Phylogenetic position of Neotropical *Bursera*-specialist mistletoes: the evolution of deciduousness and succulent leaves in *Psittacanthus* (Loranthaceae)

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Author Contributions: Andrés Ernesto Ortiz Rodriguez and Juan Francisco Ornelas conceived the ideas, designed the study, analyzed data, conducted fieldwork, and led the writing of the paper. Eydi Yanina Guerrero conducted fieldwork in Honduras and reviewed drafts of the paper. All authors contributed critically to the drafts and gave final approval for publication.

Abstract

Background: The phylogenetic relationships of the *Bursera*-host specialists *Psittacanthus nudus*, *P. palmeri* and *P. sonorae* (Loranthaceae) remain uncertain. These mistletoe species exhibit morphological and phenological innovations probably related to their dry habitats, so that determining their phylogenetic position is key on the understanding of factors associated with the morphological evolution within *Psittacanthus*.

Questions: (1) Is the evolution of some morphological innovations in the *Bursera*-host specialists associated with the ecological conditions linked to host diversification? (2) Does time of diversification in both lineages coincide?

Study species: Fourteen species of *Psittacanthus*.

Methods: Sequences of nuclear (ITS) and plastid (*trnL-trnF*) markers are analyzed with Bayesian inference, maximum likelihood and maximum parsimony methods, and molecular dating under a Bayesian approach estimated to elucidate the phylogenetic position and divergence timing of the *Bursera*-host specialists.

Results: The *Bursera*-host specialists form a strongly supported clade, named here the '*Bursera* group'. The divergence time for the *Bursera*-host specialists was estimated at 7.89 Ma. Interestingly, phylogenetic relationships between *P. nudus* and *P. palmeri*, as currently circumscribed, were not fully resolved, making *P. palmeri* paraphyletic.

Conclusions: Based on these results, the plants collected by the type locality of *P. nudus* in Honduras should be named *P. palmeri*. The seasonal deciduousness of *P. palmeri* (including *P. nudus*) and morphology of *P. sonorae* (small size, fleshy leaves) are clearly adaptations to dry ecosystems where these species have lived for a long time. In parallel, the evolutionary history of these mistletoes seems to be correlated with the evolutionary history and diversification patterns of *Bursera*.

Keywords: *Bursera,* divergence time, Honduras, ITS, Loranthaceae, mistletoes, molecular clock, Neotropics, phylogeny, *Psittacanthus*, systematics, trnL-trnF

Resumen

Antecedentes: Las relaciones filogenéticas de los muérdagos especialistas de *Bursera*, *Psittacanthus nudus*, *P. palmeri* y *P. sonorae* (Loranthaceae), se mantienen inciertas. Estas especies en muérdago exhiben innovaciones fenológicas y morfológicas que están probablemente relacionadas a sus hábitats secos, por lo que determinar su posición filogenética es clave en el entendimiento de los factores asociados con la evolución morfológica en *Psittacanthus*.

Preguntas: (1) ¿Existe una asociación entre la evolución de algunas innovaciones morfológicas en los especialistas de *Bursera* y las condiciones ecológicas en las que ocurrió la diversificación de los hospederos? (2) ¿Coincide el tiempo de diversificación de ambos linajes?

Especies de estudio: Catorce especies de *Psittacanthus*.

Métodos: Secuencias de marcadores nucleares (ITS) y plástidos (*trnL-trnF*) se analizaron con métodos de inferencia Bayesiana, máxima verosimilitud y máxima parsimonia, y tiempo de divergencia usando un enfoque bayesiano estimado para elucidar la posición filogenética y tiempo de divergencia de estos especialistas de *Bursera*.

Resultados: Los especialistas de *Bursera* forman un clado bien soportado, denominado aquí como 'grupo *Bursera*'. El tiempo de divergencia del 'grupo *Bursera*' se estimó en 7.89 millones de años. Las relaciones filogenéticas entre especies, indicaron que *P. palmeri* es parafilético con respecto a *P. nudus*.

Conclusiones: Basados en estos resultados, las plantas colectadas cerca de la localidad tipo de *P. nudus* en Honduras deberían ser nombradas *P. palmeri*. La pérdida estacional de hojas en *P. palmeri* (incluyendo *P. nudus*) y la morfología de *P. sonorae* (tamaño pequeño, hojas suculentas) son claramente adaptaciones a los ecosistemas secos en donde estas especies han habitado desde hace mucho tiempo. En paralelo, la historia evolutiva de estos muérdagos parece estar correlacionada con la historia evolutiva y patrones de diversificación de *Bursera*.

Palabras clave: *Bursera,* filogenia, Honduras, ITS, Loranthaceae, muérdagos, Neotrópicos, *Psittacanthus*, reloj molecular, sistemática, tiempos de divergencia, trnL-trnF

Psittacanthus Mart. (Loranthaceae), with *ca.* 120 species, is the most species-rich genus of mistletoes in the family, ranging from Baja California in Mexico to northern Argentina (Kuijt 2009, 2014). *Psittacanthus* species occur in almost all vegetation types and parasitize a wide variety of host tree species (Kuijt 2009). Phylogenetic analyses based on molecular characters and a limited sampling (mostly Mexican species) indicate that *Psittacanthus* is monophyletic (Ornelas *et al*. 2016), and sister to the Andean genus *Aetanthus* (Vidal-Russell & Nickrent 2008a, Su *et al*. 2015). The absence of endosperm in the fruit is considered a synapomorphy of *Psittacanthus* and *Aethanthus* that together with its large flowers, anthers thicker than the filaments, and bulky haustorial connections to the host trees, clearly distinguish the clade from the rest of the Neotropical genera of Loranthaceae (Kuijt 2009).

The present study focuses on three species of *Psittacanthus*, *Psittacanthus palmeri* (Watson) Barlow & Wiens (Figure 1A), *P. nudus* (Molina) Kuijt & Feuer (Figure 1B) and *P. sonorae* (S.Watson) Kuijt, which are the smallest plants in the genus, and only parasitize *Bursera* species (Kuijt 2009). These three mistletoe species were originally placed in the polyphyletic genus *Phrygilanthus* Eichler (Standley 1919), which at the time also included many Old and New World mistletoe taxa. Given the heterogeneous assemblage of species with both Old and New World mistletoe species, *Phrygilanthus* was thus split into ten genera, including *Psittacanthus* (Barlow & Wiens 1973). Based on the lack of endosperm, inflorescence organization, pollen characteristics and other similarities of flower features, *Phrygilanthus nudus*, *P. palmeri* and *P. sonorae* were then transferred to *Psittacanthus* (Kuijt 1971, 1973, 1981, Kuijt & Feuer 1982).

Psittacanthus sonorae is easily recognized by its terete and fleshy leaves, and by the dry viscin area forming a sealed, resinous capsule around the developing seedling; these characteristics are unique among Neotropical Loranthaceae and are considered adaptations to the desert environments in northwestern Mexico (Sonora and Sinaloa) and the southern Baja California Peninsula (Kuijt 2009). Plants of *P. palmeri* and *P. nudus* are small and share the lateral inflorescences with sessile dyads (Figure 1A–B), characters not observed in other species of *Psittacanthus* (Kuijt 2009). Further differences between these two species include the inflorescences, in which *P. palmeri* has two dyads and *P. nudus* three or more dyads, and the presumed leafless habit of *P. nudus*. The Honduran *P. nudus*, only known from the type specimen and possibly a synonym of *P. palmeri*, was described as a leafless species (Molina 1952), whereas the Mexican *P. palmeri* is more widely distributed and considered a seasonally deciduous species based on herbarium collections. The leaflessness or deciduousness of both species needs to be confirmed in the field (Kuijt & Feuer 1982, Kuijt 2009); this is relevant, since no other deciduous mistletoes have been reported in the tropics and subtropics, even on deciduous hosts (Glatzel *et al*. 2017). Interestingly, two records of leafless mistletoes exist between the type locality of *P. nudus* in Honduras (Kuijt & Feuer 1982) and the range of *P. palmeri* from Sonora south to Oaxaca (Kuijt 2009). One is a specimen from Chiapas, in the municipality of La Trinitaria near the border with Guatemala, cited as *P. palmeri* (*Davidse & al. 29967*; MEXU, MO). The second record is a photograph taken by D. Lorence of a leafless flowering individual growing on *Bursera* in the area of Juchitán, Oaxaca (Kuijt 2009), a few kilometers away from where specimens of *P. palmeri* had also been collected (*Cedillo T. & Lorence 482*; MEXU). With this evidence, Kuijt (2009) discussed that *P. palmeri* and *P. nudus* might be conspecific. It is possible that *P. nudus* does not represent a valid species, but rather is a form of *P. palmeri* where samples were collected during the leafless period. Alternatively, it is possible that *P. nudus* is not restricted geographically to Honduras, and that populations of this species exist in southern Mexico and Guatemala.

