Transitions between biomes are common and directional in Bombacoideae (Malvaceae)

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Aim: To quantify evolutionary transitions between tropical evergreen rain forest and seasonally dry biomes, to test whether biome transitions affect lineage diversification and to examine the robustness of these results to methodological choices.

Location: The tropics.

Time period: The Cenozoic.

Major taxa studied: The plant subfamily Bombacoideae (Malvaceae).

Methods: We inferred ancestral biomes based on a fossil-dated molecular phylogeny of 103 species (59% of the clade) and recorded the number of transitions among biomes using biogeographical stochastic mapping based on the dispersal-extinction-cladogenesis model. We then estimated diversification rates using state-specific speciation and extinction rate (SSE) methods. Furthermore, we tested the sensitivity of the results to model choice, phylogenetic uncertainty, measurement error and biome definition.

Results: We found numerous transitions from evergreen rain forest to seasonally dry biomes, and fewer in the opposite direction. These results were robust to methodological choices. Biome type did not influence diversification rates, although this result was subject to uncertainty, especially related to model choice and biome definition.

Main conclusions: Our results contradict the idea of evolutionary biome conservatism in Bombacoideae, and support previous findings that evergreen rain forests serve as a source for the flora of seasonally dry biomes. The impact of biome classification and biome definition on the results suggest caution when using a biome concept for biogeographical reconstruction and diversification rate analysis.

KEYWORDS: biome connectivity, biome shift, diversification, rain forest, seasonality, seasonally dry biomes, tropical biodiversity
Evolutionary transitions among biomes have been suggested as critical for the generation of plant diversity (Donoghue & Edwards, 2014). In particular, a high connectivity of biota among biomes in tropical America (the Neotropics) might have been essential for the assembly of its globally outstanding diversity (Antonelli et al., 2018; Zizka, 2019). Biomes are vegetation units defined by functionally similar plant groups and similar environmental conditions (Moncrieff, Hickler, & Higgins, 2015). Although the definition, meaning and delimitation of biomes remain controversial (Moncrieff et al., 2015; Mucina, 2019), they are often used to understand how broad-scale ecological niches change in evolutionary time (e.g. Bacon, 2013).

Generally, species tend to retain their ancestral ecological niche over time (Wiens & Donoghue, 2004). Therefore biome transitions – the shift of evolutionary lineages into new biomes – have been considered rare, especially in some biomes such as seasonally dry tropical forests (Crisp & Cook, 2012; Gagnon, Ringelberg, Bruneau, Lewis, & Hughes, 2019). Environmental dissimilarity might be a major factor limiting interchange of plant lineages among biomes (Crisp et al., 2009) and within the frost-free tropics, seasonal water availability may be particularly important constraining factor (Hughes, Pennington, & Antonelli, 2013; Olmstead, 2013).

Three major lowland tropical biomes are evergreen tropical rain forests, seasonally dry tropical forest and savanna (including tropical grass- and shrublands). Despite key differences in some ecological drivers (e.g. fire frequency), savanna and seasonally dry forest share a marked seasonality in water availability, which sets them apart from evergreen rain forest (Pennington, Lehmann, & Rowland, 2018). This raises a broader question of transitions into and out of regions with a seasonally dry climate (Pennington et al., 2018).

Previous studies give a mixed picture of the prevalence of biome transitions among seasonally dry biomes and evergreen tropical rain forests in plants. On the one hand, the phylogenetic composition of seasonally dry and rain forest sites across the tropics suggests a separation of these environments through evolutionary time (Slik et al., 2018). This could reflect that rainfall seasonality is a major determinant of the floristic composition of tropical forests (e.g. Morley, 2000), and dry climate is an important phylogenetic constraint to plant evolution for many lineages (Cássia-Silva, Freitas, Alves, Bacon, & Collevatti, 2019; Olmstead, 2013; Qian, Jin, & Ricklefs, 2017). However, transitions from evergreen rain forest to seasonally dry biomes, especially savanna, are known from plant lineages in the Neotropics (Bacon, Moraes R., Jaramillo, & Antonelli, 2017; Lohmann, Bell, Calió, & Winkworth, 2013), Africa (Estrella, Forest, Wieringa, Fougère-Danezan, & Bruneau, 2017; Monthe et al., 2019; Veranso-Libalah, Kadereit, Stone, & Couvreur, 2018), and Australia (Crisp et al., 2019) and the floristic study of Dexter et al. (2015) suggests permeability of lowland tropical biomes globally.

