

Is chiropterophily an evolutionary dead end? A phylogenetic test in the pantropical genus *Parkia* (Leguminosae, Caesalpinioideae, mimosoid clade)

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Background and aims – Pollination systems often reflect adaptations to specific groups of pollinators, and these morphological specialisations have been important in the diversification of the angiosperms. Here, we study the evolution of the capitulum and pollination system in the pantropical genus *Parkia*, which comprises 35 species of trees distributed largely in the forests of South and Central America, Africa, Madagascar, and the Indo-Pacific. The flowers are grouped into capitula that are composed of one, two, or three distinct morphological types, and are principally pollinated either by insects or by bats.

Material and methods – Using BEAST, we estimated the ages of nodes in a phylogeny based on four chloroplast regions (*matK*, *trnL*, *psbA-trnH*, and *rps16-trnQ*) and the nuclear region ITS/18S/26S. This analysis also enabled us to reconstruct the ancestral state of the capitulum and hence infer the ancestral pollination system. Euclidean distance-based cluster analysis was performed to determine which characters are consistently related to a specific pollination system.

Key results – Our results indicate that the ancestral capitulum in the genus had three types of flowers and a morphology associated with bat-pollination in both the Paleotropics and Neotropics. In one derived Neotropical clade, the number of floral types in each capitulum was reduced to two (capitulum also bat-pollinated) or one (insect-pollinated). Thus, entomophily, as seen in some Neotropical species of *Parkia*, has been derived from a bat-pollinated ancestor. Cluster analysis showed that the floral characters were mostly consistent with pollination systems.

Conclusion – Chiropterophily is not an evolutionary dead end in *Parkia* because during the evolutionary history of the genus there has been at least one transition to entomophily. *Parkia* provides a unique example of evolutionary transitions from chiropterophily to entomophily in a pantropical genus of trees.

Keywords – Chiropterophily; entomophily; evolutionary dead end; Fabaceae; legumes; pantropical.

INTRODUCTION

Pollination systems involving animals are characterized as a set of floral attributes with convergent adaptations that often reflect features of specific groups of pollinators and

these adaptations have been important in the diversification of angiosperms (Faegri & van der Pijl 1966; Rech & Brito 2012; Ashworth et al. 2015). A floral morphology that allows a better fit between the flower and the pollinator's body can generate greater reproductive success (Kay et al.

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2006). Therefore, floral characteristics tend to be shaped by those pollinators that are the most effective in pollen transfer (Fenster et al. 2015). Any floral specialization involving adaptations to new pollinators is intriguing because it causes changes in the ecological niche, and is potentially linked to reproductive isolation, and so can directly affect speciation (Armbruster et al. 2010; Rosas-Guerrero et al. 2011; Willmer 2011; van der Niet & Johnson 2012).

The direction and lability of character transitions are frequently studied in floral evolution (Pérez et al. 2006; Whittall & Hodges 2007; Tripp & Manos 2008; Brito et al. 2016). A common hypothesis is that specialized pollination systems are usually derived and generalized ones are more likely to be ancestral (Ollerton et al. 2009, 2015; Rosas-Guerrero et al. 2014). Highly specialized floral attributes, such as those seen in many chiropterophilous flowers, allow a precise adjustment with the morphology of the pollinator, thus restricting pollination to a single functional group (Muchhala & Thomson 2010). Such morphological restrictions make it difficult for other functional groups of visitors to act as pollinators, and thereby exercise selective pressure on the structure of the flower (Ollerton et al. 2007, 2011; Ashworth et al. 2015).

Pollination syndromes are defined by Fenster et al. (2004) as suites of floral traits associated with attracting efficient pollinators, and reflect adaptations to a particular pollinators or functional groups of pollinators. Chiropterophily is a specialised, derived pollination system, associated with a particular syndrome of characters. Faegri & van der Pijl (1979) list a set of floral characters associated with bat-pollination as nocturnal anthesis; flowers with less conspicuous colours and no nectar guides; large and robust flowers or inflorescences in the form of a brush exposed beyond the foliage; strong nocturnal odour and large amount of nectar and pollen. Because the syndrome associated with bat-pollination is so specialised, chiropterophily has been considered an evolutionary “dead end”, which means that it can rarely be replaced by another pollination system (Tripp & Manos 2008; Muchhala & Thomson 2010; Gómez et al. 2014). The reverse transition, from entomophily or ornithophily to chiropterophily, appears to be a more common evolutionary event (Tripp & Manos 2008; Muchhala & Thomson 2010), due to the fact that many plants pollinated by bats have a probable ancestor that is pollinated by bees, moths, or birds (Vogel 1969; Rosas-Guerrero et al. 2014).

Phylogenetic studies in plants offer a means of approaching these issues through the reconstruction of floral features (Armbruster 1992, 1993; Armbruster & Baldwin 1998; Wilson et al. 2004), tracing the historical course of relative specialization and generalization in lineages, and thus potential transitions between different pollinators. The frequency of evolutionary transitions (Armbruster & Baldwin 1998) and the identification of morphological characteristics that are more labile can also indicate how the level of specialization is maintained during changes between functional groups of pollinators, and whether evolutionary specialization is associated with floral diversification (Fenster et al. 2004).

Parkia is a pantropical genus with approximately 35 tree species that show high regional endemism, occurring in the tropical forests and savannas of South and Central America, Africa, Madagascar, and the Indo-Pacific region. The monophyly of *Parkia* has been supported both by morphological (Luckow & Hopkins 1995) and molecular data (Oliveira 2015, 2020; Oliveira et al. 2021). *Parkia* species have flowers grouped into capitula, which are arranged in panicles. Each capitulum is composed of many small, tubular flowers inserted on a receptacle with a globular or ellipsoid apex, and each is composed of up to three functional types of flowers: fertile flowers, sterile flowers specializing in nectar production, and sterile staminal flowers. These are arranged in different ways to produce three different capitular morphologies, and the structure of the capitulum is the basis for the current infrageneric classification of the genus, which recognises three taxonomic sections (Hopkins 1986) whose characters are summarized in table 1.

Two principal pollination systems occur in *Parkia*: entomophily and chiropterophily. Entomophily has been found only in the Neotropics (Hopkins et al. 2000; Chaves 2015), except for cases of primarily chiropterophilous species that are pollinated by bees in the absence of bats (e.g. Lassen et al. 2012). Chiropterophily as the principal mode of pollination occurs in both Neotropical and Paleotropical species. In the Neotropics, the bat pollinators are species of Phyllostomidae (fig. 1F–G), and in the Paleotropics, they belong to the family Pteropodidae (fig. 1H) (e.g. Baker & Harris 1957; Vogel 1968; Hopkins 1983, 1984, 1986, 1998; Grünmeier 1990; Singaravelan et al. 2007; Bumrungsri et al. 2008; Piechowski et al. 2010; Vololona et al. 2020). The two families of bats are only distantly related to each other, being in different sub-orders, suggesting that flower-visiting by bats evolved independently in the Neotropics and Paleotropics (Fleming et al. 2009).

