Mistletoes are considered the most damaging pathogens to attack commercially important coniferous and hardwood timber stands (Mathiasen et al., 2008). Despite their negative economic impact, mistletoes are ecologically important in forest ecosystems as they provide food, cover, and nesting sites for a variety of birds, mammals, and insects (Watson, 2001). The geographic range of mistletoes is related to the availability of suitable host trees, and the genetic structuring of mistletoe populations is potentially influenced by the distribution of host populations (Norton and Carpenter, 1998). The genus *Psittacanthus* Mart. (c. 119 species; Loranthaceae), an aerial hemiparasite distributed throughout the Neotropics on a wide range of tree hosts, is distinguished by its large and conspicuous red, yellow, or orange flowers, bulky haustorial connections to the host trees, and large fruits with seeds that lack endosperm (Kuijt, 2009). *Psittacanthus schiedeanus* (Cham. & Schlecht.) G. Don is characteristic of the canopy in the cloud forest edges in Mesoamerica and often parasitizes tall trees (López de Buen and Ornelas, 2002). The hermaphroditic, hummingbird-pollinated flowers are self-compatible (Ramírez and Ornelas, 2010), and ripe, lipid-rich, purplish-black fleshy fruits are dispersed by a variety of resident and migratory bird species (López de Buen and Ornelas, 1999, 2001; Ramírez and Ornelas, 2009). The foraging and flocking behavior and local abundance of birds differ widely (López de Buen and Ornelas, 2001), and consequently affect the spatial patterns of mistletoe seed deposition (López de Buen and Ornelas, 1999; López de Buen et al., 2002). At a local scale, mistletoes can develop specificity on particular host trees depending on the heterogeneity of host patches, which may lead to gene flow changes and the eventual formation of mistletoe races (Overton, 1997; Norton and Carpenter, 1998). Cross-infection experiments, which have proven useful to demonstrate host specificity in other mistletoes (Overton, 1997; Lara et al., 2009), have shown local host adaptation of *P. schiedeanus* on *Liquidambar styraciflua* L. (Ramírez and Ornelas, 2012). However, the parasite-host interaction is predominantly on other host species in areas where *L. styraciflua* is not distributed. Thus, geologic- and climate-driven processes implicated in the fragmentation of the Mesoamerican cloud forests and the distribution of potential host species across a geographic range could have influenced the distribution of genetic variation among populations of *P. schiedeanus*.

Our aim is to determine to what extent the historically fragmented distribution of cloud forest in Mesoamerica and the distributions of host species have affected the spatial genetic variability of *P. schiedeanus* and interactions with its hosts, pollinators, and seed dispersers. For these purposes, we isolated and characterized 10 polymorphic nuclear microsatellite loci that are being successfully applied to describe spatial patterns of genetic structure. To date, microsatellite primers have not been developed for this mistletoe species.
METHODS AND RESULTS

Microsatellite isolation was performed by the simple sequence repeat (SSR) development company Genetic Marker Services (Brighton, United Kingdom; http://www.geneticmarkerservices.com). We extracted genomic DNA from a single *P. schiedeanus* (PSI) individual collected in Jardín Botánico Francisco Xavier Clavijero, near the city of Xalapa, Veracruz, Mexico (Appendix 1), with the DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA) to develop an enriched library, and to design and test primer pairs for microsatellite-containing loci. Enrichment involved incubating adapter-ligated restricted DNA with filter-bonded synthetic repeat motifs: (AG)_{17}, (AC)_{17}, (AAC)_{10}, (CCG)_{10}, (CTG)_{10}, and (AAT)_{10}. We detected and sequenced 29 microsatellite-positive *Escherichia coli* clones, of which 27 contained repeat motifs, and 19 of these loci had sufficient flanking regions to design F/R primer pairs using the primer design software Primer3 (Rozen and Skaletsky, 2000). All repeat motifs were perfect dinucleotides.