1. Transitions from evergreen rain forests into seasonally dry biomes occur multiple times and are more common than vice-versa. We expect this because of the older age of evergreen rain forests and results from prior studies identifying savannas as lineage sinks, especially in the Neotropics (Donoghue & Edwards, 2014; Freitas, Bacon, Souza-Neto, & Collevatti, 2019; Pennington & Hughes, 2014).

2. Transitions from evergreen rain forest to seasonally dry biomes increased net diversification due to competitive release and adaptive radiation. An increased diversification after biome transitions has been documented in other lineages (Cardillo et al., 2017; Souza-Neto, Cianciaruso, & Collevatti, 2016).

2 | MATERIALS AND METHODS

2.1 | Study group

The Bombacoideae is distributed across all tropical regions, with approximately 90% of its species richness in the Neotropics, thus reflecting the general pattern of globally outstanding plant diversity in this region. Furthermore, the Bombacoideae occur in a variety of different habitats suggesting multiple biome transitions ensuring a sufficiently large sample to estimate directionality in transitions.

We followed the taxonomy of Bombacoideae by Robyns (1963) updated with recent revisions when necessary (www.tropicos.
comprising (Lima & Salard-Cheboldaeff, 1981) at the crown node of the clade
Herrera, Jaramillo, Wing, & Callejas, 2011); and (b) a flower fossil
crown node of the clade comprising Malvoideae and Bombacoideae
We dated the phylogeny using two fossil calibrations: (a) a macro-fossil of
Donoghue, 2015). We dated the phylogeny using two fossil calibra-
tions are most often used (e.g. Warnock, Parham, Joyce, Lyson, &
age, where non-uniform (e.g. exponential) prior probability den -
Pseudobombax and Bombacoideae (Carvalho-Sobrinho et al., 2016), we constrained
more distant outgroups to root the tree. To enable dating of the
Neobuchia lata (Figure S2.1, Appendix S2).
We used BEAST v. 1.8.1 (Drummond, Suchard, Xie, & Rambaut, 2012) for phylogenetic inference and divergence time estimation,
with an MCMC of 200 million generations, sampling every 10,000
with a burn-in of 10% chosen after checking chain convergence. We
used an uncorrelated lognormal molecular clock model, a Yule pure-
birth speciation model with no starting tree, the GTR + γ model of nucleotide substitution with four rate categories, and default oper-
ators. We chose an uncorrelated clock, due to the combination of
chloroplast and nuclear markers. We included Chiranthodendron
pentadactylon, Fremontodendron californicum, Hampea appendicu-
lata, Patinoa sphaerocarpa, Pentaplaris doroteae and Phragmoretheca
ecuadorensis; as well as Sterculia lanceolata and Sterculia nobilis as
more distant outgroups to root the tree. To enable dating of the
cladest as presented in the most recent phylogenetic revision of the
Bombacoideae (Carvalho-Sobrinho et al., 2016), we constrained the
monophyly of three clades: (a) Ceiba, (b) the clade comprising Pseudobombax and Pochota, and (c) the clade comprising Ceiba, Neobuchia, Pochota and Pseudobombax (Figure S2.1, Appendix S2).
The use of fossils allows for a minimum constraint on a clade’s
age, where non-uniform (e.g. exponential) prior probability dens-
ities are most often used (e.g. Warnock, Parham, Joyce, Lyson, &
Donoghue, 2015). We dated the phylogeny using two fossil calibra-
tion points: (a) a macro-fossil of Malvaciphyllum macondicus (Wing,
Herrera, Jaramillo, Gómez-Navarro, & Labandeira, 2009) at the
crown node of the clade comprising Malvoideae and Bombacoideae
(exponential prior, mean 0.7 and an offset of 58 mya; Carvalho,
Herrera, Jaramillo, Wing, & Callejas, 2011); and (b) a flower fossil
(Lima & Saldar-Cheboldaeff, 1981) at the crown node of the clade
comprising Ceiba, Eriotheca, Pachira, Pochota, Pseudobombax and
Spirotheca (exponential prior, mean of 2.3, offset 41.3 mya, Figure
S2.1 in Appendix S2).

