MORUS MURRAYANA (MORACEAE): A NEW MULBERRY FROM EASTERN NORTH AMERICA

Stephanie J. Galla, Brittney L. Viers, Paul E. Gradie, and Dayle E. Saar

Department of Biological Sciences, Murray State University, Murray, KY 42071 USA dayle.saar@murraystate.edu

ABSTRACT

Mulberry trees (*Morus*: Moraceae) growing in relatively undisturbed, open woodland areas of western Kentucky exhibit exceptionally large leaves (blades often >15 cm long). Fruit size is also longer than reported for other species, and leaf vein patterns are unique. Field observations, combined with the use of herbarium specimens and molecular data warrant the establishment of a new species designation, *Morus murrayana* D.E. Saar and S.J. Galla (Murray State's Mulberry). *Phytologia* 91(1):105-116 (April, 2009).

KEY WORDS: *Morus*, Moraceae, mulberry, *murrayana*, Kentucky, internal transcribed spacer, ITS

Mulberry trees (*Morus* L.: Moraceae) in western Kentucky and the surrounding states, identified as *M. rubra* L., were observed with exceptionally large leaves. In a search of plant keys (Jones, 2005; Mohlenbrock, 2002; Wunderlin, 1997; Swink & Wilhelm, 1994; Gleason & Cronquist, 1991; Radford et al., 1968; Steyermark, 1963; Britton & Brown, 1913) and detailed, authoritative books (Kurz, 2003; Dirr, 1998; Elias, 1987), only Wunderlin (1997) reported leaves of *M. rubra* over 15 cm in length. He listed the usual size as 7.5-18 cm but occasionally to 36 cm. Wunderlin assumed, based on his examination of herbarium specimens, that the large leaves were due to shade/sun forms or were perhaps associated with other growing conditions (pers. comm. to DES). It should be noted that leaf size is only mentioned in Wunderlin's (1997) species description for *M. rubra* and he did not include this feature in the diagnostic characters given in the keys for species identification. For the current study, the authors had the advantage of first-hand field observations of these trees growing in their natural habitat. Additional field data and DNA analysis demonstrate that this is a separate species from *M. rubra* and all others previously recognized.

MORUS MURRAYANA D.E. Saar and S.J. Galla, sp. nov. Fig. 1.

Arboles ad 20 m alto; folia alternatum, unifolius-quinquelobus, lamina ad 38 cm longus, serrulatus; fructus ad 4 cm longus, nigellus purpureus.

TYPE: **USA. KENTUCKY: Calloway Co.** Frequent in open mesic woodlands dominated by *Quercus* spp. and *Carya* spp. along both sides of Watersport Rd. between gate to Racer Point and boat landing on Kentucky Lake, near Hancock Biological Station, Murray State University, ca. 25 km NW of Murray, KY (36° 43.87' N; 088° 07.35' W), 13 May 2006, *Dayle E. Saar 3606* (Holotype: MUR; isotypes, BEREA, BRIT, EKU, F, MO, NCU, NY, TENN, US).

Trees to 20 m tall with a single trunk, open crown vase-shaped to rounded. Sap milky. Bark on saplings smooth, medium brown with tan lenticels, becoming gravish-brown with very thin, long and narrow scaly plates. Winter buds with pseudoterminal present, dull brown, scales glabrous with minutely ciliated margins. Leaves alternate, simple; stipules light brown, membranous to 1.1 cm long and early deciduous; petioles 2.5-6 cm long; blades to 38 cm long, widest at or below the middle, unlobed to as many as five lobes, caudate at tip and oblique at base, serrate but occasionally double serrate, scabrous above and softly pubescent on veins and lamina below, pubescence on larger veins generally restricted to sides of veins, versus the dorsal peak (Fig. 1B); basal lateral veins larger than other laterals but smaller than the midvein, veins branching from two large lower laterals (tertiary) and other laterals from midvein (secondary) curve towards the tip as they approach the blade margin without entering a tooth and only the finest veins end in a tooth (Fig. 2C). Inflorescences of unisexual flowers axillary on short peduncles; individuals varying from monoecious to polygamodioecious to dioecious. Fruit multiple, blackish purple with dark reddish-purple juice at maturity, to 4 cm long and 1.5 cm wide but often thinner, with much variation in size on a single individual. The