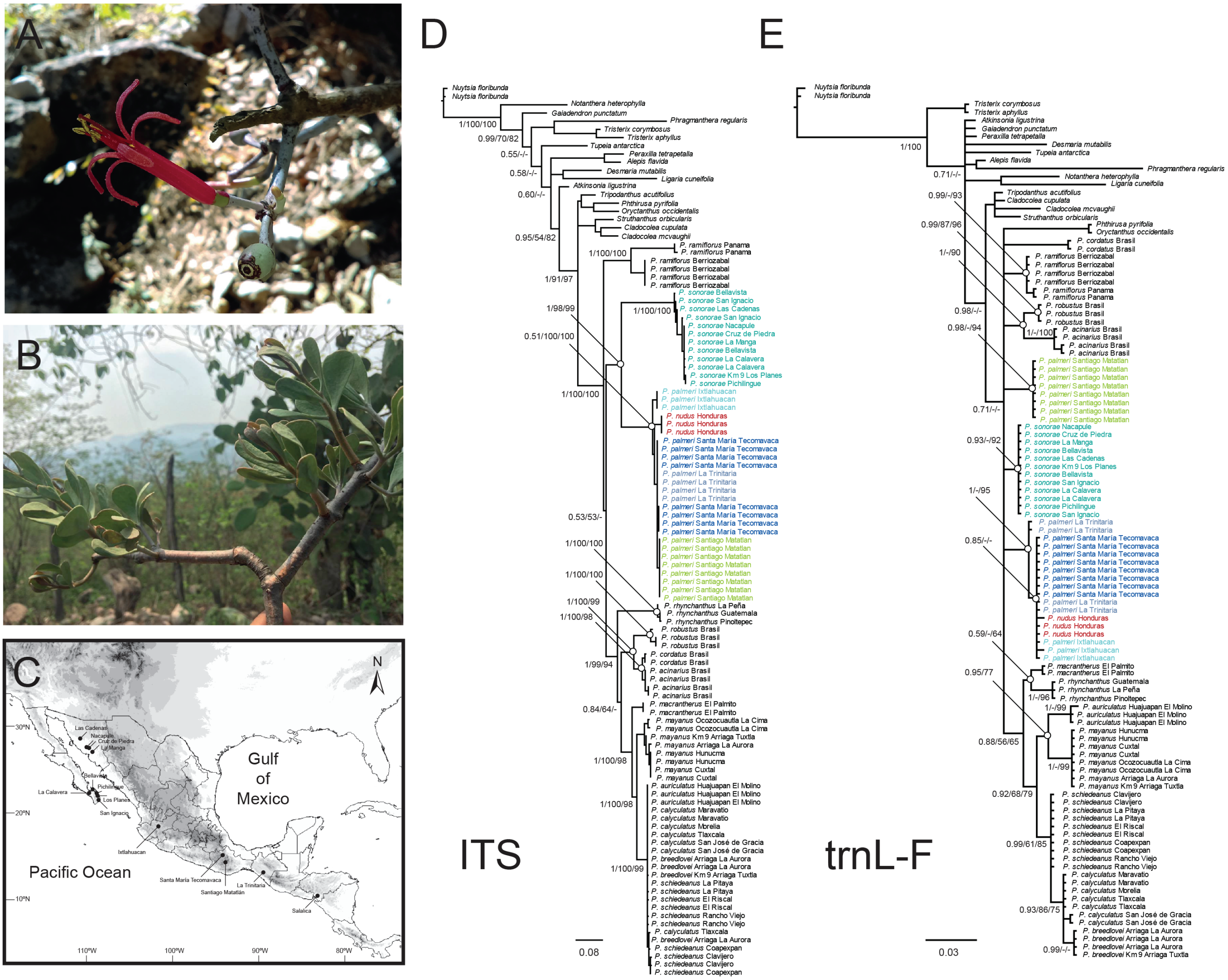

Figure 1. A) *Psittacanthus palmeri*, La Trinitaria, Chiapas (Photo: Andrés Ernesto Ortiz-Rodriguez); **B)** *P. nudus*, Honduras (Photo: Eydi Yanina Guerrero); **C)** Collection sites of *P. sonorae*, *P. palmeri* and *P. nudus* on a relief map. Detailed geographic information of collection sites is provided in table 1. Comparison of tree topologies of *Psittacanthus* for single gene partitions using Bayesian inference (BI). Posterior probabilities (PP) for Bayesian inference and bootstrap support (BS) values for maximum likelihood (ML) and maximum parsimony (MP) methods are shown above or below the nodes. **D)** BI trees derived from the ITS, **E)** *trnL-F* (B).

A previous molecular phylogenetic analysis of *Psittacanthus* sequences of nuclear (ITS) and chloroplast (*trnLF*) DNA markers (Ornelas *et al*. 2016) included 13 species, with three samples of *P. sonorae* from the Sonoran Desert and three samples of *P. palmeri* from xeric scrubland in Oaxaca. That study showed that these two species were part of a well-supported clade sister to *P. ramiflorus* G. Don. However, *P. nudus* samples have not been included in previous phylogenetic studies. The type locality of *P. nudus* is Honduras, Departamento de Francisco Morazán, SW of the Yeguare Valley, banks of Río La Orilla at 900 m (Molina 1952). Since its publication, no further material has been collected and the trees from which the original collections were made have long since been felled (Kuijt & Feuer 1982). Plant collections we made (*E. Y. Guerrero 1632,* CURLA) are 300 m lower than that reported for the *P. nudus* holotype, near the type location in the Yeguare Valley (also known as Valle del Zamorano): Aldea de Salalica, road from Zamorano to Sanbuena Ventura, Municipio de Nueva Armenia, Departamento de Francisco Morazán at 600 m elevation and about 50 km SE of Tegucigalpa (Table 1). An additional

Table 1. Geographic information of *Psittacanthus nudus, P. palmeri* and *P. sonorae* samples studied here.

Location	*n*	Altitude (m)	Latitude (N)	Longitude (W)
Psittacanthus nudus				
Honduras, Francisco Morazán, road El Zamorano-Aldea de Salalica	1	600	13° 50´ 38´´	87° 07´ 02´´
Honduras, Francisco Morazán, Nueva Armenia, Aldea de Salalica	2	500	13° 52´ 13´´	87° 06´ 36´´
Psittacanthus palmeri				
Mexico, Jalisco, Barranca de Huentitán, Ixtlahuacan del Río	3	1,770	21° 00´ 42´´	103° 09´ 17´´
Mexico, Oaxaca, Sta. Ma. Tecomavaca, Cañón del Sabino	8	725	17º 51´ 53´´	97º 02´ 10´´
Mexico, Oaxaca, Santiago Matatlán	8	1,830	16° 50´ 28´´	96° 22´ 26´´
Mexico, Chiapas, La Trinitaria	4	1,475	16° 04´ 55´´	92° 02´ 06´´
Psittacanthus sonorae				
Mexico, Baja California Sur, San Bartolo, Bellavista	2	37	23° 43´ 55´´	109° 47´ 46´´
Mexico, Baja California Sur, La Paz, Cerro La Calavera	2	36	24° 04´ 15´´	110° 16´ 19´´
Mexico, Baja California Sur, 2 km NW Pichilingue	1	23	24° 11´ 10´´	110° 17´ 58´´
Mexico, Baja California Sur, road 286, km 9 Los Planes-La Paz	1	262	24° 05´ 03´´	110° 13´ 41´´
Mexico, Baja California Sur, road La Paz-Los Cabos, Rancho San Ignacio	2	57	23° 59´ 22´´	110° 00´ 44´´
Mexico, Sonora, Cañón de Nacapule	1	83	27° 59´ 04´´	111° 02´ 40´´
Mexico, Sonora, Ejido Cruz de Piedra	1	25	27° 57´ 25´´	110° 40´ 51´´
Mexico, Sonora, Paraiso La Manga	1	7	27° 53´ 43´´	111° 06´ 55´´
Mexico, Sonora, Las Cadenas	1	12	28° 17´ 48´´	111° 25´ 37´´

specimen deposited at the herbarium Paul C. Standley de la Escuela Agrícola Panamericana Zamorano (EAP) was collected and photographed in the same area (*J. L. Linares & al. 13530*, EAP): San Antonio de Oriente, 18 km S of Zamorano, road to Salalica, Departamento de Francisco Morazán (13º 53´ 49″ N; 89º 04´ 50″ W at 750 m elevation).

We obtained sequence data from ITS and *trnF* DNA markers to address the taxonomic status and phylogenetic position of recently collected samples near the type locality of *P. nudus* in Honduras. Phylogenetic and molecular dating methods were used to (1) infer the phylogenetic position of these samples within *Psittacanthus*, and (2) to estimate the time of divergence between these and other samples of putative relatives. Our aim is to provide a phylogenetic framework to link morphological traits (*e.g.,* small size, leaf morphology and deciduousness; traits that in theory could be related to drought tolerance) to the host-mistletoe context and the associated ecological conditions at the time of diversification. Specifically, we asked: Are some morphological traits of *Psittacanthus* associated with the diversification of *Bursera*? How was the *Bursera*'s mistletoes speciation? Does time of diversification in both lineages coincide?

