Research Advance on *Metasequoia*: Applications of New Technology

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The plant genus Metasequoia Miki, 1941 and its sole living relict species Metasequoia glyptostroboides Hu et Cheng, 1948 have been of special interest for both the public and scientists since 1941 when the genus was established. Due to its unique discovery history (fossils discovered earlier than the living species) and incomparable scientific value in the research of plant evolution and its relationship with environmental and climatic changes, Metasequoia becomes arguably the most comprehensively studied higher plant in both fossil and living forms. This paper summarized recent advance in Metasequoia research by reviewing the research history of Metasequoia and the scientific value of this genus, while paid special attention to the application of new methods and techniques in the research field of Metasequoia in the recent decades. The application of biogeochemical (organic geochemistry and stable isotope) analysis as well as the new and innovated methods of preparing large-sized cuticular membrane from leaves with originally thin and fragile cuticles further secure Metasequoia's super star status for the research of palaeoclimatic reconstruction.

Key words *Metasequoia*, living and fossil material, biogeochemistry, stable isotope analysis, cuticule analysis, palaeoclimatic reconstruction

Among more than 250 000 species of modern land plants, *Metasequoia glyptostroboides* Hu et Cheng, 1948 has received exclusively special attention from both the public and scientists alike: It is known by a number of common names (including nicknames) such as "dawn redwood," "water fir," "panda in plant kingdom," "living fossil," "dinosaur-age tree," "national treasure tree (of China)," etc. It was elected by the Arnold Arboretum at Harvard University as the "Tree of the Twentieth Century" [1]. It has received the highest conservation status at both the national (China) and international levels: It has been always ranked at the highest class of protection in any formal and/or informal plant protection strategies and documentation since the People's Republic of China was established in 1949 and in 1999, it was listed among the nine conifer species that need protection by international efforts by the International Union for the Conservation of Nature and Natural Resources (IUCN). It is the only tree species in China with its own conservation organization—the "Save the Dawn Redwoods League" (also translated as "*Metasequoia* Conservation Committee"). Along with its fossils, *M. glyptostroboides* is among those very rare plant taxa on which specific scientific meetings are held periodically—since 2002, three International *Metasequoia* Symposia had been held once every four year: 2002 in China, 2006 in the US, and 2010 in Japan. After each symposium, a book or a special issue of a journal was (will be) published as the proceedings [2—4].

These seemingly exceptional attentions and privileges that *Metasequoia* has received are at least in part due to its unique discovery history and important scientific values. The genus *Metasequoia* Miki, 1941 was established to accommodate fossil conifer material discovered from Pliocene sediments near Osaka, Japan, and it was believed to be an extinct plant group at the time of fossil discovery. When several years later trees of its living

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species (Type tree see Figure 1) were found alive in South-Central China (now the border region formed by western Hubei, eastern Chongqing, and northern Hunan), it was considered "as remarkable as discovering a living dinosaur" (Ralph W. Chaney) [1] and immediately aroused wide international interests. To save the endangered "living fossil"—the sole living species of the genus, *Metasequoia glyptostroboides* became the hottest topic among both the public and scientists in the late 1940s. Through international efforts, particularly the collaboration between Chinese and American botanists and palaeobotanists, seeds of *M. glyptostroboides* were collected and distributed outside of China for cultivation. The U. S. became the country where the majority of the cultivated trees are systematically planted, and these trees with known genetic resources have been growing under various geographical, climatical, and environmental conditions across the whole country of the U. S. from East Coast to West Coast and from Alaska to the North to Florida to the South (one big tree is shown in Figure 2).

***Figure 1** The type tree of Metasequoia glyptostroboides in Moudao, Lichuan, Hubei, China. Please note persons underneath the tree as scales. Photo taken in August, 2002.*

***Figure 2** A big Metasequoia glyptostroboides tree growing in Bailey Arboretum, Long Island, New York, the U.S. from a seed collected in the late 1940's from China. Please note the person (about 1.8m high) underneath the tree as a scale. Photo taken in August, 2006.*

