

Montsechia, an ancient aquatic angiosperm

 Bernard Gomez^{a,1}, Véronique Daviero-Gomez^a, Clément Coiffard^b, Carles Martín-Closas^c, and David L. Dilcher^{d,1}

^aCNRS-UMR 5276 Laboratoire de Géologie de Lyon-Terre, Planètes, Environnement, Université Lyon 1 (Claude Bernard), 69622 Villeurbanne, France; ^bMuseum für Naturkunde, Leibniz Institute for Evolution and Biodiversity Science, 10115 Berlin, Germany; ^cDepartament d'Estratigrafia, Paleontologia i Geociències marines, Facultat de Geologia, Universitat de Barcelona, 08028 Barcelona, Catalonia, Spain; and ^dDepartment of Geological Sciences, Indiana University, Bloomington, IN 47405

Contributed by David L. Dilcher, July 3, 2015 (sent for review May 20, 2015; reviewed by Donald H. Les and Gregory Retallack)

The early diversification of angiosperms in diverse ecological niches is poorly understood. Some have proposed an origin in a darkened forest habitat and others an open aquatic or near aquatic habitat. The research presented here centers on *Montsechia vidalii*, first recovered from lithographic limestone deposits in the Pyrenees of Spain more than 100 y ago. This fossil material has been poorly understood and misinterpreted in the past. Now, based upon the study of more than 1,000 carefully prepared specimens, a detailed analysis of *Montsechia* is presented. The morphology and anatomy of the plant, including aspects of its reproduction, suggest that *Montsechia* is sister to *Ceratophyllum* (whenever cladistic analyses are made with or without a backbone). *Montsechia* was an aquatic angiosperm living and reproducing below the surface of the water, similar to *Ceratophyllum*. *Montsechia* is Barremian in age, raising questions about the very early divergence of the *Ceratophyllum* clade compared with its position as sister to eudicots in many cladistic analyses. Lower Cretaceous aquatic angiosperms, such as *Archaeofructus* and *Montsechia*, open the possibility that aquatic plants were locally common at a very early stage of angiosperm evolution and that aquatic habitats may have played a major role in the diversification of some early angiosperm lineages.

Montsechia | *Ceratophyllum* | *Archaeofructus* | aquatic angiosperm | Lower Cretaceous

When did early angiosperms begin to diversify ecologically? This question is currently unanswered. Age estimates of the divergence of crown-group angiosperms using molecular clock data vary considerably, although it is in the range of (max. 210–) often accepted, 150–140 (min. 130) million years (1–7). Parsimony reconstruction of early angiosperm habit suggests that they may have been shrubs living in “damp, dark, and disturbed” habitats (8). In contrast, many living aquatic angiosperms are basal in angiosperm phylogenies [e.g., Nymphaeales in Amborella, Nymphaeales and Illiciales, Trimeniaceae-Austrobaileya (ANITA) or Ceratophyllales with the eudicots as commonly understood]. In the fossil record, we have found an aquatic angiosperm, *Montsechia vidalii* (Zeiller) Teixeira, which is an atypical plant fossil found in the Barremian (130–125 million years ago) freshwater limestone in the Pyrenees and Iberian Range in Spain. *Montsechia* (Fig. 1) lacks roots (no proximal or adventitious roots were found in more than 1,000 shoots examined) and shows flexible axes and two types of phyllotaxy and leaf morphology. The cuticle is very thin with rare stomata. The fruit is closed with a pore near the distal tip, indehiscent, and contains one unitegmic seed developed from an orthotropous and pendent ovule (Figs. 2 and 3). Cladistic analysis of these characters places *Montsechia* on the stem lineage basal to extant *Ceratophyllum* or a clade formed by *Ceratophyllum* and Chloranthaceae (Fig. 4) suggesting that mesangiosperms (non-ANITA angiosperms) existed 125 million years ago, as indicated by the tricolpate pollen record. *Montsechia* is well-adapted to a submerged aquatic habit. *Montsechia* is contemporaneous with another aquatic plant fossil, *Archaeofructus*, indicating that some of the earliest angiosperms were fully aquatic very early in their ecological diversification.

Materials and Methods

Two fossil areas yielding *Montsechia* are known (*Supporting Information*). From the late eighteenth century to the end of the 1990s, hundreds of specimens were collected from the lower Barremian limestones of the Pedrera quarry and the La Cabróa locality in the Montsec chain, western Spanish Pyrenees, Lleida Province, Spain. Another locality, Las Hoyas, was found in the early 1980s and is still excavated today in the Serranía de Cuenca, southwestern Iberian chain, Cuenca Province, Spain. Fossils are housed in various institutions in England, France, Germany, and Spain.

