

## ALLOPATRIC SPECIATION IN ASIA CONTRIBUTED TO THE DIVERSITY ANOMALY BETWEEN EASTERN ASIA AND EASTERN NORTH AMERICA: EVIDENCE FROM ANCHORED PHYLOGENOMICS OF *STEWARTIA* (THEACEAE)

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Editor: Hervé Sauquet

**Premise of research.** The disjunct distribution of plant genera between eastern Asia (EA) and eastern North America (ENA) has long attracted the attention of biologists and biogeographers. For most genera that have been studied, there are more species in EA than in ENA, and the diversity anomaly may have resulted from the greater physiographical heterogeneity in EA than in ENA in conjunction with climate and sea level changes. However, few empirical studies have explicitly tested the association between species diversity and allopatric speciation events. The genus *Stewartia* (Theaceae) displays this diversity anomaly, with two species in ENA and 21 species in EA, but the phylogeny of this group has not been resolved because of insufficient data.

**Methodology.** Here, we sampled 15 species of *Stewartia* (65%) and generated data from over 500 nuclear loci using the anchored phylogenomic approach to produce a robust phylogeny of *Stewartia*. In addition, biogeographical analyses were performed to elucidate the natural history of *Stewartia*, including estimated times of divergence, ancestral areas, and speciation patterns.

**Pivotal results.** Our parsimony, Bayesian, and species tree analyses produced congruent phylogenies with high resolution of the interspecific relationships within *Stewartia*. Speciation in Asia was mostly allopatric between the Japanese Islands and the Asian continent during the Miocene and the early Pliocene, while the two ENA species represent lineages from different times, with *S. malacodendron* being the first lineage to split off from the remaining species and *S. ovata* coming later as sister to the deciduous species of Asian *Stewartia*.

**Conclusions.** The results provide direct evidence for the importance of allopatry in the differential diversity between EA and ENA.

**Keywords:** *Stewartia*, diversity anomaly, eastern Asia, eastern North America, phylogenomics.

**Online enhancement:** appendix. *Dryad data:* <https://doi.org/10.5061/dryad.n4gt2qv>.

### Introduction

Intercontinental disjunction of plants represents one of the most important geographic patterns of biodiversity and has been studied extensively in terms of phylogenetic relationships, diversification patterns, and how and when the disjunction was formed (Thorne 1972; Raven and Axelrod 1974; Wen 1999; Milne 2006; Li et al. 2014b, 2018). Such studies have enriched theories and practices in multiple disciplines such as biogeography, ecology, and evolutionary biology (Hong 1993; Donoghue and Smith 2004; Xiang et al. 2004). Eastern Asia (EA) and eastern North America (ENA) are similar in climate and vegetation

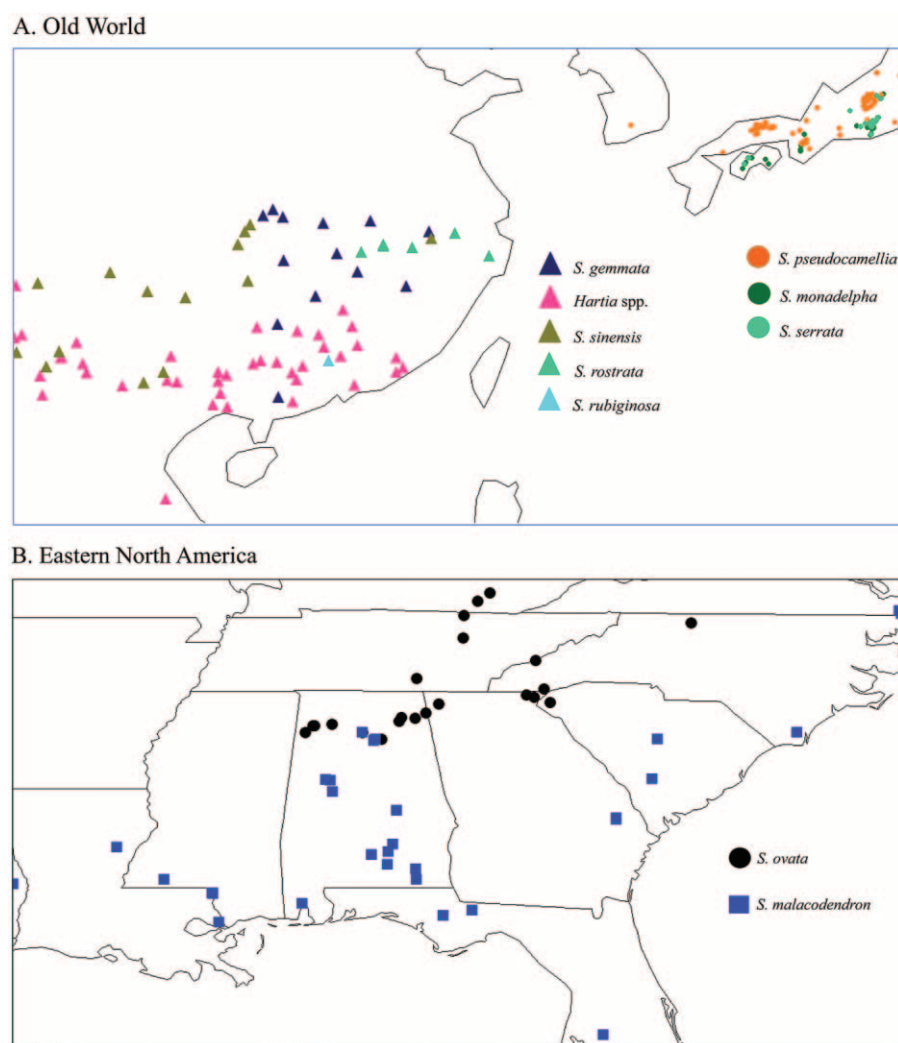
types (Qian et al. 2017) and share numerous disjunct lineages at various taxonomic levels. At the generic level, over 65 plant genera show disjunct distribution between the two continents (Li 1952; Boufford and Spongberg 1983; Wu 1983; Wen 1999). Nevertheless, taxonomic studies of the disjunct genera have concluded that the number of species in EA is almost twice that in ENA (Li 1952; Hong 1993), a diversity anomaly between two regions with similar environmental conditions that have different species richness (Xiang et al. 2004). Various factors have been proposed to explain the diversity anomaly between EA and ENA: (1) uninterrupted connection with a tropical region in EA (Sargent 1913), (2) lack of glaciation in China proper (Hu 1935), (3) greater topographic and ecological heterogeneity (Sargent 1913; Li 1952; Axelrod et al. 1998; Qian and Ricklefs 2000; Qian 2002), (4) longer evolutionary time (Donoghue and Smith 2004), and (5) faster rate of molecular evolution (Xiang et al. 2004). Though these factors may have acted alone or in combination to affect the

