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What reproductive traits tell us about the evolution and diversification of the tree-of-heaven family, Simaroubaceae

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Abstract

Floral features contribute with remarkable additions to morphological studies and are widely used to address questions about the evolution and diversification of several groups of plants. Even though Simaroubaceae are a small monophyletic family, the few detailed structural analyses of reproductive organs and the floral diversity and variations already described in their members stimulate novel structural studies. In this study, we investigate the evolution of reproductive features of Simaroubaceae by means of a combination of original data and a review of the literature, aiming to elucidate which floral characters are most informative for a better understanding of the evolutionary history of the group. We analyzed 21 out of the 23 genera of Simaroubaceae, plus six from Rutaceae and seven from Meliaceae as outgroups. We used a Bayesian method and the Parsimony optimality criterion to reconstruct ancestral reproductive character states using a re-analyzed phylogenetic tree of Sapindales. Here, we combined available molecular sequences to have the largest sample of Simaroubaceae genera. We found that the ancestral flowers of Simaroubaceae were probably polygamous or dioecious plants, with free carpels united only distally, with divergent, elongated stigmas, and with drupaceous, laterally flattened to lenticular fruits. The latter feature plus apocarpous carpels are putative synapomorphies of the family retrieved in this study. Imbricate petals and a diplostemonous androecium were recovered as conditions found in the ancestor of Simaroubaceae but also shared with the ancestors of Meliaceae and Rutaceae. Our findings were mostly in accordance with previous evolutionary studies on genera of Simaroubaceae and with other families of Sapindales.

Keywords Flower morphology · Macroevolution · Rosids · Sapindales · Sexual dimorphism

1 Introduction

Simaroubaceae are a small family with 23 genera and around 117 species distributed across the tropics, belonging to the order Sapindales (Devecchi and Pirani 2020). The circumscription of the family as a monophyletic group and phylogenetic relationships within its members were clarified after macromolecular analyses were carried out (Fernando et al. 1995). Despite being very well supported by macromolecular (plastid and nuclear DNA) and micromolecular chemical data (their quassinoids are exclusive), Simaroubaceae

are morphologically diverse and do not have a single structural feature common among all genera that is not present in related families (Cronquist 1944a; Fernando et al. 1995; Clayton 2011). Current phylogenetic evidence strongly supports Meliaceae and Rutaceae as sister clades to Simaroubaceae, although the relationships among the three families still lack support (Fernando et al. 1995; Gadek et al. 1996; Muellner-Riehl et al. 2016). These three families share the presence of unusual bitter compounds, the limonoids and quassinoids, which are based on degraded forms of triterpenes, the nortriterpenoids, compounds that are uncommon in other Angiosperms (Kubitzki and Gottlieb 1984; Gadek et al. 1996; Kubitzki 2011).

After the global-level monograph of Simaroubaceae provided by Engler (1931a), who recognized six subfamilies, the most comprehensive treatment of the group is that of Clayton (2011), which includes an overall review and keys to the genera and their description. Simaroubaceae may be

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characterized mainly by the quassinoids in the bark, mostly pinnate to imparipinnate leaves, filaments usually appendiculate at base, and especially by their free or slightly united carpels (at base and/or at the styles), which develop into free fruitlets. The family as currently redefined consists almost exclusively of the genera previously treated in the subfamily Simarouboideae by Engler (1931a), excluding *Harrisonia* (transferred to Rutaceae) and with the inclusion of the monospecific genus *Nothospondias* (formerly in Anacardiaceae) and also *Leitneria* (formerly Leitneriaceae, Clayton 2011). The latter genus was traditionally treated as monospecific until an additional species was described by Schrader and Graves (2011). *Leitneria* was the sole genus of Leitneriaceae, a family with controversial position (order Leitneriales of subclass Hamamelidae in the systems of Cronquist 1981 and Takhtajan 1997), until molecular data indicated that this genus is embedded in Simaroubaceae (Clayton et al. 2007).

Even though of great economic and medicinal potential, the representatives of Simaroubaceae have been the object of few detailed morphological and anatomical studies. As well as for some other families in Sapindales, Simaroubaceae displays remarkable structural features, such as different types of glands, various degrees of connation of floral parts, an impressive variation in floral merism and complex sexual systems, including cryptic dioecy (Engler 1931a; Clayton 2011; Alves et al. 2017). In the last few decades, some structural studies have improved our knowledge of the micro- and macromorphological features of the flowers. Ramp (1988) studied the structure (including ontogeny), function and importance of the gynoecium in Simaroubaceae classification. Nair and Joshi (1958) discussed many variations of the floral features within the family, ranging from dialysepaly to gamopetaly, bisexual (monoclinous) to unisexual (diclinous) flowers, diplostemony to haplostemony, apocarpy to syncarpy and reductions in floral merism. *Leitneria floridana* Champ., corkwood, was anatomically investigated by Abbe and Earle (1940) and Tobe (2013). Embryological studies of *Leitneria* corroborate its inclusion in Simaroubaceae (Tobe 2011), and structural studies of male flowers and inflorescences of *Leitneria* provided insights about these reduced, wind-pollinated and atypical flowers within the family (Tobe 2013). In fact, flowers of many genera of Simaroubaceae present organ reduction leading to abortion or formation of vestigial structures and cases of monoecy, dioecy and polygamy are prevalent in the family. Even in genera traditionally described as hermaphroditic, such as *Simaba* (and its recently segregated *Homalolepis,* see Devecchi et. al. 2018a), detailed anatomical analysis has revealed the occurrence of functionally unisexual flowers, with abortive ovules (Alves et. al. 2017).