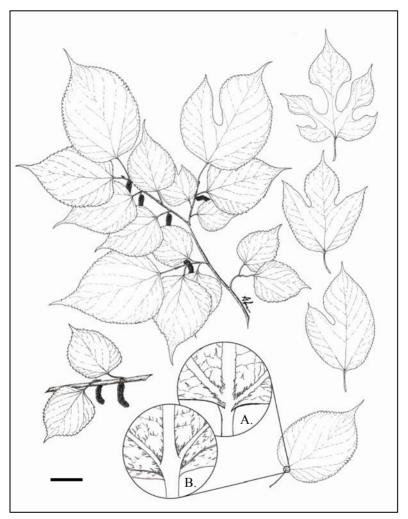


Figure 1. *Morus murrayana* with mature fruit showing variation in leaf morphology (leaves not at maximum size when fruit begins to mature); A. abaxial surface; B. adaxial surface. Scale bar = 5.0 cm.

specific epithet, *murrayana*, was chosen to honor Murray State University, Murray, KY (Murray State's Mulberry).

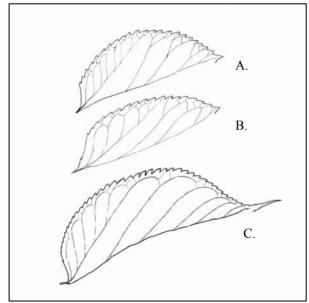


Figure 2. Leaves of A. *Morus rubra*; B. *M. alba*; and C. *M. murrayana* showing differences in veining patterns. Leaves not drawn to scale with each other. See text for further descriptions.

GENERAL MORPHOLOGY AND NATURAL HISTORY

Many individuals of *Morus murrayana* were observed growing in wet-mesic to dry-mesic open woodlands, in partly shaded to sunny locations; the few individuals located in heavy shade were growing poorly. They are virtually absent where Red Maple (*Acer rubrum* L.) has come in under overstories dominated by oaks (*Quercus* L. spp.) and hickories (*Carya* Nutt. spp.) and completely filled-in the canopy. *M. murrayana* occurs in natural as well as mildly disturbed localities, but generally is not in high disturbance places such as fence rows. Individuals observed for this study ranged in age from saplings to large trees; none were stump-sprouts. In other words, no correlation has been detected between the large leaves and the trees' age, condition, and/or habitat including soil type.

The morphological extremes in blade shape can be observed on the same branch next to each other. However, almost all trees produce either >80% lobed leaves or are >80% unlobed; few individuals display anything that approaches equal proportions of lobed and unlobed leaves. In fall, the leaves turn medium yellow, the smaller internal leaves falling first.

Trees produce either predominately staminate or carpellate inflorescences, but the presence of some staminate inflorescences on carpellate trees and vise-versa is common, especially on large saplings and older individuals. Both staminate and carpellate inflorescences may occur on the same large branch, usually separated on different twigs. This is in contrast with *M. rubra*, which is monoecious (Elias, 1987; Jones, 2005). Fruit ripens in western Kentucky during June and early July.