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diversification (speciation minus extinction) of lineages in EA and ENA, Qian and Ricklefs (2000) suggested that the higher number of species in EA might be associated with the greater allopatric speciation created by the isolation of populations between the Asian mainland and the Japanese Islands during the glacial and interglacial cycles and by populations adapting to different climatic zones. Phylogeographic studies of species spanning the Asian mainland and the Pacific Islands support the genetic differentiation of populations between the islands and the mainland (Qiu et al. 2009, 2011). Nevertheless, empirical evidence has rarely been used to evaluate the relative significance of the allopatric speciation and climatic adaptation hypotheses (Qian and Ricklefs 2000). In this study, therefore, we aimed at testing this hypothesis using the plant genus *Stewartia* L. not only because it has more species in EA than in ENA but also because it is distributed across a variety of different climatic zones in both continental Asia and the Japanese Islands.

Species of *Stewartia* are small to large trees (Spongberg 1974) with showy white flowers, and some are popular landscape

ornamentals in the temperate regions of Eurasia and North America (Dirr 1998). Li (1996) recognized 23 species in *Stewartia* sensu lato (*Hartia* Dunn included), of which two are distributed in ENA: *S. ovata* (Cav.) Weatherby in the mountains of Alabama, Georgia, North Carolina, South Carolina, Tennessee, and Kentucky, and *S. malacodendron* L. in the piedmont of Alabama, Louisiana, Texas, Arkansas, Georgia, Florida, North Carolina, and South Carolina (fig. 1). The two ENA species differ in bracteole number and in style, leaf, and seed wing morphology (Spongberg 1974). Eight to 21 species of *Stewartia* have been recognized in EA, depending on (1) the number of species recognized within the *S. sinensis* Rehder & Wilson complex in China (Spongberg 1974; Min and Bartholomew 2007) and (2) whether species of *Hartia* are included in *Stewartia* (Ye 1982; Li 1996). Three species of *Stewartia* are native to the Japanese Islands: *S. monadelphica* Siebold & Zucc., *S. pseudocamellia* Maxim., and *S. serrata* Maxim. *Stewartia koreana* Nakai ex Rehder is endemic to the Korean peninsula and differs from *S. pseudocamellia* in Japan in leaf and flower morphology and fall color



**Fig. 1** Geographic distribution of species of *Stewartia* and *Hartia* based on specimen information downloaded from the Global Biodiversity Information Facility (<http://www.gbif.org>).

(Rehder 1926). In China, *S. rubiginosa* H. T. Chang is endemic to southern Hunan and northern Guangdong, while *S. rostrata* Spongberg is distributed in Jiangxi, Hunan, and Zhejiang. *Stewartia sinensis* Rehder & E. H. Wilson is widespread in central, northern, and southern China, and its variants have sometimes been recognized as separate species, forming a taxonomically controversial species complex (see Li et al. 2002 and references therein).

Li (1996) recognized 14 evergreen species of *Stewartia* in Southeast Asia. These species have naked buds (vs. deciduous species of *Stewartia* with bud scales) and have been recognized as a separate genus, *Hartia* (Ye 1982). *Hartia* has been divided into two morphological groups: one with solitary flowers and the other with flowers forming a racemose inflorescence (Ye 1982). Recent molecular studies support the derivation of *Hartia* from within *Stewartia* (Li et al. 2013a; Lin et al. 2019) and the taxonomic fusion of *Hartia* and *Stewartia* (Li 1996). Based on this evidence, the number of species of *Stewartia* in EA is over 10 times that in ENA when *Hartia* is included in the genus (Li 1996) and at least four times that in ENA if only deciduous species are considered (Spongberg 1974; Li et al. 2002; Min and Bartholomew 2007). In this study, we treat “*Hartia*” as a clade of *Stewartia*.

If allopatric speciation has played a role in generating species of *Stewartia* in EA, as implied by Qian and Ricklefs (2000), we would expect a sister relationship for each island species with its respective mainland species. Similarly, if climatic disparities have selected adaptive lineages of *Stewartia*, it can be hypothesized that the species limited to climatic zones are closely related and share common ancestry, reflecting phylogenetic niche conservatism (Donoghue 2008). Alternatively, if sister lineages have experienced adaptations to different climatic zones, this would suggest phylogenetic overdispersion.

A well-resolved species phylogeny is essential for testing the abovementioned hypotheses, but neither nuclear internal transcribed spacers (Li et al. 2002) nor plastid genes (Li et al. 2013a) have resolved the phylogenetic relationships of the Asian species of *Stewartia*. Massive amounts of DNA sequence data have been shown to be informative in resolving relationships among closely related species and rapidly diversifying lineages (Pyron et al. 2014; Prum et al. 2015; Mitchell et al. 2017). The technique of anchored phylogenomics, which gathers data from hundreds of loci (Lemmon et al. 2012), has been used successfully in systematic studies of flowering plants (Buddenhagen et al. 2016; Mitchell et al. 2017). In this study, we generated large amounts of DNA sequence data via anchored phylogenomics (Lemmon et al. 2012), resolved relationships among species of *Stewartia*, and explored the implications of this robust phylogeny in relation to the role that allopatric speciation and climatic adaptation play in generating the diversity patterns of disjunct genera between EA and ENA.