The presence of both entomophily and chiropterophily in *Parkia* allows us to examine changes in the structure of the capitulum and its association with different pollinators within a phylogenetic context. In this study, we combine phylogenetic and morphological data in order to do so. We tested three hypotheses about the origins of the two main pollination systems and comment on the lability and directionality of associated character transitions. These hypotheses are: (1) a capitulum composed of only fertile flowers is the ancestral state and one with three types of flowers is derived; (2) that the two pollination systems, chiropterophily and entomophily, are specific by capitulum type and are each monophyletic within *Parkia*; and (3) that entomophily is ancestral and chiropterophily is derived.

MATERIAL AND METHODS

Taxon sampling

We sampled 30 of the 35 species currently recognised in the genus. We sampled 17 of the 19 Neotropical species; for the Paleotropics we sampled all four African species (including Madagascar), and nine of the 12 species for the Indo-Pacific region. The outgroups were chosen on the basis of previous phylogenetic studies of the mimosoid clade (Luckow et al.

Table 1 – Characters of the three taxonomic sections of *Parkia*.

| | Taxonomic section | | |
|---------------------------------|--|---|---|
| | <i>Parkia</i> | <i>Platyparkia</i> | <i>Sphaeroparkia</i> |
| Clades (from fig. 2) | 3, 4, 5 | 2 | 1 |
| Number of species | ca 29 | 3 | 3 |
| Distribution | Pantropical | Neotropical | Neotropical |
| Number of types of flowers | Three | Two | One |
| Shape and structure of capitula | Clavate or biglobose; composed of apical fertile flowers, intermediate nectar-secreting flowers, and basal sterile flowers with staminodia | Oblate; composed of basal fertile flowers and apical nectar-secreting flowers | Spherical; composed of only fertile flowers |

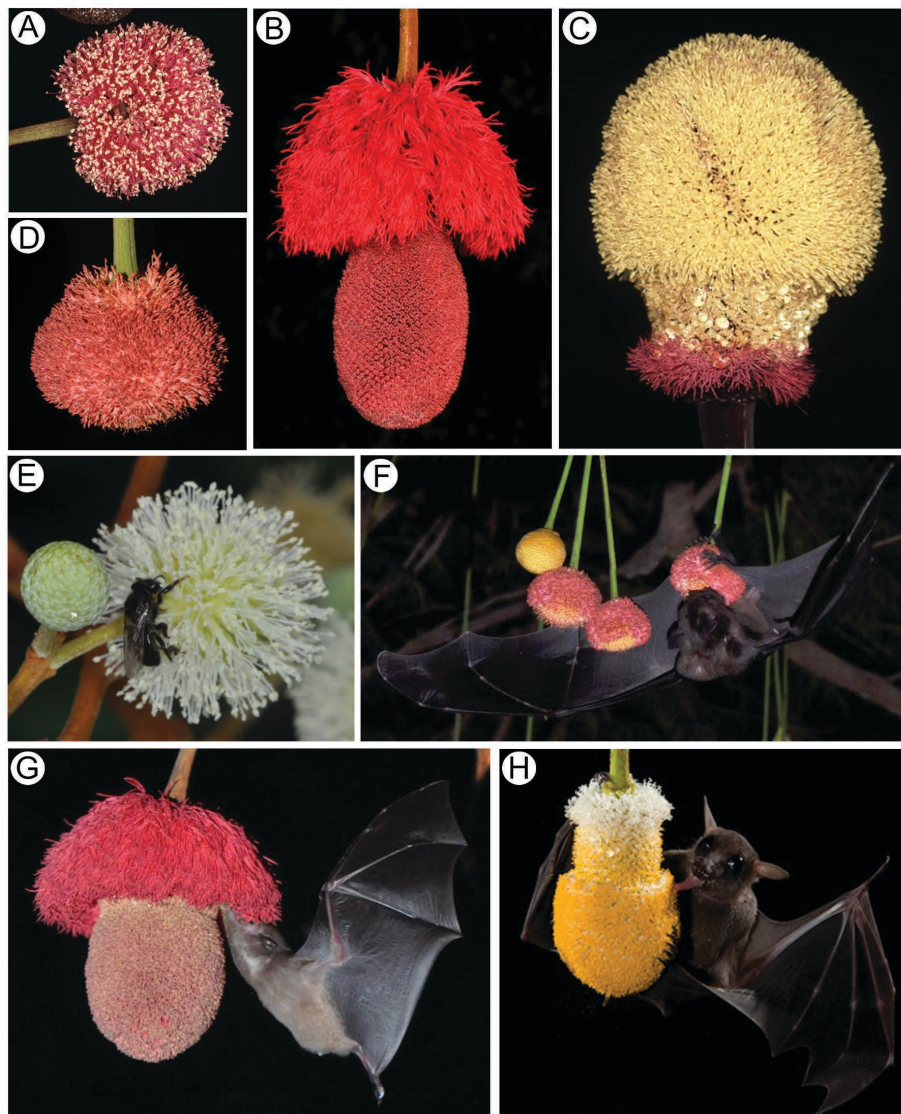


Figure 1 – A–D. Morphology of capitula in *Parkia* (Leguminosae, Caesalpinioideae, mimosoid clade). **A.** *P. velutina*, spherical capitulum comprised only of fertile flowers. **B.** *P. pendula*, oblate capitulum with fertile flowers in the middle and basal portion and nectar-secreting flowers at the apex (note visible nectar droplets). **C.** *P. discolor*, capitulum with fertile flowers forming a ball at the apex, nectar-secreting flowers in the middle portion (beneath the fringe and therefore not visible) and staminodial flowers at the base, their staminodes forming a fringe. **D.** *P. decussata*, with short staminodes not forming a fringe. **E–H.** Pollinators of *Parkia*. **E.** *Trigona* sp. bees on *P. ulei*. **F.** *Phyllostomus* sp. on *P. igneiflora*. **G.** *Glossophaga* sp. on *P. pendula*. **H.** *Eonycteris spelaea* (Pteropodidae) on *P. speciosa*. Photographs: A, D, and F by Michael Hopkins; B and C by Francisco Farroñay; F and G by Luiz Melo; H by Merlin Tuttle.