Morus murrayana has been sighted by one or more of the authors in Kentucky, Tennessee, Missouri, and Illinois, Herbarium specimens were also used to locate additional occurrences. Specimens of *M. murrayana* were readily recognizable among the accessions based on overall leaf size, caudate blade tips, and the venation pattern. Specimens confirm that this species has existed or currently exists in the four states personally documented by the authors, plus an additional six states that include Indiana, Mississippi, Louisiana, Virginia, North Carolina, and Alabama. The oldest specimen of M. murrayana (identified as *M. rubra*) was collected in the southern Illinois city of Carbondale in 1870. Other historical specimens date back to 1889, 1919, and 1937, in addition to those collected more recently. None of the label descriptions indicate a disturbed habitat, although habitat details were omitted on many labels. Most large-leafed specimens were identified as *M. rubra*, presumably due to the similar pattern of leaf However, these trees were clearly problematic to pubescence. taxonomists. Some specimens were identified as M. alba L., a few were listed as M. rubra x M. alba, and some had notations of "Morus sp." or "could not be determined." Many had annotations different than the original label identification (*M. alba* to *M. rubra* and vise-versa).

OTHER SPECIES OF MORUS IN NORTH AMERICA

Morus consists of about 12 species (Mabberley, 1997), only two of which (*M. rubra* and *M. microphylla* Buckley) are native to the United States, Canada, and Mexico; other species are native to Asia with some ranges extending west into Europe. *M. microphylla* is a small shrub or small tree with leaves to 5 cm in length. *M. murrayana* is most often confused with *M. rubra*. It can be distinguished from *M. rubra* based on leaf vein pattern (Fig. 2), leaves longer than 15 cm with caudate tips (vs. leaves <15 cm with cuspidate to broadly acute or acuminate tips), and fruits longer than 3 cm (vs. \leq 3 cm).

Another species, *Morus alba*, is naturalized from Asia and is widespread in many areas of North America, as a result of escapes from cultivation as a street planting and from an unsuccessful attempt in the 1830s to establish a silk industry in the United States (Federico, 1997), as the leaves are used to feed larvae of the silkworm, *Bombyx mori* L. *M. alba* has leaves that are shiny above and pubescence below is either absent or scattered in vein axils or sparse along the larger veins.

Morus nigra L. and M. alba var. multicaulis (Perr.) Loudon (syn. M. multicaulis Perr.) also have been documented in the US (Wunderlin, 1997; Jones, 2005). Unlike M. murrayana, M. nigra has dark brown bark and elliptic fruit to 2.5 cm long and wide (H. Sang, 2003). Further, Wunderlin (1997) reports that M. nigra, occasionally cultivated in North America, is not known to naturalize. M. alba var. multicaulis has leaves to 30 cm (L. Sang, 2003). However, the blades are thick and wrinkled, which does not describe M. murrayana, and the pattern of restricted pubescence below and glabrous above is consistent with M. alba (not M. murrayana). Also, live specimens of M. murrayana show no tendency for multiple trunks.

Plants of *Morus rubra* and *M. alba* are known to hybridize and produce intermediate pubescence patterns on leaves, but they did not produce large leaves and fruit (Burgess et al., 2005). *M. murryana* was not observed with *M. rubra*, but in the few instances where it occurred in the vicinity of *M. alba*, intermediates were not present.

MOLECULAR ANALYSIS AND COMPARISONS

Materials and Methods:

The entire herbarium collection of *Morus* at the Missouri Botanical Garden (MO) was inspected for similar leaf and fruit characteristics by DES.

Sequences for the internal transcribed spacer region (ITS) of nuclear ribosomal DNA (nrDNA) are available in GenBank (www.ncbi.nlm.nih.gov) for the species of Morus native to the Eastern Hemisphere. For this study, DNA was extracted from two individuals of *M. rubra* and three of *M. murrayana* using Quagen DNeasy kits. Amplification was carried out following the protocol detailed in Saar et al. (2003). DNA was sequenced in the DNA Core Facility at Northern Illinois University, DeKalb, Illinois, on a Beckman-Coulter capillary sequencer. All sequences of *M. rubra* and *M. murravana* were aligned with Clustal X software (Thompson et al., 2003). There were differences between the sequences of M. rubra and M. murrayana, but there was no intraspecific variation. Therefore, only one sequence of M. murrayana was necessary for comparison to existing GenBank accessions using a BLAST search (Altschul et al., 1990). The closest matches from GenBank, together with the new sequences of *M. rubra* and *M. murrayana*, were also aligned in Clustal X.