Materials and methods

Taxon sampling. A total of 37 samples were collected, three putatively of *P. nudus*, 23 of *P. palmeri* and 12 of *P. sonorae* (Figure 1A–C, geographic information for samples studied in Table 1, voucher information and GenBank accession numbers in Appendix 1). Twelve other widespread *Psittacanthus* species (52 samples) and other representatives of Loranthaceae (19 samples) downloaded from GenBank (accession nos. in Appendix 1) were used as outgroups according to Wilson & Calvin (2006), Amico *et al*. (2007), Vidal-Russell & Nickrent (2007, 2008a,b), Díaz-Infante *et al*. (2016), Ornelas *et al*. (2016), and Pérez-Crespo *et al*. (2017).

DNA extraction and gene amplification. For the newly obtained samples in this study, genomic DNA was extracted from silica dried plant tissues using a standard CTAB method (Doyle & Doyle 1987) or the DNeasy Plant Mini kit (Qiagen, Valencia, CA, USA) using the manufacturer's protocol. Nuclear ribosomal DNA internal transcribed spacer (hereafter ITS) was amplified with the primers ITS-F2-Psitta (5′-TCGCAGTATGCTCCGTATTG-3′) and ITS-R2-Psitta (5′-TCGTAACAAGGTTTCCGTAGG-3′) designed for the genus (Ornelas *et al*. 2016), whereas for the plastid *trnF* intergenic spacer region (hereafter *trnL-F*) we used the universal primers 'e' and 'f' (Taberlet *et al*. 1991). Protocols for PCR reactions and for sequencing the PCR products were described in Ornelas *et al*. (2016).

Alignment and phylogenetic analyses. Edited sequences using Sequencher 4.1.4 were imported into Se-Al 2.0a111 (Rambaut 2007) and aligned manually. For *trnL-F*, indels were introduced during alignment, but the resulting gaps were not coded. For ITS, the alignment was guided by reference to published studies of Loranthaceae (Wilson & Calvin 2006, Vidal-Russell & Nickrent 2008a,b, Ornelas *et al*. 2016, Pérez-Crespo *et al*. 2017), and indels were not coded. Polymorphic sites in the ITS region were coded as ambiguous. It is possible that divergent ITS paralogues may have been amplified in this study, including pseudo genes and recombinants. However, other phylogenetic studies with different Loranthaceae using ITS did not encounter evidence of paralogy or mention evidence of paralogues (Wilson & Calvin 2006, Vidal-Russell & Nickrent 2008a,b, Ornelas *et al*. 2016, Pérez-Crespo *et al*. 2017, Lopez-Laphitz *et al*. 2018). There is almost no missing data in the matrices, with 0.6 % of incompletely sequenced genes in ITS (404), 1.8 % in *trnL-F* (689), and 1 % in the combined ITS+*trnL-F* matrix (1,102). Individual gene alignments were saved as NEXUS files and then concatenated using Mesquite 3.01 (Maddison & Maddison 2011). The newly generated sequences were submitted to GenBank (Appendix 1).

The ITS and *trnL-F* datasets and the combined dataset were analyzed using Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP) methods. For the BI and ML analyses, appropriate substitution models were estimated using jMODELTEST 2.1.7 (Darriba *et al*. 2012), F81 + G (*trnL-F*) and GTR + I + G (ITS). The BI analyses were performed using MrBAYES 3.12 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). BI analyses were run using the CIPRES Science Gateway (Miller *et al*. 2010) for the ITS, *trnL-F* and the combined datasets. Two parallel Markov chain Monte Carlo (MCMC) analyses were executed simultaneously, and each was run for 10 million generations, sampling every 1,000 generations. Bayesian posterior probability values were calculated from the sampled trees remaining after 2,500 samples were discarded as burn-in (Huelsenbeck & Ronquist 2001) to only include trees after stationary distribution was reached. The remaining trees were used to generate a 50 % majority-rule consensus tree, showing nodes with a posterior probability (PP) of 0.5 or more. We consider nodes significantly supported if posterior probabilities were ≥ 0.95 (Huelsenbeck & Ronquist 2001).

ML analyses were performed in RAxML 8.2.4 (Stamatakis 2014) under the general time-reversible nucleotide substitution model (GTR) and 1,000 non-parametric regular bootstraps using the CIPRES Science Gateway (Miller *et al*. 2010). Bootstrap support values were interpreted as indicating weak (50–70 %), moderate (71–80 %) and strong support (81–100 %).

The most parsimonious trees were obtained using the ratchet strategy (Nixon 1999) in Winclada 1.0000 (Nixon 1999–2002), running NONA 2.0 (Goloboff 1993), with nucleotide characters treated as unordered and equally weighted, 1,000 iterations, holding 10 trees per iteration with 10 % of the nodes constrained, and all other parameters set to default. Branch support was assessed using bootstrap resampling, 1,000 bootstrap-resampled pseudoreplicate matrices were each analyzed using 100 random addition sequences (multi*100). Ten trees were retained during TBR swapping after each search initiation (hold/10) using NONA and performed in WinClada, with the same interpretations of support level as in the ML analyses.

Divergence time estimation. We estimated divergence time under a Bayesian approach using the concatenated ITS and *trnL-F* sequence dataset. The ingroup comprised all sequences of the *Psittacanthus* '*Bursera* group', and sequences from other *Psittacanthus* species and other representatives of Loranthaceae downloaded from GenBank were used as multiple outgroups (Vidal-Russell & Nickrent 2008a). Divergence time estimation was performed with BEAST 1.6.1 (Drummond & Rambaut 2007) using the uncorrelated lognormal relaxed molecular clock and the nucleotide substitution model GTR+G+I for the ITS sequence dataset and GTR+G for the *trnL-F* sequence dataset, suggested by jMODELTEST 0.1.1 (Posada 2008). The tree prior model was set using a coalescent approach assuming constant population size. To calibrate the root node, we constrained *Nuytsia floribunda* (Labill.) G. Don as sister to the aerial parasites based on Vidal-Russell & Nickrent (2008b). The divergence time between *Nuytsia* and the aerial parasites clade was used as secondary calibration, approximating a median age of 48.9 Ma (normal distribution, mean 48.9, SD 3.9, range 56.5–41.2 Ma) using pollen fossil of Loranthaceae according to Grímsson *et al*. (2017). The geometric mean of 5.798×10^{-9} substitutions per neutral site per year (s/s/y) was used to calibrate the tree based on the mean mutation rates of 4.13×10^{-9} s/s/y for ITS of herbaceous annual/perennial plants (Kay *et al*. 2006) and 8.24×10^{-9} s/s/y for *trnL-F* estimated for annual or perennial herbs (Richardson *et al*. 2001). The BEAST analysis was run two times for 100 million generations, sampling every 10,000 steps. We combined the log and trees files from each independent run using LOGCOMBINER 1.8.0 (Drummond & Rambaut 2007), then viewed the combined log file in TRACER 1.6 (http://tree.bio.ed.ac.uk/software/tracer/) to ensure that ESSs for all priors and the posterior distribution were > 200, making sure that parameter values were fluctuating at stable levels. Based on these results, the first 10 % of trees were discarded as burn-in, and the remaining trees were annotated and summarized as a maximum clade credibility tree with mean divergence times and 95 % highest posterior density (HPD) intervals of age estimates using TREEANNOTATOR 1.8.0 (Drummond & Rambaut 2007) and visualized in FIGTREE 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

Results

Molecular data. The ITS partition had 645 aligned positions (109 samples) and contained few ambiguous positions. This partition including the outgroup taxa had 348 variable characters (59.5 %), 324 (50.2 %) parsimony-informative, while considering only the ingroup had 102 variable characters (15.8 %), 98 (15.1 %) parsimony-informative. With outgroups included, the plastid *trnL-F* intergenic spacer region had 377 aligned positions (109 samples) with 173 (46.3 %) variable sites, 117 (31.3 %) parsimony informative. The alignment of this region considering only the ingroup had 17 parsimony informative sites (4.5 %). The concatenated alignment of the 109-taxon dataset consisted of 1020 aligned positions of which 557 (54.6 %) were variable sites and 441 (43.2 %) were parsimony-informative. Considering just the ingroup, the number of variable sites was 119 (11.6 %), of which 115 (11.2 %) were parsimony-informative.