Some *Montsechia* shoots and fruits were removed from the rock by applying hydrochloric acid on a drop-by-drop basis. The cuticles were bleached using a mixture of nitric acid and potassium chlorate followed by a water bath with a few drops of ammonia (a two-step treatment commonly called “Schulze reagent”). Specimens were examined under a stereomicroscope, light microscope, and scanning electron microscope.

Cladistic analyses were performed with or without backbone using Mesquite. For more details see *SI Materials and Methods*.

Results

Montsechia vidalii (Zeiller) Teixeira is a locally abundant plant fossil found in the Barremian-age sediments of two well-known localities, El Montsec in the Pyrenees and Las Hoyas in the Iberian Range, Spain. *Montsechia* is an atypical aquatic fossil plant that was poorly understood systematically and morphologically. Its affinities have been suggested to be a liverwort (9), horsetail (10), conifer (11), Gnetales (12), and an angiosperm (13, 14). Basal angiosperms, as currently understood, are rooted in the terrestrial extant plant *Amborella* (refs. 15 and 16 and references therein). Extant Nymphaeales, containing mainly aquatic plants, often is considered a sister group. The aquatic nature of early angiosperms and how many times the angiosperms have moved into aquatic environments is unknown. However, the presence of the aquatic plant *Archaeofructus*, contemporaneous with *Montsechia*, indicates that some early angiosperms were tied to aquatic environments and must be considered as significant elements with an important influence during early angiosperm evolution. Many of the

Significance

The importance of very early aquatic flowering plants is not well understood currently and is poorly documented. Here we present details of the morphology and reproductive biology of *Montsechia*, an extremely early fossil angiosperm that, because it is so ancient and is totally aquatic, raises questions centered on the very early evolutionary history of flowering plants. This paper challenges the paradigm of how we view the early evolution of basal angiosperms and particularly the role of aquatic habitats in the very early evolution and diversification of flowering plants.

Author contributions: B.G. and D.L.D. designed research; B.G., V.D.-G., C.C., C.M.-C., and D.L.D. performed research; B.G., V.D.-G., C.C., and D.L.D. analyzed data; and B.G. and D.L.D. wrote the paper.

Reviewers: D.H.L., The University of Connecticut; and G.R., University of Oregon.

The authors declare no conflict of interest.

See Commentary on page 10825.

¹To whom correspondence may be addressed. Email: bernard.gomez@univ-lyon1.fr or dilcher@indiana.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1509241112/-DCSupplemental.

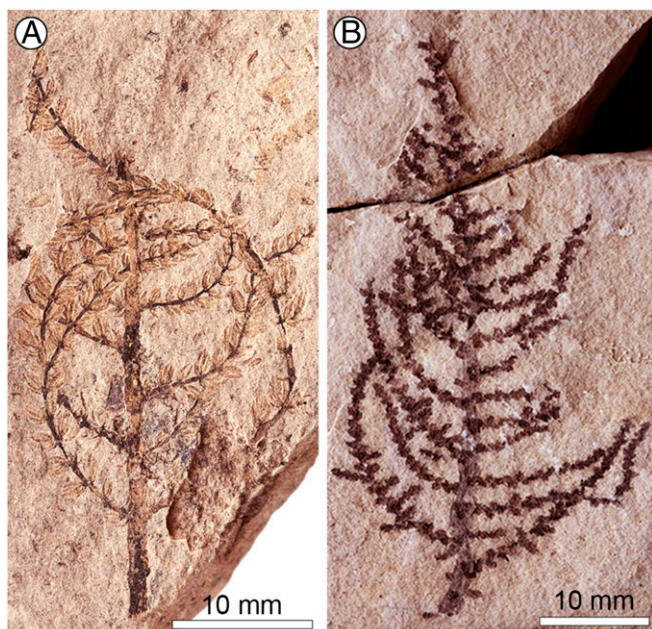


Fig. 1. Long- and short-leaved forms of *Montsechia vidalii*. (A) The long-leaved specimen shows very flexuous branches and opposite, long leaves. LH02556. (Scale bar, 10 mm.) (B) The short-leaved specimen shows regularly developed lateral branches and tiny leaf rosettes. LH07198. (Scale bar, 10 mm.)

characters of *Montsechia* are similar to, but are not exactly the same as, those found today in the extant aquatic, monotypic *Ceratophyllum*. It is generally accepted that aquatic angiosperms were derived from plants that previously lived in terrestrial environments (17). Only about 2% of angiosperms are aquatic today (18). The data presented here raise questions about the aquatic nature of some of the earliest angiosperms and their place in angiosperm history.