### TABLE 1. Characteristics of 17 microsatellite loci developed in *Psittacanthus schiedeanus*.

| Locus | Primer sequences (5′–3′) | Repeat motif | Allele size range (bp) | Fluorescent dye | GenBank accession no. |
|-------|--------------------------|--------------|------------------------|----------------|----------------------|
| Psi1  | F: GGTGAAATGTGGAATAATTGGA<br>R: GCACATTGTGCTCTGCTTG | (AG)_{18} | 95–131 | 6FAM | KP027826 |
| Psi29 | F: CCAGATTGAGATAGATCCAG<br>R: TCAATTGCTCTTCTTAACCA | (AG)_{14} | 139–145 | VIC | KP027827 |
| Psi6  | F: CATCTGTTTGGAGAAC<br>R: CTTCTCTCTCTCTCACTCA | (AG)_{12} | 145–199 | NED | KP027828 |
| Psi8  | F: TGCACTTTCTCTCTGATT<br>R: TCTTTATCATACGCCTTCA | (GA)_{13} | 209–247 | PET | KP027829 |
| Psi15 | F: AGAAAGAGAGACCTCAACC<br>R: TTTATCAAAAGAGGGCTTATAATG | (AG)_{14} | 78–102 | PET | KP027830 |
| Psi16 | F: TGAAGGTTTGGAGAGAAGA<br>R: ACACATATACAGATTTGC | (AG)_{21} | 88–130 | 6FAM | KP027831 |
| Psi17 | F: ACCAGAACACACCGCTC<br>R: CACAGGGACCAACAGATCC | (AG)_{12} | 196–208 | NED | KP027832 |
| Psi19 | F: GTGTGTGTGTGTGTAAGA<br>R: CCGGAAACCTTTATCACTT | (GA)_{17} | 145–179 | PET | KP027833 |
| *Psi7 | F: TGGGGTTTTGATGTTAATGAAA<br>R: GAGAAGGGATCAGTTTCA | (GA)_{12} | 192 | — | KP027834 |
| *Psi18 | F: GGGGTCATTTGTTTTGAGAG<br>R: TAGAGGGGCTCAGAATTC | (CT)_{9} | 163 | — | KP027835 |
| *Psi21 | F: GCTCAACAGCTGCTTTAC<br>R: TGGCAAAATTTGTAGCATAG | (AC)_{9} | 107 | — | KP027836 |
| *Psi22 | F: TCGGCAAGGAAGGAGTGC<br>R: CTCAGCCACCTCCTCTCTT | (AC)_{9} | 122 | — | KP027837 |
| *Psi24 | F: CTGCACTTGGGGATGTTT<br>R: TAGAGGGAAGGAGGGTCA<br>R: AAGAGACCACCCAGGAC<br>R: CTGCTCTCTCTGAC<br>R: ACCAGGGAGGAGGAGGAGGAGGAG<br>R: TGGCACTATCGACCTGAC<br>R: CACAGGGACCAACAGATCC | (AG)_{10} | 192 | — | KP027838 |
| *Psi27 | F: ACCAGACTTCCAAACCAAG<br>R: CTCGTATCTGCTCACCTCA<br>R: TCTGGATGTTCCTTAAAATT<br>R: ACCAGTTTCTCCAAAACCAAG<br>R: CACAGGGACCAACAGATCC | (GA)_{12} | 122 | — | KP027839 |

*Loci untested for polymorphism, probably monomorphic.

