Phylogenetics of *Morus* (Moraceae) Inferred from ITS and *trnL-trnF* Sequence Data

Madhav P. Nepal\(^1\) and Carolyn J. Ferguson\(^2\)

\(^1\)Department of Biology and Microbiology, South Dakota State University, Brookings, South Dakota 57007-1898 U. S. A.

\(^2\)Herbarium and Division of Biology, Kansas State University, Manhattan, Kansas 66506-4901 U. S. A.

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**Abstract**—*Morus* (Tribe Moreae, Moraceae) consists of ca. 13 species of trees distributed in Asia, Africa, Europe, and North, Central, and South America. The broad geographical distribution of the genus, overlapping ranges of many taxa, and documented hybridization between some species present interesting questions of taxonomy, phylogeny, and biogeography. Phylogenetic data for *Morus* also contribute to higher level taxonomic work in the family. We used sequence data from ITS of the nrDNA and the chloroplast *trnL-trnF* intergenic spacer to study phylogenetic relationships of *Morus*. Phylogenies based on separate data sets were not statistically incongruent, and the combined tree reveals that *Morus*, as currently circumscribed, is non-monophyletic. Subgenus *Morus* (sensu Leroy) is resolved as a clade and consists of two well-supported clades: one of Asian taxa and one of North American taxa. Sampled members of the genus *Trophis* (two, including the type) form a clade sister to subgenus *Morus*. *Morus mesoazzia* (Africa; subgenus *Afromorus*) and *M. insignis* (Neotropics; subgenus *Comphomorus*), which have not been included to date in other phylogenetic studies of the family, are placed outside the subgenus *Morus-Trophis* clade. This work is an important step in elucidating relationships of *Morus* and along with other recent phylogenetic studies in Moraceae, underscores the need for further work within Tribe Moreae to clarify natural generic relationships.

**Keywords**—ITS, Moraceae, Moreae, *Morus*, phylogeny, *trnL-trnF*.

*Morus* L. (Moraceae) comprises 10–13 species (Berg 2001, 2005a) distributed in Asia, Africa, Europe, and North, Central, and South America. *Morus* species are economically important to the silk industry, as they are host plants for the silkworm (*Bombyx mori* L.) larvae (Watanabe 1958). Additionally, species have been cultivated in many parts of the world for their edible fruits and as ornamental trees. *Morus* is the type genus of the cosmopolitan family Moraceae, which includes 37 genera, some of which have been subjects of recent phylogenetic work (e.g. *Artocarpus*, Zerega et al. 2010; *Ficus*, Ronsted et al. 2008). Tribal classification within Moraceae has received much attention in recent years, with both taxonomic and phylogenetic study spurring realignments (Berg 2001, 2005a; Datwyler and Weiblen 2004; Clement and Weiblen 2009). Closest relatives to *Morus* include *Bagassa* AUBL., *Milicia* Sim, *Sorocea* A. St.-Hil., *Streblus* Lour. (in part), and *Trophis* P. Br. (in part), tribe Moreae of Clement and Weiblen (2009). However, Moreae remains paraphyletic pending further study and recircumscription of *Streblus* and *Trophis*, each of which include species more closely related to tribe Durstenieae Gaudich. (based on *ndhF* and 26S data; Datwyler and Weiblen 2004; Zerega et al. 2005; see also Clement and Weiblen 2009); and Weiblen and colleagues call for further work on these genera (Datwyler and Weiblen 2004; Clement and Weiblen 2009). Tribe Moreae exhibits pleisiomorphic characters including a simple inflorescence, tetramerous flowers and usually equally branched stigmas, fleshy perianth in the fruit, and a berry-like syncarp (see Berg 2001; Nepal 2008). Species are considered wind pollinated (based on morphology; Berg 2001) and fruits are often dispersed by birds (Stapanian 1982). Characters including morphology of the leaf, winter bud, bark, pistil, and syncarp have generally been employed in species recognition. The base chromosome number is \(x = 14\) (Janaki-Ammal 1948; Chen et al. 1993; Azizan and Sonboli 2001; Awasthi et al. 2004), and polyploids with counts as high as \(2n = 308\) (probably in cultivars; e.g. Azizan and Sonboli 2001) have been reported. The genus is interesting for systematic study because of its wide geographical distribution, morphological plasticity (Gray and Gray 1987), hybridization (Burgess et al. 2005), long history of domestication, and introduction and naturalization of species in areas remote from their native ranges (Tojyo 1985).

