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# *Thismia americana*, the 101st anniversary of a botanical mystery

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## THISMIA AMERICANA, THE 101ST ANNIVERSARY OF A BOTANICAL MYSTERY

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**Premise of research.** One hundred and one years ago, *Thismia americana* was discovered in a prairie near Chicago in the United States. This tiny mycoheterotrophic plant was observed at the type locality for a few years but is now considered to be extinct. The occurrence of the mainly tropical genus *Thismia* in temperate North America remains a mystery, particularly because *T. americana*'s putative most closely related species occurs in Australia and New Zealand. Here we evaluate the affinities of *T. americana* using morphological data and the relationships of the two other species in *Thismia* subgen. *Thismia* sect. *Rodwaya* with molecular data.

**Methodology.** A 21-character morphological data set of 50 *Thismia* species was analyzed with parsimony and Bayesian phylogenetic analyses. A data set of eight species of *Thismia* and allied taxa containing sequence data of the nuclear 18S and mitochondrial *atp1* regions was analyzed with maximum likelihood and Bayesian phylogeny inference methods, including a Bayesian relaxed molecular clock analysis.

**Pivotal results.** Morphological data indicate that *T. americana* is most closely related to the Old World species of *Thismia*. Nuclear and mitochondrial data suggest that Old World species of *Thismia* are part of a single clade but also show that the section *Rodwaya* is not a monophyletic group.

**Conclusions.** The results suggest that *T. americana* diverged from an Old World ancestor, but the morphological similarity of *T. americana* to other species in the section *Rodwaya* may result from the presence of plesiomorphic characters or convergent evolution rather than close phylogenetic affinity. Northern Hemisphere species of *Thismia* may be more plausible close relatives of *T. americana*. Under the latter scenario, *T. americana* would be part of an Amphi-Pacific Northern Hemisphere disjunction, a more commonly observed distribution pattern in plants.

**Keywords:** *Thismia americana*, Thismiaceae, Burmanniaceae, disjunct distribution, mycoheterotrophy, extinct species, phylogeny, biogeography, molecular clock dating.

**Online enhancement:** appendix.

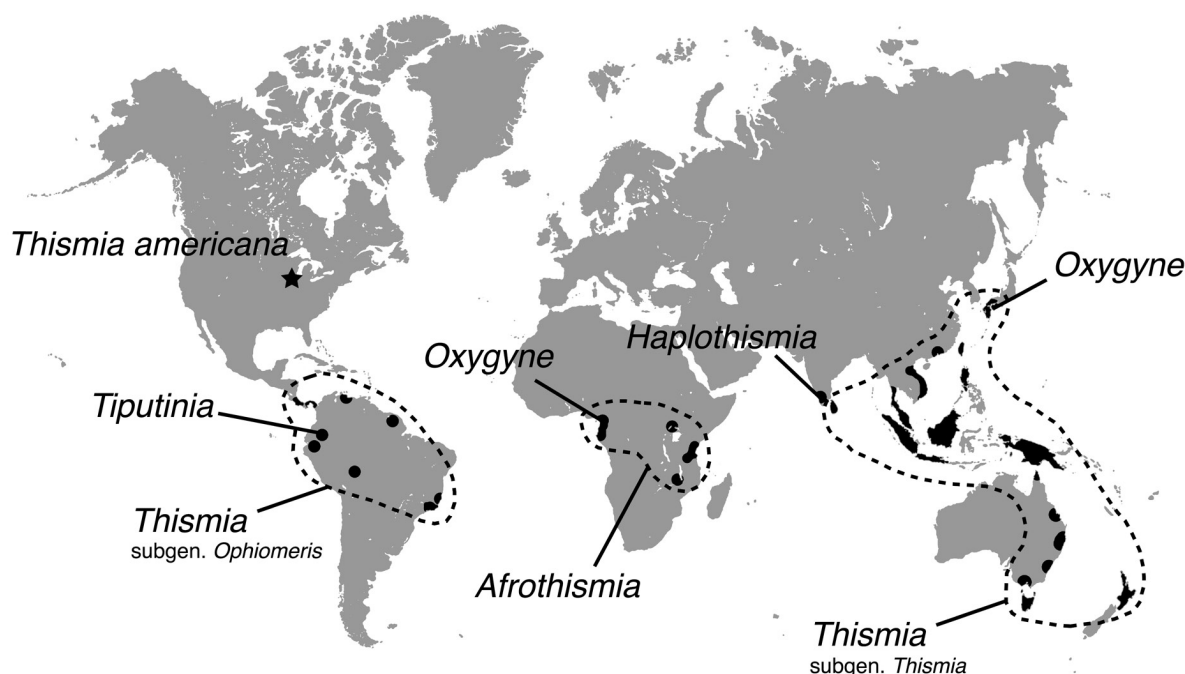
### Introduction

*Thismia* Griff. is part of the intriguing family Thismiaceae J.G. Agardh. The family consists of ~69 species in five genera (Merckx et al. 2013). All species of Thismiaceae are mycoheterotrophic; they are nonphotosynthetic and obtain carbon and nutrients from surrounding plants through shared arbuscular mycorrhizal fungi (Merckx and Bidartondo 2008; Merckx et al. 2012). The largest genus, *Thismia*, comprises ~50 species of which the majority are known from tropical America and Asia, but some species from Asia extend into the subtropics (southern Japan, Australia, and New Zealand; Thiele and Jordan 2002; Yang et al. 2002) and temperate zones (*Thismia rodwayi* F. Muell. in Tasmania; Roberts et al. 2003; Wapstra et al. 2005; fig. 2). *Afrothismia* Schltr. (~12 species)

occurs throughout tropical Africa, although most of the species are known only from Cameroon. *Tiputinia* P.E. Berry & C.L. Woodw. (one species) is endemic to Ecuador, and *Haplothismia* Airy Shaw (one species) occurs at only two sites in the Western Ghats, India (Merckx et al. 2013). Last, the cryptic genus *Oxygyne* Schltr. consists of two species from Cameroon and three from southern Japan (fig. 1).