After about 60 years of growing they now become a rare and valuable resource for various scientific endeavors, particularly the research on the correlation between plant traits and climatic parameters. The U. S. thus is known as the second motherland of *Metasequoia*. Palaeobotanists' revision on previously described conifer fossils revealed that *Metasequoia* used to be a broadly distributed fossil taxon in the Northern Hemisphere. So far, more than 500 fossil localities yielding *Metasequoia* fossils have been documented and brought to light that soon after *Metasequoia* firstly appeared in the fossil record of Late Cretaceous (Cenomanian) it became one of the dominant conifers in Paleogene and Neogene temperate floras of the Northern Hemisphere till its last fossil record appeared in Pleistocene sediments in Japan (Some fossils are shown in Figures 3—5). What is even more intriguing is that during the past ~100 million years the gross morphology of *Metasequoia* hasn't changed much, implying a stasis evolutionary history for this genus. Therefore, as a plant taxon with 1) a long evolutionary history of about 100 million years with continuous fossil records, 2) a wide range of fossil distribution in the Northern Hemisphere, 3) a morphological stasis from the first appearance till modern time, 4) a living representative species to act as the comparison for fossils, and 5) ~60 years of cultivation under various environmental conditions, *Metasequoia* is truly an ideal "model plant" for the studies of plant evolution, phylogeny, phytogeography, plant morphological, anatomical, and physiological adaptation toward environmental changes, and paleoenvironmental and paleoclimatic reconstruction, etc.

Figure 3 ***Left:*** ***Three-dimensionally preserved fossil Metasequoia (M. occidentalis) leaves and leafy branches unearthed from the Member 2 of the Late Paleocene Iceberg Bay Formation at Ellesmere Island of the Canadian Arctic. Showing the extraordinary preservation condition Scale bar = 1 cm. Right: Dissecting microscope photograph of leaves and leafy branchlets of material shown in left figure isolated by HCl-HF-HCl treatment. Scale = 1 mm.***

Figure 4 ***Dissecting microscope photograph of a part of a three-dimensionally preserved fossil Metasequoia (M. occidentalis) leafy branchlet unearthed from the Middle Eocene Buchanan Lake Formation at Axel Heiberg Island of the Canadian Arctic. The material has been gold-coated for scanning electron microscope observation. Scale = 1 mm.***

Since 1941, an exceptionally large pile of literature on this arguably best-known genus has been published all over the world in various languages, which was first summarized and reviewed by Edmund H. Fulling at the Thirty Anniversary of the establishment of the genus *Metasequoia* (1941—1971) [5]. Fulling collected almost all references available to him before he passed away in 1975 and his almost finished manuscript was published in 1976 with an additions published in 1977 in the journal "The Botanical Review".

The second comprehensive summary and review on previous research on *Metasequoia* was carried out by Arnold Arboretum of the Harvard University at the Fiftieth Anniversary of the official publication of the living species *Metasequoia glyptostrobus* (1948—1998). "Arnoldia, the Magazine of the Arnold Arboretum" combined its Issue 4 of Volume 58 (1998) and Issue 1 of Volume of 59 (1999) to reprint some classical papers on *Metasequoia* and publish a series of new research and/or review articles to celebrate the anniversary [1].

The last two decades have witnessed a sharply increased attention toward climate changes which were mainly caused by human activities. More con-

Figure 5 A part of a leafy branchlet of fossil Metasequoia (M. occidentalis) collected from the Middle Miocene Clarkia fossil bed of Idaho, the U.S. Scale = 1 mm.

cerns have been expressed to the trend and magnification of climate changes in the future. A better understanding on the scale and rate of future climate changes can be achieved by our knowledge on the mechanism of paleoclimate. Plant fossil is one of the most efficient proxies for the reconstruction of paleoclimates, particularly plants with long evolutionary history, abundant fossil records, and living representatives (even better, representative of close affinity) as a comparison reference. *Metasequoia* not only meets all these preferable criteria, its predominant role in floras of the high latitude areas of the Northern Hemisphere during Paleogene global warming period and the extraordinary preservation condition of *Metasequoia* fossils unearthed in these cold areas (refer to figures 3—4) made this genus a "super model" for the research of paleoclimate reconstruction. *Metasequoia*'s legend has been not only continued but also enhanced in the past two decades with scientists beyond botanists and paleobotanists joining the "*Metasequoia* research club."