Some Simaroubaceae taxa were studied by Endress et al. (1983) who comparatively studied the formation of the apocarpous gynoecium among different families of eudicots. This study together with more recent studies by Bachelier and Endress (2008, 2009) has analyzed the floral structure of Simaroubaceae and the clade of Anacardiaceae, Burseraceae and Kirkiaceae, which usually have apocarpy combined with a postgenital fusion of the apex of the carpels. They highlighted the implication of this phenomenon in the evolution of secondary apocarpy as well as in the development and dispersal of fruits in representatives of other Sapindales.

Morphological characters are very informative and correspond to the type of data most used in plant classification (Stuessy 2009), and they may also be very helpful in resolving phylogenetic questions. Studies involving reproductive characters on all families of Sapindales have been done in a comparative context and have mostly contributed to the systematics of the groups (Bachelier and Endress 2008, 2009; Pirani et al. 2010; Bachelier et al. 2011; El Ottra et al. 2013, 2019; Alves et al. 2017; Devecchi et al. 2018a,b; Gama et al. 2021a).

In this study, we present a morphological analysis of reproductive features in Simaroubaceae representatives and the most significant results of ancestral character state reconstructions of reproductive characters using the broadest phylogenetic framework made so far for the family. Our main goals are the following: (1) to provide a wide and detailed revision of morphological variation in the family and analyze these data from an evolutionary context, using the most complete phylogenetic inference for Simaroubaceae taxa, by combining two molecular phylogenies available for this group and other taxa of Sapindales, notably the closely related Meliaceae and Rutaceae; and (2) to infer and discuss the main macroevolutionary pathways in reproductive structure that may have taken place throughout the history of the Simaroubaceae and the closely related Meliaceae and Rutaceae lineages.

2 Materials and methods

Botanical material and macromorphological data – For the elaboration of a macromorphological character matrix we used bibliographic data on the flower morphology of Simaroubaceae genera. The data were complemented with studies of fresh and dried materials (voucher list in Table 1). Floral buds and anthetic flowers were obtained from species available in the collections of the Herbaria MG, R, RB and SPF, with additions from live plants collected during field expeditions to states of the Central and South-West regions of Brazil, through the usual methods of collection preparation for spirit and herbarium samples (Forman and Bridson 1992; Simpson 2010). The respective vouchers are deposited at the Herbarium SPF, of the Botany Department of the Institute of Biosciences at the University of São Paulo

Table 1 List of vouchers of Simaroubaceae species used in morphological analysis. Characters on the remaining taxa on the matrix were described with data from the literature

Taxa	Voucher	Herbarium
Ailanthus altissimus (Mill.) Swingle	Alves, G.G.N., 76—XII/2018	SPF
Brucea antidysenterica J.F.Mill.	Hort. Paris L. Netto. Abyssinia. R71159	R
Castela coccinea Griseb.	Beck, S.G. 9392—X/1983	SPF
	Bourdy, G. 1967—XI/1998	SPF
	Bourdy, G. 2026—IX/1998	SPF
	Hahn, W., 1682—XIII/1983	SPF
	Molas, P, 1096—IX/1986	SPF
	Vavrek, I.M.,335—IX/1981	SPF
	Beck, S.G., 9392—X/1983	SPF
Castela tweedii Planch.	Estevan, D.A., 238—VII/2003	SPF
	Hatschbach, G. 72,435—IX/2001	SPF
Eurycoma longifolia Jack	Mohd.S., 1515—III/1968	RB
Hannoa klaineana Pierre & Engl.	Dobois, J., 298—05/1958	R
Homalolepis arenaria Devecchi & Pirani (Devecchi & Pirani)	Santana, M.C., 210—IV/1984	SPF
	Devecchi, M.F., 398—II/2015	SPF
	Devecchi, M.F., 400—II/2015	SPF
Homalolepis bahiensis (Moric.) Devecchi & Pirani	Jardim, J.G., 5831—IX/2010	SPF
Homalolepis cedron (Planch.) Devecchi & Pirani	Devecchi, M.F.,238—IX/2013	SPF
Homalolepis cuneata (A.St-Hil. & Tul.) Devecchi & Pirani	Devecchi, M.F.,189—VII/2013	SPF
	Devecchi, M.F.,191—VII/2013	SPF
Homalolepis ferruginea (A.St-Hil. & Tul.) Devecchi & Pirani	Devecchi, M.F.,306—VII/2014	SPF
Homalolepis glabra (Engl.) Devecchi & Pirani	Devecchi, M.F.,322—IX/2014	SPF
Homalolepis intermedia (Mansf.) Devecchi & Pirani	Thomas, W., 4301—X/1985	SPF
	Moresco, M.C., 124—X/2005	SPF
Homalolepis insignis (A.St-Hil. & Tul.) Devecchi & Pirani	Alves, G.G.N. 71, XII/2014	SPF
Homalolepis paraensis (Ducke) Devecchi & Pirani	Van der Werff, H., 18,028—VII/2003	SPF
Homalolepis planaltina Devecchi & Pirani	Devecchi, M.F.,320—IX/2014	SPF
Homalolepis pohliana (Boas) Devecchi & Pirani	Zappi, D.C., 4493—VII/2018	MG
Homalolepis pumila Devecchi & Pirani	Devecchi, M.F.,323—IX/2014	SPF
	Devecchi, M.F., 467—VII/2017	SPF
	Antar, G.M., 232—VIII/2013	SPF
Homalolepis rigida Devecchi	Devecchi,M.F.,308—VII/2014	SPF
Homalolepis salubris (Engl.) Devecchi & Pirani	Devecchi, M.F., 321—IX/2014	SPF
		SPF
Homalolepis suffuticosa (Engl.) Devecchi & Pirani	Devecchi, M.F.,216—IX/2013	SPF
	Faria, J.E.Q. 9562—XIII/2019	UB
	Devecchi, M.F.,226—IX/2013	SPF
Homalolepis trichilioides (A.St-Hil. & Tul.) Devecchi & Pirani		
Homalolepis warmingiana (Engl.) Devecchi & Pirani	Devecchi, M.F.,432—XI/2016	SPF
Picrasma crenata (Vell.) Engl.	Alves, G.G.N., 77, X/2018	SPF
	Alves, G.G.N., 78, X/2018	SPF
	Klein, R.M., 3976, XII/1962	SPF
Picrasma excelsa (Sw.) Planch.	Plowman, T., 14,286—VII/1986	SPF
Picrolemma sprucei Hook.f.	Ribeiro, J.E.L.S., 988—VII/1993	SPF
	Cid. C.A.,665—VIII/1979	RB
Quassia amara L.	Frazão, A., 226—IX/2015	SPF
	Devecchi, M.F., 260—XII/2013	SPF