Phylogenetic analyses. Tree topologies for single (ITS, *trnL-F*) gene partitions of the BI analysis, summarizing the clade support for all methods, are shown in Figure 1. Figure 2 shows the tree topologies using maximum likelihood (ML; Figure 2A–B), and strict consensus maximum parsimony (MP; Figure 2D–E). The ITS dataset showed that *Psittacanthus* species are monophyletic (BI = 1.0 PP, MLBS = 100 % bootstrap support, MPBS = 100 % bootstrap support; Figure 1D, Figure 2A, D), and that individuals from Honduras, *P. palmeri* and *P. sonorae* are closely related, forming a well-supported clade (1.0 PP, 98 % MLBS, 99 % MPBS). In the *trnL-F*, *Psittacanthus* was not monophyletic and provided very low resolution within *Psittacanthus*, with unresolved relationships in the separate trees (Figure 1E, Figure 2B, E). Despite this, the *trnL-F* trees suggest that samples from La Trinitaria (Chiapas) provisionally determined as *P. palmeri* and those from Honduras are closely related to individuals of *P. palmeri* from Santa María Tecomavaca (Oaxaca) and individuals from Ixtlahuacan (Jalisco) by all three methods (BI, ML, and MP; Figure 1E and Figure 2B, E). However, these samples and those of *P. palmeri* from Santiago Matatlán arise from a polytomy. Although the level of resolution between the ITS and *trnL-F* datasets and phylogenetic methods differed, the relationships among individuals from Honduras, *P. palmeri* and *P. sonorae* were largely congruent in trees generated using ITS and the combined ITS+*trnL-F* datasets (Figures 1 and 2).

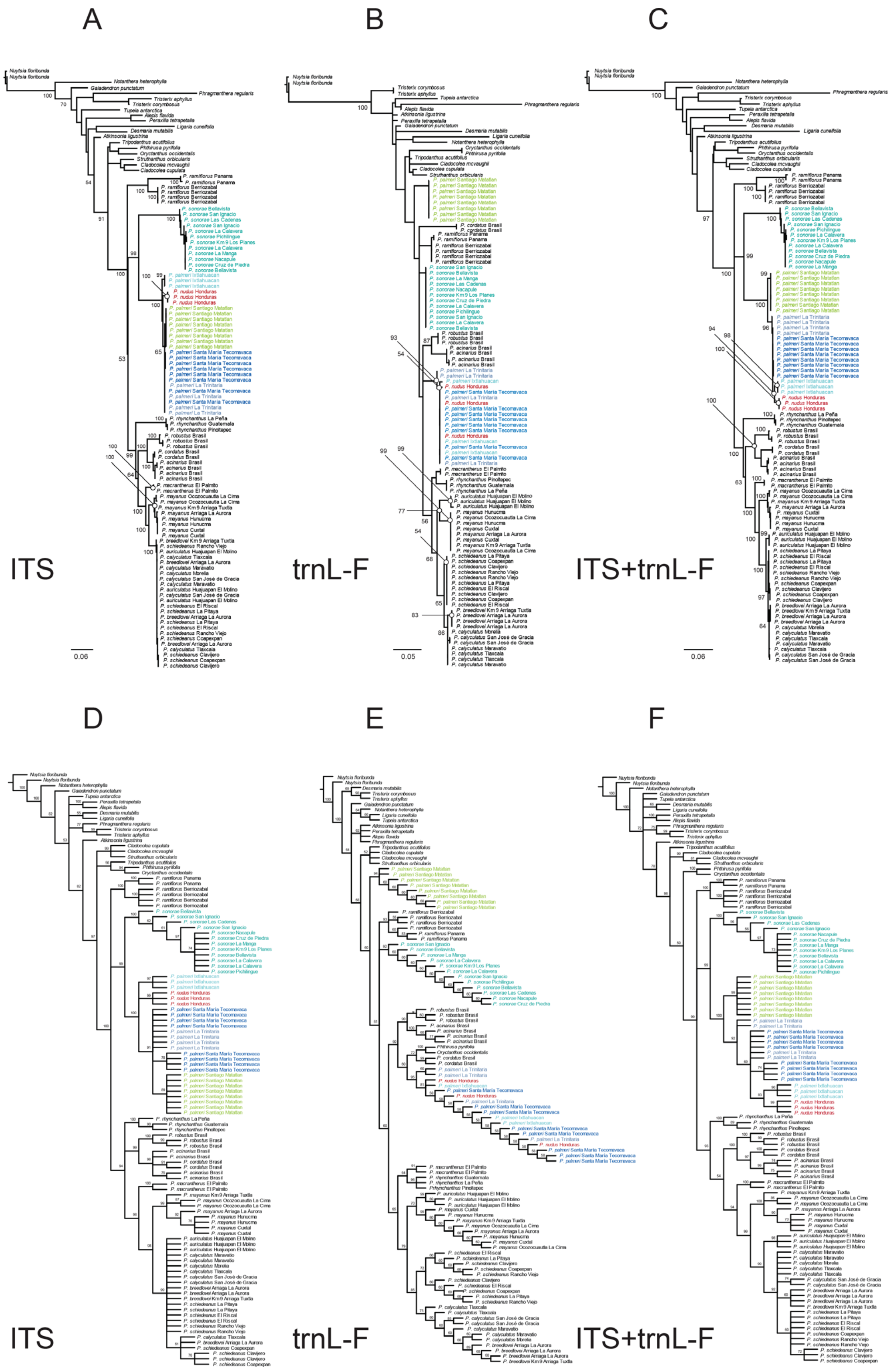

Figure 2. Comparison of tree topologies of *Psittacanthus* for single (ITS, *trnL-F*) and combined (ITS+*trnL-F*) gene partitions using maximum likelihood (ML) and strict consensus maximum parsimony (MP). Bootstrap support values are shown above or below the nodes. ML and MP trees derived from the ITS (**A**, **D**), *trnL-F* (**B**, **E**) and ITS+*trnL-F* datasets (**C**, **F**), respectively.

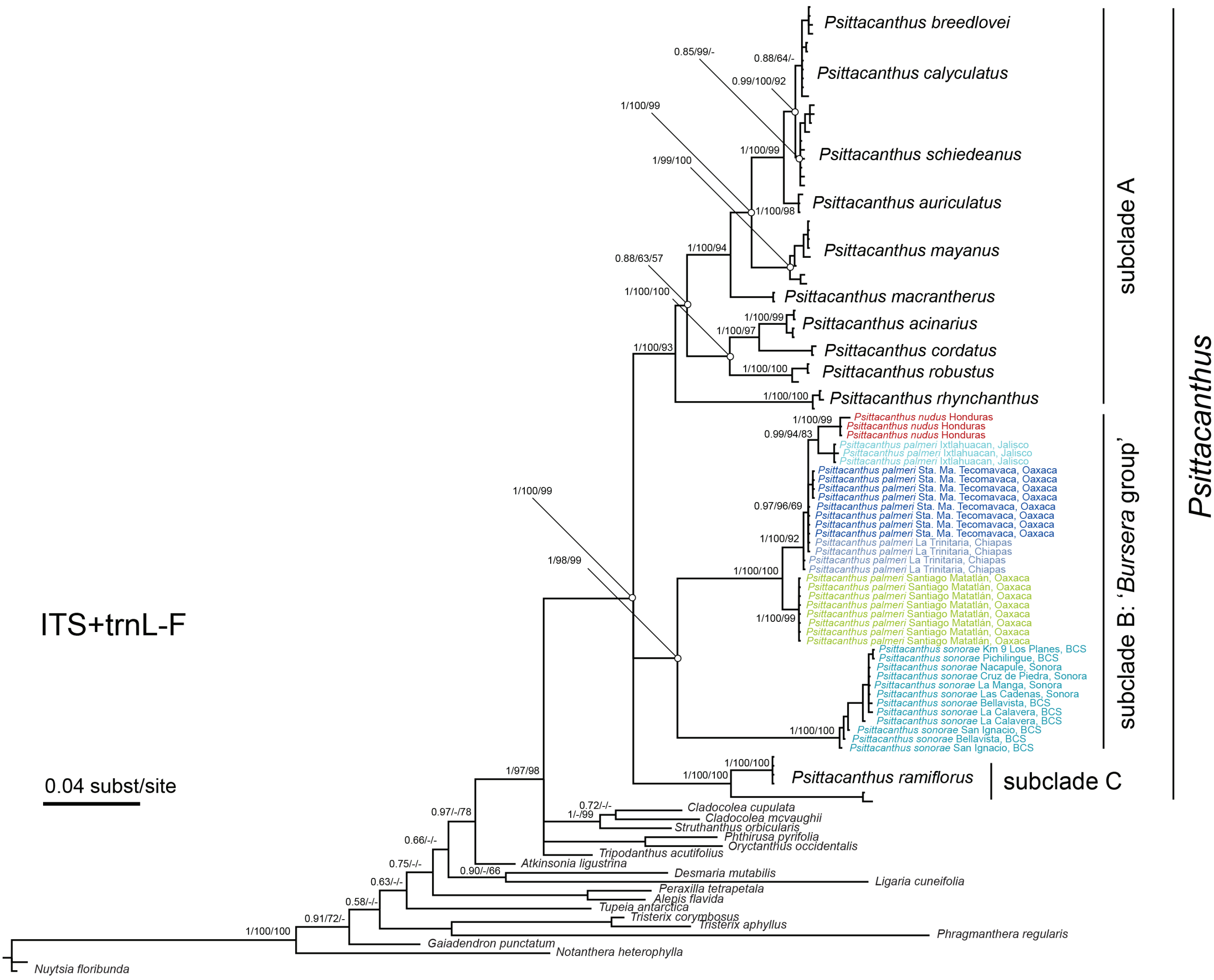

Figure 3. Bayesian inference (BI) tree (50 % majority-rule consensus) based on combined (ITS+*trnL-F*) gene partitions for 37 samples of the *Psittacanthus* '*Bursera* group' clade. The values above the branches denote posterior probabilities (PP) followed by the bootstrap support values of ML and MP analyses.