Table 2 – Name and sequence of the primers used in this study.

| Primer name | Primer sequence (5'– 3') | Reference |
|-------------|----------------------------|-----------------------------|
| matKM | TCGACTTTCTGGGCTATC | Tate & Simpson 2003 |
| trnK-2R | AACTAGTCGGATGGAGTAG | Johnson & Soltis 1994 |
| trnL A | CATTACAAATGCGATGCTCT | Taberlet et al. 1991 |
| trnL B | TCTACCGATTTCGCCATATC | Taberlet et al. 1991 |
| trnQ | GCGTGGCCAAGYGGTAAGGC | Shaw et al. 2007 |
| rsp16x1 | GTTGCTTTYTACCACATCGTTT | Shaw et al. 2007 |
| psbA | GTTATGCATGAACGTAATGCTC | Sang et al. 1997 |
| trnH2 | CGCGCATGGTGGATTCAACAATCC | Tate & Simpson 2003 |
| ITS18 | GTCCACTGAACCTTATCATTAGAGG | Delgado-Salinas et al. 2006 |
| ITS26 | GCCGTTACTAAGGGAATCCTTGTTAG | Delgado-Salinas et al. 2006 |

2003; Luckow 2005; Ribeiro et al. 2018; Simon et al. 2016) and included species of the genera placed in the Piptadenia group: *Anadenanthera*, *Lachesiodendron*, *Microlobius*, *Mimosa*, *Parapiptadenia*, *Piptadenia*, *Pityrocarpa*, *Pseudopiptadenia*, and *Stryphnodendron*.

DNA extraction, amplification, and sequencing

We extracted total genomic DNA from silica-gel dried leaves or herbarium material using the 5% CTAB protocol described by Oliveira et al. (2017) in the Laboratório de Evolução Aplicada of the Universidade Federal do Amazonas (LEA-UFAM). Other samples were extracted using DNeasy Plant Mini Kit (QIAGEN, CA, USA) or 2% CTAB (Doyle & Doyle 1987) in the Jodrell Laboratory, Royal Botanic Gardens, Kew (see <https://dnabank.science.kew.org/>).

Polymerase chain reactions (PCR) were conducted for all samples on a total volume of 20 µL, containing the final concentration of ~ 10 ng of each DNA sample, 1X buffer, 1 mmol/L of MgCl₂, 10 mmol/L of dNTPs, 1 pmol/L of each primer, and 1.5 U Taq polymerase (Kapa Biosystems, Wilmington, USA). We amplified four chloroplast regions (*matK*, *trnL*, *psbA-trnH*, and *rps16-trnQ*) and the nuclear region ITS/18S/26S, with the primers listed in table 2. The PCR cycling conditions for *matK*, *psbA-trnH*, and *trnL* were an initial denaturation for 4 min at 95°C; followed by 35 cycles consisting of denaturation for 45 s at 94°C, annealing for 1 min at 56°C (50°C for *trnL*), 1 min at 72°C; and finally 10 min at 72°C. For *rps16-trnQ*, the process was an initial denaturation at 80°C for 5 min followed by 30 cycles of denaturation at 95°C for 1 min, and annealing at 50°C for 1 min, followed by a ramp of 0.3°C/s to 65°C, and primer extension at 65°C for 4 min; followed by a final extension step of 5 min at 65°C (Shaw et al. 2007). For ITS, the process was 4 min at 95°C; followed by 35 cycles consisting of denaturation for 45 s at 94°C, annealing for 1 min at 56°C, 1 min at 72°C; and finally 10 min at 72°C. PCR products were purified by treatment with ExoSAP enzyme (Fermentas, St. Leon-Rot, Germany) and sequenced in an automatic sequencer ABI 3730 by capillary electrophoresis with ABI BigDye Terminator version 3.1 kit (Applied Biosystems Inc., Foster City, CA, USA).

The consensus sequences were assembled using the Sequencer v.4.1 (GeneCodes Corporation, Ann Arbor, MI, USA), the alignment was done using Mafft-win v.7.221 (Katoh & Standley 2013). Estimation of variables and nucleotide composition sites were made using the program MEGA v.7.0.26 (Kumar et al. 2016). All sequences used as outgroups and four sequences of *Parkia* were downloaded from GenBank from previous studies of the mimosoid clade (Luckow et al. 2003; Simon et al. 2016; LPWG 2017; Ribeiro et al. 2018). Voucher information, taxon author, and GenBank accession numbers of all sequences used in this study are provided in supplementary file 1. For species not included in the analysis, the names of the authors are provided when first quoted.

Divergence time estimates

Lineage divergence times were estimated using Bayesian inference as implemented in BEAST v.2 (Bouckaert et al. 2014). A dataset of 30 accessions of species *Parkia* was generated for the BEAST analysis, consisting of one accession per species. All species used were considered to be monophyletic based on analyses of Maximum Parsimony, Maximum Likelihood, and Bayesian Inference (Oliveira 2015, 2020; Oliveira et al. 2021). Accessions with sequences for all markers were preferentially selected over less complete accessions with only one or two marker sequences available. Sequence data were partitioned into both nuclear and plastid sequences, with the GTR+I+Γ nucleotide substitution model used for both partitions, selected using the Akaike information criterion (AIC) implemented in jModeltest v.2.1.7 (Posada 2008; Darriba et al. 2012). We chose to generate a single tree for the two datasets of nuclear and chloroplast DNA, based on previous analyses recover the same topology for different data sets (Oliveira 2015, 2020; Oliveira et al. 2021). The Calibrated Yule model was used as the tree prior and the Uncorrelated Lognormal Distribution (UCLD) was used as the clock model prior. The tree was calibrated using a calibration point at the root obtained from Simon et al. (2009) (node “Q” in their analysis). This is derived from the age of the oldest known fossil pollen polyads for the Acacieae-Ingeae tribes, and has been used in dating phylogenies in previous studies of the mimosoids (Lavin et al. 2005; Bruneau et al. 2008; Simon et

Table 3 – Observations and references to known floral visitors/pollinators to species of *Parkia*. An asterisk indicates pollinator has been physically seen. Question mark indicates unobserved pollinator inferred from inflorescence morphology.

