# 1 Relationships of Angiosperms to Other Seed Plants

Seed plants are of fundamental importance both evolutionarily and ecologically. They dominate terrestrial landscapes, and the seed has played a central role in agriculture and human history. There are five extant lineages of seed plants: angiosperms, cycads, conifers, gnetophytes, and *Ginkgo*. These five groups have usually been treated as distinct phyla—Magnoliophyta (or Anthophyta), Cycadophyta, Coniferophyta, Gnetophyta, and Ginkgophyta, respectively. Cantino et al. (2007) used the following "rank-free" names (see Chapter 12): *Angiospermae*, *Cycadophyta*, *Coniferae*, *Gnetophyta*, and *Ginkgo*. Of these, the angiosperms are by far the most diverse, with ~14,000 genera and perhaps as many as 350,000 (The Plant List 2010) to 400,000 (Govaerts 2001) species. The conifers, with approximately 70 genera and nearly 600 species, are the second largest group of living seed plants. Cycads comprise 10 genera and approximately 300 species (Osborne et al. 2012; Fragnière et al. 2015). Gnetales consist of three morphologically disparate genera, *Gnetum*, *Ephedra*, and *Welwitschia* (~90 species total) that are so distinctive that each has been placed in its own family (Gnetaceae, Ephedraceae, and Welwitschiaceae). There is a single living species of *Ginkgo*, *G. biloba*. Each of these extant lineages has a rich fossil history (T. Taylor et al. 2009; Friis et al. 2011); we cover the fossil record of the angiosperms in more detail in Chapter 2 and also in those chapters focused on specific angiosperm clades (Chapters 4–10).

There are also many extinct lineages of seed plants (Crane 1985; Decombeix et al. 2010; E. Taylor and T. Taylor 2009; T. Taylor et al. 2009). Although extant gymnosperms appear to be monophyletic (below and Chapter 2), all gymnosperms (living and extinct) together are not monophyletic. Importantly, several fossil lineages, Caytoniales, Bennettitales, Pentoxylales, and Glossopteridales (glossopterids), have been proposed as putative close relatives of the angiosperms based on phylogenetic analyses (e.g., Crane 1985; Rothwell and Serbet 1994; reviewed in Doyle 2006, 2008, 2012; Friis et al. 2011). These fossil lineages, sometimes referred to as the para-angiophytes, will therefore be covered in more detail later in this chapter. Another fossil lineage, the corystosperms, has been proposed as a possible angiosperm ancestor as part of the "mostly male hypothesis" (Frohlich and Parker 2000), but as reviewed here, corystosperms usually do not appear as close angiosperm relatives in phylogenetic trees.

The seed plants represent an ancient radiation, with the first seeds appearing near the end of the Devonian (~370 million years ago; mya). By the Early to Middle Carboniferous, a diversity of seed plant lineages already existed (e.g., *Cordaites* and walchian conifers; Thomas 1955; Bhatnagar and Moitra 1996; Kenrick and Crane 1997; Davis and Kenrick 2004; T. Taylor et al. 2009). Heterospory, prerequisite to evolution of the seed, evolved in parallel in different major clades, including lycophytes, water ferns (e.g., *Marsilea*), sphenophytes, and aneurophytes, and seed-like structures, with a retained endosporic megagametophyte nearly surrounded by an integument-like covering, occur in some lycophytes (e.g., *Lepidocarpon*). *Lepidocarpon* is not considered a true seed, but is an example of convergence. Importantly, phylogenetic analyses that include the five clades of living seed plants show that they indeed form a clade, indicating that all have inherited seeds from a common ancestor—and that these seeds did not evolve in parallel. Phylogenetic analyses including extinct seed plants also place these groups in the same clade as extant seed plants (see below). Thus, analyses support the hypothesis that fossil and extant seed plants (Spermatophyta) had a single origin.

The first seed-like structures, observed in the Late Devonian to early Carboniferous, apparently ancestral to true seeds, had free integumentary lobes and lacked a micropyle; the pollen-receptive structure, lagenostome, was formed by the nucellus rather than the integument. The fusion of integumentary lobes, except for a micropylar channel, led to the formation of true seeds as in lyginopterid seed ferns.

The fossil record of conifers dates to the Late Carboniferous and that of true cycads to the Early Permian. Available data indicate that by the Permian (~299–251 mya), at least three (cycads, conifers, *Ginkgo*) of the five extant lineages of seed plants had probably diverged (Kenrick and Crane 1997; Donoghue and Doyle 2000). In contrast, the angiosperms are relatively young—their earliest unambiguous fossil evidence is from the Early Cretaceous (~130 mya) although molecular dating methods infer older dates for their origin (see Chapter 2).

Relationships among the lineages of extant seed plants, as well as the relationships of living groups to fossil lineages, have been issues of longstanding interest and debate. A topic of particular intrigue has been the closest relatives of the angiosperms. Angiosperms are responsible either directly or indirectly for the majority of human food and account for a huge proportion of photosynthesis and carbon sequestration. They have diversified to include 350,000–400,000 species in perhaps 130–170 myr and now occur in nearly all habitable terrestrial environments and many aquatic habitats. Understanding how angiosperms accomplished this is of fundamental evolutionary and ecological importance.

At some point, nearly every living and fossil group of gymnosperms has been proposed as a possible ancestor of the angiosperms (e.g., Wieland 1918; Thomas 1934, 1936; Melville 1962, 1969; Stebbins 1974; Meeuse 1975; Long 1977; Doyle 1978, 1998a,b; Retallack and Dilcher 1981; Crane 1985; Cronquist 1988; Crane et al. 1995; reviewed in Doyle 2006, 2008, 2012; Friis et al. 2011). Among extant seed plants, the relationship between angiosperms and Gnetales has received considerable attention.

Ascertaining the closest relatives of the angiosperms is not only of great systematic importance but also critical for assessing character evolution. For example, the outcome of investigations of character evolution among basal angiosperms, including studies focused on the origin and diversification of crucial angiosperm structures (e.g., floral organs, endosperm, vessel elements), may be influenced by those taxa considered their closest relatives. The effect of outgroup choice on the reconstruction of character evolution within angiosperms is readily seen via the widespread use of Gnetales as an outgroup for angiosperms. As reviewed below, for nearly two decades beginning in the 1980s, Gnetales were considered by many to represent the closest living relatives of the angiosperms. The use of Gnetales as an angiosperm outgroup profoundly influenced character-state reconstruction within the flowering plants (see "The Anthophyte Hypothesis" section).

Clarifying relationships among seed plants, both extant and fossil, has been extremely difficult. Factors that have contributed to the difficulties in phylogeny reconstruction of seed plants (living and extinct) include the great age of these groups and the considerable morphological divergence among them, as well as the extinction of many lineages. The tremendous morphological gap among extant and fossil seed plant lineages has complicated and ultimately compromised efforts to reconstruct relationships with morphology because of homoplasy and uncertainty about the homology of structures (e.g., Doyle 1998a, 2006, 2012; Donoghue and Doyle 2000; Soltis et al. 2005b, 2008b; Friis et al. 2011).

Although progress has been made in elucidating relationships among extant seed plants using DNA sequence data, relationships remain problematic. Even with the addition of more taxa and more genes representing all three plant genomes, issues remain. Resolution of relationships among extant seed plants with DNA sequence data has also been difficult because some lineages have relatively short branches (e.g., angiosperms or Pinaceae), whereas other clades (e.g., Gnetales) have long branches. This problem is further compounded by the presence in most analyses of long branches to the sister group of seed plants (monilophytes). In groups such as the angiosperms and conifers, more taxa can be added to break up long branches, but this is not possible across seed plants as a whole given the considerable extinction that has occurred. Another concern given the ancient divergences in seed plants is multiple substitutions per site leading to saturation of base substitutions. Hence, whereas the use of morphological characters has been criticized in seed plant phylogeny (and in a global sense by Scotland et al. 2003), DNA has its own problems and certainly has not been a consistent solution to resolve relationships among extant seed plants (see Burleigh and Mathews 2004, 2007; Mathews 2009; Mathews et al. 2010; Soltis et al. 2005b, 2008b).

As stressed by other investigators, a complete understanding of seed plant phylogeny is not possible without the integration of fossils. Many investigations have attempted this integration (e.g., Crane 1985; Doyle and Donoghue 1986; Doyle 1996, 1998a,b, 2001, 2006, 2008, 2012; Frohlich 1999; Donoghue and Doyle 2000; Hilton and Bateman 2006; Magallón 2010); we cover these analyses in more detail later in this chapter. Despite these efforts, the integration of fossils into studies of seed plant phylogeny remains an area where more research is needed. Seed plant relationships and the closest relatives of the angiosperms have been the focus of many reviews (e.g., Crane 1985; Doyle and Donoghue 1986; Doyle 1996, 1998a, b, 2001, 2006, 2008, 2012; Frohlich 1999; Donoghue and Doyle 2000; Mathews 2009; Friis et al. 2011) and continue to spawn new analyses (e.g., Hilton and Bateman 2006; Doyle 2008, 2012; Magallón 2010; Mathews et al. 2010). We will consider seed plant relationships in general (living and extinct), but a major focus of this chapter is discussing the closest relative(s) of the angiosperms.

## PHYLOGENETIC STUDIES: EXTANT TAXA

We first review the considerable effort devoted to reconstructing the phylogeny of living seed plants. Given the immense debate regarding the relationships of Gnetales, we also provide a brief history of the placement of Gnetales relative to the angiosperms. We then focus on cladistic analyses that include fossil as well as extant seed plants.

Molecules and morphology have so far yielded different conclusions about the relationships of Gnetales and angiosperms. Whereas analyses of morphology have consistently placed Gnetales sister to angiosperms (but see review by Rothwell et al. 2009), molecular data support alternative placements (see below). We are strong advocates for the use of morphology in phylogenetic analyses. However, based on the morphological characters so far used, the coding employed, and analyses now available, one could legitimately conclude that to this point seed plants represent an example in which cladistic analyses of morphological characters alone have failed to resolve major relationships in congruence with molecular-informed analyses.

## PLACEMENT OF GNETALES

A close relationship of angiosperms and Gnetales was first proposed by Wettstein (1907) and by Arber and Parkin (1908) based on several shared features: vessels, net-veined leaves (present in *Gnetum* as well as angiosperms), and “flower-like” reproductive organs (Fig. 1.1) (see also reviews by Doyle 1996; Frohlich 1999). However, the reasoning that Wettstein (1907) and Arber and Parkin (1908) each used to explain the close relationship of Gnetales and angiosperms differed dramatically. Wettstein (1907) proposed that Gnetales were ancestral to the angiosperms based on the view that the formerly recognized angiosperm group Amentiferae, a group that included wind-pollinated taxa such as Juglandaceae, Betulaceae, and Casuarinaceae, are the most “primitive” living angiosperms. We stress throughout that which extant group exhibits the most “primitive” morphological traits and which is sister to all others are not equivalent, but these statements are often confounded. We can infer ancestral character states via character-state reconstruction using the best estimate of phylogeny, as we have done throughout (Chapter 6). Wettstein maintained that the distinctive inflorescences (termed aments) of Amentiferae, consisting of simple, unisexual flowers, are homologous with the unisexual strobili of Gnetales. Arber and Parkin (1908) also proposed a close relationship of angiosperms and Gnetales, but, in contrast, argued that the reproductive structures of Gnetales are not primitively simple, but reduced, derived from ancestral lineages having more parts.

By the mid-1900s, most authors no longer considered Gnetales and angiosperms closest relatives. Bailey (1944b, 1953) noted that the vessels in the two groups are derived from different kinds of tracheids and hence are not homologous. In addition, Gnetales bear ovules directly on a stem tip, whereas in angiosperms, the ovules are produced within the carpel, the latter structure possibly representing a modified leaf. Views on the earliest angiosperms also changed, with Magnoliaceae and other angiosperms with large, strobiloid flowers considered most ancient, whereas the simple flowers found in Amentiferae were considered secondarily reduced rather than ancestrally simple (e.g., Arber and Parkin 1907; Cronquist 1968; Takhtajan 1969). This new view disrupted the link between Gnetales and angiosperms (via a basal Amentiferae) envisioned by Wettstein.