Tree topologies for combined (ITS+*trnL-F*) gene partitions of the BI analysis, summarizing the clade support for all methods, are shown in Figure 3, and Figure 2 shows the tree topology using maximum likelihood (ML; Figure 2C), and the strict consensus maximum parsimony (MP; Figure 2F), that produced similar tree topologies (Figure 2C, F). The 50 % majority-rule consensus BI tree is shown in Figure 3 also including ML and MP bootstrap support values. All phylogenetic analyses indicate with strong support that the *Psittacanthus* species in our dataset are monophyletic (1.0 PP, 100 % MLBS, 99 % MPBS), with samples of *P. palmeri* and *P. sonorae* forming a strongly supported clade (1.0 PP, 99 % MLBS, 99 % MPBS), named here the '*Bursera* group'.

Within the '*Bursera* group', samples of *P. sonorae* form a clade (1.0 PP, 100 % MLBS, 100 % MPBS) that is sister to *P. nudus* and *P. palmeri* (1.0 PP, 99 % MLBS, 99 % MPBS). However, phylogenetic relationships within the group formed by *P. nudus* and *P. palmeri*, as currently circumscribed, were not fully resolved. Both species, including individuals of *P. palmeri* from Jalisco, Oaxaca and Chiapas and the samples collected near the type locality in Honduras of *P. nudus,* formed a strongly supported clade (1.0 PP, 100 % MLBS, 100 % MPBS); the relation-

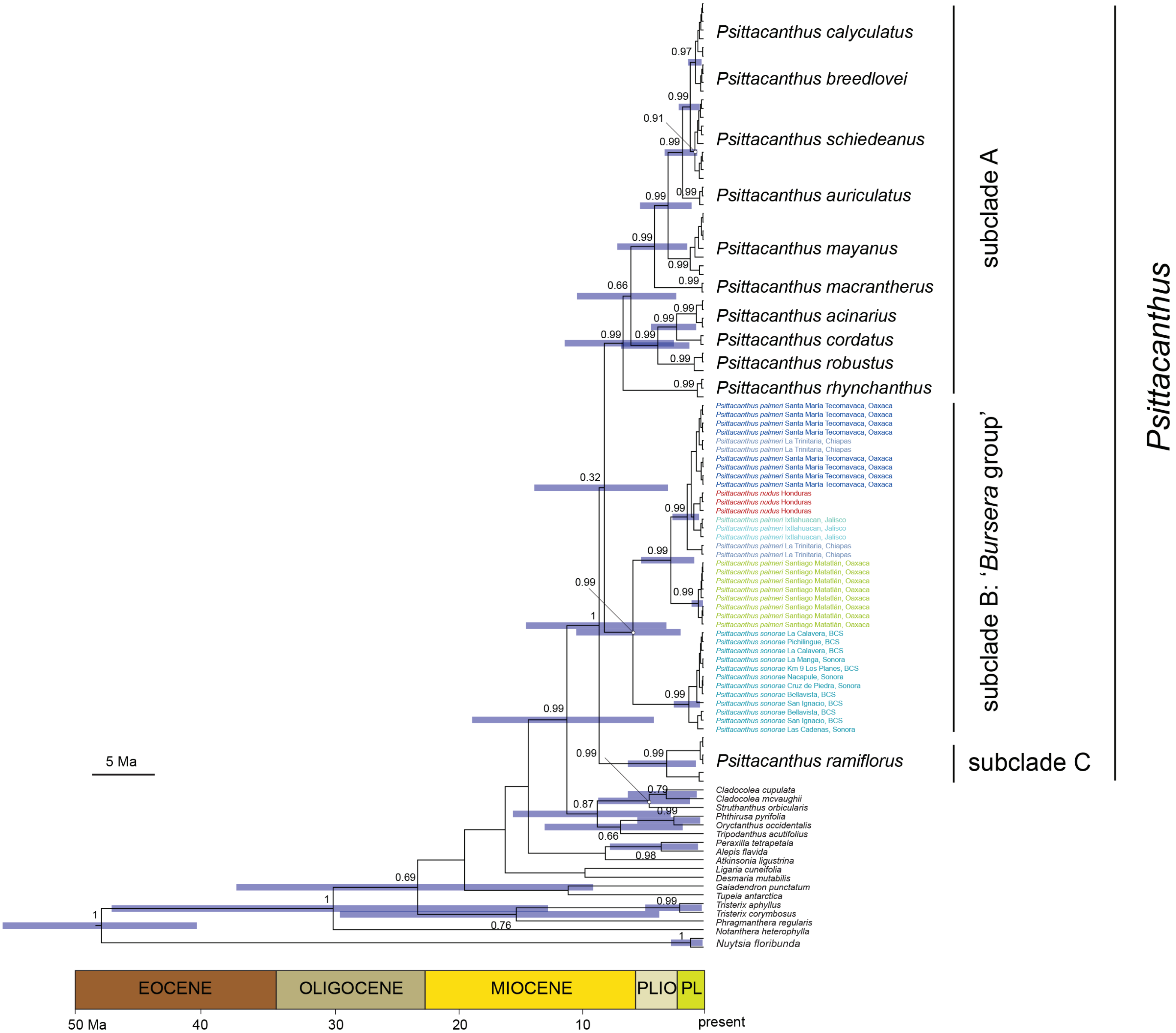

Figure 4. Chronogram of *Psittacanthus* based on a maximum clade credibility tree derived from the BEAST analysis using a coalescent prior under an uncorrelated lognormal relaxed clock model and assuming constant population size. Nodes are posterior mean ages (Mya), with blue node bars representing the 95% highest posterior density (HPD) intervals for nodes with posterior probabilities above 0.6. Geological time-scale abbreviations: PLIO, Pliocene; PL, Pleistocene.

ship between a well-supported, monophyletic group formed by individuals of *P. nudus* (1.0 PP, 100 % MLBS, 99 % MPBS) with other members of *P. palmeri* is supported, with the latter clearly paraphyletic with respect to the former. Within the '*Bursera* group' two noteworthy structural changes were detected in the *trnL-F* region. One corresponds to a deletion of 7 bases (5' GCCTAAAT-------ATGAAAAA 3'), and, within the same span of DNA, the other one to a 62-bp deletion (5' AAAATAAA-------AACTAATT 3') shared by all individuals in the ingroup, except individuals of *P. palmeri* from Santiago Matatlán (Oaxaca).

Divergence time estimation. The BEAST analyses placed the split between *Psittacanthus* and other Loranthaceae at 10.87 Ma (95 % HPD 18.42–3.94 Ma, Figure 4) and the origin of the *Psittacanthus* crown group in the Late Miocene, separating the *P. ramiflorus* clade from other

Psittacanthus (8.31 Ma, 95 % HPD 14.14–2.95 Ma, Figure 4). The '*Bursera* group' formed by samples of *P. nudus*, *P. palmeri* and *P. sonorae* split from the other *Psittacanthus* at 7.89 Ma (95 % HPD 13.47–2.83 Ma). Within the '*Bursera* group', the early split between *P. sonorae* from *P. palmeri*/*P. nudus* clade was estimated at 5.62 Ma (95 % HPD 10.13–1.82 Ma) and the crown age of *P. sonorae* was estimated at 1.17 Ma (95 % HPD 2.38–0.28 Ma). Lastly, within *P. palmeri*, the age for the group of *P. nudus* samples from Honduras was estimated at 0.28 Ma (95 % HPD 0.64–0.02 Ma).

Discussion

Phylogenetic methods and markers. Phylogenetic results obtained in this study are consistent with previous phylogenetic analyses: the plastid marker *trnL-F* showing low resolution at species level in Loranthaceae (*e.g.*, Vidal-Russell & Nickrent 2008a, Ornelas *et al*. 2016), whilst the ITS sequences have proved to be more informative showing greater resolution at species and genus level (Vidal-Russel & Nickrent 2008a, Zanjanchi & Saeidi-Mehrvarz 2015, Ornelas *et al*. 2016, Lopez-Laphitz *et al*. 2018). The trees obtained in this study showed topological differences between different partitions and very low resolution among *Psittacanthus* species using only the *trnL-F* dataset, with most of the branches poorly supported. Unlike the plastid tree, the trees retrieved using the nuclear ITS dataset showed better resolution of the phylogenetic relationships among species.