| Species | Location | Pollinator | Reference |
|----------------------------|------------------|---|---|
| <i>Parkia bahiae</i> | Brazil | Phyllostomidae bats | Hopkins 1986 |
| <i>P. barnebyana</i> | Brazil | Phyllostomidae bats (?) | Hopkins 2000b |
| <i>P. bicolor</i> | Ghana | Pteropodidae bats | Grünmeier 1990 |
| <i>P. biglandulosa</i> | India | Pteropodidae bats | Singaravelan et al. 2007 |
| <i>P. biglobosa</i> | Burkina Faso | Pteropodidae bats | Baker & Harris 1957; Hopkins 1983 |
| <i>P. cachimboensis</i> | Brazil | Phyllostomidae bats* | Carvalho 1960; Hopkins 1984; Vogel 1968 |
| <i>P. decussata</i> | Brazil | Phyllostomidae bats | Carvalho 1960; Hopkins 1984; Vogel 1968 |
| <i>P. discolor</i> | Brazil | Phyllostomidae bats | Carvalho 1960; Vogel 1968; Hopkins 1986 |
| <i>P. filicoidea</i> | Kenya | Pteropodidae bats | Baker & Harris 1957; Hopkins 1983 |
| <i>P. gigantocarpa</i> | Brazil | Phyllostomidae bats | Carvalho 1960; Vogel 1968; Hopkins 1986 |
| <i>P. igneiflora</i> | Brazil | Phyllostomidae bats* | Carvalho 1960; Vogel 1968; Hopkins 1986 |
| <i>P. leiophylla</i> | Thailand | Pteropodidae bats | Gould 1978 |
| <i>P. lutea</i> | Brazil | Phyllostomidae bats (?) | Hopkins 2000c |
| <i>P. madagascariensis</i> | Madagascar | Pteropodidae bats | Vololona et al. 2020 |
| <i>P. multijuga</i> | Brazil | nocturnal microcoleoptera Thysanoptera* | Chaves 2015 |
| <i>P. nitida</i> | Brazil | Phyllostomidae bats* | Hopkins 1986 |
| <i>P. paya</i> | Borneo | Pteropodidae bats (?) | Hopkins 2000a |
| <i>P. panurensis</i> | Brazil | Phyllostomidae bats* | Carvalho 1960; Vogel 1968; Hopkins 1986 |
| <i>P. paraensis</i> | Brazil | Phyllostomidae bats | Carvalho 1960; Vogel 1968; Hopkins 1986 |
| <i>P. parvifoliola</i> | Palau | Pteropodidae bats | Gould 1978 |
| <i>P. pendula</i> | Brazil | Phyllostomidae bats* | Carvalho 1960; Vogel 1968; Hopkins 1986; Fischer 2000; Piechowski 2007; Piechowski et al. 2010 |
| <i>P. platycephala</i> | Brazil | Phyllostomidae bats* | Carvalho 1960; Vogel 1968; Hopkins 1986 |
| <i>P. reticulata</i> | Brazil | Phyllostomidae bats | Carvalho 1960; Vogel 1968; Hopkins 1986 |
| <i>P. singularis</i> | Brunei | Pteropodidae bats | Gould 1978 |
| <i>P. speciosa</i> | Borneo | Pteropodidae bats | Danser 1929; van Heurn 1929; Docters van Leeuwen 1933, 1938 |
| <i>P. sumatrana</i> | Sumatra | Pteropodidae bats | Gould 1978 |
| <i>P. timoriana</i> | Indonesia | Pteropodidae bats | Gould 1978 |
| <i>P. ulei</i> | Brazil | <i>Trigona</i> sp., <i>Apis mellifera</i> * | Chaves 2015 |
| <i>P. velutina</i> | Brazil | <i>Megalopta</i> sp.* | Hopkins et al. 2000 |
| <i>P. versteeghii</i> | Papua New Guinea | Pteropodidae bats | Gould 1978 |

al. 2009). A normal distribution with a mean of 0.5, standard deviation (SD) value of 1, and the offset value give the age of these fossils as 45 Ma.

The Markov Chain Monte Carlo simulations (MCMC) were performed in two runs of 10,000,000 generations, recording the tree parameters every 1000th generation. Tracer v.1.6 (Rambaut & Drummond 2009) was used to check if sample sizes and estimated values (ESS) were > 200, and the results were then assessed to have reached stationarity and convergence. Data from multiple runs were combined after exclusion of burn-in trees. A maximum clade credibility (MCC) tree was calculated in TreeAnnotator v.1.8.0 (Drummond & Rambaut 2010). The MCC tree with 95%

highest posterior density (HPD) was visualized in FigTree v.1.4.2 (Rambaut 2014).

Ancestral state estimations

A Bayesian approach to reconstruct discrete ancestral states was implemented in BEAST and performed simultaneously with divergence time estimates according Lemey et al. (2009). The character states were coded as follows: (A) capitula with one flower type (fig. 1A); (B) capitula with two flower types (fig. 1D), (C) capitula with three flower types (fig. 1B–C), and (D) spike (for outgroups). We categorize the three types of capitula because different capitula have different syndromes. Pollination coding was as follows: (EP) entomophily and (CP) chiropterophily. The information

Table 4 – Characters used in the cluster analysis.

| Characters | |
|--------------------------|-------------------------------|
| Peduncle | 1. Erect 2. Pendent |
| Peduncle | 1. Up to 6 cm 2. 10 cm to 6 m |
| Staminodes | 1. Absent 2. Present |
| Staminodial fringe | 1. Absent 2. Present |
| Nectar-secreting flowers | 1. Absent 2. Present |
| Corolla tube | 1. 6–16 mm 2. 20–29 mm |
| Stamens | 1. Up to 6 mm 2. 7 to 31 mm |
| Anthers | 1. Dorsifixed 2. Basifixed |

on the character states for morphology and pollination systems was compiled from the literature (including Baker & Harris 1957; Vogel 1968; Hopkins 1983, 1984, 1986, 1994, 2000a, 2000b; Grünmeier 1990; Fischer 2000; Hopkins et al. 2000; Singaravelan et al. 2007; Bumrungsri et al.

2008; Piechowski et al. 2010; Chaves 2015; Vololona et al. 2020) and observations in the herbarium. Table 3 provides information and references of the visitors for 30 species used in the analyses. Species whose pollinators have not been studied in the field were scored as either chiropterophilous or entomophilous on the basis of their morphology, primarily the structure of the capitulum, and hence their similarity to known bat or insect-pollinated species.

Morphological cluster analyses

To determine the extent to which various characters that are related to pollination consistently indicate the same groups that were found by the phylogenetic analysis, we scored character states for seven floral features (table 4) for all 30 species included in our study. The information on the character states were observed in material collected by us and preserved in 70% alcohol, in material from the herbarium, and compiled from the literature (Hopkins 1983, 1984, 1986, 1994, 2000a, 2000b). We then performed a Euclidean