There has long been great taxonomic interest in *Morus*, at least in part due to its economic importance. Linnaeus (1753) established the genus and described seven species: *M. alba* L., *M. indica* L., *M. nigra* L., *M. papyrifera* L., *M. rubra* L., *M. tartarica* L. and *M. tinctoria* L. Of these, *M. papyrifera* and *M. tinctoria* were later moved to the genera *Broussonetia* L’Hér. ex Vent. (*B. papyrifera* (L.) L’Hér. ex Vent.) and *Maclura* Nutt. (*M. tinctoria* (L.) D. Don ex Steud.), respectively. Bureau (1873) recognized five species, and also described 21 varieties and 13 subvarieties (with taxonomic emphasis on syncarp shape and style length within *M. alba*). Greene (1910) treated *Morus* in the southwestern U. S. A., dividing *M. microphylla* into 13 species. Koidzumi (1917), who presented the most recent genus-wide treatment, recognized 24 species under two major sections: Section *Macromorus* Koidz. (= Sect. *Morus*; species with short styles, < 0.5 mm) and Section *Dolichoystyle* Koidz. (species with long styles, > 1 mm in length). Thus, in his classification, Koidzumi promoted some of Bureau’s varieties to species (and some of Bureau’s conspecific varieties became species classified into separate sections within the genus). Leroy (1949), in his article on sericulture in the tropics, provided a classification of *Morus* dividing the genus into three subgenera, each with geographic integrity: *Eumorus* J. F. Leroy (= Subg. *Morus*; Asian and North American *Morus*, including one species also ranging into Central America), *Comphomorus* J. F. Leroy (*M. insignis* from Central and South America; and *M. trianae* J. F. Leroy, now considered a synonym of the former [Berg 2001]), and the monotypic *Afromorus* A. Chev. (*M. lactea* (Sim) Midlbr., now considered a synonym...
of *M. mesozygia* Stapf., from Africa). Hotta (1954) studied variation in shape and position of leaf cystolith cells in *M. alba*, *M. australis*, and *M. mongolica*. Katsumata (1971) studied size and shape of leaf ideoblasts and used these data to classify several races of *M. alba* and *M. australis*. Other important taxonomic studies on *Morus* include description of new species from China (Chang 1984; Wu and Chang 1989; Cao 1991), lectotypification of *M. alba* (Rao and Jarvis 1986), an overview of *Morus* distribution (Sanjappa 1989), and revision of *Morus* in the *Flora of China* (Zhukov and Gilbert 2003). Zhekun and Gilbert (2003) recognized 12 species in China alone, although workers outside of China have generally not concurred with recognition of such great diversity at the species rank.

Taxonomic and floristic work on Moraceae worldwide has been conducted by Berg (e.g. Berg 2001, 2005a, 2005b). He has most recently estimated the number of species in *Morus* at 10–13 or 12 (2001, 2005); however, he has not enumerated those species, and has noted the need for taxonomic revision of the genus. Based on synthesis of taxonomic literature on *Morus* and examination of herbarium specimens (> 1,500 specimens from U. S. A. herbaria; Nepal 2008), 13 distinctive *Morus* species can be recognized: eight species native to Asia (see Appendix 1 for authorities; *M. alba*, *M. australis*, *M. cathayana*, *M. macroura*, *M. mongolica*, *M. nigra*, *M. notabilis*, and *M. serrata*), four New World species (*M. celtidifolia*, *M. insignis*, *M. microphylla*, and *M. rubra*), and one species occurring in Africa (*M. mesozygia*). Three Asian species (*M. australis*, *M. notabilis*, and *M. mongolica*) have long styles (sensu Koidzumi; range 1–11 mm), while the remaining species have short styles (range 0–0.5 mm). Species with the longest catkins (6–16 cm, versus 0.5–2 cm in other species) are *M. macroura* (occurring in tropical cloud forests in East Asia) and *M. insignis* (in tropical cloud forests in Central and South America). The African *M. mesozygia* differs notably from all other species in its distinctly tri-nerved leaf lamina with scalariform secondary veins from the midrib, and a peduncle longer than the infructescence. Given diversity of the genus *Morus*, development of a phylogenetic framework in the context of family relationships will be valuable (family-wide phylogenetic studies have included at most two species of *Morus*; Datwyler and Weiblen 2004; Zerega et al. 2005; Clement and Weiblen 2009).