The trees obtained from the Bayesian analyses of the individual molecular markers (ITS and *trnL-F*) and the combined dataset differ in topology and degree of resolution. The *trnL-F* gene produced the poorest resolved tree (Figure 1D), and also contained the smallest number of nodes with posterior probability (PP) values above 0.90. The combined ITS and *trnL-F* dataset produced the most resolved tree, including the highest number of strongly supported nodes (Figure 3). The phylogenetic relationships of the major *Psittacanthus* clades based on ML and MP analyses of ITS+*trnL-F* showed some incongruence between the ML and MP topologies and the BI tree topology (*e.g.*, split between *P. rhynchanthus* (Benth.) Kuijt and other *Psittacanthus* species), but most of these conflicted relationships received weak bootstrap support.

All BI, ML and MP analyses of the combined dataset resulted in similar tree topologies, showing strong support for the monophyly of *Psittacanthus* and phylogenetic relationships between species of the '*Bursera* group' (Figure 3). Given that coalescence occurs four times faster in maternally inherited chloroplast genes than in biparentally inherited nuclear genes (Hudson 1990), this result is not unexpected (see also Amico *et al*. 2007).

Psittacanthus *lineages, phylogeny and the status of* P. nudus. The three used analytical methods (BI, ML, and MP) of the combined ITS+*trnL-F* analyses resolved the conflicts between the single-gene trees, and topologies were more congruent with previous phylogenetic analysis of *Psittacanthus*, including the unresolved relationships between members of subclade C (*P. ramiflorus* and allies) and those in suclade B ('*Bursera* group') and subclade A (Ornelas *et al*. 2016, Pérez-Crespo *et al*. 2017, Licona-Vera *et al*. 2018). Based on this we considered the 'ITS+*trnL-F* tree' to be the best estimate of the phylogeny relationships among samples for further discussion and taxonomic considerations, until further phylogenetic analysis includes more species and markers, treating gaps (generally representing an insertion or deletion; *i.e.*, indels) with the input sequence alignment as missing data and coded characters in phylogeny estimation.

The '*Bursera* group' (subclade B) is composed of two strongly supported clades: *P. sonorae* and *P. palmeri* (five populations, from Jalisco, Oaxaca, and Chiapas) plus samples of *P. nudus* from Honduras. The ITS+*trnL-F* tree (Figure 3) supports a topology in which the population from Honduras is more closely related to the Ixtlahuacan (Jalisco) population of *P. palmeri* than to the other population of that species (Oaxaca and Chiapas). Here, the strongest phylogenetic signal for these relationship derives from the ITS partition, as the chloroplast gene failed to distinguish this clade (Figure 1D–E). The lack of congruence between the two gene phylogenies could be due to hybridization events or to incomplete lineage sorting in which reciprocal monophyly has not been reached (Ornelas *et al*. 2016, Pérez-Crespo *et al*. 2017, Licona-Vera *et al*. 2018). Although we cannot exclude any of the two processes, the ITS phylogenetic pattern is partial evidence that the *trnL-F* undifferentiated populations could be incipiently divergent. A complete geographic sampling of *P. palmeri* and genotyping using next-generation sequencing (*e.g.*, SNPs) will be necessary to address these issues. Strong support for the paraphyly of *P. palmeri* and putative samples of *P. nudus* from Honduras is seen with the nuclear and chloroplast partitions (Figure 1D–E) and in the combined analysis (Figures 2 and 3). However, the combined analysis does not resolve the relationship between Mexican populations of *P. palmeri* and samples of *P. nudus* from Honduras.

The data presented herein shows that samples from Honduras are not genetically distinct from those of *P. palmeri*, suggesting that *P. palmeri* and *P. nudus* do indeed represent the same species (possibly a species complex), or that the sampled population in Honduras derived from populations representing part of the distribution of a wider-ranging taxon, *P. palmeri*. Based on a recent specimen from Guatemala (*Mario Véliz Pérez 18578*; BIGU, MO), the distribution range of *P. palmeri* would not be restricted to Mexico. Furthermore, the collector of the holotype (A. Molina) believes that the species no longer exists at the type locality (Kuijt 2009).

The question of whether there could have been two species (*P. nudus* and *P. palmeri*) sympatric in this area may only be answered with the inclusion of the holotype or isotypes of *P. nudus* in the molecular analysis, but samples of the *P. nudus* holotype were not available. The present study suggests the combination of both taxa. However, a more thorough geographic and molecular sampling to be analyzed using a detailed phylogeographic approach, in combination with species distribution modeling would be needed to delimit geographic lineages.

Molecular dating of Psittacanthus *and of the* ‘Bursera *group*’. Most of our estimated ages for clades within *Psittacanthus* correspond to the Pliocene-Pleistocene epochs. Using a similar dataset that included the same *Psittacanthus* species and multiple samples for *P. schiedeanus* (Schltdl. & Cham.) G. Don, Ornelas *et al*. (2016) identified a mean of 5.41 Ma with a 95 % HPD time interval of 6.75–1.11 Ma for the age of the *Psittacanthus* crown, while the estimated di-

Table 2. Estimated divergence times between the main *Bursera* hosts and its corresponding closest relatives for *Psittacanthus nudus*, *P. palmeri* and *P. sonorae* according to De-Nova *et al*. (2012).

Mistletoe species	*Bursera* node	Divergence time (Ma)
Psittacanthus nudus	*B. simaruba* Sarg. / *B. itzae* Lundell	8.4
Psittacanthus palmeri	*B. cuneata* Engl. / *B. biflora* Standl.	10.15
	B. heteresthes Bullock / *B. fragrantissima* Bullock	6.35
	B. infernidialis Guevara & Rzed. / *B. penicillata* Engl.	6.55
	B. palmeri S. Watson / *B. stenophylla* Sprague & L. Riley	7.52
	B. bippinata Engl. /*B. vejar-vazquezii* Miranda-*B. submoniliformis* Engl.	7.05
	B. sarukhanii Guevara & Rzed.-*B. velutina* Bullock / *B. bicolor* Engl.	5.63
	B. copallifera (Sessé & Moc. ex DC.) Bullock / *B. excelsa* Engl.	4.63
	B. grandifolia Engl. / *B. instabilis* McVaugh & Rzed.	5.45
	B. multijuga Engl. / *B. confusa* (Rose) Engl.	10.95
	B. galeottiana Engl. / *B. arida* Standl.-*B. suntui* C.A. Toledo-*B. morelense* Ramírez	11.17
	B. fagaroides Engl. / *B. aptera* Ramírez	6.32
	B. schlechtendalii Engl. / *B. medranoana* Rzed. & E. Ortiz	4.33
Psittacanthus sonorae	*B. microphylla* A. Gray / *B. multifolia* (Rose) Engl.	7.72
	B. hindsiana Engl. / *B. cerasifolia* Brandegee	4.56
	B. laxiflora S. Watson / *B. filicifolia* Brandegee	7.08
	B. fagaroides Engl. / *B. aptera* Ramírez	6.32

vergence ages by Pérez-Crespo *et al*. (2017) using the same *Psittacanthus* species and multiple samples of *P. calyculatus* G. Don placed the origin of the *Psittacanthus* crown clade latter, in the Late Miocene (7.36 Ma, 95 % HPD 11.12–3.75 Ma). Here, samples of *Psittacanthus* formed a well-supported monophyletic clade, with diversification also occurring during the Late Miocene, with an even older mean age, but a wider HPD (8.31 Ma, 95 % HPD 14.14–2.95 Ma). Differences in age constraints (median age of 48.9 Ma in this study using pollen Loranthaceae fossils, *vs.* 28 Ma in other studies using substitution rates) for the tree root during dating analyzes can be attributed to discrepancies among these studies.

Within *Psittacanthus*, the group formed by samples of *P. sonorae* formed a well-supported monophyletic group, with a split between *P. sonorae* and *P. palmeri*/*P. nudus* samples occurring during the Pliocene (5.62 Ma). Thus, the divergence dates within the *Psittacanthus* '*Bursera* group' appear to be related with those of its main host species (De-Nova *et al*. 2012), where the divergence of the host necessarily precedes the origin of the parasite. Divergence times for *Bursera* species parasitized by *P. sonorae* and its corresponding closest relatives were estimated between 7.7 and 4.6 Ma, for those parasitized by *P. palmeri* between 11.2 and 4.3 Ma, and for the only known host species for *P. nudus* (*B. simaruba* L. Sarg.) and its closest relative (*B. itzae* Lundell) the divergence time was estimated as 8.4 Ma (De-Nova *et al*. 2012; Table 2).

Based on the *Bursera* phylogenetic tree (De-Nova *et al*. 2012), *P. palmeri* and *P. sonorae* could have colonized during the Late Miocene both ancestors of the Section *Bursera* and Section *Bullockia*, after having a significant increases in their diversification from Early to Middle Miocene (25–18 Ma), and ecological shifts from their ancestral seasonally dry tropical forest to xerophytic scrub (*e.g.*, *B. microphylla* A. Gray). More recent lineages of *Psittacanthus* (*P. mayanus* Standl. & Steyerm., *P. rhynchanthus*, Figure 2) invaded ancestors of the Simaruba clade in the Middle Miocene, after shifting from the seasonally dry tropical forest to the tropical rain forest (De-Nova *et al*. 2012).