## Material and Methods

### Samples

Sixty-one samples were included in this study (app. A), representing the morphological and geographic diversity of *Stewartia* (seven out of nine species) and “*Hartia*” (eight out of 14 species)

and outgroups *Gordonia lasianthus* (L.) Ellis, *Franklinia alatamaha* Bartr. ex Marshall, and *Schima* (Li et al. 2013a). Whenever possible, multiple samples were used to account for intraspecific variation and the potential existence of incomplete lineage sorting of the nuclear loci used in the study.

### Molecular Techniques

We followed Cardillio et al. (2017), Fragoso-Martinez et al. (2017), Mitchell et al. (2017), and Léveillé-Bourret et al. (2018), who targeted ~500 anchored hybrid enrichment loci using a probe kit developed by Buddenhagen et al. (2016). Briefly, genomic DNA was extracted from silica gel-dried leaves or fresh leaves using a DNeasy plant mini kit following the manufacturer’s instructions (Qiagen, Valencia, CA). After quantifying DNA concentrations using Qubit fluorometric quantitation, libraries were prepared and enriched at the Florida State University (FSU) Center for Anchored Phylogenomics (Tallahassee, FL; <http://www.anchoredphylogeny.com>) following the methods of Lemmon et al. (2012) and Prum et al. (2015). Library preparation involved sonicating DNA to 200–800-bp fragments using a Covaris E220 focused-ultrasonicator (Woburn, MA) and ligating adapters (with 8-bp indexes) using a Beckman-Coulter Biomek FXP liquid-handling robot (Indianapolis, IN). Library pools containing approximately 16 samples each were then enriched for the anchor regions using the Agilent Angiosperm version 1 kit described in Buddenhagen et al. (2016). Enriched libraries were sequenced on two paired-end 150-bp sequencing lanes on an Illumina HiSeq2500 at the FSU Translational Science Laboratory.

### Data Analysis

After sequencing, reads passing the CASAVA high-chastity filter were demultiplexed then merged following Rokyta et al. (2012). The process employed also removed sequencing adapters. The quasi-de novo assembler described by Hamilton et al. (2016) was then used to assemble reads to *Arabidopsis*, *Billbergia*, and *Carex* reference sequences derived from the alignments used for probed design by Buddenhagen et al. (2016). In order to avoid the effects of possible contamination and/or misindexing, assembled contigs derived from fewer than 24 reads were removed from further analysis. Pairwise distances were then used to establish the orthology of homologous contigs passing this filter, as described in Hamilton et al. (2016). After aligning sets of orthologous sequences using MAFFT (ver. 7.023b; Katoh and Standley 2013), alignments were trimmed and masked with MINGOODSITES = 14, MINPROPSAME = 0.4, and MISSING-ALLOWED = 67 (see Hamilton et al. 2016 for details). Misaligned regions identified upon inspection in Geneious R9 (Biomatters; Kearse et al. 2012) were masked.

Phylogenetic trees were constructed based on the concatenated data sets of all loci using the SVDquartets method (Chifman and Kubatko 2014), as implemented in PAUP\* (Swofford 2002) on the CIPRES Science Gateway with 100 bootstrap replicates (Miller et al. 2010). Partitions of our data set were assessed, and their optimal models of evolution were selected using PartitionFinder (Frandsen et al. 2015). However, we did not partition our data by codon position because identifying coding regions and codon positions is difficult for this type of data (Mitchell et al. 2017). For Bayesian tree inference, two Markov chain Monte Carlo (MCMC)

runs with four chains each were done for 10 million generations using MrBayes, as implemented on the CIPRES Science Gateway, and trees were sampled every 10,000 generations. We plotted the generations and posterior likelihood scores to ensure that the Bayesian runs had enough generations for the likelihood scores to reach the stationarity. The first 25% of generations were discarded as burn-ins. The majority consensus of the remaining trees was generated to show posterior probability support for individual clades. Species trees were inferred without concatenation of the loci using the Accurate Species Tree Algorithm (ASTRAL-II; ver. 4.9.7; Mirarab and Warnow 2015; default parameters) under the coalescent model and multilocus bootstrapping of 200 replicates from the RAxML-estimated gene trees (Feng et al. 2017). The locus-specific gene trees were estimated using RAxML version 8.1.21 (Stamatakis 2014) with GTRGAMMA model with default run settings, and branch support values were estimated using 100 bootstrap replicates.

### Molecular Dating

After reviewing fossils assigned to Theaceae, Grote and Dilcher (1989) concluded that the family existed with some diversity by the Late Cretaceous. The oldest reliable fossils of *Gordonia* and *Polyspora* have been found in the Eocene of Europe and North America (Grote and Dilcher 1992). Grote and Dilcher (1992) described *Gordonia lamkinensis* and *Gordonia warmanensis* from the Middle Eocene of the Claiborne Formation in Kentucky and Tennessee, which ranges from 37.8 to 47.8 Ma (Walker et al. 2012). We used the middle Eocene (ca. 40.4 Ma) as the minimal divergence age of the tribes Gordonieae and Stewartieae, as in Li et al. (2013a). Fruits of *Schima* were recently reported from the Nanlin Formation in Longchuan Basin, Yunnan, China, suggesting the minimum divergence time of *Franklinia* and *Schima* in the early to middle Miocene (ca. 17.3 Ma; Li et al. 2013b). Although fossil leaves, fruits, and seeds of “*Hartia*” (Mai 1975) and *Stewartia* (van der Burgh 1978) have been reported from the late Tertiary of Europe and Asia, none of them can be confidently identified as closely related to an extant species (Grote and Dilcher 1989). Therefore, we did not attempt to use the fossils as calibration points. Our geological timescales followed Walker et al. (2012).

