RESEARCH ARTICLE

Character evolution and biogeography of *Casearia* (Salicaceae): Evidence for the South American origin of a pantropical genus and for multiple migrations to the Caribbean islands

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Abstract *Casearia* (Salicaceae) is a pantropical genus of circa 200 species, around half of which dwell in the Neotropics. Despite the availability of phylogenetic studies that suggest that *Casearia* sensu Sleumer is not monophyletic, a strong phylogenetic framework was still lacking for this genus. We tested the monophyly of *Casearia* and examined the relationships of its species to other taxa of the tribe Samydeae, including *Laetia*, *Samyda* and *Zuelania*, which recently have been sunk into *Casearia*, as well as *Euceraea*, *Lunania*, *Neoptychocarpus*, *Ryania* and *Tetrathylacium*. We further put a focus on the Neotropical taxa since *Casearia* and allies are speciose both on the Caribbean islands and adjacent mainlands, thus providing an interesting group to address the origin of the Caribbean and Cuban flora. Our phylogenetic analyses based on four combined rapidly evolving plastid regions (*petD*, *rpl16*, *rps4-trnT-L-F*, *trnK-matK-psbA*) as well as nuclear ITS revealed *Casearia* as monophyletic with high support, including not only the former members of *Laetia*, *Samyda* and *Zuelania* but also *Euceraea* and *Neoptychocarpus*. *Casearia* is constituted by several major clades, mostly being entirely Neotropical, one of which exclusively comprises species endemic to the Caribbean islands. Another clade, which includes all Palaeotropical species, is nested among Neotropical lineages. Our divergence date estimates using the plastid dataset and fossil calibration points in Salicaceae indicate that the *Casearia* crown group started to diversify during the late Eocene, approximately 39 Ma. The stem of the Old World clade diverged from Neotropical ancestors around 27 Ma, in the Oligocene. We used BayesTraits to reconstruct the evolution of seven characters commonly used to define *Casearia* and allied genera. We found morphological characters, such as branched inflorescences (fasciculate, glomerulous, cymose) or uniseriate stamen series, that work well to circumscribe the genus, whereas dioecy, which was used to diagnose *Neoptychocarpus*, or higher stamen numbers (>12), found in *Laetia* and *Zuelania*, are homoplastic in Salicaceae, the latter character derived within *Casearia* from ancestors with 7–12 stamens. Pellucid dots appear to have evolved earlier than the divergence of the *Casearia* clade in Samydeae, and were lost in *Ryania* and *Tetrathylacium*, and thus are no synapomorphy for *Casearia*. In order to establish a monophyletic genus concept for *Casearia*, we propose to also merge *Euceraea* and *Neoptychocarpus*. Our reconstruction of ancestral areas using BioGeoBears indicate that South America is the ancestral area of *Casearia*. From there, multiple migrations occurred to Mesoamerica and the Caribbean islands. The Caribbean that comprises nearly all Caribbean endemics started to diversify around 9.5 Ma. Our trees depict *C. corymbosa*, which exhibits significant infraspecific phylogenetic structure for the sampled Mexican and Colombian individuals, as the sister to the Caribbean clade. The other clade, with Cuban endemics (*C. ternstroemioides*) but also Mesoamerican and South American taxa, is not sufficiently resolved internally, to allow biogeographic conclusions. The Old World clade of *Casearia* provides another example for a late Laurasian migration starting in the Neotropics.

Keywords ancestral area reconstruction; ancestral character state reconstruction; *Euceraea*; *Neoptychocarpus*; Neotropics; Samydeae

Supporting Information may be found online in the Supporting Information section at the end of the article.

■ INTRODUCTION

The genus *Casearia* Jacq. (Salicaceae) is pantropical with approximately 200 species (Sleumer, 1980), among which ca. 90 are found in the Neotropics (Gentry, 1996). Formerly, the genus formed part of the widely polyphyletic family Flacourtiaceae Rich. ex DC. (Warburg, 1895; Sleumer, 1980; Lemke, 1988). Phylogenetic studies by Chase & al. (2002)

revealed the Flacourtiaceae to consist of different clades located in distant positions within Malpighiales, which were then recognized as part of Achariaceae Harms and Salicaceae Mirb. (Bremer & al., 2003; Chase & al., 2016). The latter includes *Casearia*. Based on *rbcL* sequences, the authors inferred *Casearia sylvestris* Sw. as sister to all remaining Salicaceae (Chase & al., 2002), followed by *Scyphostegia* Stapf. This topology was confirmed by subsequent studies using 82 plastid genes (Xi & al., 2012) and 17 genes from all three genomic compartments (Soltis & al., 2011). Whereas the Salicaceae as a whole and its first two branches were well supported, all these studies were limited in taxon sampling. Alford (2005) carried out a phylogenetic analysis of the former Flacourtiaceae, with a much better representation of taxa, in particular of the tropical members of the Salicaceae clade sensu lato (Chase & al., 2002). Using the plastid *trnL-F* spacer and the *trnL* intron (here referred to as "*trnL-F*" region), *ndhF* sequence data and a comprehensive morphological dataset, the resulting trees provided good support for three clades within Salicaceae s.l. He recognized these three clades at family level, namely as Samydaceae Vent. (including *Casearia*), Scyphostegiaceae Hutch. and Salicaceae (Alford, 2005) to account for their morphological differences. However, the plastid trees alone did not show Samydaceae as a clade but indicated a clade comprising *Casearia* and allied genera (see Alford, 2005: fig. 2.7).

The species of *Casearia* are trees or shrubs with pellucid dots or striations on the leaves, which in most cases have serrate margins (Hutchinson, 1967; Gentry, 1996). The flowers are axillary and apetalous, with twice as many (or more) stamens than sepals (Fig. 1). The stamens are more or less perigynous, uniseriate and the ovary is unilocular with three parietal multi-ovulate placentas (Warburg, 1895). Identification of *Casearia* species is generally difficult, particularly in the field, as the commonly used diagnostic characters are mainly floral features no bigger than a few millimetres (Sleumer, 1980). The first comprehensive treatment of *Casearia* and relatives can be attributed to Warburg (1895), who dealt with the genus in the context of the whole Flacourtiaceae. He divided the family into 11 tribes, one of which was "Caseariae Benth." (not validly published), including *Casearia*, *Euceraea* Mart., *Laetia* Loef. ex L., *Lunania* Hook., *Osmelia* Thwaites, *Patrisia* J.St.-Hil. (syn. *Ryania* Vahl.), *Samyda* Jacq., *Tetrathylacium* Poepp. and *Zuelania* A.Rich. He defined the tribe as having perigynous flowers and no petals. The leaves present pellucid dots, and there are twice or more stamens than sepals, apart from *Tetrathylacium*, which possess both in equal numbers. Plus, all members of the "Caseariae" tribe have staminodes. Warburg then presented the genus *Casearia* with the following diagnostic characters: 4 to 6 persistent sepals, 6 to 15 uniseriate stamens and the same number of staminodia alternating with them, being free or merged at the base, punctate leaves and a simple or 3–4-parted style (Warburg, 1895). The pellucid dots and striation of *Casearia* leaves are ducts and cavities present in the limb. They are often secretory structures that play a role in the chemical defence of the plant (Roupert, 1926; Evert, 2006). In the case of *Casearia*, their structure has been studied but their function remains unknown (Ferreira Fernandes & al., 2018). Hutchinson (1967) largely adopted Warburg's classification but added new genera to the tribe "Casearieae", namely *Neoptychocarpus* Buch., *Ophiobotrys* Gilg. and *Pseudosmelia* Sleumer. The "Casearieae" was considered as a well-defined natural group in the classification system of Lemke (1988), who defined 10 tribes of Flacourtiaceae by a set of morphological, wood anatomical and secondary compound characters (Lemke, 1988). The tribe "Casearieae" was characterized to have small or obsolete floral bracts, lacking petals and vessels without spiral thickenings and simple perforation plates (Lemke, 1988). Lemke also described the tribe to have a half-inferior ovary, which probably is a confusion as *Casearia* possess a superior ovary (Warburg, 1895; Alford, 2005). His circumscription corresponded to the "Casearieae" of Hutchinson (1967) but additionally included *Bivinia* Jaub. ex Tul. (later transferred to Homalieae; Chase & al., 2002) and *Synandrina* Standl. & Steyerm. (now synonym of *Casearia*). Chase & al. (2002) pointed out that the name "Casearieae" was not validly published according to Art. 31(1)b of the *Code* then in effect, so that Samydeae has to be used.

Phylogenetic studies by Samarakoon (2015) focused on the Samydeae (there classified as Samydaceae at the family level) and provided well-resolved trees based on combined plastid *ndhF + matK* and nuclear *EMB2765 + GBSSI* sequence data. The author included 21 species of *Casearia*, most of which appeared in a core clade called Samydeae, whereas *C. commersoniana* Cambess. and *C. javitensis* Kunth, together with *Ryania* and *Trichostephanus*, were depicted as the sister clade called Ryanieae. Those two species were part of *C.* sect. *Piparea* (Aubl.) Benth. Recently, the members of this section were put into the genus *Piparea* Aubl. On the basis of those phylogenetic results (Samarakoon & Alford, 2019). Within the narrowly defined Samydeae, the core of *Casearia* appeared paraphyletic to the small segregated genera *Hecatostemon* S.F.Blake, *Laetia*, *Samyda* and *Zuelania*, which were merged with *Casearia* (Samarakoon & Alford, 2019). Warburg (1895) recognized five sections in *Casearia*, of which only one possesses species from the Old World, together with species from the New World. Sleumer (1980) recognized six sections in *Casearia*. First is *C.* sect. *Guidonia* (DC.) Eichler with three species and characterized by an undivided style, formed by the upper part of the ovary, and staminodes fused to the corona. The type of this section is *C. spinescens* (Sw.) Griseb. Second is *C.* sect. *Endoglossum* Sleumer containing only *C. tremula* (Griseb.) Griseb. ex C.Wright, characterized by an undivided style and free staminodia that are disposed in a distinct row as the row of stamen. Third is *C.* sect. *Casearia*, which is the largest of all sections, comprising all species of the Old World, around 100, together with about 60 species from the New World. This section was divided into six informal groups including the group Ilicifoliae, with species endemic to the Caribbean islands Cuba and Hispaniola. Similar to *C.* sect. *Endoglossum*

it is distinguished by having an undivided style, but the free staminodes are arranged in the same row as the stamens, and alternating with them. Fourth is *C.* sect. *Gossypiospermum* Griseb. with around three species characterized by hairy seeds, a trifid style and free staminodes. The type is *C. praecox* Griseb. The fifth section is *C.* sect. *Crateria* Benth., with around three species, and defined in having a trifid style and staminodes alternating with the stamens. The type is *C. carpinifolia* Benth. (= *C. sylvestris* var. *lingua* (Camb.) Eichler as currently

accepted name). Finally, he recognized *C.* sect. *Piparea*, the species of which also have a trifid style but staminodes placed in the same row as the stamens, with *C. commersoniana* as the type. However, as mentioned above this section was recently excluded from *Casearia* (Samarakoon & Alford, 2019). According to the same study (Samarakoon & Alford, 2019), *Casearia* now includes further nine species formerly known as *Samyda*, characterized by perigynous stamens, lacking staminodes, and sepals fused into a tube. The authors also
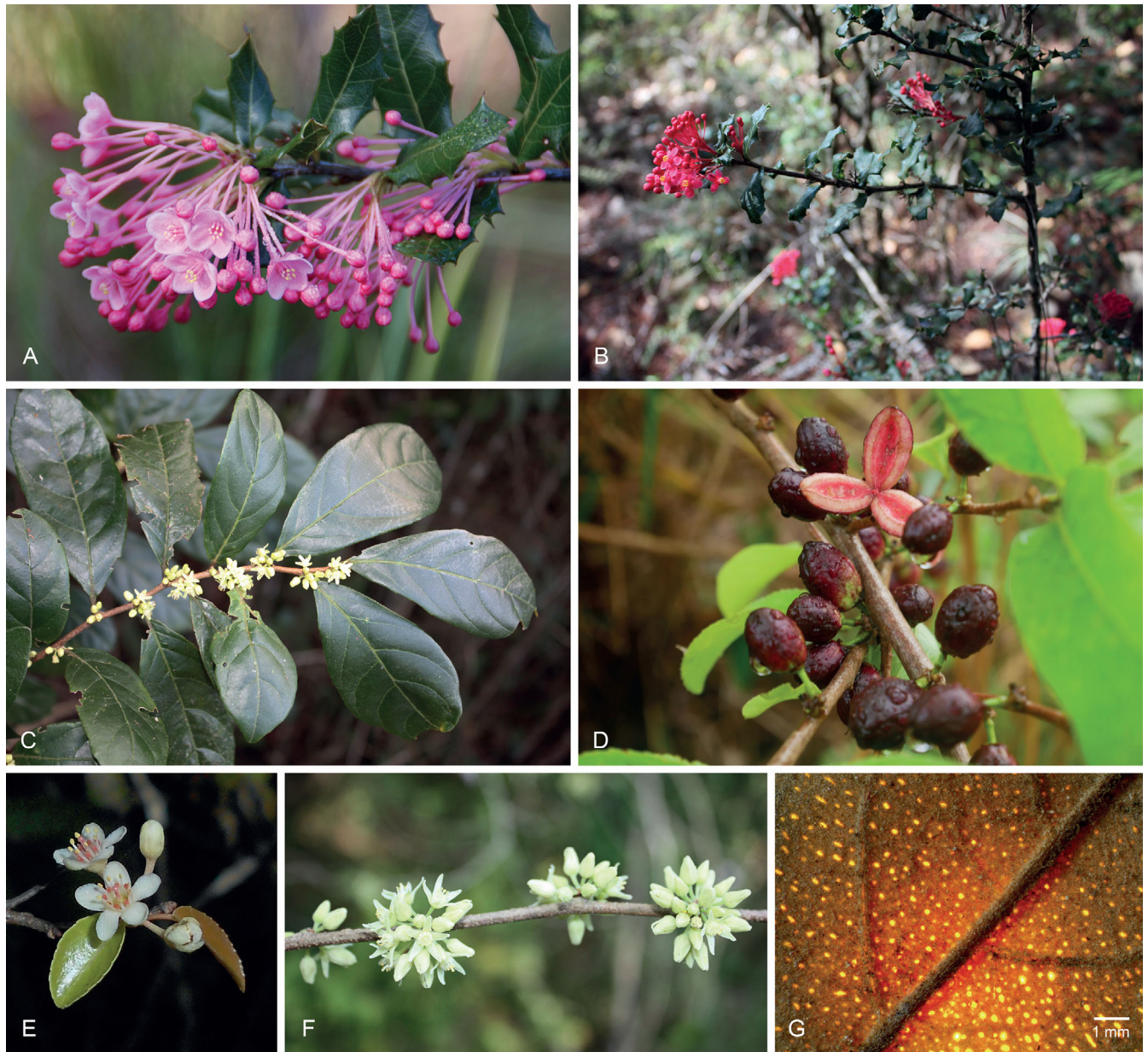

Fig. 1. Morphology and species diversity of *Casearia*. **A**, Inflorescence of *C. bissei* from Cuba, Guantanamo (*Borsch & al. 4428* [B, HAJB; laboratory code: SAL012]); **B**, Flowering branch with coriaceous leaves of *C. crassinervis* from Cuba, Holguin (*Borsch & al. 4056* [B, HAJB; SAL002]); **C**, Axillary flowers of *C. aculeata* from Cuba, Villa Clara (*Borsch & al. 5243* [B, HAJB; SAL014]); **D**, Fruits of *C. aculeata* from Colombia; **E**, Flowers of *C. nitida*, Cuba; **F**, Flowers in glomerules on a branch of *C. mollis* from Cuba (*Borsch & al. 5138* [B, HAJB; SAL013]); **G**, Pellucid dots and lines of *C. arborea*, Cuba (*T. Borsch & al. 4845* [B, HAJB]). — Photos: A by M. Ackermann; B, C & F by T. Borsch; D by O. Rojas-Zamora; E by J.L. Gómez; G by A. de Mestier.

merged the eight species of the genus *Laetia* (which are also characterized by perigynous, albeit free stamens and the absence of staminodes) with *Casearia*. Furthermore, they merged *Casearia* with the monotypic genera *Hecatostemon*, which was originally diagnosed as distinct because of its numerous (90–100) stamens, and *Zuelania*, characterized by numerous stamens and an absent style.

