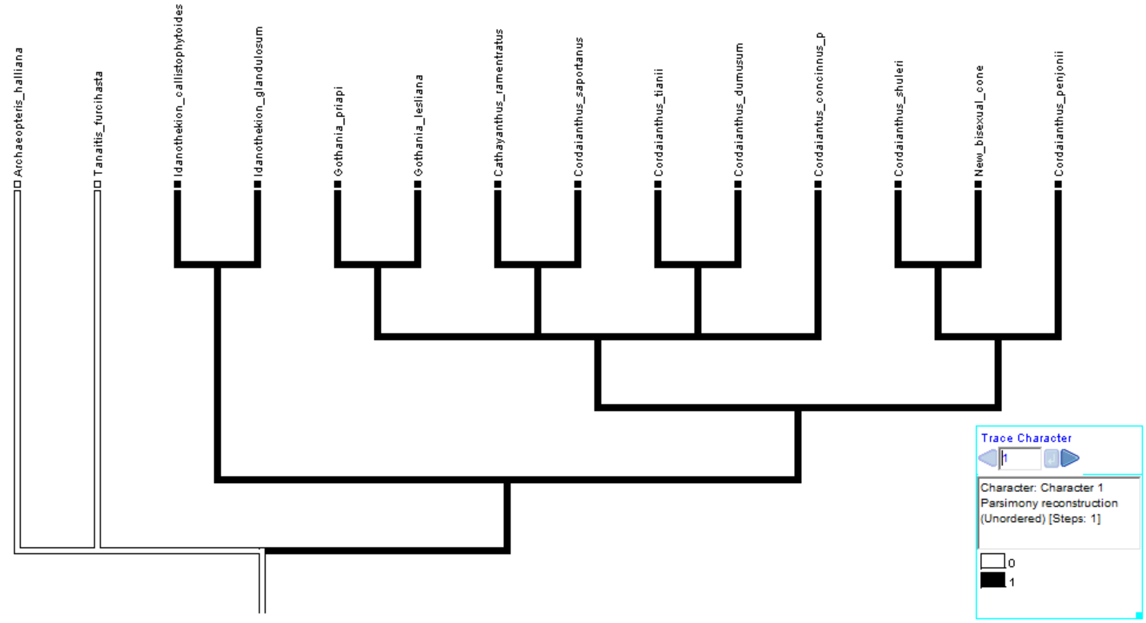
APP4. 9 – Analysis including *Cordaianthus ximinensis* & *Cordaianthus xixhanensis*, male-fe male cones – PAUP*.



APP4. 10 – Analysis including *Cordaianthus ximinensis* & *Cordaianthus xixhanensis*, plant l ineage analysis – PAUP*.

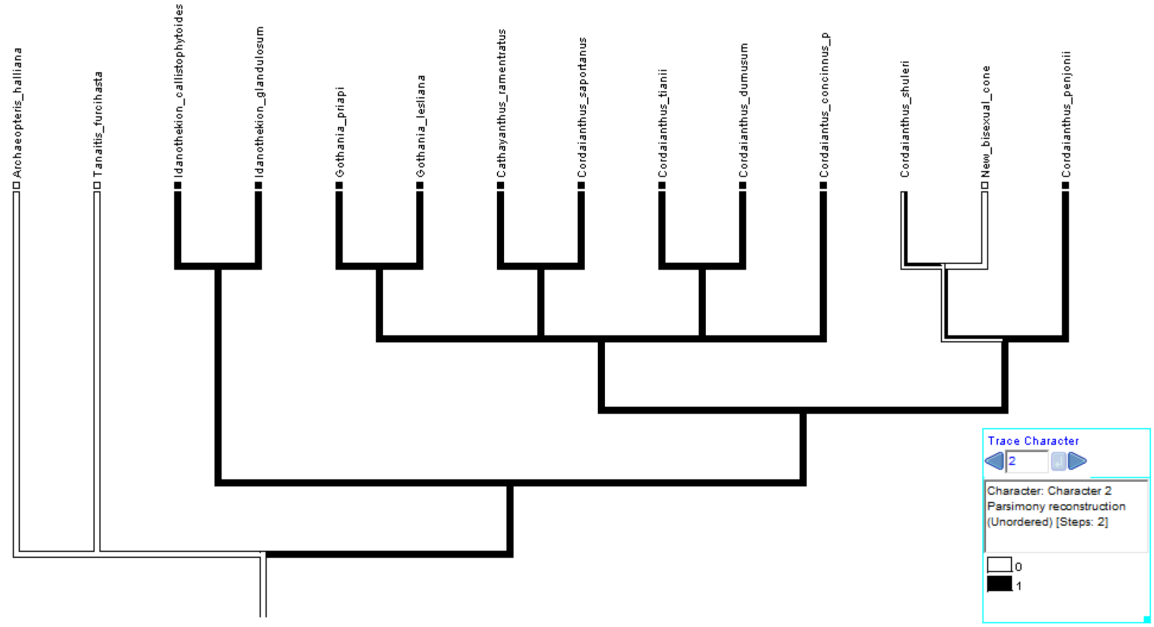
APPENDIX 5

Mesquit – interesting traits mapped to the male-only cones. Compare to TNT results above.



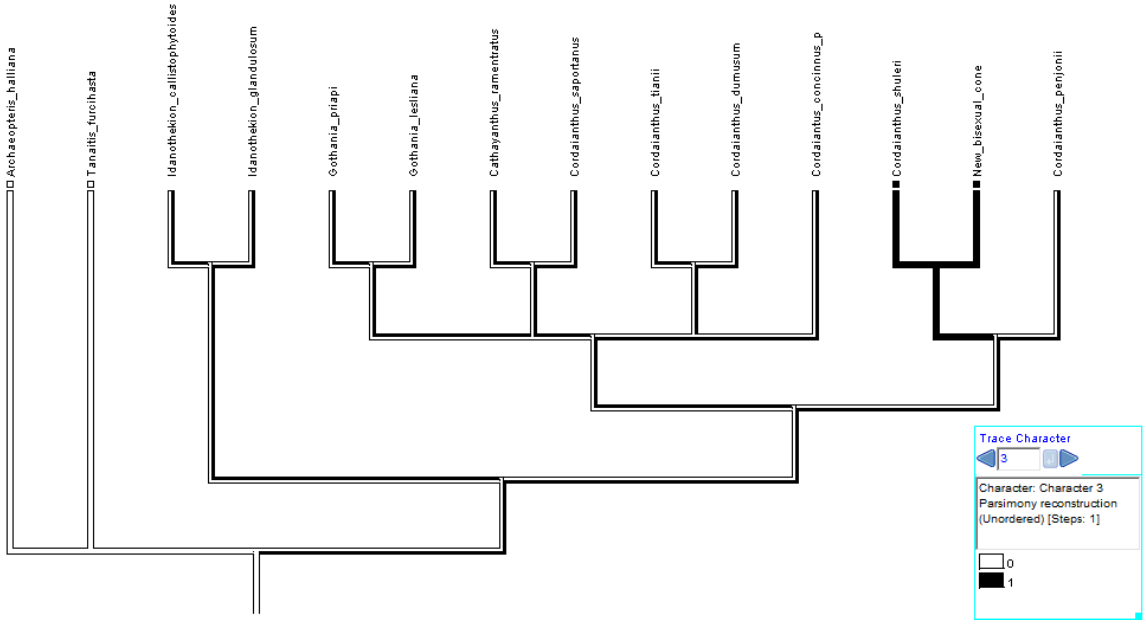
Character: Character 1
Parsimony reconstruction
(Unordered) [Steps: 1]

App. 5 1– Strict consensus tree from PAUP* male-only analysis mapping character 1.

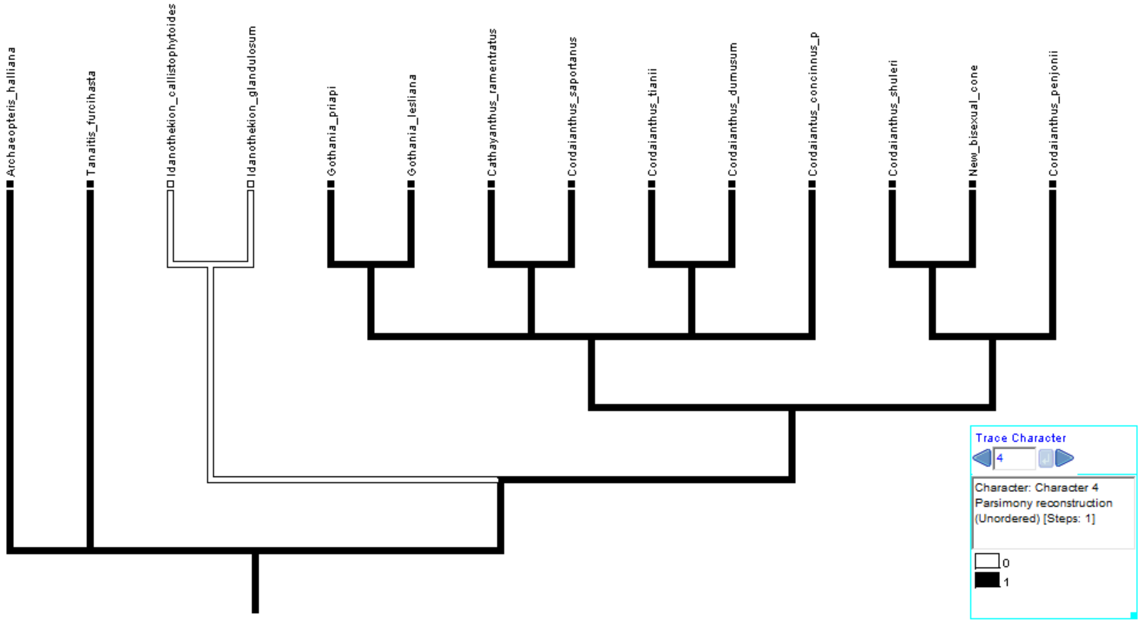


Character: Character 2
Parsimony reconstruction
(Unordered) [Steps: 2]