Both semiparametric and parametric methods (Sanderson 2002; Drummond et al. 2016) have been widely used to estimate the time of divergence of lineages. Here, we used the r8s-PL (penalized likelihood) method (Sanderson 2002, 2003) to estimate the times of divergence of lineages within *Stewartia* using 100 Bayesian trees and the smoothing factor determined via cross evaluation (Sanderson 2006). The minimum age of Gordonieae and Stewartieae was set at 40.4 Ma and that of *Franklinia* and *Schima* at 17.3 Ma. For parametric estimates of divergence times using the computer package BEAST (Drummond et al. 2016), we reduced the original data set by including a single sample for individual populations of each species and limiting the number of sites to about 50,000. The informativeness of each partition, as defined by the PartitionFinder, was evaluated and ranked in PhyDesign (Lopez-Giraldez and Townsend 2011). The top 25 partitions were used in BEAST for the dating exercises. The age priors for Gordonieae-Stewartieae and *Franklinia-Schima* were set at an exponential distribution with an offset of 40.4 and 17.3 Ma, respectively, and a mean of 1.0. Sites were set up with the substitution model GTRGAMMA+I with four gamma

categories and estimated base frequencies. Both Yule and birth-death speciation processes were used as tree prior models, with one of the Bayesian trees as the initial tree. Operator mix was set to fixed tree topology with tree estimates including subtreeSlide, narrowExchange, wideExchange, wilsonBalding, and subtreeLeap deselected. For the BEAST analysis, we set the length of the MCMC to 200 million generations with parameters sampled every 1000 generations to make sure that the effective sample size was over 200, as determined by Tracer (Drummond et al. 2016). The first 25% of the trees were discarded as burn-ins. The remaining trees were summarized and annotated with posterior probabilities and with highest posterior density node heights and rates using TreeAnnotator in BEAST (ver. 1.8.4; Drummond et al. 2016).

### Biogeographic Analysis

Because *Stewartia* shows a disjunct distribution between EA and ENA and the focus of the study was on the diversification of the island lineages, we included three areas of endemism in the biogeographic analysis: continental Asia, the Japanese archipelago, and ENA. In order to infer the ancestral area of the three Japanese species, we used both statistical dispersal and vicariance analysis (S-DIVA; Yu et al. 2010) and dispersal and extinction cladogenesis (DEC; Ree and Smith 2008) analysis, as implemented in RASP (Yu et al. 2015). S-DIVA penalizes dispersal and extinction and takes into account the uncertainty of phylogenies, while the DEC method infers the likelihood of ancestral areas by incorporating models of range changes and branch lengths of the phylogeny (Ree and Smith 2008). For S-DIVA, we conducted the analysis using 100 trees randomly selected from the Bayesian trees. The maximum credibility tree produced from a sample of trees in BEAST was used for the DEC analysis, wherein we assumed an equal probability of migration across the three areas of endemism in all periods of geological times because of the lack of information about the dispersal potential of seeds and fruits of *Stewartia* and the impact of the formation of the Sea of Japan during the early Miocene on the dispersal of *Stewartia*. Matzke (2014) proposed a DEC+J model to infer biogeographic history, introducing the J parameter for “founder event” or “jump dispersal” speciation, which creates a new colonization without the existence of a widespread ancestor. However, concerns about the validity of the DEC+J model have been raised (Ree and Sanmartín 2018), and the model was not used in this study.

## Results

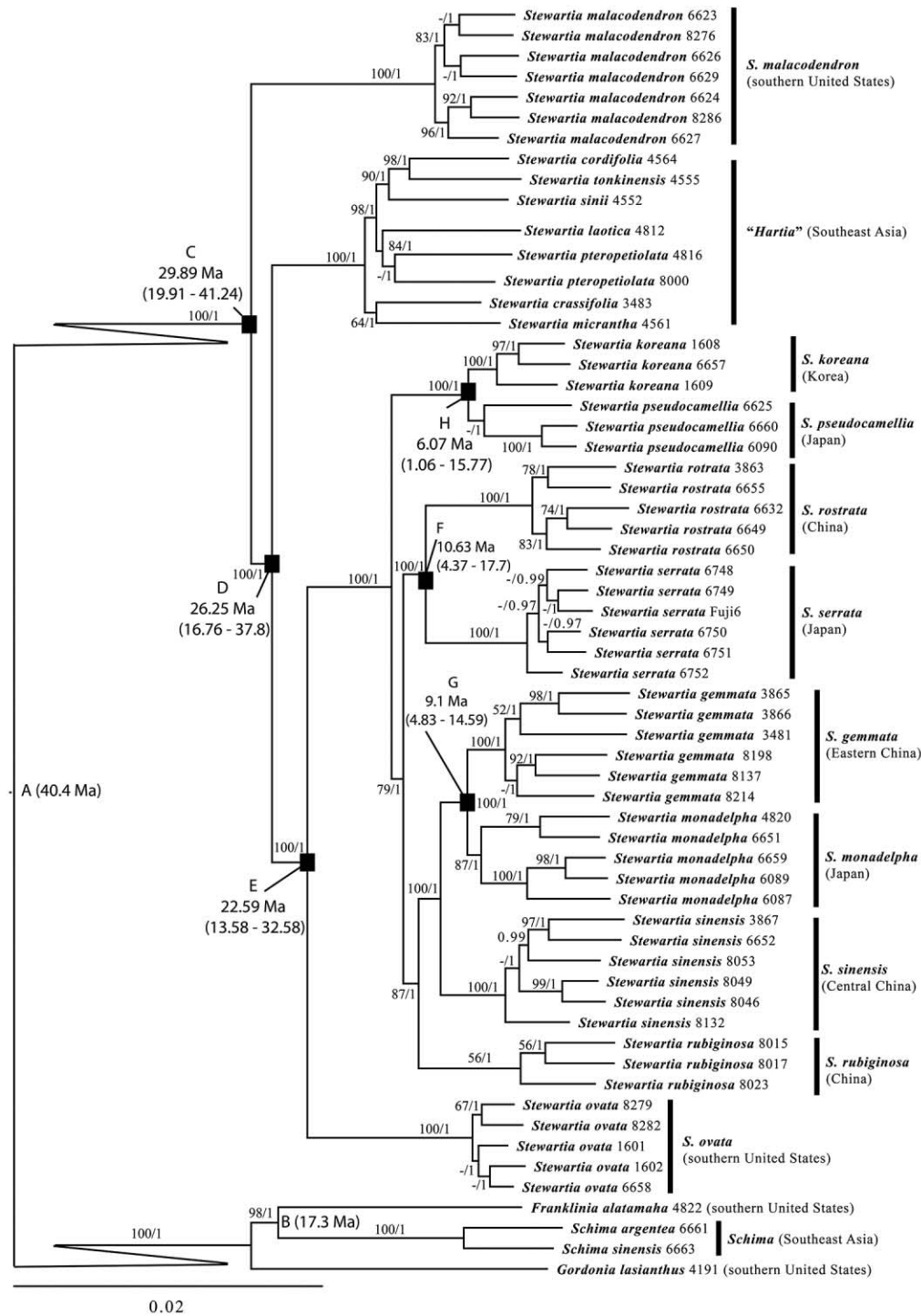
### Sequence Data and Phylogenetic Relationships within *Stewartia*