Table 1 (continued)

Taxa	Voucher	Herbarium
Simaba guianensis Aubl.	Ducke, A., s/n—VIII/1923	SPF
	de Lima, M.P.M., 123—VI/1986	SPF
	Miranda, I.P.A. 43—XI/2000	SPF
Simaba obovata Spruce ex Engl.	Devecchi, M.F., 425—IV/2015	SPF
Simaba orinocensis Kunth	Devecchi, M.F., 422—IV/2015	SPF
	Devecchi, M.F., 423—IV/2015	SPF
Simaba polyphylla (Cavalcante)W.W.Thomas	Ribeiro, J.E.L.S.1924—IX/1997	SPF
Simaba new sp.	Assunção, P.A.C., 357—VI/1996	SPF
Simarouba amara Aubl.	Goes, B.T.M., 61—VIII/2015	SPF
	Pirani, J.R., CFCR2069—IX/1981	SPF
	Alves, G.G.N. 85—VII/2019	SPF
	Alves, G.G.N. 86—VII/2019	SPF
	Alves, G.G.N.87—VII/2019	SPF
Simarouba versicolor A.St-Hil.	Rizzo, J.A., 10,423—VIII/84	SPF
	Proença, C., 858—VII/1993	SPF
	Aparecida da Silva, M., 2301—IX/1994	SPF
	Aparecida da Silva, M., 3177—IX/1996	SPF
	Carvalho, J.G., 2384—IX/1909	SPF

(see Table 1). Newly collected flowers were conserved and analyzed in 70% ethanol, and dried flowers were rehydrated before examination under a stereomicroscope.

We built a matrix with 20 macromorphological characters from 21 out of 23 genera of Simaroubaceae, six genera of Rutaceae and seven from Meliaceae. The list of descriptions and encoding of characters is given in Table 2. The expression of these character states in each taxon is provided in Table 3. Only the monospecific genera *Laumoniera* Noot. and *Iridosma* Aubév. & Pellegr. were not included, since they were not sampled in the available phylogenetic studies used here.

The characters were encoded following comprehensive studies within Sapindales, and Simaroubaceae (Engler 1931a; Cronquist 1944a, b; Aubréville 1962; Noteboom 1962a, b; Pennington and Styles 1975; Clayton 2011); genus-specific descriptions and notes (Pirani 1987a, b; Alves et al. 2017; Devecchi et al. 2018a, b) and new observations of species placed in *Ailanthus, Castela, Eurycoma, Homalolepis, Picrasma, Simaba* and *Simarouba*.

The characters of the outgroups were encoded according to the Rutaceae monographs by Engler (1931b) and Kubitzki et al. (2011), plus some more detailed structural studies by Gut (1966), Ramp (1988), Caris et al. (2006) and Beurton (1994). For Meliaceae, characters were encoded according to the monographs by Pennington and Styles (1975), Mabberley (2011), and also from data analyzed by Gama et al. (2021a). General morphological concepts and terminology follow Bawa and Beach (1981), Weberling (1989) and Sakai and Weller (1999).

