

## **Characterization of Microsatellite Markers for *Broussonetia papyrifera* (Moraceae)**

Authors: Peñailillo, Johany, Kuo, Wenhshi, Olivares, Gabriela, Silva-Poblete, Gerardo, Peña-Ahumada, Barbara, et al.

Source: Applications in Plant Sciences, 5(8)

Published By: Botanical Society of America

URL: <https://doi.org/10.3732/apps.1700044>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

## CHARACTERIZATION OF MICROSATELLITE MARKERS FOR *BROUSSONETIA POPYRIFERA* (MORACEAE)<sup>1</sup>

JOHANY PEÑAILILLO<sup>2</sup>, WENHSI KUO<sup>3</sup>, GABRIELA OLIVARES<sup>2</sup>, GERARDO SILVA-POBLETE<sup>2</sup>,  
BARBARA PEÑA-AHUMADA<sup>2</sup>, SOFÍA MUÑOZ<sup>2</sup>, XIMENA MONCADA<sup>4</sup>, KUO-FANG CHUNG<sup>3</sup>,  
DANIELA SEELENFREUND<sup>2</sup>, AND ANDREA SEELENFREUND<sup>5,6</sup>

<sup>2</sup>Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago, Chile; <sup>3</sup>Biodiversity Research Center, Academia Sinica, Nankang, Taipei, Taiwan; <sup>4</sup>Centro de Estudios Avanzados en Zonas Áridas (CEAZA), La Serena, Chile; and <sup>5</sup>Escuela de Antropología, Facultad de Ciencias Sociales, Universidad Academia de Humanismo Cristiano, Santiago, Chile

- **Premise of the study:** *Broussonetia papyrifera* (Moraceae) is native to Asia and is used as a medicinal plant and as a source of fiber for making paper. It was dispersed into the Pacific region as a fiber source for making nonwoven textiles (barkcloth). Microsatellites were developed to trace the human-mediated dispersal of this species into the Pacific region.
- **Methods and Results:** A set of 36 microsatellites was isolated and initially assayed on 10 accessions to assess polymorphism. We found that 20 markers were polymorphic, with the number of alleles per marker ranging from four to 35 in 70 accessions genotyped from three Asian populations. Observed and expected heterozygosities ranged from 0.04 to 0.85 and from 0.19 to 0.94, respectively. These markers were tested in four Moraceae species and one Rosaceae species.
- **Conclusions:** These markers will be useful for the assessment of genetic diversity in *B. papyrifera*. They show low transferability to other species tested.

**Key words:** *Broussonetia papyrifera*; genetic diversity; microsatellite; Moraceae.

Paper mulberry (*Broussonetia papyrifera* (L.) L'Hér. ex Vent.), belonging to the family Moraceae, is a multifunctional tree of cultural importance in Asia that has been used for centuries in the manufacture of high-quality paper. *Broussonetia papyrifera* is native to southern and central China, Vietnam, Thailand, and Taiwan (Matthews, 1996; Chang et al., 2015), where it is common in secondary forests growing at moderate elevations. *Broussonetia papyrifera* was intentionally transported into the Pacific region by prehistoric voyagers for making barkcloth, a nonwoven textile, and several centuries ago to Japan as a high-quality fiber source (Whistler, 2009).

The genetic diversity of *B. papyrifera* has been studied using intersimple sequence repeat (ISSR) markers (Ho and Chang, 2006; Liao et al., 2014; González-Lorca et al., 2015), sequence-related amplified polymorphism (SRAP) markers (Liu et al.,

2009), hypervariable chloroplast DNA (cpDNA) sequences, and internal transcribed spacer (ITS) sequences of ribosomal DNA (Chang et al., 2015). These molecular markers have been useful for characterizing the genetic diversity and population structure of this species within its native range. However, studies using ITS sequences and ISSR data (Seelenfreund et al., 2011; González-Lorca et al., 2015) and a sex marker (Peñailillo et al., 2016) in *B. papyrifera* in the introduced range in the Pacific region have not provided the resolution to understand its dispersal across this geographic area where it has been propagated asexually (Peñailillo et al., 2016). Therefore, the development of microsatellite markers is crucial to genotype the species' genetic diversity in Japan and the Pacific region. Here, we present the isolation and characterization of 20 microsatellite markers that will provide information on the fine structure of *B. papyrifera* populations both in its native and introduced range.

<sup>1</sup>Manuscript received 1 May 2017; revision accepted 15 June 2017.