Several samples were sequenced twice to ensure the accuracy of the data generated, and the sequence data were deposited in the Dryad Digital Repository (<https://doi.org/10.5061/dryad.n4gt2qv>; Li et al. 2019). Our sequencing targeted 500 nuclear loci and produced data from 532 orthologous loci including the flanking regions of some target loci. The concatenated data set contained 386,131 aligned nucleotide sites, with missing data ranging from 2% to 76% (mean = 20%). Repeated sequencing of the same samples produced identical results. PartitionFinder



recognized 206 partitions in the data set. Both SVDquartets analysis of the concatenated data set and Bayesian analyses of 206 partitions identified by the PartitionFinder produced congruent and well-supported phylogenetic trees (fig. 2). The trees

are congruent with the species tree (fig. B3; figs. B1–B3 are available online) generated with ASTRAL under the coalescent model. When *Gordonia*, *Franklinia*, and *Schima* were used as outgroups to root the trees, *Stewartia malacodendron* was sister to the



**Fig. 2** Phylogeny of *Stewartia* generated from maximum parsimony and Bayesian analyses. Numbers at the nodes represent bootstrap percentages and posterior probabilities. Highlighted letters A and B represent nodes with fossil dates used for calibration. Letters C–H indicate nodes, with estimated times of divergence in parentheses. Japanese island species diverged from the mainland ancestral populations by the early Pliocene.

clade containing the remaining species of *Stewartia*. Species of “*Hartia*” formed a clade and were derived from within *Stewartia*. *Stewartia ovata* was sister to the remaining deciduous Asian species of *Stewartia*. The deciduous Asian species of *Stewartia* formed a grade in the order of *S. pseudocamellia* + *S. koreana*, *S. rostrata* + *S. serrata*, *S. rubiginosa*, *S. gemmata* + *S. sinensis*, and *S. monadelphica*. Each of the three Japanese species of *Stewartia* formed a sister relationship with a continental species or lineage: *S. pseudocamellia* with *S. koreana*, *S. serrata* with *S. rostrata*, and *S. monadelphica* with *S. gemmata*.

#### Biogeographic Patterns and Divergence Time Estimates

Biogeographic analyses using both S-DIVA and DEC algorithms as implemented in RASP inferred that the Asian continent was the ancestral area of all three Japanese species and that three vicariance events resulted in the allopatric speciation of the Japanese lineages (fig. 3).

The cross evaluation of smoothing factors from 1 to 20 with an interval of 0.5 based on one of the Bayesian trees suggested that the optimal smoothing value for the data set was 1. When the smoothing value and 100 Bayesian trees were used in r8s analyses, our estimated times of divergence of lineages of *Stewartia* ranged from 4.13 to 14.19 Ma (table B1, available online). Specifically, the disjunction between North America and eastern Asia was formed at 11.41 Ma (10.91–11.97 Ma, SD = 0.2 Ma), and the evergreen “*Hartia*” evolved at 12.75 Ma (12.22–13.25 Ma, SD = 0.22 Ma). *Stewartia serrata* and *S. rostrata* diverged by the middle Pliocene at 6.95 Ma (6.47–7.47 Ma, SD = 0.16 Ma), *S. monadelphica* and *S. gemmata* at 5.49 Ma (5.2–5.88 Ma, SD = 0.14 Ma), and *S. pseudocamellia* and *S. koreana* at 4.13 Ma (3.86–4.4 Ma, SD = 0.12 Ma).

Dating using BEAST with Yule and birth-death tree models produced age estimates with a wide range of variation for EA-ENA disjunction and the three species pairs between the Japanese Islands and the Asian continent (table B1). *Stewartia ovata* diverged from the Asian species at 22.59 Ma (13.58–32.58 Ma; Yule) and 22.41 Ma (13.24–32.41 Ma; birth-death model), while *S. rostrata* and *S. serrata*, *S. gemmata* and *S. monadelphica*, and *S. pseudocamellia* and *S. koreana* were separated at 10.63 Ma (4.37–17.7 Ma) and 10.46 Ma (4.26–18.09 Ma), 9.1 Ma (4.83–14.59 Ma) and 9.09 Ma (4.18–15.26 Ma), and 6.07 Ma (1.06–15.77 Ma) and 6.11 Ma (1.23–14.42 Ma), respectively (figs. B1, B2, table B1).

