STATUS OF *MORUS MURRAYANA* (MORACEAE)

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ABSTRACT

A reexamination of *Morus murrayana* with more individuals from a wider geographic range, coupled with an additional molecular marker, has led to the conclusion that *M. murrayana* should be revised as ***M. rubra var. murrayana***. Leaf vein patterns are shown to be a much more accurate character for species delineation between *M. rubra* and *M. alba* than the commonly used comparisons of leaf pubescence, as verified by DNA-identified individuals. *Phytologia 94(2): 245-252 (August 1, 2012).*

KEY WORDS: Moraceae, mulberry, *Morus*, vars. *murrayana*, *rubra*, *alba*, Kentucky, internal transcribed spacer, ITS, *trn*L-F

Morus murrayana D.E. Saar & S.J. Galla (Murray State's Mulberry) was named (Galla et al., 2009) based on unique morphological characters and sequences from nuclear DNA (internal transcribed spacer region (ITS) of nuclear ribosomal DNA (nrDNA)). Distinctions between native *M. rubra* L. (Red Mulberry) and the invasive, non-native *M. alba* L. (White Mulberry) continue to be blurred due to the almost exclusive use of pubescence as the diagnostic character in plant keys (e.g., Jones, 2005; Mohlenbrock, 2002; Wunderlin, 1997; Swink & Wilhelm, 1994; Gleason & Cronquist, 1991; Elias, 1987; Radford et al., 1968; Steyermark, 1963; Britton & Brown, 1913). *M. alba* is a highly variable species, even within its native range in Asia (Chen Renfang, Southwest University, China, pers. com. to DES). This variability includes leaf pubescence, with the result

that many pubescent individuals of *M. alba* have been incorrectly identified as *M. rubra*, as is evident on many herbarium specimens. This includes individuals of *M. alba* where the top surface of the leaves are somewhat scabrous. The taxonomy of *M. murrayana* was further complicated by its large leaves and long fruits, neither of which is described in the literature. Further, *M. murrayana* does not have straight secondary veins that end in a marginal tooth, as is illustrated in the literature for *M. rubra* (e.g., Wunderlin, 1997; Britton & Brown, 1913).

Further studies utilizing more individuals from a much larger distribution area, with the additional molecular data from a chloroplast marker (*trn*L-F intergenic spacer), have led us to the conclusion that *M. murrayana* is a variety of *M. rubra*. We also found that the leaf vein pattern is a much more reliable diagnostic character than pubescence, when compared to DNA-identified individuals.

Morus rubra* var. *murrayana (D.E. Saar & S.J. Galla) D.E. Saar, **comb. & stat. nov.**

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 County.** 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: F, MO, NY, TENN, US).

Basionym: *Morus murrayana* D.E. Saar & S.J. Galla. Phytologia 91: 105-116. 2009. Holotype: as listed above; isotypes: as listed above.

MATERIALS AND METHODS

Trees of *M. rubra* and *M. alba* were observed and sampled from KY, TN, AL, NC, VA, OH, PA, NY, MA, MI, IL, WI, IA, MN,

and ON, Canada, during the summers of 2009, 2010, and 2011. Plant material was stored in silica gel and herbarium vouchers are at MUR.

DNA was extracted using Quagen DNeasy kits. Potential chloroplast markers were screened from these chloroplast regions: *ndh*C-*trn*V (Timme et al., 2007), *ndh*G-*ndh*I (Panero & Crozier 2003), *rbc*L-*acc*D (Panero & Crozier, 2003), *rpl*16 (Crawford & Mort, 2005), *trn*L-*rpl*32 (Timme et al., 2007), *trn*L-F (Taberlet et al., 1991), and *trn*Y-*trn*E (Timme et al., 2007). DNA was sequenced in the DNA Core Facility at Northern Illinois University, DeKalb, Illinois, on a Beckman-Coulter capillary sequencer. All sequences were aligned with Clustal X software (Thompson et al., 2003). Resulting sequences, along with the nuclear markers (ITS) utilized in Galla et al. (2009), were compared to the voucher specimens collected during field work.

RESULTS

Most primer pairs screened in this study either do not amplify despite several modifications to the PCR parameters, do not demonstrate informative interspecific differences, or produce multiple bands on electrophoresis gels. A six-base insertion occurs in sequences of *M. rubra* in the *trn*L-F intergenic spacer, which was used to differentiate it from *M. alba*, along with additional ITS sequences generated from this study.