2.3 | Geographical distribution
We compiled geographical localities of Bombacoideae from our own
fieldwork and public databases (www.gbif.org, biendata.org, Gilles et
al., 2016; Conservatoire et Jardin Botaniques de la Ville de Genève &
South African National Biodiversity Institute Pretoria, 2017; Schmidt
et al., 2017). We used the rgbif package v1.1.0 (Chamberlain, 2017)
in R (R Core Team, 2019) to obtain records from www.gbif.org (GBIF.
org, 2018). We only included records filed as the accepted species names and used the taxize R-package v0.9.5 (Chamberlain & Szöcs,
2013) to resolve spelling errors in the species names. We merged
sub-specific ranks under the accepted species name, and restricted
species’ occurrences to the native species range on a regional level
based on our field experience and the literature. We retained only
one record per species per site and cleaned occurrence records geo-
graphically using the CoordinateCleaner R-package v2.0–7 (Zizka et
al., 2019). To visualize the global species richness of Bombacoideae,
we generated species ranges from the occurrence records using
geospheric convex hulls clipped to coastlines using the CalcRange
function of the speciesgeocodeR R-package v2.0–10 (Töpel et al.,
2017), using a 50km buffer for species with less than 3 occurrences.

2.4 | Biome classification
Based on the occurrence records and a widely used global biome
definition (Olson et al., 2001), we classified species as: evergreen
rain forest present in ‘Tropical and subtropical moist broadleaf for-
est'; seasonally dry forest present in ‘Tropical and subtropical dry
broadleaf forests' or ‘Deserts and xeric shrublands'; or savanna pre-
sent in ‘Tropical and subtropical grasslands, savanna and shrubland'.
To account for outlier individuals and imprecision in geographical co-
ordinates, we counted a species as present in a biome, if at least 5% of
its records occurred there. We treated seasonally dry forest and
savanna differently for the ancestral state reconstruction, because
they differ in their ecology (Pennington et al., 2018, i.e. the presence
of fire) and because they might differ in their affinities with evergreen
rain forest. However, since we were interested in transitions among
evergreen rain forest and seasonally dry biomes we combined these
two biomes as seasonally dry biomes (SDB) for the estimation of di-
versification rates. We justify this with the potential importance of
rainfall seasonality in the diversification of flowering plants (Areces-
Berazain & Ackerman, 2017) and because Bombacoideae are often
used as indicator species of evergreen rain forest in the fossil record
(Morley, 2000). Furthermore, a more fine-scale biome classification
would lead to reduced statistical power and classification accuracy
of species to biomes (Silva de Miranda et al., 2018).

2.5 | Ancestral biome estimation
We used biogeographical stochastic mapping based on the dis-
persal-cladogenesis-extinction model (DEC) as implemented in
BioGeoBEARS v1.1.2 (Dupin et al., 2017; Matzke, 2016) to recon-
struct ancestral biomes on the phylogeny. Because there is good
evidence for an older age of evergreen forests as compared to sa-
vanna and seasonally dry tropical forest, as well as fossil evidence
that the Bombacoideae are ancestrally a rain forest group (Wing
et al., 2009), we used a time-stratified model together with an
areas-allowed-matrix, and limited the group to evergreen rain forest before the Miocene (23.03 mya). We used 1,000 stochastic replicates on the maximum clade credibility tree from the BEAST analysis. To quantify the number of biome transitions (Hypothesis 1), we counted the number of transitions from evergreen rain forest into either seasonally dry biomes and vice versa, inferred by the biogeographical stochastic mapping.