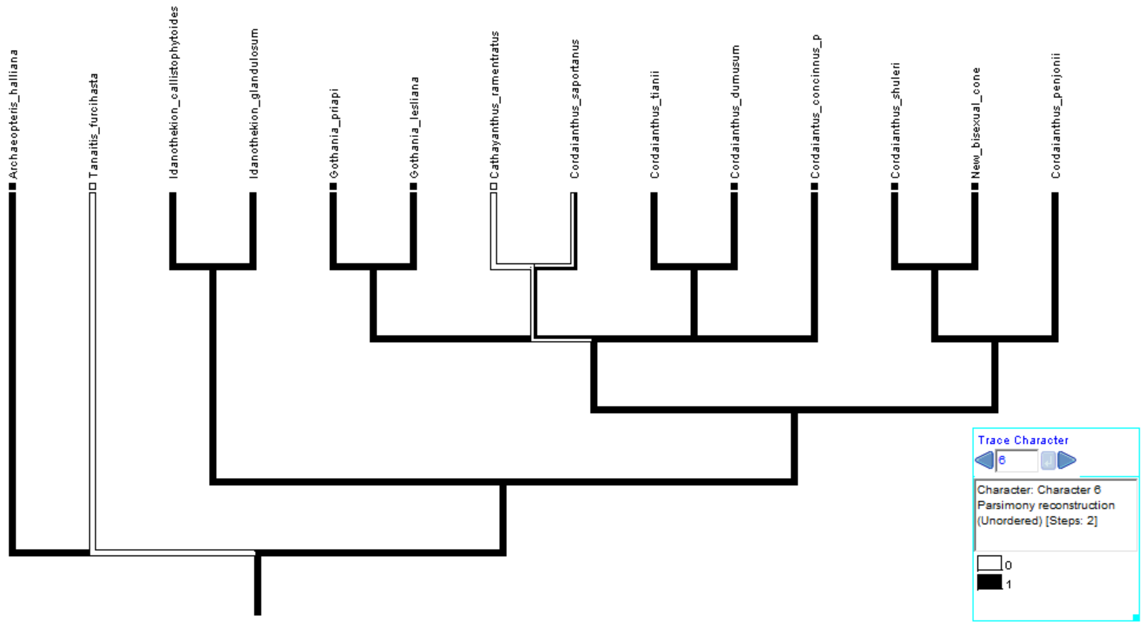
App. 5 2 – Strict consensus tree from PAUP* male-only analysis mapping character 2.



App. 5 3 – Strict consensus tree from PAUP* male-only analysis mapping character 3.

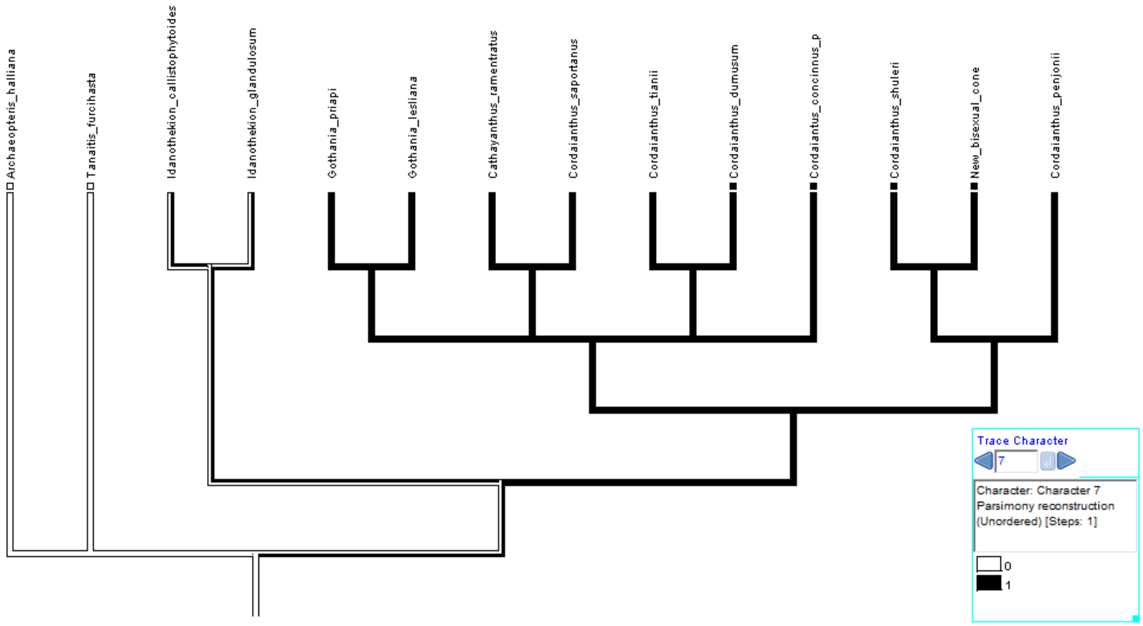


App. 5 4 – Strict consensus tree from PAUP* male-only analysis mapping character 4.



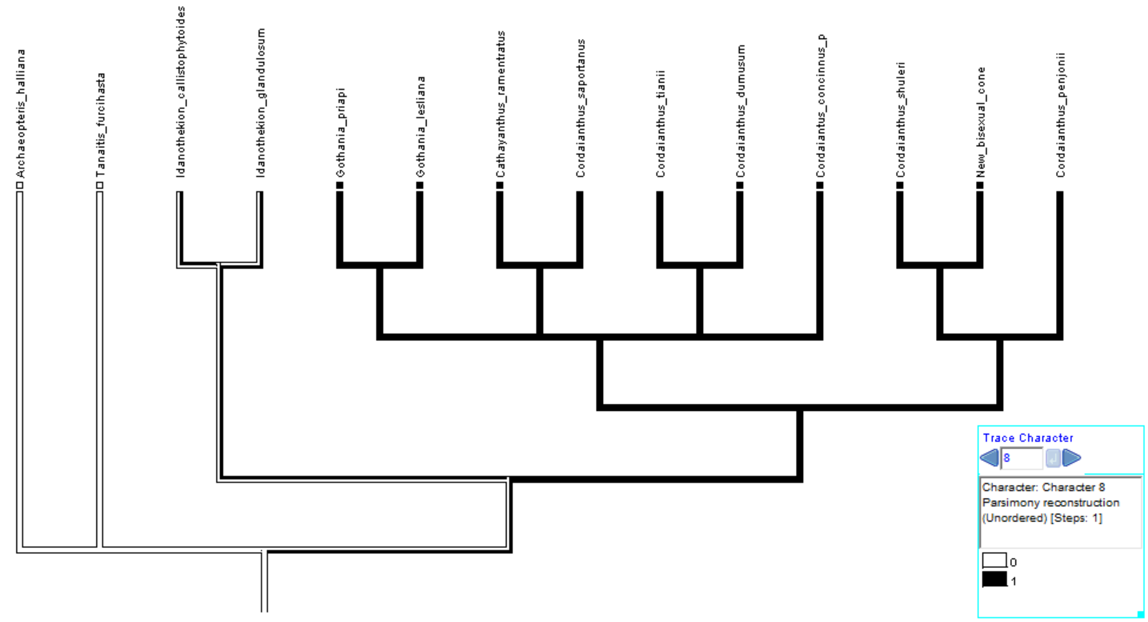
Character: Character 6
Parsimony reconstruction
(Unordered) [Steps: 2]

App. 5 5 – Strict consensus tree from PAUP* male-only analysis mapping character 6.

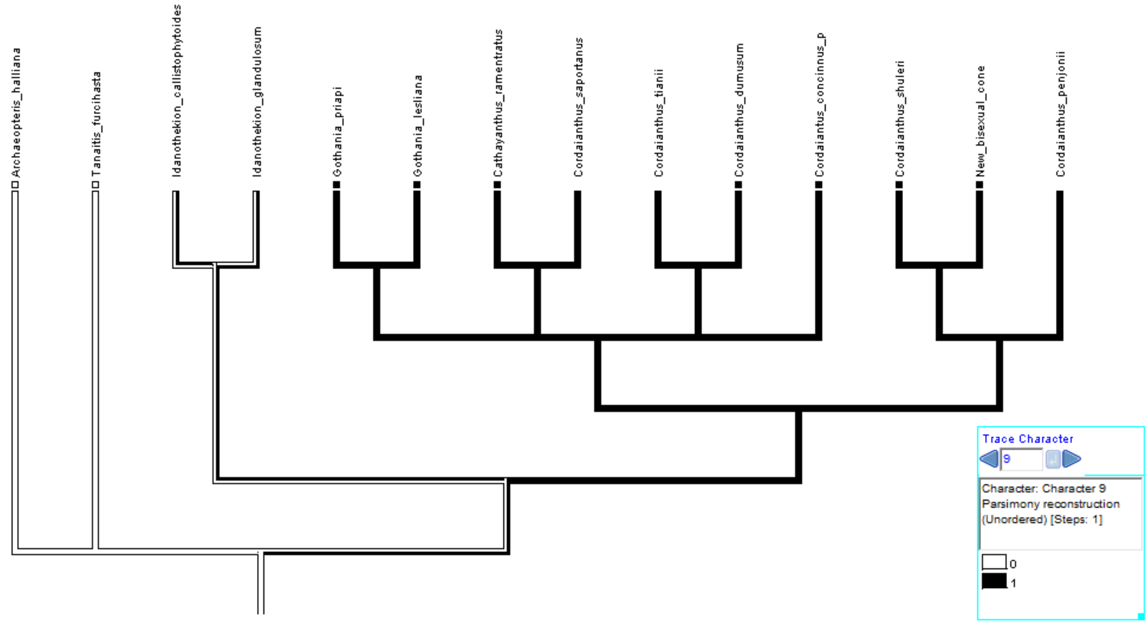


Character: Character 7
Parsimony reconstruction
(Unordered) [Steps: 1]