Most of the species diversity of the pantropical genus *Casearia* is centred in the Neotropics. In the circumscription of Samarakoon & Alford (2019), the genus comprises some 220 species including the four above-mentioned segregate genera that only occur in the Americas, totalling to about 110 species in the Americas. Asia and Oceania comprise about 100 species, whereas Africa only has some 15–30 species, depending on their circumscription (Breteler, 2008; African Plant Database, 2020). Of the 110 American species, about 70 are found in continental South America and 50 are restricted to that area (70%) based on Sleumer (1980) and later descriptions of species (Marquete & Mansano, 2010, 2012, 2013; Alford, 2015; Nepomuceno & Alves, 2017), whereas Mesoamerica and Mexico possess around 30 species, of which 15 are endemics (50%), based on Sleumer (1980) and later descriptions of species (Castillo-Campos & Medina Abreo, 2003; Linares & Angulo F., 2005). The Caribbean islands harbour about 30 species, of which 15 to 20 are endemic (50%; including many of the former genus *Laetia*), based on Sleumer (1980) and later descriptions of taxa (Gutiérrez, 1980). These include two taxa endemic to Cuba, which are recognized subspecies of widely distributed species: *C. arborea* subsp. *occidentalis* J.E.Gut. and *C. sylvestris* subsp. *myricoides* (Griseb.) J.E.Gut.

Although less pronounced than in other genera, *Casearia* belongs to those Neotropical lineages with significant species diversity and endemism in the Caribbean in relation to adjacent mainland, like *Acalypha* L. and other Acalyphoideae, Euphorbiaceae (Cervantes & al., 2016); *Brunfelsia* L., Solanaceae (Filipowicz & Renner, 2012); the tribe Miconieae, Melastomataceae (Michelangeli & al., 2008); *Phyllanthus* L., Phyllanthaceae (Falcón Hidalgo & al., 2020); or *Rondeletia* L., Rubiaceae (Torres-Montúfar & al., 2020). In addition to species restricted to either the islands or the mainland, there are eight species in *Casearia* with a distribution shared between the Colombian mainland and the Caribbean islands.

Casearia species grow in Neotropical dry forests, in humid rain forests and savanna habitats. They mostly occur at low elevations but can go up as far as 2000 m (Sleumer, 1980). Another interesting feature is that the genus comprises some widely distributed species occurring in a broad range of habitats, whereas others show more restricted distribution or ecological niches (Gutiérrez, 2000; Gonzalez, 2007; Breteler, 2008). *Casearia* is therefore an interesting model to study both the origin of Neotropical taxa in the context of the evolution of a pantropical genus as well as biogeographic relationships and species diversification in northern South America adjacent to the Caribbean and on the islands themselves.

Biogeographic relations between the Caribbean islands and adjacent mainland have been the subject of several recent studies. The evolution of plant diversity in the Caribbean was strongly influenced by a complex geological history (tectonic movements, volcanism, formation and submersion of islands), as well as the proximity of continental land masses with an exceptionally rich flora, as a source for plants to colonize the islands through time (Santiago-Valentin & Olmstead, 2004). There are three major geological scenarios hypothesized that potentially have influenced the distribution patterns of the flora.

First, the Greater Antilles Aves Ridge (GAARlandia; Iturralde-Vinent, 1999), thought to have connected the islands with Mesoamerica in the Eocene from 35 to 32 Ma., has been put forward to explain some diversification patterns such as in *Copernicia* Mart. ex Endl. (Bacon & al., 2012). The respective lineages were supposed to have colonized the Caribbean from northern parts of South America via the GAARlandia bridge, as in other genera such as *Croton* L. and *Styrax* L. (Fritsch, 2003; Van Ee & al., 2008) and also animals (Dávalos, 2004; Deler-Hernández & al., 2018). However, this hypothesis remains controversial, and some authors doubt that it played a major role to support plant migrations to the Caribbean islands. Cervantes & al. (2016) analyzed the speciose subfamily Acalyphoideae (Euphorbiaceae) and found repeated arrivals of the ancestors of Caribbean island endemic lineages only since the Miocene (approx. 9 Ma and younger), many of which came from Central America and Mexico, thus not being compatible with the GAARlandia hypothesis. Nieto-Blázquez & al. (2017) looked at divergence times of 32 Caribbean endemic genera using published sequence data, indicating younger stem and crown nodes than the GAARlandia time frame in 22 of these, albeit not all lineages were appropriately sampled taxonomically. More recently, Roncal & al. (2020) reviewed so far published phylogenetic studies, which essentially confirmed the picture envisaged by Cervantes & al. (2016) in that Central and South America are important ancestral areas of Caribbean endemic lineages most of which diversified well after the Eocene. Ali (2012) even questioned the existence of a GAARlandia land bridge from a geological point of view.

The second important geological event is the closure of the Panama Isthmus that connects South America to Mesoamerica, and was fundamental for the migration of animals and plants between the Americas, including South American ancestors of Mesoamerican and Mexican species, which later reached Cuba and other islands over a near sea distance from the West. However, the exact timing is still debated. The latest review of all available data (geological, palaeontological and molecular record) concluded a closure in the Late Pliocene (O'Dea & al., 2016), although Bacon & al. (2015) had suggested from a macroecological study and reviewing biogeographic data from both plants and animals that the exchange between South America and Mesoamerica started as early as the Oligocene–Miocene boundary.

The third complex of geological and palaeoenvironmental factors are major sea level changes shaping intra-archipelagic

connectivity (Caribbean islands) and thus, speciation through vicariance (Weigelt & al., 2016; Heads, 2018). Sea level changes also affect the outline of islands. They could cause the extinction of plant populations that can no longer function as the source for radiations, for example when an area of distribution became submerged (Alonso & al., 2012). According to Iturralde-Vinent (1999), the sea level raised considerably in the Caribbean at 30 Ma, thus reducing the surface of land available for plants and creating barriers. However, sea level changes and their impact on land plants are still poorly studied in the Caribbean, and this includes the much more recent Pliocene fluctuations that included lower sea levels and potentially increased connectivity in periods of glaciation. On the other hand, overwater dispersal is also proposed to explain how plants migrated in the wider Caribbean, such as in Acalyphoideae (Cervantes & al., 2016) or palms of the tribes Cryosophyleae and Sabaleae (Cano & al., 2018). Such dispersal is supposed to be facilitated by hurricanes, which happen repeatedly in the region (Lugo & al., 2000; Hedges, 2001; Andraca-Gómez & al., 2015). The more recent investigation of species-rich lineages such as *Phyllanthus* or the Acalyphoideae (Euphorbiaceae) with a distribution on all adjacent mainland (South America, Mesoamerica, Mexico) as well as the islands even suggested more frequent migrations between Mesoamerica and Mexico and the Caribbean islands, with continuous arrivals since the Miocene, often followed by speciation on the islands (Cervantes & al., 2016; Falcón Hidalgo & al., 2020).

Well-resolved and dated molecular phylogenies are crucial to understand the origins and diversification of the Caribbean flora. However, a thorough analysis of these patterns also requires extensive knowledge of species-level taxonomy. Currently, detailed revisions or monographs covering both the islands and adjacent mainland are rare, as are species-level phylogenetic analyses with such a geographic coverage.

This study has therefore two major objectives: the first is to provide a phylogenetic framework for *Casearia* and relatives in order to better understand the phylogenetic position of Neotropical taxa in this pantropical genus, which includes an evaluation of the evolution of key morphological characters for a monophyletic circumscription of the genus. We therefore sampled five of the nine genera of the Salicaceae tribe Samydeae. The phylogenetic analysis of Samarakoon (2015) provided important insights towards a monophyletic circumscription of the genus *Casearia*. However, the relationships between *Casearia* and allied genera remained still uncertain due to the limited resolution and node support in her molecular trees, and also, contrary to Asian species, Neotropical taxa were only sparsely sampled. Based on our phylogenetic hypothesis of *Casearia* and relatives, which provides the first well-supported molecular trees for this group of plants, our second major objective is to unravel the origin of the species of *Casearia* in the Caribbean and in particular to test for biogeographic relationships with entities occurring in the adjacent South American mainland. We therefore estimated divergence times and ancestral areas of the *Casearia* clade in order to provide a first picture of its divergence in time and space.

■ MATERIALS AND METHODS

Taxon sampling. — In this study, 103 samples corresponding to 54 species were included, of which 42 belong to *Casearia* in the circumscription of Samarakoon & Alford (2019) including the segregate genera *Laetia*, *Samyda* and *Zuelania* (voucher information in Appendix 1). From the Samydeae, we further sampled *Euceraea*, *Lunania*, *Neoptychocarpus*, *Piparea*, *Ryania*, and *Tetrathylacium*; seven genera representing other lineages of Salicaceae were also selected as outgroup. We also included *Lacistema* (Lacistemataceae) as outgroup, considering the topology of Malpighiales published by Xi & al. (2012). Plant material was collected in the Colombian departments Atlántico, La Guajira, Magdalena and Bolívar between 2017 and 2019 (32 accessions) as well as in Cuba and the Dominican Republic between 2010 and 2017 (29 accessions). We further sampled herbarium specimens from B, BR, and P from other parts of the Neotropics, Africa and Asia (8 accessions). Field-collected plant tissue was silica-dried, and corresponding vouchers were deposited in the herbaria B, HAJB, and UNO. Information about localities, collectors etc. can be found in Appendix 1. Recently, plastid genomes of *Casearia decandra* Jacq. and *C. velutina* Blume were published (Li & al., 2019), so we also included the respective genomic regions from these sequences in our analyses (Li & al., 2019). It is to be noted that for them the vouchers are not available, but we were able to confirm their identification with barcode sequences available in GenBank for the same species.

DNA isolation, sequencing, alignment and indel coding. — Genomic DNA was extracted from herbarium specimens following the modified CTAB protocol (with triple extraction) from Borsch & al. (2003). After a chloroform extraction step, the DNA was precipitated with isopropanol, resuspended in TE and purified by ammonium acetate and sodium acetate washing steps followed by ethanol precipitation. Extraction of genomic DNA from silica-dried leaf material was achieved using the NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany). Four plastid genomic regions (*petD*, *rpl16*, *rps4-trnT-L-F*, *trnK-matK-psbA*) and one nuclear region (nrITS) were selected based on their utility to resolve relationships at the genus and species levels (Borsch & Quandt, 2009). The *trnLF* region was extended by the two adjacent spacers. Universal primers were used to amplify these regions as much as possible, but also new internal primers were designed in this study (suppl. Table S1) for more fragmented DNAs from herbarium specimens. The larger *rps4-trnT-L-F* and *trnK-matK-psbA* regions were generally amplified in two overlapping halves. Most primers (suppl. Table S1) were also used for sequencing. Amplifications were made with a peqStart Thermocycler (PeqLab Biotechnologie, Erlangen, Germany). Each tube contained 4 μl of DNA with a concentration of 25 ng/μl, 19.2 μl water, 0.3 μl DNA polymerase Hot start (PeqLab), 5 μl Taq Buffer S, 2.5 μl MgCl$_2$, 10 μl dNTP, 5 μl betaine and 2 μl forward and 2 μl reverse primers. In some cases, an enhancer was added, enhancer

solution P (PeqLab). The plastid regions were amplified using a program with initial denaturation (1′30 min at 95°C); 34 cycles of denaturation (0′30 min at 95°C), annealing (1 min at 52°C, *rpl16*: 55°C), extension (1 min at 72°C); and a final extension step (10 min at 72°C). For ITS, we used a program with 35 cycles of denaturation (1 min at 96°C), annealing (1 min at 48°C), extension (1′45 min at 60°C); and a final extension step (7 min at 72°C). PCR products were stained with 100× SYBR Green nucleic acid stain and electrophoresed on a 1.5% NEEO agarose gel (Carl Roth, Karlsruhe, Germany) running for 2 hours at 100 volts. When excised from the gel the products were purified using the GEL/PCR DNA Fragment Extraction Kit (AveGene Life Science, Taipei, Taiwan), or PCR products were cleaned directly using the Stratec Kit (Invitek Molecular, Berlin, Germany). After a concentration adjustment, all PCR products were sent to Macrogen Europe (Amsterdam, the Netherlands) and sequenced on an ABI 3730 XL capillary sequencer using the KB3730-Pop7-BDTv3 dye set.

Contiqs were assembled in PhyDE v.09971 after prior inspection of pherograms for erroneous base calls. A motif-based approach (Löhne & Borsch, 2005) was used to align the sequences using PhyDE (Müller & al., 2010) after initial pre-alignment with the MUSCLE plug in. Short regions of uncertain homology (hotspots) were excluded from the analyses. Gaps were coded using the simple indel coding method (Simmons & Ochoterena, 2000) as implemented in SeqState v.1.4.1 (Müller, 2005). Consensus DNA sequences were submitted to ENA (European Nucleotide archive, www.ebi.ac.uk/ena/) using the software tool annonex2embl v.1.0.3 (Gruenstaeudl, 2020). The combined multiple sequence alignment with annotated hotspots is provided in suppl. Appendix S1

and the corresponding matrix used for analysis in suppl. Appendix S2; the ITS alignment is found in suppl. Appendix S3 and the corresponding matrix in suppl. Appendix S4.

Phylogenetic analyses. — Parsimony analysis (MP) was performed in PAUP* v.4.0b10 (Swofford, 2008) using the commands obtained from the parsimony ratchet (Nixon, 1999) as implemented in PRAP (Müller, 2004). The files generated by PRAP included all characters with equal weight and treated the gaps as missing characters. Ratchet settings included 200 iterations, unweighting 25% of the positions randomly (weight = 2) and 100 random additional cycles. Jackknife (JK) support was obtained by a single heuristic search in PAUP within each of 10,000 JK pseudo-replicates, tree bisection-reconnection branch swapping, and 36.79% of characters being deleted in each replicate.

Bayesian inference (BI) was performed in MrBayes v.3.2.7.a (Ronquist & al., 2011) using the CIPRES portal (Miller & al., 2010). We chose the optimal nucleotide substitution models for our matrices using jModelTest v.2.1.7 (Darriba & al., 2012) under the Akaike information criterion (AIC). Matrices were obtained from partitions corresponding to individual genomic regions (Table 1, also for the best-fit models). Regarding the indels, we used the model proposed by Ronquist & al. (2011), which is the F81 like model. We realized four runs each with four chains performed for 50 million generations, for the plastid dataset and 20 million generations for the nuclear dataset, sampling every 10,000th generation. We verified the convergence of the runs using the average standard deviation of split frequencies and post burn-in effective sampling size (ESS). The first 10% of trees were discarded as burn-in, and the remaining trees were used to construct a 50% majority-rule consensus tree.

Table 1. Summary of character statistics, evolutionary models and tree statistics for each dataset under parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI).

	rps4-trnL-F	*trnK-matK*	*rpl16*	*petD*	Combined plastid	ITS
Number of taxa	103	103	103	103	103	66
Aligned length (bp)	2133	3138	1128	1333	7732	761
Parsimony-informative characters	356	529	237	265	1036	236
Consistency index	0.813	0.789	0.768	0.812	0.689	0.567
Retention index	0.897	0.901	0.866	0.933	0.828	0.767
Tree length	889	1376	604	532	3923	993
Partition	spacer *rps4*, *trnT* exon	*trnK* intron, *trnK* exon	*rpl16* intron	spacer *petD*, *petD* exon		
	spacer *trnT-trnL*, *trnL* exon	*matK*		*petD* intron		
	trnL intron, spacer *trnL-trnF*, *trnF*	*trnK* intron 2, *trnK* exon 2, spacer, *psbA*				
Substitution model	GTR + Γ GTR + Γ GTR + Γ	GTR + Γ GTR + Γ GTR + Γ	GTR + Γ	GTR + I GTR + Γ	partitioned	GTR + I + Γ

Maximum likelihood (ML) analyses were performed using RAxML v.8.2.12 in CIPRES. Rapid bootstrap support (BS) was estimated based on the majority-rule consensus tree from 1000 pseudo-replicates with 200 searches. The models general time-reversible (GTR) + Γ and binary (BIN) + Γ were used for the nucleotide and indel partitions, respectively.

The 50% majority-rule consensus tree obtained in MrBayes was processed in TreeGraph v.2.14.0-771 (Stöver & Müller, 2010).