Montsechia shows no roots, consists of flexible axes, and has shoots of two types of phyllotaxy and leaf morphology (Figs. 1 and 3 A and B). Although there are thousands of *Montsechia* fragments in 10 beds, these two shoot types are never attached to each other in the field, but they are always collected together from the same bed and are about equally abundant in these beds. One type has opposite-decussate branches with awl-shaped linear leaves and is rarely fruit-bearing (Figs. 1A and 3A); the other has spiral branches and short scale-shaped leaves and is commonly fruit-bearing (Figs. 1B and 3B and [Supporting Information](#)). However, these two types of shoots probably belong to the same species because they have the same microstructure and female organs. The cuticle is thin with anomocytic stomata. Ascidiolate, nonornamented fruits are borne in pairs on indeterminate inflorescences; each fruit has a pore near the distal tip (Figs. 2 and 3B and [Supporting Information](#)) and bears one unitegmic seed which is borne inverted with the micropyle proximal; a hilum is located ventrally with the funiculum running from the proximal placenta, at the base of the distal attachment, to the seed (Figs. 2 and 3 C and D). Thus the ovule is orthotropous and pendent. The fruits most often are borne in pairs, terminally on an axis.

No male reproductive organs or scars of such were found on any of the axes or dispersed with them.

Discussion

Montsechia and *Ceratophyllum* share many similarities, including hydrophily (water pollination), which is a rare event occurring in less than 5% of aquatic flowering plants (in nine families) (17, 19–21). Hydrophily is suggested because both genera have a pore

in the fruit wall through which the pollen tube may enter, and other common features such as an orthotropous pendent ovule, a single-seeded fruit, and nonornamented unisexual flowers and lack roots. These characters when analyzed in a morphological cladistic dataset place the fossil on the stem group basal to extant *Ceratophyllum* or a clade formed by *Ceratophyllum* and Chloranthaceae (Fig. 4). Historically, based on molecular data, *Ceratophyllum* was placed basal to all angiosperms (22). Currently, based on molecular data, *Amborella* appears to be basal; the position of *Ceratophyllum* as basal to all eudicots is generally accepted (15, 23) but is not strongly supported (16). However, a few analyses (24, 25) propose that *Ceratophyllum* is basal to all angiosperms. In other analyses the Ceratophyllales are placed as sister to the eudicots (15, 16, 23) or as sister to Chloranthaceae in the analysis including Cretaceous fossils unrelated to *Montsechia* (26). This difference in placement depends on the backbone used for the analysis. All the analyses place *Montsechia* as sister to *Ceratophyllum*. The great age of *Montsechia*, and thus the great age of the *Ceratophyllum* clade, supports the molecular results placing *Ceratophyllum* in a rather basal position in angiosperm phylogeny.

The very ancient age of *Montsechia* and the close similarity of so many reproductive characters suggest that it is part of an early stem lineage of Ceratophyllales. Just as in extant *Ceratophyllum*, *Montsechia* has no roots and has a pore in the carpel wall that would allow pollen entry under water rather than a functional stigma typical of pollination in the majority of angiosperms. Aquatic plants often modify their vegetative form to accommodate an aquatic environment; however, the reproductive organs of aquatic plants are more conservative and often reflect remnants of the morphology of their terrestrial ancestry (17, 27). Many aquatic plants have emergent reproductive organs little modified from their terrestrial ancestors. *Ceratophyllum* is very different morphologically and also has a little understood long-branch molecular placement with extant angiosperm taxa. Therefore it has been difficult to place *Ceratophyllum* systematically. Even with the large dataset used by Ruhfel et al. (16), there is only 50–75% certainty in its relationships. The detailed



Fig. 2. Fruit and seed of *Montsechia vidalii*. The fruit shows a small apical pore (po). The funicle (f) of the single, upside-down seed (orthotropous pendent) is attached from the hilum (h) to the placenta (pl). (Scale bar, 500 μ m.)