Historically, Thismiaceae were considered a family closely related to Burmanniaceae (Schlechter 1921; Hutchinson 1934, 1959; Dahlgren et al. 1985; Takhtajan 1997) or the genera of Thismiaceae were included as the tribe Thismieae in a broadly defined Burmanniaceae (Miers 1847; Jonker 1938; Stevenson and Loconte 1995; Maas-van de Kamer 1998). DNA data have convincingly shown that the genera of Thismiaceae are not part of Burmanniaceae, but this has yet to be formally established. However, the analyses are still somewhat inconclusive about the monophyly and exact phylogenetic position of Thismiaceae in Dioscoreales (Merckx et al. 2009, 2010). The latest hypotheses based on nuclear 18S rDNA and mitochondrial

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**Fig. 1** Global distribution of Thismiaceae, based on multiple sources. *Thismia* in South America consists of *Thismia* subgen. *Ophiomeris*. *Thismia* species in Southeast Asia are part of *Thismia* subgen. *Thismia*.

*atp1* and *nad1 b-c* data suggest that *Thismia*, *Tiputinia*, and *Haplothismia* form a clade sister to *Tacca* J.R. Forst. & G. Forst, and the clade of those four genera is in turn the sister group of *Afrothismia* (Merckx et al. 2010). These phylogenies further suggest that *Thismia* is paraphyletic because it includes *Haplothismia*, *Tiputinia*, and perhaps also *Oxygyne* (Yokoyama et al. 2008). But because of the low number of sampled species, limited available molecular data, and extremely high DNA substitution rates observed in Thismiaceae, these phylogenetic hypotheses should be treated with caution.

Many species of Thismiaceae are known from very few collections and are thus considered to be extremely rare. For example, a few Neotropical species of *Thismia* have not been seen for more than a century and are possibly extinct (Merckx et al. 2013). However, for some species, this apparent rarity may be influenced by their hidden nature and ephemeral occurrence. In at least one case, *T. rodwayi* in Tasmania, it has been shown that the plants' tiny habit, ephemeral occurrence, and ability to flower under leaf litter explain the paucity of encounters. Since its discovery in 1890, the species had been recorded only about six times in Tasmania over a period of 112 yr (Roberts et al. 2003). However, because of recent targeted surveys, *T. rodwayi* is now known from more than 26 sites in east and central Tasmania (Roberts et al. 2003; Wapstra et al. 2005; M. Wapstra, personal communication).

The most remarkable species of *Thismia* was discovered on August 1, 1912, by Norma E. Pfeiffer, then a botany student at the University of Chicago, near Lake Calumet, Chicago (Mohlenbrock 1985). In an open prairie "in a small space along the margin of a grass field" (Pfeiffer 1914, p. 124) she

found a tiny achlorophyllous plant, which she would later describe as *Thismia americana* N. Pfeiff. (Pfeiffer 1914; fig. 2A). At the time of the discovery, only about 15 other species of *Thismia* were known, and most had been collected in tropical rain forests. Remarkably, Pfeiffer concluded that the specimens she found in the Lake Calumet area seemed most closely related to *Thismia* species from South East Asia, New Guinea, Australia, and New Zealand (Pfeiffer 1914). Furthermore, in his revision of the Thismieae, the German botanist Rudolf Schlechter (1921) classified *T. americana* (as *Sarcosiphon americanus* Schltr.) in *Sarcosiphon* section *Rodwaya* together with *Sarcosiphon rodwayi* (F. Muell.) Schltr. from Australia and *Sarcosiphon hillii* Schltr. from New Zealand (both species are now considered to be *T. rodwayi*). He noted that he had little doubt ("ganz außer Zweifel") that the South Australian species are closely related to the specimens Pfeiffer found in North America. In his monograph of the Burmanniaceae, Frederik Jonker even considered the possibility that *T. americana* may be conspecific with *T. rodwayi*, and he classified both species as the only members of *Thismia* sect. *Rodwaya*. He further noted, "It is hardly [*sic*] to believe that Chicago is the normal area for this species, but I cannot give a satisfactory explanation why it occurs there" (Jonker 1938, p. 12). Robert Thorne (1972, p. 407) declared the intriguing antipodal distribution pattern that follows from the presumed close relationship between *T. americana* and *T. rodwayi* as the "most anomalous disjunction of all." Unfortunately, the occurrence of *T. americana* in the Chicago area remains an unsolved mystery because the plant has not been collected for almost a century. After her discovery in 1912, Pfeiffer later recollected

specimens at the same locality in the summers of 1913 and 1914 (Pfeiffer 1918; Tardy 1952) and some authors suggest also in 1915–1916 (Williams 1973; Maas et al. 1986; Masters 1995; Lewis 2002). However, in a letter to Robert Mohlenbrock, Pfeiffer claims that by 1914 “a barn had been built on this particular area,” destroying the type locality of *T. americana* (Mohlenbrock 1985, p. 37). This contradicts her statement that the sporangia of *T. americana* were studied using material collected in 1913 and 1914 (Pfeiffer 1918, p. 357). Interestingly, in the same letter, Pfeiffer recalls finding a few *T. americana* specimens at another location nearby (presumably also in 1912), “about a third of a mile away” from the original site (Mohlenbrock 1985, p. 36). The type locality is now a suburban area, and despite intensive searches in similar habitats nearby, the plant has never been seen since (Bowles et al. 1994). Consequently, *T. americana* is considered to be possibly extinct (Lewis 2002). Recently, a new species of *Thismia* was described from material collected in Taiwan (Chiang and Hsieh 2011). This species closely resembles both *T. americana* and *T. rodwayi* and is thus considered a member of *Thismia* subgen. *Thismia* sect. *Rodwaya* as well (fig. 2; for notes about the current taxonomic subdivision of *Thismia*, see app. B [available online]).

The disjunct distribution pattern that results from the presumed sister group relationship of *T. americana* and *T. rodwayi* has been considered as one of the strangest and largest disjunctions in flowering plants (Thorne 1972). Several competing biogeographic scenarios may explain this remarkable distribution pattern, including a long-distance dispersal event and migration of *Thismia* through Beringia or even through Europe into North America across the Atlantic land bridge. Here we evaluate these various hypotheses on the relationships and biogeographic history of *T. americana*, using a morphological data set and the available molecular data of *Thismiaceae*. The morphological data were acquired from published species descriptions rather than specimen observations. Although the latter is preferable, collections of *Thismia* are very rare, material is often poorly preserved or too scarce to allow dissecting (Maas et al. 1986), and—for at least one species—no material is available (Saunders 1996).