Assessment of morphological characters and ancestral character state reconstruction. — Morphological characters were selected that have been used to diagnose genera and sections in the Samydeae. The characters and their states were the following: (1) sexual system (0 = bisexual, 1 = dioecious) and (2) calyx lobes fused into a tube (0 = absent, 1 = present). Characters 1 and 2 were used to diagnose *Neoptychocarpus* (Buchheim, 1959; Sleumer, 1980; Gentry, 1996). We also assessed diagnostic characters for the genus *Casearia*, which were (3) presence of pellucid dots on the leaves (0 = absent, 1 = present; see Fig. 1), (4) the number of stamens (1 = 1–6, 2 = 7–12, 3 >12), as the number of stamens was used to distinguish *Casearia* species with usually 7 to 12 stamens, whereas species of *Laetia*, *Samyda* and *Zuelania* possess more (Sleumer, 1980), and (5) the presence of staminodes (0 = absent, 1 = present) (Warburg, 1895; Hutchinson, 1967; Sleumer, 1980). We also looked at (6) the arrangement of the stamens (0 = spiral, 1 = uniseriate) and (7) the inflorescence type (0 = panicle, 1 = catkin, 2 = raceme/corymb, 3 = fascicle/glomerule, 4 = cyme, 5 = solitary). In order to limit the number of states that can be handled by BayesTraits (Pagel & Meade, 2006), we grouped together the states "raceme" and "corymb" because a corymb can be understood as a specialized raceme. We also combined "fascicle" and "glomerule" since both are very similar and differ solely in the presence (fascicle) or absence of peduncles (glomerule) (Beentje, 2016). The information on characters and states was obtained from the literature (Warburg, 1895; Hutchinson, 1967; Sleumer, 1980; Gutiérrez, 2000; Marquete & Mansano, 2012), herbarium vouchers and protologues. The resulting morphological character matrix can be found in Appendix 2.

For the reconstruction of ancestral character states, a reduced plastid matrix (suppl. Appendix S5) with one sample per species was employed. From the post burn-in trees inferred with MrBayes, using the above stated specifications, a maximum credibility tree was generated with Mesquite v.3.6 (Maddison & Maddison, 2018), and a population of 1000 trees from the MrBayes analysis were randomly selected to consider topological variation. Probabilities for ancestral states were estimated in BayesTraits v.2.0.2 (Pagel & Meade, 2006). Therefore, we generated a command file in TreeGraph v.2.14.0-771 (Stöver & Müller, 2010) using the function AddNodes including all relevant nodes for the analysis. We then used a reverse jump Markov chain Monte Carlo (rjMCMC) approach to consider both the phylogenetic uncertainty and the ancestral state uncertainty. An exponential hyperprior with the mean on a uniform interval [0–100] was employed for each rjMCMC run in order to reduce the problem of assigning arbitral values to the parameters of the prior distribution. We then visualized the results using the Import BayesTraits data option in TreeGraph.

Estimation of divergence time. — Divergence time was estimated using BEAST v.2.6.1, setting up the input file with BEAUTi (Drummond & al., 2012). We used a log normal, uncorrelated relaxed clock (UCLN) model with three calibration points (Drummond & al., 2006) and applied a GTR + Γ model to the plastid matrix (suppl. Appendix S1). The first calibration point was applied to the *Salix-Idesia* clade using a fossil of *Pseudosalix*, which was found in the U.S.A. and dated 48 Ma (Boucher & al., 2003). The second calibration point was applied to the *Casearia* clade, as defined above, including *Euceraea* and *Neoptychocarpus* using a fossil of *Casearia*. It is a pollen fossil found in Panama and dated 37 Ma (Graham, 1985). The pollen of *Casearia* has been studied by Keating (1973) and this particular pollen was already used in other studies (Davis & al., 2005; Xi & al., 2012). As it cannot be identified at the species level, we assigned it to the crown node of our *Casearia* clade. Indeed, it seems that this fossil could be attributed with certainty to the *Casearia* genus and it is most similar to *C. sylvestris* Sw. Attributing it to the crown group of *Casearia* allows to not underestimate the age of the crown group. Fossil calibrations are used as a minimum age, and we selected a lognormal distribution, which gives higher probabilities to older ages. We respectively selected 37 Ma and 48 Ma as the minimum age (*Casearia* and *Pseudosalix* fossil) and used the age of the Eocene, to which the fossil was dated as a maximum age. Details of fossil calibration points are found in suppl. Table S2. We also gave a maximum age to the root of the tree, using the 92.78 Ma crown group age of the Malpighiales (Foster & al., 2017). The effect of the Yule speciation model versus the Birth-Death model as speciation priors was tested using a stepping-stone sampling (SSS), with 150 path steps, each with a chain length of one million iterations. The other parameters were set by default in BEAST v.2.6.0. We then calculated the log-Bayes factor (BF; suppl. Table S3) and found that the Yule model fitted best.

We used BEAST v.2.6.1 under the relaxed clock normal and Yule model with 50 million generations (logging parameters every 10,000 generations). We used Tracer v.1.7.1 (Rambaut & al., 2018) to check for convergence using the effective sample size (ESS). The first 10% of the Markov chain Monte Carlo (MCMC) generations were removed as a burn-in and the post burn-in MCMC runs were summarized using TreeAnnotator v.2.6.0 (Drummond & Rambaut, 2007) to generate a maximum clade credibility tree visualized in FigTree v.1.4.4 (Rambaut, 2010).

Ancestral area reconstruction. — Areas were scored following Cervantes & al. (2016) as (A) Mexico, (B) Mesoamerica, (C) South America, (D) Caribbean Islands and (E) Africa. The only difference in our area definitions used here is that (F) stands for Asia, and a separate area is defined

for New Caledonia (G). Since species limits in *Casearia* are in many cases not fully understood and distribution areas depend on currently accepted names with which specimens are identified, we preferred to code all samples by their respective areas from which they were collected. Thus, we were able to test for phylogeographic patterns in presumably widespread taxa and to properly code samples in the case currently accepted species were not resolved as monophyletic. This was particularly relevant for our goal to analyse species diversification between the Caribbean islands and adjacent mainlands. Consequently, the area of occurrence of each individual was coded rather than the area of distribution of the respective species corresponding to the currently applied taxon concept. The ML tree from the analysis of suppl. Appendix S2 was used in BioGeoBears implemented in RASP v.4 (Yu & al., 2020). BioGeoBears estimates the ranges of the areas taking into account processes such as dispersal, extinction, founder-event speciation and vicariance. We evaluated the models Bayarea-like, DIVA and DEC and compared the AIC values and likelihood ratio tests (LRTs).

■ RESULTS

Phylogenetic analyses. — For this study we generated 230 new sequences from four plastid genomic regions. The concatenated multiple sequence alignment of the four plastid genomic regions had 8228 positions, of which *rps4-trnT-L-F* contributed 2316, *trnK-matK-psbA* 3260, *petD* 1395 and *rpl16* 1257 positions. The matrix used for tree inference had 7733 positions, after exclusion of hotspots (for *rps4-trnL-F*: three poly-A microsatellites in alignment positions 434–443, 651–663, 1392–1403, one poly-AT microsatellite in 864–879, seven hotspots of other AT-rich sequence elements of unclear homology in 980–1000, 1055–1063, 1160–1172, 1609–1627, 1873–1881, 1923–1958, 2220–2234, one poly-T microsatellite in 2039–2047; for *trnK-matK*: five poly-A microsatellites in 2565–2578, 2661–2666, 2908–2919, 4910–4921, 5311–5325, two poly-T microsatellites in 2785–2793, 2891–2894, one poly-AT microsatellite in 3102–3125, one hotspot of other AT-rich sequence elements of unclear homology in 5110–5136; for *petD*: one poly-A microsatellite in 5881–5888, three poly-T microsatellites in 6038–6047, 6223–6236, 6528–6538, and one hotspot of other AT-rich sequence elements of unclear homology in 6366–6384; and for *rpl16*: three poly-A microsatellites in 7057–7067, 7271–7274, 7364–7372, two hotspots of other AT-rich sequence elements of unclear homology in 7804–7819, 7925–7981, one poly-AT microsatellite in 8063–8094. In addition, 381 indels were coded. The multiple sequence alignment is provided as suppl. Appendix S1 and the matrix in suppl. Appendix S2 including the indels. Sequence statistics, models of sequence evolution, and tree statistics for the individual genomic regions and concatenated matrices are presented in Table 1. The plastid trees obtained with BI, ML and MP are mostly congruent and the ML and MP trees are presented in suppl.

Figs. S1 and S2. On average, most nodes received significant support, with PP > 0.95, and BS as well as JK values >75 % (Fig. 2).

Phylogenetic relationships inferred from the plastid genome. — Plastid sequences revealed a well-supported clade of Samydeae (BI-PP: 1, ML-BS: 100, MP-JK: 99.9), including *Tetrathylacium* as well as a *Lunania* and a *Ryania + Piparea* lineage, as successive sisters to a *Casearia* clade (PP: 1, BS: 100, JK: 100) that includes the genera *Laetia*, *Samyda*, and *Zuelania* (Fig. 2, clades B3 & B6), which recently were merged into *Casearia*. One species of *Euceraea* and two species of *Neoptychocarpus* were found as nested well inside the *Casearia* clade (Fig. 2, clade B2). The other genera of Salicaceae appear in an also well-supported sister-clade to the Samydeae.

Within the *Casearia* clade we found two well-supported clades (Clades A and B, Fig. 2). Clade A (PP: 0.98, BS: 60, JK: 63.9) includes species from South America, Mexico and the Caribbean islands. Two subclades, clade A1 (PP: 1, BS: 92, JK: 91.2) and clade A2 (PP: 1, BS: 79, JK: 92.3) were revealed, the latter of which comprises the samples of *C. sylvestris* with the different subspecies from Colombia, Cuba and Venezuela, which constitute a paraphyletic group since this clade also includes *C. zizyphoides* Kunth and *C. selloana* Eichler. Clade B (PP: 0.99, BS: 69, JK: 63.4) comprises species both from the New and the Old World. Seven well-supported subclades can be distinguished: Clade B1 (PP: 0.72, BS: 52, JK: 64.2) with all *C. arborea* (Rich.) Urb. samples from Colombia, Cuba and Jamaica as well as *C. manausensis* Sleumer as one lineage (PP: 1, BS: 97, JK: 63.6) that is sister to *C. obliqua* Spreng. plus *C. ulmifolia* Vahl ex. Vent. (PP: 1, BS: 97, JK: 98.1). Clade B2 (PP: 0.94, BS: 42, JK: 52.3) comprises the largely Amazonian *Euceraea nitida* and two species of the Neotropical genus *Neoptychocarpus*. Clade B3 (PP: 0.53, BS: 29, JK: [100]) consists of *C. tremula* together with species that formerly were part of the genera *Laetia* and *Zuelania*. Clade B4 (PP: 1, BS: 99; JK: 95.6) harbours all individuals of *C. corymbosa* Kunth. from the Colombian Caribbean as sister to a sample from Mexico. Clade B5 (PP: 1, BS: 100; JK: 99.7) comprises species from the Caribbean islands, *C. comocladifolia* Vent. from Hispaniola and *C. nitida* Jacq., *C. crassinervis* Urb., *C. moaensis* Vict., *C. ophiticola* Vict. and *C. bissei* J.E.Gut. from Cuba. Clade B6 (PP: 0.83, BS: 65; JK: [97.4]) includes *C. deplanchei* Sleumer from New Caledonia together with species from the Old World, in two well-supported lineages. One (PP: 1, BS: 73, JK: 60.8) includes three species from Asia and the other (PP: 0.99, BS: 44, JK: [60.8]) four species from Africa. Finally, Clade B7 (PP: 1, BS: 71, JK: 74.1) comprises taxa from Colombia, Ecuador, the Dominican Republic and Cuba. *Casearia dodecandra* (Jacq.) T.Samar. & M.H.Alford (formerly *Samyda*) is sister to a broadly paraphyletic assemblage of individuals from *C. aculeata* Jacq. that includes individuals currently identified as *C. spinescens* from Cuba.

Phylogenetic relationships inferred from ITS. — The ITS dataset focuses on the Samydeae clade with *Xylosma*
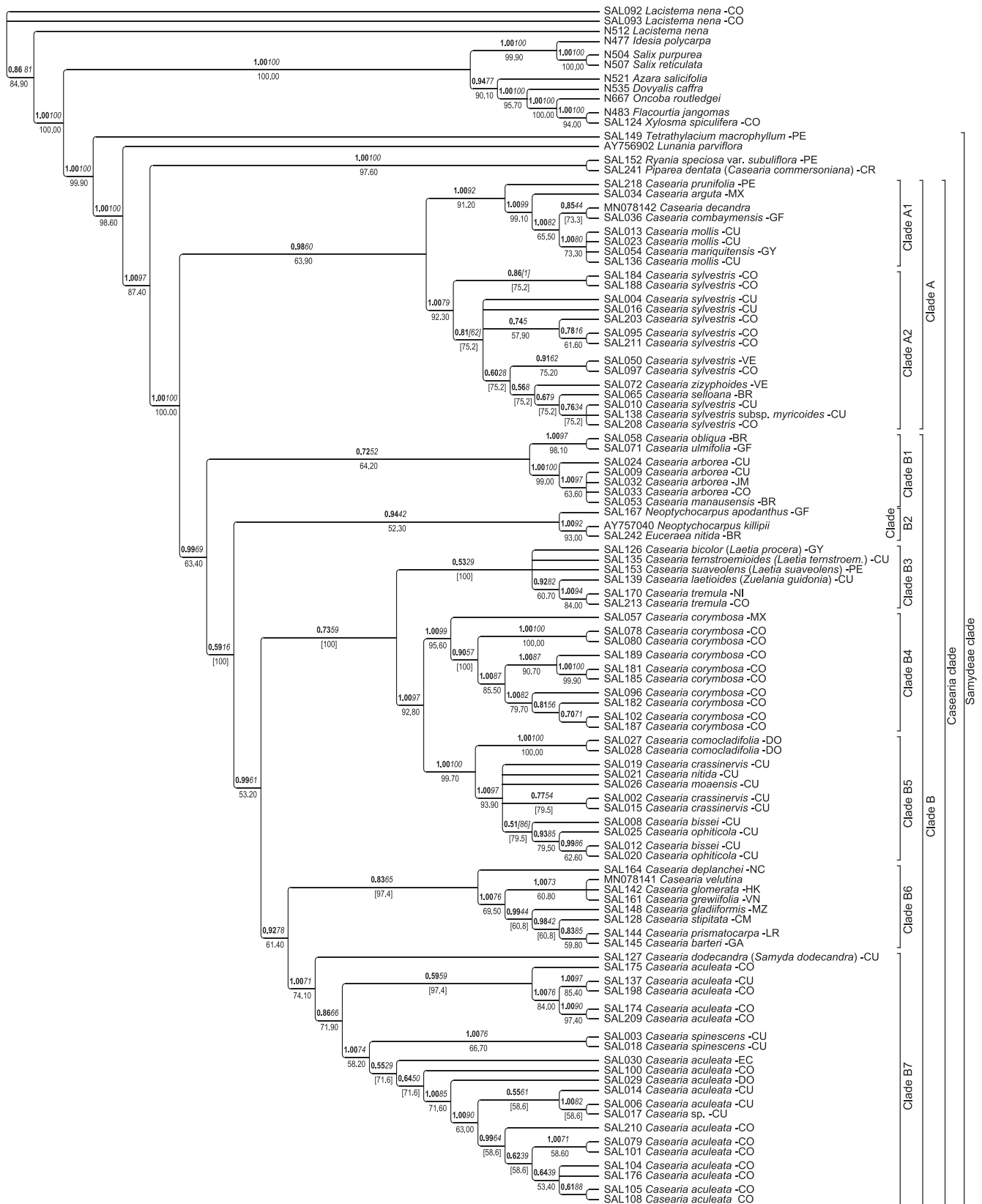
Fig. 2. Bayesian 50% majority-rule consensus tree of *Casearia* based on four plastid markers (*rps4-trnL-F*, *trnK-matK*, *rpl16*, *petD*). Values above the node indicate posterior probability (PP, bold) and bootstrap support (BS, italics), and Jackknife (JK) support is indicated below the node. Values in square brackets indicate conflicting topologies between Bayesian analysis and maximum likelihood or Bayesian analysis and parsimony. At the tip of the node is the DNA number followed by the species name and the country code from where the individual was collected.

G.Forst as outgroup. This was due to the strongly deviant ITS sequences in the other lineages of Salicaceae and Lacistemata-ceae that were not reliably alignable. The multiple sequence alignment of ITS (suppl. Appendix S3) resulted in 860 positions, and 63 ITS sequences were newly generated in this study. The matrix had 763 positions, after exclusion of hot-spots (alignment positions 128–148, 162–174, 296–305, 350–359, 395–409, 589–601, 606–621). In addition, 140 indels were coded (suppl. Appendix S4). Sequence statistics and models of sequence evolution are presented in Table 1.