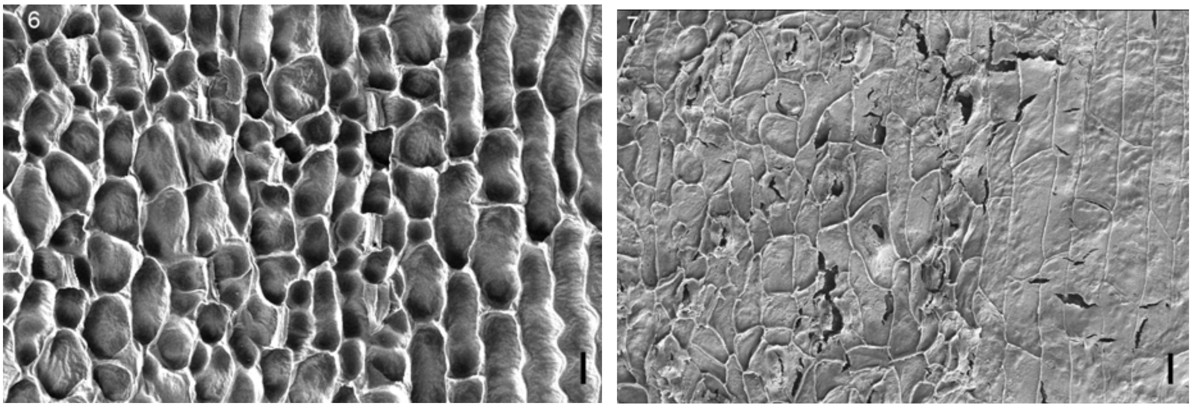
In 2002, scientists from 4 countries who were actively involved in the study of fossil and living *Metasequoia* gathered together to participate in the First International *Metasequoia* Symposium held in China, the mother land of *M. glyptostroboides*. After exchanging scientific ideas through presentations and workshop discussions in Wuhan, participants took a field trip to *Metasequoia* Valley in Lichuan, Hubei Province to visit the type tree (Figure 1) and other native trees of *M. glyptostroboides*. A proceedings including some of the presentations was published [2], serving as an important reference of the updates of the research on *Metasequoia* in the fields of history, conservation, paleobiology, paleoecology, and modern ecology. In addition, the symposium participants' effort of conserving *Metasequoia* led to the final establishment of the Xingdoushan National Nature Reserve (2003), which encompasses the distribution center area of native *M. glyptostroboides* trees.

The second symposium, entitled "*Metasequoia* and Associated Plant: Evolution, Physiology, Horticulture, and Conservation" took place in 2006 in the U.S., the second mother land of *M. glyptostroboides* where most cultivated trees are growing. Again, after exchanging ideas in various research fields and field visits to some of the cultivated trees (one of the trees is shown in Figure 2) which have been growing healthily on the land of North America, a proceedings was published in the journal "Bulletin of the Peobody Museum of Natural History, Yale University" [3]. One of the highlights of this symposium was the application of biomolecular (biomarker) and stable isotope techniques in the research field of *Metasequoia* trying to recover paleophysiological and paleoclimatic information from *Metasequoia* fossils that conventional methods/techniques failed to obtain previously. For example, Yang, Leng, and LePage (refer to Ref [3]) detected *in situ* preservation of polysaccharides and cutin acids in three-dimensionally preserved *Metasequoia* leaves from Member 2 of the Late Paleocene Iceberg Bay Formation at Ellesmere Island of the Canadian Arctic (~60 million years, Figure 3) using a modified version of the online pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) technique involving the use of tetramethylammonium hyroxide (TMAH). These biomolecules are the oldest of these types of biomolecules so far documented in the fossil record. Along with evidence from SEM observations, this study proposed a significant role for these labile biomolecules in the fossilization and preservation of three-dimensional leaf tissues.

Since the appearance of this article and other related papers in the proceedings [3], a series of publications using molecular and stable isotope techniques on *Metasequoia* material, both fossil and living, to reveal physiological and climatic information emerged. Using *Metasequoia* as an example, the critical issues concerning with material, technology, and applications of molecular hydrogen isotope values were first discussed and a model of how hydrogen is incorporated into *Metasequoia*, the apparent hydrogen fractionation factors between *Metasequoia* lipid molecules and source water ($\varepsilon_{[\text{water-lipid}]}$) were proposed [6]. Many other research followed. For example, an analysis of environmentally decayed *Metasequoia* leaves revealed that guaiacyl lignin units and cellulose were degraded more rapidly relative to vinyl phenol, a primary pyrolysis product that is thought to have derived from cutin and plant cuticles, suggesting that cutin is likely more stable than lignin [7]. These evidences along with the observed changes in the cellular structure and cuticle of the modern, decayed, and fossil *Metasequoia* leaves under SEM, offered molecular explanation for the long held observation of the abundance of cuticle preservation in the fossil record. The evidence was also supported by a heating experiment of modern *Metasequoia* needle in confined conditions that generated a macromolecular composition similar to those present in the fossils. Results of the experimental heating also implicated the contributions of cutin and its diagenetically altered products to the presence of aliphatic components in terrestrially derived sedimentary organic matter.