2.6 | Diversification rate estimation

We used the GeoHiSSE model (Caetano, O’Meara, & Beaulieu, 2018) as implemented in the GeoHisse function of the hisse v.1.9.6 R-package (Beaulieu & O’Meara, 2016), to estimate state-specific diversification and extinction rates from the phylogeny, and hence the impact of biomes on diversification (Hypothesis 2). GeoHiSSE estimates speciation and extinction rates dependent on geographical trait states, as well as transition rates among states while allowing for widespread ancestors and taking sampling frequencies into account. We chose GeoHiSSE above other SSE methods, since it (a) can account for widespread species, (b) can include ‘concealed traits’ and therefore is less prone to false positives (Caetano et al., 2018), and (c) has a limited number of parameters suited for our moderate-sized phylogeny. To account for incomplete phylogenetic sampling, we calculated the fraction of evergreen rain forest and seasonally dry biome species sampled in the phylogeny from the total number of Bombacoideae species and included this information using the sampling.f argument of the GeoHisse function (\( f_{EB} = 0.438, f_{SE} = 0.938, f_{widespread} = 0.767 \)).

To test the effect of biome state on diversification rate, we fitted five different models to the maximum clade credibility phylogeny, and compared their fit using the Akaike Information Criterion corrected for small sampling size (AICc): (a) a null model with no concealed trait and all rates equal, (b) a model with no concealed trait and all speciation and extinction rates equal, but different transition rates, (c) a canonical GeoSSE model with no concealed traits, but different speciation, extinction and transition rates, (d) a GeoHiSSE model with one concealed trait, equal speciation and extinction rates but different transition rates, and (e) a full GeoHiSSE model with one concealed trait and different speciation, extinction and transition rates.

2.7 | Reliability of the results

We tested the sensitivity of our conclusions to four potential caveats and sources of uncertainty.

1. Model choice. Model choice can affect the ancestral biome reconstruction and diversification rate estimates (Davis, Midford, & Maddison, 2013; Herrera-Alsina, Els, & Etienne, 2019). To test its effect on ancestral biome reconstruction, we reconstructed ancestral biomes using stochastic character mapping and the model-averaged marginal reconstructions from the GeoHiSSE models which we used to estimate diversification rates. To test the effect of model choice on diversification rate estimates, we ran analyses using the BiSSE (Maddison, Midford, & Otto, 2007), HiSSE (Beaulieu & O’Meara, 2016) and FiSSE (Rabosky & Goldberg, 2017) models.

2. Phylogenetic uncertainty. Phylogenetic relationships are never known with certainty, especially in groups undergoing rapid radiations. To test the effect of phylogenetic uncertainty we ran analyses, reconstructing ancestral biomes and estimating state-dependent diversification rates on 100 randomly sampled phylogenies from the posterior distribution of the phylogenetic reconstruction, using the same biome classification and model as for the main analyses.

3. Measurement error/biome classification. Assigning species to biomes is challenging, because it is unclear when a species should be considered as present in a biome (is one record sufficient?) and because of the low precision of biome delimitations, the uncertainty of geographical coordinates and interdigitation of biomes (e.g. gallery forests (rain forest) within savanna). To test the impact of these issues, we repeated the ancestral biome reconstruction and the diversification rate estimation across 100 stochastic replicates. For each replicate, we classified the recent species (the tips of the phylogeny) into biomes based on the fraction of collection records assigned to each biome, and otherwise used the same specifications as for the main analysis (‘record-based’ biome classification hereafter).