The trees obtained with BI, ML and MP are mostly congruent and presented in suppl. Figs. S3 and S4. The Bayesian majority-rule consensus tree based on the ITS dataset is shown in Fig. 3. All three tree inference methods resolved a *Casearia* clade (PP: 1, BS: 77, JK: 79.31). We found the same nine subclades as in the plastid phylogeny, however, with a lower resolution of the early branching in the *Casearia* clade (Fig. 3). *Euceraea nitida* is retrieved together with *Neoptychocarpus* with good support (PP: 1, BS: 91, JK: 89.17) and within the *Casearia* clade, like in the plastid tree. However, this lineage, which appears within clade B in the plastid trees, is here depicted in a polytomy with other subclades of clade B and a weakly supported clade A. In the ITS tree, all samples of *C. aculeata* appear in a clade with *C. spinescens* as sister. Compared to the plastid dataset, the ITS tree presents four major sublineages of clade B, namely clade B1, clade B2, clade B3 p.p. and a clade consisting of clades B3 p.p., B4, B5, B6, B7, respectively, in a polytomy. *Casearia bicolor* (formerly *Laetia procera* (Poepp.) Eichler) is found as sister of *C. suaveolens* (formerly *L. suaveolens* (Poepp.) Benth.) in a polytomy with clade A and clade B of the ITS tree, and *C. ternstroemioides* (= *L. ternstroemioides*) is part of clade B6 in ITS (Fig. 3) along with taxa from the Old World.

Ancestral character states. — The matrix of morphological characters is provided in Appendix 2. The ancestral character states are visualized as pie charts in Figs. 4 and 5, and the exact posterior probabilities calculated with Bayes Traits for each node can be found in suppl. Table S4 (see suppl. Fig. S5 for node numbers). The reconstructions in Figs. 4 and 5 show that the number of stamens between 7 and 12, the presence of staminodes, one series of stamen and the fasciculate/glomerulate inflorescence are ancestral characters of *Casearia* with high probability (respectively Bayesian posterior probability [BPP] = 1, BPP = 1, BPP = 1 and BPP = 0.98, see suppl. Table S4). However, clade B3 with old Neotropical *Laetia* and *C. tremula* seemed to have gained a number of stamens, as it has more than 12. The analyses indicate that a plesiomorphic character for the clade is the presence of staminodes, but they are lost in some species of *Casearia* (formerly *Laetia*) and *Neoptychocarpus*. The fasciculate/glomerulate state is also plesiomorphic for the genus, although *Euceraea* presents a paniculate inflorescence. The analyses indicate that more strongly ramified inflorescences are gained in this lineage and also in clade B4. The presence of pellucid dots on the leaves has been considered a key character in the identification of *Casearia* species. However, our

ancestral character state reconstruction (Fig. 4) shows the presence of pellucid dots not only in *Casearia* but also in *Lunania* and *Piparea*, indicating that it arose earlier than the divergence of the actual *Casearia* clade but was lost again in *Ryania*. Interestingly, the vast majority of *Casearia* species and all other species of the Samydeae are bisexual. However, the lineage of *Neoptychocarpus* evolved dioecy, and *Euceraea*, which also belongs to this lineage, has an unknown sexual system but it is suspected to be dioecious or androdioecious (Berry & al., 1998). Contrary to the Samydeae, the other Salicaceae are largely dioecious, and dioecy apparently has originated early on (Fig. 4).

Divergence time estimates. — The crown group of the *Casearia* clade has an Eocene age of 39 Ma (95% highest posterior density [HPD]: 37.04–46.22), whereas the stem dates back to the Paleocene 50 Ma (95% HPD: 45.09–67.52; Fig. 6). The African/Asian clade (clade B6) started to diverge from Neotropical ancestors in the Oligocene (25 Ma; 95% HPD: 18.01–31.01), and the crown age of the group is Miocene (20 Ma; 95% HPD: 13.6–28.79, node XI). The crown group of clade A (37 Ma; 95% HPD: 32.32–45.29) is slightly older than that of clade B (34 Ma; 95% HPD: 26.3–40.47). The most complex Caribbean clade (clade B5) originated around 15 Ma (95% HPD: 12.32–24.28) from South American ancestors, and its crown group started to diversify around 9 Ma (95% HPD: 5.57–15.35). The other Caribbean lineages are all younger. The precise ages estimated for all nodes can be found in suppl. Table S5.

Ancestral areas. — We found the BayArea model with founder speciation event (BAYAREALIKE+J) to be the most accurate model to reconstruct the ancestral areas of *Casearia* and its relatives. Supplementary Table S6 shows the log-likelihood (LnL) values and LRT results across all tested models. The Samydeae including the *Casearia* clade originated in South America with multiple migrations to the Caribbean Islands and one towards the Old World (Fig. 7). The most comprehensive Caribbean clade (clade B5) is sister to a lineage (clade B4) composed of Colombian and Mexican samples of *C. corymbosa*, and these two clades are sister to a mixed Mesoamerican, Caribbean, South American (Colombia and Guyana) clade B3. Notably, the ancestral areas reconstructed (Fig. 7) are always in South America, indicating that the most recent common ancestors of species in the Caribbean islands came from there. Subclade B4 further shows *C. comocladifolia* from the Dominican Republic as sister to a lineage with endemic taxa from Cuba such as *C. bissei* or *C. ophiticola*, thus suggesting a single common ancestor for the Cuban species and an early split in *Casearia* diversification on different Caribbean islands soon after a Miocene (crown group age of 9 Ma of clade B5) arrival. The Old World clade clearly has South American ancestors (Fig. 7) that apparently split into an African and an Asian lineage at the beginning of their crown group diversification. The obtained probabilities for the respective ancestral areas in each node of the tree are provided in suppl. Table S7.
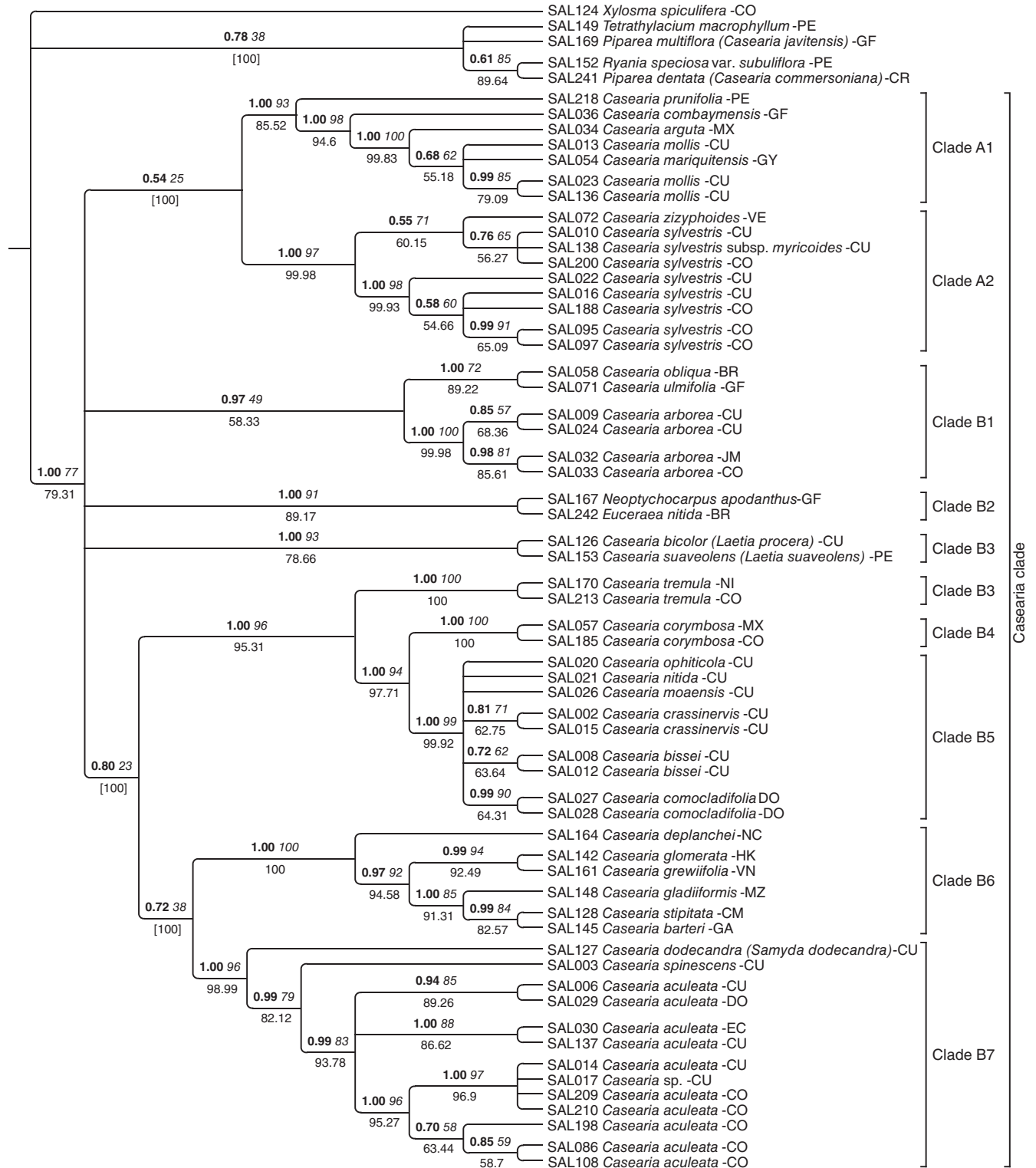
Fig. 3. Bayesian 50% majority-rule consensus tree of *Casearia* based on the nuclear marker ITS. Values above the node indicate posterior probability (PP, bold) and bootstrap support (BS, italics), and Jackknife (JK) support is indicated below the node. Values in square brackets indicate conflicting topologies between Bayesian analysis and maximum likelihood or Bayesian analysis and parsimony. At the tip of the node is the DNA number followed by the species name and the country code from where the individual was collected.
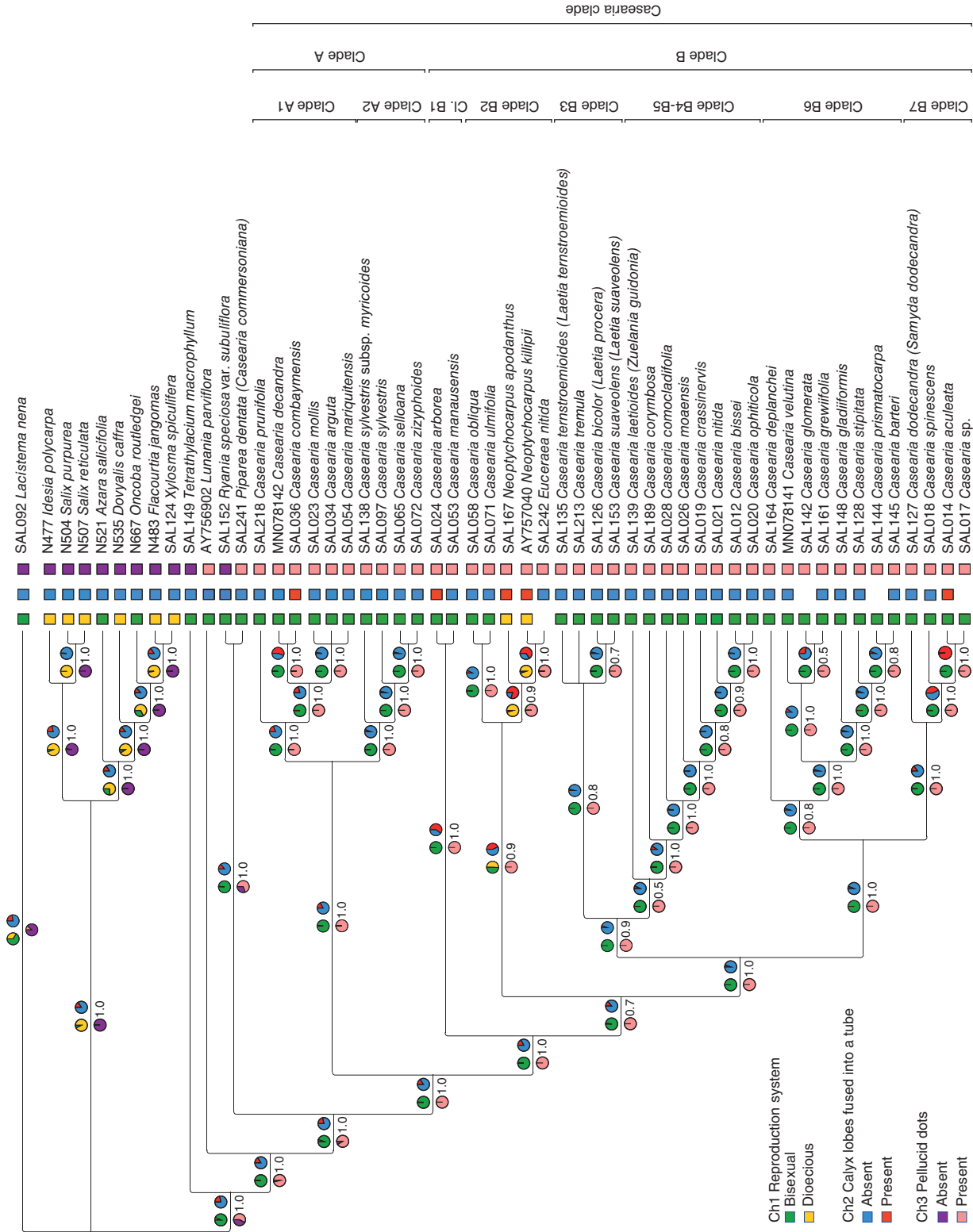
Fig. 4. Bayesian inference of ancestral state reconstruction of three morphological characters plotted on the Bayesian majority-rule consensus tree. Reconstructed ancestral character state is represented as pie charts at the stem node. The legend indicates the state of each character. Squares indicate the state of character for each species, white squares represent missing data.