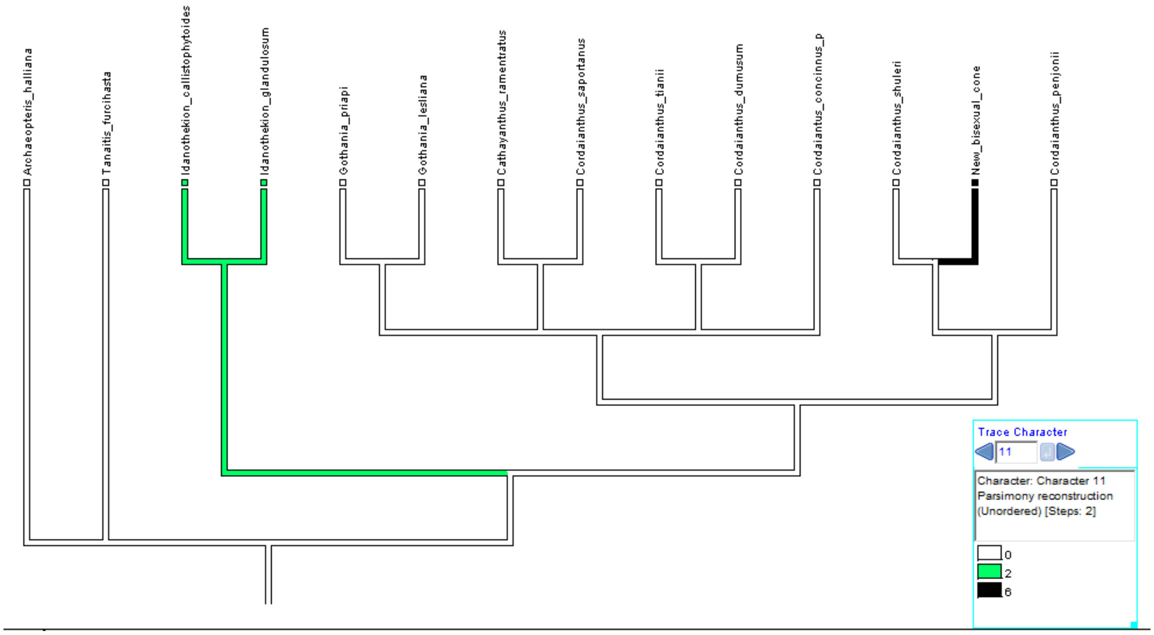
App. 5 6 – Strict consensus tree from PAUP* male-only analysis mapping character 7



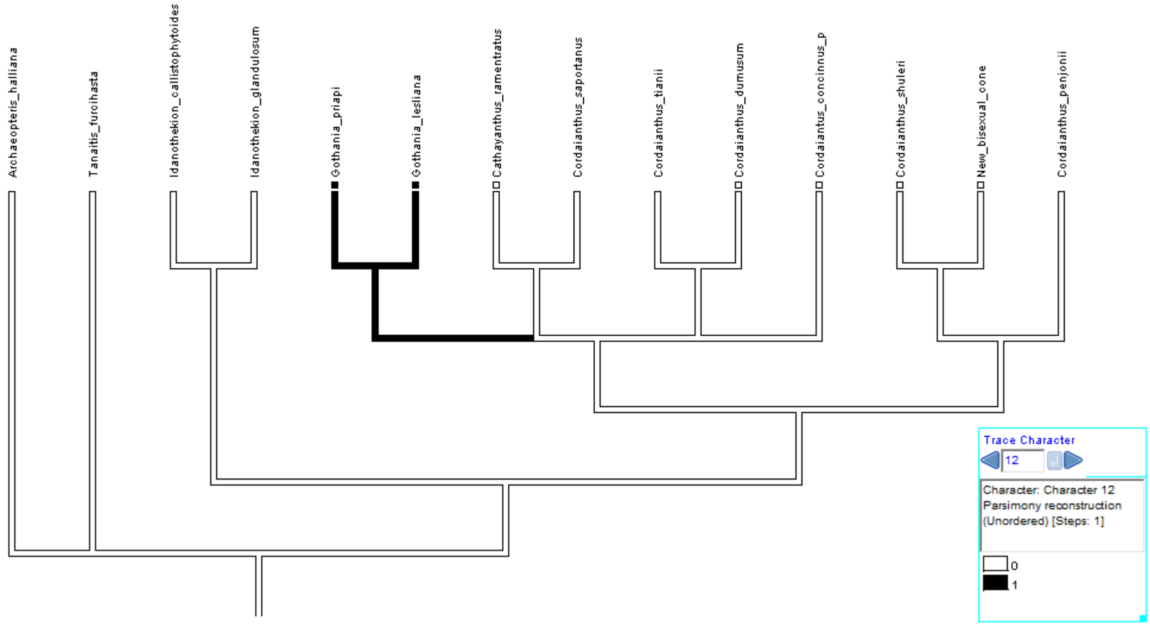
App. 5 7 – Strict consensus tree from PAUP* male-only analysis mapping character 8.



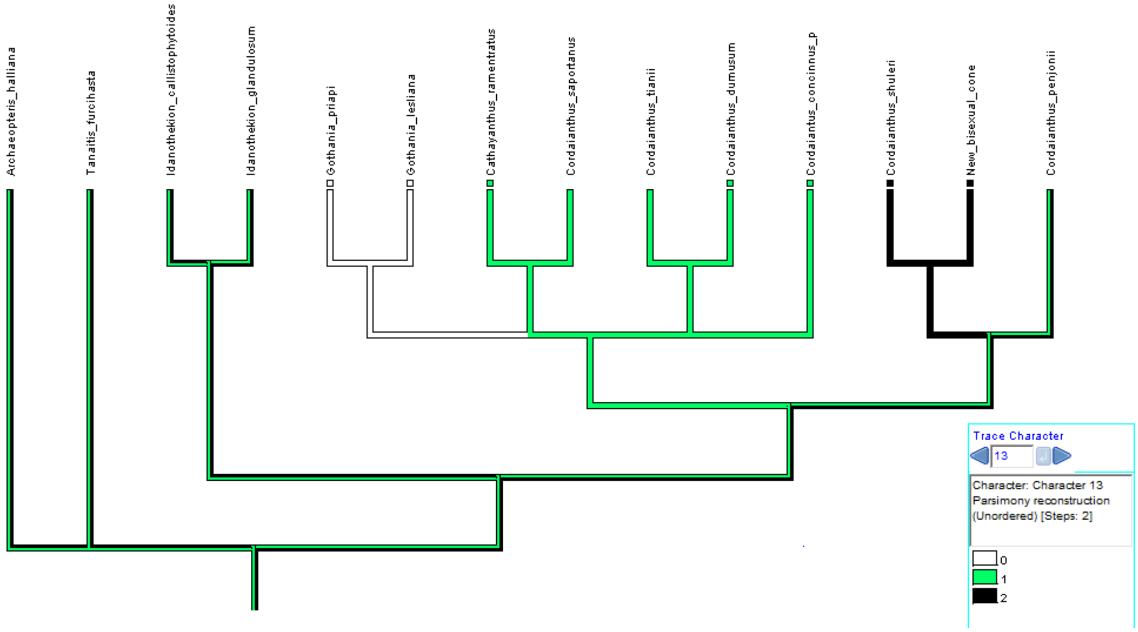
App. 5 8 – Strict consensus tree from PAUP* male-only analysis mapping character 9.



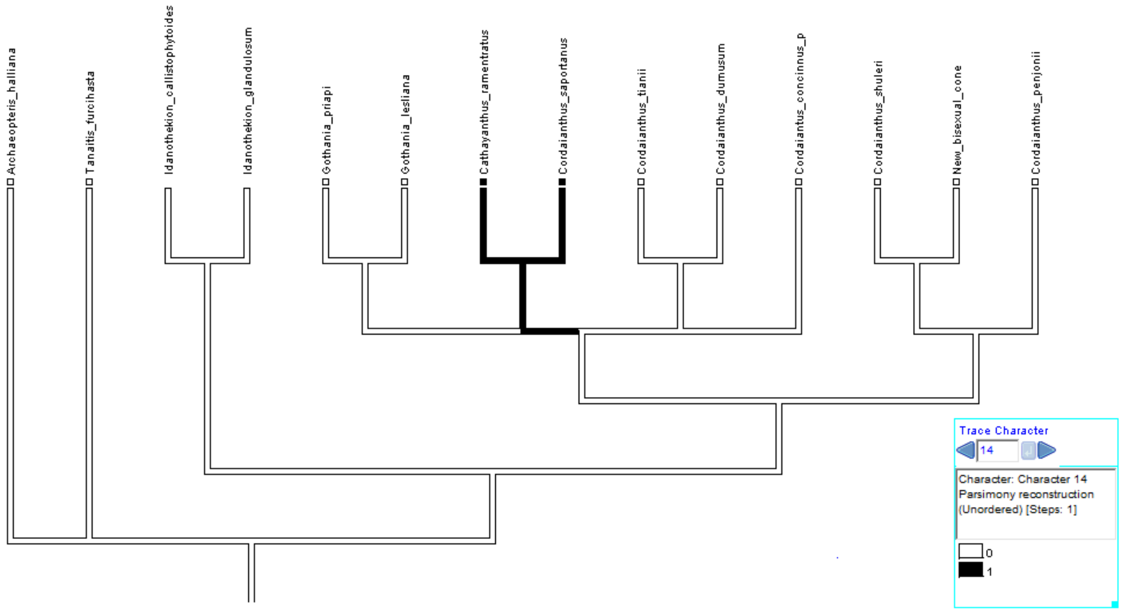
App. 5 9 – Strict consensus tree from PAUP* male-only analysis mapping character 11.



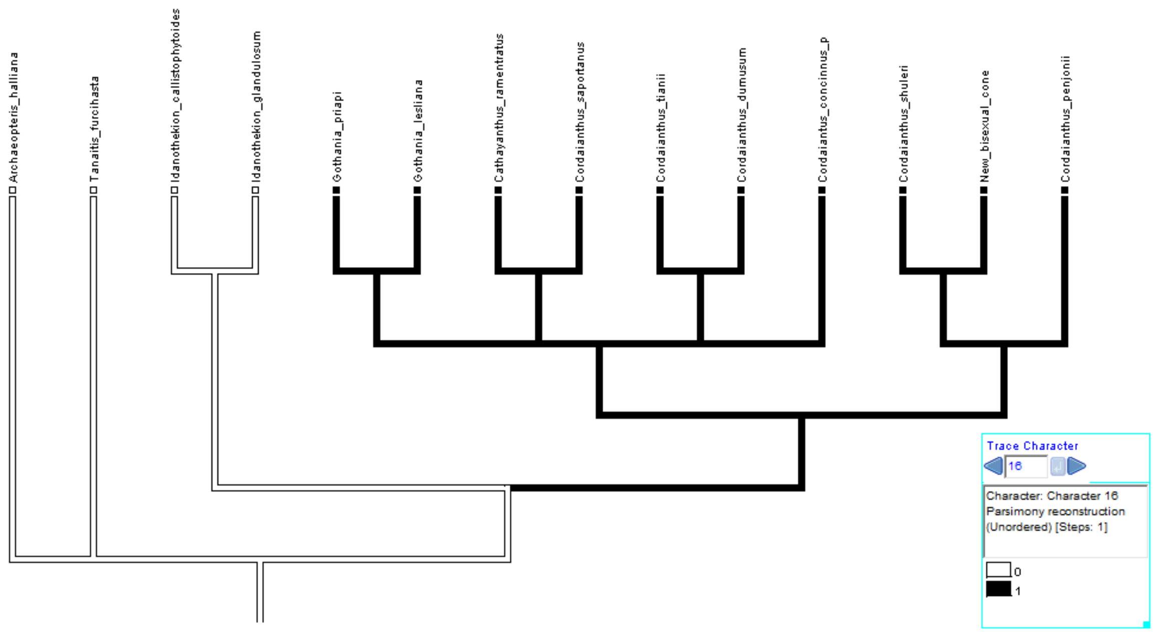
App. 5 10 – Strict consensus tree from PAUP* male-only analysis mapping character 12.