In cases of genera with polymorphic characters, all applicable states were scored. For the androecium, we consider the arrangement type (number of stamens in relation to petals and sepals) and recognize just three character states: isostemonous (including obhaplostemonous), diplostemonous (including obdiplostemonous) and polystemonous. Simaroubaceae are mostly haplostemonous; as obhaplostemony is observed in a sole terminal (*Picrolemma*), we did not include it as a distinct state of isostemony. Even though flowers of most families of Sapindales are usually described as obdiplostemonous (with the carpels in an antepetalous position, Kubitzki 2011; Kubitzki et al. 2011), a review by Ronse De Craene and Bull-Hereñu (2016) demonstrates that this condition has long been controversial and that it "represents an amalgamation of at least five different floral development pathways, all of them leading to the external positioning of the alternisepalous stamen whorl within a two-whorled androecium." Additionally, those authors distinguish primary and secondary obdiplostemony and advocate that these arrangements can be seen as transitional stages from diplostemony to either haplostemony or obhaplostemony. Thus, as detailed studies of androecium development are still scarce for most Simaroubaceae, it is impossible to accurately encode the state obdiplostemonous in our analysis.

Filament union was encoded with four states: free from each other, basally connate, connate up to three-quarters of the filament length forming a staminal tube, and coherent only by the intertwining trichomes of basal appendages forming a pseudotube (according to Alves et al. 2017). The

Table 2 List of morphological characters and their character state coding used for the ancestral state reconstruction in selected taxa of Meliaceae, Rutaceae and Simaroubaceae

1. Inflorescence type: thyrse (0); thyrsoid (1); panicle (2); rounded to corymbiform cymoid (3); fascicle (4); raceme (5); catkin (6); botryoid (7)

2. Sexual system: monoecious plants (only unisexual flowers, male and female on a same individual) (0); dioecious plants (only unisexual flowers, on distinct individuals) (1); polygamous plants (male, female and bisexual flowers) (2); hermaphroditic plants (only bisexual flowers) (3)

3. Sepal number: three (0); four (1); (four-)five(-six) (2); five or more (3)

4. Sepal union: free (0); basally connate (1); union up to three-quarters (2); completely connate (3)

5. Corolla aestivation: imbricate (0); contorted (1); induplicate-valvate (2); valvate (3)

6. Petal number: three (0); four (1); (four-)five(-six) (2); seven(-eight) (3)

7. Androecium: isostemonous (stamens uniseriate, equal in number in relation to petals and sepals) (0); diplostemonous (stamens biseriate, double in number in relation to petals and sepals) (1); polystemonous (numerous stamens) (2)

8. Stamen number: four (0); (four-)five (1); six (2); eight (3); ten (4); (eight-)ten(-twelve) (5); more than twelve (6)

9. Filaments appendages: absent (0); present (1)

10. Filament union: free (0); connate only at the base (1); connate (union up to three-quarters) (2); coherent by intertwining trichomes of basal appendages (3)

11. Staminodes: absent (0); present and very reduced (1); present and similar to a stamen (2)

12. Pistillodes: absent (0); present (reduced/vestigial, lacking ovules) (1); present (like a fertile gynoecium) (2)

13. Carpels union: free (united only at the apex of the ovary) (0); united only at the base (1); united at the base and at the apex of the ovary (2); totally united (at least at ovary level) (3)

14. Style–stigma proportion: style absent (0) style larger than stigma (1); style smaller than stigma (2); style–stigma with the same size (3)

15. Style union: free (0); united (1); slightly united (only at the base of the styles) (2)

16. Stigma union: united (0); separated (1)

17. Stigma type: punctiform, without conspicuous lobes (0); with conspicuous lobes (1); conical (2); discoid (3); elongate and divergent (4); claviform (5); single branch distally expanded (6); reniform (7)

18. Stalk-like elongations: absent (0); present as a gynophore (1); present as an androgynophore (2)

19. Intrastaminal disk: absent (0); present (1)

20. Fruit—general structure related to dispersal (either syncarpic fruits or separated fruitlets): (sub)globose drupaceous (0); laterally flattened to lenticular drupaceous (1); berry-like (2); samaroid (winged) (3); capsule (4); follicle (5)

gynophore and androgynophore were defined as "stalk-like" elongations (Leins and Erbar 2010) of the floral axis below the fertile part of the carpels (i.e., ovary), or of both carpels and stamens, respectively. We treated the nectariferous disk character based on macromorphological grounds, that is when a conspicuous disk could be discernable, which is generally a widespread and common feature among Sapindales. Also, when the entire surface of the gynophores is known to be nectariferous from anatomical analysis (Alves et al. 2017), it was encoded accordingly. We encoded as "indistinct" all cases where a nectary is not morphologically visible with the naked eye or with a stereomicroscope and does not have anatomical evidence. We followed the staminode definition as proposed by Ronse De Craene and Smets (2001) and considered three character states: absent, present and very reduced, and present and similar to a stamen. Given the scarcity of anatomical and developmental data on the gynoecium of most genera, we encoded union of parts of the carpels simply as free or "united," rather than using terms related to development, such as congenital or postgenital.

Since there are no sufficient anatomical data available for all taxa with drupes and the divergences in the literature about the definition of this type of fruit (Clifford and Dettmann 2001), we applied drupaceous fruits in the broadest sense, that is fleshy fruits with one or more pyrenes in which the woody portion is constituted by a lignified endocarp and/or inner mesocarp.