Several studies have used genetic approaches to study diversity within *Morus*. Zhao et al. (2005) sequenced the ITS region of nrDNA and the chloroplast *trnL* intron to investigate genetic distances among some Asian taxa (including *M. alba*, *M. australis*, *M. macroura*, and *M. mongolica*). Molecular markers widely used in population genetic studies including randomly amplified polymorphic DNA (RAPDs) and inter simple sequence repeats (ISSRs) have also been employed to study patterns of genetic relationships among species of Asian *Morus* (e.g. Sharma et al. 2000; Bhattacharya and Ranade 2001; Awasthi et al. 2004; Vijayan et al. 2004; Vijayan et al. 2006; Zhao et al. 2007). More than thirteen microsatellite markers are available for Asian *Morus* species (see Aggarwal et al. 2004; Tani et al. 2005). Most of these studies focused primarily on the cultivated species in Asia with an aim toward improving cultivars for the silkworm industry.

The objective of the present study was to develop phylogenies for *Morus* to advance taxonomic study of the genus and to place findings within the context of taxonomic and phylogenetic work on the family. In this study we present phylogenies for the genus *Morus* based on sequence data from ITS and the chloroplast *trnL-trnF* intergenic spacer region, and discuss the implications.

**Materials and Methods**

**Taxon Sampling**—Thirteen species of *Morus* (Appendix 1) as well as one species of *Sonora* and two species of *Trophiis* (Appendix 2) were sequenced and used as ingroup taxa, and *Artocarpus heterophyllus*, *Tribe Artocarpaceae R. Br.*, was selected as an outgroup (see Clement and Weiblen 2009; Zerega et al. 2010). Additional available Tribe Moroideae sensu Clement and Weiblen (2009) were included as part of the ingroup for phylogenetic analyses, as possible: *Bogassia* (one species; ITS and *trnL-trnF*), *Milicia* (one; ITS); *Sonora* (two additional species; ITS and *trnL-trnF*); and *Streblus* (five; four ITS and three *trnL-trnF*; Appendix 2).

**DNA Isolation and PCR Amplification**—Total DNA was extracted from leaf material (dried in silica gel or from herbarium specimens) following the CTAB protocol of Loockerman and Jansen (1996; modified from that of Doyle and Doyle [1987]), or using a DNeasy plant mini kit (Qiagen Corp., Valencia, California). The ITS region (ITS1, the 5.8S coding region and ITS2) and the chloroplast intergenic spacer region *trnL-trnF* (partial *trnL-CAA* intron, *trnl* gene, intergenic spacer between *trnL* and *trnF*; and partial *trnF* intron) were amplified with the primers *ITS4* (White et al. 1990) and modified ITS5 (Downie and Katz-Downie 1996), and the primers ‘c’ and ‘f’ (Taberlet et al. 1991), respectively. The PCR was conducted in a reaction mixture of 50 µl containing ~25 ng genomic DNA, 2 mM of PCR buffer, 0.4 µM of primer, 0.2 mM of each dNTP and 1 unit of Taq polymerase with 1.25 mM and 2.5 mM of MgCl₂ for ITS and *trnL-trnF*, respectively. “Hot start” PCR conditions were: 1) for ITS amplification: initial denaturation at 94°C for 5 minutes by following 1 minute at 72°C during which time Taq polymerase was added; and 35 cycles of 1 minute denaturation at 94°C, 1 minute annealing at 50°C, and 2 minutes elongation at 72°C; and a final elongation of 5 minutes at 72°C; and 2) for *trnL-trnF*: initial denaturation at 94°C for 5 minutes followed by 1 minute at 72°C during which time *Taq* polymerase was added, and 28 cycles of 1 minute denaturation at 94°C, 1 minute annealing at 55°C, and 2 minutes elongation at 72°C; and a final elongation of 10 minutes at 72°C. The PCR products were purified using a QIAquick purification kit (Qiagen Corp.).