The study received financial support from the Comisión Nacional de Investigación Científica y Tecnológica (CONICYT) and Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) (grants 1080061 and 1120175 to A.S.) from Chile, and from the Biodiversity Research Center, Academia Sinica, Taiwan (to K.F.C.). The authors thank Dr. K.-Y. Ho (National Chiayi University, Taiwan) for providing some of the Taiwanese samples, P. Matthews (National Museum of Ethnology, Osaka, Japan) for providing samples of *B. papyrifera* from Japan, and M. Floor for valuable assistance.

<sup>6</sup>Author for correspondence: aseelenfreund@gmail.com

doi:10.3732/apps.1700044

## METHODS AND RESULTS

To isolate microsatellites, genomic DNA from 10 samples of *B. papyrifera* collected in Taiwan, Japan, Chile, and several islands in Oceania was used (Appendix 1). These samples are deposited at the University of Chile, and one sample (BQUCH0152) has a voucher (SGO162505) at the herbarium of the National Museum of Natural History, Chile. Total DNA was extracted from young silica gel-dried leaves following the cetyltrimethylammonium bromide (CTAB) extraction protocol (Lodhi et al., 1994) and modified as described in Moncada et al. (2013). Approximately 1 cm<sup>2</sup> of tissue was homogenized, mixed with extraction buffer (50 mM EDTA, 100 mM Tris-HCl, 0.3 M NaCl, 2.0% [w/v] CTAB, 0.5% [v/v] 2-mercaptoethanol [pH 8.0]), and incubated at 65°C for

*Applications in Plant Sciences* 2017 5(8): 1700044; <http://www.bioone.org/loi/apps> © 2017 Peñailillo et al. Published by the Botanical Society of America. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC-BY-NC-SA 4.0), which permits unrestricted noncommercial use and redistribution provided that the original author and source are credited and the new work is distributed under the same license as the original.

25 min, followed by organic extraction. Total DNA was precipitated and stored at  $-20^{\circ}\text{C}$  until analysis. Purified DNA was quantified by spectrophotometric absorbance (Nanodrop, ThermoFisher Scientific, Wilmington, Delaware, USA) and Picogreen (Synergy H1, Winooski, Vermont, USA) and its integrity verified by 0.8% agarose gel electrophoresis.

Ecogenics GmbH (Zurich, Switzerland) constructed an enriched library using magnetic streptavidin beads and biotin-labeled CT and GT repeat oligonucleotides to develop size-selected fragments of genomic DNA for enrichment of microsatellite sequences. The microsatellite-enriched library was analyzed with GS FLX Titanium chemistry on a Roche 454 platform (454 Life Sciences, a Roche Company, Branford, Connecticut, USA). A total of 32,947 reads was found with an average length of 321 bp. Microsatellite simple sequence repeats (SSRs) with tri- or tetranucleotide motifs, repeated at least six times, and dinucleotides of at least 10 repeat units, were found in 10,024 reads. Primers were designed for 190 of these reads, and 36 were tested for the presence of polymorphisms on 10 samples from different geographic areas.

Polymorphisms in these 36 loci were assessed using the procedure described by Schuelke (2000). A universal 18-bp fluorophore-labeled M13 tail (5'-TGTA-AAACGACGGCCAGT-3') was incorporated into the PCR products during the first PCR cycles. In subsequent cycles, these products function as templates for the fluorophore-labeled universal M13 primer to produce fluorescent PCR products. PCR reactions were performed in a reaction volume of 10  $\mu\text{L}$  containing 2–10 ng of genomic DNA, 1 $\times$  buffer with 15 mM  $\text{MgCl}_2$ , 200  $\mu\text{M}$  dNTP mix, 0.04  $\mu\text{M}$  forward primer, 0.16  $\mu\text{M}$  of the reverse and the M13 primer, and 0.05  $\mu\text{L}$  of HotStarTaq DNA polymerase (QIAGEN, Hilden, Germany). PCR

amplifications were conducted under the following conditions: an initial denaturation of 15 min at  $95^{\circ}\text{C}$ ; 30 cycles at  $95^{\circ}\text{C}$  for 30 s, at an annealing temperature specific for each primer for 45 s, and at  $72^{\circ}\text{C}$  for 45 s; followed by eight cycles at  $95^{\circ}\text{C}$  for 30 s, at  $53\text{--}55^{\circ}\text{C}$  for 45 s, and at  $72^{\circ}\text{C}$  for 45 s; and a final extension step at  $72^{\circ}\text{C}$  for 30 min.

The amplified products were analyzed on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Waltham, Massachusetts, USA) with GeneScan 500 ROX Size Standard (Applied Biosystems). Genotypes were determined using GeneMapper version 3.2 (Applied Biosystems) with default settings. Due to the M13 tail attached to each forward primer, 18 bp were subtracted from the experimentally determined amplicons to obtain the length of actual alleles.