Phylogenetic analyses – For this study, we combined molecular sequences made available by Muellner-Riehl et al. (2016) and Clayton et al. (2007) to infer an updated chronogram of Sapindales that includes all genera of Simaroubaceae with available DNA sequences. We used published sequence data from the plastid regions *atp*B, *mat*K, *rbc*L, and *trn*L-F (Clayton et al. 2007; Muellner-Riehl et al. 2016) and the nuclear region *Phy*C (Clayton et al. 2007). With this strategy, we combined the species-level sampling of Simaroubaceae (Clayton et al. 2007) with the wide sampling of the order Sapindales (Muellner-Riehl et al. 2016). It is noteworthy to highlight two taxonomic issues at the genus level: Five species formerly treated as *Simaba* are currently included in *Homalolepis* (Devecchi et al. 2018b); *Holacantha* and *Castela* are herein kept as distinct taxa because they were treated this way in Clayton's (2007) tree and monograph (Clayton 2011), although the former is best merged within *Castela* (Moran and Felger 1968; Majure et al. 2021a, 2021b; Pirani et al. 2021).

Table 3 Matrix of taxa/morphological character states of Simaroubaceae studied here, and outgroups (selected genera from Meliaceae and Rutaceae)

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Ailanthus	1	1	3	1	2	2	1	4	0	0	1	1	0 1	1	0 1	0	4	02	01	3
Amaroria	0	1	2	1	0	2	1	5	0	0	1	1	?	0	?	0	7	0	1	1
Brucea	0	0 1	1	1	0	1	0	0	0	0	1	1	1	2	2	1	4 5	0	1	1
Castela	4	1	2	1	0	2	1	5	0	0	1	1	0	2	1	0	4	0	1	1
Eurycoma	1	2	2	1	2	2	0	1 2	1	0	1	1	0	1	1	0	4	0	0	1
Gymnostemon	1	2	3	2	0	2	1	4	0	0	0	0	0	1	1	0	0	0	1	1
Hannoa	1	2	2	1 2	0	2	1	4	1	0	0	1	0	1	1	0	1	0	1	1
Holacantha	1	1	3	1	0	2 3	1	5	0	1	2	1	1	2	2	1	4	0	1	1
Homalolepis	0 1	2	2	1 2	1	2	1	5	1	1 3	0	2	2	1	1	0	0 1	1	0	0 1
Leitneria	6	2	?	?	?	?	?	1	0	0	0	0	?	2	?	?	6	0	0	1
Nothospondias	1	2	1	2	1	1	1	3	0	0	0	1	0	1	0	0	5	1	0	1
Odyendea	1	3	2	2	0	2	1	5	1	0	0	0	1	1	1	0	1 4	1	0	1
Perriera	1	2	2	1	2	2	1	5	0	0	0	1	1	3	1	1	4	0	1	1
Picrasma	1	2	2	0 1	3	2	0	1	0	0	1	1	0	1 3	2	1	4	0 1	0 1	1
Picrolemma	1	1	2	2	0	2	0	1	0	0	1	0	0	1	0	0	3	0 1	0 1	1
Pierreodendron	1	2	2	2	0 1	2	1 2	5 6	1	0	0	1	0	1	1	0	3 4	0	1	1
Quassia	1 5	3	2	0	1	2	1	4	1	0	0	0	0	1	1	0	0	1	0	1
Samadera	3	3	0 2	2	0 1	0 2	1	2 5	1	0	0	0	0	1	1	0	0	1	0	1
Simaba	7	2	2	01	1	2	1	2 5	1	3	1	0	2	1	1	1	0 1	1 4	1	1
Simarouba	1	1	2	1	0 1	2	1	5	1	0	1	1	0 1	2	1	1	4	1	0	1
Soulamea	1	2	0 2	1	0	0 2	1	0 5	0	1	1	1	3	0	?	1	7	0	1	3
Outgroup																				
Rutaceae	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Atalantia	234	3	0 2	1 2	0	123	1	234	0	012	0	0	3	1	1	0	0	0	1	2
Cneorum	3	2	0 1	0	0	12	0	0?	0	0	0	0 1	3	1	1	1	4	2	0	0
Correa	3	3	1	2	3	2	1	3	0	0	0	0	2	1	1	0	0	0 1	0 1	5
Murraya	1	3	2	1	0	3	1	4	0	0	0	0	3	1	1	0	0	0 1	0 1	2
Ruta	1	3	2	1	0	23	1	3 4	0	0	0	0	3	1	1	0	0	0 1	0 1	4
Zanthoxylum	245	012	023	0 1	0 3	123	0	0123	0	0	1	1	012	1	0 1	0 1	0	0 1	0 1	5
Meliaceae	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Aglaia	0	1	0 2	2	0	1 2	012	3	0	2	2	2	3	0 2	1	0	0 2	0	0	4 2
Carapa	0	0	2	1 2	1	2	1	5	1	2	2	2	3	3	1	0	3	0	1	4
Cedrela	0	0	2	1 2	0	2	0	1	0	0	2	2	3	1	1	0	3	2	0	4
Lepidotrichilia	0	2	3	2	3	2	1	4	1	2	0	0	3	1	1	0	0	0	0	0
Melia	1	2	3	1 2	0	2	1	5	1	2	0	0	3	1	1	0	0 1	0	1	0
Swietenia	0	0	2	2	1	2	1	5	1	2	2	2	3	3	1	0	3	0	1	4
Trichilia	1	2	0 2	023	0 3	0 2	0 1	1 4	0	012	0	0	3	1	1	0	0 2	0	1	4