Fig. 5. Bayesian inference of ancestral state reconstruction of four morphological characters plotted on the Bayesian majority-rule consensus tree. Reconstructed ancestral character state is represented as pie charts at the stem node. The legend indicates the state of each character. Squares indicate the state of character for each species, white squares represent missing data.
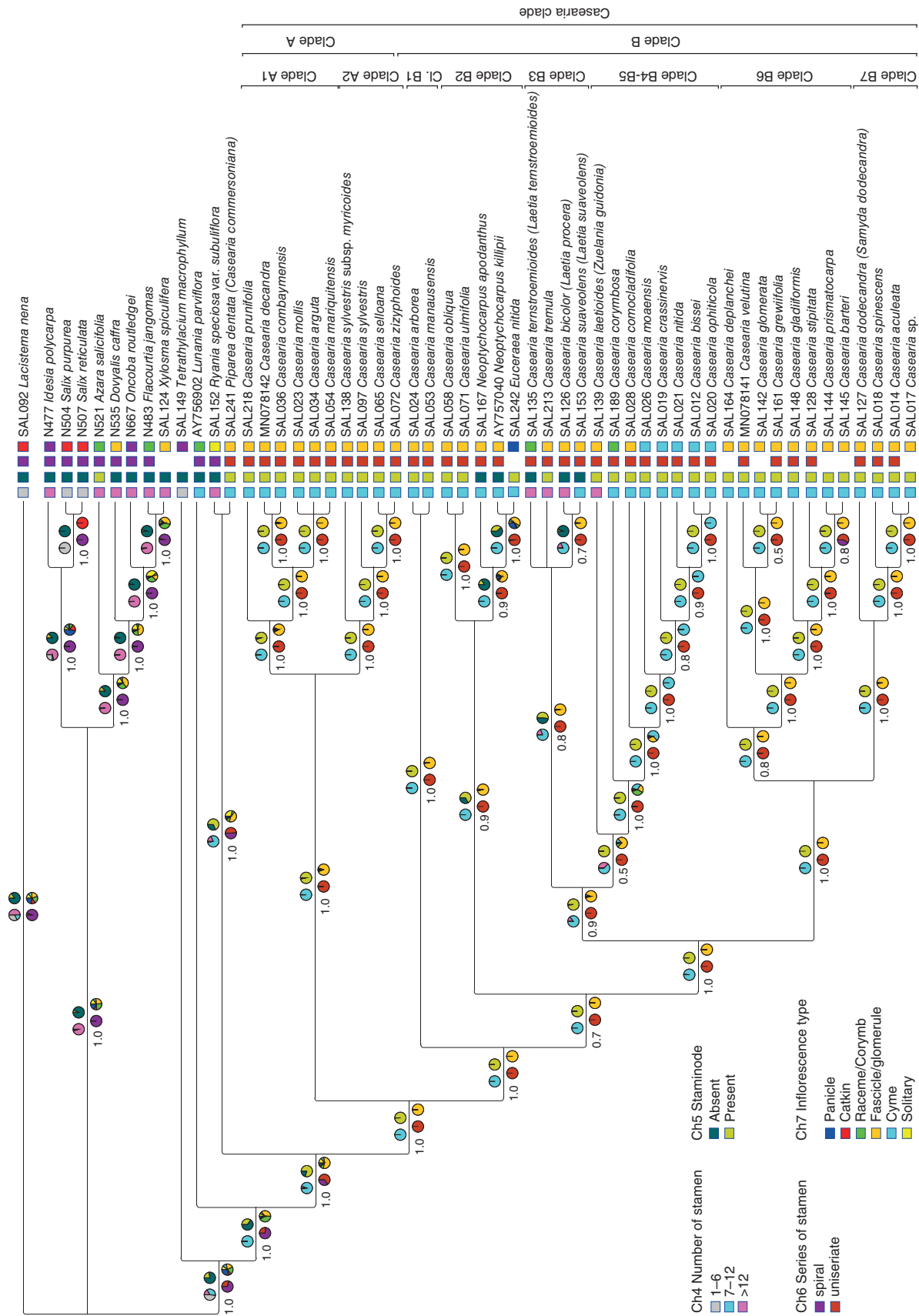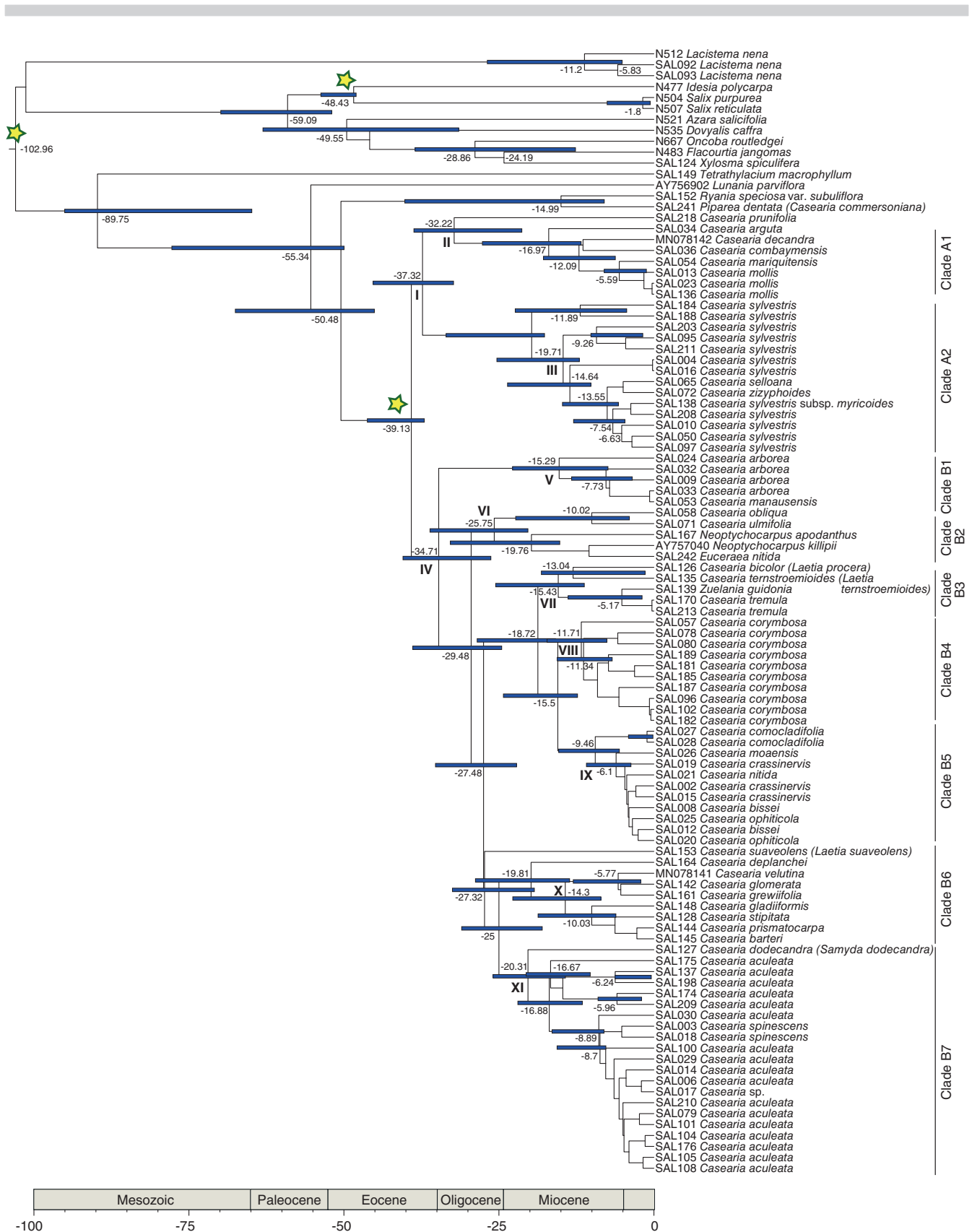
Fig. 6. Maximum clade credibility (MCC) chronogram obtained in BEAST based on four plastid markers (*rps4-trnL-F*, *trnK-matK*, *rpl16*, *petD*) with age estimate using three calibration points. Time interval is indicated in the scale in millions of years ago, yellow stars correspond to the calibration points. Blue bars represent 95% highest posterior density (HPD) intervals of the divergence times, the number at each node represents the median age of the most recent common ancestor (MRCA), and the roman numbers correspond to the clades for which ages are given in Table S5.

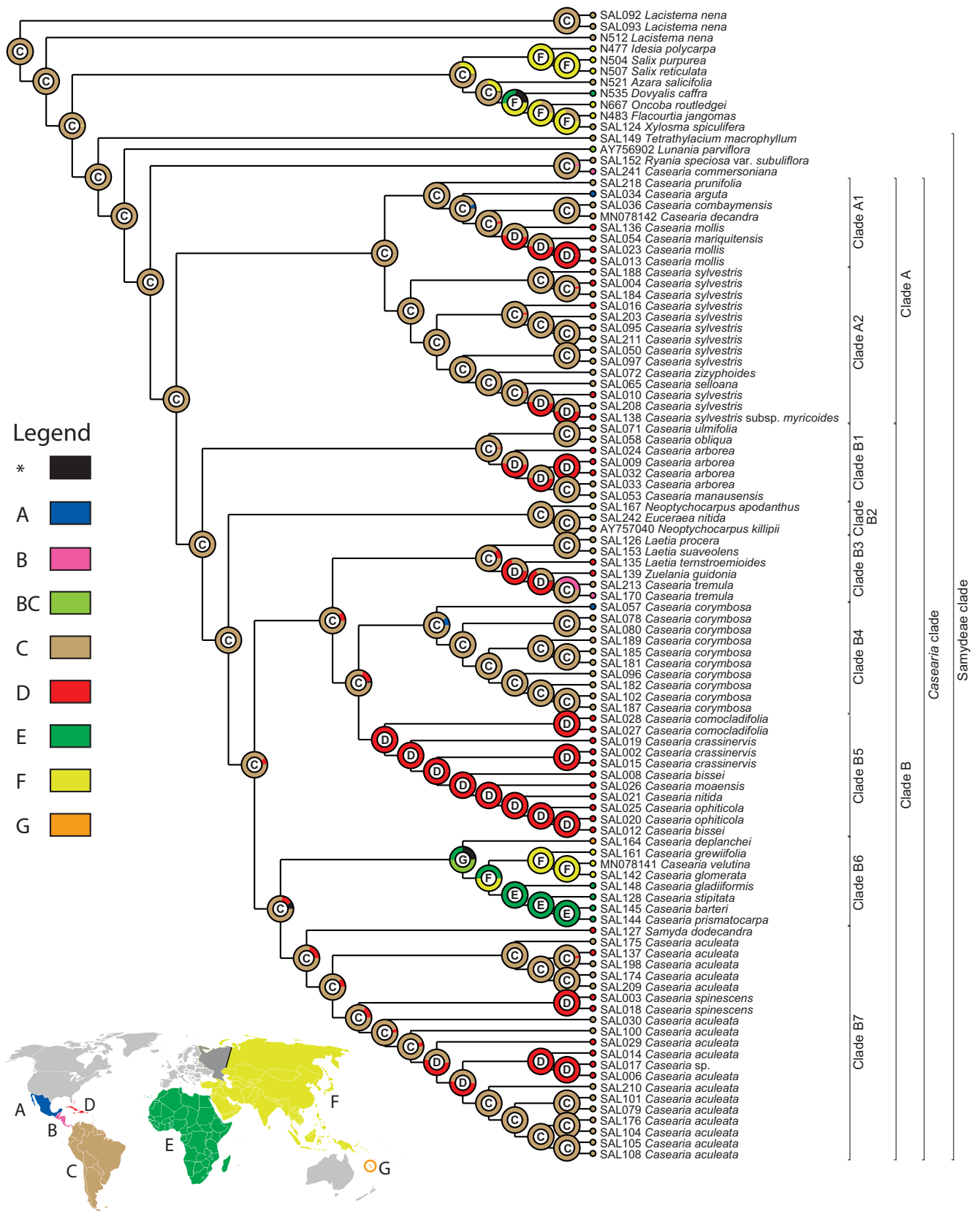**Fig. 7.** Result of the Bayesian binary method (BBM) analysis for the ancestral area reconstruction obtained with RASP. The pie chart at each node gives insight into the ancestral geographic range at the different nodes, black represents other ancestral ranges.

■ DISCUSSION

Phylogenetic relationships of Samydeae. — With this investigation we were able to recover a well-supported Samydeae clade (PP: 1, BS: 100, JK: 99.9). This clade includes *Tetrathylacium*, *Lunania*, *Ryania*, *Piparea* and a more inclusive also well-supported *Casearia* clade (Figs. 2, 3). Our molecular trees therefore considerably advance the previously existing knowledge (Alford, 2005; Samarakoon, 2015) about phylogenetic relationships in this part of the Salicaceae. The *Casearia* clade includes not only the genera *Laetia*, *Samyda* and *Zuelania* that were recently merged into *Casearia* (Samarakoon & Alford, 2019) but also the Neotropical genera *Euceraea* and *Neoptychocarpus*. The Samydeae are retrieved as sister to a well-supported clade (Fig. 2) comprising all other sampled genera of the Salicaceae with *Idesia* Maxim. and *Salix* L. in one subclade and *Azara* Ruiz & Pav., *Flacourtia* Comm. ex L'Hér., *Oncoba* Forssk. and *Xylosma* in another. These findings are in line with the results of Chase & al. (2002), and Xi & al. (2012) depicting two major lineages within Salicaceae, although we did not sample *Scyphostegia* that appeared as sister to them. More recently, Li & al. (2019) inferred a lineage of *C. decandra* and *C. velutina* as sister to other Salicaceae in their maximum likelihood analysis of 63 plastid gene sequences, in which other taxa of Samydeae were not included (Li & al., 2019). We retrieved *Tetrathylacium macrophyllum* Poepp. as sister to all other Samydeae with high support based on our combined plastid matrix and with all three inference methods (Fig. 2). This topology was also shown by Alford (2005) after combining *trnL-F* and *ndhF* sequence data with his morphological matrix and *Lunania parviflora* Spruce ex Benth. alone forming the second branch (Alford, 2005; his fig. 2.8). Also, *Ryania speciosa* Vahl and *Piparea dentata* Aubl. were here inferred as sisters, as third branch in Samydeae (Fig. 2). The *Casearia* clade, retrieved in our plastid tree with high support (PP: 1, BS: 100, JK: 100; Fig. 2), was also found in the combined morphological analysis by Alford (2005) with a JK value of 100%, although the internal relationships of the *Casearia* clade remained largely unresolved in his study. Now, we found *Samyda dodecandra* Jacq. as first branch of the well-supported subclade B7 of the *Casearia* clade in our plastid and ITS trees (Figs. 2, 3). The segregate genus *Laetia* is monophyletic within the *Casearia* clade, with *L. procera*, *L. suaveolens* and *L. ternstroemioides* retrieved together, however in a weakly supported subclade B3, along with *Zuelania guidonia* (Sw.) Britton & Millsp. and *C. tremula* (Fig. 2). Thus, our results support the recent nomenclatural changes made by Samarakoon & Alford (2019). However, *Casearia* still appears paraphyletic to *Euceraea* and *Neoptychocarpus*, which were retrieved with good support within the *Casearia* clade, in both the plastid and the nuclear trees (Figs. 2, 3).

Phylogenetic relationships of *Casearia*. — With the results of this study (Figs. 2, 3) we present the most comprehensive species-level phylogeny of *Casearia* to date, sampling 42 currently accepted taxa. Within the monophyletic genus *Casearia* (including *Euceraea* and *Neoptychocarpus*), nine subclades are retrieved that are mostly consistent in composition when the plastid (Fig. 2) and ITS trees (Fig. 3) are compared. Relationships between these subclades are better resolved in the plastid trees than with ITS, probably caused by considerable homoplasy and even alignment uncertainty due to predominant indels in ITS at larger distances as also observed in other datasets (Fuentes-Bazan & al., 2012; Falcón Hidalgo & al., 2020). Subclades A1 and A2 are consistently supported as sister by plastid and ITS data, both comprising South American, Mexican and Caribbean island taxa. The Palaeotropical species were retrieved together in a well-supported clade (subclade B6) nested among New World *Casearia*. Direct sister is subclade B7 including all samples of *C. aculeata* as well as *C. spinescens* from Cuba and *C. dodecandra* (formerly *Samyda dodecandra*) branching first. The subclade B5 with species from the Caribbean islands is sister to *C. corymbosa*, a species very common in the Caribbean part of Colombia but also distributed in Meso- and South America. Subclade B5 is constituted of species that were informally called the Ilicifoliae group defined by Sleumer (Sleumer, 1980), together with one new species from Cuba, *C. bissei*, that are morphologically different from other *Casearia* species by forming thick coriaceous and mostly spiny leaves and having pink or white flowers (Fig. 1). The Ilicifoliae group is endemic to Hispaniola and Cuba, with *C. comocladifolia*, occurring on the coasts of Cuba and Hispaniola (Gutiérrez, 2000), as sister to a lineage of Cuban serpentine endemics (*C. bissei*, *C. crassinervis*, *C. moaensis*, *C. ophiticola*). Although *C. nitida* is not in the Ilicifoliae group sensu Sleumer, Gutiérrez (1998) already suggested its affinity with it, which is confirmed by the molecular results. The well-supported serpentine clade is in line with what has been found in other flowering plant genera such as *Buxus* (Gonzalez Gutierrez, 2014) or *Phyllanthus* (Falcón Hidalgo & al., 2020). In these cases, the serpentine-adapted species often also differ by coriaceous and smaller leaves from their next relatives growing on limestone (Brady & al., 2005; Anacker, 2014). *Casearia* sect. *Casearia*, with *C. nitida* as type, is paraphyletic to the former segregate genera *Laetia*, *Samyda* and *Zuelania* and also to *Euceraea* and *Neoptychocarpus*. *Casearia* sect. *Guidonia*, with the type *C. spinescens*, appears to be very closely related to *C. aculeata* and is nested within the *C.* sect. *Casearia*, as is the monotypic *C.* sect. *Endoglossum* with *C. tremula*. Also, subclade A1 with *C. mollis* Kunth, *C. prunifolia* Kunth and relatives belongs to *C.* sect. *Casearia*, as currently classified, so that sect. *Crateria* with *C. sylvestris* and relatives becomes deeply nested in sect. *Casearia*. Thus, the currently used sectional classification (Sleumer, 1980) is highly artificial.

Some reticulation or incomplete lineage sorting becomes evident by comparing plastid and nuclear ITS topologies. Apparently, this only applies to individual taxa or samples within terminal subclades such as *C. spinescens* from Cuba, nested among samples of *C. aculeata* in the plastid tree (B7, Fig. 2), whereas it is sister to *C. aculeata* in the ITS tree (Fig. 3). At this level, speciation is still ongoing with some

probability that our dichotomous trees do not fully describe evolutionary relationships, but also that an even more complete sampling of individuals and populations across the range of species will yield further insights.