## Material and Methods

### Analyses of Morphological Data

On the basis of the available literature, we scored 21 characters for 50 species of *Thismia* and for *Oxygyne*, *Tiputinia foetida*, *Haplothismia exannulata*, and *Afrothismia* as outgroups (for character list, comments, and data set, see app. B). Character scoring procedure followed Brazeau (2011). Safe taxonomic reduction (Wilkinson 1995) was then applied to the data set using PerIEQ (<http://www.molekularesystematik.uni-oldenburg.de/download/goto.php?w=/Programs/PerIEQcmd.zip>). This method identifies taxa that can be deleted a priori without having an impact on the inferred relationships of the remaining taxa. Cladistic analysis on the resulting data set with 21 taxa was performed with PAUP\*4.10b (Swofford 2002), using the branch and bound search option with the MulTrees option in effect. All characters were set unordered. Bootstrap analysis was performed using 100 bootstrap pseudoreplicates

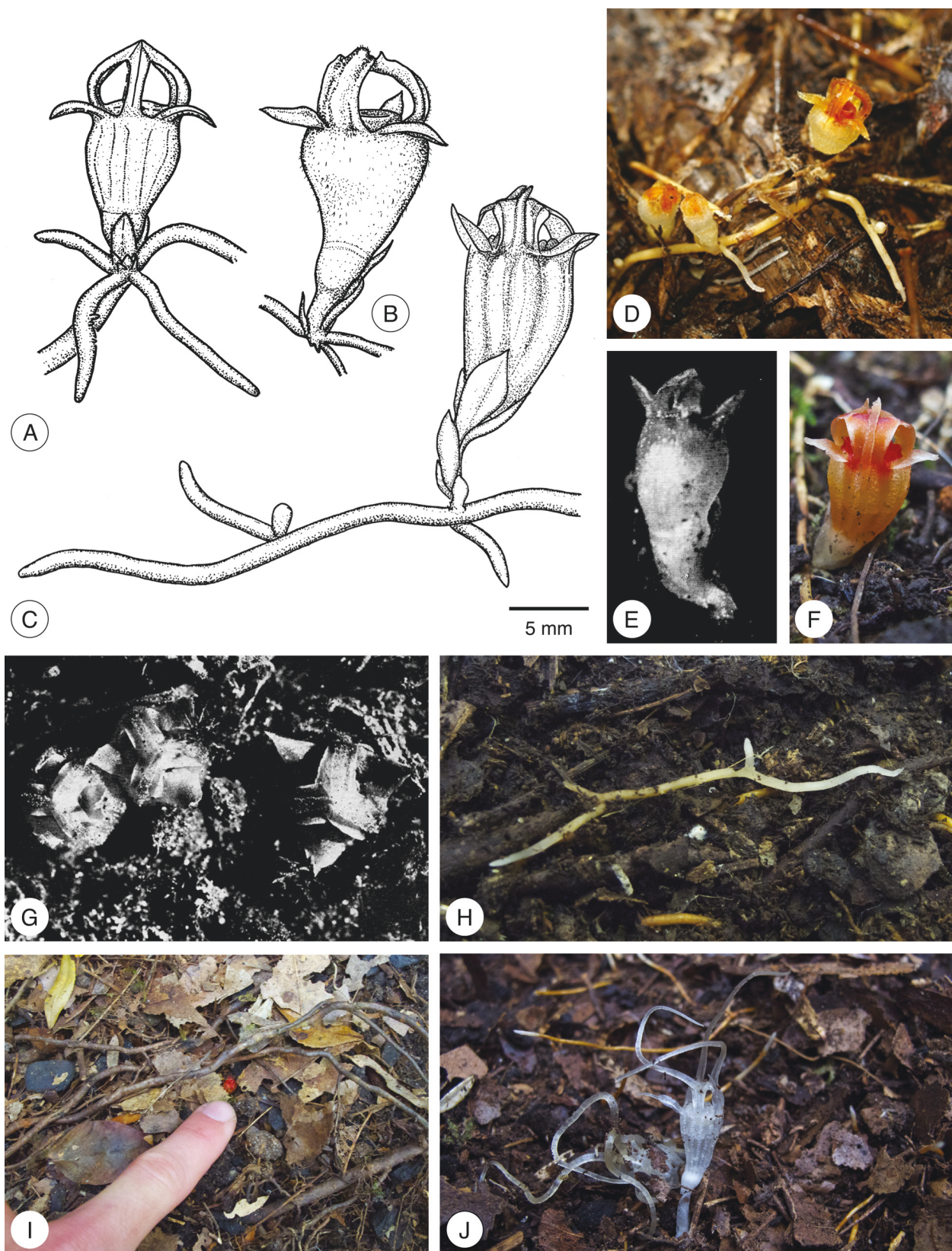
and branch and bound search with the MulTrees option in effect. Bayesian inference (BI) analysis used a Markov K model with a gamma-distributed rate variation (Mk + G; Lewis 2001) with  $1 \times 10^7$  generations sampling every 2000 generations (MrBayes, ver. 3.2.1; Ronquist et al. 2012). The 100,000-generation burn-in period was discarded and the majority-rule consensus tree computed for the sampled generations.