We included 252 sequences available for *atp*B, 77 sequences for *mat*K, 71 sequences for *Phy*C, 252 sequences for *rbc*L, and 211 sequences for *trn*L-*trn*F. To obtain an ultrametric tree for further evolutionary analyses, the phylogenetic tree was inferred using BEAST v2.6.2 (Bouckaert et al. 2019), which is available at CIPRES (http://www.phylo.org/index.php/). We used Yule tree prior and previous evolutionary models inferred for each partition following Muellner-Riehl et al. (2016) for *atp*B, *rbc*L and *trn*L-F and Clayton et al. (2007) for *mat*K and *Phy*C. Stem and crown nodes of families and less inclusive clades were constrained using age intervals inferred by Muellner-Riehl et al. (2016). A total of 14 nodes were constrained, including all family-level clades, and five comprehensive clades within the order with maximum support (Muellner-Riehl et al. 2016). A relaxed clock with lognormally distributed rate changes was used. These secondary calibrations used normally distributed priors with

95% confidence intervals lying between the maximum and minimum values of the 95% intervals.

The tree search followed Muellner-Riehl et al. (2016). We ran 12 analyses with 20 million generations of MCMC each and assessed convergence and burn-in threshold using Tracer v1.7 (Rambaut et al. 2018). Sample sizes of all parameters were also evaluated. All analyses reached convergence and were combined using LogCombiner (v1.7) with 10% burn-in and sampling every 2,000th generation with TreeAnnotator (v1.7). A maximum clade credibility tree was recovered using the R package "phangorn" (Schilep 2011). The final ultrametric mcc (maximum clade credibility) and posterior trees were edited (Supplementary Material 1) and non-focal families removed (Supplementary Material 2 shows the node numbers), leading to a final tree with 34 genera sampled for Simaroubaceae, Meliaceae and Rutaceae.

Ancestral character state reconstruction – The maximum clade credibility (mcc) tree and posterior probability trees (Supplementary Material 3) were used to infer the ancestral character states for the 34 genera included in our analysis, using parsimony and Bayesian criteria. For the parsimony reconstruction, we used Mesquite 3.61 (Maddison and Maddison 2019). For the Bayesian analysis, we used the software BayesTraits 3.0 (Pagel et al. 2006); this program enables the inclusion of polymorphic characters with missing data. All characters studied have three or more states and used the reversible jump MCMC strategy (Pagel and Meade 2006). Node uncertainty was incorporated using 1000 trees randomly selected from the posteriors. Bayesian ancestral state inference used exponential hyper-priors and ran for 5 million generations, sampling parameters every 1000 generations. Two runs were performed for each character with convergence and stationary distribution assessed using the R package "coda" (Plummer et al. unpublished). Log-likelihood and other parameters were marginalized, and stationary distribution was evaluated against the number of generations. For each dataset, a burn-in of 10% was sufficient and applied. For all nodes, the state with probability above 70% was considered the most probable, and results above 90% of probability were considered robust. During phylogenetic searches, tree topology was constrained in key points outside Simaroubaceae, and ages of the families followed the ones previously inferred (Muellner-Riehl et al. 2016). As a consequence, the tree topology and branch lengths largely resemble previous phylogenetic findings. The focal clade comprising the families Simaroubaceae, Meliaceae and Rutaceae was pruned and reduced at genus level for further evolutionary analyses.

3 Results

Phylogenetic relationships – The mcc reduced tree sampled 34 genera from the three families. Maximum support values of posterior probabilities were recovered for most clades (22 nodes with PP = 1), and only four branches had support lower than 0.9 PP. This robust phylogenetic tree was used in all comparative analyses. Using an ultrametric tree, the Bayesian method infers ancestral states without major distortions related to differences in molecular evolutionary rates.

Ancestral morphological character state reconstructions – Our comprehensive overview of flower and fruit trait variation of almost all genera of Simaroubaceae shows a remarkable diversity of floral features, including some examples from the outgroups (Figs. 1, 2, 3, 4, 5). Ancestral states were inferred for all clades, but we highlight five clades (Fig. 6) that received particular emphasis in our discussion and present results of the reconstruction of selected ones (Figs. 7, 8, 9, 10, 11, 12). Both Bayesian and Parsimony reconstructions showed similar results, although the former enables one to estimate the probability of ancestry percentage (compare characters "inflorescence type" and "sexual system," Fig. 7), Thus, we present only Bayesian results for the remaining characters (Figs. 8, 9, 10, 11, 12), while the respective results from Parsimony analysis are found in Supplementary materials 4–8.

Inflorescence type (Fig. 7a). We retrieved thyrsoid as the most likely ancestral type of inflorescence for Simaroubaceae (PP = 79.8). Thyrsoids are widespread also in the Meliaceae and Rutaceae. Transitions to many other inflorescence types occurred, and these are very heterogeneous. The botryoids of *Simaba* (clade 1), the catkin-like inflorescence of *Leitneria* (clade 3), fascicles of *Castela* (clade 4), cymoids of *Samadera*, racemes of *Quassia* and thyrses of *Homalolepis* (member of clade 1 of Fig. 6), *Amaroria*, and *Brucea* (clade 3) seem to have evolved independently, but mostly from ancestors with thyrsoids. It is noteworthy the variation of inflorescence types observed among distinct species of *Quassia* (one with thyrsoids, the other with racemes), and of *Homalolepis,* which is the largest genus in the family (most species with thyrsoids, some with thyrses).