Evolution of morphological characters and circumscription of *Casearia*. — The ancestral character state reconstruction showed that the crown group of the *Casearia* clade (including the segregate genera *Euceraea*, *Laetia*, *Neoptychocarpus*, *Samyda* and *Zuelania*) is marked by a number of character state transitions. Several of the states are almost unique or completely unique to the members of this clade and thus support the corresponding monophyletic circumscription of *Casearia*. The pellucid dots on the leaves (Fig. 4) are a useful character to recognize *Casearia* in the field. However, the results of our analysis show that it is not a synapomorphy for the genus. Pellucid dots already evolved before the divergence of *Lunania* although they were lost in *Ryania* (Fig. 4). Fasciculate and glomerulate inflorescences are almost a synapomorphy of the *Casearia* clade within the Samydeae, although corymbs and cymose inflorescences, respectively present in *C. corymbosa* and Cuban endemic species, are derived from a fasciculate state. The first is independently gained in *C. corymbosa* and *C.* (= *Laetia*) *ternstroemioides*, and the cymes within the Caribbean subclade B4 (Fig. 5). The presence of one series of stamens is also almost a synapomorphy for the *Casearia* clade, although it got lost again in the *Neoptychocarpus* sublineage. *Laetia* and *Samyda* were recognized as distinct from *Casearia* and long accepted as separate genera on the basis of a few characters such as the absence of staminodes and a higher stamen number (Warburg, 1895; Sleumer, 1980; Samarakoon, 2015). However, our ancestral character state reconstruction shows that the presence of staminodes is homoplastic as they were lost two times, both in *Neoptychocarpus* and in *Laetia* (Fig. 5; the species *C. bicolor* (= *L. procera*), *C.* (= *L.*) *suaveolens* and *C.* (= *L.*) *ternstroemioides* in clade B3). The position of *C. tremula* in clade B3 is not resolved so that no conclusion is possible whether staminodes were re-gained in *C. tremula* or lost after its divergence. Furthermore, the stamen number is also homoplastic as it increased independently in subclade B3 (*C. tremula*, *C. bicolor* (= *L. procera)* and *C.* (= *L.*) *ternstroemioides* and in *C. laetioides* (= *Z. guidonia*), which are deeply nested in *Casearia*. The genus *Zuelania* can therefore not be distinguished from *Casearia* based on a higher number of stamens (de la Sagra, 1845; Sleumer, 1980). *Neoptychocarpus* is a South American genus with three species. It was first described by Buchheim (1959) and separated from *Casearia* for being dioecious, having the calyx lobes fused into a tube in addition to lacking staminodes; however, the transition from bisexual flowers to a dioecious arrangement of unisexual flowers is frequent in flowering plants (Renner & Ricklefs, 1995). Within Neotropical Samydeae, dioecy is only present in *Neoptychocarpus* and is also suspected to occur in the genus *Euceraea*, although its mode of reproduction remains unclear (Berry & al., 1998). Two Old World Samydeae, *Osmelia* and *Pseudosmelia*, also are dioecious (Alford, 2005) The transition from free to fused calyx lobes happened multiple times in the evolution of the *Casearia* clade, apart from *Neoptychocarpus* in *C. arborea*, *C. combaymensis* Tul. and the *C. aculeata–C. spinescens* lineage. Finally, some *Casearia* species (formerly *Laetia*) also lack staminodes, but this is a secondary loss (Fig. 5). Therefore, both morphology and the molecular phylogeny support the inclusion of *Neoptychocarpus* into *Casearia*. *Euceraea* is a South American genus of three species, first described by Martius (1831) and separated from *Casearia* for having a paniculate inflorescence, four perianth parts and a sessile stigma (Sleumer, 1980; Berry & al., 1998). However, like *Casearia* it possesses pellucid dots on the leaves, has eight stamen and staminodes. Individuals of *Casearia* can present four sepals; the paniculate inflorescence has been independently gained in the taxon, and other species also present a sessile stigma such as *C. laetioides* (= *Z. guidonia*). Therefore, both morphology and the molecular phylogeny support the inclusion of *Euceraea* into *Casearia*.

Biogeography of the pantropical genus *Casearia* at a continental level. — The Salicaceae and the Samydeae are part of the rapid radiation of the Malpighiales that started in the Cretaceous (Davis & al., 2005). The origin of the Samydeae here estimated as 102 Ma (95% HPD: 86.05–102.08, stem) and 90 Ma (95% HPD: 64.86–95.04, crown) falls into a time when the breakup of Gondwana could still have resulted in vicariant biogeographic patterns. The Samydeae clade is unambiguously South American in origin (Fig. 7). Our ancestral area reconstruction infers this even for the Salicaceae, although additional taxon sampling will be necessary to better understand the diversification in the other tribes of Salicaceae. Apparently, there was no migration into or out of South America within the Samydeae at Gondwanan times. The *Casearia* clade started to diversify during the Eocene (40 Ma, 95% HPD: 37.04–46.22, crown group age), and from the perspective of the diversification of the pantropical genus *Casearia* it is noteworthy that there is a single Old World subclade (B6, labelled as node X in Fig. 7) that diverged from Neotropical ancestors at the Oligocene/Miocene boundary (stem age 25 Ma, 95% HPD: 18.01–31.01). In our trees, the crown group of the Old World subclade is split into an African and an Asian lineage at 14 Ma (95% HPD: 8.55–22.76). Although our estimation of the crown group age may be slightly too young, considering that a denser sampling of species from this subclade may break down the rather long stem, it can be considered as Miocene with confidence (Fig. 7).

Casearia therefore shows a clear pattern of a South American ancestral area, from where migration or dispersal must have occurred towards Africa and Asia. There are two main hypotheses. One is migration across Laurasia via a series of connections that may have acted as a huge bridge (the "North Atlantic Land Bridge") for plant migrations, at a time with Eocene paratropical climates (Tiffney, 1985), considering that the breakup of Gondwana started in the Southern Hemisphere. Such a scenario was assumed for Malpighiaceae by Davis & al. (2002), supported by fossils found in North America and Europe. Our case of the late

Oligocene/early Miocene stem age (25 Ma, 18.01–31.01 HPD) of the Old World clade (B6) of *Casearia* is at the verge of supporting a scenario of Laurasian migration or being too young. The split into an African and an Asian sublineage of the Old World subclade of *Casearia* could be consistent with ancestors having migrated eastwards, first reaching Africa and then Asia. However, considering that the African and Asian species of *Casearia* sampled so far are resolved into two sister clades, any further conclusions on the directionality based on the sampling of this investigation are limited. It is noteworthy that the New Caledonian *C. deplanchei* is sister to the African plus Asian clade (Figs. 2, 3) and has diverged already in the early Miocene (stem age of 19.8 Ma, Fig. 6), whereas the African-Asian split of the crown group occurred much later (14.3 Ma). It is therefore questionable whether *C. deplanchei* represents a "Gondwanan relic" or an arrival from other continents after the breakup of Gondwana. It requires additional sampling, in particular of SE Asian species of *Casearia*, considering that New Caledonian biota have very different origins (Pillon, 2012).

There are other plant groups for which South America is the ancestral area, such as the Solanaceae (Dupin & al., 2017). In that case, many dispersal events from South America to other continents were dated to have occurred well after the breakup of Gondwana, starting at the end or later than the Oligocene, mostly into Central and North America. While the authors do not reject a migration through Beringian land bridge (Dupin & al., 2017), they favour a transoceanic long-distance dispersal due to the variety of fruits from dry to fleshy that can easily be transported through currents or migrating animals. Contrary to the Solanaceae, which include a large number of taxa growing in temperate climates, a migration of *Casearia* via the Beringia land bridge does not seem to be likely in *Casearia*, which is exclusively constituted by tropical shrubs and trees, for which the climate probably was too cold. There are other cases in which plants from the Neotropics migrated to the Old World, through long-distance dispersal as in the African *Tragia/Tragiella* lineage (Euphorbiaceae; Cervantes & al., 2016), the *Cissus trianae* clade (Vitaceae; Rodrigues & al., 2014), or *Jacquemontia* (Convolvulaceae; Namoff & al., 2010). Long-distance dispersal is also considered to explain the relatively recent origin of Neotropical Acanthaceae from Old World ancestors (Tripp & McDade, 2014) and it is another hypothesis for *Smilax* and Solanaceae (Zhao & al., 2013; Dupin & al., 2017) or *Phyllanthus procerus* and relatives (Falcón Hidalgo & al., 2020). *Casearia* fruits are small and fleshy, dispersal by birds is therefore a possible mechanism given the colourful arils (Howe & Primack, 1975; Howe & Estabrook, 1977).

Biogeographic relations between the Caribbean islands and adjacent mainland. — The largest Caribbean subclade with mostly Cuban endemics (e.g., *C. crassinervis*, *C. ophiticola*) and the Dominican *C. comocladifolia* started to diversify during the late Miocene (9 Ma, 95% HPD: 5.57–15.35, Figs. 6, 7). Studies on other genera that comprise endemic Caribbean subclades also found similar divergence times of their crown groups (Appelhans & al., 2012; Filipowicz & Renner, 2012; Cervantes & al., 2016; Machado & al., 2018). Indeed, during the late Miocene–Pliocene, the islands were separating from each other, which likely triggered speciation (Alonso & al., 2012). Whereas Santiago-Valentin & Olmstead (2004) summarized that most of the mainland areas adjacent to the Caribbean islands could have been places of origin for the ancestors of island taxa, some better-resolved and supported phylogenetic trees point to South American origins as in *Brunfelsia* (Filipowicz & Renner, 2012), which has not reached Mesoamerica, and *Philodendron* (Canal & al., 2019). However, Cervantes & al. (2016) showed through their biogeographic analysis of the subfamily Acalyphoideae (Euphorbiaceae), a speciose group of flowering plants on the Caribbean islands and as well on all adjacent mainland, that multiple colonization events from Mexico and Mesoamerica to the islands occurred since the Miocene.

In the case of *Casearia*, our results depict multiple migrations of the genus towards the Caribbean from South America, followed by speciation within the islands, especially Cuba and Hispaniola. The Caribbean clade is sister to *C. corymbosa*, here represented by several samples from Colombia and one from Mexico (Fig. 2). Due to the current sampling, the origin of the Caribbean *Casearia* clade is inferred as South American with the only Mexican individual appearing as sister to a Colombian clade. Our divergence time estimate indicates that stem nodes relevant for the split of Caribbean Island lineages are not older than 12 to 15 Ma (Fig. 6). This also the case for the shallow clade with *C. mollis* from Cuba and *C. mariquitensis* Kunth from Guyana, which are sister to *C. arguta* from Mexico (Figs. 2, 3, clade A1). Further sampling of individuals within widespread species and Mesoamerican-Mexican taxa will be needed to test if the ancestor of the Cuban-Hispaniolan *Casearia* clade B5 in the Miocene really arrived from the South American continent and to better resolve relationships in clade A1.

On the other hand, the stem and crown of the Caribbean subclade B5 are far too young to assume a migration via a GAARlandia land bridge that was advocated to have existed in the early Eocene ca. 40 Ma (Iturralde-Vinent, 1999). The debate if such a land bridge existed and if it facilitated the direct migration of plants to the Caribbean islands from South America is still ongoing (Nieto-Blázquez & al., 2017; Roncal & al., 2020), and our results add further evidence of Caribbean plant migrations that do not support the GAARlandia hypothesis.

Contrary to earlier ideas, the closure of the Isthmus of Panama is often reported as early as Miocene (Bacon & al., 2015; Cervantes & al., 2016; Sosa & al., 2018) and could have facilitated the subsequent crossing of relatively short marine distances. The timing and extent of this American biotic interchange is therefore relevant for Caribbean *Casearia* as a potential migration route rather than arrivals on Caribbean islands directly from South America. About 24% of *Casearia* species occur in Mesoamerica and Mexico, with eight species occurring only in that area (e.g., *C. bartletii* Lundell, *C. elegans* Standl., *C. williamsiana* Sleumer). However, these are

still underrepresented in our taxon sampling. Looking at widespread species, there are more taxa shared only between South America and the Caribbean islands than taxa shared only between Mesoamerica and the Caribbean. Our analysis includes two widely distributed species of *Casearia*, namely *C. aculeata* and *C. sylvestris*, which occur on most of the Caribbean Islands, in southern Mexico, Mesoamerica and large parts of South America (GBIF, 2020). The split into the respective lineages containing these species was inferred to have occurred 20 Ma (95% HPD: 14.28–25.95, stem age; Fig. 6) and 37 Ma (95% HPD: 32.32–45.29, stem age; Fig. 6), respectively.

Our current ancestral area reconstruction (Fig. 7) identifies South American ancestors for the sampled Caribbean plants. Whereas the existence of multiple migrations between mainland and Caribbean islands is suggested by our data, further analyses are required to understand the precise patterns, also because the currently applied species concepts appear to be very wide, with multiple heterotypic synonyms in both of these species (Sleumer, 1980; WFO, 2021).

Our gene trees do not entirely correspond to current species circumscriptions, which may indicate incomplete lineage sorting and reticulate speciation. Another explanation may be that the currently accepted taxa based on few deviating morphological characters (alpha-taxonomy) do not reflect meaningful biological entities. Interestingly, Cuba has a morphologically well-defined endemic subspecies *Casearia sylvestris* subsp. *myricoides*, found on serpentine soil (Gutiérrez, 2000), which appears nested within the *C. sylvestris* subclade, although it is distant from Cuban samples of *C. sylvestris* subsp. *sylvestris*. Interestingly, the individual of *C. arborea* from Jamaica is found sister to the plant from Colombia in our ITS tree (Fig. 3), but further sampling of this widespread species also from the Lesser Antilles and Mesoamerica, as well as phylogeographic methods applied to species complexes within *Casearia*, will be needed to illuminate their biogeographic history, which is likely to include more recent dispersals in addition to migration events in the Miocene. Considering that the majority of endemic Caribbean species belongs to more widespread and not endemic genera, further detailed analyses of such bigger genera will be instrumental to obtain a full picture of the evolution and diversification of Caribbean plants in time and space.

Implications for species delimitation. — Our molecular phylogenetic results reveal the need to clarify species limits within *Casearia*, as some species were retrieved paraphyletic to other species currently accepted. This regards to the widespread *C. sylvestris* in subclade A2, which is one of the most common species of the New World in the currently accepted species classification, ranging from Argentina and Uruguay to Mexico and to all Caribbean islands. *Casearia sylvestris* is retrieved paraphyletic to two South American taxa: *Casearia selloana*, the type of which is from Bahia, Brazil, and which was already considered to be a possible variant of *C. sylvestris* in very dry habitats (Sleumer, 1980).

The second taxon is *Casearia zizyphoides*, which was originally described from Venezuela (where our sample is from) but was also reported from Trinidad and Tobago and was considered a dry-adapted relative of *C. sylvestris* (Sleumer, 1980). It can be morphologically distinguished by the mucronate leaves and the simple style (branched in *C. sylvestris*). It should be examined in more detail if the individuals morphologically matching *C. zizyphoides* are closely related to the populations of *C. sylvestris* subsp. *myricoides* that occur as xerophytic shrubs over serpentine in Cuba (Gutiérrez, 2000), given that they appear closely related in our ITS trees, albeit without statistical support.

Another species retrieved as paraphyletic is *Casearia arborea*, a widely distributed species from Mexico to northern Argentina, including the Caribbean islands. It is retrieved together with *C. manausensis*, described in 1980 and so far only reported from Manaus, Brazil (Sleumer, 1980). It appears in the same group of *C. arborea* and is morphologically very similar. Its leaves differ in being hirsutulous beneath at the midrib and lateral nerves, and *C. arborea* leaves are reported to be entirely glabrous. We also retrieved a *C. aculeata* clade including *C. spinescens* in the plastid tree (Fig. 2), whereas *C. spinescens*, a widespread species here represented by two Cuban samples, appears as sister to all other samples from *C. aculeata* (Caribbean islands, Colombia, Ecuador) in the ITS tree (Fig. 3). The topological differences between plastid and nuclear trees may indicate plastid capture in *C. spinescens* and warrant the addition of further material matching the morphotype of *C. spinescens* to test if this evolved once or if the less numerous flowers in the inflorescences of *C. spinescens*, the smaller fruits compared to *C. aculeata* and a different distribution of spines on the plant (Gutiérrez, 2000; Sleumer, 1980) are more an adaptive homoplastic trait. *Casearia aculeata* is a widespread species that has more than 20 synonyms and was first described by Jacquin (1760) from a plant collected in Haiti. No authentic specimen is known for this species, and an illustration from Plumier (1757: t. 147, fig. 1) was later designated by Sleumer (1980) as a lectotype.