Issues became more complex when Eames (1952) proposed that the three lineages of Gnetales were not monophyletic. Eames considered *Ephedra* to be related to the fossil group Cordaites and conifers while *Gnetum* and *Welwitschia* were thought to be closer to another extinct lineage of seed plants, Bennettitales. Although morphology and DNA later confirmed the monophyly of Gnetales (below), the work of Eames (1952) shifted interest away from Gnetales as an angiosperm relative. Concomitantly, paleobotanists focused attention on fossils such as *Caytonia* and Glossopteridales as the closest relatives of angiosperms

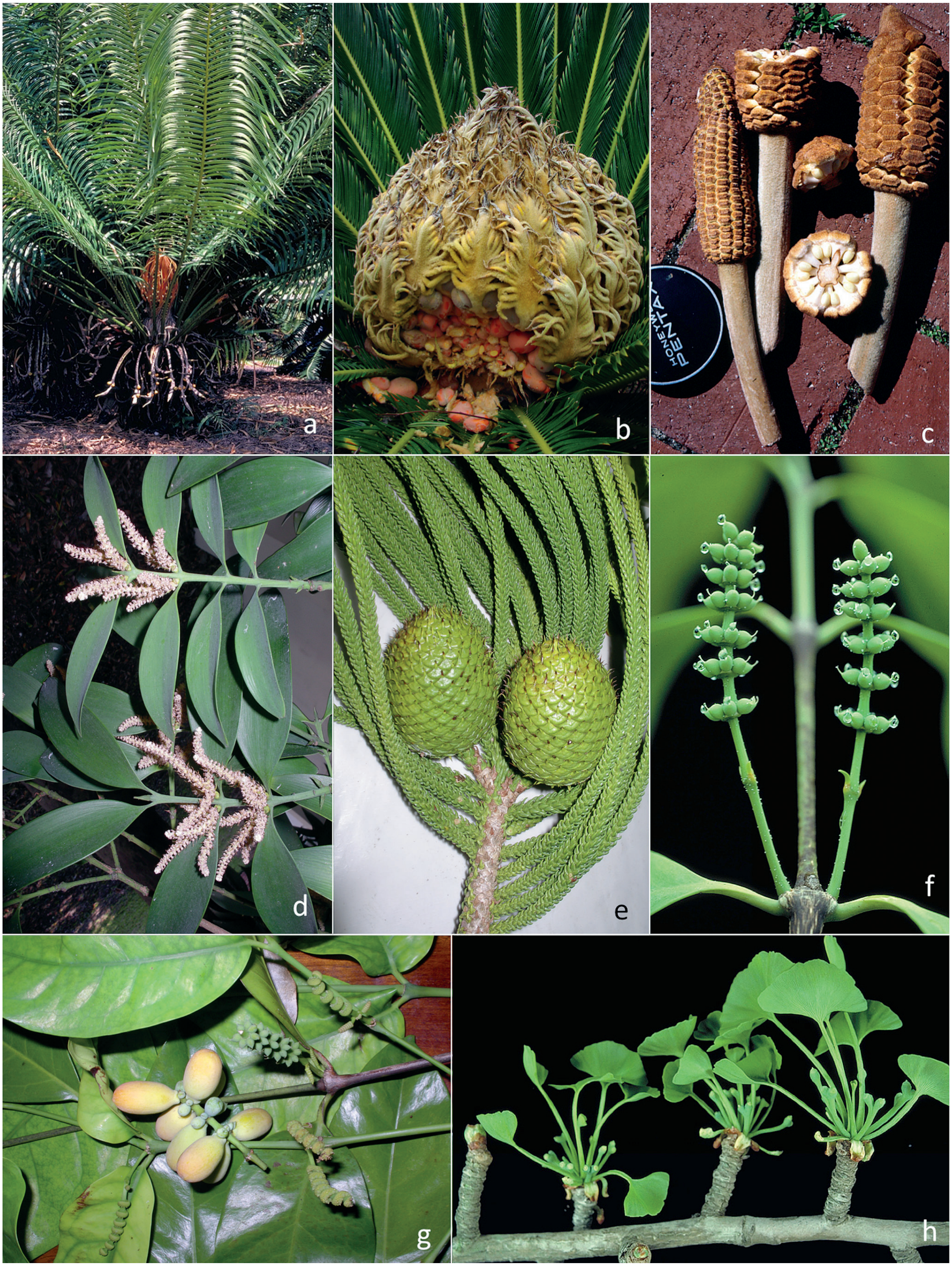

**Figure 1.1.** Extant gymnosperms: Araucariaceae, Cycadaceae, Ginkgoaceae, Gnetaceae, Podocarpaceae, and Zamiaceae. a. *Cycas circinalis* L. (Cycadaceae), whole plant with megasporophylls. b. *Cycas circinalis*, leaflike megasporophylls and pinnately compound leaves. c. *Zamia furfuracea* L. (Zamiaceae), three ovulate strobili. d. *Nageia nagi* Kuntze (Podocarpaceae), simple microsporangiate strobili and multi-veined leaves. e. *Araucaria subulata* Vieill. (Araucariaceae), female “cones.” f. *Gnetum gnemon* L. (Gnetaceae), compound ovulate “cones.” g. *Gnetum gnemon*, with multi-veined leaves, young compound ovulate “cones,” mature compound ovulate “cones,” and young compound microsporangiate “cones” from another plant. h. *Ginkgo biloba* L. (Ginkgoaceae), short shoots with foliage and young ovules.

(Doyle 1996; Frohlich 1999; see below), further diverting attention from Gnetales as possible close relatives of the angiosperms. Gnetales re-emerged, however, as putative close relatives of angiosperms when cladistic approaches were first used to investigate seed plant relationships (below).

## THE ANTHOPHYTE HYPOTHESIS

Seed plant relationships were first assessed by cladistic methodology using morphological characters in the 1980s. Several of these early studies included both extant and fossil taxa (e.g., Crane 1985; Doyle and Donoghue 1986). These studies revealed that the three morphologically disparate members of Gnetales (*Ephedra*, *Gnetum*, and *Welwitschia*) are monophyletic (illustrated in Fig. 1.1), a finding now well supported by both morphology and molecules. Only Nixon et al. (1994) found Gnetales not to be monophyletic. However, Doyle's (1996) subsequent reanalysis of the data used in Nixon et al. (1994) found a monophyletic Gnetales.

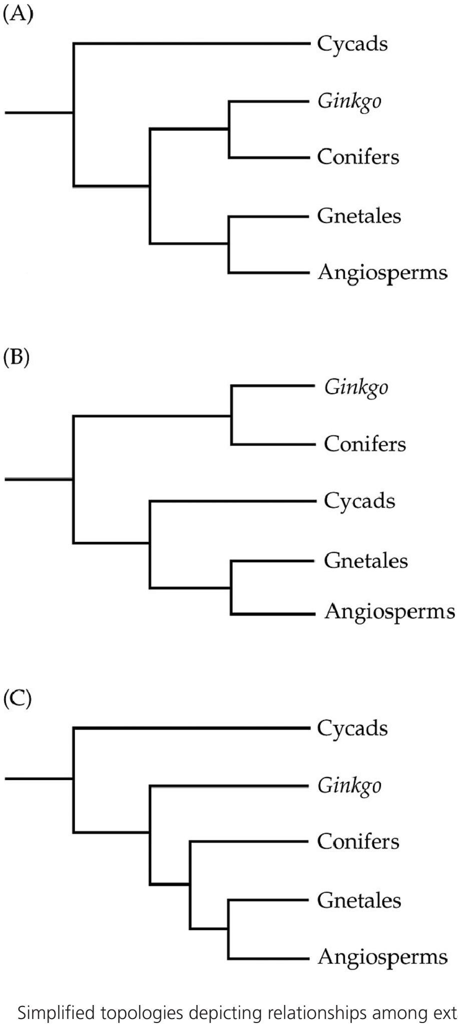

**Figure 1.2.** Simplified topologies depicting relationships among extant seed plants based on phylogenetic analyses of morphological data. Fossil taxa have been removed from these topologies. (A) Parenti (1980); Crane (1985); Doyle and Donoghue (1986, 1992); Doyle (1996). (B) Doyle and Donoghue (1986, 1992); Doyle (1996). (C) Loconte and Stevenson (1990).

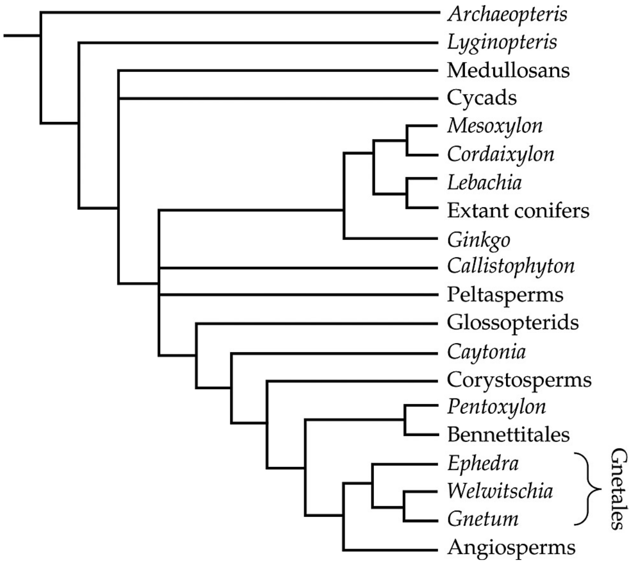

**Figure 1.3.** One of two shortest trees recovered by Crane (1985) in a cladistic analysis of extant and fossil seed plants (representing the "preferred topology" of Crane 1985). Redrawn from Crane (1985).

Early phylogenetic studies relying on morphological characters (Parenti 1980; Hill and Crane 1982; Crane 1985; Doyle and Donoghue 1986) recovered Gnetales as the closest living relatives of angiosperms (Fig. 1.2). Perhaps the best known is Crane (1985), which also included fossil seed plants. Crane (1985) recovered Gnetales as the sister group to angiosperms (Fig. 1.3). Subsequent phylogenetic analyses of morphological characters (e.g., Loconte and Stevenson 1990; Doyle and Donoghue 1992; Doyle 1994, 1996; Hilton and Bateman 2006), some of which also included fossils, continued to recover this Gnetales + angiosperm relationship (Fig. 1.2); as summarized by Rothwell et al. (2009; p. 296), "the anthophyte topology of the seed plant tree continues to be supported by morphological analyses of living and extinct taxa."

However, these same early cladistic studies often differed in the relationships suggested among extant seed plants (see Fig. 1.2). In morphological cladistic analyses, extant gymnosperms do not form a clade distinct from the angiosperms, and the positions of some lineages were unstable. Considering extant seed plant lineages, Crane (1985) found that cycads are sister to other extant seed plants and that conifers + *Ginkgo* form a clade that is sister to angiosperms + Gnetales (Fig. 1.2A). In contrast, the shortest trees of Doyle and Donoghue (1986) indicated that conifers + *Ginkgo* are sister to a clade in which cycads are the sister to angiosperms + Gnetales (Fig. 1.2B). Loconte and Stevenson (1990) found cycads followed by *Ginkgo*, then conifers, to be subsequent sisters to Gnetales + angiosperms (Fig. 1.2C).

Crane (1985) conducted two cladistic analyses of extant and fossil seed plants and in one analysis recovered a clade of Bennettitales, *Pentoxylon*, and Gnetales + angiosperms; a second analysis recovered a clade of Glossopteridales, *Caytonia*, corystosperms, Bennettitales + *Pentoxylon*, and Gnetales + angiosperms (Fig. 1.3). Doyle and Donoghue (1986, 1992) similarly found shortest trees in which Gnetales and angiosperms appeared in a clade with the fossil taxa Bennettitales and *Pentoxylon*. However, when fossil lineages were considered, Gnetales were not the immediate sister group to angiosperms (Fig. 1.4). Angiosperms and then *Pentoxylon* and Bennettitales were subsequent sisters to Gnetales (Fig. 1.4). Doyle and Donoghue (1986) named this clade of angiosperms, Gnetales, Bennettitales, and *Pentoxylon* the "anthophytes" in reference to the flower-like reproductive structures shared by all members (see Fig. 1.1 for Gnetales and sections below for fossil groups). Rothwell and Serbet (1994) later recovered the same anthophyte clade. The repeated recognition of this clade resulted in the formulation of the anthophyte hypothesis—that angiosperms are sister to Gnetales within a clade that also included Bennettitales and *Pentoxylon*. As reviewed below, the anthophyte hypothesis subsequently influenced interpretation of character evolution in seed plants and thus had a profound and long impact on studies of angiosperm evolution.

Doyle and Donoghue (1992) and Doyle (1996) recovered Glossopteridales and then *Caytonia* as subsequent sisters to their anthophyte clade (see Doyle 1996). However, Doyle (1996) did not expand the definition of the anthophyte clade to include *Caytonia* or Glossopteridales. This ultimately resulted in confusion given that in most subsequent studies (reviewed below), phylogenetic analyses consistently recovered a revised or modified anthophyte clade that includes Glossopteridales, *Caytonia*, *Pentoxylon*, and Bennettitales as sister groups to the angiosperms, but with Gnetales more distantly related (e.g., Hilton and Bateman 2006; Magallón 2010; Doyle 2008, 2012). For clarity, we refer to this modified or revised anthophyte clade as the "para-angiophytes" (see Doyle 2012), encompassing Glossopteridales, *Caytonia*, *Pentoxylon*, Bennettitales, and angiosperms, but not Gnetales (see below). Hilton and Bateman (2006) refer to this same clade as the "glossophytes." Earlier (pre-cladistic) investigations had also pointed to most of these same fossil groups as close relatives of angiosperms. Arber and Parkin (1907) proposed that Bennettitales and angiosperms shared a common ancestor. Several early workers also suggested *Caytonia* as a close angiosperm relative (Thomas 1925; Harris 1941; Gaussen 1946; see also Stebbins 1974; Doyle 1978). Stebbins (1974) and Retallack and Dilcher (1981) pointed to similarities between Glossopteridales and angiosperms.

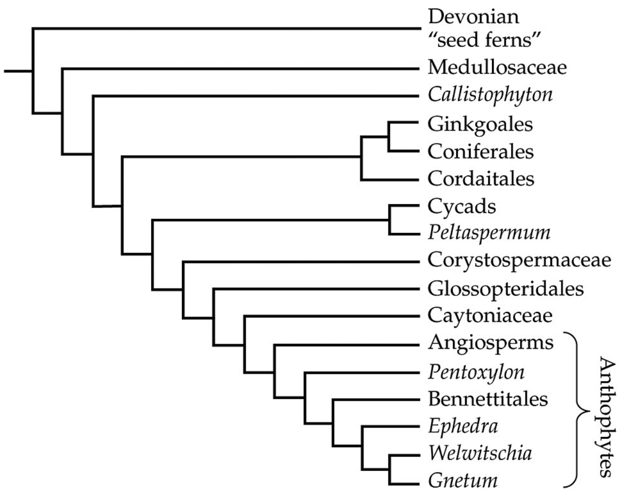

**Figure 1.4.** Shortest tree recovered by Doyle and Donoghue (1992). Note the composition of the anthophyte clade. Redrawn from Doyle and Donoghue (1992).