**Sequencing and Alignment**—Sequencing reactions in both directions were performed using Big Dye V3.1 kit (Applied Biosystems Inc., Framingham, Massachusetts; following the manufacturer’s instructions, except that quarter reactions were used). The sequencing reaction conditions were as follows: 96°C for 2 minutes; followed by 25 cycles of 96°C for 15 sec, 50°C for 1 sec, and 60°C for 4 minutes; and subsequent storage at 4°C. Sequencing reactions were purified through Sephadex (Sigma, St. Louis, Missouri) columns, dried using a vacuum-centrifuge, and sent to one of two sequencing and synthesis facilities at Iowa State University where gels were run using an ABI 3700 automated sequencer. Resulting sequences were aligned and edited using Sequencer 4.5 (Gene Codes Corp., Ann Arbor, Michigan), and the data matrices constructed (with inclusion of additional sequences from GenBank) and aligned manually using Se-Al (Rambaut 2002).

**Phylogenetic Analyses**—Data sets were analyzed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI) methods. The MP and ML analyses were performed in PAUP* 4.0b10 (Swoford 2002) using branch and bound and heuristic searches, respectively. All characters were treated as equally weighted and unordered. Parsimony analyses were performed treating gaps as both missing data and as a fifth character state. Modelltest (version 3.06; Posada and Crandall 1998; Posada and Crandall 2004) was used to determine the substitution model that best fit the *Morus* sequence data. The parameters of the best model under the Akaike Information Criterion (AIC) were used in ML and BI analyses. The PAUP* “command block” resulting from Modelltest was appended to each data matrix for specifying the evolutionary model during ML analysis. Bootstrapping (Felsenstein 1985) was performed to assess support for branches (20,000 full heuristic replicates for MP and 1,100 replicates for ML analyses).

The BI analyses were performed in MrBayes (ver. 3.1.2; Huelsenbeck and Ronquist 2001). Model parameters for the substitution model K81uf + G were specified and each analysis was conducted with two independent runs with four (three heated and one cold) Markov Chain Monte Carlo (MCMC) chains for two million generations starting from a random tree. The number of generations required to bring the standard deviation of split frequencies between runs below 0.01 was identified as the minimum number of generations required for the analysis. Trees were saved every 2012
RESULTS

Data Matrices—Sequences of the ITS region in *Morus* ranged from 611–635 base pairs (bp), and from 936–944 bp for *trnL-trnF*. GenBank accession numbers are provided in Appendices 1 and 2, and data matrices are available on TreeBase (study number 11391). There were 666 bp and 25 taxa in the complete, aligned ITS data matrix (including 13 indels one bp in length, and eight indels > one bp), 981 bp and 21 taxa in the *trnL-trnF* data matrix (including seven indels of one bp and six indels > one bp), and 1,640 bp and 20 taxa in the combined data matrix. A 17 bp region of ITS1 was excluded from analysis due to ambiguous alignment. Analysis of the ITS data revealed that ITS1 (not counting the excluded characters) and ITS2 are equally variable, but ITS1 has more parsimony informative characters than ITS2 (Table 1). The 5.8S region within most of *Morus* is not variable, but there are eight variable sites in the 5.8S region for the ingroup taxa as a whole, two of which are parsimony informative. Analysis of the *trnL-trnF* data matrix revealed that the *trnL* intron has fewer variable sites than the *trnL-trnF* spacer, but the former has slightly more informative sites than the latter, which is in contrast to findings for these regions in many taxa for which comparisons have been made (see Shaw et al. 2005).

Phylogenetic Analyses—Statistics for the MP analyses are presented in Table 1. MP analysis of the ITS data set resulted in 77 most parsimonious trees when gaps were treated as missing, and eight most parsimonious trees with gaps coded as a fifth character state. The tree topology in the latter case did not change except in the additional resolution of a clade containing *M. australis* and *M. notabilis*. Overall bootstrap (BS) support was also higher with gaps coded as a fifth character state. The strict consensus tree is presented in Fig. 1. The MP analysis of the *trnL-trnF* data set yielded 87 most parsimonious trees when gaps were treated as missing, and 18 most parsimonious trees with gaps treated as a fifth character state (Fig. 1). The ML and BI analyses of each data set with the common substitution model (*K81uf + G*) yielded similar results to the MP analyses; BI posterior probabilities are reported in Fig. 1. The parameter values for the substitution model were: base frequencies for A, C, T and G as 0.35, 0.17, 0.16 and 0.30, respectively; rate matrix *R[a], R[b], R[c], R[d], R[e]* and *R[f]* as 1.00, 2.47, 0.25, 2.47 and 1.00, respectively; and gamma shape of 0.1005. The -lnL, K, and ΔAIC values for the *K81uf + G* model were 1,828.86, 53 and 2.4326, respectively.