### TABLE 2. Genetic properties of the 10 newly developed polymorphic microsatellites of *Psittacanthus schiedeanus*.

| Locus | Jitotol (n = 5) | Motozintla (n = 7) | Rancho Viejo (n = 19) | Xilitla (n = 8) |
|-------|----------------|------------------|----------------------|----------------|
| Psi1  | A | H_o | H_e | HWE | A | H_o | H_e | HWE | A | H_o | H_e | HWE | A | H_o | H_e | HWE |
| Psi29 | 4 | 0.600 | 0.822 | 0.1959 | 2 | 0.000 | 0.263 | 0.0771 | 11 | 0.631 | 0.829 | 0.1824 | 7 | 1.000 | 0.875 | 0.6202 |
| Psi16 | 3 | 0.000 | 0.666 | 0.0043* | 3 | 0.000 | 0.666 | 0.0043* | 10 | 0.555 | 0.792 | 0.0021* | 8 | 1.000 | 0.857 | 0.1551 |
| Psi8  | 4 | 0.750 | 0.821 | 0.3172 | 3 | 0.200 | 0.511 | 0.1105 | 8 | 0.705 | 0.798 | 0.5665 | 3 | 0.375 | 0.675 | 0.1994 |
| Psi25 | 4 | 0.600 | 0.777 | 0.6951 | 6 | 0.571 | 0.868 | 0.0012* | 6 | 0.473 | 0.605 | 0.0359 | 5 | 0.500 | 0.725 | 0.1767 |
| Psi15 | 6 | 0.200 | 0.911 | 0.0009* | 4 | 0.333 | 0.651 | 0.0699 | 4 | 0.473 | 0.613 | 0.3026 | 3 | 0.375 | 0.425 | 0.3854 |
| Psi6  | 4 | 0.600 | 0.911 | 0.0009* | 4 | 0.428 | 0.648 | 0.2545 | 10 | 0.842 | 0.832 | 0.0093 | 5 | 0.500 | 0.533 | 0.5869 |
| Psi16 | 3 | 0.600 | 0.511 | 1.000 | 2 | 0.428 | 0.362 | 1.000 | 5 | 0.052 | 0.482 | 0.0002* | 3 | 0.500 | 0.425 | 1.000 |
| Psi17 | — | — | — | — | 1 | — | — | — | 1 | — | — | — | 1 | — | — | — |
| Psi19 | 4 | 0.000 | 0.800 | 0.0034* | 9 | 0.571 | 0.912 | 0.0058 | 12 | 0.500 | 0.892 | 0.0008* | 6 | 0.625 | 0.816 | 0.0678 |

Note: A = number of alleles sampled; H_o = expected heterozygosity; H_e = observed heterozygosity; HWE = P values of the exact test of Hardy–Weinberg equilibrium; n = number of individuals sampled.

*All four populations are located in Mexico. See Appendix 1 for geographic coordinates and voucher information.

* Locus showed significant deviations from Hardy–Weinberg equilibrium after Bonferroni correction (P < 0.005).
CONCLUSIONS

The 10 microsatellites described here are the first to be developed for *P. schiedeanus* and the genus *Psittacanthus*. These polymorphic loci will be useful in studies of genetic diversity and genetic population differentiation and will provide valuable information to understand the importance of host distribution and abiotic factors involved in geographic variation and structure of this widespread mistletoe in Mesoamerica. Cross-species amplifications were successful in closely related *P. calyculatus* and *P. angustifolius*, but unsuccessful in most of the studied *Psittacanthus* species, likely due to their high genetic divergence.

LITERATURE CITED

EXCOFFIER, L., G. LAVAL, AND S. SCHNEIDER. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47–50.

KUIJT, J. 2009. Monograph of *Psittacanthus* (Loranthaceae). *Systematic Botany Monographs*, vol. 86. American Society of Plant Taxonomists, Laramie, Wyoming.

LARA, C., G. PEREZ, AND J. F. ORNELAS. 2009. Provenance, guts, and fate: Field and experimental evidence in a host-mistletoe-bird system. *Science* 16: 399–407.

LÓPEZ DE BUEN, L., AND J. F. ORNELAS. 1999. Frugivorous birds, host selection and the mistletoe *Psittacanthus schiedeanus*, in central Veracruz, Mexico. *Journal of Tropical Ecology* 15: 329–340.

LÓPEZ DE BUEN, L., AND J. F. ORNELAS. 2001. Seed dispersal of the mistletoe *Psittacanthus schiedeanus* by birds in central Veracruz, Mexico. *Biotropica* 33: 487–494.

LÓPEZ DE BUEN, L., AND J. F. ORNELAS. 2002. Host compatibility of the cloud forest mistletoe *Psittacanthus schiedeanus* (Loranthaceae) in central Veracruz, Mexico. *American Journal of Botany* 89: 95–102.

LÓPEZ DE BUEN, L., J. F. ORNELAS, AND J. G. GARCÍA-PRENO. 2002. Mistletoe infection of trees located at fragmented forest edges in the cloud forests of central Veracruz, Mexico. *Forest Ecology and Management* 164: 293–302.