### Analyses of Molecular Data

Appendix A lists all taxa included in the molecular phylogenetic analysis with voucher information and GenBank accession numbers. For sequences newly obtained for this study, DNA was extracted from silica gel-dried plant material with the DNeasy Plant Mini Kit (QIAGEN, Venlo), following the manufacturer's instructions. Amplification of the 18S rDNA region was carried out with the primers NS1, NS2, NS3, NS4, NS5, and NS8 (White et al. 1990) under the following conditions: 1 min at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at 44°C, and 1 min at 72°C, followed by 7 min at 72°C. Mitochondrial *atp1* sequences were amplified with the primers and conditions described by Eyre-Walker and Gaut (1997). Sanger sequencing was performed by the MacroGen sequencing facilities (Amsterdam). Sequences were assembled and edited with Geneious Pro (ver. 5.5.6; Biomatters, New Zealand). Sequence alignments were obtained with the MAFFT (ver. 6.814b) alignment tool (Katoh et al. 2002) implemented in Geneious Pro. Phylogenetic reconstructions were conducted using both maximum likelihood (ML) and BI optimality criteria. First, each of the DNA data sets was analyzed separately. The substitution model for both DNA regions was selected with jModeltest (ver. 2.3.1; Darriba et al. 2012) under the Akaike Information Criterion. The best-fitting model for both data sets is the GTR + I + G model. Searches for the best likelihood tree were performed with RAxML (ver. 7.2.8; Stamatakis 2006). Clade support was estimated by nonparametric bootstrap analyses on 500 pseudo-replicate data sets. An ML analysis on the combined data was performed using the same settings as described above, with a GTR + I + G model applied to the total data set. We choose to apply a single model to the complete data set rather than a separate model on the two partitions because for some taxa, no *atp1* data were available. In addition, BI analyses were conducted on the combined data set with MrBayes (ver. 3.2.1) with a GTR + I + G model to the total data set. Four analyses, each consisting of four Markov chains starting with a random tree, were run simultaneously for  $1 \times 10^7$  generations, sampling trees at every 2000th generation. The first 500 sampled trees of each analysis were regarded as burn in and discarded. A majority-rule consensus tree was calculated using the remaining 4500 trees.

In addition, the same data set was used for a phylogenetic analysis enforcing a relaxed molecular clock with BEAST (ver. 1.7.5; Drummond et al. 2012). We applied a GTR + I + G model with four gamma categories on the total DNA data set. The uncorrelated lognormal clock model (Drummond et al. 2006) was selected, and a prior with a normal distribution of  $123 \pm 8$  Ma was assigned to the root node of the tree (crown node of Dioscoreales). This prior encapsulates previous estimates for crown node of the Dioscoreales (Janssen and Bremer





**Fig. 2** A, *Thismia americana*. Redrawn from Pfeiffer (1914). B, *Thismia huangii*. Redrawn from Chiang and Hsieh (2011). C, *Thismia rodwayi*. Drawn from a specimen collected by V. S. F. T. Merckx in Tasmania. D, *Thismia huangii*. Flowers in bud on the left, open flower on

2004; Merckx and Bidartondo 2008; Merckx et al. 2008). The distribution of all other priors was set to uniform, except for the uncorrelated relaxed clock standard deviation, to which an exponential prior was assigned. Posterior distributions of parameters were approximated using two independent Markov chain Monte Carlo analyses of 20,000,000 generations followed by a discarded burn-in of 2,000,000 generations (10%). Convergence of the chains was checked by evaluating the effective sampling size values of each parameter with TRACER (ver. 1.4).

## Results

### *Analyses of Morphological Data*

Three characters were found to be parsimony uninformative. On the basis of the remaining 18 characters, one most parsimonious tree with a total length of 32 steps was found (fig. 3). This topology has a consistency index of 0.688 and a retention index of 0.839. Bayesian analysis recovered a similar topology (not shown). In the obtained topology, *Thismia* is a paraphyletic group with respect to *Tiputinia*, *Haplothismia*, and *Oxygyne*, but branch support is low (bootstrap percentage, 52; Bayesian posterior probability, 0.50). *Thismia* subgen. *Ophiomeris* is not a monophyletic group, with section *Pyramidalis* more closely related to *Thismia* subgen. *Thismia* than to other species of the subgenus *Ophiomeris*. *Thismia* subgen. *Thismia* forms a monophyletic group with low branch support (bootstrap percentage, 56; Bayesian posterior probability, 0.50). Within subgenus *Thismia*, sections *Euthismia*, *Glaziocharis*, *Rodwaya*, and *Sarcosiphon* are paraphyletic groups. Sections *Geomitra* and *Scaphiophora* are monophyletic. *Thismia americana* is placed as the sister group of a clade that consists of the sections *Geomitra*, *Sarcosiphon*, and *Scaphiophora*.

### *Analyses of Molecular Data*

BI analysis of 18S rDNA recovered a topology (fig. B1 [figs. B1–B4 available online]) that did not show highly supported incongruence with the topology reconstructed with *atp1* data (fig. B2), except for the position of *Trichopus*. ML and BI analyses on the combined data resulted in very similar topologies. The highest-likelihood tree is shown in figure 4; the majority-rule consensus tree of the BI analysis is provided as figure B3. *Thismiaceae* are a paraphyletic group with respect to *Tacca*. The sister group relationship between *Tacca* and the clade consisting of *Thismia*, *Oxygyne*, *Haplothismia*, and *Tiputinia* receives significant Bayesian posterior probability (0.98) and moderate bootstrap percentage (77). Within the latter clade, *Oxygyne* is sister to the rest of the taxa, and *Thismia* is not a monophyletic group with respect to *Haplothismia* and *Tiputinia*; however, these relationships are not well supported. All *Thismia* subgen. *Thismia* species are part

of a well-supported monophyletic group (bootstrap percentage, 100; Bayesian posterior probability, 1.00). Within this clade, the two species from Taiwan—*Thismia taiwanensis* (sect. *Glaziocharis*) and *Thismia huangii* (sect. *Rodwaya*)—are well-supported sister taxa. This clade is sister to a clade that consists of taxa from Borneo (*Thismia clavigera* [sect. *Geomitra*], *Thismia aseroe*, and *Thismia javanica* [both sect. *Euthismia*]) and Australia (*Thismia rodwayi* [sect. *Rodwaya*] and *Thismia clavarioides* [sect. *Glaziocharis*]). Specimens of *T. rodwayi* are not placed in a monophyletic group because of the sister group relationship between *T. clavarioides* and *T. rodwayi* specimens from Australia.

The phylogenetic relationships recovered by the Bayesian relaxed clock analysis do not show well-supported incongruences with the BI and ML trees (fig. B4). The mean stem group age of the *Thismiaceae* (without *Afrothismia*) is estimated at 84 Ma, with a 95% confidence interval (CI) of 61–106 Ma. The crown node age of this clade is estimated at 73 Ma (95% CI, 51–94 Ma). The stem and crown node age estimates of the *Thismia* subgen. *Thismia* clade are, respectively, 67 Ma (95% CI, 46–89 Ma) and 51 Ma (95% CI, 32–72 Ma; fig. 5).