Twenty primer pairs were successfully amplified, with the expected sizes and banding patterns displaying clear polymorphisms in *B. papyrifera* (Table 1). Polymorphisms were evaluated in samples of *B. papyrifera* from its native range (three Asian populations,  $n = 70$ ). Table 2 shows the number of alleles per marker, observed and expected heterozygosity ( $H_o$  and  $H_e$ ), polymorphism information content (PIC), coefficient of inbreeding ( $F_{IS}$ ), null allele frequency ( $r$ ), and Hardy–Weinberg equilibrium (HWE) of the analyzed samples from three populations.  $H_o$ ,  $H_e$ , and  $F_{IS}$  were estimated using Arlequin 3.5.2.2 (Excoffier and Lischer, 2010). CERVUS 3.0.7 was used to calculate PIC (Kalinowski et al., 2007). Null allele frequency was calculated using MICRO-CHECKER (van Oosterhout et al., 2004) and HWE with GenAlEx 6.502 (Peakall and Smouse, 2006, 2012). The total number of alleles ranged from four to 35 with a mean of 19.2 (Table 1).  $H_o$  and  $H_e$  ranged from 0.038 to 0.846 and from 0.191 to

TABLE 1. Characteristics of 20 polymorphic microsatellite loci developed in *Broussonetia papyrifera*.

| Locus        | Primer sequences (5'–3')                                  | Repeat motif       | Allele size range (bp) | A  | $T_a$ ( $^{\circ}\text{C}$ ) | GenBank accession no. |
|--------------|---|--------------------|------------------------|----|------------------------------|-----------------------|
| Bropap_01931 | F: GGCATCGTTTTGACTGTTGG<br>R: GCAGTGAGGTAAGGCCAG          | (AC) <sub>16</sub> | 170–219                | 20 | 54                           | KU598930              |
| Bropap_02075 | F: GGTGGTGTGATGCAGAACATGC<br>R: TTTCCCTCGCGTCAAGATCG      | (TAG) <sub>8</sub> | 224–256                | 12 | 55                           | KU598931              |
| Bropap_02214 | F: CGGAAGAGAGAGATTACAACTAGC<br>R: ACTTGACCATTCTAAGCAAGACC | (GA) <sub>19</sub> | 220–262                | 24 | 55                           | KU598932              |
| Bropap_02359 | F: ATTTGTTGCTGCCCAAGGTC<br>R: TTCGCTCCCTTCCCTATTGC        | (AGA) <sub>8</sub> | 168–183                | 4  | 56                           | KU598933              |
| Bropap_02560 | F: CATGTGGACCCGTGAAAACC<br>R: AGAAGCCGCTTACTGGGAG         | (AC) <sub>15</sub> | 179–254                | 21 | 55                           | KU598934              |
| Bropap_02801 | F: GACATCTCATAAAATGTTTAAATCCAG<br>R: AATTGCCCTGTAGCATTCGG | (AC) <sub>11</sub> | 102–179                | 20 | 56                           | KU598935              |
| Bropap_03147 | F: CAGACTAGGTTGACCGCAAG<br>R: CACGTTTTGAACCTGGGAGC        | (GT) <sub>11</sub> | 192–201                | 8  | 55                           | KU598936              |
| Bropap_07697 | F: GGTGCATCTGCTGATTTTTCG<br>R: CCTGCCAAATACACCAACCG       | (AC) <sub>13</sub> | 185–256                | 20 | 55                           | KU598937              |
| Bropap_15123 | F: TTACGTTTCCCTCCACGTCC<br>R: CGCCATTACAAAGTAACTGCC       | (GA) <sub>15</sub> | 147–184                | 19 | 55                           | KU598938              |
| Bropap_16591 | F: CCTCCTCATATAACAGGCAG<br>R: TCGCGATCTGTAGCCTTCG         | (AG) <sub>17</sub> | 62–89                  | 23 | 56                           | KU598939              |
| Bropap_20558 | F: TCCACCATCAACGATGAAG<br>R: GGGCTAAACTACCTTGTCCG         | (TAT) <sub>7</sub> | 214–248                | 13 | 55                           | KU598940              |
| Bropap_20939 | F: GACACATGCTCACAATGTC<br>R: GGGGCGATGAAAGACTTGAG         | (AG) <sub>13</sub> | 119–178                | 31 | 56                           | KU598941              |
| Bropap_21091 | F: TGCTAGTTTGGTTTAAAGACAGG<br>R: CAAGGGACAACAGCAATGAAAG   | (AC) <sub>22</sub> | 146–191                | 30 | 55                           | KU598942              |
| Bropap_22348 | F: CCTCCAAGCCTTTTAGGC<br>R: CGTACGTTGAACAAAATGGACAC       | (TC) <sub>12</sub> | 110–116                | 5  | 54                           | KU598943              |
| Bropap_23758 | F: CGTCAATTTTGCTTGCCCTTC<br>R: AACAAACCACCAAGATGCC        | (AGA) <sub>7</sub> | 199–225                | 14 | 55                           | KU598944              |
| Bropap_23810 | F: AAAATGCCTCGCCATTGACC<br>R: CCCACCAACCTCGAAAACC         | (GA) <sub>12</sub> | 156–197                | 20 | 55                           | KU598945              |
| Bropap_25444 | F: TCACACTTACACACGGAGGG<br>R: GGTACGTAATTCACCACC          | (GA) <sub>14</sub> | 146–212                | 28 | 55                           | KU598946              |
| Bropap_26773 | F: CCTGCATCTGACACCAAGTC<br>R: ATTGCTCTCTGAGGGGTGG         | (TC) <sub>13</sub> | 169–264                | 29 | 56                           | KU598947              |
| Bropap_26985 | F: AGAATCACCACTCTCCCTTGG<br>R: TGGTTTGTTCATTCAAAAAGTG     | (TG) <sub>12</sub> | 178–191                | 8  | 56                           | KU598948              |
| Bropap_30248 | F: AGAGCAGGGCAAGCAATATC<br>R: GGTCAATCCATTTGTCTGAACCTC    | (GA) <sub>14</sub> | 83–195                 | 35 | 56                           | KU598949              |