When DNA-identified individuals were plotted on a map of the US, it appears that the actual range for *M. rubra* is smaller than published range maps (e.g., Wunderlin, 1997; Elias, 1987) (unpublished climate/species correlations forthcoming). This is probably due to the incorporation of individuals of *M. alba* that were misidentified as *M. rubra* when developing the current maps.

DISCUSSION

Comparisons between DNA-identified individuals and their respective vouchers indicate that *M. alba* is a highly variable species. While most leaves are glabrous and often lustrous on the upper side, this species can also exhibit leaves that are slightly scabrous. Field observations also identified this condition, particularly on vigorous

growth produced after the initial early season leaf-out. When this scabrous feature was encountered (MOS & DES, pers. ob.), leaves produced earlier in the season were often glabrous and moderate to highly lustrous on the same tree.

As mentioned previously, current keys focus on pubescence as the diagnostic character for distinguishing *M. rubra* from *M. alba*. Plant key descriptions are summarized as follows:

- *M. alba* leaves are glabrous above and often lustrous, glabrous below or pubescence restricted to scattered hairs in vein axils or scattered along larger veins.
- *M. rubra* leaves are scabrous above and undersides are pubescent throughout.

Given the above descriptions, individuals of *M. alba* with scabrous and/or pubescent leaves would be identified as *M. rubra*. This is particularly true in areas where *M. rubra* does not actually occur but is thought to be present, based on inaccurate range maps. This was the case with the presumed *M. rubra* sequenced in the study of *M. murrayana* by Galla et al. (2009). The individuals of *M. rubra* sequenced in the 2009 study for comparison to *M. murrayana* were from northeastern IL, where we now believe "true" *M. rubra* var. *rubra* does not exist, although they were from an area well within the published range. The identity of these trees as *M. rubra* was also confirmed locally by leading professional botanists, further underscoring the need for reliable field characters.

Based on our comparisons between DNA sequences and their respective herbarium vouchers, we have concluded that leaf vein patterns are more accurate for species delineation than pubescence. Laterals from the midvein, or secondary veins, for *M. rubra* curve towards the tip of the leaf as they approach the leaf margin and connect to the next lateral. The Leaf Architecture Working Group (LAWG) (1999) describes this pattern as the brochidodromous subtype of pinnate venation. The secondary veins of *M. alba* are fairly straight and terminate in a marginal tooth. This is pattern is classified as the craspedodromous subtype of pinnate venation (LAWG, 1999). The large tertiary veins off the lowest lateral vein can occupy up to a third of the blade surface and curve to the next tertiary vein on both *M. rubra*

and *M. alba*, and therefore are not useful for species delineation. Both varieties of *M. rubra* have the curved brochidodromous vein pattern. *M. rubra* var. *murrayana* is differentiated from *M. rubra* var. *rubra* by its longer fruits and tendency for much larger leaves.

M. rubra var. *murrayana* occurs in open mesic to wet-mesic woodlands in western KY, NW TN, and S IL. It rarely occurs in deep shade or in areas with moderate to high disturbance. Additional field work is needed to better define its geographic range.

Key to Native, Invasive, and Hybrid Species in North America:

1. Leaves (without petioles) 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. Secondary leaf veins, sometimes branched, ending in a marginal tooth, never curving to connect with the next secondary vein distal to the leaf base; leaf surface glabrous and often lustrous to slightly scabrous above, glabrous to variously pubescent below, the latter more often on later growth; mature fruit short cylindrical to slightly ovoid, white through pink to blackish purple. . . . . *M. alba*
    2. Secondary veins curve before reaching margins and connect to next secondary vein distal to the leaf base, only tiniest veinlets end in a tooth, or with both straight and curved vein patterns present, usually on different leaves; mature fruit short to long cylindrical, pink through blackish purple. 3

3. Both vein patterns present, or with curved vein pattern (only) and at least a slight luster on upper leaf sides; mature fruit to 3 cm long but usually shorter, pink to blackish purple. . . . . hybrid of *M. rubra* and *M. alba**
3. Leaves completely without luster, curved vein pattern present on all leaves; mature fruit blackish purple. 4

4. Leaves to 16 cm long but often <10 cm, acute to acuminate at tip, mature fruit to 3 cm long but usually shorter. . . . . *M. rubra* var. *rubra*

4. Leaves to 38 cm long, outer three leaves on branchlets almost always ≥ 16 cm, fruit to 4 cm long and 1.5 cm wide but often thinner, with some size variation on a single individual. . . . . *M. rubra* var. *murrayana*

* Hybrid individuals matching this description may be *Morus rubra* x *M. alba*, *M. alba* x *M. rubra*, or a hybrid of *M. rubra* and *M. alba* followed by back crosses with one or both species, or with other hybrids.

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