4. Biome definition. To evaluate the sensitivity of our conclusions to alternative biome definitions, we repeated the ancestral biome estimation and the diversification rate estimation using: (a) a biome definition based on remote sensing of phenology and leaf area index (Buitenwerf & Higgins, 2016, ‘phenology-based’ hereafter), where we considered species as occupying the evergreen rain forest biome if they occurred in pixels classified as the ‘Evergreen high leaf area index biome’ or ‘Mixed’ and as occupying seasonally dry biomes if they occurred in ‘Deciduous’ pixels, and (b) a classification based on our own experience in the field (‘expert-based’ hereafter). See Appendix S1 for the expert-based classification and Figures S2.2 and S2.3 in Appendix S2 for a map of the ecoregion-based and phenology-based definitions.

See Appendix S4 for more detail on the tests related to the ancestral biome reconstruction and Appendix S5 for more detail on the tests related to diversification rate estimation.

3 | RESULTS

3.1 | Temporal evolution and recent biogeography

We inferred the root age of the Bombacoideae between 53.5 and 59.3 mya (Ma), close to the fossil constraint for the crown node of Bombacoideae + Malvoideae (58 Ma). Most branches in the
reconstructed phylogeny were well supported, with some exceptions in *Ceiba*, *Eriotheca* and *Pachira* (Figure S2.3 in Appendix S2). The results suggested recent radiations (in the last 2.6 Ma) within *Ceiba* (including *C. crispiflora*, *C. erianthos*, *C. glaziovii*, *C. pubiflora*, *C. rubriflora*, *C. speciosa* and *C. ventricosa* and *Pseudobombax* (including *P. longiflorum*, *P. campestre*, *P. majus* and *P. petropolitanum*). We found 14,865 high-quality occurrence records for 172 species (98% of the Bombacoideae; max. 3,062 records for *C. pentandra*; median of 19 per species; Figure S2.4, Appendix S2 for geographical sampling intensity; Appendix S3 for species range maps). The range maps confirmed Amazonia and the Atlantic forest as centres of Bombacoideae diversity (Figure 1).

We found the majority of species to be evergreen rain forest biome (EFB) specialists (73, 42% all species with occurrence information) or generalists occurring in the EFB and seasonally dry biomes (SDB, 73 species). Some genera were enriched in rain forest specialists: *Catostemma* (12/71% of the genus), *Scleronema* (4/67%) and *Pachira* (25/56%). *Ceiba* (14/73%) was especially enriched in generalists. Fewer species were specialists in SDB (16/9%), especially *Adansonia* (4/50%) and *Pseudobombax* (5/20%). The results were dependent on the biome classification and definition. The proportion of specialists increased when using the record-based biome classification (generalist: 6%; EFB: 58%; SDB: 22%), and the expert-based biome definition (generalist: 2%; EFB: 49%; SDB: 50%, Table 1).

3.2 | Ancestral biome reconstruction and biome transitions

Biome transitions occurred multiple times in different groups of Bombacoideae (Figure 2), for instance, *Eriotheca*, *Ceiba* and *Pseudobombax*. The 95% quantile of recorded transitions from EFB to SDB ranged from 38–52 (mean 46) and 11–25 transitions from SDB to EFB (mean = 18; Table 1). The findings of multiple transitions between EFB and SDB, and more transitions from SDB to EFB were consistent across all uncertainty tests (Table 1, Appendix S4).

We found transitions towards SDB shortly after they were allowed in the model, in the early Miocene, with shifts to seasonally dry forest beginning slightly earlier (17.3–23.0 Ma) than to savanna (15.3–23.0 Ma). The first shifts back to EFB occurred later (2.5–17.4 Ma). The timing of the first transitions to SDB in the early Miocene were consistent across phylogenetic and biome classification uncertainty as well as biome definitions (Table 1). Alternative ancestral biome reconstructions using different methodology do not exclude transitions to SDB specialists already in the early Miocene, but suggests most transitions during the Late Miocene.