Although the anthophyte clade as originally defined by Doyle and Donoghue (1992) remained a focal point of study and controversy for about 15 years, the close relationship inferred between Gnetales and angiosperms was not well supported in any morphological cladistic analysis. Doyle and Donoghue (1986, 1992), for example, found topological differences in trees that were only one or two steps longer than the shortest trees they obtained (i.e., suboptimal trees). In some trees only one step longer than the shortest trees, angiosperms appeared as sister to *Caytonia* and Glossopteridales, rather than with Gnetales, Bennettitales, and *Pentoxylon*. In other one-step-longer trees, the anthophyte clade was retained, but relationships among anthophyte taxa varied (Doyle and Donoghue 1992; reviewed in Doyle 1996). In some studies, Gnetales appeared sister to the angiosperms even when data for fossils were included (e.g., Crane 1985), whereas in others (e.g., Doyle and Donoghue 1986) the sister relationship between Gnetales and angiosperms emerged only when the fossils were removed. Another fossil group that may deserve more attention is Erdtmanithecales, a fossil group putatively closely related to Gnetales or Bennettitales (Friis et al. 2007, 2011), although the group is contentious (Rothwell et al. 2009).

One limitation of early cladistic studies of morphology is that investigators often treated the angiosperms as a single terminal rather than employing multiple representatives. This approach required assumptions about the ancestral states of the angiosperms. Criticism of this approach prompted additional analyses in which several different, putatively basal angiosperm lineages were represented (e.g., Doyle et al. 1994; Nixon et al. 1994; Doyle 1996, 1998a). Although the sister relationship of Gnetales and angiosperms was again recovered in these analyses, strong bootstrap support for this relationship was still lacking, and suboptimal trees again included diverse topologies (the importance of suboptimal trees is noted in Chapter 3).

Despite the lack of internal support and some concerns regarding character homology, the anthophyte hypothesis quickly became widely accepted. Concomitantly, acceptance of the anthophyte hypothesis and a sister relationship between angiosperms and Gnetales had a profound and broad impact. This acceptance stimulated the reinterpretation of character evolution in angiosperms (reviewed in Frohlich 1999; Donoghue and Doyle 2000; Soltis et al. 2005b, 2008b; Doyle 2008, 2012), including the origin of the carpel, the angiosperm leaf (Doyle 1994, 1998a), and double fertilization (Friedman 1990, 1992, 1994). For example, the "double fertilization" process in Gnetales was considered a possible precursor to the double fertilization of angiosperms (Friedman 1990, 1992, 1994) and ultimately a putative synapomorphy for Gnetales + angiosperms (Doyle 1996).

## DEMISE OF THE ANTHOPHYTE HYPOTHESIS

Numerous studies (molecular and molecular + morphological) have tried to resolve the relationships among living seed plants—cycads, *Ginkgo*, Gnetales, conifers, and angiosperms—with the caveat that some analyses were much broader in scope, focusing on all land plants or all green plants (e.g., Hamby and Zimmer 1992; Albert et al. 1994; P. Soltis et al. 1999a; Goremykin et al. 1996; Malek et al. 1996; Chaw et al. 1997, 2000; Qiu et al. 1999; Bowe et al. 2000; Nickrent et al. 2000; Magallón and Sanderson 2002; D. Soltis et al. 2002b, 2005b; Burleigh and Mathews 2004; Rydin et al. 2002; Rai et al. 2008; Hajibabaei et al. 2006; Hilton and Bateman 2006; Wu et al. 2007; Zhong et al. 2010; Magallón 2010; Finet et al. 2010; Lee et al. 2011; reviewed in Doyle 2008, 2012). Although considerable progress has been made in resolving relationships (reviewed below), these studies yielded a diversity of results, which highlights the difficulties inherent in resolving relationships among extant seed plants, as well as seed plants in general (reviewed in Burleigh and Mathews 2004; Mathews 2009; Soltis et al. 2005b, 2008b; Doyle 2008, 2012). In this section, we summarize the many molecular phylogenetic analyses of seed plants and discuss the uncertain position of Gnetales, as well as our best current estimate of phylogeny among extant seed plants.

Numerous molecular and morphological phylogenetic studies have provided strong support for the monophyly of Gnetales, despite the pronounced morphological differences among the three genera *Ephedra*, *Gnetum*, and *Welwitschia* (e.g., Hamby and Zimmer 1992; Hasebe et al. 1992; Chase et al. 1993; Albert et al. 1994; Goremykin et al. 1996; Chaw et al. 1997, 2000; Stefanović et al. 1998; Hansen et al. 1999; Winter et al. 1999; Qiu et al. 1999; P. Soltis et al. 1999a,b; Bowe et al. 2000; D. Soltis et al. 2000, 2007c, 2011; Burleigh and Mathews 2004; Magallón 2010; Zhong et al. 2010). The fossil record has brought forth additional extinct genera, indicating a greater diversity of the Gnetales during the Mesozoic (Crane and Upchurch 1987; Friis et al. 2011). However, ascertaining the relationships of Gnetales to other seed plants, as well as determining seed plant relationships in general, has been more problematic. Nonetheless, a close relationship of angiosperms and Gnetales has not been recovered by molecular studies.

As single-gene phylogenetic trees began to appear, it became apparent that they did not support placement of Gnetales as sister to the angiosperms, although the position of Gnetales among other seed plant lineages varied from study to study. Single-gene investigations of plastid (ITS, *rpoC1*), nuclear (18S rDNA), and mitochondrial (*cox1*) sequences indicated a sister-group relationship between Gnetales and conifers (Fig. 1.5). Some analyses of *rbcL* alone and some analyses of 18S and 26S rRNA sequences placed Gnetales as sister to all other seed plants, with angiosperms as sister to the remaining gymnosperms (i.e., a clade of cycads, *Ginkgo*, and conifers; Hamby and Zimmer 1992; Albert et al. 1994; Fig. 1.5A). One parsimony analysis of *rbcL* placed angiosperms as sister to a clade of gymnosperms; within the latter clade, Gnetales were sister to cycads plus (*Ginkgo* + conifers) (Hasebe et al. 1992; Fig. 1.5B). Maximum likelihood analysis of *rbcL* also placed angiosperms as sister to the monophyletic gymnosperms, but relationships among gymnosperms were different than in the parsimony topology (Hasebe et al. 1992; compare Figs. 1.5C and 1.5B). A study using partial 26S rDNA data (Stefanović et al. 1998) did recover a topology with angiosperms sister to Gnetales, but subsequent analyses with more complete 26S rDNA data did not recover this same topology (e.g., Soltis et al. 2011).

Few of these single-gene studies provided strong internal support for relationships. As exceptions, two analyses of 18S rDNA sequences provided some support for a Gnetales + conifers sister-group relationship, with bootstrap percentages of 84 and 64, depending on size of the dataset (Fig. 1.5, E and F; Chaw et al. 1997; P. Soltis et al. 1999b). However, few other studies using single genes provided support > 60% for relationships (Fig. 1.5). Other early DNA sequence analyses provided additional evidence for the monophyly of the living gymnosperms and for a close

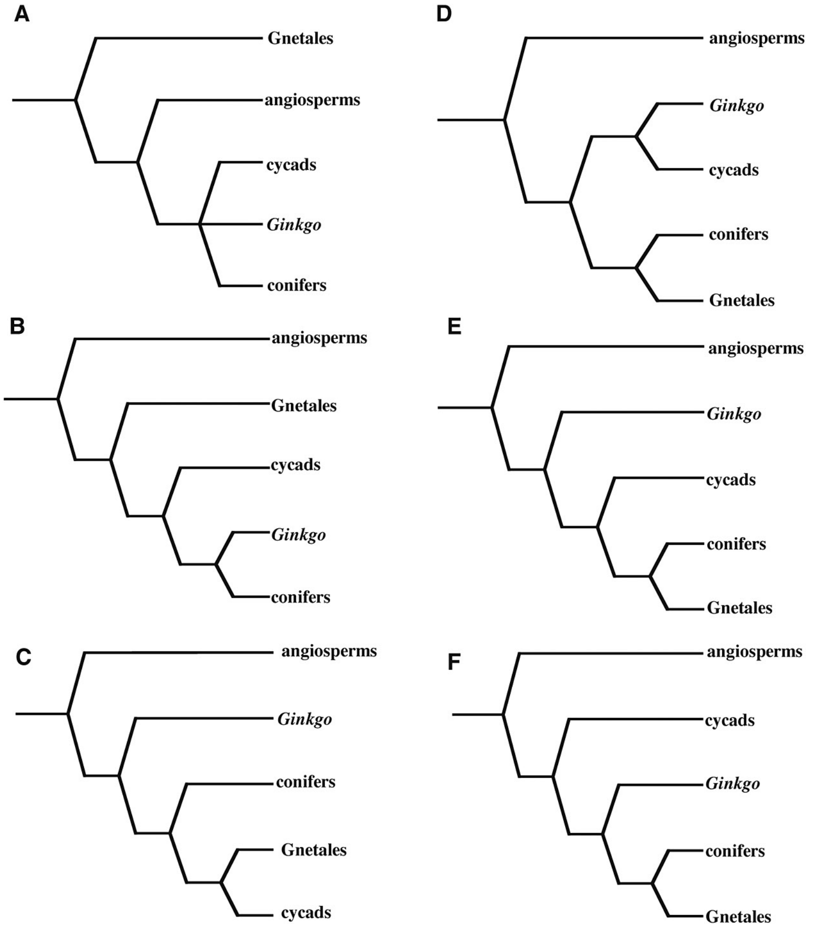

**Figure 1.5.** Simplified topologies depicting relationships among extant seed plants based on early phylogenetic analysis of gene sequence data using single genes showing diverse topologies. (A) rRNA sequence data, Hamby and Zimmer (1992); *rbcL*, Albert et al. (1994). (B) *rbcL* with parsimony, Hasebe et al. (1992). (C) *rbcL* with maximum likelihood, Hasebe et al. (1992). (D) Plastid ITS (= cpITS), Goremykin et al. (1996). (E) 18S rDNA, Chaw et al. (1997). (F) 18S rDNA, P. Soltis et al. (1999a).

relationship of Gnetales and conifers. However, taxon sampling in early studies was often sparse (e.g., Hansen et al. 1999; Winter et al. 1999; Frohlich and Parker 2000). For example, Hansen et al. (1999) obtained sequence data for a 9.5-kb portion of the plastid genome, but included only *Pinus*, *Gnetum*, and three angiosperms, and used *Marchantia* (a liverwort, distantly related to seed plants) as the outgroup.

Despite limitations of single-gene studies, the anthophyte hypothesis was not supported. However, because sample sizes were often small and internal support low, these analyses were considered equivocal (Doyle 1998a; Donoghue and Doyle 2000). Nonetheless, the results of these single-gene analyses posed a serious challenge to the widespread acceptance of the anthophyte hypothesis.

The lack of support for Gnetales + angiosperms increased with the addition of taxa and the use of multiple genes (see reviews by Doyle 2008, 2012). Analyses in which multiple genes were combined (Fig. 1.6) have repeatedly indicated, with strong support, that Gnetales are not closely related to angiosperms (e.g., Qiu et al. 1999; P. Soltis et al. 1999a; Bowe et al. 2000; Chaw et al. 2000; Graham and Olmstead 2000; Nickrent et al. 2000; D. Soltis et al. 2000, 2002b, 2007c, 2011; Pryer et al. 2001; Magallón and Sanderson 2002; Rydin et al. 2002; Burleigh and Mathews 2004, 2007; Rai et al. 2008; Hajibabaei et al. 2006; Wu et al. 2007; Zhong et al. 2010; S. A. Smith et al. 2010; Magallón 2010; Lee et al. 2011; Ruhfel et al. 2014). These molecular analyses resulted in the rapid demise of the anthophyte hypothesis. However, the placement of Gnetales varied among these analyses. Also, gymnosperm taxon sampling was sparse in studies aimed at the angiosperms (e.g., Qiu et al. 1999; P. Soltis et al. 1999a; D. Soltis et al. 2000, 2007c, 2011; Graham and Olmstead 2000) or vascular plants (Pryer et al. 2001), or all land plants (e.g., Qiu et al. 2007), or even all green plants (e.g., Wickett et al. 2014; Ruhfel et al. 2014).

To summarize, although many different topologies have now been obtained for extant seed plants, several features typically are recovered. Angiosperms are sister to extant gymnosperms; among gymnosperms, cycads or cycads plus *Ginkgo* are sister to the remainder. Placement of Gnetales remains problematic; four hypotheses typically emerge from multi-gene datasets (Fig. 1.6 A-D). Gnetales are 1) sister to all other seed plants, as in some analyses of D. Soltis et al. (2002b), Burleigh and Mathews (2004), and Magallón and Sanderson (2002); 2) sister to all conifers (Gnetifer hypothesis), as in some analyses of Chaw et al. (2000), some analyses of D. Soltis et al. (2002b), Rydin et al. (2002), and S. A. Smith et al. (2010); 3) within conifers, sister to Pinaceae (Gne-pine hypothesis, which was first seen in single-gene trees, such as Chaw et al. 1997; P. Soltis et al. 1999a), as in Qiu et al. (1999), Bowe et al. (2000), some analyses of D. Soltis et al. (2002b), some analyses of Burleigh and Mathews (2004), Chaw et al. (2000), Hajibabaei et al. (2006), Magallón (2010), some analyses of Zhong et al. (2010), and Wickett et al. (2014); and 4) within conifers, sister to cupressophytes or conifers other than Pinaceae (the Gne-cup hypothesis), as in Nickrent et al. (2000), some analyses of Zhong et al. (2010), Doyle (2006), and Ruhfel et al. (2014).