Another example evolves the relationships between hydrogen isotope values and latitude as well as climate. Statistical analyses found a strong negative correlation between *n*-alkane δD (stable isotope ratios of hydrogen) and latitude as well as statistically significant correlations between δD and both annual mean temperature (AMT) and spring (February-May) mean temperature (SMT). A weaker but still significant correlation between δD and spring mean precipitation (SMP) was also detected. These results bear strong implications for using δD values obtained from *Metasequoia* fossils as paleoclimatic and paleoenvironmental proxies. These results are consistent with the model of multiple controls for the variability of hydrogen isotopic compositions in higher plant *n*-alkanes based upon a global data set from modern ecosystems [8].

While new technology at the molecular and isotope levels is playing an increasing role in using *Metasequoia* as a proxy for climate study, conventional methods are continuing to perform well in the research field of *Metasequoia* from new perspectives. One of the conventional botanical and paleobotanical techniques is cuticle analysis which has been used as one of the most important tools for plant identification, classification, and correlation for almost two centuries and in recent decades has played a key role in the reconstruction of atmospheric CO_2 concentration. However, the thin and fragile nature of cuticles of *Metasequoia*, a deciduous conifer, impeded the application of cuticle analysis in using the abundant *Metasequoia* fossils to the research field of paleo-CO_2 reconstruction. New and innovated methods developed in recent two year [9,10] now make the recovery of large-sized clean cuticular membranes from fossil *Metasequoia* that recorded paleo-CO_2 information available (Figures 6—7). This breakthrough open doors to more precise calculation of stomata on fossils like *Metasequoia* that has long and well documented fossil record but thin cuticles for the reconstruction of ancient atmospheric CO_2 concentration.



Figures 6—7 Scanning electron microscope photographs of the inner surface of cuticle of the lower epidermis of living Metasequoia (M. glyptostroboides, Fig. 1, leaf sampled from a mature tree cultivated in Nanjing, China) and fossil Metasequoia (M. occidentalis, Fig. 2, sampled from Member 2 of the Late Paleocene Iceberg Bay Formation at Ellesmere Island of the Canadian Arctic), showing both the stomatal zone (left and middle area) and non-stomatal zone (right area). Scale bar = 20 μm.

Recently, the Third International *Metasequoia* Symposium was organized in 2010 in Osaka, Japan, where fossils based on which the genus name *Metasequoia* was established were discovered. During this symposium, more molecular and stable isotope techniques have been applied to resolve questions related to *Metasequoia* as its applications as a climate change indicator. For example, to further investigate the link between hydrogen isotope signals from *Metasequoia* leaves and climate factors, Yang, Blais, and Leng measured δD values from south facing leaf *n*-alkanes of 27 *Metasequoia* trees cultivated across the United States under different latitudes and climatic regions. These trees grown from seeds derived from a single genetic source in China in the late 1940s. These stable isotope records were cross analyzed with climate data over the past 50 years (1950—2009) compiled from stations near each site where these *Metasequoia* trees have been growing [11].

While more *Metasequoia* fossils are to be discovered and analyzed, the living species *M. glyptostrobides* will continue to be treasured in its native habitat in South-Central China as well as in botanical gardens around the globe. With more new technologies applied to both fossil and living forms, scientists will no doubt uncover more characters toward its unique evolutionary history as well as its applications toward a better understanding of the co-evolving earth environment. *Metasequoia* as a vigorous and beautiful plant genus is taking on a new scientific journey on the path of solving new challenging biological and geological questions.

This work was funded in part by the CAS/SAFEA International Partnership Program for Creative Research Teams, the Pilot Project of Knowledge Innovation, CAS (KZCX2-YW-105), the Major Basic Research Projects (2006CB806400), the National Science Foundation of China (40402002, 40872011), Bryant University summer research stipend, and a NASA RI Space Grant. This paper is the contribution 201103 for the Laboratory of Terrestrial Environment of Bryant University.

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(22 October 2010; accepted 28 December 2010)