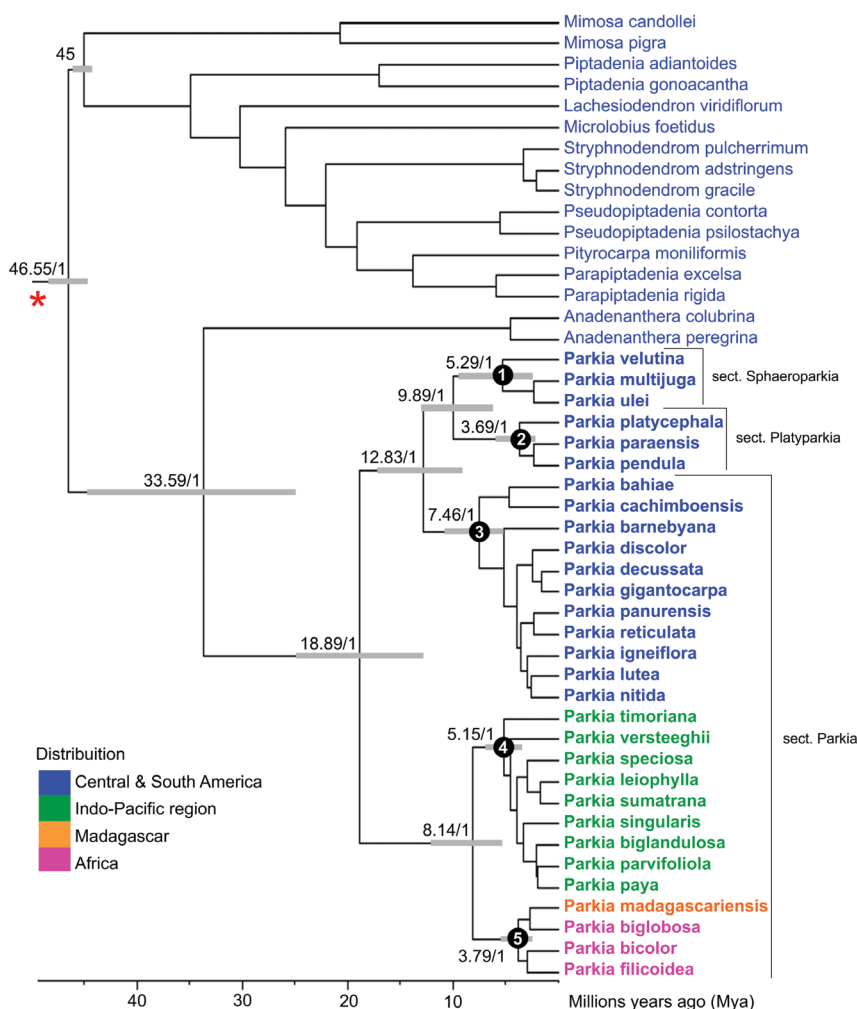


Figure 2 – Chronogram with the divergence time estimations of 30 species of *Parkia* and 16 outgroups based on DNA sequences of the *matK*, *trnL*, *psbA-trnH*, and *rps16-trnQ* non-coding plastid loci and ITS/18S/26S nuclear region. Numbers are age (Ma) and Bayesian posterior probability (PP), respectively. Gray bars corresponds to the 95% highest posterior density (HPD).

distance-based cluster analysis for the data matrix using the software R v.4.0.3 (R Core Team 2020). A table with the raw data of each character for each species used in this study are provided in supplementary file 2.

RESULTS

Divergence time and geographical occurrence

The divergence time estimations are shown in fig. 2. Our analysis indicates that *Parkia* last shared a common ancestor with any of the mimosoid outgroups that we included at 33.59

Ma (95% HPD 35.33–22.4 (fig. 2). The node representing the last common ancestor (LCA) of all the currently extant ancestral species of the genus is dated at 18.89 Ma (95% HPD 13.44–24.31 Ma; fig. 2). The Neotropical and Paleotropical clades diverged at around 18.89 Ma (95% HPD 13.44–24.31 Ma) and so this is the likely date of dispersal from the Neotropics to the Paleotropics. The Neotropical clade subsequently split into clades (1 + 2) and clade 3 at 12.83 Ma (95% HPD 9.13–16.87 Ma). The split between clade 1 and clade 2 is estimated to have occurred at 9.89 Ma (95% HPD 6.78–13.18 Ma), and the divergence of extant lineages in clade 3 started at 7.46 Ma (95% HPD 4.82–10.35 Ma). In