Results:

There were no herbarium specimens at MO with similar morphological characteristics from any continent, except those identified as *Morus rubra*.

The BLAST search indicates the closest species to *M. murrayana* are *M. macroura* Miq., *M. lhou* Koidz., *M. bombycis* Koidz., *M. cathayan* Hemsl., *M. atropurpurea* Roxb., *M. alba*, *M. nigra*, *M. australis* Poir., and *M. multicaulis*. Nucleotide polymorphisms are summarized in Fig. 3.

DISCUSSION

From the list of species whose sequences most closely match *M. murrayana* (Fig. 3), *Morus atropurpurea*, *M. bombycis*, *M. lhou*,

		18S	ITS 1	5.8S ITS 2	
Base Number (:	reading dn.)→	000000000000000000000000000000000000000	000000000112222 777788888891235	/	
¢GenBank No.(species)↓	species)ț	6017823456	890123490109	6017823456789012349010932124301681325334675	
AM042006 (M.)	bombycis)	CAT	CCCP	CATCCCA-CTCCAC-CGCGCCC-TTC	
AM042001 (M. 0	(M. cathayana)	CAT	CCCP	CATCCCA-CTCCAC-CGCGCCC-TTC	
AM042004 (M. 8	australis)	CAT	CCCP	CATCCCA-aaCCAC-CGCGCCC-TTC	
AM041999 (M.	lhou)	CAT	CCCP	CATCCCA-CTCCAC-CGCGCCC-TTC	
AM041998 (M. 3	alba)	CAT	CCCP	CATCCCA-CTCCAC-CGCGCCC-TTC	
AM042002 (M. J	nigra)	CAT		CCCA-CTCCACCGCGCAC-TTC	
AM042000 (M. 1	macroura)	CAT	CCCP	CCCA-CTCCAC-CGCGCCC-TTC	
AM042003 (M. 1	multicaulis)	CAcaaT	CCCP	CCCA-CTCCACCGCGCCCCCTTC	
FJ605516 (M. :	rubra)	CAT		CCCA-CTCCACCGCG-CC-TTC	
AY345145 (M. a	atropurpurea)	T	CCCP	-ATCCCA-CTCCAC-CGCGCCTTC	
FJ605515 (M. 1	(M. murrayana)	Cgcgtgc	Jcaatgcgctttg	CgcgtgcgcaatgcgctttgtCTttt-gttataCCGct	
	Notes:	****	******	***#* *********	
Figure 3. Summary of single nucleotide polyr <i>murrayuna</i> , based on a BLAST search of Gen others from GenBank accessions. In this align begins at base 402; 26S gene begins at base 625	of single nucleotide a BLAST search o accessions. In this S gene begins at bas	polymorphisms if GenBank. Se alignment, ITS se 625.	from 627 aligned quences of <i>M. ru</i> 1 begins at base :	Figure 3. Summary of single nucleotide polymorphisms from 627 aligned bases from closest sequences to <i>Morus murrayana</i> , based on a BLAST search of GenBank. Sequences of <i>M. rubra</i> and <i>M. murrayana</i> from this study; others from GenBank accessions. In this alignment, ITS 1 begins at base 53; 5.8S gene begins at base 241; ITS 2 begins at base 402; 26S gene begins at base 625.	to <i>Morus</i> his study; 41; ITS 2
Notes: * unique base	Notes: * unique base is M. murrayana; # unique base is M. murrayana and one other species.	≠ unique base is .	<i>M. murrayana</i> and	one other species.	