Note: A = number of alleles;  $T_a$  = annealing temperature.

TABLE 2. Genetic properties of the 20 newly developed polymorphic microsatellites of *Broussonetia papyrifera*.<sup>a</sup>

| Locus        | Taiwan (n = 24) |                |                |       |                 |        | China-Guangdong (n = 26) |    |                |                |       |                 | China-Yunnan (n = 20) |                       |    |                |                |       |                 |        |                       |
|--------------|-----------------|----------------|----------------|-------|-----------------|--------|--------------------------|----|----------------|----------------|-------|-----------------|-----------------------|-----------------------|----|----------------|----------------|-------|-----------------|--------|-----------------------|
|              | A               | H <sub>o</sub> | H <sub>e</sub> | PIC   | F <sub>IS</sub> | r      | HWE <sup>b</sup>         | A  | H <sub>o</sub> | H <sub>e</sub> | PIC   | F <sub>IS</sub> | r                     | HWE <sup>b</sup>      | A  | H <sub>o</sub> | H <sub>e</sub> | PIC   | F <sub>IS</sub> | r      | HWE <sup>b</sup>      |
| Bropap_01931 | 5               | 0.083          | 0.684          | 0.618 | 0.880           | 0.396  | 82.206***                | 13 | 0.731          | 0.778          | 0.736 | 0.061           | 0.016                 | 95.908 <sup>ns</sup>  | 9  | 0.579          | 0.811          | 0.762 | 0.292           | 0.137  | 44.281 <sup>ns</sup>  |
| Bropap_02075 | 2               | 0.083          | 0.284          | 0.239 | 0.711           | 0.243  | 11.760***                | 9  | 0.231          | 0.587          | 0.541 | 0.612           | 0.288                 | 147.262***            | 5  | 0.158          | 0.602          | 0.532 | 0.743           | 0.327  | 39.474***             |
| Bropap_02214 | 17              | 0.792          | 0.931          | 0.905 | 0.152           | 0.064  | 152.385 <sup>ns</sup>    | 10 | 0.769          | 0.830          | 0.795 | 0.075           | 0.031                 | 41.515 <sup>ns</sup>  | 12 | 0.684          | 0.834          | 0.792 | 0.183           | 0.083  | 54.562 <sup>ns</sup>  |
| Bropap_02359 | 2               | 0.125          | 0.191          | 0.169 | 0.349           | 0.117  | 2.617 <sup>ns</sup>      | 3  | 0.192          | 0.242          | 0.217 | 0.209           | 0.075                 | 1.669 <sup>ns</sup>   | 3  | 0.263          | 0.317          | 0.275 | 0.174           | 0.061  | 0.971 <sup>ns</sup>   |
| Bropap_02560 | 5               | 0.208          | 0.660          | 0.608 | 0.689           | 0.317  | 46.039***                | 13 | 0.692          | 0.823          | 0.792 | 0.161           | 0.088                 | 113.967***            | 10 | 0.526          | 0.839          | 0.796 | 0.379           | 0.182  | 61.204 <sup>ns</sup>  |
| Bropap_02801 | 8               | 0.542          | 0.677          | 0.636 | 0.204           | 0.101  | 42.756**                 | 9  | 0.692          | 0.680          | 0.610 | -0.018          | -0.037                | 37.294 <sup>ns</sup>  | 11 | 0.684          | 0.853          | 0.811 | 0.203           | 0.083  | 99.592***             |
| Bropap_03147 | 4               | 0.458          | 0.646          | 0.577 | 0.295           | 0.144  | 35.458***                | 5  | 0.423          | 0.613          | 0.545 | 0.314           | 0.140                 | 14.972 <sup>ns</sup>  | 6  | 0.474          | 0.728          | 0.676 | 0.356           | 0.164  | 21.984 <sup>ns</sup>  |
| Bropap_07697 | 10              | 0.417          | 0.760          | 0.708 | 0.457           | 0.226  | 101.345***               | 14 | 0.346          | 0.911          | 0.885 | 0.625           | 0.296                 | 219.180***            | 11 | 0.474          | 0.856          | 0.817 | 0.454           | 0.215  | 105.556***            |