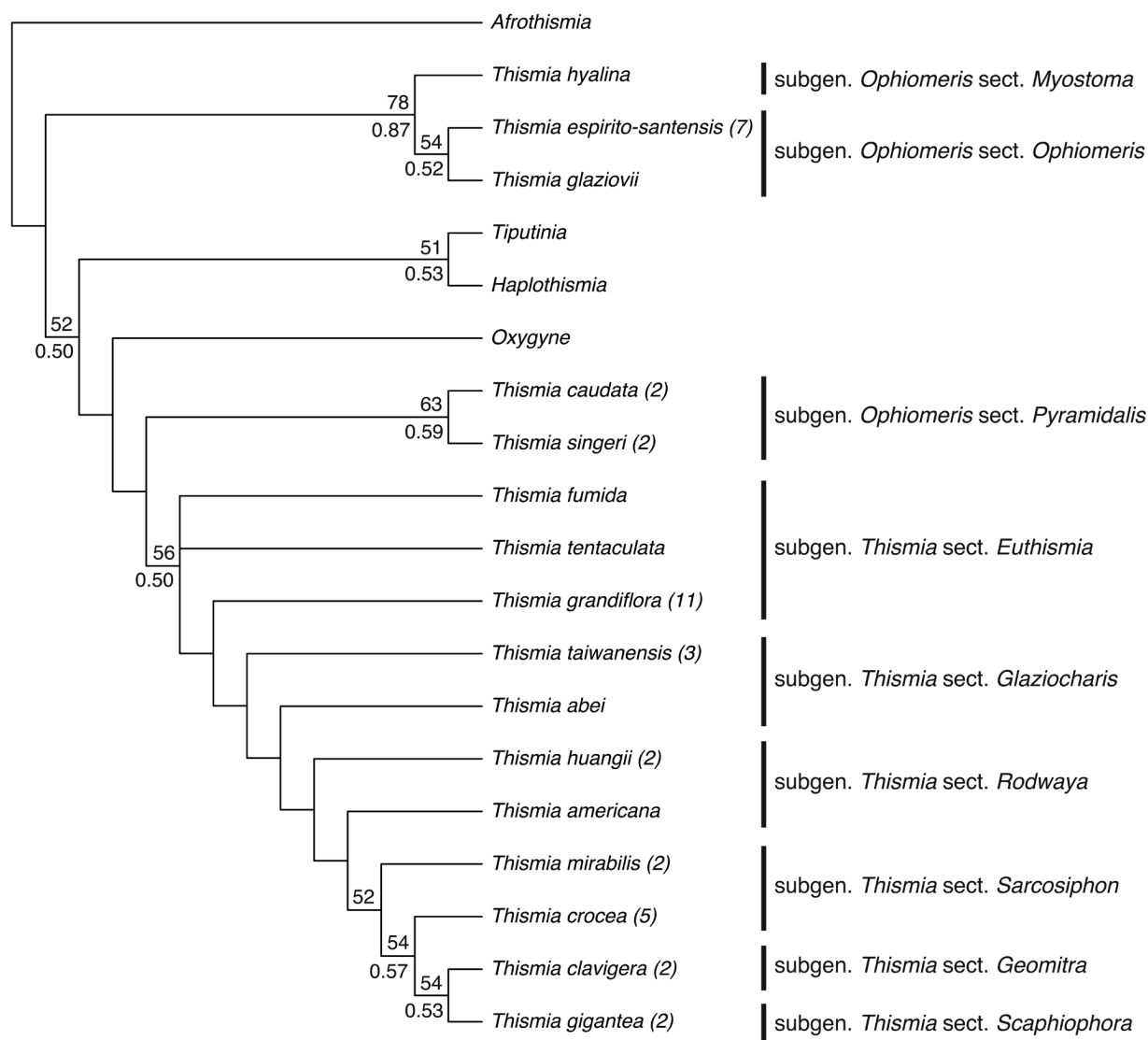
## Discussion

### *Phylogenetic Affinities*

As noted by Pfeiffer (1914) and subsequent authors (Schlechter 1921; Jonker 1938), morphology suggests that *Thismia americana* is most closely related to Old World species of *Thismia*. It closely resembles *Thismia rodwayi* and *Thismia huangii* but lacks the short dorsal mitre appendages observed in these two species. Therefore, in the phylogeny, on the basis of morphological data, *T. americana* is placed sister to a clade with species of the sections *Sarcosiphon*, *Geomitra*, and *Scaphiophora* because of its resemblance with *Thismia mirabilis* and *Thismia angustimitra*. These two species also lack mitre appendages and have, similar to *T. americana*, creeping vermiform roots. All other species in the sections *Sarcosiphon*, *Geomitra*, and *Scaphiophora* have coralloid roots.

Phylogenetic relationships of Dioscoreales obtained with the molecular data are consistent with those found in previous studies (Merckx and Bidartondo 2008; Merckx et al. 2009). This phylogeny suggests that variation in perianth appendages may bear little phylogenetic information in *Thismia*. *Thismia clavarioides*, a species with prominent appendages on both the mitre and the outer perianth lobes, is closely related to *T. rodwayi*, which has short mitre appendages and lacks appendages on the outer perianth lobes. Apart from the differences in perianth appendages, the flower morphology of *T. rodwayi* and *T. clavarioides* is very similar (Thiele and Jordan

the right. Reused with permission from Chiang and Hsieh (2011). E, *Thismia americana*. Image from Pfeiffer (1914), used with permission. F, Open flower of *T. rodwayi*, pictured on Mount Wellington, Tasmania, Australia. Photo by V. S. F. T. Merckx. G, Three open flowers of *T. americana*, viewed from the top. Image from Pfeiffer (1914), used with permission. H, *Thismia rodwayi* in vegetative stage. Blue Mountains, New South Wales, Australia. Photo by V. S. F. T. Merckx. I, Open flower of *T. rodwayi*, growing in the leaf litter in Morton National Park, New South Wales, Australia. Photo by V. S. F. T. Merckx. J, *Thismia clavarioides* pictured close to the type locality in Morton National Park, New South Wales, Australia. Photo by V. S. F. T. Merckx.