Table 1. Statistics from parsimony analyses of different data sets including consistency index (CI) and retention index (RI). The CI values listed are excluding uninformative characters. Number of taxa included in analyses were 25, 21 and 20 for ITS, *trnL-trnF* and combined data set, respectively.

<table>
<thead>
<tr>
<th>Gap handling</th>
<th>Description</th>
<th>ITS (ITS1, 5.8S, ITS2 data set)</th>
<th>trnL-trnF (*trnL intron, IGS) data set</th>
<th>Combined data set</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gap as missing</strong></td>
<td>Parsimony informative characters</td>
<td>144 (84, 8, 49)</td>
<td>15 (9,6)</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>Number of most parsimonious trees</td>
<td>142</td>
<td>186</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Tree lengths</td>
<td>469</td>
<td>113</td>
<td>443</td>
</tr>
<tr>
<td></td>
<td>CI</td>
<td>0.6130</td>
<td>0.5932</td>
<td>0.6325</td>
</tr>
<tr>
<td></td>
<td>RI</td>
<td>0.8027</td>
<td>0.8437</td>
<td>0.8267</td>
</tr>
<tr>
<td><strong>Gap as fifth character state</strong></td>
<td>Parsimony informative characters</td>
<td>202 (132, 8, 62)</td>
<td>27 (17,10)</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>Number of most parsimonious trees</td>
<td>8</td>
<td>18</td>
<td>7</td>
</tr>
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<td>Tree lengths</td>
<td>692</td>
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<td></td>
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<tr>
<td></td>
<td>RI</td>
<td>0.8307</td>
<td>0.8598</td>
<td>0.8452</td>
</tr>
</tbody>
</table>
Separate analyses of ITS and trnL-trnF, each with slightly different sampling outside of Morus, do not resolve *M. mesozygia* and *M. insignis* with the subgenus Morus-Trophis s. s. clade (Fig. 1). In the ITS MP strict consensus, *M. mesozygia* and *Milicia excelsa* are sister (with strong MP BS support, 97%, but without PP support), and that clade together with *M. insignis* form a poorly supported clade (MP BS 62%) which is in turn sister to a *Streblus* clade (MP BS 81%; Fig. 1). Relationships of the subgenus Morus-Trophis s. s. clade, the *M. mesozygia*-*Milicia excelsa*-M. *insignis*-Streblus clade and a clade of *Sorocea* are unresolved, while *Bagassa guianensis* falls as sister to that entire clade (MP BS 80%; Fig. 1). There is less sampling of Tribe Moreae for *trnL-trnF*, and *trnL-trnF* is generally less resolved and supported (Fig. 1). The subgenus Morus-Trophis s. s. clade forms a polytomy with *Bagassa guianensis* and a clade of *Sorocea* (with additional population sampling relative to the present study), but parsimony analysis of their sequences (obtained from GenBank; data not shown) yields unresolved relationships. Future studies employing more variable DNA regions with extensive sampling (including the breadth of diversity considered here) will be valuable.

**Discussion**

**Phylogenetic Relationships of Morus**—Most of Morus forms a well-supported monophyletic group which corresponds to *Morus* subgenus *Morus* sensu Leroy (1949). Within this core *Morus* group, all Asian species (including the type of *Morus*, *M. nigra*) form a clade, and the native North American taxa (including *M. Celtidifolia*, which ranges into Central America) form a clade. Given our sampling of Tribe Moreae, *Trophis* s. s. (*T. racemosa* is synonymized with *T. americana* L., which is the type of *Trophis* [Berg 2001]) is sister to *Morus* subgenus *Morus* (a close relationship between *Morus* and some members of *Trophis* has previously been documented by Datwyler and Weiblen [2004] and Zerega et al. [2005]). The African *M. mesozygia* (Leroy’s Subg. *Afromorus*) and the Neotropical *M. insignis* (Leroy’s Subg. *Gomphomorus*) are more distantly related to subgenus *Morus*, rendering *Morus* non-monophyletic.

