COMMENTARY

Water from the rock: Ancient aquatic angiosperms flow from the fossil record

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The world of 120 million years ago was one of dynamic biological processes. During that time the flowering plants emerged as the dominant global floristic element, a transformative event that ultimately altered the character of the entire planet. Understandably, the rapid rise of angiosperms has intrigued paleoecologists and evolutionary biologists, who have strived to elucidate underlying explanations for their successful radiation. Lamented by Charles Darwin as "an abominable mystery" (1), the abrupt appearance of angiosperms and their sudden burst of diversification in the fossil record continues to captivate contemporary plant systematists, who have sought a solution by incorporating massive amounts of DNA sequence data in comparative phylogenetic studies of extant species. However, despite extensive technological advances in genetics, genomics, and bioinformatics, which have revolutionized plant research over the past decades, our comprehension of life on earth during such ancient times continues to rely primarily on one data source: the fossil record. In PNAS, Gomez et al. (2) uncover an important clue to Darwin's mystery by their analyses of *Monteschia vidalii*, an enigmatic plant fossil from Barremian deposits in Spain. Their reinterpretation of these fossils provides a new perspective that allies *Monteschia* not only with the angiosperms, but specifically with extant members of the order Ceratophyllales, a group of considerable interest in debates concerning the origin of flowering plants. The characterization of these early plants as highly specialized aquatics also offers novel interpretations regarding the ecological characteristics of early angiosperms.

Fig. 1. *Ceratophyllum submersum* (shown here) is an extant aquatic species of Ceratophyllales, the order to which Barremian fossils of *M. vidalii* have been assigned.

Prior ecological interpretations of early Cretaceous environments have been based primarily on terrestrial plant species, which vastly dominate the paleobotanical record. In some fossil floras, aquatic angiosperms do not appear until the middle to late Albian stage (3) and these typically depict floating-leaved plants similar in habit to that of the modern order Nymphaeales, which resolves phylogenetically at or near the base of the angiosperm tree of life. Because floating-leaved species are thought to represent an intermediate evolutionary stage between terrestrial plants and true hydrophytes, one

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See companion article on page 10985.

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might reasonably conclude that entirely submersed species did not evolve until later. Indeed, fossils similar to the submersed genus *Ceratophyllum* (Fig. 1), another ancestral angiosperm candidate, first appear in the late Albian (4). However, an earlier presence of hydrophytes was documented by the discovery of the aquatic angiosperm *Archaefructus* from Barremian deposits (5). The contemporary occurrence of *Monteschia* from that same time period not only indicates an early derivation of the aquatic and submersed habits, but also that some of the very earliest angiosperms were already well adapted to aquatic conditions. Furthermore, by their association with *Ceratophyllum*, the *Monteschia* fossils rekindle an interesting question regarding the ultimate age of the Ceratophyllales.

Interestingly, the phylogenetic analysis of extant plant DNA sequences initially resolved *Ceratophyllum* as the sister group of all other angiosperms (6), a result hypothesized earlier by analysis of nonmolecular data (7). However, the recognition of *Ceratophyllum* as the earliest diverging angiosperm lineage met with much skepticism, due in large part to its aquatic habit, which was believed to represent a highly derived condition evolutionarily. Subsequent molecular phylogenetic studies also yielded inconsistent placements of *Ceratophyllum*, which consistently was characterized by a long branch in the analyses. As more DNA data were analyzed, it was the terrestrial genus *Amborella*, not *Ceratophyllum*, that most studies resolved as the sister group of angiosperms. Consequently, despite the lack of a fossil record, the ancestral position of *Amborella* has become accepted by many as fact and has been used even in the interpretation of genome-scale evolutionary factors (8). However, the unusual features shared by *Monteschia* and *Ceratophyllum* (e.g., rootlessness, unitegmic ovules, and carpellate pore associated with water pollination) indicates that the Ceratophyllales must have diverged from other flowering plants considerably earlier than the Barremian, which suggests that the question of the most ancestral angiosperm lineage deserves renewed scrutiny.

Two factors complicate the molecular phylogenetic analysis of ancestral angiosperms, especially in the context of different datasets. First, the phylogenetic placement of *Amborella* is not as consistent as many seem to believe, and second, different data resolve *Ceratophyllum* in various phylogenetic positions, often with weak internal support, which provides no definitive answer to where the group actually belongs. The enormity of many phylogenetic datasets often results in the presentation of results without adequate analysis of the characters included. However, more critical evaluation of DNA characters has reduced confidence in the basal placement of *Amborella* (9) and has demonstrated that the incorporation of highly saturated sites can alter the position of *Amborella* and *Ceratophyllum* in phylogenetic analyses (10, 11). The following inconsistencies are particularly noteworthy. Analyses of protein-coding gene sequences from chloroplast DNA (cpDNA) data place *Amborella* as the sister to other angiosperms while placing *Ceratophyllum* either with Piperales or distant from Piperales, depending on whether different codon positions or amino acid sequences are analyzed (12). Nuclear ribosomal DNA data also are problematic. Analyses of 18s rDNA sequences resolve a basal angiosperm grade comprising the monocots and *Ceratophyllum*, whereas 26s rDNA place *Amborella* in that position; combined 18s + 26s sequences yield yet another result where *Amborella* and Nymphaeales comprise a sister clade to other angiosperms whereas *Ceratophyllum* plus monocots resolve as a clade (13). Analysis of low-copy nuclear genes strongly associates Ceratophyllaceae and Chloranthaceae rather than Piperaceae (14). Moreover, a position of *Ceratophyllum* (rather than *Amborella*) as the angiosperm sister group is maintained in analyses of xanthine dehdrogenase data (15) and selected cpDNA data in large-scale taxonomic analyses (16). Given these inconsistencies, it seems premature to simply dismiss other potential candidates such as Ceratophyllales (e.g., *Ceratophyllum* and its fossil ally *Monteschia*) as the sister lineage of angiosperms. Certainly, it is clear that a satisfactory answer to the question of earliest diverging angiosperms cannot be attained until the phylogenetic placements of both *Amborella* and *Ceratophyllum* have been established much more confidently.

Fossils such as those reported for *Monteschia* can only help to unravel the evolutionary and ecological events that accompanied the rise of flowering plants to global prominence.

It is important to emphasize that all proposed schemes of angiosperm relationships represent hypotheses, not facts (17). Although they cannot be falsified, these hypotheses must be tested further, and not simply by the exclusive incorporation of molecular data. Furthermore, comparative morphological analyses provide the only practical means of exploring phylogenetic relationships among long-extinct plants (18), where no DNA is available for molecular analysis. For this reason, the continued quest for fossils such as those reported for *Monteschia* can only help to unravel the evolutionary and ecological events that accompanied the rise of flowering plants to global prominence.

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