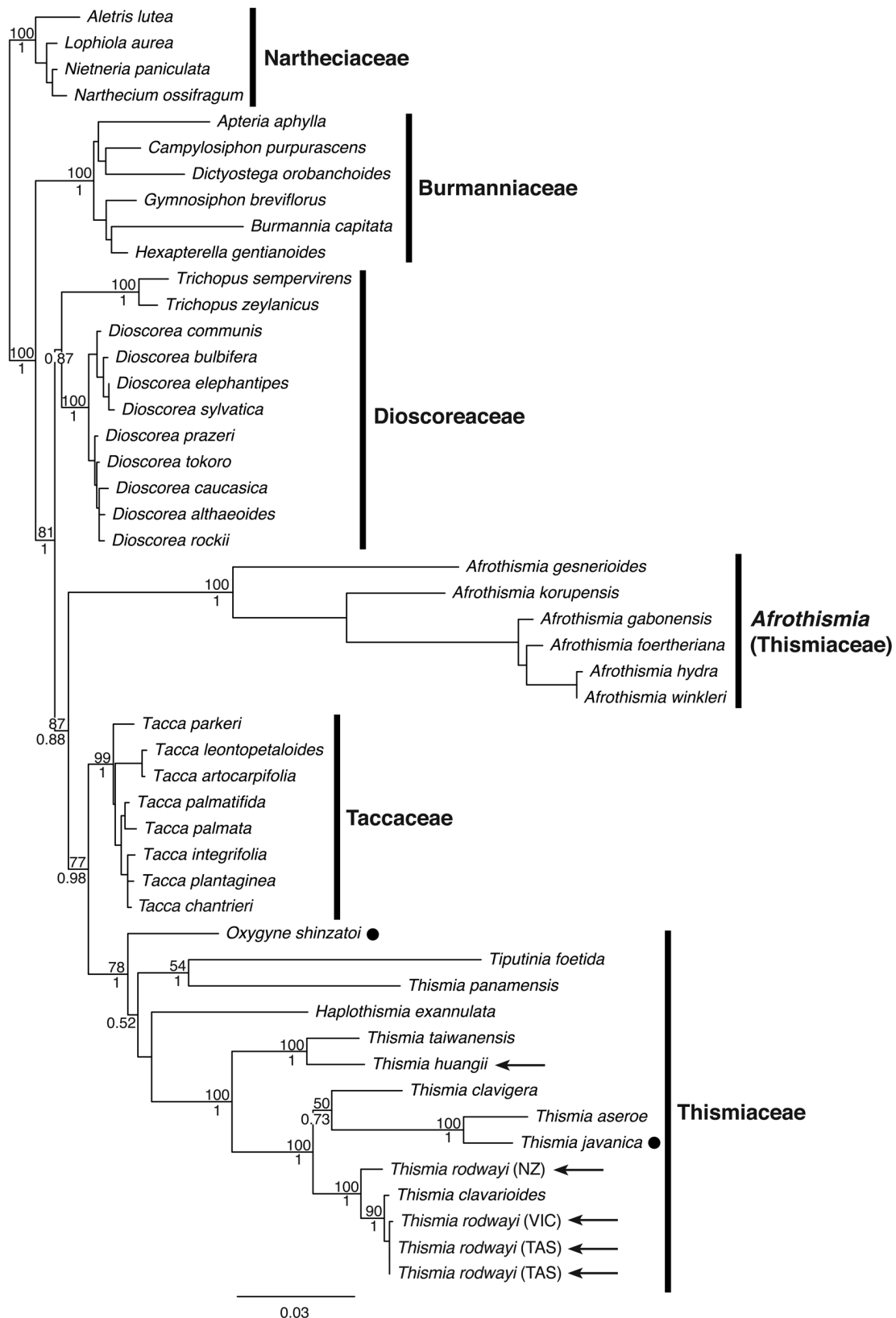


**Fig. 3** Single shortest tree (length, 32; consistency index, 0.688; retention index, 0.839) obtained with parsimony analysis of the morphological data. Bootstrap support values of  $\geq 50$  are shown above branches. Bayesian posterior probabilities of  $\geq 0.50$  are given below the branches. Numbers in parentheses indicate the total number of species a taxon represents, which were reduced to a single taxon after safe taxonomic reduction.

2002); therefore, their close phylogenetic relationship is not unexpected.

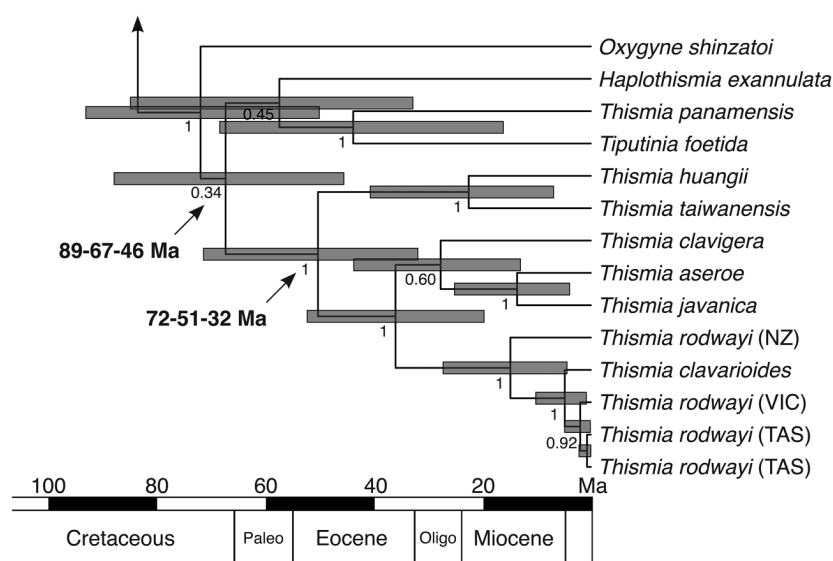
Apart from the perianth appendages, other differences between species of the section *Rodwaya* include the presence of short hairs on the flower tube of *T. huangii*, which are absent in the two other species, and the color of the flower (whitish-green in *T. americana*, orange in *T. rodwayi*, and white and orange in *T. huangii*). In addition, *T. americana* lacks anther connective appendages that are present in *T. rodwayi* and *T. huangii* (Thiele and Jordan 2002; Chiang and Hsieh 2011). Therefore, the suggestion that *T. americana* and *T. rodwayi* are conspecific (Jonker 1938) is very unlikely. Moreover, molecular data clearly show that the section *Rodwaya* is not monophyletic and that the resemblance of *T. rodwayi* and *T.*