| Bropap_15123 | 7               | 0.500          | 0.791          | 0.742 | 0.373           | 0.168  | 32.367 <sup>ns</sup>     | 12 | 0.538          | 0.766          | 0.735 | 0.301           | 0.134                 | 107.792***            | 8  | 0.263          | 0.720          | 0.675 | 0.641           | 0.295  | 65.363***             |
| Bropap_16591 | 11              | 0.667          | 0.855          | 0.817 | 0.224           | 0.106  | 54.653 <sup>ns</sup>     | 16 | 0.462          | 0.928          | 0.903 | 0.507           | 0.243                 | 201.769***            | 12 | 0.316          | 0.890          | 0.853 | 0.652           | 0.315  | 148.766***            |
| Bropap_20558 | 5               | 0.458          | 0.682          | 0.607 | 0.332           | 0.150  | 20.954*                  | 8  | 0.577          | 0.676          | 0.634 | 0.149           | 0.076                 | 58.285***             | 9  | 0.684          | 0.752          | 0.698 | 0.093           | 0.041  | 30.828 <sup>ns</sup>  |
| Bropap_20939 | 9               | 0.333          | 0.560          | 0.520 | 0.410           | 0.199  | 41.092 <sup>ns</sup>     | 23 | 0.769          | 0.920          | 0.895 | 0.167           | 0.074                 | 328.229**             | 12 | 0.842          | 0.859          | 0.819 | 0.020           | -0.004 | 73.210 <sup>ns</sup>  |
| Bropap_21091 | 17              | 0.833          | 0.922          | 0.895 | 0.098           | 0.038  | 155.884 <sup>ns</sup>    | 19 | 0.692          | 0.891          | 0.863 | 0.226           | 0.111                 | 215.801*              | 14 | 0.789          | 0.913          | 0.880 | 0.139           | 0.052  | 89.625 <sup>ns</sup>  |
| Bropap_22348 | 4               | 0.125          | 0.487          | 0.428 | 0.747           | 0.304  | 28.744***                | 4  | 0.038          | 0.360          | 0.321 | 0.895           | 0.320                 | 52.015***             | 2  | 0.211          | 0.273          | 0.231 | 0.234           | 0.088  | 0.825 <sup>ns</sup>   |
| Bropap_23758 | 5               | 0.667          | 0.652          | 0.571 | -0.024          | -0.027 | 18.217 <sup>ns</sup>     | 9  | 0.654          | 0.732          | 0.693 | 0.109           | 0.050                 | 36.555 <sup>ns</sup>  | 8  | 0.421          | 0.458          | 0.434 | 0.083           | 0.056  | 37.515 <sup>ns</sup>  |
| Bropap_23810 | 7               | 0.625          | 0.647          | 0.601 | 0.035           | 0.011  | 32.872*                  | 13 | 0.731          | 0.798          | 0.763 | 0.086           | 0.023                 | 140.218***            | 10 | 0.579          | 0.829          | 0.784 | 0.308           | 0.149  | 52.140 <sup>ns</sup>  |
| Bropap_25444 | 14              | 0.542          | 0.691          | 0.658 | 0.219           | 0.126  | 124.820*                 | 15 | 0.423          | 0.900          | 0.873 | 0.535           | 0.260                 | 176.389***            | 12 | 0.579          | 0.879          | 0.842 | 0.348           | 0.167  | 100.278**             |
| Bropap_26773 | 9               | 0.708          | 0.843          | 0.805 | 0.163           | 0.069  | 49.938 <sup>ns</sup>     | 19 | 0.846          | 0.934          | 0.911 | 0.096           | 0.040                 | 180.859 <sup>ns</sup> | 19 | 0.737          | 0.935          | 0.904 | 0.216           | 0.099  | 179.181 <sup>ns</sup> |
| Bropap_26985 | 5               | 0.667          | 0.715          | 0.651 | 0.068           | 0.016  | 19.153*                  | 6  | 0.269          | 0.609          | 0.539 | 0.563           | 0.255                 | 43.514***             | 4  | 0.263          | 0.587          | 0.496 | 0.559           | 0.242  | 27.144***             |
| Bropap_30248 | 14              | 0.458          | 0.881          | 0.852 | 0.485           | 0.237  | 165.102***               | 16 | 0.308          | 0.902          | 0.876 | 0.663           | 0.319                 | 281.041***            | 15 | 0.526          | 0.927          | 0.895 | 0.439           | 0.206  | 219.606***            |