■ TAXONOMIC TREATMENT

The genera *Euceraea* and *Neoptychocarpus* are nested inside the *Casearia* clade, and the morphological characters that were used to distinguish it from *Casearia* are homoplastic or can be well-explained as part of the evolutionary diversification of *Casearia*. In order to make *Casearia* monophyletic, the genera *Euceraea* and *Neoptychocarpus* have to be merged.

1. ***Casearia apodantha*** (Kuhlm.) de Mestier, Celis & Borsch, **comb. nov.** ≡ *Ptychocarpus apodanthus* Kuhlm. in Arch. Jard. Bot. Rio de Janeiro 4: 358 1925 ≡ *Neoptychocarpus apodanthus* (Kuhlm.) Buchheim in Taxon 8: 76. 1959 – Lectotype (designated by Sleumer in Fl. Neotrop. Monogr. 22: 255. 1980): Brazil, Parà, Peixe Boi, fr., *Huber s.n.*

(MG barcode MG0007811; isolectotype: MG barcode MG000930 [image!]).

2. ***Casearia chocoensis*** (A.H.Gentry & Forero) de Mestier, Celis & Borsch, **comb. nov.** ≡ *Neoptychocarpus chocoensis* A.H.Gentry & Forero in Phytologia 55(6): 368–370. 1984 – Holotype: Colombia, Choco, road (under construction) to Lloro (2 km S of Yuto) from km 27 of Quibdó–Istmina road, alt. ca. 100 m, tropical pluvial forest, 05°30′ N, 76°37′W, 1982, *Gentry & J. Brand 36955* (COL n.v.; isotypes: F barcode V0060274F [No. 1921760; image!], K barcode K000471345 [image!], L barcodes L 0539777 [image!] & L 0539778 [image!], MO barcodes MO-279911 [No. 3008215; image!] & MO-279912 [No. 3189823; image!], NY barcodes 00108117 [image!] & 00108118 [image!], US barcode 00114918 [image!]).

3. ***Casearia euceraea*** de Mestier, Celis & Borsch, **nom. nov.** ≡ *Euceraea nitida* Mart., Nov. Gen. Sp. Pl. 3: 90, t. 238. 1831, non Jacq. 1760 – **Lectotype (designated here):** Colombia, Amazonas, Rio Caqueta, falls of Aracuara & Montes Cupati, 1885, *Martius 3202* (M barcode M-0113016 [image!]; isolectotypes: M barcodes, M-0113013 [image!], M-0113014 [image!] & M-0113015 [image!]).

Note: The name *Casearia nitida* Jacq. was validly published by Jacquin (1760), and the respective taxon, a species endemic to Cuba, is accepted (Gutiérrez, 2000). A new name for this largely Amazonian species had to be found. The lectotype is here designated, following the original description of Martius, and the locality was precised following Dugand (1948).

4. ***Casearia killipii*** (Monach.) de Mestier, Celis & Borsch, **comb. nov.** ≡ *Ptychocarpus killipii* Monach. in Phytologia 2. 432. 1948 ≡ *Neoptychocarpus killipii* (Monach.) Buchheim in Taxon 8: 76. 1959 – Holotype: Peru, Loreto, Mishuyacu near Iquitos, 1929, *Killip & Smith 29936* (NY barcode 00108135 [image!]; isotypes: F barcode V0041365F [No. 615917; image!], L barcode L 0011146 [image!], US barcode 00114915 [image!]).

5. ***Casearia rheophytica*** (P.E.Berry & M.E.Olson) de Mestier, Celis & Borsch, **comb. nov.** ≡ *Euceraea rheophytica* P.E. Berry & M.E.Olson in Brittonia 50(4): 493–496. 1998 – Holotype: Venezuela, Amazonas: cerro de la Neblina, camp IV, 15 km NNE of Pico Phelps, N branch of river in canyon, 780 m, 00°51′N, 65°57′W, 1984, *R. Liesner 16820* (MO barcode MO-1921543 [No. 5998402; image!]).

6. ***Casearia sleumeriana*** (Steyerm. & Maguire) de Mestier, Celis & Borsch, **comb. nov.** ≡ *Euceraea sleumeriana* Steyerm. & Maguire in Mem. New York Bot. Gard. 23: 870, fig. 10. 1972 – Holotype: Venezuela, Bolivar, occidental border of the Meseta de Sarisarinama, 1967, *Steyermark 97851* (VEN n.v.; isotypes: L barcode L 0010830 [image!]; NY barcode 00107406 [image!]; US barcode 00114815 [image!]).

■ CONCLUSION

We presented here the most comprehensive study to date of the tribe Samydeae and more precisely of *Casearia*, based on five molecular markers from plastid and nuclear DNA. Samydeae was retrieved as a well-supported clade as was a *Casearia* clade with nine subclades, including accessions from *Euceraea* and *Neoptychocarpus*. The combination of rapidly evolving plastid genomic regions with high levels of hierarchical phylogenetic signal resulted in an overall well-resolved and supported phylogenetic tree of the Samydeae. Considering that the topology is largely consistent with the trees inferred from nuclear ITS, our study provides the first picture on the evolution of *Casearia* and relatives through time and space. Our study unravelled that some of the currently accepted taxon concepts at species level are in conflict with a more evolutionary based delimitation of these species, in particular of the taxa considered to be more widespread with ranges shared between the Caribbean islands and Mesoamerica, Mexico and South America. Further work is needed that uses a geographically representative sampling within these taxa, and employs further molecular markers to increase resolution within shallow terminal clades and also network analyses. Our inference of ancestral areas underscores the importance of both a dense taxon sampling and a thorough knowledge of species-level taxonomy to illuminate the origin of the Caribbean flora and its biogeographic relationships. We have used an approach in which the geographic origin of each specimen included in the molecular trees was coded, and thus were able to avoid bias that could have been introduced by extrapolating distributions of taxa without prior clarification of species limits. Our study shows once again that migrations between the Caribbean islands and adjacent mainland have been frequent within genera since the Miocene, and that including or not including some species may be fundamental to correctly infer to geographic origin of the Caribbean flora.

■ AUTHOR CONTRIBUTIONS

Study conception and design: AM, TB; performance of the research: AM, BFH, GB, JG, MC, TB; acquisition of material in the field and identification of species: AM, BFH, GB, JG, MC, TB; acquisition of molecular data: AM; analysis of the data: AM, TB; drafting of manuscript: AM, TB; critical revision: AM, BFH, GB, JG, MC, TB. All authors contributed to and accepted the final manuscript. — AM, https://orcid.org/0000-0002-9976-8196; GB, https://orcid.org/0000-0001-5395-7533; MC, https://orcid.org/0000-0002-4298-2052; BFH, https://orcid.org/0000-0003-2519-3278; JG, https://orcid.org/0000-0003-2868-6254; TB: https://orcid.org/0000-0002-5724-2786

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Appendix 1. Taxa used for molecular data.

Name and author, labnumber, DNA bank number, locality, collector and collector number, herbarium voucher and NCBI accession number for *rps4-trnL-F*, *trnK-matK*, *rpl16*, *petD* and ITS, respectively. Species names are used following Sleumer (1980). Accessions are listed in alphabetic order. [1]Korotkova & al. (2009), [2]Li & al. (2019), [3]Alford (2005), all others are newly sequenced for this study; "–" indicates missing sequences.

Outgroups: *Azara salicifolia* Griseb., N521, Bolivia, *G. Torrico & C. Peca 204* (LPB), FM178041[1], –, –, –, –; *Casearia commersoniana* Cambess., SAL241, Costa Rica, *Salaza 2638* (BH), OU452357, –, OU452355, OU452353, OU452365; *Dovyalis caffra* (Hook.f. & Harv.) Warb., BG Bochum, *T. Borsch* (B), FM178039[1], –, –, –, –; *Flacourtia jangomas* (Lour.) Raeusch., BG Bonn 12841, *N. Korotkova 59* (BONN), FM178042[1], –, –, –, –; *Idesia polycarpa* Maxim., BG Bonn 15364, *N. Korotkova 12* (BONN), FM178040[1], –, –, –, –; *Lacistema nena* J.F.Macbr., SAL092, Colombia: La Guajira, *M.C. Martinez-Habibe & al. 2552* (UNO), HG997980, OV302621, HG997900, HG998084, FR990812; *Lacistema nena* J.F.Macbr., SAL093, Colombia: La Guajira, *M.C. Martinez-Habibe & al. 2553* (UNO), HG997981, OV302622, HG997901, HG998085, FR990813; *Lacistema nena* J.F.Macbr., N512, Bolivia, *P. Espinoza 3* (B, LPB), FM178032[1], –, –, –, –; *Ryania speciosa* var. *subuliflora* (Sand.) Monach., SAL152, DB 44793, Peru, *M. Rimachi Y. 8081* (BR), HG998004, OV302639, HG997922, HG998104, FR990829; *Salix purpurea* L., BG Bonn 17982, *A. Worberg 30* (BONN), FM178037[1], –, –, –, –; *Salix reticulata* L., Germany: Bavaria, *T. Borsch 3825* (B), FM178038[1], –, –, –, –; *Tetrathylacium macrophyllum* Poepp., SAL149, DB 44790, Peru: Loreto, *M. Rimachi Y. 8224* (BR), HG997921, OV302638, HG998103, –, FR990828; *Xylosma spiculifera* (Tul.) Triana & Planch., SAL124, Colombia: Cundinamarca, *Mariasole Calbi & al. 205* (JBB), HG997991, OV302628, –, HG998094, FR990817. — **Ingroups:** *Casearia aculeata* Jacq., SAL006, DB 44708, Cuba: Guantanamo, *T. Borsch 4284* (HAJB, B), HG997945, OV302588, HG997867, –, FR990780; *Casearia aculeata* Jacq., SAL014, DB 44716, Cuba: Villa Clara, *T. Borsch 5243* (HAJB, B), HG997951, OV302593, HG997871, HG998057, FR990786; *Casearia aculeata* Jacq., SAL029, DB 27861, Dominica: Pedernales, *W. Greuter 26587* (B), HG997964, OV302606, HG997884, HG998070, FR990798; *Casearia aculeata* Jacq., SAL030, DB 27862, Ecuador: Guayas, *E. Asplund 15213* (B), HG997965, OV302607, HG997885, HG998071, FR990799; *Casearia aculeata* Jacq., SAL079, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 2510* (UNO), HG997978, OV302619, HG997898, HG998082, –; *Casearia aculeata* Jacq., SAL086, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 2538* (UNO), –, –, –, –,FR990811; *Casearia aculeata* Jacq., SAL100, Colombia: La Guajira, *M.C. Martinez-Habibe & al. 2591* (UNO), HG997985, –, HG997905, HG998088, –; *Casearia aculeata* Jacq., SAL101, Colombia: La Guajira, *M.C. Martinez-Habibe & al. 2593* (UNO), HG997986, OV302624, HG997906, HG998089, –; *Casearia aculeata* Jacq., SAL104, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 2605* (UNO), HG997988, OV302625, HG997908, HG998091, –; *Casearia aculeata* Jacq., SAL105, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 2606* (UNO), HG997989, OV302626, HG997909, HG998092, –; *Casearia aculeata* Jacq., SAL108, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 2611* (UNO), HG997910, HG998093, FR990816; *Casearia aculeata* Jacq., SAL137, DB 44778, Cuba: Artemisa, *S. Fuentes & al. 1977* (HAJB, B), HG997997, OV302632, HG997916, HG998098, FR990823; *Casearia aculeata* Jacq., SAL174, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 2929* (UNO), HG998009, OV302645, –, HG998109, –; *Casearia aculeata* Jacq., SAL175, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 2938* (UNO), HG998010, OV302646, HG997928, HG998110, –; *Casearia aculeata* Jacq., SAL176, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 2940* (UNO), HG998011, OV302647, –, HG998111, –; *Casearia aculeata* Jacq., SAL198, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 3000* (UNO), HG998019, –, HG997935, HG998119, FR990838; *Casearia aculeata* Jacq., SAL209, Colombia: La Guajira, *M.C. Martinez-Habibe & al. 2745* (UNO), HG998021, OV302655, HG997937, HG998121, FR990840; *Casearia aculeata* Jacq., SAL210, Colombia: La Guajira, *M.C. Martinez-Habibe & al. 2756* (UNO), HG998022, OV302656, HG997938, HG998122, FR990841; *Casearia arborea* (Rich.) Urb., SAL009, DB 44711, Cuba: Guantanamo, *T. Borsch & al. 4845* (HAJB, B), HG997947, OV302590, –, HG998055, FR990782; *Casearia arborea* (Rich.) Urb., SAL024, DB 44726, Cuba: Holguin, *S. Fuentes & al. 1370* (HAJB, B), HG997959, OV302601, HG997880, HG998065, FR990794; *Casearia arborea* (Rich.) Urb., SAL032, DB 27864, Jamaica: Parish of Clarendon, *E. Köhler 231* (HAJB, B), HG997966, OV302608, HG997886, HG998072, FR990800; *Casearia arborea* (Rich.) Urb., SAL033, DB27865, Colombia: Antioquia, *R. Fonnegra 6711* (B), HG997967, –, –, –, FR990801; *Casearia arguta* Kunth, SAL034, DB27866, Mexico: Chiapas, *E. Martinez M-26281* (B), HG997968, OV302609, HG997887, HG998073, FR990802; *Casearia barteri* Mast., SAL145, DB 44786, Gabon: Moyen-Ogooué, *F.J. Breteler 13106* (BR), HG998001, OV302636, HG997919, HG998102, FR990826; *Casearia bicolor* Urb., SAL126, DB33090, Guyana: Rupununi, *M.J. Jansen-Jacobs 2495* (B), HG997992, –, HG997911, –, FR990818; *Casearia bissei* J.E.Gut., SAL008, DB 44710, Cuba: Guantanamo, *M. Ackermann 862* (HAJB, B), HG997946, OV302589, HG997868, HG998054, FR990781; *Casearia bissei* J.E.Gut., SAL012, DB 44714, Cuba: Guantanamo, *T. Borsch & al. 4428* (HAJB, B), HG997949, OV302591, HG997869, HG998056, FR990784; *Casearia combaymensis* Tul., SAL036, DB27868, French Guyana, *J.J. de Granville 13157* (B), HG997969, OV302610, HG997888, HG998074, FR990803; *Casearia comocladifolia* Vent., SAL027, DB 44729, Cuba: Independancia, *S. Fuentes*

Appendix 1. Continued.