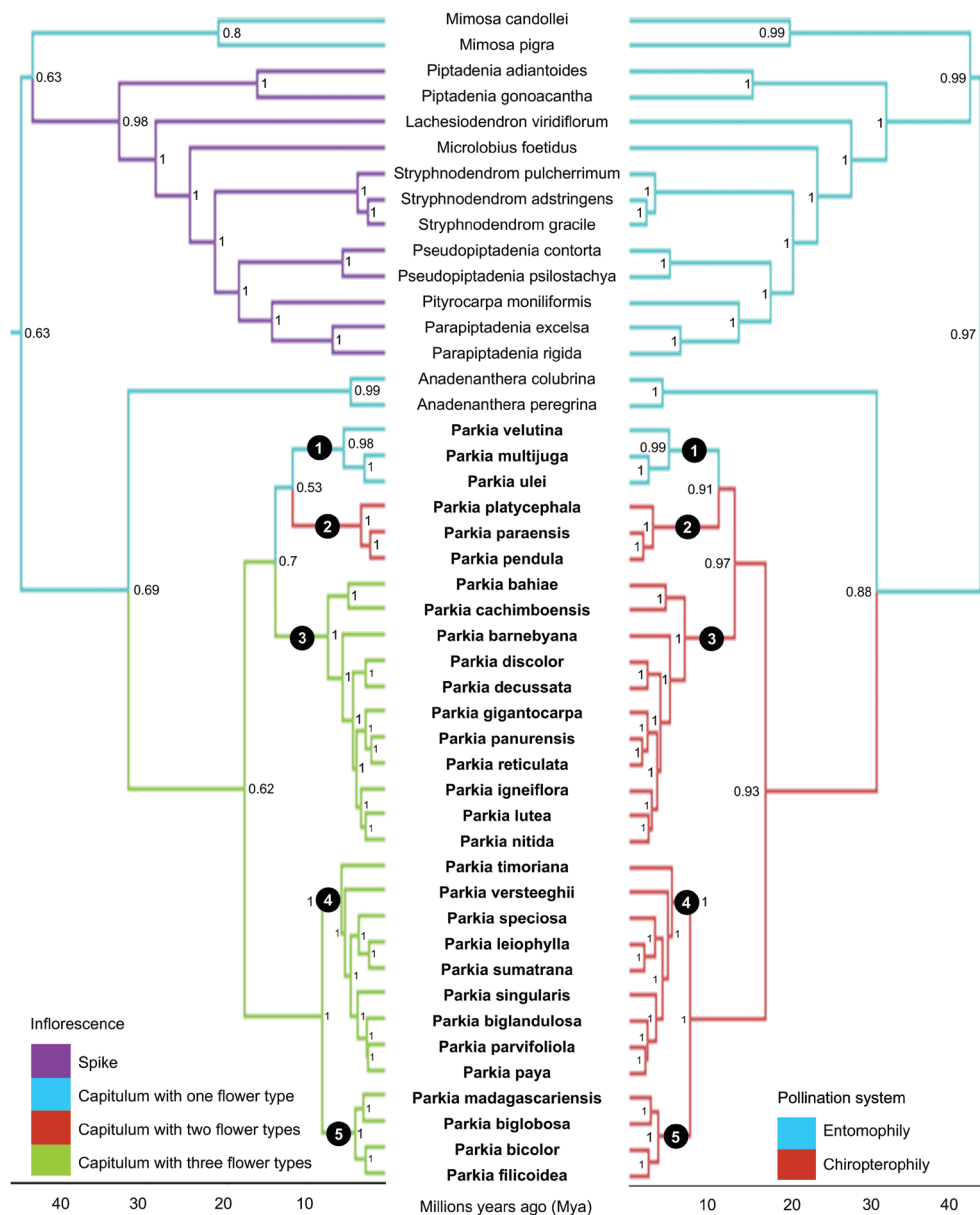


Figure 3 – Ancestral state reconstruction on the chronogram with the divergence time estimations of 30 species of *Parkia* and 16 outgroups based on DNA sequences of the *matK*, *trnL*, *psbA-trnH*, and *rps16-trnQ* non-coding plastid loci and ITS/18S/26S nuclear region. Colours of the lines and numbers are Bayesian posterior probability (PP) corresponds to the type of inflorescence and the pollination syndrome. The enumeration of clade 1 to 5 corresponds to the phylogenetic clades in fig. 2.

the Paleotropics, clades 4 and 5 diverged at around 8.14 Ma (95% HPD 5.6–10.85 Ma), with the current diversification in clade 4 occurring around 5.15 Ma (95% HPD 3.39–6.9 Ma) and in clade 5 at around 3.79 Ma (95% HPD 2.02–5.81 Ma).

Ancestral state estimation

The reconstruction of ancestral character states for the capitula are shown in fig. 3 (left). Our analyses suggest that the last common ancestor of extant *Parkia* species had a capitulum consisting of three types of flowers: fertile flowers, nectar-secreting flowers, and sterile flowers with staminodia; present in all species in clades 3, 4, and 5. A transition to a capitulum composed of only fertile flowers occurred in clade 1. A capitulum consisting of fertile flowers and nectar-secreting flowers, which is present in clade 2, is also a derived condition from a three-flowered ancestor.

Ancestral character states for the pollination system are shown in figure 3 (right). Chiropterophily is the ancestral

condition present in all species in clades 2 to 5. The entomophily a derived condition present in three species in clade 1.

Morphological cluster analyses

The cluster analysis recovered four groups that can be morphologically characterised as follows (fig. 4):

Cluster 1: Neotropical species grouped in phylogenetic clade 3; pendent peduncle 10 cm to 6 m, nectar-secreting and staminodial flowers, staminodial fringe well developed, corolla tube 20–29 mm, stamens up to 6 mm and anthers basifixed.

Cluster 2: Paleotropical species in phylogenetic clades 4 and 5; pendent peduncle 10 cm to 6 m, nectar-secreting and staminodial flowers, corolla tube 20–29 mm, stamens up to 6 mm and anthers basifixed.

Cluster 3: Neotropical species in phylogenetic clade 2; pendent peduncle 10 cm to 6 m, nectar-secreting and staminodial flowers, corolla tube 20–29 mm, stamens 7–31 mm and anthers basifixed.

Cluster 4: Neotropical species in phylogenetic clade 1; erect peduncle up to 6 cm, corolla tube 6–16 mm, stamens up to 6 mm and anthers dorsifixed.

In our cluster analysis, the characters that are clearly associated with chiropterophily are: pendent peduncle up to 6 m (except *Parkia decussata*); nectar-secreting and staminodial flowers; staminodial fringe well developed; corolla tube long (20–29 mm); stamens 7–31 mm and anthers basifixed. In contrast, the characters that are clearly associated with entomophilous species are: erect peduncle up to 6 cm, corolla tube short (6–16 mm), stamens up to 6 mm and anthers dorsifixed.

The morphological character states were specific for each pollination system and the morphological groups were mostly (except *Parkia decussata*) consistent with the relationships between species found in the phylogenetic analysis. The morphological grouping of the Neotropical species *Parkia decussata* in the clade of Paleotropical species can be explained by the absence, in this species, of the staminodial fringe (see Discussion).

DISCUSSION

Evolution of capitular morphology

The evolution of capitular morphology in *Parkia* show a transition of complex capitula consisting of three types of flowers towards simpler capitula composed of only fertile flowers. The capitula with two types of flowers (fertile flowers and nectar-secreting flowers), before considered as an intermediary form between the simpler and more complex type (Luckow & Hopkins 1995), can now be interpreted as derived from the more specialized capitula with three types of flowers. The evolution of floral morphology in *Parkia* has been directional. The three distinct types of capitula or flowers have evolved according to their pollinators. This is expected in flowers pollinated by animals. According to Stebbins (1974), the floral phenotype of a plant will evolve

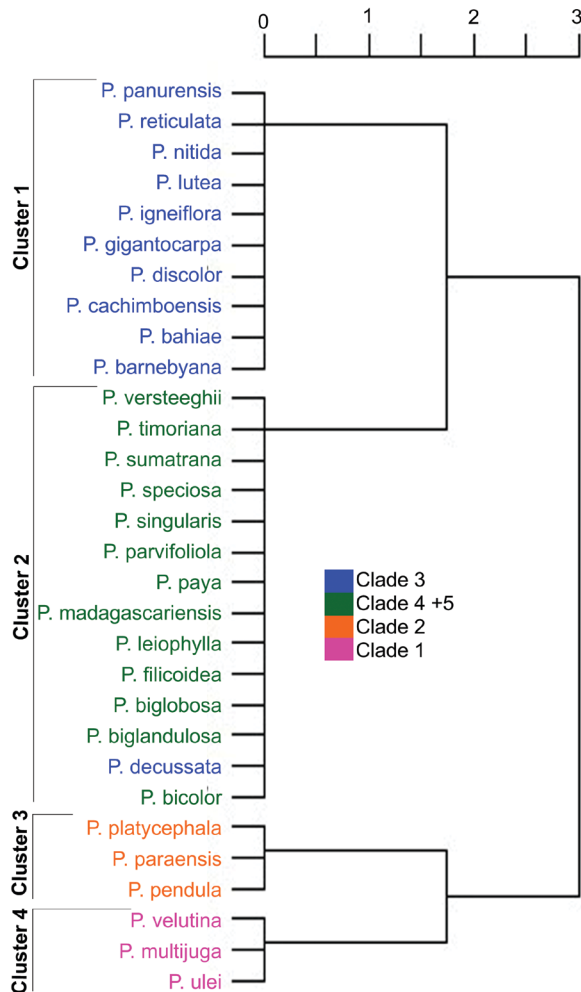


Figure 4 – Euclidean distance-based cluster dendrogram of morphological characters (see table 4) calculated in R. Height corresponds to the average distance between clusters. The colours of the species correspond to clade 1 to 5 of the phylogenetic clades in figs 2 and 3.

in response to the most frequent and effective pollinator, this is because for successful animal pollination the flower must become a highly integrated structure, with all of its parts precisely adjusted to one another. Our results contradict the popular belief in pollination biology, although floral morphology is directly associated with the morphology of its pollinators, in *Parkia*, specialization is not an evolutionary end (Tripp & Manos 2008).