#### Discussion

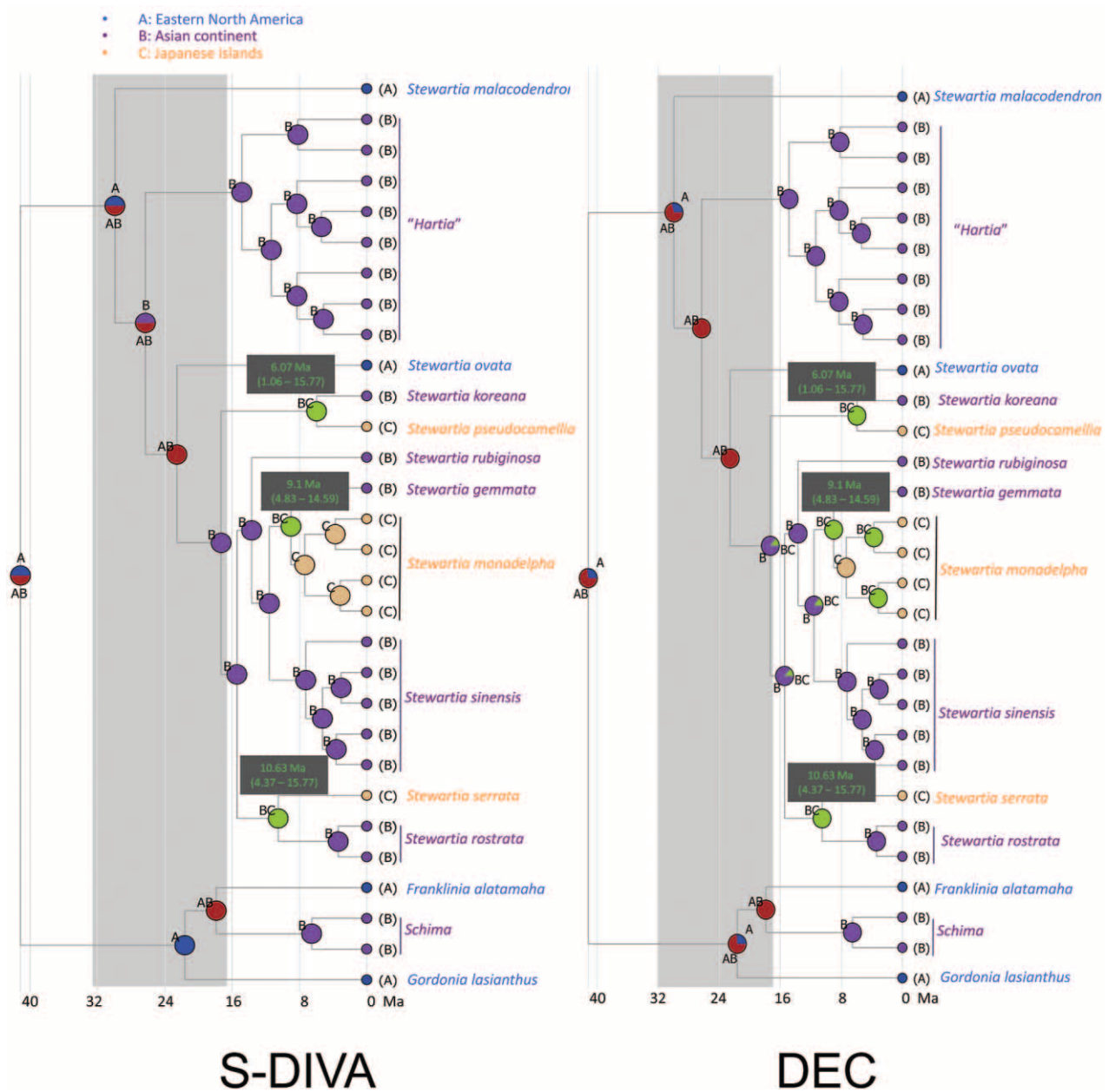
The diversity anomaly between EA and ENA has been noted in the 1900s (Li 1952; Hong 1993; Wen 1999) and quantitatively confirmed in the 2000s (Qian and Ricklefs 2000; Qian et al. 2017). The “out of Asia” hypothesis (Donoghue and Smith 2004) suggests that the anomaly may have resulted from more ancient ages for the Asian lineages to diversify in EA than in ENA. The higher diversity in EA may also be associated with the higher genetic diversity (Xiang et al. 2004) and/or the local origin of the EA endemic species (Harris et al. 2013), partially due to the greater physical heterogeneity of the region (Qian and Ricklefs 1999). The allopatric speciation between the Pacific

Islands and the Asian continent may have contributed to the higher diversification of species in EA than in ENA (Qian and Ricklefs 2000). Robust phylogenies of the EA-ENA disjunct genera are needed for testing the allopatry hypothesis.

The ancestral populations of *Stewartia* were inferred to have a widespread distribution in EA and in ENA (fig. 3). *Stewartia malacodendron* is the first lineage of the genus, while *S. ovata* represents one of the more recent lineages and is more closely related to the Asian species of *Stewartia*. Thus, the two North American species evolved at different times. Also, *S. ovata* occurs in mountains and adjacent piedmont with higher elevation and drier conditions, while *S. malacodendron* is native to the coastal plain and piedmont of lower elevation (Spongberg and Fordham 1975). Geographically, *S. ovata* is distributed in slightly higher latitude (35°N) than *S. malacodendron* (32°N; fig. 1B). Therefore, the two North American species arose at different times with distinctive ecology and morphology.