Note: A = number of alleles; F<sub>IS</sub> = coefficient of inbreeding; H<sub>e</sub> = expected heterozygosity; H<sub>o</sub> = observed heterozygosity; HWE = Hardy-Weinberg equilibrium; PIC = polymorphism information content; r = null allele frequency.

<sup>a</sup> Voucher and locality information are provided in Appendix 2.

<sup>b</sup> Deviations from HWE using  $\chi^2$  tests: \*P ≤ 0.05, \*\*P ≤ 0.01, \*\*\*P ≤ 0.001; ns = not significant.

TABLE 3. Transferability of the 20 microsatellite markers developed in *Broussonetia papyrifera* across three Moraceae and one Rosaceae species.

| Locus       | <i>B. papyrifera</i> | <i>Morus</i><br>sp. <sup>a</sup> | <i>Ficus</i><br><i>elastica</i> <sup>a</sup> | <i>Ficus</i><br><i>carica</i> <sup>a</sup> | <i>Prunus</i><br><i>dulcis</i> <sup>a</sup> |
|-------------|----------------------|----------------------------------|--|--|---|
| Bropap01931 | +                    | —                                | —  | +  | *   |
| Bropap02075 | +                    | +                                | *  | +  | *   |
| Bropap02214 | +                    | —                                | *  | +  | +   |
| Bropap02359 | +                    | —                                | *  | *  | *   |
| Bropap02560 | +                    | —                                | *  | +  | *   |
| Bropap02801 | +                    | —                                | —  | —  | —   |
| Bropap03147 | +                    | —                                | *  | —  | *   |
| Bropap07697 | +                    | —                                | —  | —  | —   |
| Bropap15123 | +                    | —                                | *  | +  | —   |
| Bropap16591 | +                    | —                                | *  | *  | +   |
| Bropap20558 | +                    | —                                | —  | *  | —   |
| Bropap20939 | +                    | +                                | *  | *  | +   |
| Bropap21091 | +                    | —                                | —  | *  | —   |
| Bropap22348 | +                    | —                                | —  | —  | —   |
| Bropap23758 | +                    | —                                | —  | —  | —   |
| Bropap23810 | +                    | —                                | +  | +  | +   |
| Bropap25444 | +                    | —                                | —  | —  | —   |
| Bropap26773 | +                    | —                                | —  | —  | —   |
| Bropap26985 | +                    | —                                | —  | —  | —   |
| Bropap30248 | +                    | —                                | —  | —  | —   |

Note: + = successful amplification; — = failed amplification; \* = amplification in fewer than four of five samples.

<sup>a</sup>Transferability was tested on five independent specimens from Santiago, Chile.

0.935 with averages of 0.495 and 0.722, respectively. PIC values ranged from 0.169 to 0.911 with averages of 0.678 and  $F_{IS}$  values ranged from -0.024 to 0.895 with an average of 0.329. Null allele frequency values ranged from -0.037 to 0.396 (Table 2). No significant deviation of HWE in terms of heterozygosity deficiency was detected for four markers (Bropap02214, Bropap02359, Bropap23758, Bropap26773) in the three populations (Table 2). Across the three populations (in Guangdong and Yunnan in southern China and Taiwan), 384 alleles were scored. Samples were from nonadjunct individuals because *B. papyrifera* is a widely distributed and common species in East Asia.

The reported genetic diversity for *B. papyrifera* represents only part of the diversity found in Asia. The transferability of these markers was tested in three additional Moraceae and one Rosaceae species (Table 3, Appendix 2). Some of the developed markers exhibited limited interspecific transferability. Six markers showed transferability to *Ficus carica* L. Eight markers exhibited no transferability to any of the tested species (Table 3).

## CONCLUSIONS

We identified and characterized 20 highly polymorphic and informative microsatellite markers for *B. papyrifera*, presenting an average of 19.2 alleles per marker. Some of the markers show limited transferability to other Moraceae and one Rosaceae species. The described genetic diversity represents a subset of the genetic diversity in the native range. These microsatellite markers may be able to serve as useful tools to analyze genetic diversity, population genetic structure, and gene flow of *B. papyrifera* in its native range, to trace its worldwide dispersal history, and to help in germplasm conservation.