MATHIASSEN, R. L., D. L. NICKRENT, D. C. SHAW, AND D. M. WATSON. 2008. Mistletoes: Pathology, systematics, ecology, and management. *Plant Disease* 92: 988–1006.

NORTON, D. A., AND M. A. CARPENTER. 1998. Mistletoes as parasites: Host specificity and speciation. *Trends in Ecology and Evolution* 13: 101–105.

OVERTON, J. M. 1997. Host specialization and partial reproductive isolation in desert mistletoe (*Phoradendron californicum*). *Southwestern Naturalist* 42: 201–209.

RAMÍREZ, M. M., AND J. F. ORNELAS. 2009. Germination of *Psittacanthus schiedeanus* (mistletoe) seeds after passage through the gut of cedar waxwings and grey silky-flycatchers. *Journal of the Torrey Botanical Society* 136: 322–331.

Table 3. Cross-species amplifications of microsatellite primers developed for *Psittacanthus schiedeanus*.

| Species                     | Psi1 | Psi29 | Psi6 | Psi8 | Psi5 | Psi15 | Psi2 | Psi16 | Psi17 | Psi19 |
|-----------------------------|------|-------|------|------|------|-------|------|-------|-------|-------|
| *Psittacanthus robustus*    | –    | –     | –    | –    | –    | –     | –    | –     | –     | –     |
| *Psittacanthus acinarius*   | –    | –     | –    | –    | –    | –     | –    | –     | –     | –     |
| *Psittacanthus cordatus*    | –    | –     | –    | –    | –    | –     | –    | –     | –     | –     |
| *Psittacanthus sonorensis*  | –    | –     | –    | –    | –    | –     | –    | –     | –     | –     |
| *Psittacanthus ramiflorus*  | –    | –     | –    | –    | –    | +     | –    | –     | +     | –     |
| *Psittacanthus mayanus*     | +    | –     | –    | –    | –    | –     | –    | –     | –     | +     |
| *Psittacanthus macranthus*  | +    | +     | +    | +    | +    | +     | +    | +     | +     | +     |

Note: + = successful amplification; ~ = amplification of multiple bands; – = failed amplification.
APPENDIX 1. Voucher, number of individuals sampled, and location information for *Psittacanthus* species in this study.