App. 5 11 – Strict consensus tree from PAUP* male-only analysis mapping character 13.

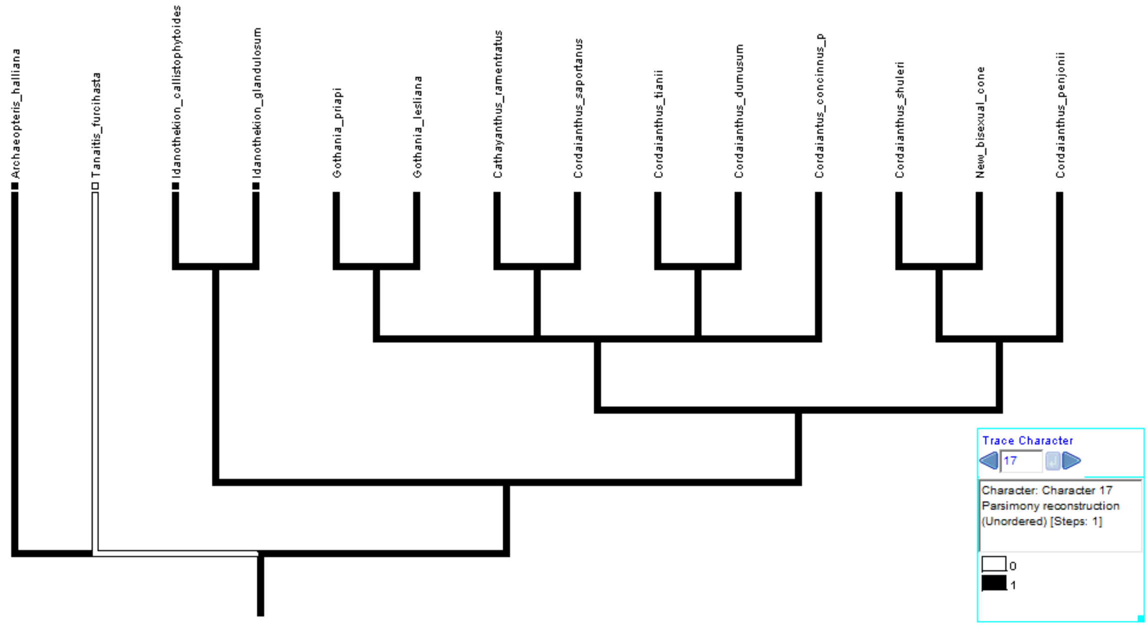


App. 5 12 – Strict consensus tree from PAUP* male-only analysis mapping character 14.



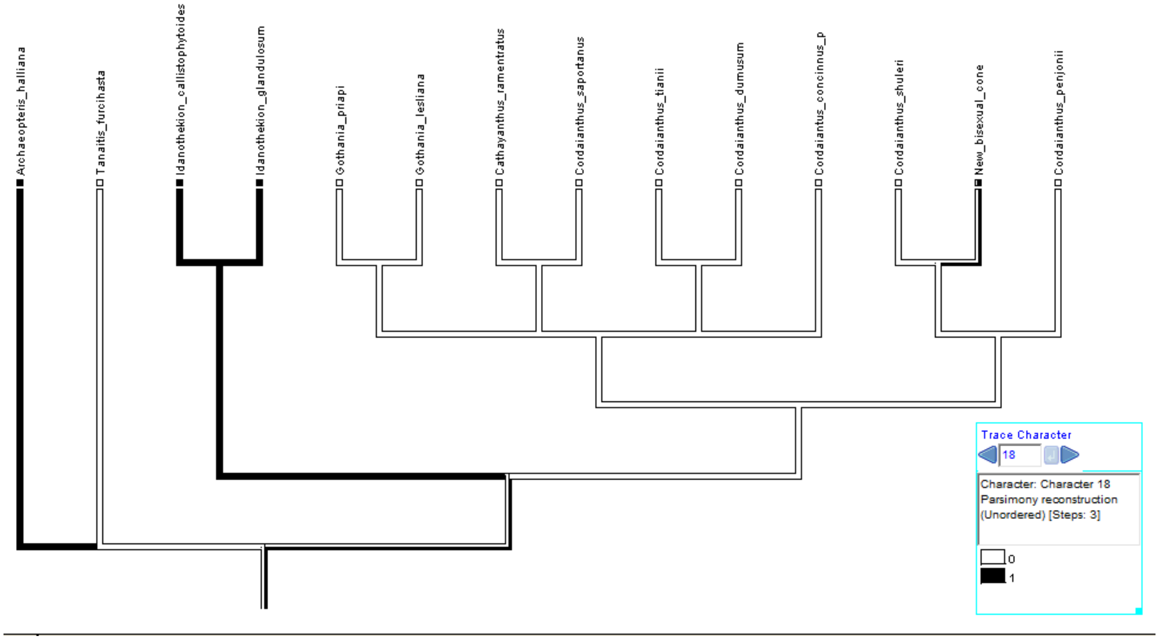
Character: Character 16
Parsimony reconstruction
(Unordered) [Steps: 1]

App. 5 13 – Strict consensus tree from PAUP* male-only analysis mapping character 16.

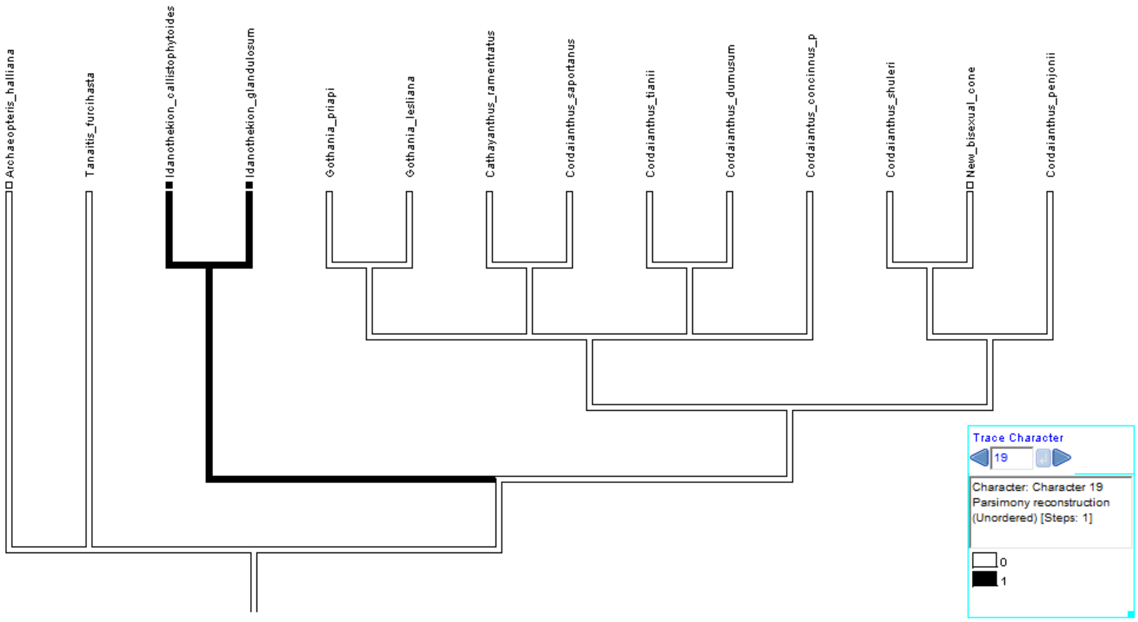


Character: Character 17
Parsimony reconstruction
(Unordered) [Steps: 1]

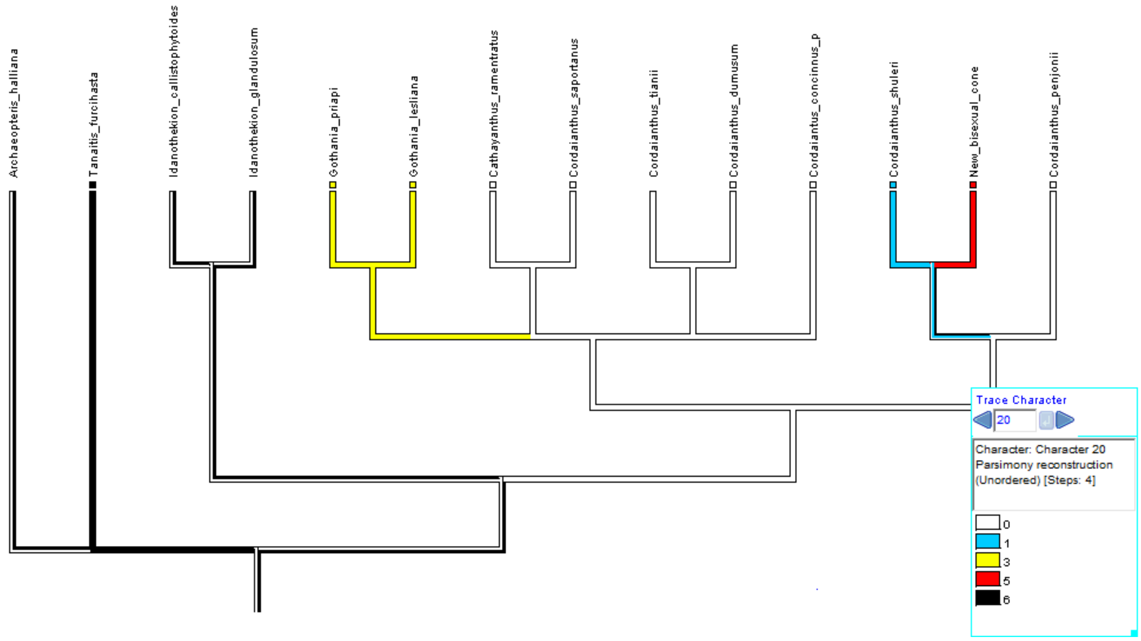
App. 5 14 – Strict consensus tree from PAUP* male-only analysis mapping character 17.