This study represents the most comprehensive phylogenetic study of *Morus* to date. The thirteen species of *Morus* included in the phylogeny are morphologically distinct from one another and are representative of the diversity of the genus. Indeed, sampling of *Morus* herein may be considered comprehensive (see Berg 2001, 2005a; Nepal 2008), although some workers have split the genus more extensively (particularly in Asia, Zhekun and Gilbert 2003; see also Greene’s treatment of variation in the southwestern U. S. A. [synonymized within *M. microphylla*], Greene 1910) and, as noted by Berg (2005a), a modern worldwide monograph is lacking. The ITS and *trnL-trnF* regions do not provide sufficient variation to resolve relationships at the “tips” of the tree within *Morus*. Zhao et al. (2005) used sequences of ITS and the *trnL* intron to infer relationships based on genetic distance among some Asian taxa (with additional population sampling relative to the present study), but parsimony analysis of their sequences (obtained from GenBank; data not shown) yields unresolved relationships. Future studies employing more variable DNA regions with extensive sampling (including the breadth of diversity considered here) will be valuable.

**Subgenus Morus, Asian Clade**—Relationships within the Asian clade, which harbors the most taxonomic diversity of the genus, are largely unresolved. Of the eight species, three...
are characterized by a long style (> 1 mm; *M. australis*, *M. mongolica*, and *M. notabilis*), and the remaining five have a short style (< 0.5 mm; *M. alba*, *M. serrata*, *M. cathayana*, *M. macroura*, and *M. nigra*; as do other *Morus* species outside of the Asian clade). The phylogeny is consistent with a hypothesized single origin of the long style (sensu Koidzumi; > 1 mm) are designated “long” in parentheses following the taxon name. Relationships are unresolved in the combined tree but *M. australis* groups with and *M. notabilis* in the ITS tree and with *M. mongolica* in the trnL-trnF tree, though with weak support in each case. *Morus mongolica* differs from the other long-styled species in having a long pointed apiculum on the leaves; and *M. australis* and *M. notabilis* are differentiated from each other in leaf shape, leaf apex, and infructescence...
length characters. *Morus cathyana* and *M. macroura*, which are grouped with weak support (Fig. 2) share a long catkin length (> 2 cm) and larger axillary buds (0.2–1 cm × 0.5 cm) relative to other Asian species (see Nepal 2008). The known variation in ploidy levels within *Morus* (from 2x to 22x, x = 14; Janaki-Ammal 1948; Chen et al. 1993; Azizan and Sonboli 2001) occurs among the Asian species and thus could not be assessed in light of the current phylogeny. Similarly, classification based on leaf cystolith cells (Hotta 1954) could not be fully evaluated. Interestingly, all of the taxa in the Asian clade correspond to only two species sensu Bureau (1873), who would have recognized *M. nigra* and *M. alba*, with all other taxa recognized herein as varieties of the latter.

Several population genetic studies have been conducted on Asian species of *Morus*, investigating genetic variation between genotypes and varieties, and even exploring interspecific relationships (e.g. Sharma et al. 2000; Bhattacharya and Ranade 2001; Awasthi et al. 2004; Vijayan et al. 2006;
Zhao et al. 2007). Of these, the only study to sample outside of subgenus Morus was that of Sharma et al. (2000; using AFLPs). They included one sample of the African M. mesozygia (discussed below; M. insignis was not included), and, interestingly, it grouped with a large cluster of highly similar samples including M. microphylla, M. celtidifolia, and M. nigeriformis (≡ M. alba), while samples they listed as M. laevigata (≡ M. macroura), M. tiliaefolia (≡ M. macroura), and M. boninensis (≡ M. australis) were more genetically distant. It is important to note that phenograms depicting genetic distance may not correspond to phylogenetic relationships; furthermore, ascertaining evolutionary relationships was not an aim of most of these studies, rather, the focus was generally on genetic similarity, often for breeding purposes.

**Subgenus Morus, North American Clade—**Our findings support a single origin of native New World species (M. rubra, M. microphylla, and M. celtidifolia), sister to the Asian clade within subgenus Morus. The southwestern species M. microphylla (North America) and M. celtidifolia (Central America) are sister species, with that clade sister to the widespread eastern North American M. rubra. Intriguing ecological and population genetic work in North America demonstrates that the native M. rubra hybridizes naturally with the introduced M. alba in some areas; moreover the introduced species may represent a threat to the increasing ecological and population genetic work in North America introduced species may represent a threat to the increasing (Datwyler and Weiblen 2004; Zerega et al. 2005; Clement and Weiblen 2009); it is now clear that this work must take into consideration relationships of the genus Morus as well.