& al. 1164 (HAJB, B), HG997962, OV302604, HG997882, HG998068, FR990796; ***Casearia comocladifolia*** Vent., SAL028, DB 44730, Cuba: Barahona, *S. Fuentes & al. 1212* (HAJB, B), HG997963, OV302605, HG997883, HG998069, FR990797; ***Casearia corymbosa*** Kunth, SAL057, DB27889, Mexico: Oaxaca, *Misael Elorsa C. 478* (B), HG997973, OV302613, HG997892, HG998077, FR990805; ***Casearia corymbosa*** Kunth, SAL078, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 2502* (UNO), OV302618, –, HG997897, HG998081, –; ***Casearia corymbosa*** Kunth, SAL080, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 2512* (UNO), HG997979, OV302620, HG997899, HG998083, –; ***Casearia corymbosa*** Kunth, SAL096, Colombia: La Guajira, *M.C. Martinez-Habibe & al. 2572* (UNO), HG997983, –, HG997903, HG998086, –; ***Casearia corymbosa*** Kunth, SAL102, Colombia: La Guajira, *M.C. Martinez-Habibe & al. 2598* (UNO), HG997987, –, HG997907, HG998090, –; ***Casearia corymbosa*** Kunth, SAL181, Colombia: Magdalena, *M.C. Martinez-Habibe & al. 2973* (UNO), HG998012, OV302648, HG997929, HG998112, –; ***Casearia corymbosa*** Kunth, SAL182, Colombia: Magdalena, *M.C. Martinez-Habibe & al. 2976* (UNO), HG998013, OV302649, HG997930, HG998113, –; ***Casearia corymbosa*** Sw., SAL185, Colombia: Magdalena, *M.C. Martinez-Habibe & al. 2982* (UNO), HG998015, OV302651, HG997932, HG998115, FR990836; ***Casearia corymbosa*** Kunth, SAL187, Colombia: Magdalena, *M.C. Martinez-Habibe & al. 2986* (UNO), HG998016, OV302652, HG997933, HG998116, –; ***Casearia corymbosa*** Kunth, SAL189, Colombia: Magdalena, *M.C. Martinez-Habibe & al. 2988* (UNO), HG998018, OV302654, HG997934, HG998118, –; ***Casearia crassinervis*** Urb., SAL002, DB 44704, Cuba: Holguin, *T. Borsch & al. 4056* (HAJB, B), HG997942, OV302586, –, HG998052, FR990778; ***Casearia crassinervis*** Urb., SAL015, DB 44717, Cuba: Holguin, *N. Köster & al. 2724* (HAJB, B), HG997952, OV302594, HG997872, HG998058, FR990787; ***Casearia crassinervis*** Urb., SAL019, DB 44721, Cuba: Holguin, *S. Fuentes & al. 453* (HAJB, B), HG997956, OV302598, HG997876, HG998062, –; ***Casearia decandra*** Jacq., MN078142[2] (complete genome); ***Casearia deplanchei*** Sleumer, SAL164, DB 44875, New Caledonia, *G. Dagostini 783*, P 04819071, HG998006, OV302642, HG997925, HG998106, FR990832; ***Casearia dodecandra*** (Jacq.) T.Samar. & M.H.Alford, SAL127, DB 33091, Dominica: Pedernales, *W. Greuter 26585* (B), HG997993, OV302629, HG997912, HG998095, FR990819; ***Casearia gladiiformis*** Mast., SAL148, DB 44789, Mozambique, *P.C.M. Jansen 7670*, BR 0000015960818, HG998003, OV302637, HG997920, –, FR990827; ***Casearia glomerata*** Roxb., SAL142, DB 44783, Hong Kong, *L. Tsuen 12687* (BR), HG998000, OV302635, HG997918, HG998101, FR990825; ***Casearia grewiifolia*** Vent., SAL161, DB 44874, Vietnam, *U. Swenson 1541* (P), OV302641, HG997924, –, HG998105, FR990831; ***Casearia sp.***, SAL017, DB 44719, Cuba: Holguin, *S. Fuentes & al. 442* (HAJB, B), HG997954, OV302596, HG997874, HG998060, FR990789; ***Casearia javitensis*** Kunth, SAL169, DB 44877, French Guyana, *J.J. de Granville 17165* (P), –, –, –, –, FR990834; ***Casearia laetioides*** (A.Rich.) Northr., SAL139, DB 44780, Cuba: Pinar del Rio, *T. Borsch & al. 5954* (B), HG997999, OV302634, –, HG998100, –; ***Casearia manausensis*** Sleumer, SAL053, DB27885, Brasil: Amazonas, *J.L. Santos 855* (B), HG997971, OV302612, HG997890, HG998076, –; ***Casearia mariquitensis*** Kunth, SAL054, DB27886, Guyana: Rupununi, *M.J. Jansen-Jacobs 4348* (B), HG997972, –, HG997891, –, –; ***Casearia moaensis*** Vict., SAL026, DB 44728, Cuba: Holguin, *S. Fuentes & al. 1689* (HAJB, B), HG997961, OV302603, –, HG998067, FR990795; ***Casearia mollis*** Kunth, SAL013, DB 44715, Cuba: Villa Clara, *T. Borsch & al. 5138* (HAJB, B), HG997950, OV302592, HG997870, –, FR990785;***Casearia mollis*** Kunth, SAL023, DB 44725, Cuba: Matanzas, *T. Borsch & al. 5611* (HAJB, B), –, –, HG997879, HG998064, FR990793; ***Casearia mollis*** Kunth, SAL136, DB 44777, Cuba: Artemisia, *S. Fuentes & al. 1976* (HAJB, B), HG997996, OV302631, HG997915, HG998097, FR990822; ***Casearia nitida*** Jacq., SAL021, DB 44723, Cuba: Las Tunas, *S. Fuentes & al. 841* (HAJB, B), HG997958, OV302600, HG997878, –, FR990791; ***Casearia obliqua*** Sprengel, SAL058, DB27890, Brasil: Parana, *R. Kummrow 3000* (B), HG997974, OV302614, HG997893, –, FR990806; ***Casearia ophiticola*** Vict., SAL020, DB 44722, Cuba: Holguin, *S. Fuentes & al. 656* (HAJB, B), HG997957, OV302599, HG997877, HG998063, FR990790; ***Casearia ophiticola*** Vict., SAL025, DB 44727, Cuba: Holguin, *S. Fuentes & al. 1645* (HAJB, B), HG997960, OV302602, HG997881, HG998066, –; ***Casearia prismatocarpa*** Mast., SAL144, Liberia, *C.C.H. Jongkind 9804* (BR), –, –, –, –, –; ***Casearia prunifolia*** Kunth, SAL218, DB 44851, Peru: Loreto, *A. Gentry 29158* (JBGP), HG998025, –, HG997941, –, FR990843; ***Casearia selloana*** Eichler, SAL065, DB27897, Brasil: Paraiba do Sul, *Schwacke 3215* (B), HG997975, OV302615, HG997894, HG998078, –; ***Casearia spinescens*** (Sw.) Griseb., SAL003, DB 44705, Cuba: Holguin, *T. Borsch & al. 4115* (HAJB, B), HG997943, OV302587, HG997866, HG998053, FR990779; ***Casearia spinescens*** (Sw.) Griseb., SAL018, DB 44720, Cuba: Holguin, *S. Fuentes & al. 445* (HAJB, B), HG997955, OV302597, HG997875, HG998061, –; ***Casearia stipitata*** Mast., SAL128, DB33096, Cameroon, *A.J.M. Leeuwenberg 9884* (B), HG997994, –, HG997913, HG998096, FR990820; ***Casearia suaveolens*** (Poepp.) T.Samar. & M.H.Alford, SAL 153, Peru, *M. Rimachi Y. 7666* (BR), HG998005, OV302640, HG997923, –, FR990830; ***Casearia sylvestris*** Sw., SAL016, DB 44718, Cuba: Holguin, *SF 441* (HAJB, B), HG997953, OV302595, HG997873, HG998059, FR990788; ***Casearia sylvestris*** Sw., SAL050, DB27882, Venezuela: Guerico, *H. & E. Walter 236* (B), HG997970, OV302611, –, HG998075, –; ***Casearia sylvestris*** Sw., SAL095, Colombia: La Guajira, *M.C. Martinez-Habibe & al. 2569* (UNO), HG997982, –, HG997902, –, FR990814; ***Casearia sylvestris*** Sw., SAL097, Colombia: La Guajira, *M.C. Martinez-Habibe & al. 2581* (UNO), HG997984, OV302623, HG997904, HG998087, FR990815; ***Casearia sylvestris*** Sw., SAL184, Colombia: Magdalena, *M.C. Martinez-Habibe & al. 2981* (UNO), HG998014, OV302650, HG997931, HG998114, –; ***Casearia sylvestris*** Sw., SAL188, Colombia: Magdalena, *M.C. Martinez-Habibe & al. 2987* (UNO), HG998017, OV302653, –, HG998117, FR990837; ***Casearia sylvestris*** Sw., SAL200, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 3002* (UNO), –, –, –, –, FR990839; ***Casearia sylvestris*** Sw., SAL203, Colombia: Bolivar, *M.C. Martinez-Habibe & al. 3005* (UNO), HG998020, –, HG997936, HG998120, –; ***Casearia sylvestris*** Sw., SAL211, Colombia: La Guajira, *M.C. Martinez-Habibe & al. 2764* (UNO), HG998023, OV302657, HG997939, HG998123, –; ***Casearia sylvestris*** Sw., SAL022, DB 44724, Cuba: Pinar del Rio, *T. Borsch & al. 5784* (HAJB, B), –, –, –, –, FR990792; ***Casearia sylvestris*** subsp. ***myricoides*** (Griseb.) J.E.Gut., SAL010, DB 44712, Cuba: Guantanamo, *T. Borsch & al. 4901* (HAJB, B), HG997948, –, –, –, FR990783; ***Casearia sylvestris*** subsp. ***myricoides*** (Griseb.) J.E.Gut., SAL138, DB 44779, Cuba: Sancti Spiritu, *S. Fuentes & al. 1727* (HAJB, B), HG997998, OV302633, HG997917, HG998099, FR990824; ***Casearia ternstroemioides*** (Griseb.) T.Samar. & M.H.Alford, SAL 135, DB 44776, Cuba: Guantanamo, *T. Borsch & al. 4425* (HAJB, B), HG997995, OV302630, HG997914, –, FR990821; ***Casearia tremula*** (Griseb.) Griseb. ex C.Wright, SAL 170, DB, Nicaragua: Boaco, *J.B. Quezada 242* (P), HG998008, OV302644, HG997927, HG998108, FR990835; ***Casearia tremula*** (Griseb.) Griseb. ex C.Wright, SAL 213, DB 44846, Colombia: Bolivar, *V. Londono 408* (JBGP), HG998024, –, HG997940, HG998124, FR990842; ***Casearia ulmifolia*** Vahl ex Vent., SAL071, DB27903, French Guyana, *S. Mori 21177* (B), HG997976, OV302616, HG997895, HG998079, FR990807; ***Casearia velutina*** MN078141[2] (complete genome); ***Casearia zizyphoides*** Kunth, SAL072, DB27904, Venezuela: Bolivar, *J.A. Steyermark 88293* (B), HG997977, OV302617, HG997896, HG998080, FR990808; ***Euceraea nitida*** Mart., SAL242, Brazil, *G. Hobbes 213* (BH), OU452358, –, OU452356, OU452354, –, OU452366; ***Neoptychocarpus apodanthus*** (Kuhlm.) Buch., SAL167, DB 44802, French Guyana, *O. Lachenaud 2143* (P), HG998007, OV302643, HG997926, HG998107, FR990833; ***Neoptychocarpus killipii*** (Monach.) Buch., *Alford & Grandez 3119*, AY757040[3]

Appendix 2. Matrix of coded of morphological characters. The characters are 1: sexual system (0 = bisexual, 1 = dioecious), 2: calyx lobes fused into a tube (0 = absent, 1 = present), 3: presence of pellucid dots on the leaves (0 = absent, 1 = present), 4: stamen number (1 = 1–6, 2 = 7–12, 3 >12), 5: presence of staminodes (0 = absent, 1 = present), 6: arrangement of the stamens (0 = spiral, 1 = uniseriate), 7: inflorescence type (0 = panicle, 1 = catkin, 2 = raceme/corymb, 3 = fascicle/glomerule, 4 = cyme, 5 = solitary).

Species	Code	Character 1	2	3	4	5	6	7	Source
Azara salicifolia	N521	0	0	0	3	1	0	2	Sleumer (1980), Alford (2015)
Casearia commersoniana	SAL242	0	0	1	2	1	1	3	Sleumer (1980)
Dovyalis caffra	N535	1	0	0	3	0	0	3	Protologue, herb. specimen
Flacourtia jangomas	N483	1	0	0	3	0	0	2	Sleumer (1954), Alford (2015)
Idesia polycarpa	N477	1	0	0	3	0	0	0	Yang & Zmarzty (2007)
Lacistema nena	N512	0	0	0	1	0	0	1	Sleumer (1980), Macbride (1941)
Ryania speciosa var. *subuliflora*	SAL152	0	0	0	3	0	0	5	Sleumer (1980), Alford (2015)
Salix purpurea	N504	1	0	0	1	0	0	1	Argus (1986), herb. specimen
Salix reticulata	N507	1	0	0	1	0	0	1	Argus (1997), herb. specimen
Tetrathylacium macrophyllum	SAL149	0	0	0	1	0	–	0	Sleumer (1980), Alford (2015)
Xylosma spiculifera	SAL124	1	0	0	3	0	–	3	Sleumer (1980)
Casearia aculeata	SAL014	0	1	1	2	1	1	3	Sleumer (1980), Gutiérrez (2000), herb. voucher
Casearia arborea	SAL024	0	1	1	2	1	1	3	Sleumer (1980), herb. voucher
Casearia arguta	SAL034	0	0	1	2	1	1	3	Sleumer (1980), herb. voucher
Casearia barteri	SAL145	0	0	1	2	1	–	3	Sleumer (1971), Breteler (2008)
Casearia bicolor (*Laetia procera*)	SAL126	0	0	1	3	0	1	3	Sleumer (1980), herb. voucher
Casearia bissei	SAL012	0	0	1	2	1	1	4	Gutiérrez (2000)
Casearia combaymensis	SAL036	0	1	1	2	1	1	3	Sleumer (1980)
Casearia comocladifolia	SAL028	0	0	1	2	1	1	3	Gutiérrez (2000)
Casearia corymbosa	SAL189	0	0	1	2	1	1	2	Sleumer (1980)
Casearia crassinervis	SAL019	0	0	1	2	1	1	4	Gutiérrez (2000), herb. voucher
Casearia decandra	MN078142	0	0	1	2	1	1	3	Sleumer (1980)
Casearia deplanchei	SAL164	0	0	1	2	1	–	3	Sleumer (1974), Lescot (1980)
Casearia dodecandra (*Samyda dodecandra*)	SAL127	0	0	1	2	1	1	3	Gutiérrez (2000)
Casearia gladiiformis	SAL148	0	0	1	2	1	1	3	Sleumer (1971), Alford (2015)
Casearia glomerata	SAL142	0	–	1	2	1	–	3	Yang & Zmarzty (2007)
Casearia grewiifolia	SAL161	0	–	1	2	1	1	3	Sleumer (1955)
Casearia laetioides (*Zuelania guidonia*)	SAL139	0	0	1	3	1	1	3	Sleumer (1980), Gutiérrez (2000)
Casearia manausensis	SAL053	0	0	1	2	1	1	3	Sleumer (1980), herb. voucher
Casearia mariquitensis	SAL054	0	0	1	2	1	1	3	Sleumer (1980)
Casearia moaensis	SAL026	0	0	1	2	1	1	4	Gutiérrez (2000), herb. voucher
Casearia mollis	SAL023	0	0	1	2	1	1	3	Sleumer (1980)
Casearia nitida	SAL021	0	0	1	2	1	1	4	Gutiérrez (2000), herb. voucher
Casearia obliqua	SAL058	0	0	1	2	1	1	3	Sleumer (1980)
Casearia ophiticola	SAL020	0	0	1	2	1	1	4	Gutiérrez (2000)
Casearia prismatocarpa	SAL144	0	–	1	2	1	–	3	Breteler (2008)
Casearia prunifolia	SAL218	0	0	1	2	1	1	3	Sleumer (1980)
Casearia selloana	SAL065	0	0	1	2	1	1	3	Sleumer (1980)
Casearia sp.	SAL017	0	–	1	2	1	–	3	Herb. voucher
Casearia spinescens	SAL018	0	0	1	2	1	1	3	Gutiérrez (2000)
Casearia stipitata	SAL128	0	0	1	2	1	1	3	Sleumer (1971), Breteler (2008)
Casearia suaveolens (*Laetia suaveolens*)	SAL153	0	0	1	2	0	1	3	Sleumer (1980)

(Continues)

Appendix 2. Continued.

Species	Code	Character							Source
		1	2	3	4	5	6	7	
Casearia sylvestris	SAL097	0	0	1	2	1	1	3	Sleumer (1980), Alford (2015), herb. voucher
Casearia sylvestris subsp. *myricoides*	SAL138	0	0	1	2	1	1	3	Gutiérrez (2000), herb. voucher
Casearia ternstroemioides (*Laetia ternstroemioides*)	SAL135	0	0	1	3	0	1	2	Sleumer (1980)
Casearia tremula	SAL213	0	0	1	3	1	1	3	Sleumer (1980), Gutiérrez (2000), Alford (2015), herb. voucher
Casearia ulmifolia	SAL071	0	0	1	2	1	1	3	Sleumer (1980), Marquete & Mansano (2012)
Casearia velutina	MN078141	0	0	1	2	1	1	3	Sleumer (1955), Yang & Zmarzty (2007)
Casearia zizyphoides	SAL072	0	0	1	2	1	1	3	Sleumer (1980), herb. voucher
Euceraea nitida	SAL241	–	0	1	2	1	–	0	Sleumer (1980), Alford (2015)
Lunania parviflora	AY756902	0	0	1	2	0	0	2	Sleumer (1980)
Neoptychocarpus apodanthus	SAL167	1	1	1	2	0	1	3	Sleumer (1980)
Neoptychocarpus killipii	AY757040	1	1	1	2	0	1	3	Sleumer (1980), protologue, Alford (2015)
Oncoba routledgei	N667	0	0	0	3	0	0	5	Type, herb. voucher