| Species            | Locality                     | Latitude  | Longitude  | n   | Voucher no.  | Herbarium ID* |
|--------------------|------------------------------|-----------|------------|-----|--------------|---------------|
| P. acinarus        | Brazil, Mato Grosso, Cuiaba  | -15°35'56" | -56°05'42" | 3   | G. Ceccantini 3676 | (USP)        |
| P. angustifolius   | Mexico, Chiapas, Comitán     | 16°13'46"  | -92°08'01" | 2   | A. Ortiz-Rodríguez s.n. | (XAL)  |
| P. angustifolius   | Mexico, Oaxaca, Puerto Escondido | 15°43'32" | -96°39'48" | 1   | E. Ruiz-Sánchez 448 | (XAL)  |
| P. auriculatus     | Mexico, Oaxaca, El Molino    | 17°46'14"  | -97°44'58" | 3   | A. Ortiz-Rodríguez s.n. | (XAL)  |
| P. calycatus       | Mexico, Michoacán, Maravatio | 19°54'00"  | -100°27'00" | 1  | E. Ruiz-Sánchez 414 | (XAL)  |
| P. calycatus       | Mexico, Michoacán, Morelia   | 19°60'05"  | -101°23'00" | 1  | A. González s.n. | (XAL)  |
| P. calycatus       | Mexico, Tlaxcala, Tlaxcala   | 19°17'00"  | -98°14'00" | 1   | C. Lara s.n. | (XAL)  |
| P. coryanus        | Brazil, Mato Grosso, Cuiabá  | -15°35'56" | -56°05'42" | 3   | G. Ceccantini 3671 | (USP)  |
| P. macrantherus    | Mexico, Sinaloa, El Palmito  | 23°33'00"  | -105°50'00" | 1  | E. Ruiz-Sánchez 348 | (XAL)  |
| P. mayanus         | Mexico, Yucatán, Unucmá      | 21°02'58"  | -89°54'38" | 1   | Nonvouched    |                |
| P. mayanus         | Mexico, Yucatán, Cuxtal      | 20°54'37"  | -89°37'15" | 1   | Nonvouched    |                |
| P. mayanus         | Mexico, Chiapas, Ocozocuaua  | 16°47'47"  | -93°24'30" | 1   | A. Ortiz-Rodríguez s.n. | (XAL)  |
| P. ramiflorus      | Mexico, Chiapas, Berriozaab  | 16°50'21"  | -93°18'11" | 3   | A. Ortiz-Rodríguez s.n. | (XAL)  |
| P. rynchanthus     | Guatemala, Patutul            | 14°22'24"  | -91°08'18" | 3   | J. J. Vega s.n. | (UVAL) |
| P. robustus        | Brazil, Minas Gerais, Serra do Cipó | -19°18'26" | -43°52'33" | 3   | G. Ceccantini 3589 | (USP)  |
| P. schiedeanus     | Mexico, San Luis Potosí, Xilitla | 21°22'39"  | -98°59'35" | 8   | E. Ruiz-Sánchez 281 | (XAL)  |
| P. schiedeanus     | Mexico, Veracruz, Clavijero  | 19°30'47"  | -96°56'28" | 1   | M. T. Mejía 2036 | (XAL)  |
| P. schiedeanus     | Mexico, Veracruz, Rancho Viejo | 19°31'11"  | -96°58'22" | 19  | M. T. Mejía 362 | (XAL)  |
| P. schiedeanus     | Mexico, Veracruz, Coacoatzintla | 19°37'41"  | -96°52'56" | 1   | M. T. Mejía 2043 | (XAL)  |
| P. schiedeanus     | Mexico, Veracruz, Tlalnelhuayocan | 19°34'47"  | -96°57'38" | 1   | M. T. Mejía 2041 | (XAL)  |
| P. schiedeanus     | Mexico, Veracruz, Actopán    | 19°23'13"  | -96°36'56" | 1   | M. T. Mejía 2049 | (XAL)  |
| P. schiedeanus     | Mexico, Veracruz, La Mancha  | 19°20'43"  | -96°36'05" | 1   | M. T. Mejía 2050 | (XAL)  |
| P. schiedeanus     | Mexico, Chiapas, Motorizinta | 15°21'21"  | -92°14'54" | 7   | E. Ruiz-Sánchez 261 | (XAL)  |
| P. schiedeanus     | Mexico, Chippas, Jitotol     | 17°01'47"  | -92°50'46" | 5   | E. Ruiz-Sánchez 263 | (XAL)  |
| P. sonorae         | Mexico, Sonora, Nacapule     | 27°59'04"  | -111°02'40" | 1  | Nonvouched    |                |
| P. sonorae         | Mexico, Sonora, Cruz de Piedra | 27°57'25"  | -110°40'51" | 1   | Nonvouched    |                |
| P. sonorae         | Mexico, Sonora, Paraiso La Manga | 27°53'43"  | -111°06'55" | 1   | Nonvouched    |                |

*IDs reported below refer to accession numbers in the Instituto de Ecología, A.C. (XAL), Universidad del Valle de Guatemala (UVAL), and the Universidad de São Paulo (USP) herbaria.

**References**

Ramírez, M. M., and J. F. Ornelas. 2010. Pollination and nectar production of *Psittacanthus schiedeanus* (Loranthaceae) in central Veracruz, Mexico. *Boletín de la Sociedad Botánica de México* 87: 71–77.

Ramírez, M. M., and J. F. Ornelas. 2012. Cross-infection experiments of *Psittacanthus schiedeanus*: Effects of host provenance, gut passage and host fate on mistletoe seedling survival. *Plant Disease* 96: 780–787.

Rozen, S., and H. Skalletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. In S. Misener and S. A. Krawetz [eds.], Methods in molecular biology, vol. 132: Bioinformatics methods and protocols, 365–386. Humana Press, Totowa, New Jersey, USA.

Watson, D. M. 2001. Mistletoe—A keystone resource in forests and woodlands worldwide. *Annual Review of Ecology and Systematics* 32: 219–249.

http://www.bioone.org/loi/apps