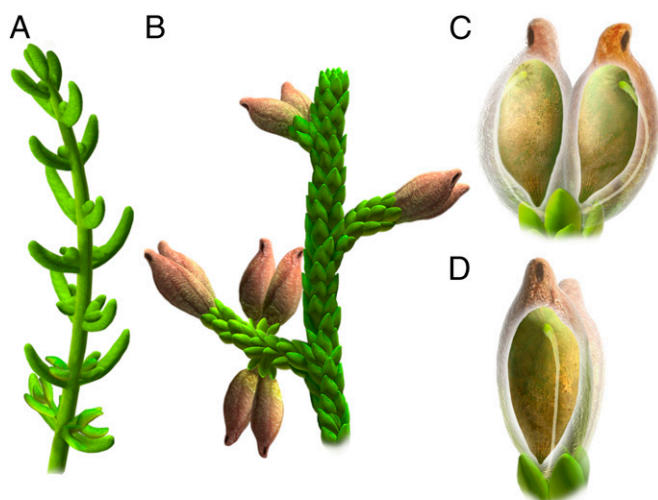


Fig. 3. Reconstructions of *Montsechia vidalii*. (A) The long-leaved form shows the opposite leaves and branches. (B) The short-leaved form shows the alternate phyllotaxy of leaves and branches bearing pairs of ascidiate, nonornamented fruits. (C and D) The fruit shows a small apical pore and a single seed developed from an orthotropous pendent ovule. The funicle arises from the placenta (near the micropyle) to the hilum (near the pollination pore). (C) Lateral view. (D) Front view. Diagram by O. Sanisidro, B.G., and V.D.-G.

analysis of *Montsechia* presented here demonstrates an extremely long and independent history for the stem lineage of *Ceratophyllum*. *Montsechia*, with characteristics typical of a plant living submerged in water, demonstrates that the ancestral stock of this clade lived and reproduced submerged in water more than 125 million years ago. The aquatic environments of the Lower Cretaceous probably were not very different from those in later ages, so there was little pressure for change over time. We find fossils even more similar to the extant *Ceratophyllum* in the late Albian (28). These fossils also support an ancient age for the *Ceratophyllum* lineage and suggest a more basal position for this lineage, as found in some recent phylogenetic analyses (24, 25).

Because of the differences between *Montsechia* and *Ceratophyllum*, we construct a new family, Montsechiaceae, for these fossils. Family Montsechiaceae Gomez, Daviero-Gomez, Coiffard, Martín-Closas et Dilcher fam. nov. Description: Herbaceous,

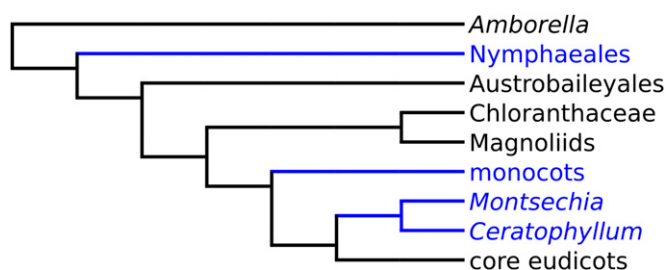


Fig. 4. Most parsimonious position of *Montsechia* in a simplified tree derived from the matrix by Endress and Doyle (26) using the J & M backbone. Taxa in blue are considered ancestrally water-related (27). Diagram by C.C. and B.G.

aquatic/submerged plants with branching stems. Branches originate as axillary shoots terminating in reproductive organs. Stems are slender, flexible and dimorphic bearing simple, cylindrical leaves arranged in alternate or opposite phyllotaxy. Cuticle thin; stomata rare, anomocytic. Fruits paired on indeterminate inflorescences, ascidiate, closed except a pore near the distal tip. Seed unique, orthotropous and pendent, unitegmic. This new family should be included in the Ceratophyllales. This new family is extinct and basal in this order.

Montsechia, the fossil angiosperm presented here, raises questions centered on the very early evolutionary history of angiosperms. The importance of very early aquatic flowering plants, perhaps basal to all angiosperms, as previously proposed (29), merits serious consideration and reevaluation. Clearly, *Montsechia* was very well adapted to a submerged aquatic habit and lived during an early stage of angiosperm evolution. Now it is time for the fossil angiosperm families Montsechiaceae (30) and Archaeofractaceae (29) to become a part of the phylogenies presented in our current angiosperm literature.