*huangii* is explained by the presence of plesiomorphic characters or the result of convergent evolution. Remarkably, molecular data also suggest that despite their close morphological similarity, *T. rodwayi* specimens used in this study are not placed in a monophyletic group due with respect to *T. clavarioides*. Thus, one option to retain *T. rodwayi* as a monophyletic species is to reduce *T. clavarioides* to the latter. However, because of clear macromorphological differences between *T. rodwayi* and *T. clavarioides* (Thiele and Jordan 2002), we advocate for the alternative option: the recognition of a *Thismia* species endemic to New Zealand. The taxon from New Zealand was initially described as a distinct species, *Bagnisia hillii* Cheeseman, and thus the combination *Thismia hillii* should be used. This combination has already been validly



**Fig. 4** Highest-likelihood tree ( $-\ln L: 12,909.56$ ) obtained with maximum likelihood analysis of the combined molecular data set. Bootstrap support values are shown above the branches. Bayesian posterior probabilities are indicated below the branches. Support values are shown for only major Dioscoreales clades and clades that are relevant for this study. Circles indicate taxa for which only 18S rDNA data were available. Arrows indicate specimens that are classified as *Thismia* subgen. *Thismia* sect. *Rodwaya*.





**Fig. 5** *Oxygyne-Haplothismia-Tiputinia-Thismia* clade isolated from the Bayesian relaxed clock analysis (for complete tree, see fig. B4, available online). Numbers under nodes indicate posterior probabilities. Gray bars encompass 95% confidence intervals of the age estimates. Age estimates (97.5% maximum-mean-2.5% minimum) are shown for the stem and crown nodes of *Thismia* subgen. *Thismia*.

published by Pfeiffer (1914): *Thismia hillii* (Cheeseman) N. Pfeiff.

The polyphyly of the section *Rodwaya* shows that the current classification of *Thismia*, at least for the species in this section, does not reflect their natural relationships. Therefore, the affinities of the enigmatic *T. americana* remain unclear, although morphology suggests that it diverged from an Old World rather than a New World ancestor. Additional DNA sampling is needed to evaluate the relationships and taxonomy of *Thismia*. A phylogenetic hypothesis based on more taxa will also identify morphological characters with high phylogenetic information and thus help to elucidate the affinities of *T. americana*. Similarly, such phylogeny will be a useful tool to evaluate biogeographic scenarios that have shaped the distribution of this group.

### Biogeography

On the basis of the morphological data presented in this study, *T. americana* diverged from an Old World species of *Thismia* (*Thismia* subgen. *Thismia*), but its close relationship with *T. rodwayi* remains questionable. Close relationships between plant species in eastern Asia and eastern North America are not uncommon and have been highlighted by several authors (Li 1952; Graham 1972; Wood 1972; Tiffney 1985b; Wen 1999; Donoghue et al. 2001). There are several competing biogeographic scenarios that explain the links between the flora of North America and Southeast Asia. We discuss each scenario below in relation to the divergence time estimates we obtained. These estimates are based on a very limited sampling of *Thismia* and result from a single secondary calibration point placed quite distantly from the clade of interest. Also, the high substitution rates observed in *Thismia* and related mycoheterotrophic lineages may influence the age estimates and phy-

logenetic relationships. Thus, the time estimates must be considered as a very rough proxy for the divergence of *Thismia*.

The first hypothesis is that the occurrence of *Thismia* in North America is the result of a long-distance dispersal event between Southeast Asia and North America. Seeds of *Thismia* are, like most mycoheterotrophic plants, minute (Maas et al. 1986). Seed dispersal has not been studied in detail, but since fruits are cup shaped, it is assumed that seeds are dispersed by rain splash, after which they may be transported over considerable distance by water (Stone 1980). Some authors also suggested that the fruits of *Thismia* are eaten by animals (Croat 1978; Wood 2010). Seeds may also stick to the feet of ground-dwelling animals, in which case birds would be the most likely vector for transoceanic dispersal. The occurrence of *Thismia* in New Zealand, which has been physically isolated from the rest of the world for more than 55 million years (McLoughlin 2001), demonstrates that long-distance dispersal events did occur in the biogeographic history of *Thismia*. Our relaxed clock analysis suggests that the New Zealand *Thismia* shared a common ancestor with Australian species of *Thismia* at 4–27 Ma. The presence of *Thismia* in New Zealand is therefore most likely the result of a long-distance dispersal event. Current data, albeit still from a limited sampling, suggest that *Thismiaceae* species are mycorrhizal specialists (Merckx and Bidartondo 2008; Merckx et al. 2012). The occurrence of a suitable host fungus may therefore be the most crucial element to determine the success of a long-distance dispersal event for *Thismia*. We still know very little about the worldwide distribution of arbuscular mycorrhizal fungi. Yet some fungi may be widespread, facilitating long-distance dispersal of their mycoheterotrophic parasites. The fungal taxon found in roots of *T. rodwayi* (Merckx et al. 2012) has thus far been detected in Argentina and Finland and on prairie in Kansas (Öpik et al.

2010), showing a theoretical potential for wide dispersal of this mycoheterotrophic plant. A long-distance dispersal event may have occurred at any time, but since *T. americana* shows considerable morphological differences with all of the currently known *Thismia* species, it seems unlikely that such a dispersal event would have happened in historical times.

During the Tertiary, when the diversification of *Thismia* subgen. *Thismia* took place (fig. 5), various land bridges temporarily connected Eurasia and North America. Migration of *Thismia* across these land bridges offers a plausible alternative scenario for a long-distance dispersal event. One such land bridge is known as the Atlantic track, a dispersion pathway north of the Tethys seaway and across the North Atlantic through the Eocene (Tiffney 1985a; Davis et al. 2002). Eocene Atlantic track migration from the Old World into the New World has been postulated for many tropical plant families, such as Rubiaceae, Annonaceae, and Melastomataceae (Renner et al. 2001; Antonelli et al. 2009; Couvreur et al. 2011). Plant migration over the Atlantic track was possible until the Miocene (Tiffney 2000; Donoghue et al. 2001), although cooler climates probably prevented tropical and subtropical taxa from migrating over this pathway after the Eocene. However, for nontropical *Thismia* species, this route may have been a plausible option in the Miocene. According to our molecular clock estimates, *Thismia* subgen. *Thismia* originated between 46 and 89 Ma and started to diversify between 32 and 72 Ma (fig. 5). This timing is not in conflict with a possible dispersal event over the North Atlantic land bridge. However, such a scenario would involve a long migration route through southwestern Asia and Europe, followed by subsequent extinction events.