Outside of the subgenus Morus-Trophis s. s. clade, confident inference of relationships is not yet possible. In the ITS phylogeny, M. mesozygia and M. insignis are paraplethric to the single sample of Milicia, and that clade in turn is sister to a monophyletic Streblus. In considering the combined tree, it is important to note that Milicia and Streblus were not included because trnL-trnF data were unavailable. Therein, M. mesozygia and M. insignis resolved as a clade sister to subgenus Morus-Trophis s. s., with a monophyletic Soroea and the single sample of Bagassass a each subsequently basal. However, the interior region of the phylogeny is poorly supported and exhibits short branch lengths, and our study thus adds to others that have noted poor phylogenetic resolution and taxonomic challenges within this part of the Tribe Moroeae phylogeny (Datwyler and Weiblen 2004; Zerega et al. 2005; Clement and Weiblen 2009).

Our finding of a non-monophyletic Morus is novel, and recovery of particular clades with geographic integrity (the Asian and North American clades of Subg. Morus) as well as the positions of African M. mesozygia and Neotropical M. insignis lay important groundwork for further systematic study of Morus. This study also highlights the importance of ongoing work to resolve relationships and circumscribe natural genera within Tribe Moroeae.

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**Literature Cited**

Appendix 1. *Morus* species sampled, with information on general morphology (see Nepal 2008), native range, voucher information and collection location (abbreviations for herbaria follow Holmgren et al. 1990), and GenBank accession numbers for ITS and trnL-trnF.

*Morus alba* L., Leaf blade abaxially sparse pubescent along the veins, adaxially glabrous, margin irregularly dentate, apex usually obtuse, style absent or indistinct, infructescence up to 1.5 cm, Asia: South and Central China, Nepal 396, Central China, or absent, infructescence 2-5 cm, Asia: China, Japan, Korea, Nepal 396, Yunnan, China (MO), HM747167, HM747183; M. *notabilis* C. K. Schneid., Leaf abaxially densely pubescent, glabrous adaxially, leaf margin with wider teeth, slightly drooping branches, style long, infructescence cymidc, 2.5-4 cm, Asia: South and Central China, *Heng* 11734, Yunnan, China (GH), HM747175, HM747191; M. *rubra* L., Leave ovate with often corymb base, surface abaxially densely pubescent, adaxially often scabrous, stem with horizontally spreading (slightly drooping) branches, style short, infructescence cymidc, up to 2 cm, North America: Eastern United States to southeastern Canada, Nepal 701, Kansas, United States (KSC), HM747165, HM747181; M. *serrata* Roxb., Leaf margin with evenly spaced triangular teeth, semi persistent bud scales and stipules, style short, stigma densely pubescent, infructescence less than 2 cm, Asia: China, India, Nepal, *Blattariai* 1, Ilam, Nepal (KSC), HM747176, HM747192.

Appendix 2. Information for additional ingroup samples of Tribe Moroeae sensu Clement and Weiblen (2009), and outgroup taxon *Artocarpus heterophylla*, including voucher information for new sequences reported in the present study, and GenBank accession numbers for ITS and trnL-trnF. Taxon names for sequences obtained from GenBank follow GenBank listings (differences in determination listings in publication are noted in brackets).

Bagassa guianensis Aubl., no voucher, FJ917001, FJ917061; Milicia excelsa (Welw.) C. C. Berg, no voucher, MEU93585,—; Soroea affinis Hemsl., Weiblen 1437, Costa Rica (MIN), HM747179, HM747195; Soroea muriculata Miq. [S. steinbachii C. C. Berg; Zerega et al. 2010], no voucher, FJ916998, FJ917063; Soroea pilatea Burger [S. briquetii J. F. Macbr.; Zerega et al. 2010], no voucher, FJ916999, FJ917084; Streblus banksii (Cheesem.) Webb, no voucher, EF635452,—; Streblus glaber (Merr.) Corner, no voucher, DQ499105,—; Streblus heterophyllus (Blume) Corner, no voucher, DQ499106,—; Streblus smithii (Cheesem.) Corner, no voucher, EF635447,—; Streblus pulausinau (Endl.) F. Muell., no voucher,—; AF501669; Trophis involucrata W. Burger, Weiblen 1405, Costa Rica (MIN), HM747177, HM747199; T. racemos (L.) Urban, Weiblen 1400, Costa Rica (MIN), HM747178, HM747194;

Outgroup: Artocarpus heterophyllus Lam., no voucher, FJ917052, FJ917113.