For a relatively small genus, *Parkia* shows considerable variation in the structure, shape, and colour of the capitulum. This degree of variability is probably greater than in other genera of similar size in the mimosoid clade. Figure 3 shows that *Parkia* is differentiated by the structure of its inflorescences, from the most closely related genera in Leguminosae. For example, the capitular structures with two and three types of flowers appear to be unique among the mimosoid legumes. The mimosoid genus *Dichrostachys* has entomophilous inflorescences with staminodial flowers at the base and fertile flowers at the distal end and are bicolored as are some in *Parkia*, but lack the nectar-secreting flowers between the staminodial and fertile ones in *Parkia*.

The flower morphological characters and their implications for pollination

Our results of the cluster analysis show that floral morphology of *Parkia* is indicative of different pollination syndromes. Fenster et al. (2004) described pollination syndromes as a suite of floral traits associated with attraction, handling and resource use, and reflect adaptations to a particular pollinator or functional group of pollinators.

Among the characters listed by Faegri & van der Pijl (1979), large and robust inflorescences exposed beyond foliage (flagelliflory), nocturnal anthesis, strong nocturnal scent, and large amount of nectar are associated with pollination by bats in *Parkia*. In all chiropterophilous species, the capitula are held free from the foliage, on a long peduncle of up to 6 m in length, which projects beyond the crown. According to Tschapka & Dressler (2002), chiropterophilous flowers tend to grow very exposed and accessible to animals, as in *Mucuna holtonii* (Kuntze) Moldenke (Leguminosae), *Markea dressleri* D'Arcy (Solanaceae), and *Marcgravia nervosa* Triana & Planch. (Macgraviaceae). In *Parkia*, bat-adapted flowers show nocturnal anthesis and emit a strong, distinctive scent (Hopkins 1998). The nectar-secreting flowers produce large amounts of nectar (5–12 ml per night), with a high concentration of sugar (Grünmeier 1990; Piechowski 2007), this is the principal recompense for bat-pollinators in these species. Most chiropterophilous species (29 of the 32 species) have staminodial flowers. In the Neotropical species, staminodial flowers form a fringe (except in *P. decussata*; fig. 1C). According to Hopkins (1983) the staminodial fringe changes the shape of the capitulum, perhaps allowing bats to differentiate between open capitula and buds. In addition, it provides a landing pad for bats, as it would be more easily grabbed by the bats' feet than a thin, waving peduncle. Furthermore, hiding the nectar in a distant floral type forces the bat to bend over the fertile flowers, and thus forces contact with the fertile flowers. In Paleotropical species, the widest part of the capitulum is

composed of fertile flowers providing the landing platform. In these species, the bats land on the capitula head upwards, grabbing the broad, fertile part of the capitulum with their feet, so that a fringe as a landing pad is unnecessary (fig. 1H). In the chiropterophilous capitula without staminodes, the bats land on the capitula head downwards, grabbing the fertile part of the capitulum (fig. 1F). A character that appears directly related with pollination in *Parkia*, is the form of anther insertion in the stamens. Luckow & Hopkins (1995) were the first to relate basifixed anthers with chiropterophily in *Parkia*. According to these authors basifixed anthers may be more resistant to mechanical damage by large mammalian pollinators.

The bat-pollinated species have distinct capitula, although it can be predicted that the different types of capitula would correspond to different pollinators, current data do not support this hypothesis (Hopkins 1984). There is an overlap of pollinators between the two types of capitula in the Neotropics, and the change in pollinators, occurs within of capitulum with three types of flowers.

Entomophilous capitula differ from bat-adapted species mainly in the absence of flowers specialized in the production of nectar and staminodes. Some characters were retained from the ancestral syndrome, such as nocturnal anthesis (except in *Parkia ulei*) and strong scent. The peduncles are short and erect; the capitula are minors and delicate (Hopkins et al. 2000). There is no record of nectar production in these species; pollen is the main, and perhaps the only floral reward for pollinators. Unlike chiropterophilous species, the anthers are dorsifixed in the entomophilous species. According to Luckow & Hopkins (1995), dorsifixed anthers in mimosoids tend to be quite fragile and break off easily at the point of attachment to the filaments.

Whether syndromes accurately reflect true pollinators is the subject of debate. While several studies have found support for the traditional view that syndromes do reflect pollination by particular animal groups (Wilson et al. 2004; SanMartin-Gajardo & Sazima 2005; Whittall & Hodges 2007) some studies have questioned the value of recognising systems and using them to predict pollinators. According to Faegri & van der Pijl (1979), the different characters of a particular syndrome are not necessarily exclusive, thus can appear in more than one syndrome. Other studies again, concluded that generalization in plant-pollinator interactions is more common than previously thought (Armbruster 1996; Waser et al. 1996; Olesen & Jordano 2002; Zhang et al. 2005; Linder et al. 2006; Smith et al. 2008). The pattern found in *Parkia* mostly supports the traditional hypothesis.

Reconstruction of the ancestral pollination system

Our reconstruction of the ancestral pollination system in *Parkia* showed that the last common ancestor of the extant clades had a chiropterophilous syndrome (with three functional types of flowers per capitulum), and there has been at least one transition from chiropterophily to entomophily, in which the specialised nectar-secreting and staminodial flowers were lost.

Although a transition to entomophily has occurred, pollination by bats is maintained in most lineages of the

genus, occurring in 16 Neotropical species, and retained in the four African and 12 Asian species. In the Neotropical region, *Phyllostomus discolor* Wagner, 1843 is the most important pollinator in different habitats. In Indo-Pacific region, *Eonycteris spelaea* (Dobson, 1871) is considered to be the main bat visitor, and probably the most effective pollinator in the region (Acharya et al. 2015). These bats resemble species of *Phyllostomus* in terms of their size and behaviour at flowers (Docters van Leeuwen 1933; Gould 1978). In Africa, flower visitors include *Epomophorus gambianus* (Ogilby, 1835), *Nanonycteris veldkampii* (Jentink, 1888), *Eidolon helvum* (Kerr, 1792), and *Micropteropus pusillus* (Peters, 1868) (Baker & Harris 1957; Hopkins 1983), as well as the more specialised *Megaloglossus woermanni* Pagenstecher, 1885 (Grünmeier 1990). These bat pollinators, for the most part, are less specialised and more opportunistic bats. *Parkia* has diversified in the tropics, and according to Ollerton (2017), tropical communities possess, on average, a greater diversity of functionally specialized plant-pollinator interactions, possibly because of the relatively low bee diversity in the tropics.