3.3 | Diversification rate analyses

We found no significant effect of biome state on the diversification rates. The best-fitting GeoHiSSE model had no trait-dependent speciation, unequal transition rates and one concealed trait. The lack of significant difference in diversification rates between EFB and SDB in the Bombacoideae was robust to phylogenetic uncertainty and biome classification (Table 2). However, this conclusion was sensitive to (a) model choice (HiSSE suggested higher diversification rates in SDB, whereas FiSSE rejected state-dependent diversification), (b) phylogenetic uncertainty (only 65% of the replicates agreed on the same model), and (c) biome definition (the phenology-based definition suggested higher rates in EFB whereas the expert-based definition suggested higher rates in SDB).
All analyses suggested unequal transition rates between EFB and SDB. However, the direction and strength of the difference varied strongly with all methodological choices (Table 2). While our main analyses, and the additional HiSSE analyses suggested higher transition rates from SDB to EFB, the direction was reversed when using a phenology-based biome definition. For phylogenetic uncertainty and biome classification, the direction was variable and the absolute diversification and transition rates varied by orders of magnitude among the individual replicates (Figure S5.10 in Appendix S5).

### 4 | DISCUSSION

Here, we tested hypotheses on the role of biome transitions in the evolutionary of the tropical plant group Bombacoideae. We found support for multiple independent transitions among the evergreen rain forest (EFB) and seasonally dry biomes (SDB) and more transitions from EFB to SDB than vice-versa (Hypothesis 1). We rejected the hypothesis that biomes differ in net diversification (Hypothesis 2).

#### 4.1 | Temporal evolution

The root-age of Bombacoideae is consistent with the dating of its sister Malvoideae to the late Cretaceous (Areces-Berazain & Ackerman, 2017). Furthermore, a Paleocene or earlier origin of the Malvatheca (Bombacoideae + Malvoideae) and the Bombacoideae clade, as we inferred, is supported by fossils of those groups in North America and South America at least since the Paleocene (Carvalho et al., 2011) and possibly much earlier (Vega, García-Barrera, Perrilliat, & Coutiño, M. A., & Mariño-Pérez, R., 2006). However, there is uncertainty on the placement of some fossils (either within Bombacoideae or within the former Bombacaceae, a broader group including some lineages now considered Malvoideae). Furthermore, our use of exponential priors on the two macrofossil calibrations constrained by the age of the strata they were derived from implies high confidence in their age, which seems justified in this case because the fossil specimens are well-preserved and present clear morphological synapomorphies allowing for their assignment to a specific nodes on the phylogeny. Additionally, the stratum the Malvaciphyllum fossil is derived from is temporally well-defined (Wing et al., 2009). Using less informative priors, at least with respect to maximum age, would likely shift divergences in the group to somewhat older ages.

The Bombacoideae have a relatively rich fossil record compared to other plant groups, especially for pollen (Jaramillo, Rueda, & Torres, 2011). Unfortunately, it is generally difficult to place pollen fossils in relation to recent taxa, due to high homoplasy in Malvaceae pollen morphology (Saba, 2007), making the full integration of fossil and molecular data challenging. However, our results on the number and direction of biome shifts should be robust to uncertainties in phylogenetic dating.

#### 4.2 | Biome transitions

Our findings suggest caution using Bombacoideae fossils as indicators of past evergreen rain forests (e.g. Morley, 2000; Pross et al., 2012), unless these fossils have biome-specific traits. The number

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**Table 1**: Biome transitions in Bombacoideae among evergreen rain forest (EFB) and seasonally dry biomes (SDB), the latter including seasonally dry forest (SDF) and savanna (SAV).