Other, more unusual, relationships based on DNA sequence data have sometimes been recovered for extant gymnosperms, such as cycads + angiosperms (Mathews et al. 2010). The molecular-only analysis of Magallón (2010) recovered a Gne-pine tree. However, a cycad + angiosperm relationship was obtained in a maximum parsimony total evidence analysis (DNA + morphology) of living and fossil seed plants, but only if fossils were subsequently removed from the tree (Magallón 2010) (Fig. 1.7; the fossils Glossopteridales, *Pentoxylon*, Bennettitales, and *Caytonia* are immediate sisters to angiosperms, followed by cycads). Several studies of numerous nuclear genes suggested a placement of Gnetales as sister to all other living gymnosperms, with angiosperms still sister to all living gymnosperms (de la Torre-Barcena et al. 2009; Lee et al. 2011). However, both analyses have important caveats (see below).

Most molecular phylogenetic studies (and molecular + morphology) suggest that Gnetales were derived from within conifers and are sister to Pinaceae (Gne-pine). Analyses of 18S rDNA alone first recovered this topology with BS > 50% (Chaw et al. 1997; P. Soltis et al. 1999b). Multi-gene analyses subsequently recovered this topology (Fig. 1.6), including Qiu et al. (1999, 2007), Bowe et al. (2000), Chaw et al. (2000), Burleigh and Mathews (2004, 2007), Magallón (2010), and Wickett et al. (2014). Bowe et al. (2000) analyzed a four-gene dataset (*rbcL*, 18S rDNA, and mitochondrial *atpA* and *cox1*) and found strong support for Pinaceae + Gnetales. Analysis of combined sequences of mitochondrial small subunit (SSU) rDNA, 18S

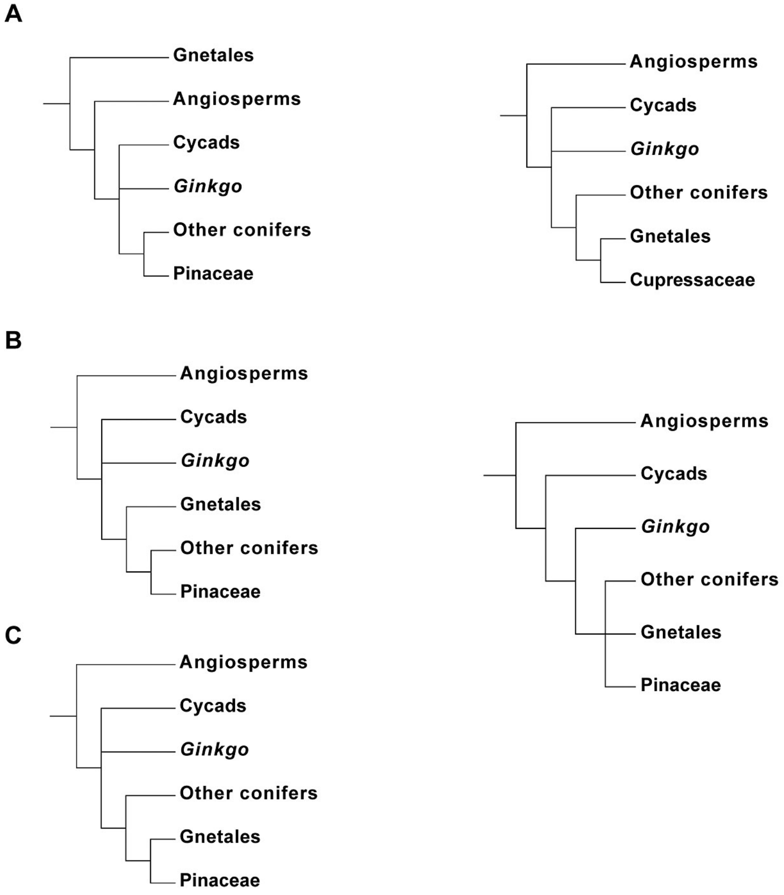

**Figure 1.6.** Summary of commonly recovered relationships among extant seed plants with an emphasis on the placement of Gnetales. A. Gnetales sister to other seed plants. B. Gnet-ifer topology with Gnetales sister to conifers. C. Gne-pine topology with Gnetales sister to Pinaceae. D. Gne-cup topology with Gnetales sister to Cupressaceae. E. Conservative seed plant summary tree.

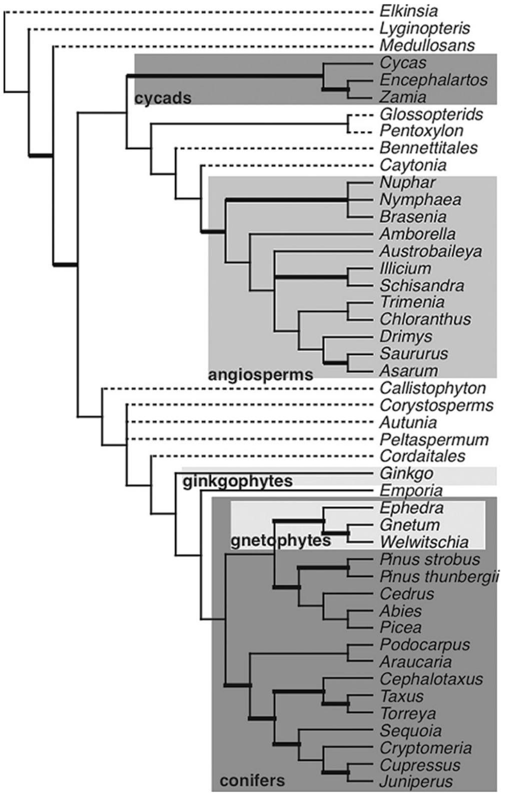

**Figure 1.7.** Total evidence tree (DNA + morphology) for seed plants based on analyses of Magallón (2010). Modified from Magallón (2010, Fig. 2).

rDNA, and *rbcL* similarly provided strong internal support for Gnetales + Pinaceae (Chaw et al. 2000; Fig. 1.6). An eight-gene analysis (D. Soltis et al. 2002b), involving four plastid genes, three mtDNA genes, and one nuclear gene, also provided strong support for Gnetales + Pinaceae. Qiu et al. (2007) examined seven plastid genes, three mtDNA genes, and nuclear 18S rDNA from 192 land plants and found Gnetales sister to Pinaceae, but with low support (BS = 67%). Burleigh and Mathews (2004) examined a 13-gene dataset representing all three genomes using various partitioning schemes and methods of analysis (see below). ML analyses of the combined datasets and from each partition recovered well-supported Gne-pine trees. Removal of rapidly evolving sites also favored Gne-pine trees.

Although multiple analyses of plastid, mitochondrial, and nuclear genes support the Gne-pine hypothesis, most molecular studies of seed plants included few conifers. With a dataset that included 15 conifers and 13 loci, Burleigh and Mathews (2004) found strong support for Gnetales + Pinaceae. However, Rydin et al. (2002), using 30 conifers and a four-gene dataset, found strong support for Gnetales as sister to conifers (Gnet-ifer hypothesis) (see Rydin and Korall 2009), suggesting that the Gne-pine hypothesis might be an artifact of inadequate taxon sampling. Furthermore, use of the parametric bootstrap in analyses of a dataset involving many base pairs but few taxa revealed that the placement of Gnetales as sister to conifers (rather than as sister to Pinaceae) could not be rejected (D. Soltis et al. 2002b).

A structural mutation in the plastid genome (Raubeson and Jansen 1992) also indicates that a Gnetales-conifer sister-group relationship may be a more parsimonious explanation of the data than a placement of Gnetales within conifers. Most land plants, including Gnetales, have two copies of the ribosomal genes in the plastid genome (the inverted repeat region), but conifers have only a single ribosomal coding region. Placement of Gnetales within conifers (e.g., either Gne-pine or Gne-cup) would necessitate that the ribosomal genes were lost in the conifers and then subsequently regained in Gnetales, which is less parsimonious. However, Wu and Chaw (2014) recently proposed independent losses of the inverted repeat in Pinaceae and cupressophytes.

Some fossil evidence also supports a close relationship of Gnetales and conifers (Wang 2004). However, most morphological and anatomical characters are so different between these groups that morphological data to this point have had little impact in resolving these relationships. Nonetheless, we stress that the Gne-pine hypothesis would necessitate either that the cone evolved twice (once in Pinaceae, and again in all other conifers) or that the cone was lost in Gnetales. The cone is a fairly complex morphological structure (with bracts associated with ovulate scales), and there is no indication that such a structure is present in the very different reproductive axes of Gnetales. Thus, in some ways morphology argues more strongly for the Gnetifer hypothesis.

The Gne-cup topology (Fig. 1.6) was recovered by Nickrent et al. (2000), Zhong et al. (2010), and Ruhfel et al. (2014). The last study employed nearly complete plastid genomes for 360 species of green plants, or *Viridiplantae*, including 311 seed plants. Ruhfel et al. (2014) found strong support for a placement of Gnetales with Cupressaceae (BS = 87%) and BS = 100% for a sister group of this Gne-cup clade with all remaining conifers. As a point of caution, however, Zhong et al. (2010) suggested in another analysis of plastid genomes (but with limited taxon sampling) that support for Gne-cup may be the result of long-branch attraction; by removing rapidly evolving proteins, support for Gne-cup decreased (see also Yang and Rannala 2012). Furthermore, by removing what they considered parallel substitutions between lineages leading to Gnetales and to *Cryptomeria* (the sole cupressophyte in their analyses), a Gne-pine topology was recovered by Zhong et al. (2010).

Trees based on large numbers of nuclear genes (de la Torre-Barcena et al. 2009; Lee et al. 2011; Wickett et al. 2014) merit more discussion. The first two of these studies recovered Gnetales sister to all other living gymnosperms—an unusual placement. However, de la Torre-Barcena et al. (2009) employed only 16 taxa. Using a large number of nuclear genes (22,833 sets of orthologs) from 101 land plant genera, Lee et al. (2011) found strong support for Gnetales sister to remaining all gymnosperms ([cycads + *Ginkgo*] + conifers); this gymnosperm clade is then sister to angiosperms. In another independent analysis of hundreds of nuclear genes, Wickett et al. (2014) found strong support for the monophyly of extant gymnosperms and for the Gne-pine hypothesis. The Lee et al. (2011) dataset is characterized by extensive missing data, whereas in Wickett et al. (2014), more data cells are filled; this difference could result in different topologies.

Resolving seed plant relationships with DNA data is difficult because the signal in datasets may be complex. Sanderson et al. (2000), Magallón and Sanderson (2002), and Rydin et al. (2002) reported conflict between first and second versus third codon positions in plastid genes. Although third codon positions of plastid genes generally have most of the phylogenetic signal (e.g., Källersjö et al. 1998; Olmstead et al. 1998), the third positions may be saturated in some instances (Rydin et al. 2002), depending on taxon sampling. These results may also reflect short branches within the seed plant radiation, as well as high rates of molecular evolution in Gnetales and the outgroups (reviewed in Palmer et al. 2004). Adding to the complexity of the conflict between first plus second versus third positions is the fact that transitions within each codon position conflict with transversions (Chaw et al. 2000; Rydin et al. 2002). Conflicting signal in the datasets could also be explained by differences in trees obtained with rapidly versus slowly evolving sites. Burleigh and Mathews (2004) found that trees in which Gnetales are sister to all other seed plants appear to be the result of signal in the most rapidly evolving sites, whereas when these sites are excluded, Gne-pine trees are obtained.

Given the diversity of studies, in both genes and taxa, as well as the diversity of results, can we make any firm statements regarding the position of Gnetales? In addition, what do most analyses suggest regarding extant seed plant relationships in general? Most analyses now favor some type of a relationship of Gnetales with conifers—a close relationship with angiosperms can be ruled out. Many analyses, including those of numerous nuclear genes (e.g., Wickett et al. 2014), as well as studies involving particularly rigorous examination of the underlying molecular data and signal (e.g., Burleigh and Mathews 2004), appear to favor Gne-pine. However, the largest plastid datasets so far employed favor Gne-cup (e.g., Ruhfel et al. 2014), so different genomes may be telling different stories.

Can we provide any firm summary regarding the overall picture of relationships among extant seed plants (see Fig. 1.6)? It is clear that angiosperms appear sister to a clade of extant gymnosperms. Within living gymnosperms, cycads and *Ginkgo* are then sisters to conifers + Gnetales, but the exact placement of Gnetales to the conifers remains unclear. Similarly, the relationship between cycads and *Ginkgo* also remains uncertain. Some analyses indicate that cycads and *Ginkgo* are successive sisters to other living gymnosperms (e.g., Burleigh and Matthews 2004, 2007; Graham and Iles 2009; Nickrent et al. 2000; Qiu et al. 2007; Ran et al. 2010), often with strong internal support. Other analyses indicate, however, that cycads and *Ginkgo* form a clade that is sister to other living gymnosperms. Internal support for the latter relationship is weak in some studies (e.g., Rydin and Källersjö 2002; Qiu et al. 2006), but in other multigene analyses it is very strong (e.g., Wu et al. 2007; Finet et al. 2010; Zhong et al. 2010; Lee et al. 2011; Ruhfel et al. 2014; Wickett et al. 2014). In still other studies, cycads and *Ginkgo* form a trichotomy with a clade of other living gymnosperms (Rydin and Korall 2009). Accordingly, we have depicted the relationship of cycads and *Ginkgo* as uncertain in a conservative summary tree (Fig. 1.6E).