In EA, the Japanese island species are derived from the ancestral populations in the Asian continent (fig. 3). In two of the three cases, however, the island species occur in slightly higher latitudes than the continental sister species; the average latitudes for *S. monadelphica* and *S. serrata* are 34.8° and 35°N, respectively, whereas those of *S. gemmata* of eastern and southern China and *S. rostrata* are 28.5° and 29°N, respectively. Nevertheless, the ecological conditions of the Japanese Islands and the Asian continental species of *Stewartia* are similar, with warm, temperate, moist conditions (Qian and Ricklefs 2000). Therefore, adaptations to different climates may not have played an important role in the diversification of species of *Stewartia* in EA or in ENA. *Hartia* occurs in warmer regions of the Asian continent (fig. 1), but it does not form a sister relationship with any of the other Asian species of *Stewartia* in the northern latitudes (fig. 3). Thus, climatic zoning in Asia does not seem to have had an evident impact on the diversification of lineages of *Stewartia*. In contrast, the geographic barriers and connections associated with the formation of the Sea of Japan and the glacial/interglacial cycles may have been more important in the generation of greater species diversity in EA than in ENA.

Geological studies have shown that the Sea of Japan was formed in the early Miocene (18–32 Ma; Tamaki et al. 1992), and the last land connections between the Japanese Islands and the Asian continent were severed in the late Pliocene because of the glacial lowering of sea levels (Hua et al. 1987; Maruyama et al. 1997). The estimated times of divergence using r8s and BEAST are different, with the former being more recent (see above), which seems to be consistent with other studies (Nie et al. 2008), as well as with simulation studies (Ho et al. 2005). Nevertheless, the estimates are generally within the range of previous age estimates and fossil dates. For example, a fossil species of *Hartia* was reported from the Upper Miocene of western Europe (Mai 1975), which agrees with our estimated divergence of “*Hartia*” at 12.75 Ma (PL) and 16.76–37.8 Ma (BEAST; figs. 2, 3). The disjunction between *S. ovata* and Asian species of *Stewartia* is estimated to have formed at 11.41 Ma (PL) and 13.58–32.58 Ma, which is similar to Li et al.’s (2013a) estimate of 11.3–29.1 Ma. Li et al. (2013a) did not estimate times of divergence among the Asian species of *Stewartia* because of the poor resolution of interspecific relationships. However, our estimates from r8s and BEAST dating exercises overlap and suggest that the Japanese species diverged from the continental ancestral populations



**Fig. 3** Inferred ancestral areas of lineages in *Stewartia* from biogeographic analyses using statistical dispersal and vicariance analysis (S-DIVA) and dispersal and extinction cladogenesis (DEC) methods. Lineages on the Japanese Islands evolved from ancestral populations in the Asian continent after the formation of the sea barrier separating the islands. The three areas of endemism are eastern North America (A), Asian continent (B), and Japanese Islands (C). The vertical, wide gray bar indicates the time of formation of the Sea of Japan, 18–32 Ma.

before the late Pliocene, the most recent time when the two areas were connected via land bridges (Maruyama et al. 1997; Kameda and Kato 2011). Therefore, our phylogenetic and biogeographic analyses recognize that allopatric speciation due to the formation of the Sea of Japan was probably the most important contributing factor to the greater diversification of *Stewartia* in Asia, while greater climatic disparity seems to have contributed little to the diversity anomaly between EA and ENA. This is consistent with

Qian and Ricklefs (2000) and the sister relationship of the Japanese species with mainland species in several other EA-ENA disjunct lineages such as *Torreya* (Li et al. 2001), *Tsuga* (Havill et al. 2008), *Pieris* (Li et al. 2009), and *Wisteria* (Li et al. 2014a). However, many EA-ENA disjunct lineages with species on the Japanese Islands and the Asian continent need to be evaluated with better phylogenetic resolution and comprehensive taxon sampling to further test the hypothesis that there is significantly more

allopatric speciation in EA than in ENA. It is worth noting that in the clade of *S. gemmata*, *S. monadelpha*, and *S. sinensis*, our DEC analysis suggests the possibility of *S. monadelpha* going back to the Asian continent from the Japanese Islands (fig. 3). Interestingly, a report of the introgression of plastid genes between the continental and the island species of *Stewartia* (Lin et al. 2019) appears to be consistent with our DEC results. Thus, a more comprehensive sampling at the population levels of all the species with molecular data from nuclear and plastid genomes will be important for gaining a better understanding of plant speciation in eastern Asia.

### Acknowledgments

We thank the Arnold Arboretum, Dr. Yoko Saito, and Wenbo Liao and Qiang Fan of Zhongshan University for providing leaf material for the study; Janine Luke and Mel Seiden for providing financial support for the project; Michelle Kortyna, Jesse Cherry, Sean Holland, Alyssa Bigelow, and Kirby Birch from Florida State University's Center for Anchored Phylogenomics for their assistance with molecular data collection and analysis; and Dr. Thomas Bultman for reading the earlier version of the manuscript and providing constructive comments.

## Appendix A

### Accession, Collection Location, and Voucher Specimen of Species and Samples Used in the Study

AA: Arnold Arboretum; SYSU: Sunyatsen University; HCHM: Hope College.