ACKNOWLEDGMENTS. We thank Oscar Sanisidro for art reconstructions. Funding for this research was provided by Unité Mixte de Recherche 5276 of Centre National de la Recherche Scientifique (B.G. and V.D.-G.); Projects CGL2011-27869, CGL2012-35199, and CGL2013-42643-P of the Ministerio de Ciencia e Innovación of the Spanish government and Project 2014SGR-251 funded by the Catalan government (B.G., V.D.-G., and C.M.-C.); Grant CO 1060/3-1 from the German Funding Agency (to C.C.); European Community-funded Project SYNTHESYS Grants GB-TAF-1038, DE-TAF-1221, and ES-TAF-3066 (to B.G.); and the Indiana Geological Survey and the Department of Geological Sciences of Indiana University (D.L.D.).

- Sanderson MJ, Doyle JA (2001) Sources of error and confidence intervals in estimating the age of angiosperms from rbcL and 18S rDNA data. *Am J Bot* 88(8):1499–1516.
- Wikström N, Savolainen V, Chase MW (2001) Evolution of the angiosperms: Calibrating the family tree. *Proc Biol Sci* 268(1482):2211–2220.
- Davies TJ, et al. (2004) Darwin's abominable mystery: Insights from a supertree of the angiosperms. *Proc Natl Acad Sci USA* 101(7):1904–1909.
- Bell CD, Soltis DE, Soltis PS (2005) The age of the angiosperms: A molecular timescale without a clock. *Evolution* 59(6):1245–1258.
- Soltis DE, Bell CD, Kim S, Soltis PS (2008) Origin and early evolution of angiosperms. *Ann N Y Acad Sci* 1133:3–25.
- Moore MJ, Soltis PS, Bell CD, Burleigh JG, Soltis DE (2010) Phylogenetic analysis of 83 plastid genes further resolves the early diversification of eudicots. *Proc Natl Acad Sci USA* 107(10):4623–4628.
- Magallón S, Gómez-Acevedo S, Sánchez-Reyes LL, Hernández-Hernández T (2015) A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. *New Phytol* 207(2):437–453.
- Feild TS, Chatelet DS, Brodribb TJ (2009) Ancestral xerophobia: A hypothesis on the whole plant ecophysiology of early angiosperms. *Geobiology* 7(2):237–264.
- Blanc-Louvel C (1991) Etude complémentaire de *Montsechia vidalii* (Zeiller) Teixeira 1954: Nouvelle attribution systématique. *Ann Paleontol* 77(3):129–141.
- Zeiller R (1902) Sur quelques empreintes végétales du Kimméridgien de Santa Maria de Meya, province de Lérida en Catalogne (Espagne). *Mem Real Acad Cienc Artes Barcelona* 4(1902):345–356.
- Lacasa-Ruiz A (1975) *Estudio del yacimiento portlandiense del Montsech de Rubiés*. (Instituto de Estudios Ilerdenses, Lérida, Catalonia, Spain), 4 pp.
- Krassilov VA (2011) On *Montsechia*, an angiospermoid plant from the Lower Cretaceous of Las Hoyas, Spain: New data and interpretations. *Acta Palaeobot* 51(2):181–205.
- Teixeira C (1954) La flore fossile des calcaires lithographiques de Santa Maria de Meyá (Lérida, Espagne). *Bol Soc Geol Portugal* 11(2-3):139–152.
- Blanc-Louvel C, Barale G (1983) *Montsechia vidalii* (Zeiller) Teixeira 1954. Nouvelles observations et réflexions sur son attribution systématique. *Ann Paleontol* 69(3):151–174.
- Moore MJ, Bell CD, Soltis PS, Soltis DE (2007) Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. *Proc Natl Acad Sci USA* 104(49):19363–19368.
- Ruhfel BR, Gitzendanner MA, Soltis PS, Soltis DE, Burleigh JG (2014) From algae to angiosperms—inferring the phylogeny of green plants (Viridiplantae) from 360 plastid genomes. *BMC Evol Biol* 14:23.
- Philbrick CT, Les DH (1996) Evolution of Aquatic Angiosperm Reproductive Systems: What is the balance between sexual and asexual reproduction in aquatic angiosperms? *Bioscience* 46(11):813–826.
- Cook CDK (1999) The number and kinds of embryo-bearing plants which have become aquatic: A survey. *Perspect Plant Ecol Evol Syst* 2:79–102.
- Cox PA (1993) Water-pollinated plants. *Sci Am* 269(10):68–74.
- Les DH (1988) The origin and affinities of the Ceratophyllaceae. *Taxon* 37(2):326–345.
- Philbrick CT (1991) The evolution of hydrophily: Ecological and phylogenetic considerations. *Rhodora* 93(873):36–50.
- Chase MW, et al. (1993) Phylogenetics of seed plants: An analysis of nucleotide sequences from the plastid gene rbcL. *Ann Mo Bot Gard* 80(3):528–580.