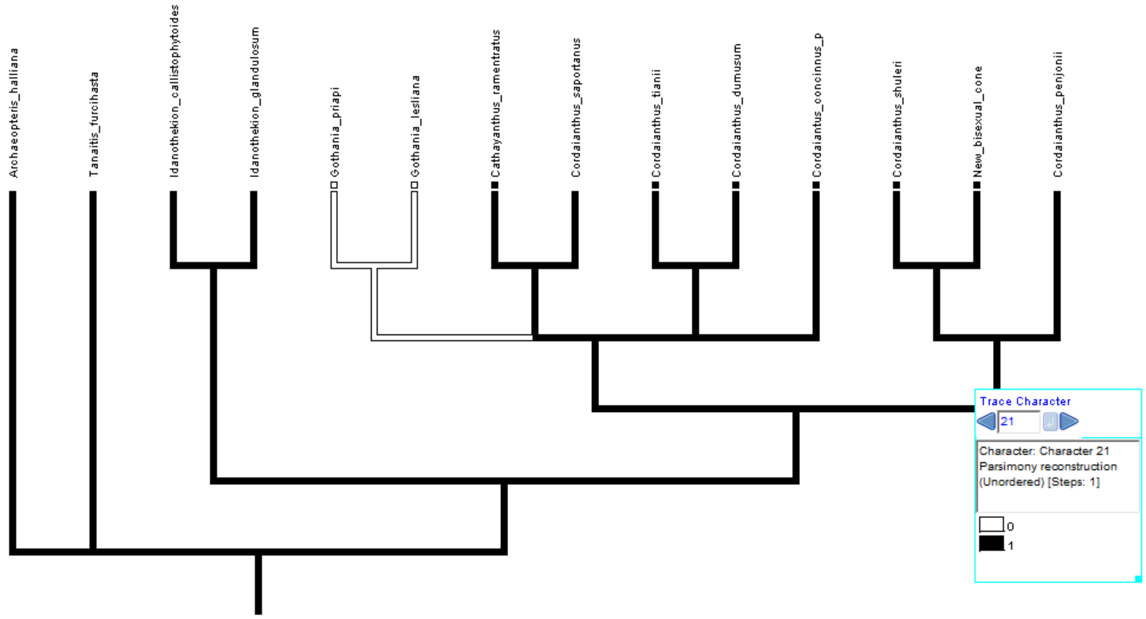
App. 5 15 – Strict consensus tree from PAUP* male-only analysis mapping character 18.



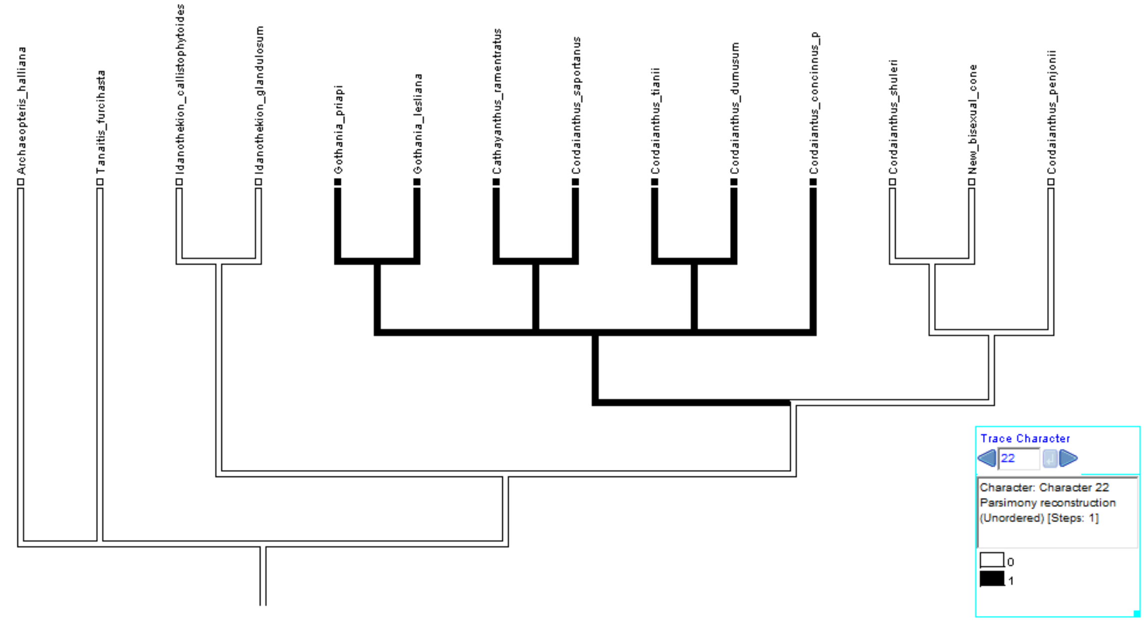
App. 5 16 – Strict consensus tree from PAUP* male-only analysis mapping character 19.



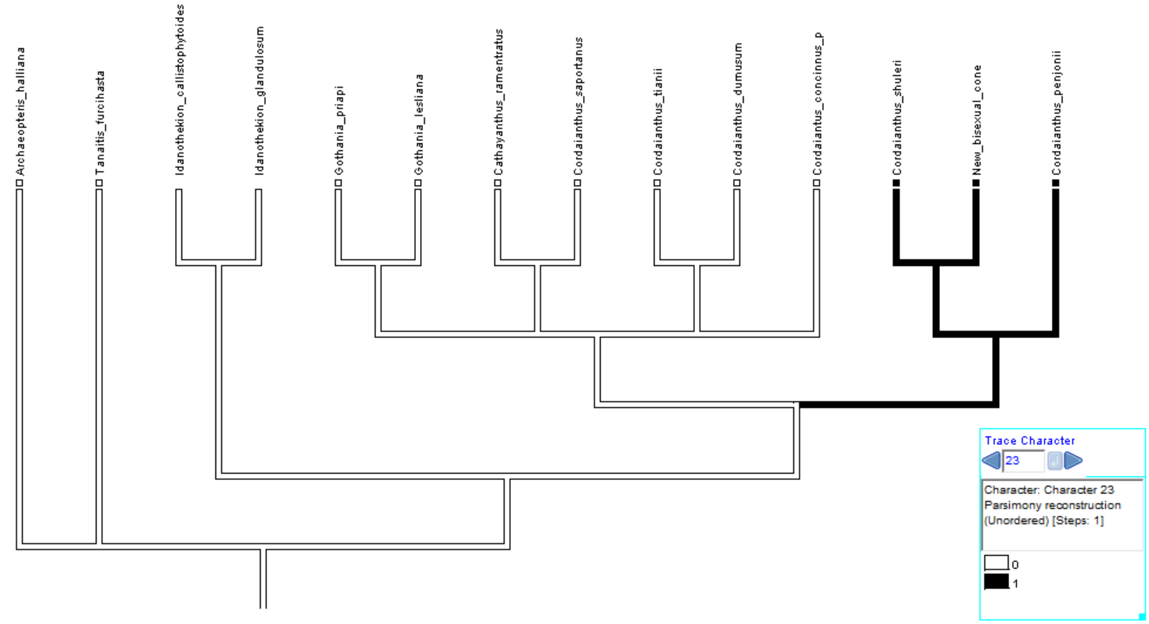
App. 5 17 – Strict consensus tree from PAUP* male-only analysis mapping character 20.



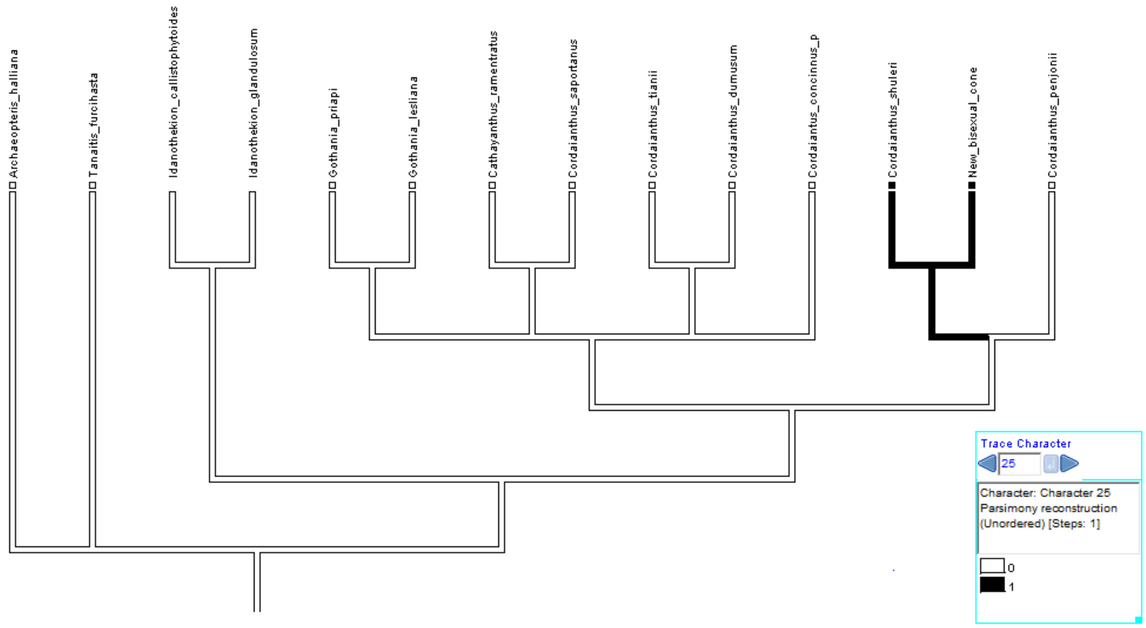
App. 5 18 – Strict consensus tree from PAUP* male-only analysis mapping character 21.