and *M. multicaulis* are synonyms or varieties of *M. alba* (Ghafoor, 1985; Shu, 2003; Index Kewensis). All species from this list can be separated from *M. murrayana* based on morphology. *M. macroura* has yellowish-white fruit when mature, 6-12 cm long (Ghafoor, 1985; N. Sang, 2003). (*M. murrayana* fruits are blackish-purple and ≤ 4.0 cm.) The Trade Winds Fruit Company (www.tradewindsfruit.com) reports that *M. macroura* is only hardy to about 18-25°F (varies by individual). Western Kentucky and southern Illinois are in USDA zone 6 (average low of -10-0°F). *M. cathayana* has leaves 8-20 cm long, but they are thick and papery and winter buds are white pubescent (S. Sang, 2003), again, unlike *M. murrayana*. Morphologic distinctions from *M. alba* and *M. nigra* have been discussed previously. The fruits of *M. australis* are <2.5 cm long and plants are shrubs or small trees (Shu, 2003; J. Sang, 2003) with twisted branches (Dirr, 1998).

In addition to *M. alba*, ten other species from Asia are described in Flora of China (Shu, 2003). All species and varieties have leaves ≤ 15 cm except for *M. nigra* with 6-12(-20) and *M. cathayana* with 8-20 cm leaves (both discussed previously). Four species are included in the Flora of Pakistan. One of these species, *M. serrata* Roxb. (syn. *M. alba* var. *serrata* (Roxb.) Bureau), which is confined to the Indo-Pakistan subcontinent, has leaves 5-15 cm long. However, this species has longer stipules (1.5-2.5 cm long) and smaller fruits (0.8-2.5 cm long) (Ghafoor, 1985) than does *M. murrayana*.

The DNA sequence data separate *M. murrayana* from all others in the GenBank database. Sequences of *M. rubra* and the Asian species are similar, whereas *M. murrayana* has a notable 13-base insertion plus five 1-2 base indels and 16 single base substitutions (Fig. 3). It is clearly the most distinctive sequence of the entire group.

Although it is somewhat unusual to describe a new, wideranging, tree-sized species from the US, it is not without precedent. Due in part to the fact that there are so few species of *Morus* found in North America, very few characters are required to separate them. For example, the most common sympatric species ranges include *M. rubra* and *M. alba*, which can be separated based on leaf pubescence, thereby eliminating the need to elaborate with additional descriptions. With so few diagnostic characters utilized, *M. murrayana* falls within the parameters of virtually all plant keys for this genus in North America and falls under *M. rubra*. Thus, skepticism may be minimal because it is seemingly a good "fit" with the key. We have not observed *M. rubra* growing with *M. murrayana*, which could have facilitated direct comparisons. Further, with only two native species, *Morus* is not a particularly attractive candidate for taxonomic study in North America. The reputation of our native *M. rubra* may suffer due to a close resemblance with its weedy, non-native congener, *M. alba*.

Further studies are underway to learn more about the natural history of this species, and to produce a more detailed and extensive distribution map.

Key to Native and Introduced Species in North America:

- Leaves 2-5 cm in length, strongly bicolored (dull dark green above, pale green below); shrubs or small straggly trees to 7 m; trees of the American SW and N Mexico. . . . M. microphylla
- 1. Leaves 3.8-14 cm long or longer, not strongly bicolored; trees. . . 2

 - 2. Leaves scabrous on upper surface and soft pubescent below. $\ . \ 3$
- 3. Leaves with cordate bases; fruits elliptic to long ovoid, ≤ 2.5 cm long, maturing from red to black; landscape plant *M. nigra*
- 3. Leaf bases variously oblique to slightly cordate; fruits cylindrical and >2.5 cm long; mature fruits blackish purple. 4
 - 4. Leaves to 15 cm long but often <10 cm, acute to acuminate at tip, lateral veins (secondary) above lowest lateral fairly straight and ending in a tooth; mature fruit to 3 cm long. *M. rubra*
 - 4. Leaves to 38 cm long, outer three leaves on branchlets almost always \geq 15 cm, caudate, lateral veins curve before reaching margins, only tiniest veins end in a tooth; mature fruit to 4 cm long and 1.5 cm wide but often thinner, with much size variation on a single individual. *M. murrayana*