23. Jansen RK, et al. (2007) Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. *Proc Natl Acad Sci USA* 104(49):19369–19374.
24. Morton CM (2011) Newly sequenced nuclear gene (*Xdh*) for inferring angiosperm phylogeny. *Ann Mo Bot Gard* 98(1):63–89.
25. Goloboff PA, et al. (2009) Phylogenetic analysis of 73 060 taxa corroborates major eukaryotic groups. *Cladistics* 25(2009):211–230.
26. Doyle JA, Endress PK (2014) Integrating Early Cretaceous Fossils into the phylogeny of living angiosperms: ANITA lines and relatives of Chloranthaceae. *Int J Plant Sci* 175(5): 555–600.
27. Sculthorpe CD (1967) *The Biology of Aquatic Vascular Plants* (St. Martin's, New York).
28. Dilcher DL, Wang H (2009) An Early Cretaceous fruit with affinities to Ceratophyllaceae. *American Journal of Botany* 96(12):2256–2269.
29. Sun G, Dilcher DL, Zheng S, Zhou Z (1998) In search of the first flower: A Jurassic angiosperm, *Archaeofructus*, from northeast China. *Science* 282(5394):1692–1695.
30. Vidal LM (1899) Compte-rendu des excursions dans la province de Lérida du 11 au 15 octobre. *Bull Soc Geol Fr* 3(26):884–899.
31. Martínez Delclòs X (1991) *The Lower Cretaceous Lithographic Limestones of Montsec. Ten Years of Paleontological Expeditions* (Institut d'Estudis Ilerdencs, Lleida, Spain).
32. Martínez-Delclòs X (1995) *Montsec and Montral-Alcover, Two Konservat-Lagerstätten, Catalonia, Spain* (Institut d'Estudis Ilerdencs, Lleida).
33. Martín-Closas C (1989) Els caròfits del Cretaci inferior de les conques perifèriques del Bloc de l'Ebre. PhD thesis (Universitat de Barcelona, Barcelona).
34. Martín-Closas C, López-Morón N (1996) The Lower Cretaceous Charophyte Flora from El Montsec (Catalonia, Spain). *El Patrimoni Natural del Montsec*, 9–19, ed Fanló E (Institut d'Estudis Ilerdencs, Lleida, Spain).
35. Martín-Closas C, Clavel B, Charollais J, Conrad M-A (2009) Charophytes from the Barremian-lower Aptian of the Northern Subalpine Chains and Jura Mountains, France: Correlation with associated marine assemblages. *Cretac Res* 30(1):49–62.
36. Sanz JL, et al. (1988) An early Cretaceous faunal and floral continental assemblage: Las Hoyas fossil site (Cuenca, Spain). *Geobios* 21(5):611–635.
37. Buscalioni AD, Fregenal-Martínez M (2010) A holistic approach to the palaeoecology of Las Hoyas Konservat-Lagerstätte (La Huérguina Formation, Lower Cretaceous, Iberian Ranges, Spain). *J Iber Geol* 36(2):297–326.
38. Diéguez C, Trinção P, Martín-Closas C, López-Morón N (1995) Paleobotany. *Las Hoyas: A lacustrine Konservat-Lagerstätte, Cuenca, Spain*, ed Meléndez N (Universidad Complutense de Madrid, 29–32, Madrid).
39. Vicente A, Martín-Closas C (2013) Lower Cretaceous charophytes from the Serranía de Cuenca, Iberian chain: Taxonomy, biostratigraphy and palaeoecology. *Cretac Res* 40: 227–242.
40. Endress PK, Doyle JA (2009) Reconstructing the ancestral angiosperm flower and its initial specializations. *Am J Bot* 96(1):22–66.
41. Maddison WP, Maddison DR (2011) Mesquite: A modular system for evolutionary analysis, version 2.75. Available at mesquiteproject.org. Accessed July 19, 2014.
42. Endress PK (2001) The flowers in extant angiosperms and inferences on ancestral flowers. *International Journal of Plant Science* 162(5):1111–1140.