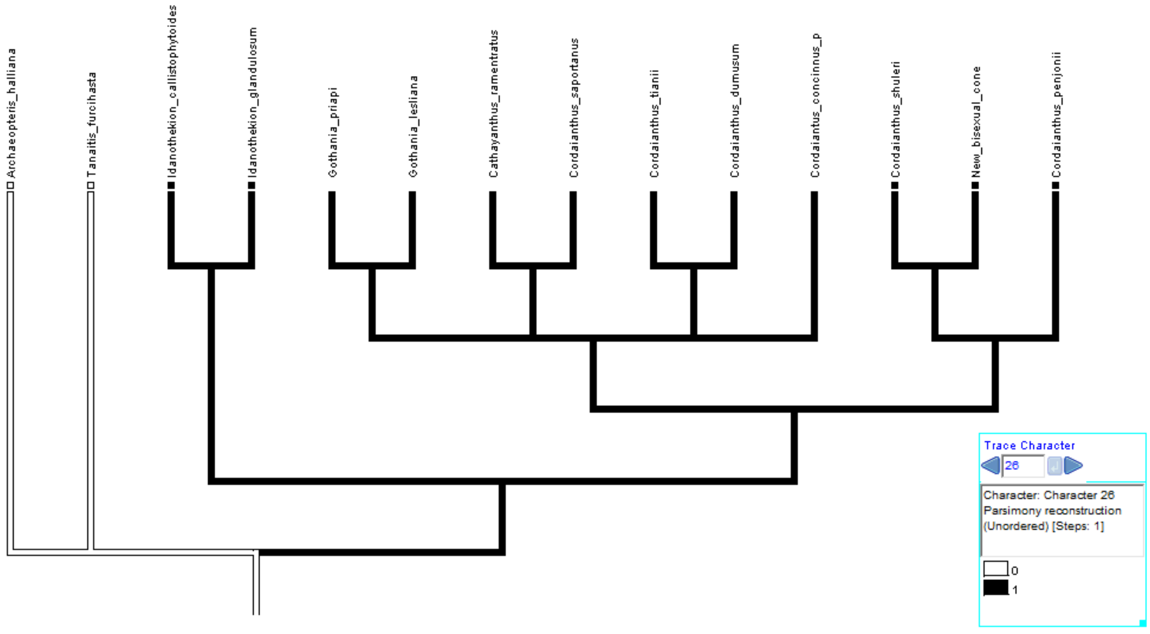
App. 5 19 – Strict consensus tree from PAUP* male-only analysis mapping character 22.



App. 5 20 – Strict consensus tree from PAUP* male-only analysis mapping character 23.



App. 5 21 – Strict consensus tree from PAUP* male-only analysis mapping character 25.



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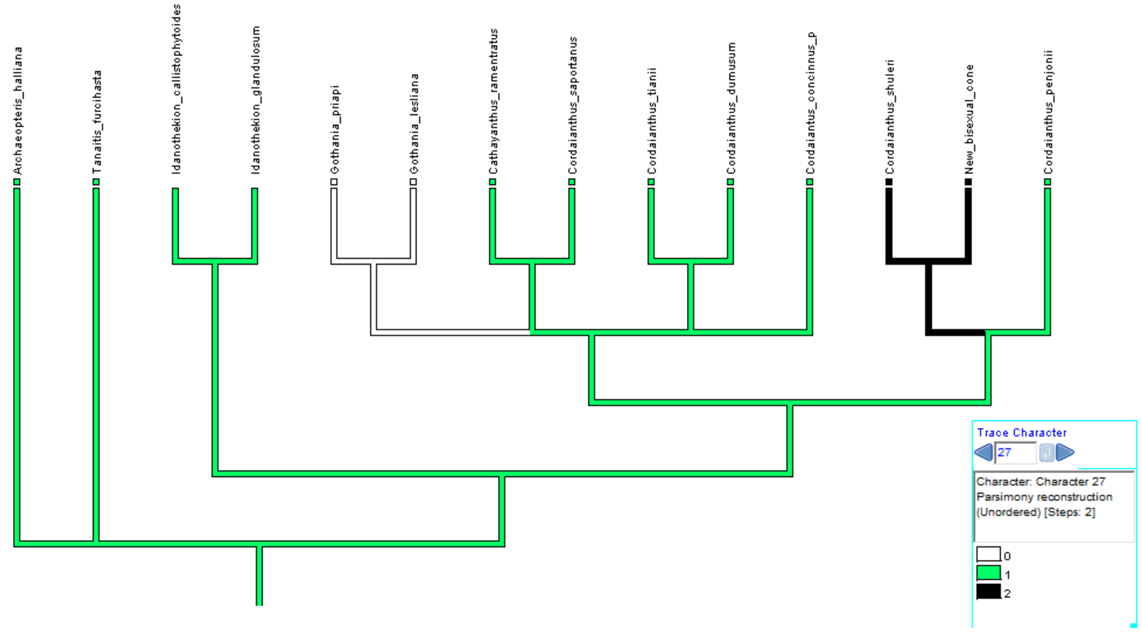
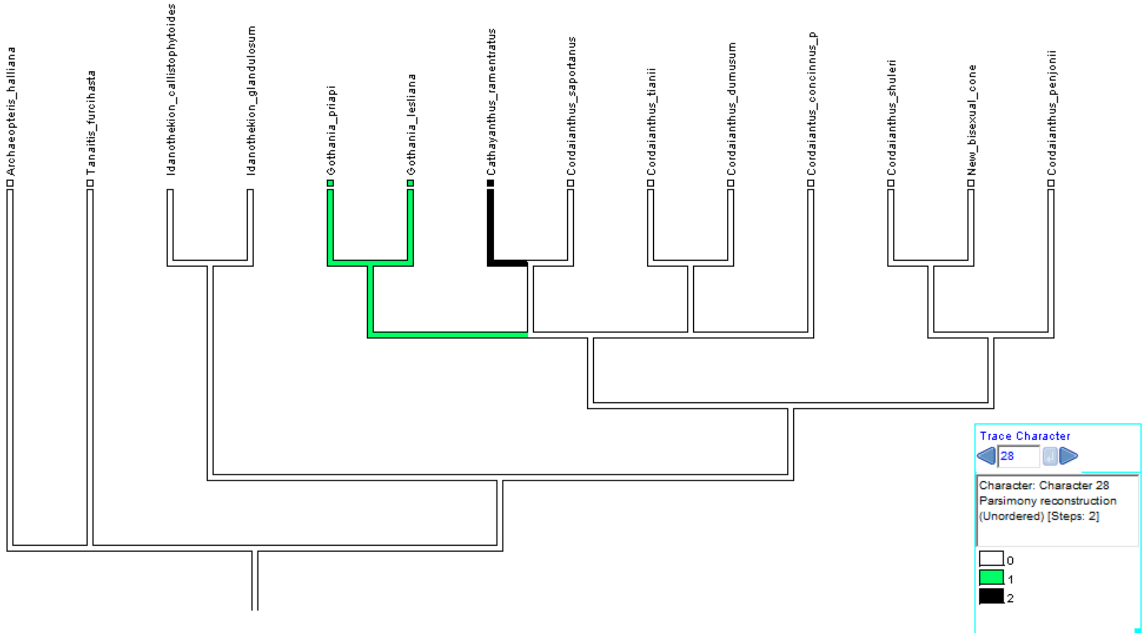
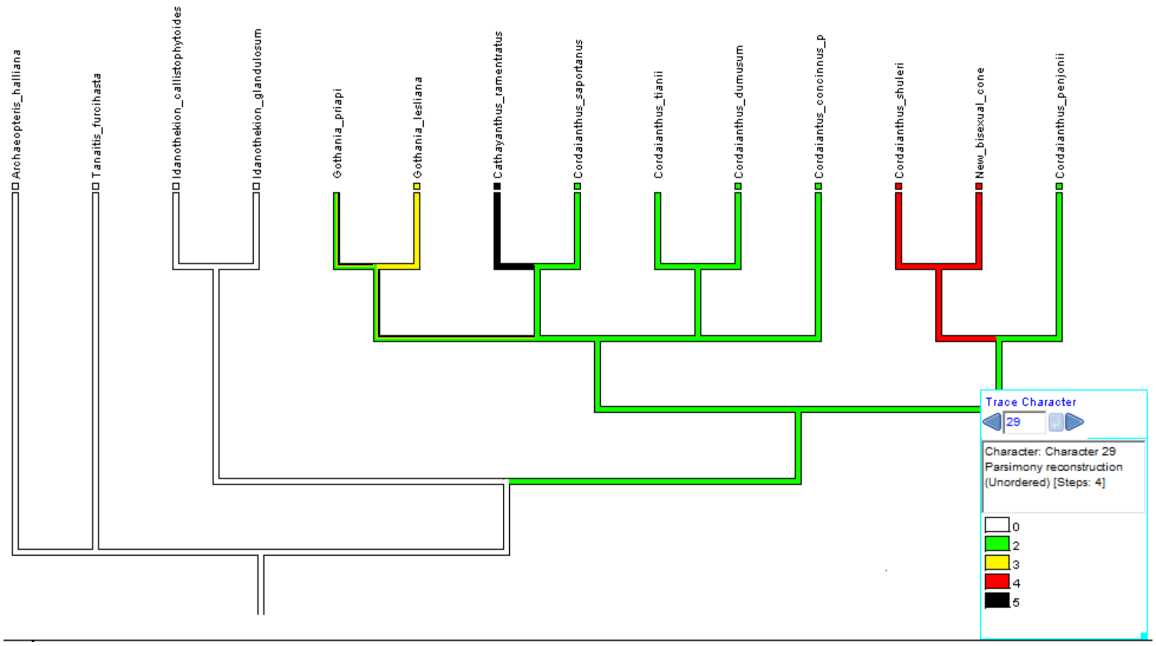
p. 5 22 – Strict consensus tree from PAUP* male-only analysis mapping character 26.



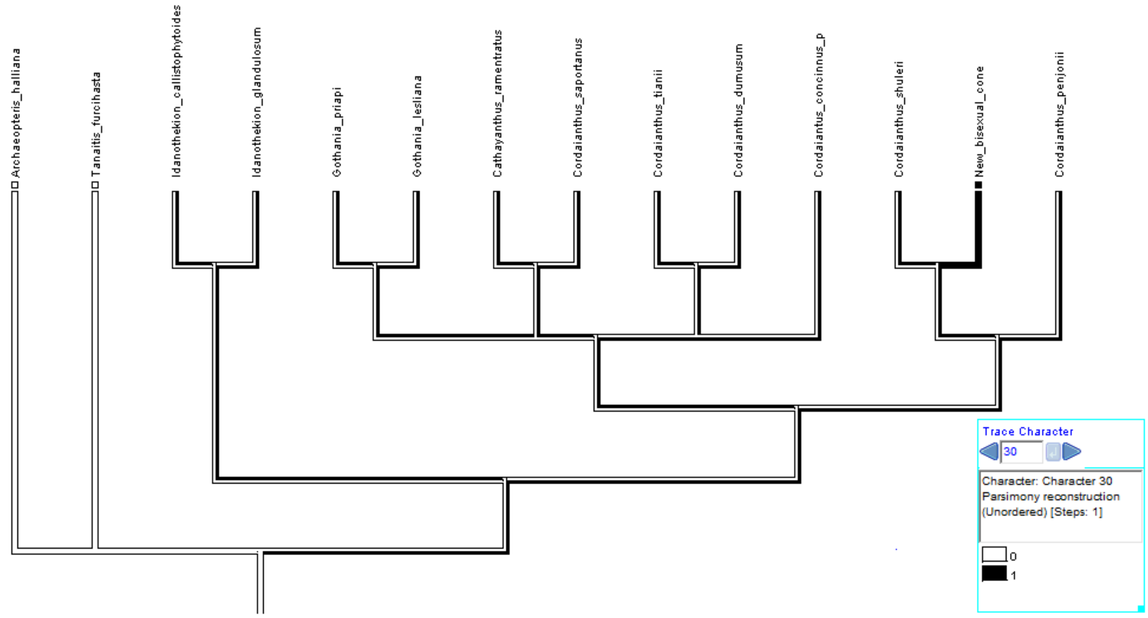
App. 5 23 – Strict consensus tree from PAUP* male-only analysis mapping character 27.