A more plausible land bridge scenario for disjunct plant distributions across eastern North America and Southeast Asia involves the Beringia land bridge, also known as the Pacific track (Donoghue et al. 2001; Sanmartin et al. 2001; Donoghue and Smith 2004). For plants, Pacific track migration is generally considered to have occurred mostly during the past 30 million years (Donoghue and Smith 2004). However, dispersal from Asia to North America over Beringia during the late Paleocene or Early Eocene is commonly accepted for mammals (Rose 2006; Beard 2008). The current Amphi-Pacific distribution of *Thismia* subgen. *Thismia* shows similarities with that of several Northern Hemisphere genera, such as *Gymnocladus* (Fabaceae), *Liriodendron* (Magnoliaceae), *Hamamelis* (Hamamelidaceae), and *Weigela-Diervilla* (Caprifoliaceae). All of these disjunct patterns have been explained by cross-Beringia migration (Donoghue et al. 2001; Donoghue and Smith 2004). This scenario involves the shortest route for *Thismia* subgen. *Thismia* migration, requires the fewest number of extinction

events, and cannot be rejected by the divergence time estimates. Therefore, it seems currently the most likely explanation for the occurrence of *Thismia* in North America.

### Conclusions

Unless *T. americana* is rediscovered or DNA is successfully obtained from a museum specimen, inferences about its evolutionary history will inevitably involve uncertainty and speculation. However, this should not prevent attempts to make critical assessment of possible scenarios for the occurrence of *Thismia* in North America. Our analyses support Pfeiffer's view that the closest relative of *T. americana* is a species from the Old World. However, the presumed close relationship between *T. americana*, *T. rodwayi*, and *T. huangii* is, at least for the latter two species, rejected by DNA data. This indicates that morphological similarity in *Thismia* may to some extent be attributed to plesiomorphic characters or convergent evolution rather than to close phylogenetic relationship. Therefore, it seems unlikely but not impossible that the closest extant relative of *T. americana* is the antipodean *T. rodwayi*. Northern Hemisphere species of *Thismia*, particularly those from subtropical Asia, may be more plausible close relatives of *T. americana*. Under the latter scenario, *T. americana* would be part of an Amphi-Pacific Northern Hemisphere disjunction, a more commonly observed distribution pattern in plants. In this case, the current distribution of *Thismia* subgen. *Thismia* would most likely result from migration over Beringia.

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## Appendix A

### Molecular Data (18S rDNA, *atp1*)

XXX = missing. For newly generated sequences, specimen voucher information is provided. Burmanniaceae: *Apteria aphylla*: DQ786034, EU421007; *Burmannia capitata*: DQ786065, EU421009; *Campylosiphon purpurascens*: EU420996, EU421024; *Dictyostegia orobanchioides*: DQ786056, EU421026; *Gymnosiphon breviflorus*: DQ786036, EU421032; *Hexapterella gentianoides*: DQ786057, EU421038; Dioscoreaceae: *Dioscorea communis*: EU186223, AY277804; *Dioscorea bulbifera*: AF069203, FJ215775; *Dioscorea elephantipes*: FJ215767, FJ215777; *Dioscorea sylvatica*: FJ215768, FJ215778; *Dioscorea prazeri*: DQ786089, EU421028; *Dioscorea tokoro*: DQ786088, FJ215776; *Dioscorea caucasica*: FJ215769, FJ215779; *Dioscorea*

*althaeoides*: EU420997, EU421027; *Dioscorea rockii*: DQ786090, EU421029; *Trichopus sempervirens*: AF309395, AY299724; *Trichopus zeylanicus*: AF309394, AY277805; Nartheciaceae: *Alettris lutea*: DQ786092, FJ215780; *Lophiola aurea*: DQ786091, EU421039; *Narthecium ossifragum*: AF309411, AY299809; *Nietneria paniculata*: EU186219, EU421041; Taccaceae: *Tacca parkeri*: EU421001, JN850562; *Tacca leontopetaloides*: EU420999, AF039252; *Tacca artocarpifolia*: AF309397, EU421043; *Tacca palmatifida*: DQ786084, FJ215774; *Tacca palmata*: EU421000, EU421046; *Tacca integrifolia*: DQ786085, EU421045; *Tacca plantaginea*: U42063, EU421047; *Tacca chantrieri*: DQ786086, EU421044; Thismiaceae: *Afrothismia gesnerioides*: EU420989, EU421003; *Afrothismia korupensis*: EU420991, EU421005; *Afrothismia gabonensis*: FJ215766, FJ215772; *Afrothismia foertheriana*: EU420988, EU421002; *Afrothismia hydra*: EU420990, EU421004; *Afrothismia winkleri*: EU420992, EU421006; *Thismia rodwayi* (New Zealand): AF309403, AY299849; *T. rodwayi* (Australia, TAS)—Merckx and Wapstra TAS1-3 (L): KF692536, KF692540; *T. rodwayi* (Australia, TAS)—Merckx and Wapstra TAS10-1 (L): KF692537, KF692542; *T. rodwayi* (Australia, VIC)—Walsh s.n. (L): KF692538, KF692541; *Thismia huangii*—T. H. Hsieh and P. Y. Chiang 3031 (TAI): KF692534, KF692543; *Thismia taiwanensis*: DQ786080, EU421051; *Thismia clavarioides*—P. Jordan NSW 447624 (NSW): KF692533, KF692539; *Thismia javanica*—Agatho-Elsener 167 (L): KF692535, XXX; *Thismia clavigera*: AF309405, EU421049; *Thismia aseroe*: AF309404, EU421048; *Thismia panamensis*: DQ786081, EU421050; *Oxygyne shinzatoi*: AB437090, XXX; *Tiputinia foetida*: FJ215764, FJ215770; *Haplothismia exannulata*: DQ786082, EU421037.

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