## LITERATURE CITED

- CHANG, C.-S., H.-L. LIU, X. MONCADA, A. SEELENFREUND, D. SEELENFREUND, AND K.-F. CHUNG. 2015. A holistic picture of Austronesian migrations revealed by phylogeography of Pacific paper mulberry. *Proceedings of the National Academy of Sciences, USA* 112: 13537–13542.
- EXCOFFIER, L., AND H. E. L. LISCHER. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564–567.
- GONZÁLEZ-LORCA, J., A. RIVERA-HUTINEL, X. MONCADA, S. LOBOS, D. SEELENFREUND, AND A. SEELENFREUND. 2015. Ancient and modern introduction of *Broussonetia papyrifera* ([L.] Vent.; Moraceae) into the Pacific: Genetic, geographical and historical evidence. *New Zealand Journal of Botany* 53: 75–89.
- HO, K., AND J. CHANG. 2006. Relationship between population genetic structure and riparian habitat of *Broussonetia papyrifera* Vent (Moraceae) on the western Taiwan. *Bioresources and Agriculture of National Taiwan University Experimental Forest* 20: 165–174.
- KALINOWSKI, S. T., M. L. TAPER, AND T. C. MARSHALL. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* 16: 1099–1106.
- LIAO, S. X., Z. H. DENG, K. CUI, Y. Z. CUI, AND C. H. ZHANG. 2014. Genetic diversity of *Broussonetia papyrifera* populations in southwest China. *Genetics and Molecular Research* 13: 7553–7563.
- LIU, Z. Y., W. H. FAN, AND S. H. SHEN. 2009. SRAP marker in *Broussonetia papyrifera*. *Scientia Silvae Sinicae* 45: 54–58.
- LODHI, M. A., G. N. YE, N. F. WEEDEN, AND B. I. REISCH. 1994. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant Molecular Biology Reporter* 12: 6–13.
- MATTHEWS, P. J. 1996. Ethnobotany and the origins of *Broussonetia papyrifera* in Polynesia: An essay on tapa prehistory. In J. Davidson, G. Irwin, F. Leach, A. Pawley, and D. Brown [eds.], *Oceanic culture history: Essays in honour of Roger Green*, 117–132. New Zealand Journal of Archaeology Special Publication. New Zealand Archeological Society, Dunedin, New Zealand.
- MONCADA, X., C. PAYACÁN, F. ARRIAZA, S. LOBOS, D. SEELENFREUND, AND A. SEELENFREUND. 2013. DNA extraction and amplification from contemporary Polynesian bark-cloth. *PLoS ONE* 8: e56549.
- PEAKALL, R., AND P. E. SMOUSE. 2006. GenA1Ex 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Resources* 6: 288–295.
- PEAKALL, R., AND P. E. SMOUSE. 2012. GenA1Ex 6.5: Genetic analysis in Excel. Population genetic software for teaching and research: An update. *Bioinformatics* 28: 2537–2539.
- PEÑAILILLO, J., G. OLIVARES, X. MONCADA, C. PAYACÁN, C.-S. CHANG, K.-F. CHUNG, P. J. MATTHEWS, ET AL. 2016. Sex distribution of paper mulberry (*Broussonetia papyrifera*) in the Pacific. *PLoS ONE* 11: e0161148.
- SCHUELKE, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* 18: 233–234.
- SEELENFREUND, D., R. PIÑA, K.-Y. HO, S. LOBOS, X. MONCADA, AND A. SEELENFREUND. 2011. Molecular analysis of *Broussonetia papyrifera* (L.) Vent. (Magnoliophyta: Urticales) from the Pacific, based on ribosomal sequences of nuclear DNA. *New Zealand Journal of Botany* 49: 413–420.
- VAN OOSTERHOUT, C., W. F. HUTCHINSON, D. P. WILLS, AND P. SHIPLEY. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535–538.
- WHISTLER, W. A. 2009. Plants of the Canoe People: An ethnobotanical voyage through Polynesia. National Tropical Botanical Garden, Hawaii, Kaua'i, Hawai'i.