In this context, the divergence and evolution of lineages within the *Psittacanthus* "*Bursera* group" could be related to the colonization of new geographic areas, where potential hosts are already occurring (occupation of novel niches).

In addition, the colonization of these sites with different selection pressures (*e.g.*, dry ecosystems) would require key morphological innovations, such as the terete, fleshy leaves unique to *P. sonorae*, small size (fruit ranges from ca. 7 mm long in *P. sonorae,* to at least 15 mm in *P. macrantherus* Eichler and 20 mm in *P. acinarius* Mart.; Kuijt 2009) and encapsulated cotyledons by dried viscin and enlarged cells of the vesicular viscin tissue (Kuijt 2009), and the seasonal deciduousness exhibited by *P. nudus* / *P. palmeri* (Appendix 2). Although morphological similarities between *P. palmeri* and *P. nudus* and their differences with *P. sonorae* are evident (Appendix 2), traits associated with host shifts leading these mistletoes to colonize and specialize on *Bursera* host species (*e.g.*, haustoria, host-compatibility mechanisms) remain to be investigated.

Evolution of deciduousness and succulent leaves in Psittacanthus*.* Of all described species of *Psittacanthus*, *P. nudus*, *P. palmeri* and *P. sonorae* are the smallest (Kuijt 2009), and according to our analyses they share a common origin. The likely conspecific *P. palmeri* and *P. nudus* share lateral and umbellate inflorescences and petals with inner vermiform appendages, but differ in the shape of the basal ligule (Kuijt 2009; Appendix 2). According to Kuijt (2009), *P. sonorae* is succulent, while *P. palmeri* is possibly seasonally deciduous and *P. nudus*, previously known only from the type collected in Honduras, is apparently leafless.

The lack of leaves in *P. nudus* was not confirmed by Kuijt (2009). The leaflessness or deciduousness of *P. nudus* has been a botanical enigma since its publication (Molina 1952). Based on our field observations of *P. palmeri* in Oaxaca and Chiapas and a leafy branch of a specimen from Huehuetenango, Guatemala (BIGU, *MV 18578*; 15º 53′ 23″ N, 91º 44′ 00″ W; at 1,088 m) photographed by Mario Véliz (14 June 2007), and a specimen with leaves photographed by Eydi Yanina Guerrero (5 May 2016) near the type locality of *P. nudus* in Honduras (Table 1, Figure 1B), we confirm that *P. palmeri* is seasonally deciduous, and based on our phylogenetic studies, argue that *P. palmeri* and *P. nudus* are the same species. However, more field observations or phenological studies in these mistletoes throughout the seasons are needed to determine the start and ending of the leaf loss stage.

It is possible that the evolution of deciduousness in *P. palmeri* and *P. nudus* and of the terete leaves of *P. sonorae* are linked to their xeric habitats: seasonally dry tropical forest and xerophytic scrubs. A seasonally dry tropical forest was reconstructed as the ancestral vegetation type of *Bursera* and the most likely state for most internal nodes, and shifts to other vegetation types always involved a change from seasonally dry tropical forest to a different vegetation type, most frequently to xerophytic scrubs (De-Nova *et al*. 2012). Another species in the genus, *P. divaricatus* (Kunth) G. Don, possibly the closest relative of *P. cordatus* (Hoffmanns.) G. Don (Kuijt 2009; subclade A), occurs in the extremely xeric coastal areas of Ecuador and northern Peru, and has cordate-clasping, bluish-grey (glaucous) leaves (Kuijt 2009). Our observations indicate that *P. palmeri* is seasonally deciduous. In Figure 1B we show a photograph of an individual in vegetative condition that was taken near the type locality of *P. nudus*, and we have observed *P. palmeri* individuals without leaves during the non-breeding season.

The above observations are important because available information on deciduous mistletoes is sparse. According to a recent review on leaf production in mistletoe-host associations (Glatzel *et al*. 2017), most of the world's mistletoes are evergreen, regardless of the foliar habit of their hosts. Deciduous mistletoes are rare and confined to a few species in Loranthaceae in Eurasia, to Misodendraceae, and the monospecific genus *Desmaria* (Loranthaceae) in southern South America. Our observations of deciduousness in *P. palmeri* and possibly *P. nudus* challenge Glatzel *et al*. (2017) conclusion that there are no deciduous mistletoes in the tropics and subtropics. Further comparative phenology of mistletoes in the '*Bursera* group' is needed to analyze whether leaf deciduousness is synchronized with the deciduousness of their *Bursera* main hosts.

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Appendix 1. Vouchers and GenBank accession numbers for taxa used in our molecular phylogenetic analysis. Following each species name is GenBank numbers of ITS and *trnL-F*, *collector number* (herbarium code of voucher), and country of origin. Sequences being reported here for the first time are indicated by *. NA = no information available.

Mistletoe species	ITS	*trnL-F*	Collector (herbarium)	Country
Ingroup				
Psittacanthus nudus (Molina) Kuijt & Feuer	*KY315964	*KY315984	E. Y. Guerrero 1632 (CURLA)	Honduras
	*MH157216	*MH157221	E. Y. Guerrero 1632 (CURLA)	Honduras
	*MH157217	*MH157222	E. Y. Guerrero 1632 (CURLA)	Honduras
Psittacanthus palmeri (Watson) Barlow & Wiens	*KY315954	*KY315974	C. Soberanes (NA)	Mexico
	*KY315955	*KY315975	C. Soberanes (NA)	Mexico
	*KY315956	*KY315976	C. Soberanes (NA)	Mexico
	*KX421357	*KX421363	C. Soberanes (NA)	Mexico
	*KY315957	*KY315977	C. Soberanes (NA)	Mexico
	*KX421358	*KY315978	C. Soberanes (NA)	Mexico
	*KY315958	*KY315979	C. Soberanes (NA)	Mexico
	*KY315959	*KY315980	C. Soberanes (NA)	Mexico
	*KX421356	*KX421362	A. E. Ortiz-Rodriguez 814 (XAL)	Mexico
	*KY315945	*KY315967	A. E. Ortiz-Rodriguez 815 (XAL)	Mexico
	*KY315946	*KY315968	A. E. Ortiz-Rodriguez 816 (XAL)	Mexico
	*KY315947	*KY315969	A. E. Ortiz-Rodriguez 817 (XAL)	Mexico
	*KY315948	*KY315970	A. E. Ortiz-Rodriguez 818 (XAL)	Mexico
	*KY315949	*KY315971	A. E. Ortiz-Rodriguez 819 (XAL)	Mexico
	*KY315950	*KY315972	A. E. Ortiz-Rodriguez 820 (XAL)	Mexico
	*KY315951	*KY315973	A. E. Ortiz-Rodriguez 821 (XAL)	Mexico
	*KY315960	*KX421364	A. E. Ortiz-Rodriguez 823 (XAL)	Mexico
	*KY315961	*KY315981	A. E. Ortiz-Rodriguez 824 (XAL)	Mexico
	*KY315962	*KY315982	A. E. Ortiz-Rodriguez 825 (XAL)	Mexico
	*KY315963	*KY315983	A. E. Ortiz-Rodriguez 826 (XAL)	Mexico
	*MH157218	*MH157223	A. E. Ortiz-Rodriguez 826 (XAL)	Mexico
	*MH157219	*MH157224	A. E. Ortiz-Rodriguez 826 (XAL)	Mexico
	*MH157220	*MH157225	A. E. Ortiz-Rodriguez 826 (XAL)	Mexico
Outgroup				
Alepis flavida Tiegh.	DQ333847	DQ340598	Calvin and Wilson NZ98-04 (NA)	New Zealand
Atkinsonia ligustrina F. Muell.	DQ333865	DQ788714	Calvin and Wilson AU00-01 (NA)	Australia
Cladocolea cupulata Kuijt	DQ333861	DQ340612	Calvin and Wilson MX03-08 (NA)	Mexico
Cladocolea mcvaughii Kuijt	DQ333860	DQ340611	Calvin and Wilson MX03-09 (NA)	Mexico
Desmaria mutabilis Tiegh.	DQ333852	DQ340603	Calvin and Wilson CL03-07 (NA)	Chile
Gaiadendron punctatum G. Don.	DQ333866	DQ340617	Calvin and Wilson CR01-08 (NA)	Costa Rica
Ligaria cuneifolia Tiegh.	DQ333853	DQ340604	Calvin and Wilson CL03-01 (NA)	Chile
Notanthera heterophylla (Ruiz & Pav.) G. Don.	DQ333855	DQ340606	Calvin and Wilson Cl03-03 (NA)	Chile
Nuytsia floribunda R. Br.	DQ788705	DQ788716	Nickrent 2747 (NA)	Australia
	-	-	Nickrent 3080 (NA)	Australia