To summarize, a conservative phylogenetic tree for extant seed plants (Fig. 1.6E) reveals major uncertainties. In fact, this overall summary tree is comparable to what was depicted more than a decade ago (Soltis et al. 2005b). Resolving seed plant relationships and the placement of Gnetales remains problematic, despite intensive study. As a caveat, although the number of base pairs included in seed plant analyses has steadily increased, taxon sampling has remained low in most of the analyses conducted to date, with a few noteworthy exceptions. This sparse sampling should be remedied in future studies. More problematic is the effect of taxa that cannot be sampled for DNA due to their extinction—a real problem in that most seed plant clades are extinct.

Whereas living gymnosperms consistently appear monophyletic, gymnosperms as a whole (fossil and extant) are clearly paraphyletic (Figs. 1.6–1.9; Doyle 2006, 2012). Some extinct gymnosperm lineages attach along the branch to angiosperms, whereas others attach near the base of the seed plant tree. In recent analyses, an "acrogymnosperm" clade (*Acrogymnospermae*; see Cantino et al. 2007) is recovered that includes some fossil gymnosperms as well as extant gymnosperms; a second subclade (sometimes called para-angiophytes) contains angiosperms and other

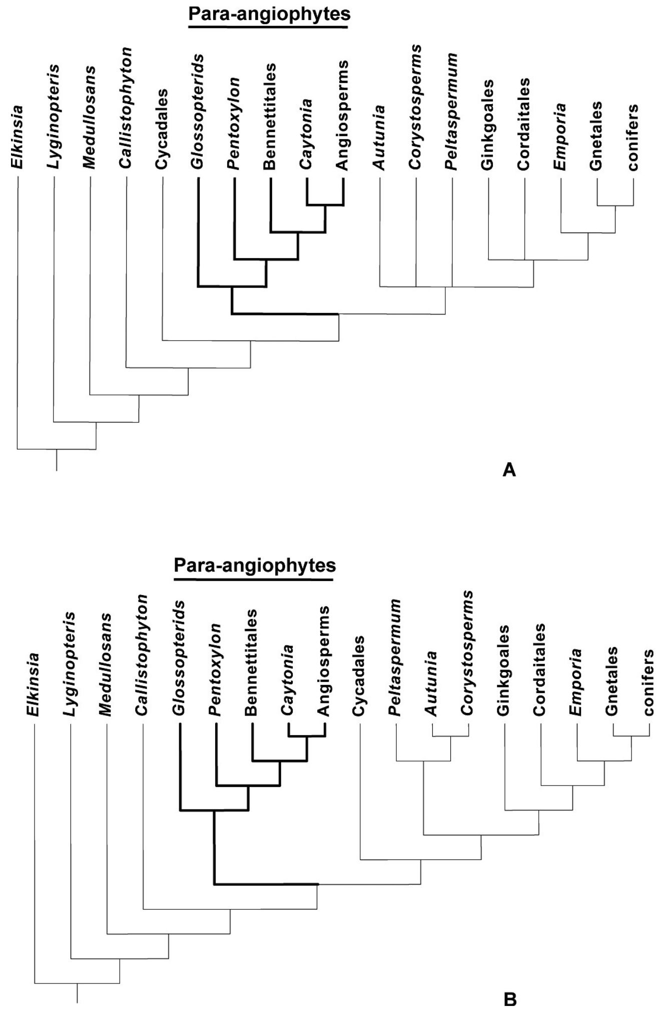

**Figure 1.8.** Revised views of the phylogeny of seed plants showing putative closest relatives of angiosperms obtained by using both the morphological matrix of Doyle (1996) and molecular data (reviewed in Soltis et al. 2005). A revised anthophyte clade—the para-angiophytes—is depicted. (A) Tree modified from Soltis et al. (2005), in which molecular data for seed plants are combined with the morphological matrix of Doyle (1996). (B). Tree modified from Doyle (2001), in which a molecular constraint was used, placing Gnetales with other extant gymnosperms. Redrawn from Soltis et al. (2008; Fig. 1).

fossil gymnosperms (e.g., Caytoniales) (Figs. 1.8–1.9). However, based on analyses conducted to date, fossil and seed plants as a whole (Spermatophyta) are monophyletic.

## MORPHOLOGY REVISITED

Whereas cladistic analyses of morphological data have indicated that angiosperms and Gnetales are closest living relatives (albeit without strong support), most studies of DNA sequences and DNA plus morphology have placed Gnetales with conifers despite their obvious morphological differences. Doyle (1998a) initially attempted to reconcile this conflict, but as more molecular studies argued against a close relationship between Gnetales and angiosperms, such efforts diminished (Donoghue and Doyle 2000).

It is instructive to reconsider those few characters that were thought to unite angiosperms and Gnetales in morphological cladistic analyses (Crane 1985; Doyle and Donoghue 1987, 1992; Loconte and Stevenson 1990; Taylor and Hickey 1992; Nixon et al. 1994; Doyle 1996). Some of these features are actually shared by angiosperms, Gnetales, Bennettitales, *Pentoxylon*, and Caytoniales. As reviewed below, careful scrutiny leads to the conclusion that the homology of many of these shared characters is, in fact, dubious.

Following Crane (1985), two key features unite angiosperms, Gnetales, and Bennettitales and *Pentoxylon*: a distinctively thin megaspore membrane and microsporophylls aggregated in a whorl, or pseudo-whorl, distinct from the helical arrangement in conifer and cycad pollen cones. Doyle and Donoghue evaluated the relationship between Gnetales and angiosperms in several papers (e.g., Doyle 1978, 1994, 1996, 1998a,b; Doyle and Donoghue 1986, 1992); we summarize here the non-DNA characters that support a sister group of Gnetales + angiosperms in Doyle's (1996) analysis. Angiosperms and Gnetales share similar lignin chemistry (i.e., the presence of a Mäule reaction, which is absent from other seed plants; McLean and Evans 1934; Gibbs 1957), double fertilization, microsporangia fused at least basally, an embryo derived from a single uninucleate cell via cellular divisions, a thin megaspore wall (as in Crane 1985 and Loconte and Stevenson 1990), siphonogamy, and a granular exine structure. Doyle (1996) also scored vessels in angiosperms and Gnetales as homologous. Loconte and Stevenson (1990) analyzed only extant taxa and provided three synapomorphies of Gnetales and angiosperms—thin megaspore wall (following Crane 1985), short cambial initials, and lignin syringial groups (equivalent to the Mäule reaction of Doyle 1996).

Some putative synapomorphies for angiosperms and Gnetales from Crane (1985), Doyle (1996), and Loconte and Stevenson (1990) are more complex than initially suggested in these analyses and may in fact not be homologous (see also Donoghue and Doyle 2000). For example, although the angiosperms and Gnetales were coded the same for the presence of a tunica layer in the vegetative shoot apex, the tunica is two cells thick in many angiosperms and only one cell thick in Gnetales. Similarly, although angiosperms and Gnetales were coded as having the same state for the thickness of the megaspore wall, the megaspore wall is thin in Gnetales and absent in angiosperms. The pollen exine character used in some studies is now known to be inappropriate because a granular exine is not ancestral in angiosperms, as once was hypothesized (e.g., Doyle and Endress 2000; Doyle 2001, 2009; see also Chapter 6). Furthermore, the homology of vessels in angiosperms and Gnetales has long been doubted (Bailey 1944b, 1953), and Carlquist (1996) concluded that they are not homologous. Angiosperms and Gnetales should therefore not be scored identically for these features of the tunica, megaspore, and vessel elements. The homology of double fertilization in angiosperms and Gnetales has also been questioned (see Friedman 1994, 1996; Doyle 1996, 2000), but this issue is complex. In most angiosperms, a second sperm nucleus fuses with two nuclei of the megagametophyte (producing triploid endosperm), whereas in Gnetales a second sperm fuses with only one nucleus of the megagametophyte, yielding a diploid nucleus. However, in the basal angiosperm clades Nymphaeales and Austrobaileyales (but not Amborellaceae), a second sperm nucleus fuses with only a single megagametophyte nucleus (Chapter 4). Furthermore, double fertilization events that seem similar to those documented for *Ephedra* (Gnetales) have been reported for conifers, including *Thuja* and *Abies* (Friedman and Floyd 2001). In addition, developmental events in cycads and *Ginkgo* are consistent with double fertilization (reviewed in Friedman and Floyd 2001). Thus, double fertilization may be a synapomorphy for all extant seed plants (Friedman and Floyd 2001), although the formation of endosperm is an exclusively angiosperm feature.

The anthophyte hypothesis continues to have an impact on interpretation of morphology. Friis et al. (2007) proposed a new synapomorphy for some of the traditional anthophytes, namely Gnetales and Bennettitales. Using phase-contrast X-ray microtomography, they found a distinctive seed architecture shared by Gnetales and Bennettitales (as well as Erdtmanithecales, another putatively closely related fossil group). Friis et al. (2007) argued that this seed feature "defines a clade containing these taxa." Nearly all recent topologies indicate a distant relationship of Gnetales and Bennettitales (Figs. 1.8, 1.9) so in our view this seed feature may best be interpreted as homoplasious, having evolved independently in Gnetales and the Bennettitales (Ertmanithecales might, however, share a common origin of the feature with either of them). However, phylogenetic analysis using the seed plant matrix of Hilton and Bateman (2006) supports the grouping of Erdtmanithecales, Bennettitales, and Gnetales (Friis et al. 2011, p. 104).

## INTEGRATING FOSSILS

We now revisit the possible close relatives of the angiosperms that are known from the fossil record. The importance of integrating fossils, and thus morphology, into datasets to understand the phylogeny of seed plants has long been noted and continues to be emphasized (e.g., Doyle 2006, 2008, 2012; Doyle and Donoghue 1987; Kenrick and Crane 1997; Donoghue and Doyle 2000; Rydin et al. 2002; D. Soltis et al. 2002b, 2005b; Crane et al. 2004; Magallón 2010; Hilton and Bateman 2006; Mathews et al. 2010). Even if DNA sequence data largely resolved relationships among living seed plant groups (this is not the case; see above), a complete understanding of seed plant relationships and the origins of angiosperm structures such as floral organs still requires the integration of fossil taxa because the extinct taxa can affect the phylogenetic placement of extant taxa. Relationships among lineages of anthophytes have varied among studies depending on whether or not fossils were included. In some cases, Gnetales were sister to the angiosperms even when fossils were included (e.g., Crane 1985); in other analyses, the sister relationship between Gnetales and angiosperms appeared only when fossils were removed from the matrix (e.g., Doyle and Donoghue 1986).

Significantly, most phylogenetic analyses that include fossils (whether morphology or morphology + DNA) reveal the same cast of characters as close, now extinct, relatives of the angiosperms: Glossopteridales, *Pentoxylon*, Bennettitales, and Caytoniales (D. Soltis et al. 2005b, 2008b), referred to as the para-angiophytes (Doyle 2012; Fig. 1.8). Initial studies of morphology alone placed Bennettitales and *Pentoxylon* as subsequent sisters to angiosperms + Gnetales (Crane 1985; Doyle and Donoghue 1986, 1992; Rothwell and Serbet 1994). Doyle (1996) found *Caytonia* sister to *Pentoxylon*, followed by Glossopteridales as sister to Gnetales + angiosperms. However, other studies suggest that Caytoniales are the immediate sister to angiosperms, with Bennettitales sister to this clade. Doyle (2001, 2008) and Soltis et al. (2005b) constructed a molecular scaffold on the basis of molecular phylogenetic analyses, constraining Gnetales to be sister to the conifers, and then analyzed Doyle's (1996) original morphological matrix. Both found a revised anthophyte clade of angiosperms + *Caytonia*, Bennettitales, and *Pentoxylon*, with Glossopteridales sister to the remaining members of this expanded anthophyte clade (Fig. 1.8). Other studies using morphology (Hilton and Bateman 2006), and morphology + DNA (Magallón 2010) (Fig. 1.7), plus studies using a molecular backbone for the living taxa (fixing Gnetales in a Gne-pine position; Doyle 2008, 2012) (Fig. 1.9), also have recovered a para-angiophyte clade with *Pentoxylon* + Glossopteridales sister to Bennettitales, followed by Caytoniales, with the last the immediate sister to angiosperms (Fig. 1.9).