The transition to entomophily occurs in only three species. *Parkia ulei* is pollinated by diurnal bees (Hopkins 1986; Chaves 2015). *Parkia velutina* is visited by nocturnal bees of the genus *Megalopta* (Hopkins et al. 2000). In *P. multijuga*, the principal visitors are nocturnal microcoleoptera and Thysanoptera (Chaves 2015). These three species seem to have retained some characters associated with chiropterophily in the genus, such as nocturnal anthesis (except in *P. ulei*) and strong scent. Changes in floral characters associated with one or another specialized pollinator can be designated via an intermediate stage, characterizing intermediate pollination systems (Stebbins 1970; Muchhala 2003). Intermediate pollination systems seem to be a relatively frequent event during the evolution of flowering plants, as in *Siphocampylus sulfureus* E.Wimm. (Lobeliaceae) (Sazima et al. 1994), *Abutilon* species (Malvaceae) (Buzato et al. 1994), species of *Burmeistera* (Campanulaceae) (Muchhala 2003), and *Paliavana tenuiflora* Mansf. (Gesneriaceae) (Ferreira 2008). However, although the entomophilous species show intermediate characters between insect and bat syndromes, these species are visited and pollinated only by insects (Hopkins 1986; Hopkins et al. 2000; Chaves 2015).

Bat pollination is one of the most specialized pollination systems, so it has been considered an evolutionary dead end, which means that it can rarely be replaced by another syndrome and, in many cases evolved secondarily from adaptation to other large pollen vectors, such as birds, sphingid moths, and carpenter bees (Stebbins 1970). The transition from entomophily or ornithophily to chiropterophily appears to be a more common evolutionary event (Stebbins 1970; Tripp & Manos 2008; Muchhala & Thomson 2010; Gómez et al. 2014). In fact, the reverse transition from chiropterophily to entomophily has been little documented. In addition to *Parkia*, only the genus *Cayapania* (Cucurbitaceae) presented twice a transition from chiropterophily to entomophily (Vogel 1969; Heithaus 1979; Ramírez 2004; Duchen & Renner 2010). Thus, disagreeing with the common hypothesis that specialized systems are

derived (Muchhala & Thomson 2010; Gómez et al. 2014), chiropterophily is not an evolutionary dead end in *Parkia*.

Pantropical bat pollination in *Parkia*

Our fossil-calibrated Bayesian analysis dated the Neotropical chiropterophilous lineage of *Parkia* at ca 18.85 Ma in the Middle Miocene. African and Asian chiropterophilous clades share an immediate common ancestor after their separation from the America lineage (at 18.85 Ma). In the Neotropics, the bat pollinators are species of Phyllostomidae, and in the Paleotropics, they belong to the family Pteropodidae (Hopkins 1986). The diversification dates for Phyllostomidae and Pteropodidae are consistent with the diversification dates of *Parkia* in the Neotropics and Paleotropics. Phyllostomidae have a crown age of ca 35 Ma (Giannini & Velazco 2020) and diversified at the end of the Middle Miocene (15–20 Ma), by when most genera had appeared (Datzmann et al. 2010; Rojas et al. 2011, 2012, 2016; Arita et al. 2014). Jones et al. (2005) detected two significant changes in the rate of diversification in Phyllostomidae in the Chiroptera supertree (Jones et al. 2002) and suggested a relationship between this diversification events and the rate of diversification in flowering plants. Pteropodidae are older than Phyllostomidae, dating from approximately 39 Ma (Teeling et al. 2005). Some lineages of fruit bats from the Indo-Pacific region date from the Oligocene at approximately 31 Ma, with diversification events from the Miocene to the Pleistocene (Almeida et al. 2009, 2016). Recent phylogenetic studies indicate that Pteropodidae probably repeatedly colonized Africa from Asian ancestors (Almeida et al. 2016). Estimates of divergence dates suggest that these events occurred in different periods and that although local diversification appears to have started in the late Miocene, the more extensive diversification that produced the modern fauna occurred much later, in the Pleistocene (Almeida et al. 2016).

Chiropterophily in Pantropical genera is very rare, and apart from *Parkia*, it is known only in *Mucuna* (Leguminosae) and *Cayapania* (Cucurbitaceae) (Duchen & Renner 2010; Moura et al. 2016). *Mucuna* is a genus largely composed of climbers that contains chiropterophilous and ornithophilous species. The genus appears to have originated in tropical Asia and achieved its wide distribution through numerous dispersal events, including ones to the Neotropics at ca 12 and 6 Ma (Moura et al. 2016). The liana genus *Cayapania* is distributed across the American continent, Africa, and Madagascar, and has pollination by bats and bees (Vogel 1969; Heithaus 1979; Ramírez 2004; Duchen & Renner 2010). Also, the tree genus *Ceiba* (Malvaceae), although largely Neotropical, contains a single amphiatlantic, bat-pollinated species, *C. pentandra* (L.) Gartn., which is native in the New World and west tropical Africa, and introduced elsewhere in the tropics, including Asia. Its amphiatlantic distribution is also the result of recent long-distance dispersal (Dick et al. 2007). The rarity of genera that contain chiropterophilous species in both the Neotropics and Paleotropics means that *Parkia* provides a unique example in which to examine the evolution of bat-pollination in a Pantropical genus of trees.

Why was bat pollination not an evolutionary dead end in *Parkia*?

Although bat-pollinated flowers belong to numerous families and cover a considerable range in morphology, size, and structure (Dobat & Peikert-Holle 1985; Fleming et al. 2009), in general, they have specialisations that result in a distinct chiropterophilous syndrome (Faegri & van der Pijl 1966; Tschapka & Dressler 2002). Genera in which chiropterophily is said to be an evolutionary dead end commonly have relatively small, tubular flowers that attract a restricted range of highly specialised nectarivorous bats as pollen vectors. These bats are small, often with long snouts and tongues and they frequently (though not always) feed while hovering. In the Neotropics, they belong to the subfamilies Glossophaginae and Lonchophyllinae (Fleming et al. 2009). The bat-pollinated species of *Parkia* also have many morphological and behavioural adaptations to chiropterophily but their capitula have brush-like structure and are, for the most part, visited by less specialised and more opportunistic bats. This may explain, at least in part, why this mode of pollen transfer has not been an evolutionary dead end in *Parkia*.

SUPPLEMENTARY FILES

Supplementary file 1 – Voucher specimens for DNA sequences used in this study. For each entry in the following list, the species name and author, collection locality, collector's name and number for the voucher, herbarium acronym, and GenBank accession numbers are reported.

<https://doi.org/10.5091/plecevo.2021.1845.2565>

Supplementary file 2 – Characters used in the cluster analysis.

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