Mistletoe species	ITS	*trnL-F*	Collector (herbarium)	Country
	DQ333867	DQ340618	Calvin and Wilson AU01-22 (NA)	Australia
Oryctanthus occidentalis Eichler	DQ333862	DQ340613	Calvin and Wilson CR01-11 (NA)	Costa Rica
Peraxilla tetrapetala (L. f.) Tiegh.	DQ333846	DQ340597	Calvin and Wilson NZ98-03 (NA)	New Zealand
Phragmanthera regularis (Steud. ex Sprague) M.G. Gilbert	DQ333830	DQ340579	Calvin and Wilson Y88-01 (NA)	NA
Phthirusa pyrifolia Eichler	DQ333857	EU544504	Calvin and Wilson CR01-03 (NA)	Costa Rica
	-	-	Nickrent 2762 (NA)	NA
Psittacanthus acinarius Mart.	KU923005	KU923279	G. Ceccantini 3670A (USP)	Brazil
	KU923006	KU923280	G. Ceccantini 3670B (USP)	Brazil
	KU923010	KU923284	G. Ceccantini 3785 (USP)	Brazil
	KU923011	KU923285	G. Ceccantini 3786 (USP)	Brazil
Psittacanthus auriculatus Eichler	KU923007	KU923281	M. J. Perez 004 (XAL)	Mexico
	KU923008	KU923282	M. J. Perez 005 (XAL)	Mexico
	KU923009	KU923283	M. J. Perez (NA)	Mexico
Psittacanthus breedlovei Kuijt	KU922961	KU923278	NA (NA)	Mexico
	*KY767938	*KY767945	NA (NA)	Mexico
	*KY767939	*KY767946	NA (NA)	Mexico
	KU922963	*KY767947	NA (NA)	Mexico
Psittacanthus calyculatus G. Don	KU923012	KU923286	E. Ruiz-Sanchez 414 (XAL)	Mexico
	KU923013	KU923287	E. Ruiz-Sanchez 415 (XAL)	Mexico
	KU923014	KU923288	NA (NA)	Mexico
	KU923015	KU923289	NA (NA)	Mexico
	KU923016	KU923290	NA (NA)	Mexico
	KU923017	KU923291	E. Ruiz-Sanchez 307 (XAL)	Mexico
	KU923018	KU923292	E. Ruiz-Sanchez NA (XAL)	Mexico
Psittacanthus cordatus (Hoffmanns.) G. Don	KU923019	KU923293	G. Ceccantini 3671 (USP)	Brazil
	KU923020	KU923294	G. Ceccantini 3672 (USP)	Brazil
Psittacanthus macrantherus Eichler	KU923021	KU923295	E. Ruiz-Sanchez 348 (XAL)	Mexico
	KU923022	KU923296	F. Rodriguez NA (XAL)	Mexico
Psittacanthus mayanus Standl. & Steyerm.	KU923023	KU923297	NA (NA)	Mexico
	KU923024	KU923298	NA (NA)	Mexico
	KU923025	KU923299	NA (NA)	Mexico
	KU923026	KU923300	NA (NA)	Mexico
	KU923027	KU923301	Y. Licona Vera 017 (XAL)	Mexico
	KU923028	KU923302	Y. Licona Vera 018 (XAL)	Mexico
	*KY767937	*KX241736	NA (NA)	Mexico
	*KY767936	*KX241737	NA (NA)	Mexico
Psittacanthus ramiflorus G. Don	KU923032	KU923306	Y. Licona Vera 013 (XAL)	Mexico

Mistletoe species	**ITS**	***trnL-F***	**Collector (herbarium)**	**Country**
	KU923033	KU923307	Y. Licona Vera 014 (XAL)	Mexico
	KU923034	KU923308	Y. Licona Vera 015 (XAL)	Mexico
	KU923035	KU923309	Y. Licona Vera 016 (XAL)	Mexico
	KU923036	KU923310	NA (NA)	Panama
	KU923037	KU923311	NA (NA)	Panama
Psittacanthus rhynchanthus (Benth.) Kuijt	KU923038	KU923312	P. Carrillo Reyes 5372 (XAL)	Guatemala
	KU923040	KU923314	E. Ruiz-Sanchez 417 (XAL)	Mexico
	KU923041	KU923315	T. Mejia-Saules 2048 (XAL)	Mexico
Psittacanthus robustus Mart.	KU923042	KU923316	G. Ceccantini 3588 (USP)	Brazil
	KU923043	KU923317	G. Ceccantini 3589 (USP)	Brazil
	KU923044	KU923318	G. Ceccantini 3596 (USP)	Brazil
Psittacanthus schiedeanus (Schltdl. & Cham.) G. Don	*KY767940	*KY767950	NA (NA)	Mexico
	KU922967	*KY767951	NA (NA)	Mexico
	*KY767941	*KY767952	NA (NA)	Mexico
	KU922972	*KY767953	NA (NA)	Mexico
	KU922979	*KY767954	NA (NA)	Mexico
	*KY767942	*KY767955	NA (NA)	Mexico
	KU922981	*KY767948	NA (NA)	Mexico
	*KY767944	*KY767957	NA (NA)	Mexico
	KU922985	*KY767956	NA (NA)	Mexico
	*KY767943	*KY767949	NA (NA)	Mexico
Psittacanthus sonorae (S. Watson) Kuijt	*KX421354	*KX421359	Y. Licona Vera 182 (XAL)	Mexico
	*KX421349	*KY315985	Y. Licona Vera 183 (XAL)	Mexico
	*KX421351	*KY315988	E. Gándara 3148 (XAL)	Mexico
	*KX421353	*KY315989	E. Gándara 3152 (XAL)	Mexico
	*KX421352	*KY315990	E. Gándara 3156 (XAL)	Mexico
	*KY315966	*KX421361	Y. Licona Vera 181 (XAL)	Mexico
	*KX421355	*KY315986	E. Gándara 3187 (XAL)	Mexico
	*KX421350	*KY315987	E. Gándara 3184 (XAL)	Mexico
	KU923045	KU923319	T. S. Rodríguez (NA)	Mexico
	KU923046	KU923320	T. S. Rodríguez (NA)	Mexico
	KU923047	KU923321	T. S. Rodríguez (NA)	Mexico
	*KY315965	*KX421360	F. Molina Freaner (NA)	Mexico
Struthanthus orbicularis (Kunth) Eichler	DQ333856	DQ340607	Calvin and Wilson CR01-02 (NA)	Costa Rica
Tripodanthus acutifolius (Ruiz & Pav.) Tiegh.	DQ333864	DQ340615	Calvin and Wilson AR03-04 (NA)	Argentina
Tristerix aphyllus Tiegh. ex Barlow & Wiens	DQ442966	DQ442919	G. Amico 166 (BRCU)	Chile
Tristerix corymbosus (L.) Kuijt	DQ333854	DQ340605	Calvin and Wilson CL03-02 (NA)	Chile
Tupeia antarctica Cham. & Schltdl.	DQ333850	DQ340601	Calvin and Wilson NZ98-02 (NA)	New Zealand

Appendix 2. Morphological characters of the *Psittacanthus* '*Bursera* group'.

Character	***Psittacanthus nudus***	***Psittacanthus palmeri***	***Psittacanthus sonorae***
Foliage	Deciduous	Deciduous	Perennial
Leaf	Obovate, 3 cm long, 1.5 cm wide	Obovate	Terete, succulent, 4 cm long, 2 mm thick
Internodes	Terete	Terete	Terete
Inflorescences	Lateral umbels, with 3 or more pairs of pedicellate flowers	Lateral umbels	Terminal raceme, 3 or 4 dyads
Floral pedicel	Terminating in a small bract	9–10 mm long	1–1.5 cm long
Ovary	Cylindrical, 4.5 mm long, 3 mm wide	Cylindrical, calyculus smooth, 4 mm long, 3 mm wide	Calyculus smooth, 3 mm long, 2 mm wide
Buds	Clavate apex, 4.5 cm long, slightly twisted	Clavate apex, 4 cm long, slightly twisted	Clavate apex, 3 cm long, slightly twisted
Petals number	6 (rarely 5 or 7)	6, 3.5 cm long	6, 3 cm long
Inner margin of petals	With vermiform appendages	With vermiform appendages	Smooth
Basal ligules	Irregularly lobed, down-turned ligule bearing vermiform appendages	Fleshy below, 3 mm long, the upper half occupied by elongated vermicular or digitate appendages	Absent
Stamens	Dimorphic, filaments attached 20–22 mm above the petal base, just below the middle of the petals	Dimorphic, filaments 10–11 mm long	Dimorphic, filaments 8 mm long
Anthers	Versatile, dorsifixed, oblong, 4.5 mm long, 1.5 mm wide	4–5 mm long	3–4 mm long
Style	Smooth, slender and straight	Straight and smooth	Straight and smooth
Fruit	Ovoid, calyculus obscure, apex truncate, 8 mm long, 6 mm in diameter	Black	Dark purple to black, 7 mm long, 6 mm in diameter