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LITERATURE CITED

- Altschul S.F., W. Gish, and W. Miller. 1990. Basic local alignment tool. J. Mol. Biol. 215: 403-410.
- Britton, N.L. and A. Brown. 1913. An illustrated flora of the northern United States and Canada, ed. 2. Dover reprint (1970), Dover Publications, Inc., New York, NY.
- Burgess, K.S., M. Morgan, L. Deverno, and C. Husband. 2005. Asymmetrical introgression between two *Morus* species (*M. alba*, *M. rubra*) that differ in abundance. Mol. Ecol. 14: 3471-3483.
- Dirr, M.A. 1998. Manual of woody landscape plants, ed. 5. Stipes Publishing L.L.C., Champaign, IL.
- Elias, T.S. 1987. The complete trees of North America field guide and natural history, Gramercy Publishing Co., New York, NY.
- Federico, G. 1997. An Economic History of the Silk Industry, 1830-1930, (Cambridge Studies in Modern Economic History) Cambridge University Press, New York, NY.
- Ghfoor, A. 1985. Moraceae. Flora of Pakistan, vol. 171, Missouri Botanical Garden Press, St. Louis, MO.
- Gleason, H.A. and A. Cronquist. 1991. Manual of vascular plants of northeastern United States and adjacent Canada, ed. 2. The New York Botanical Garden, Bronx, NY.

- Jones, R.L. 2005. Plant Life of Kentucky. University Press of Kentucky, Lexington, KY.
- Kurz, D. 2003. Trees of Missouri, C. Overby, ed. Missouri Department of Conservation, Jefferson City, MO.
- Mabberley, D.J. 1997. The plant-book, ed. 2. Cambridge University Press, New York, NY.
- Mohlenbrock, R.H. 2002. Vascular flora of Illinois. Southern Illinois University Press, Carbondale, IL.
- Radford, A.E., H.E. Ahles, and C.R. Bell. 1968. Manual of the Vascular Flora of the Carolinas. University of North Carolina Press, Chapel Hill, NC.
- Saar, D.E., N.O. Polans, and P.D. Sørensen. 2003. A phylogenetic analysis of the genus *Dahlia* (Asteraceae) based on internal and external transcribed spacer regions of nuclear ribosomal DNA. Syst. Bot. 28: 627-639.
- Sang, H. 2003. *Morus nigra*. Flora of China, vol. 5, Missouri Botanical Garden Press, St. Louis, MO.
- Sang, J. 2003. *Morus australis*. Flora of China, vol. 5, Missouri Botanical Garden Press, St. Louis, MO.
- Sang, L. 2003. *Morus alba*. Flora of China, vol. 5, Missouri Botanical Garden Press, St. Louis, MO.
- Sang, N. 2003. *Morus macroura*. Flora of China, vol. 5, Missouri Botanical Garden Press, St. Louis, MO.
- Sang, S. 2003. *Morus cathayana*. Flora of China, vol. 5, Missouri Botanical Garden Press, St. Louis, MO.
- Shu, S. 2003. *Morus*. Flora of China, vol. 5, Missouri Botanical Garden Press, St. Louis, MO.
- Steyermark, J.A. 1963. Flora of Missouri. Iowa State University Press, Ames, IA.
- Swink, F. and G. Wilhelm. 1994. Plants of the Chicago region, ed. 4. Indiana Academy of Science, Indianapolis, IN.
- Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25: 4876–4882.
- Wunderlin, R.P. 1997. *Morus* [Moraceae] *in* Flora of North America north of Mexico, vol. 3, pp. 390-392, Flora of North America Editorial Committee, eds. Oxford University Press, New York, NY.