| Index                               | Main analysis | Phylogenetic uncertainty | Measurement error | Biome definition |
|-------------------------------------|---------------|--------------------------|-------------------|-----------------|
|                                     |               |                          |                   |                 |
| EFB specialists                      | 73            | 73                       | 99.9              | 43              |
| SAV specialists                      | 2             | 2                        | 14.4              | NA              |
| SDF specialists                      | 9             | 9                        | 20.4              | NA              |
| SDB specialists (SAV or SDF)        | 16            | 16                       | 36.8              | 21              |
| Generalist species (SDB \ EFB)      | 73            | 73                       | 11.6              | 98              |
| Total number of transitions         | 80–89 (84.1)  | 79–88 (83.3)             | 34–48 (40.6)      | 61–69 (64.7)    |
| Transitions to EFB                  | 11–25 (18.0)  | 8–23 (15.4)              | 3–11 (6.82)       | NA              |
| Transitions to SDF                  | 30–42 (35.9)  | 31–44 (37.5)             | 13–23 (17.8)      | NA              |
| Transitions to SAV                  | 26–34 (30.2)  | 26–34 (30.4)             | 12–20 (16.3)      | NA              |
| EFB -> SDB                          | 38–52 (45.6)  | 40–55 (47.5)             | 16–28 (21.9)      | 38–53 (46.5)    |
| SDB -> EFB                          | 11–25 (18.0)  | 8–23 (15.2)              | 2–11 (6.57)       | 10–27 (18.2)    |

Note: The columns represent different analyses to test the effect of different types of uncertainty. Main analysis: A reconstruction of ancestral biomes using biogeographic stochastic mapping based on a DEC model. Phylogenetic uncertainty: 100 replicates of the main analyses using randomly sampled phylogenetic trees from the posterior distribution of the phylogenetic reconstruction. Biome classification: 100 replicates with stochastic assignment of tips to biomes based on the number of occurrence records available. Biome definition: replications of the main analysis using two alternative biome definitions based on remote sensing (Phenology) or expert knowledge of the study species (Expert).
FIGURE 2  Biome evolution in Bombacoideae. The evergreen rain forest is represented in purple, seasonally dry forest ('Seasonally dry tropical and subtropical forest' and 'Deserts and Xeric Shrublands', Olson et al., 2001) in blue and savanna in yellow. Dispersals into seasonally dry forest and savanna were allowed from the beginning of the Miocene onward based on fossil information. There are multiple independent transitions into seasonally dry biomes, especially in the last 10 million years.
of biome transitions among EFB and SDB are high, at least at the large scale (Figure 2, Table 1), especially compared to other similar-sized groups (Cardillo et al., 2017; Estrella et al., 2017), which is likely partly due to how we chose to account for widespread species and to our use of biogeographical stochastic mapping, which reconstructs anagenetic events along branches, rather than just counting shifts observed at nodes (which represent the minimum number of shifts).

Our results from the biogeographical stochastic mapping suggest more transitions towards seasonally dry biomes than the reverse. This fits with expectations based on the age of the biomes and observations from other lineages (Pennington & Hughes, 2014; Simon & Pennington, 2012). The evergreen rain forests of Amazonia – one of the diversity centres of Bombacoideae – have been suggested as a regional and global species source, 'pumping lineages' into other biomes (Antonelli et al., 2018, 2015). Cases in which specific lineages have shown asymmetry in the direction of biome transitions have been suggested to indicate the presence of enabler traits that facilitate biome transitions and the establishment in a new biome (Donoghue & Edwards, 2014). Thus, an interesting question is what enabler traits preadapt Bombacoideae to seasonally dry environments. A candidate is parenchymatous wood that can readily yield the succulent stem habit typical of seasonally dry tropical forests. Further possible candidates are large fruit and seeds, and high dispersal capacity.