Sexual systems (Fig. 7b). Our reconstruction retrieved polygamy (presence of unisexual and bisexual flowers) or dioecy (presence of male and female flowers in distinct individuals) as the putative ancestral state of Simaroubaceae, since both analyses retrieve a probability of PP = 58.6 for polygamy and PP = 33.8 for dioecy to be likely present in this ancestor. According to parsimony analysis, polygamous plants are likely present in the common ancestor of all three families and in the Meliaceae and Rutaceae ancestors, while through Bayesian analysis, this is uncertain. In
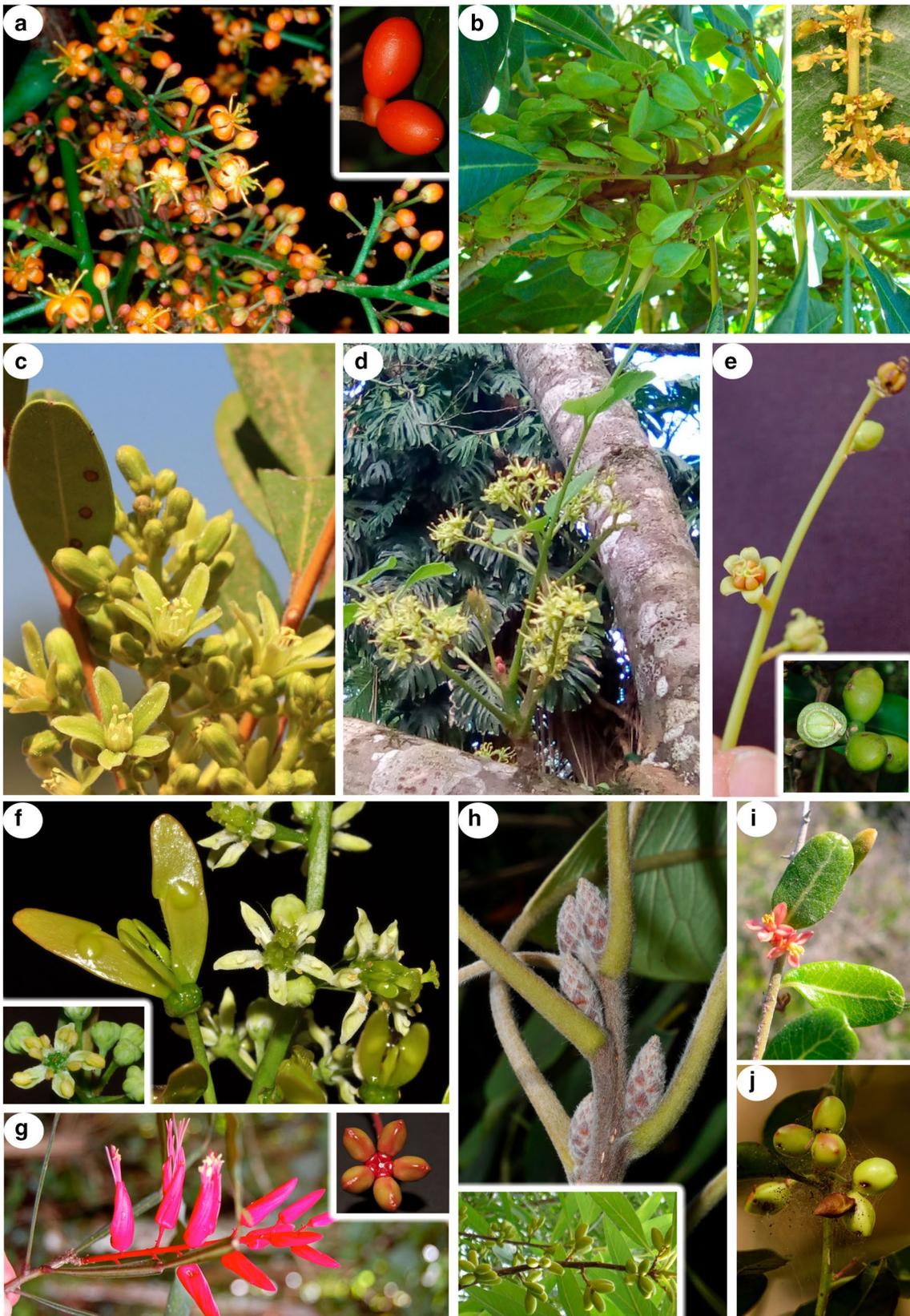
◄**Fig. 1** Morphological diversity in Simaroubaceae. **a** Flowers of *Picrolemma sprucei* in a thyrsoid inflorescence. Laterally flattened drupaceous fruitlets of *Picrolemma* sp. (inset). **b** Samaras of *Soulamea amara*. Flowers of *Soulamea amara* in a thyrsoid inflorescence (inset). **c** Flowers of *Homalolepis suffruticosa* in a thyrsoid inflorescence. **d** Male flowers of *Picrasma crenata* in a thyrsoid inflorescence. **e** Female flower of *Simarouba versicolor*. Laterally flattened drupaceous fruitlets of *Simarouba amara* (inset). **f** Samaria and female flowers of *Ailanthus altissimus* in a thyrsoid inflorescence. Male flower of *Ailanthus altissimus* (inset). **g** Flowers of *Quassia amara* in a racemoid inflorescence. Laterally flattened drupaceous fruitlets of *Quassia amara* (inset). **h** Male flowers of *Leitneria floridana* in a catkin-like inflorescence. Laterally flattened drupaceous fruitlets of *Leitneria floridana* (inset). **i** Flowers of *Castela* sp. in a fasciculate inflorescence. **j** Drupaceous fruitlets of *Castela tweedii*. Photos: Willian W. Thomas (**a**), Richard J. Abbott (**b**, **e** inset, **f**, **h**, **i**), Henrique Moreira (**c**), Pedro Acevedo-Rodriguez (**j**), M.F. Devecchi (**a** inset, **e**, **g**)