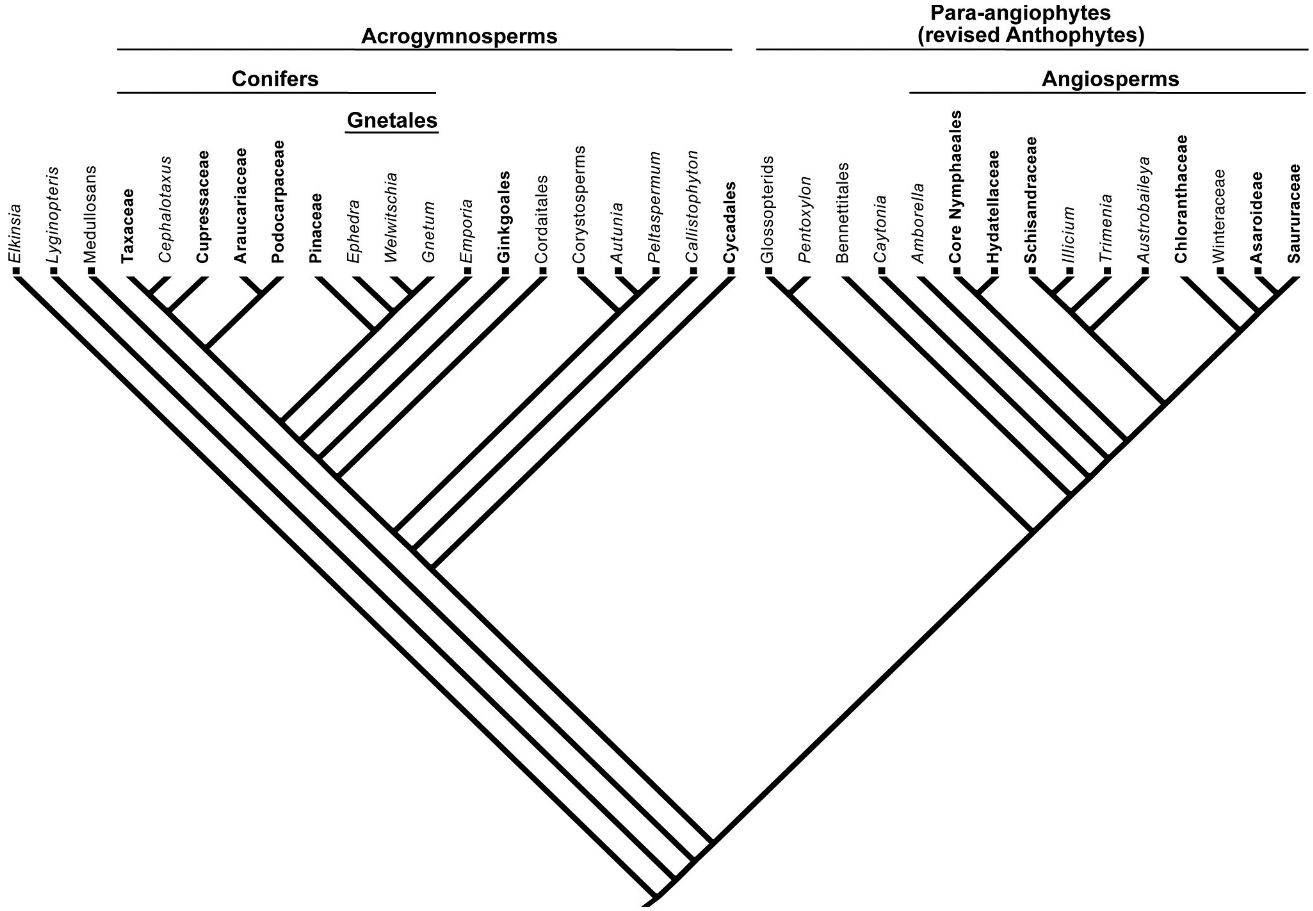

**Figure 1.9.** Seed plant phylogenetic tree (from analysis of morphological data of Doyle 2008) with the relationships of living gymnosperms constrained to show a Gne-pine backbone. Redrawn from Doyle (2012).

## PARA-ANGIOPHYTES: A NEW LOOK AT CHARACTER EVOLUTION

The phylogenetic results reviewed above are crucial for reconstructing morphological evolution of the angiosperms. Topologies that recover the para-angiophyte clade are consistent with the hypothesis that the cupule of Glossopteridales and *Caytonia* is homologous with the outer integument of the angiosperm bitegmic ovule (reviewed in Doyle 2006, 2008, 2012) (Figs. 1.10, 1.11), a hypothesis that had been proposed much earlier (e.g., Gaussen 1946; Stebbins 1974; Doyle 1978). To review, whereas angiosperm ovules generally have two protective layers (integuments), all gymnosperm ovules have a single integument (Fig. 1.10). [Some angiosperms, such as asterids, have only a single integument due either to loss of an integument or to fusion of the two integuments (Endress 2011a; see Chapters 11, 14).] Furthermore, the gymnosperm micropyle is located opposite the stalk bearing the ovule (except in Podocarpaceae), whereas in many angiosperms, the ovule is curved back on itself with the micropyle close to the stalk (i.e., anatropous rather than orthotropous; Fig. 1.10B). Angiosperm ovule types are reviewed in Chapter 4 (see Fig. 4.8).

To address the origin of the outer integument of the angiosperm ovule, Doyle (2012) reconstructed the evolution of the ovule-bearing surface across a phylogenetic tree for fossil and extant seed plants (Fig. 1.9); we summarize his findings here. In *Caytonia* and Glossopteridales, the ovule-bearing surface is the adaxial or upper surface, whereas in those other seed plants that have been considered angiosperm ancestors, such as peltasperms and corystosperms, ovules are borne on the abaxial surface (underside) (Fig. 1.11). Other seed plants bear ovules in an apical or marginal position. Hence, the reconstruction of Doyle (2012) is consistent with the hypothesis that the second or outer integument of angiosperms is homologous with the cupule in *Caytonia* and Glossopteridales—with the cupule in these plants representing a leaf or leaf segment with ovules on the upper surface.

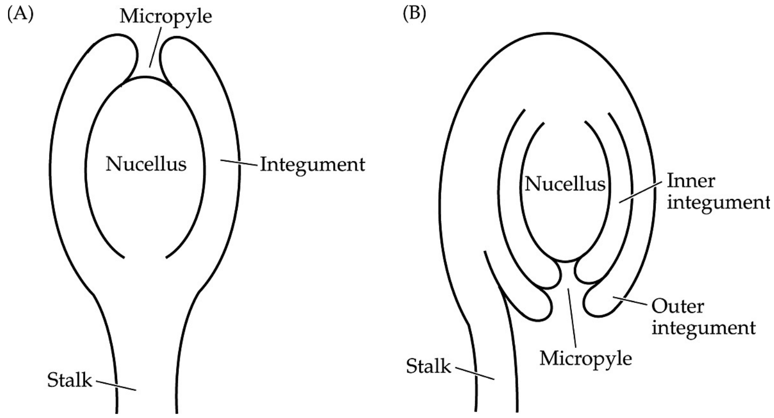

**Figure 1.10.** Ovules of gymnosperms and angiosperms. Gymnosperms (A) have one integument whereas angiosperms (B) have two—an inner and an outer integument.

The cupule of *Caytonia* is similar to the bitegmic ovule of flowering plants (Stebbins 1974; Doyle 2012). The shape of the *Caytonia* cupule resembles an anatropous ovule in angiosperms (compare Figs. 1.11, 1.12; see below). The *Caytonia* cupule contains multiple ovules, however, so ovule number would have been reduced to one with the origin of the angiosperms (see Stebbins 1974). Doyle (2012) further noted that in Glossopteridales, the cupule is "most easily interpreted" as a fertile leaf "borne on an axillary branch that is adnate to the subtending leaf"; in contrast, cupules in *Caytonia* have been interpreted as leaflets borne along the rachis of a compound leaf (see Stebbins 1974; Doyle 1978; Retallack and Dilcher 1981; Doyle 1978, 2012). Bennettitales are problematic, however, in that the female

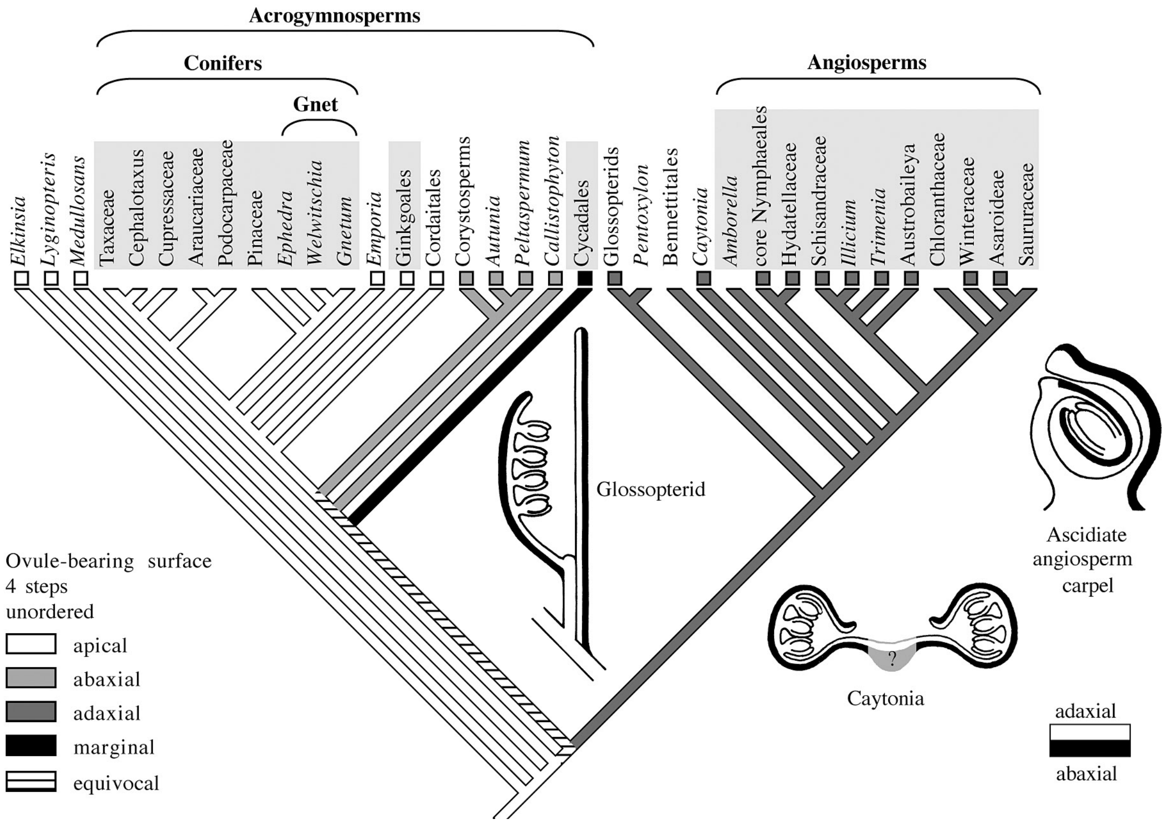

**Figure 1.11.** Putative reconstruction of ovule evolution using topology shown in Figure 1.9. Diagrams show ovulate structures in glossopeterids and *Caytonia* as well as an ascidiate angiosperm carpel. Abaxial surfaces are shown in black; ovules are borne on the adaxial surface in glossopterids, *Caytonia*, and angiosperms, but not in acrogymnosperms. Based on this reconstruction, the angiosperm bitegmic ovule and carpel may be derived from ovulate structures in glossopterids and *Caytonia*; see text (taken from Doyle 2012).

reproductive structures of this group show no clear relation to structures in these other plants (see Doyle 2012 and section on Bennettitales below).

Doyle (2008, 2012) also used these same phylogenetic results to attempt to infer the homology of the angiosperm stamen. Using the current "best" topology for angiosperms (chapter 3), which has Amborellaceae, Nymphaeales, and Austrobaileyales as basalmost branches, Endress and Doyle (2009) inferred that, in the earliest angiosperms, sporangia were originally located in a lateral or adaxial position. Doyle (2008, 2012) therefore hypothesized that the angiosperm stamen may be "comparable with male structures in Glossopteridales, which had a sporangium-bearing branch on the adaxial side of a leaf, or with those in Bennettitales, which had synangia on the adaxial side of a sporophyll" (Doyle 2012, p. 316) (see also illustrations later in this chapter).

## FOSSILS: IN SEARCH OF THE SISTER GROUP OF ANGIOSPERMS

We provide brief coverage of four fossil lineages that may be the closest relatives of angiosperms as inferred from cladistic analyses and/or noteworthy morphological similarities: Caytoniales, Bennettitales, Pentoxylales, and Glossopteridales. Traditionally, the search for fossil groups as possible angiosperm ancestors focused on features of the ovule and structures that may be homologous to the angiosperm carpel, although many other characters, such as pollen morphology, stem anatomy, and leaf venation patterns, are also important in phylogenetic considerations. Several lines of evidence indicate that the outer integument of angiosperms has a different origin from the inner integument. Developmental genetic studies indicate that the inner and outer integuments are under separate genetic control (see discussion of *INO* below and McAbee et al. 2006). Other possible evidence for this distinct origin is the mismatch of the two integuments seen in many basal angiosperms, producing a "zig-zag" micropyle (Davis 1967; Stebbins 1974). This feature is actually widely distributed in the angiosperms, occurring in diverse eudicots, including Bixaceae, various Brassicales, Fabaceae, Malvaceae, Hamamelidaceae, and Dilleniaceae (Endress 2011a). Another distinctive feature is that stomata are occasionally produced on the outer, but not the inner, integument (see Stebbins 1974; Corner 1976).

Developmental studies have shown that in many angiosperms, the outer integument is hood-shaped, indicating an origin from a leaf (Yamada et al. 2001a,b). The gene *INNER NO OUTER* (*INO*) participates in the regulation of dorsoventrality of lateral organs and is expressed in the abaxial side of leaves, as well as in the outer epidermis of the outer integument in *Arabidopsis* (e.g., Bowman 2000). This same expression pattern has been observed in ovules of *Nymphaea*, supporting the hypothesis that the outer integument is homologous with a leaf and that ovules are located on the adaxial surface, away from the zone of expression (Yamada et al. 2003). This differential pattern of *INO* gene expression in inner and outer integuments is consistent with separate origins of the two integuments of angiosperms.