APPENDIX 1. Locality and voucher information for the 10 samples of *Broussonetia papyrifera* used for microsatellite development.

| Species              | Location                                      | Range      | Voucher no. <sup>a</sup> | Collector <sup>b</sup> | Latitude <sup>c</sup> | Longitude <sup>c</sup> |
|----------------------|---|------------|--------------------------|------------------------|-----------------------|------------------------|
| <i>B. papyrifera</i> | Fatu Hiva, Marquesas Island, French Polynesia | Introduced | BQUCH0045                | AS                     | -10.512452            | -138.683993            |
| <i>B. papyrifera</i> | Waimea, Big Island, Hawaii, USA               | Introduced | BQUCH0064                | AS                     | 20.028473             | -155.660508            |
| <i>B. papyrifera</i> | Kanokupolu, Tongatapu, Tonga                  | Introduced | BQUCH0100                | AS                     | -21.075553            | -175.334735            |
| <i>B. papyrifera</i> | Votua, Viti Levu, Fiji                        | Introduced | BQUCH0115                | AS                     | -18.208704            | 177.709575             |
| <i>B. papyrifera</i> | Santiago, Chile                               | Introduced | BQUCH0134                | AS                     | -33.469052            | -170.519744            |
| <i>B. papyrifera</i> | Taichung, Taiwan                              | Native     | BQUCH0138                | KFC                    | 24.077374             | 120.664456             |
| <i>B. papyrifera</i> | Wulai District, Taiwan                        | Native     | BQUCH0140                | KFC                    | 24.853237             | 121.548910             |
| <i>B. papyrifera</i> | Kyoto, Honshu, Japan                          | Introduced | BQUCH0141                | PM                     | 35.025817             | 135.781519             |
| <i>B. papyrifera</i> | Kyoto, Botanical Garden, Honshu, Japan        | Introduced | BQUCH0144                | PM                     | 35.050188             | 135.763272             |
| <i>B. papyrifera</i> | Roiho, Easter Island, Chile                   | Introduced | BQUCH0152                | AS                     | -27.113048            | -109.404041            |

<sup>a</sup>Specimens are deposited at the University of Chile, Santiago, Chile; specimen BQUCH0152 is also deposited (voucher SGO162505) at the herbarium of the National Museum of Natural History, Santiago, Chile.

<sup>b</sup>AS = Andrea Seelenfreund; KFC = Kuo-Fang Chung; PM = Peter Matthews.

<sup>c</sup>Latitude and longitude are provided in decimal degrees.

APPENDIX 2. Locality and voucher information for samples used in this study.

| Species   | Location <sup>a</sup>     | Voucher no. <sup>b</sup> | <i>N</i> | Collectors <sup>c</sup> | Latitude <sup>d</sup> | Longitude <sup>d</sup> |
|---|---------------------------|--------------------------|----------|-------------------------|-----------------------|------------------------|
| <i>Broussonetia papyrifera</i> (L.) L'Hér. ex Vent. | Taiwan (T1–T24)           | HAST Chung 7-1           | 24       | KFC, HLL, KYH           | 24.8506600            | 121.5698400            |
| <i>B. papyrifera</i>                                | Guangdong, China (G1–G26) | HAST Chung 1742          | 26       | KFC                     | 24.4823900            | 113.7839700            |
| <i>B. papyrifera</i>                                | Yunnan, China (Y1–Y20)    | HAST Chung 016           | 20       | KFC, CSA                | 22.6588300            | 99.603500              |
| <i>Ficus carica</i> L.                              | Santiago, Chile           | BQUCHZ0010               | 5        | DS, BPA                 | -33.4229130           | -70.6529900            |
| <i>F. elastica</i> Roxb. ex Hornem.                 | Santiago, Chile           | BQUCHZ0009               | 5        | DS, BPA                 | -33.4225410           | -70.6539970            |
| <i>Morus</i> L. sp.                                 | Santiago, Chile           | BQUCHZ0011               | 5        | DS                      | -33.4132500           | -70.5568700            |
| <i>Prunus dulcis</i> (Mill.) D. A. Webb             | Santiago, Chile           | BQUCHZ0012               | 5        | DS                      | -33.4132700           | -70.5571000            |

Note: *N* = number of individuals sampled.

<sup>a</sup>Number of accessions from Taiwan, Guangdong, and Yunnan are indicated in parentheses.

<sup>b</sup>Voucher specimens identified as HAST are deposited at the Herbarium at the National Taiwan University, Taipei, Taiwan; voucher specimens identified as BQUCHZ are deposited at the University of Chile, Santiago, Chile.

<sup>c</sup>KFC = Kuo-Fang Chung; HLL = Hsiao-Lei Liu; KYH = Kuen-Yi Ho; CSA = Chi-Shan Chang; AS = Andrea Seelenfreund; PM = Peter Matthews; DS = Daniela Seelenfreund; BPA = Barbara Peña-Ahumada.

<sup>d</sup>Latitude and longitude are provided in decimal degrees. Coordinates for *B. papyrifera* samples are representative of the approximate range of the sampled populations.