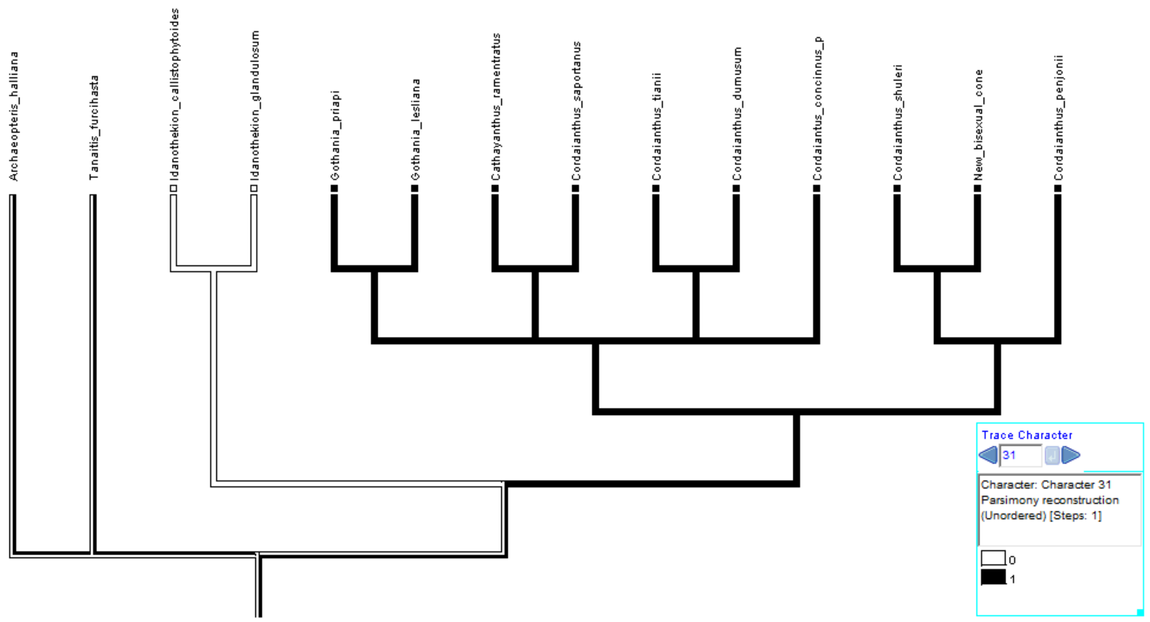
App. 5 24 – Strict consensus tree from PAUP* male-only analysis mapping character 28.



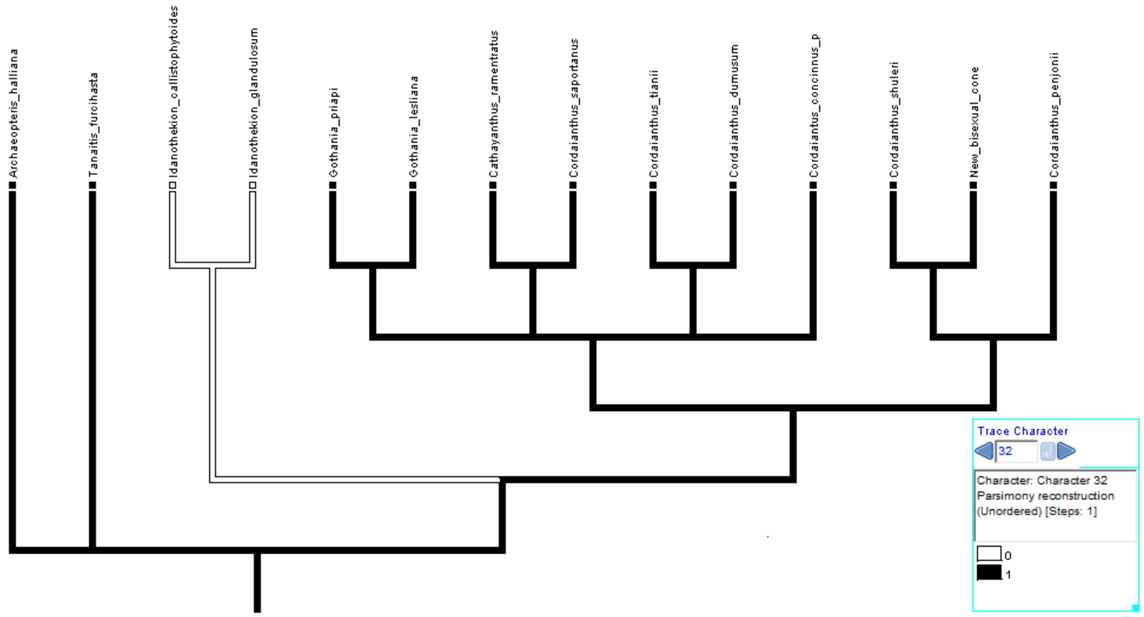
App. 5 25 – Strict consensus tree from PAUP* male-only analysis mapping character 29.



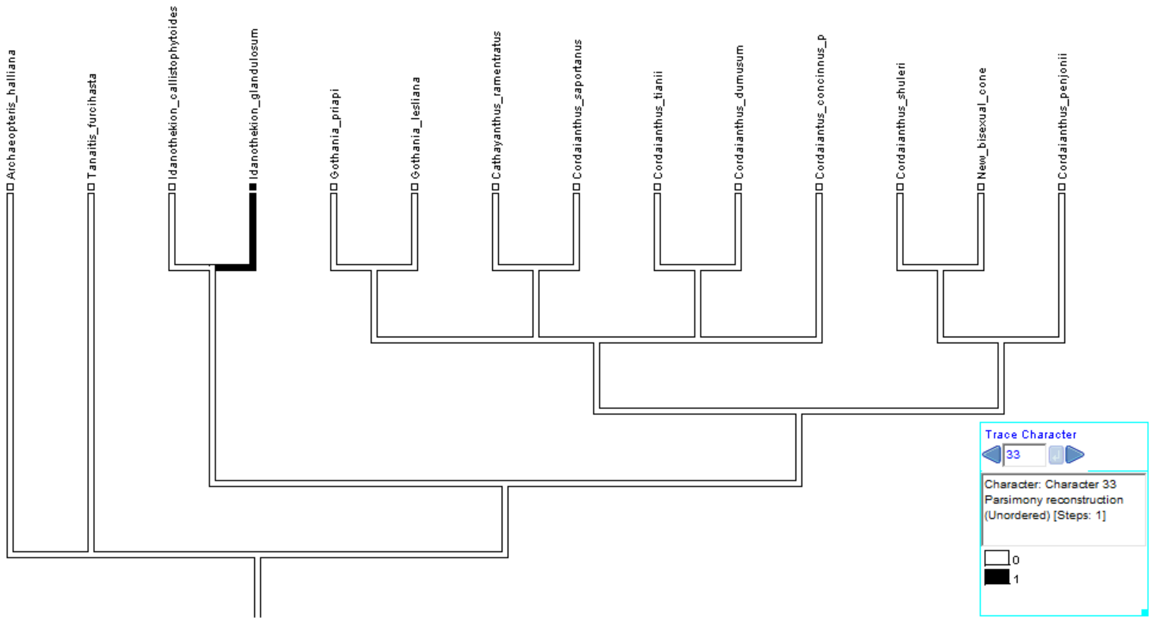
App. 5 26 – Strict consensus tree from PAUP* male-only analysis mapping character 30.



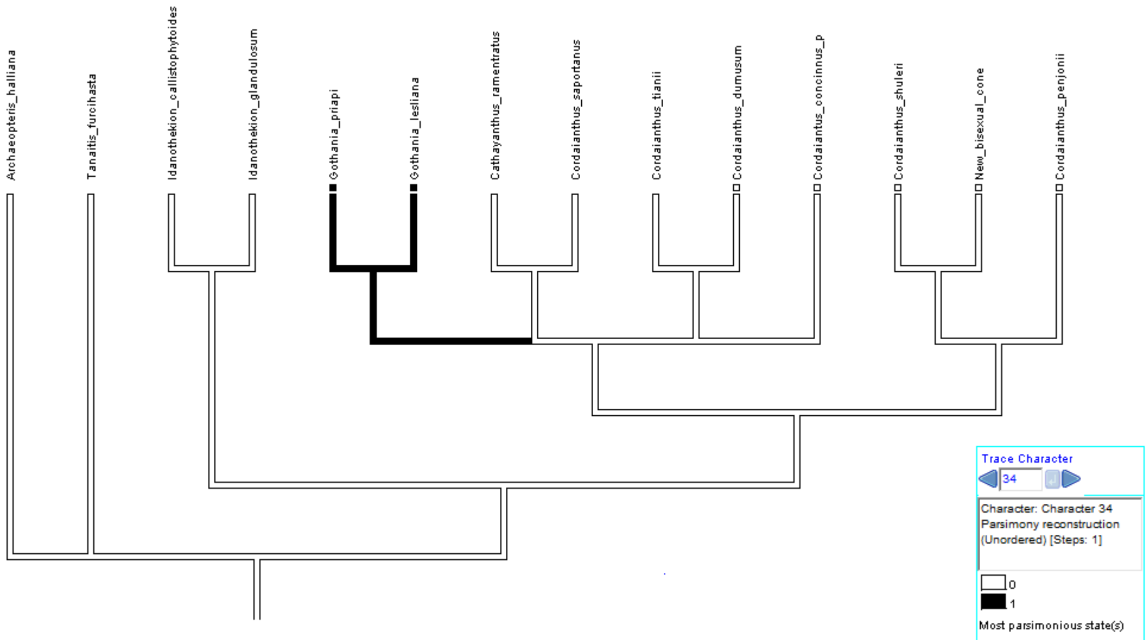
App. 5 27 – Strict consensus tree from PAUP* male-only analysis mapping character 31.