Fossil groups such as Caytoniales, Glossopteridales, *Pentoxylon*, corystosperms, and Bennettitales have been proposed as possible close relatives of angiosperms because the ovule is surrounded by a "cupule," a structure that may be homologous to the outer integument of angiosperms. In Caytoniales (and also corystosperms, which are not close angiosperm relatives in phylogenetic studies), the cupule plus ovule structure is curved, which has been considered a possible antecedent of the anatropous ovule of angiosperms. Corystosperms produced ovules on the abaxial surface of the megasporophyll and therefore probably were not angiosperm ancestors. However, *Caytonia*, *Petriellaea* (a Mesozoic seed fern; Taylor et al. 1994), and *Glossopteris* (Taylor and Taylor 1992) produced ovules on the adaxial surface of a megasporophyll and, on this basis, could be angiosperm relatives (see Fig. 1.11). The seed-enclosing structures of *Petriellaea* and *Glossopteris* seem to have evolved via different structural modifications: in *Petriellaea* by transverse folding of the leaf and in some Glossopteridales by longitudinal enrolling of the leaf margin (Taylor and Taylor 1992). The folding of the megasporophyll in both fossils differs from the presumed origin of the angiosperm carpel via longitudinal folding of a megasporophyll (this assumes plicate carpel development—the situation may be different in ascidiate carpels; see Chapter 4). This leaves *Caytonia* as a possible ancestor or close relative of angiosperms.

### CAYTONIALES

Before morphological cladistic analyses placed Gnetales as sister to the angiosperms (Crane 1985; Doyle and Donoghue 1986), Caytoniales had already received considerable attention as a possible angiosperm ancestor (e.g., Thomas 1925; Gaussen 1946; Harris 1951; Stebbins 1974; Doyle 1978, 1996). Although not initially considered an "anthophyte" in early cladistic analyses (Crane 1985; Doyle and Donoghue 1986), Caytoniales emerged as part of a clade (the para-angiophytes; see above and Figure 1.8) that included anthophytes in later analyses (Doyle and Donoghue 1992), appearing in some analyses as sister to the angiosperms (Doyle 1996).

In Caytoniales, male and female reproductive structures do not appear to have been produced together; in fact, neither has been found attached to stems (Fig. 1.12). Caytoniales were considered a possible ancestor of the angiosperms because of their cupules and ovules. The morphology of Caytoniales seemed to explain the origin of both the two integuments and the anatropous ovule characteristic of most angiosperms. In Caytoniales, each cupule contained several ovules, and the cupule enclosed these ovules, leaving only a small opening between the ovule and the stalk to the cupule. Within the cupule, numerous unitegmic ovules were present, each with a micropyle oriented toward the opening (or mouth) of the cupule (Fig. 1.12). It was argued that if the *Caytonia* cupule contained only a single ovule rather than multiple ovules, then the resultant structure would resemble a typical angiosperm ovule in being anatropous and possessing two integuments (Gaussen 1946; reviewed in Stebbins 1974; Doyle 1978; Frohlich and Parker 2000).

One criticism of Caytoniales as a close angiosperm relative or precursor is that no Caytoniales fossils have been reported that possess just one ovule per cupule. The counter argument is that only a simple reduction in ovule number (from several to one) is required. Furthermore, the origin of the angiosperm carpel from Caytoniales cannot easily be explained because the ovules of the latter were located on opposite sides of a narrow stalk, a structure that is difficult to envision as forming a carpel (Fig. 1.12B). The hypothesis put forward was that this Caytoniales stalk became wide and flat and eventually enclosed the ovules, forming the angiosperm carpel (Gaussen 1946; Doyle 1978; Crane 1985). Another difficulty with Caytoniales as a possible angiosperm ancestor is that the microsporophylls in Caytoniales were highly divided, differing greatly in morphology from angiosperm stamens, and bore bisaccate pollen, the only similarity being the occurrence of four microsporangia per structural unit (Fig. 1.12A). Leaves of *Sagenopteris*, the foliage of *Caytonia*, bore leaflets with venation resembling that of *Glossopteris* leaves, lacking the multiple orders of venation typical of angiosperms. Because of the large morphological gap between Caytoniales and angiosperms, the hypothesis that Caytoniales are closely related to angiosperms remains problematic; it is not known whether caytonialean plants produced vessels in their xylem, as do most angiosperms, or lacked them, as do Glossopteridales. However, presence of vessels in an angiosperm relative no longer seems crucial given the absence of vessels in some early-branching angiosperms (Chapter 4). Nevertheless, this group consistently appears close to angiosperms in phylogenetic analyses.

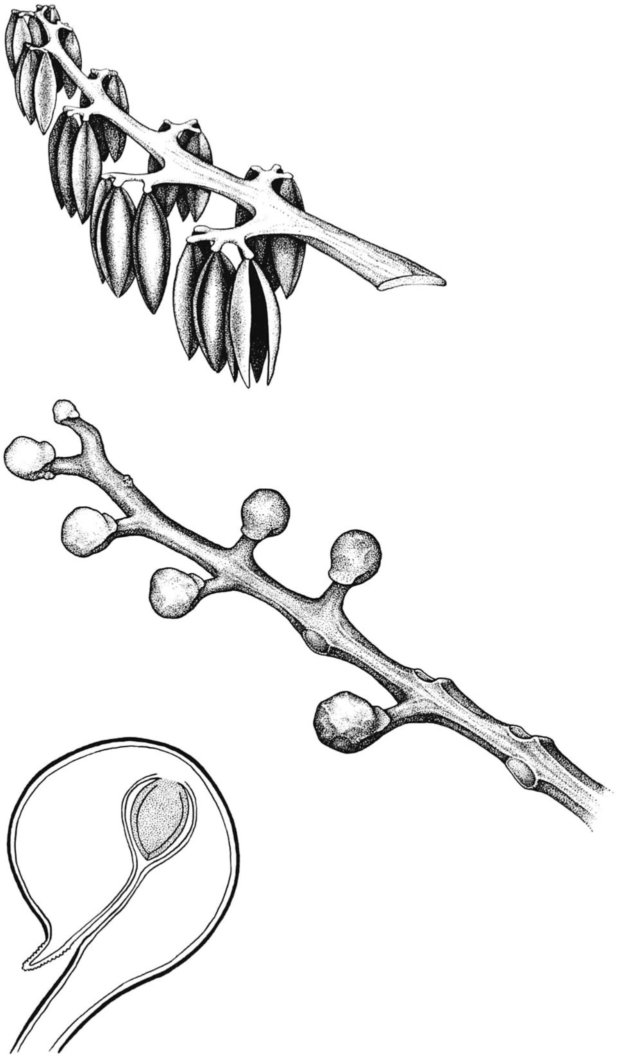

**Figure 1.12.** Reconstructions of Caytoniales (from Crane 1985). (Top) Male synangia of *Caytonanthus arberi*, based on Harris (1941). (Middle) *Caytonia nathorstii* megasporophylls, based on Harris (1964). (Bottom) *Caytonia* cupule containing seeds, based on Reymanówna (1973).

## BENNETTITALES

Bennettitales have long been considered close relatives of angiosperms. Arber and Parkin (1908) proposed a close relationship between angiosperms, Gnetales, and Bennettitales. In Crane (1985), Bennettitales and *Pentoxylon* appeared as sister to Gnetales + angiosperms. The analyses of Doyle and Donoghue (1992) and Rothwell and Serbet (1994), as well as most subsequent analyses, similarly placed angiosperms, *Pentoxylon*, Bennettitales, and Gne-

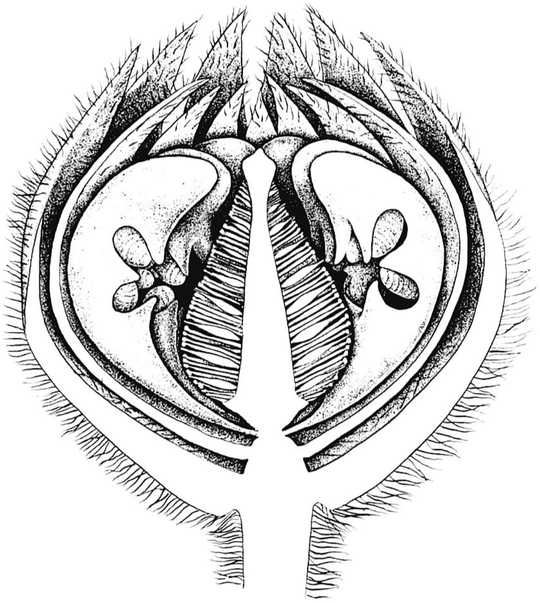

**Figure 1.13.** Reconstruction of Bennettitales (from Crane 1985). *Williamsoniella coronata*, longitudinal section through "flower," based on Harris (1964).

tales together in one clade. Rothwell et al. (2009) reviewed the unusual reproductive structures of Bennettitales.

Bennettitales possessed strobili that were unisexual in some representatives and bisexual in others. In bisexual (hermaphroditic) forms, the microsporangia and ovules were associated on lateral branches that arose among the leaf bases (Fig. 1.13). This strobiloid reproductive structure was important in the formulation of the "anthostrobilus" theory for the origin of the angiosperm flower (Arber and Parkin 1907). The strobiloid reproductive structure of Bennettitales was considered similar to the strobiloid flowers of Magnoliaceae, and this similarity was the rationale for considering members of "Ranales" (Schisandraceae, Magnoliaceae, Ranunculaceae, and their relatives) as the most "primitive" extant angiosperms (e.g., Delpino 1890; Bessey 1915; reviewed in Endress 1993). Many Bennettitales had flat microsporophylls with adaxial sporangia, containing monosulcate pollen; these structures could be considered suitable precursors to the angiosperm stamen. In other regards, Bennettitales are problematic as close relatives of the angiosperms. Ovules of Bennettitales had an orthotropous orientation rather than potentially anatropous as in Caytoniales, corystosperms, and most angiosperms (see Crane 1985; with notable exceptions including Chloranthaceae, *Ceratophyllum*, and possibly *Amborella*: Endress and Doyle 2009). Discounting Bennettitales on the basis of ovule orientation assumes, however, that the anatropous ovule is ancestral for angiosperms (see Chapters 4 and 6). Furthermore, assessing ovule orientation is problematic when carpels are not present. Finally, Bennettitales did not possess cupules (Rothwell and Stockey 2002; Rothwell et al. 2009). Rothwell et al. (2009) summarized features of Bennettitales—the seeds were produced terminally on sporophylls and were unique in having a nucellus with a solid apex, no pollen chamber, and a single integument; the seeds were not enclosed by a cupule or any other specialized structures. As a result, Bennettitales differ substantially from Gnetales, angiosperms, and *Caytonia* and have sometimes been considered part of a separate lineage, perhaps sharing a common glossopterid ancestor with the angiosperms (see "Glossopterids," below), but not as close relatives of the angiosperms. However, morphological cladistic analyses consistently place Bennettitales close to the angiosperms.

## PENTOXYLALES

Before Crane's (1985) phylogenetic analyses, *Pentoxylon* was considered an isolated gymnosperm of uncertain affinity. As background, various detached organs have been inferred to be part of the "*Pentoxylon*" plant: *Pentoxylon* (stem), *Nipaniophyllum* (leaf), *Sahnia* (male flower), and *Carnoconites* (female cone) (Bose et al. 1985). *Pentoxylon* was first associated with anthophytes in Crane (1985) because of its flower-like arrangement of microsporophylls, aggregation of ovules into a head (as in Bennettitales), and similarity of ovules to those of Bennettitales (Fig. 1.14). *Pentoxylon* was also placed in the anthophyte clade of Doyle and Donoghue (1992) and Rothwell and Serbet (1994). Bose et al. (1985) challenged some of the anthophyte-like anatomical features of *Pentoxylon*. However, when Doyle (1996) rescored *Pentoxylon* with the data of Bose et al. (1985) and Rothwell and Serbet (1994), the genus continued to show a close relationship to Gnetales, angiosperms, Bennettitales, and *Caytonia*.

In *Carnoconites*, the ovules were sessile and putatively helically arranged into compact heads. The ovulate heads were borne terminally on short shoots. One problem with Pentoxylales as a close relative of the angiosperms is that there is no clear carpel prototype in *Pentoxylon*. Furthermore, like Bennettitales, the ovules of *Carnoconites* had an orthotropous, rather than anatropous, orientation. Researchers also debate the presence of a cupule in *Carnoconites*, which would ultimately form the outer (second) angiosperm integument. Crane (1985) suggested that the ovules in *Carnoconites* were surrounded by a cupule, whereas others maintained that *Carnoconites* did not possess a cupule (Nixon et al. 1994; Rothwell and Serbet 1994). In addition, the megasporophylls of *Carnoconites* were not leaf-like in

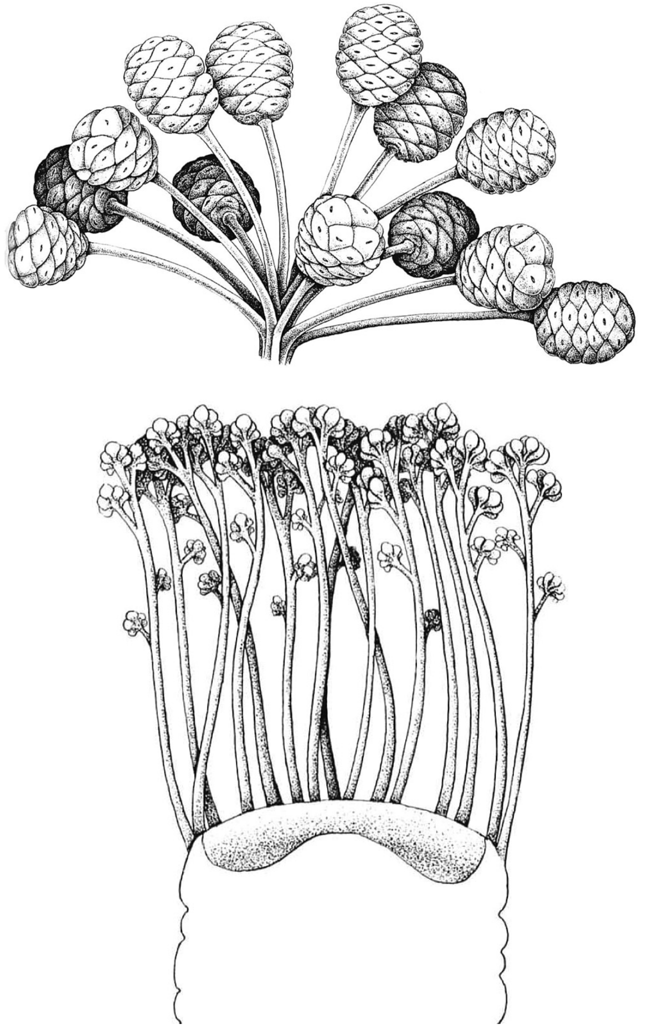

**Figure 1.14.** Reconstructions of *Pentoxylon* plants (from Crane 1985). (Top) Ovulate heads of *Carnoconites cranwelliae*, based on Harris (1964). (Bottom) Microsporangiate structures of *Sahnia*, based on Vishnu-Mittre (1953).

appearance; rather, each ovulate head bore 10 to 20 spirally arranged (note that Crane 1985, Fig. 19, depicts these as whorled), stalked, unilocular megasporangia (Fig. 1.14; Crane 1985). As a result of these concerns, Pentoxylales, like Bennettitales, have been envisioned as part of a separate lineage, perhaps sharing a common glossopterid ancestor with angiosperms (Fig. 1.14), but not as a close relative or direct ancestor of the angiosperms.