*Franklinia alata* Marshall, 4822, 2428-2A (AA), cultivated, USA; *Gordonia lasianthus* (L.) E. Ellis, 4191, North Carolina, USA, Li 4191 (AA); *Stewartia cordifolia* (Li) J. Li et Ming, 4564, Guangxi, China, Fan Q. 8181 (SYSU); *Stewartia crassifolia* (S.Z. Yan) J. Li et Ming, 3483, Guangdong, China, Li 3483 (HCHM); *Stewartia laotica* (Gagnep.) J. Li et Ming, 4812, Yunnan, China, Fan, Q. 8371 (SYSU); *Stewartia micrantha* (Chun) Sealy, 4561, Guangdong, China, Fan Q. 8150 (SYSU); *Stewartia sichuanensis* (S.Z. Yang) J. Li et Ming, 4816, Sichuan, China, Fan, Q. 8412 (SYSU); *Stewartia pteropetiolata* W.C. Cheng, 8000, Kunming, China (cultivated), Li Pan 8000 (ZJU); *Stewartia sinii* (Wu) Sealy, 4552, Guangxi, China, Fan Q. 8241 (SYSU); *Stewartia tonkinensis* (Merr.) C.Y. Wu, 4555, Guangdong, China, Fan Q. 8120 (SYSU); *Schima argentea* E.Pritz. ex Diels, 6661, Sichuan, China, 1992.381B (QA); *Schima sinensis* (Hemsl. & E.H. Wilson) Airy Shaw, 6663, Sichuan, China, 1991.339N (QA); *Stewartia gemmata* S.S. Chien & W.C. Cheng, 3865, Nanjing, Jiangsu, China, 531-34C (AA); *Stewartia gemmata*, 3481, Mangshan, Hunan, China, Li 3481 (HCHM); *Stewartia gemmata*, 8137, Kaihua, Zhejiang, China, Li 8137 (ZJU); *Stewartia gemmata*, 8198, Shangrao, Jiangxi, China, Li 8198 (ZJU); *Stewartia gemmata*, 8214, Linan, Zhejiang, China, Li 8214 (ZJU); *Stewartia gemmata*, 3866, Nanjing, Jiangsu, China, 531-34B (AA); *Stewartia koreana* Nakai, 6657, Mt. Chirisan, Korea; 11440A (AA); *Stewartia koreana*, 1609, Mt. Mudung, Korea; 1269-83A (AA); *Stewartia koreana*, 1608, Mt. Chirisan, Korea; 11440B (AA); *Stewartia malacodendron* L., 6627, Alabama, USA; 2007-149C (AA); *Stewartia malacodendron*, 6626, Alabama, USA; 2007-150C (AA); *Stewartia malacodendron*, 6624, Alabama, USA; 2007-151C (AA); *Stewartia malacodendron*, 6623, Alabama, USA; 2007-147B (AA); *Stewartia malacodendron*, 8286, Blount Co., Alabama, USA; del Tredici (AA); *Stewartia malacodendron*, 827, Blount Co., Alabama, USA; del Tredici (AA); *Stewartia malacodendron*, 6629, Virginia, USA; 2007-152C (AA); *Stewartia monadelpha* Siebold & Zucc., 6087, Yakushima, Japan; 1989.319C (QA); *Stewartia monadelpha*, 6651, Japan, 653-74B (AA); *Stewartia monadelpha*, 4820, Japan, 653-74A (AA); *Stewartia monadelpha*, 6089, Yakushima, Japan, 1989.319D (QA); *Stewartia monadelpha*, 6659, Yakushima, Japan, 1989.319D (QA); *Stewartia ovata* (Cavanilles) Weatherby, 6658, Highlands, North Carolina, USA; 18244C (AA); *Stewartia ovata*, 1602, Highlands, North Carolina, USA, 18244B (AA); *Stewartia ovata*, 1601, USA, 18847A (AA); *Stewartia ovata*, 8282, Chattooga River, South Carolina, USA, (AA); *Stewartia ovata*, 8279, Chattooga River, South Carolina, USA, (AA); *Stewartia pseudocamellia* Maxim., 6625, Honshu, Japan, 2007-502A (AA); *Stewartia pseudocamellia*, 6090, Honshu, Japan; 1989.071A (AA); *Stewartia pseudocamellia*, 6660, Honshu, Japan, 1989.071A (QA); *Stewartia rostrata* Spongberg, 6649, Lushan, Jiangxi, China, 249-2005B (AA); *Stewartia rostrata*, 3863, Lushan, Jiangxi, China, 769-36A (AA); *Stewartia rostrata*, 6655, Lushan, Jiangxi, China, 322-2004A (AA); *Stewartia rostrata*, 6632, Lushan, Jiangxi, China, 249-2005A (AA); *Stewartia rostrata*, 6650, Lushan, Jiangxi, China, 327-2004A (AA); *Stewartia rubiginosa* H. T. Chang, 8017, Yingde, Guangdong, China, Li 8017 (ZJU); *Stewartia rubiginosa*, 8023, Ruyuan, Guangdong, China, Li 8023 (ZJU); *Stewartia rubiginosa*, 8015, Yingde, Guangdong, China, Li 8015 (ZJU); *Stewartia serrata* Maxim., 6749, Fuji Mountains, Japan; Saito 01 (TI); *Stewartia serrata*, 6750, Fuji Mountains, Japan, Saito 02 (TI); *Stewartia serrata*, 6751, Fuji Mountains, Japan; Saito 03 (TI); *Stewartia serrata*, 6752, Fuji Mountains, Japan, Saito 04 (TI); *Stewartia serrata*, fuji6, Fuji Mountains, Japan, Saito 06 (TI); *Stewartia serrata*, 6748, Fuji Mountains, Japan, Saito 05 (TI); *Stewartia sinensis* Rehder & Wilson, 3867, Wudangshan, Hubei, China, 691-94A (AA); *Stewartia sinensis*, 8046, Foping, Shangxi, China, Li 8046 (HCHM); *Stewartia sinensis*, 8049, Foping, Shangxi, China, Li 8049 (HCHM); *Stewartia sinensis*, 8053, Yichang, Hubei, China, Li 8053 (ZJU); *Stewartia sinensis*, 8132, Badagongshan, Hunan, China, Li 8132 (ZJU); *Stewartia sinensis*, 6652, Shennongjia, Hubei, China, 383-2001A (AA).

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