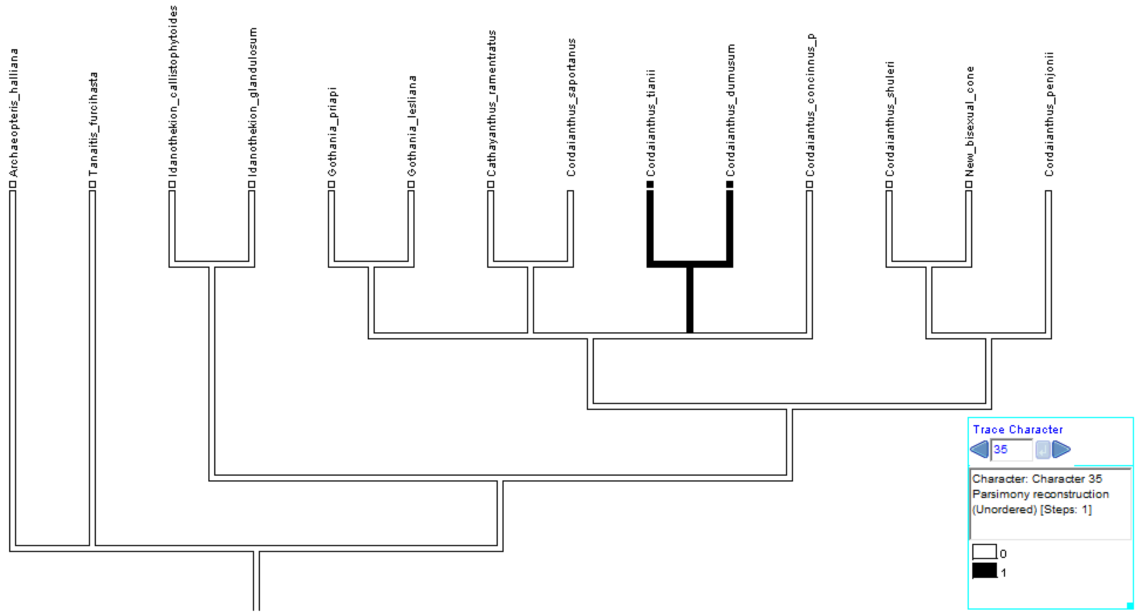
App. 5 28 – Strict consensus tree from PAUP* male-only analysis mapping character 32.



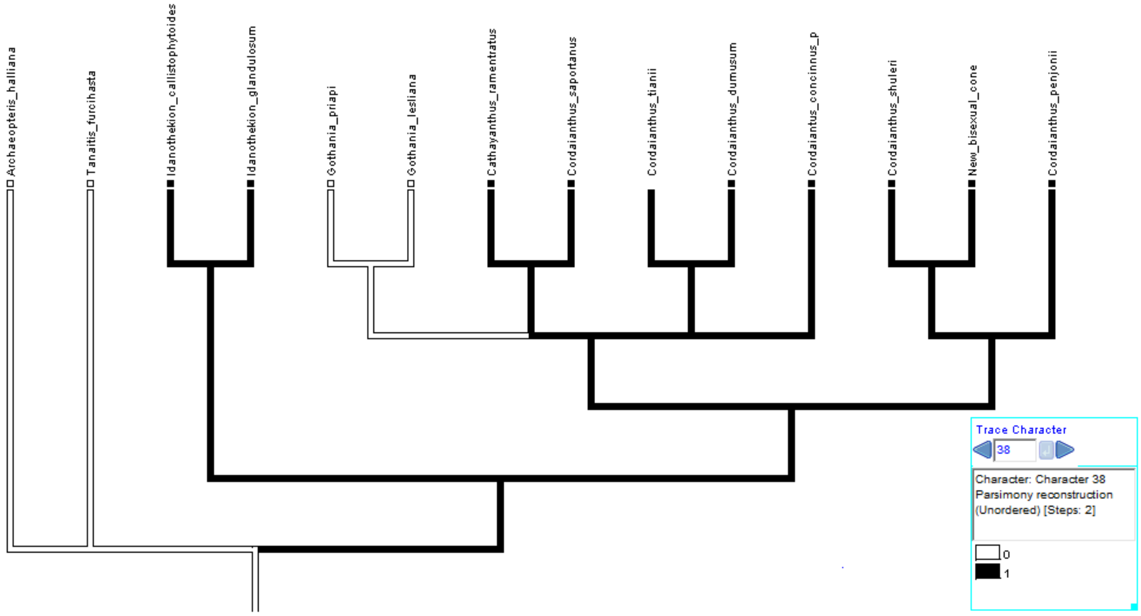
App. 5 29 – Strict consensus tree from PAUP* male-only analysis mapping character 33.



App. 5 30 – Strict consensus tree from PAUP* male-only analysis mapping character 34.



App. 5 31 – Strict consensus tree from PAUP* male-only analysis mapping character 35.



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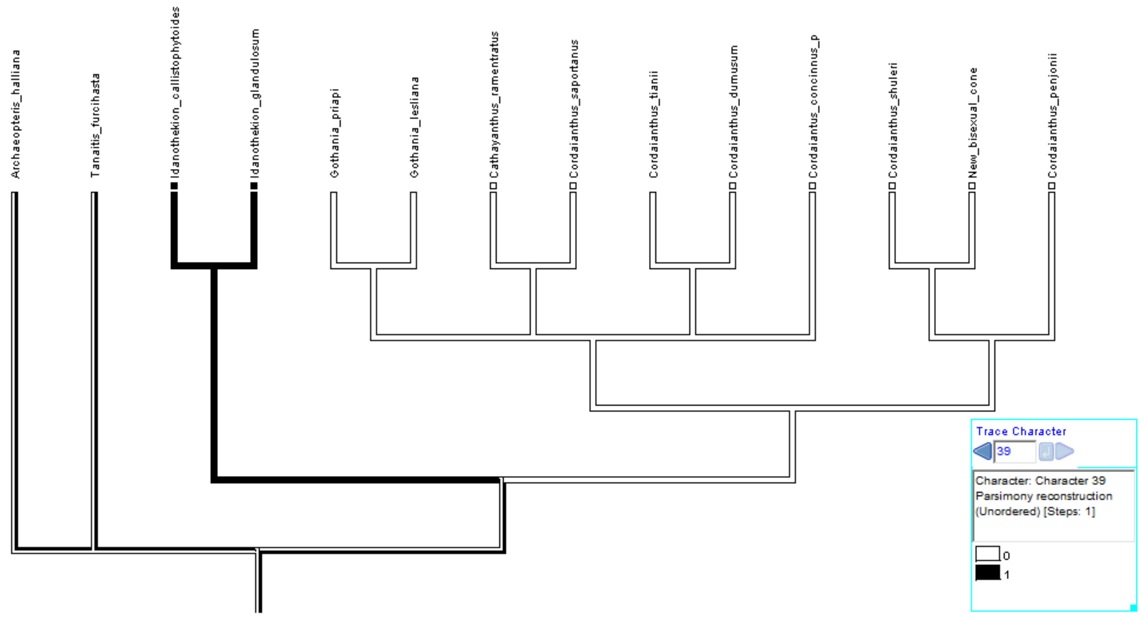
App. 5 32 – Strict consensus tree from PAUP* male-only analysis mapping character 38.



App. 5 33 – Strict consensus tree from PAUP* male-only analysis mapping character 39.

APPENDIX 6

Male-Only Character Values

Character	Steps	C.I.	R.I.	Asymmetry 2ln	Bias (Fwd/Bwd)	Mk1 Rate
Character 1	1	1	1	0.67129693	4.05089009	0.050453
Character 2	2	0.5	0.5	0.88102109	2.89776262	0.136607
Character 3	1	1	1	-1.78E-15	1.00000002	0.481212
Character 4	1	1	1	0.67129693	4.05089023	0.050453
Character 5	0	0	0	1.38629149	1.44E-06	1.06E-10
Character 6	2	0.5	0	2.46258837	3.49999993	0.246229
Character 7	1	1	1	0.28406831	1.96245186	0.185147
Character 8	1	1	1	0.40541622	2.54159567	0.101068
Character 9	1	1	1	0.40541622	2.54159567	0.101068
Character 10	0	0	0	?	?	?
Character 11	2	1	1	?	?	?
Character 12	1	1	1	0.20053942	0.52652318	0.134567
Character 13	2	1	1	12.93709972	?	0.151456
Character 14	1	1	1	0.41071068	0.35900997	0.059179
Character 15	0	0	0	1.38629149	1.44E-06	1.06E-10
Character 16	1	1	1	0.13462037	1.69581241	0.050685
Character 17	1	1	0	1.04649629	3	15.5748
Character 18	3	0.666667	0.5	?	?	?
Character 19	1	1	1	0.00621784	0.916894595	0.487778
Character 20	4	1	1	?	?	?
Character 21	1	1	1	0.26515217	2.15727265	0.104652
Character 22	1	1	1	0.010179596	0.86854875	0.04831
Character 23	1	1	1	0.22433109	0.5004683	0.063278
Character 24	0	0	0	1.38629149	1.44E-06	1.06E-10
Character 25	1	1	1	0.56552343	0.29829278	0.062876
Character 26	1	1	1	0.400261495	2.37971295	0.133618
Character 27	2	1	1	17.36643158	?	0.064238
Character 28	2	1	1	17.49845599	?	0.061874
Character 29	4	1	1	?	?	?
Character 30	1	1	0	0.33979807	0.50000001	15.36389
Character 31	1	1	1	0.40482449	2.71191123	0.055986
Character 32	1	1	1	0.67129693	4.05089023	0.050453
Character 33	1	1	0	2.31072316	0.07692308	0.048039
Character 34	1	1	1	0.31214545	0.44255645	0.124576
Character 35	1	1	1	0.41028186	0.35967223	0.059179

Character 36	0	0	0	1.38629098	1.69E-06	20
Character 37	0	0	0	1.38629098	1.69E-06	20
Character 38	2	0.5	0.666667	0.08257553	1.44949636	0.131641
Character 39	1	1	1	0.29824621	0.44217168	0.078147