Model choice partly affected the ancestral biome reconstruction, with GeoHiSSE suggesting the majority of lineages as generalist rather than rain forest specialist (Figure S4.6 in Appendix S4). This seems unlikely and might be due to the unconstrained treatment of time and biome age. The inferred importance of generalist species likely also relates to the finding of a higher transition rate from SDB to EFB suggested by the main GeoHiSSE analysis. In general, the qualitative conclusions based on the ancestral biome reconstruction using biogeographical stochastic mapping were robust to uncertainty in phylogenetic reconstruction, biome classification and biome definition. However, the number of reconstructed transitions was sensitive to biome classification and definition, and the total number of transitions was considerably lower when using a record-based biome-classification or expert-based biome definition. This change in absolute number likely reflects the lower number of recent generalist species scored by these schemes: both methods tend to favour species' classifications as specialists. In the case of the record-based biome classification, most species predominantly occur in one biome, and in the case of the expert-based biome definition, this might be caused by a focus on micro-habitat in the site classification (for instance slope and local moisture availability).

Each biome definition has advantages and challenges, but biome classifications based on spatially explicit biome definitions (the ecoregion and phenology-based definitions in our case) and specimen data are more reproducible, less influenced by researcher biases, and better suited to computational investigations of uncertainty in downstream analyses, than expert-based
approaches. Importantly, the different results for the absolute number of biome transitions and the diversification rates we observed with the expert-based classification call for further exploration of how biome delimitation and identification might influence our understanding of actual transitions in habitats and highlight the need for caution when interpreting quantitative results of biome transition analyses.

4.3 | Biomes and diversification

We did not find a significant relation between diversification rate and biome type, rejecting the hypothesis that biome transitions into seasonally dry biomes are a driver of increased diversification, although this finding is subject to uncertainty. This could reflect a lack of statistical power or indicate that other factors besides the adaptation leading to evolutionary radiations in seasonally dry conditions are the main drivers of diversification in Bombacoidae, contrasting with results from other Malvaceae (Areces-Berazain & Ackerman, 2017). Other potential drivers of diversification in Bombacoidae include plant-pollinator interactions, fruit/seed evolution and changes in ploidy level (Carvalho-Sobrinho et al., 2016; Costa, Oliveira, Carvalho-Sobrinho, & Souza, 2017).

The transition and diversification rate analyses were sensitive to all types of tested uncertainty. This was partly expected due to the different specifications of the models (especially if they account for generalist species) and the resulting changes in the classification of tip trait states, as well as the relatively small size of our phylogeny (Davis et al., 2013). However, these results were also surprising, especially concerning sensitivity to model choice on the qualitative conclusions (Table S5.2. Appendix S5) as well as the effect of phylogenetic uncertainty and biome classification on the estimated rates (Figure S5.10. Appendix S5). Reasons for the sensitivity of the diversification rate analyses to model choice and uncertainty deserve more study, but the results indicate that we currently lack evidence that biome alters diversification rate in Bombacoidae.

5 | CONCLUSIONS

We show that evergreen rain forest – seasonally dry biome transitions are common in Bombacoidae, especially within the Neotropics. These findings are robust to methodological choices and support the view that the evergreen rain forest-seasonally dry biome boundary is permeable for this plant lineage on evolutionary time scales. Furthermore, this permeability is directional with transitions from evergreen rain forest to seasonally dry biomes being more common than the reverse.

Our results also demonstrate that model choice as well as different biome classifications and biome definitions can lead to qualitatively different conclusions, stressing the importance of carefully selecting a biome-scoring scheme that is suitable for the question at hand and testing its sensitive to methodological choices and assumptions.

DATA ACCESSIBILITY STATEMENT

A species list is available in the Supporting material (Appendix S1). The DNA alignments, the shapefiles of species ranges and all analyses scripts with input data are available from zenodo (https://zenodo.org/record/2634308#.Xku300p7mHt)

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AUTHOR CONTRIBUTIONS

AZ and CDB designed the study with contribution from all authors. JCS and AZ gathered the data. JCS provided the field-based biome classification. CDB reconstructed and dated the phylogeny, AZ analysed the data, and all authors interpreted the results. AZ wrote the manuscript with contributions from all authors.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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