## GLOSSOPTERIDALES (GLOSSOPTERIDS)

Plumstead (1956) and Melville (1962) suggested a close relationship of angiosperms and Glossopteridales partly on the basis of reticulate leaf venation, although the venation of glossopterid leaves is much more like that of *Caytonia* leaflets than that of any angiosperms. Retallack and Dilcher (1981) revived interest in a Glossopteridales ancestry of angiosperms, suggesting that the ovule-bearing organs of Glossopteridales have structures that might be homologous with both the outer integument and the carpel of angiosperms (Fig. 1.15). They described the fossil glossopterid *Denkania* using terminology applied to angiosperms, stating that this fossil taxon had bitegmic, orthotropous ovules with the cupule homologous with the outer integument of angiosperms. These "bitegmic" ovules were arranged on a leaf surface but were not enclosed. The leaf surface was considered homologous with the angiosperm carpel (Fig. 1.15). This close relationship is questioned because the initial interpretations of glossopterid fructifications were found to be incorrect (see Doyle and Hickey 1976; Schopf 1976; Taylor and Taylor 1992), and the homologies inferred by Retallack and

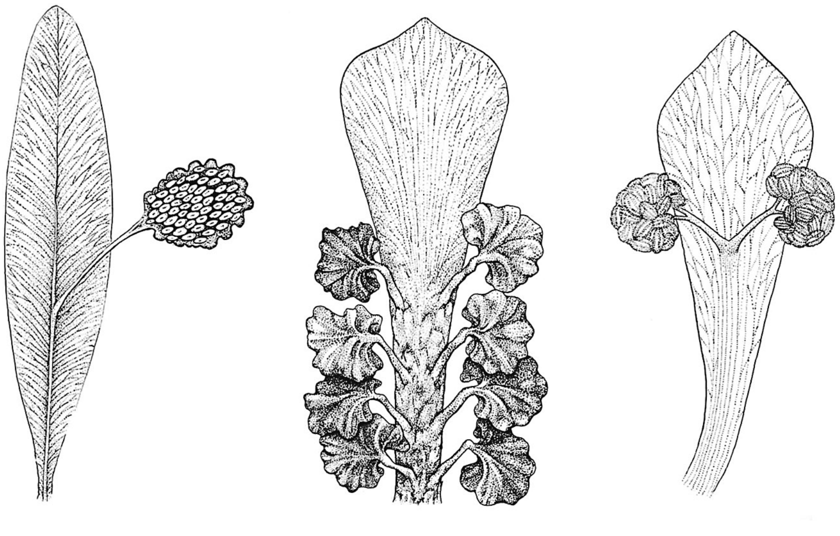

**Figure 1.15.** Reconstructions of glossopterids (from Crane 1985). (Left) Megasporophyll of *Ottokaria* and associated leaf, redrawn from Pant (1977). (Center) Megasporophyll of *Lidgettonia africana*, based on Thomas (1958). (Right) Microsporophyll of *Eretmonia*, redrawn from Surange and Chandra (1975).

Dilcher have been questioned (Taylor and Taylor 2009). Doyle (1996, 2006) also suggested a close relationship of glossopterids and angiosperms. He proposed not only that Glossopteridales might have been the ancestral group from which Gnetales arose via intermediate fossil forms such as *Piroconites* and *Dechellyia*, but also that the common ancestor of *Caytonia* and the angiosperms might have had "glossopterid-like bract-sporophyll complexes" bearing several cupules per bract (leaf) that would correspond to the "anatropous cupules" of *Caytonia*, which have often been considered homologous to the bitegmic, anatropous ovules of angiosperms (Fig. 1.11). The underlying bract would then only have to be folded lengthwise to produce a carpel or reduced to produce a *Caytonia* sporophyll. Doyle (1996) maintained that this scenario is perhaps more plausible than the origin of a carpel through the expansion and folding of the *Caytonia* rachis (Stebbins 1974; Doyle 1978). In later analyses and reviews (see above and Doyle 2012), Gnetales were removed from this evolutionary scenario, but Glossopteridales remain close to angiosperms and hence the above hypothesis retains some credibility. The bisaccate pollen produced by Glossopteridales is comparable to that in other gymnosperm groups but is not found in any angiosperms. The motile sperm documented in well-preserved *Glossopteris* seeds (Nishida et al. 2004) is a feature shared with cycads and *Ginkgo*, distinguishing them from conifers, Gnetales, and angiosperms.

Cladistic analyses have often indicated a more distant relationship between glossopterids and angiosperms than between Bennettitales, *Pentoxylon*, Caytoniales, and angiosperms (Crane 1985; Rothwell and Serbet 1994; Doyle 1996, 1998a,b; Soltis et al. 2005b). Glossopteridales were not, for example, considered part of the "anthophyte clade" as originally defined (Doyle and Donoghue 1992). However, in Doyle and Donoghue (1992), Caytoniales followed by glossopterids were the sister taxa to the anthophyte clade as originally defined. In a later analysis, Doyle (1996) recovered what he termed a "glossophytes" clade in which glossopterids were sister to angiosperms, Gnetales, Bennettitales, and *Caytonia*. This topology is consistent with the hypothesis that both angiosperms (Retallack and Dilcher 1981) and Gnetales (Schopf 1976) were derived from glossopterids with a loss, in each case, of saccate pollen and motile sperm. However, these hypotheses are now unsupported, with the molecular placement of Gnetales with conifers refuting the anthophyte hypothesis. Nevertheless, it is possible that glossopterids are the immediate sister to a clade that includes angiosperms and their closest relatives and may, therefore, have played a crucial role in angiosperm origins.

## MOSTLY MALE

The origin of the flower has long been problematic—as made famous by Darwin in his reference to the origin and early diversification of the angiosperms as "an abominable mystery" (Darwin's letter of July 22, 1879, to Joseph Hooker). Clearly, floral organs are derived from some organ in their gymnosperm precursors, but by what mechanism? Several hypotheses have been proposed and in some

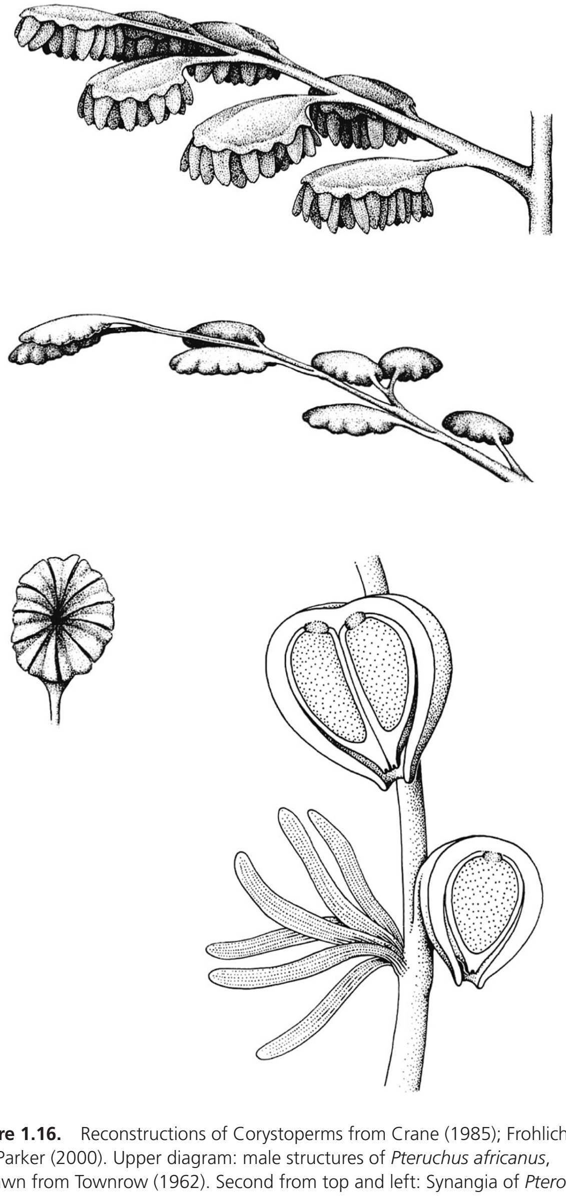

**Figure 1.16.** Reconstructions of Corystoperms from Crane (1985); Frohlich and Parker (2000). Upper diagram: male structures of *Pteruchus africanus*, redrawn from Townrow (1962). Second from top and left: Synangia of *Pteroma thomasii*, redrawn from Harris (1964). Bottom right: Cupules of *Ktalenia*, from Taylor and Archangelsky (1985).

cases bring fossil evidence from extinct gymnosperm lineages into the proposed evolutionary scenarios. We review leading hypotheses below.

Frohlich and Parker's (2000) mostly male hypothesis for the origin of the flower (Fig. 1.16) was based on their work with the homeotic gene *Floricaula/LEAFY* (*FLO/LFY*). Gymnosperms generally have two copies of the *LFY* gene (although *Gnetum* appears to have only a single copy; see Fig. 1.16A), referred to as the "needle" and "leaf" families. The function of the *LFY* genes of pine has been studied in detail. The "needle" (*NEEDLY*) paralogue is expressed only in early-developing female reproductive structures (hereafter referred to as $LFY_f$) (Mouradov et al. 1998). The "leaf" paralogue is expressed in male reproductive structures of gymnosperms (hereafter $LFY_m$). Frohlich and Meyerowitz (1997) speculated that these two gene families show such specialization because gymnosperms have had separate male and female reproductive structures since the Devonian. The *LFY* duplication in gymnosperms may have accompanied or perhaps facilitated the specialization of separate male and female reproductive structures (Fig. 1.16B).

Angiosperms have only one copy of *LFY*, which is most closely related to $LFY_m$, having apparently lost the female-specifying "needle" paralogue ($LFY_f$). These data prompted Frohlich and Parker (2000) to propose the mostly male hypothesis of floral origins (Fig. 1.16). Their hypothesis offers one developmental genetic mechanism by which a plant with separate male and female reproductive structures, a feature of all extant gymnosperms, could produce a bisexual structure such as a flower. Frohlich and Parker (2000) suggested that developmental control of floral organization derives more from systems operating in the male reproductive structures of the gymnosperm ancestor of angiosperms than from the female reproductive structures—hence, "mostly male." In the mostly male hypothesis, ovules are considered ectopic in origin on male reproductive structures (i.e., stamens) in early flowers (Fig. 1.16). An ectopic origin of angiosperm ovule position was earlier hypothesized by Meyen (1988).

Researchers have challenged the mostly male hypothesis on the basis of additional studies of *FLO/LFY*. Shindo et al. (2001) demonstrated that, although the *FLO/LFY* gene *GpLFY* from *Gnetum* is in the "leaf" clade ($LFY_m$) of Frohlich and Parker (2000), *GpLFY* is expressed in female strobili of *Gnetum*. This does not agree with the Frohlich and Parker assumption that only gene members of the "needle" clade (i.e., $LFY_f$) were expressed in gymnosperm female structures. These observations prompted Shindo et al. (2001) to conclude that the mostly male theory is not plausible.

A scenario somewhat similar to the mostly male hypothesis was proposed by Theissen et al. (2002; Theissen and Melzer 2007), termed "out of male." Following the "out-of-male" hypothesis, the bisexual flower of angiosperms originated from a male gymnosperm cone; reduction of the expression of B-class genes in the upper region of the male cone then resulted in the development of female, rather than male, reproductive units in this upper portion of the structure that became the flower.

Albert et al. (2002) provided another alternative to the mostly male hypothesis. Their model assumes that pleiotropic interactions between $LFY_m$ and $LFY_f$ were critical for stabilizing the retention of these two genes in gymnosperms and suggests that disruption of this delicate balance between the two *LFY* genes occurred in an ancestor of modern angiosperms. This ancestral taxon might have had unisexual flowers together on the same plant, or might have been loosely bisexual. $LFY_f$ would then have been lost through selection for an integrated bisexual reproductive axis.