Simaroubaceae, transitions to dioecy occurred independently at least five times within the family, while transitions to hermaphroditism (only bisexual flowers) took place three times, and only some species of *Brucea* became monoecious. Polygamous plants are more frequent in clade 1 ((*Simaba*, *Homalolepis*) (*Simarouba*, *Pierreodendron*)), in which only *Simarouba* is dioecious, and also in clade 2 (*Perriera* (*Gymnostemon*, *Hannoa*)).

Sepal and petal number Fig. 8a, b). Perianth merism is a quite variable feature in the three families studied, but calyces and corollas with (4–)5(–6) sepals or petals prevail and were retrieved as the possible ancestral character state in Simaroubaceae, especially in clades 1 and 2 with up to 94% posterior probability. A tetramerous perianth became fixed independently in *Brucea* and *Nothospondias*, while transitions to five or more sepals and petals took place in *Ailanthus* and *Gymonstemon*. Reductions to a trimerous perianth occurred in some species of *Samadera* and *Soulamea* independently, and petals were lost, while sepals became vestigial in female flowers of *Leitneria,* whose male flowers became achlamydeous.

Sepal union (Fig. 8c). In Simaroubaceae, the condition of basally connate sepals was found as the ancestral character state (PP = 73.33), and the union of sepals up to three-quarters of their length evolved independently in many genera with reversions to the plesiomorphic state. Free sepals arose only in some *Picrasma*, *Quassia* and some *Simaba* species.

Corolla aestivation (Fig. 8d). Imbricate corolla aestivation emerged as the most likely character state in the common ancestor of all three families (PP = 73.7). Transitions to contorted, induplicate valvate or valvate aestivations occurred at least three times each within Simaroubaceae. Even though imbricate is a widespread condition in our analysis, it is important to highlight that there are some subtypes under the imbricate condition that we have not considered herein due to lack of information for all taxa, such as the quincuncial, ascending and descending cochlear

types, which are common and particular to specific clades or individual genera.

Androecium (Fig. 9a). The diplostemonous androecium emerged as the plesiomorphic condition shared among the Simaroubaceae, Meliaceae and Rutaceae clade (PP = 94.89). It was conserved in most Simaroubaceae lineages with four independent transitions to an isostemonous androecium (*Brucea*, *Eurycoma*, *Picrasma* and *Picrolemma*). Polystemony was established only in *Pierreodendron,* while the achlamydeous flowers of *Leitneria* underwent a reduction to just (1–)4 stamens.

Stamen number (Fig. 9b). This character is highly variable within Simaroubaceae, but we retrieved support that flowers with (8–)10(–12) stamens could be the probable ancestral number for Simaroubaceae and Meliaceae (PP = 63). Some lineages of Simaroubaceae (e.g., clade 1) present a 92% posterior probability of this state as ancestral.

Filament appendages (Figs. 3c, e, j; 9c). Our analysis recovered filament appendages in the common ancestor of the lineage of clade 5 with PP = 99.4 plus *Picrolemma* (PP = 99.6). *Perriera* and *Gymnostemon* likely lost these structures. This large lineage (clade 5) holds the greatest number of species in the family. Appendaged stamens have evolved independently in some Meliaceae, and also in some genera of Rutaceae not included in our sampling.

Filament union (Fig. 9d). Free filaments emerged as the most likely character state in the common ancestor of all three families, but with transition to connate filaments (up to three-quarters of their length) likely occurring in the Meliaceae ancestor (PP = 75.4). Union at the base of the filaments evolved independently three times within Simaroubaceae (*Holacantha,* some species of *Homalolepis* and *Soulamea*). A peculiar arrangement of filament union by intertwining trichomes is a possible synapomorphy of the clade (*Simaba*, *Homalolepis*), as this character state is retrieved for the ancestor of this clade in both analyses.

Staminodes and pistillodes (Fig. 10a, b). The presence of very reduced to vestigial staminodes is a homoplastic feature among the Simaroubaceae taxa in both analyses. On the other hand, pistillodes (vestigial/reduced, lacking ovules) were retrieved as a putative synapomorphy for Simaroubaceae in both analyses (PP = 82.2), with subsequent and homoplastic events of loss taking place in several genera. *Homalolepis* likely has the autapomorphy of having pistillodes that are hardly distinguishable from a fertile gynoecium but have abortive ovules.

Carpel union (Fig. 10c). The Simaroubaceae ancestor most likely featured a gynoecium having free carpels (united only at the apex of the ovary), according to both analyses. Since the outgroups in our analysis share the condition of "carpels totally united," free carpels emerge as a putative synapomorphy of Simaroubaceae in our topology (PP = 79.2). In this family, at least five transitions occurred
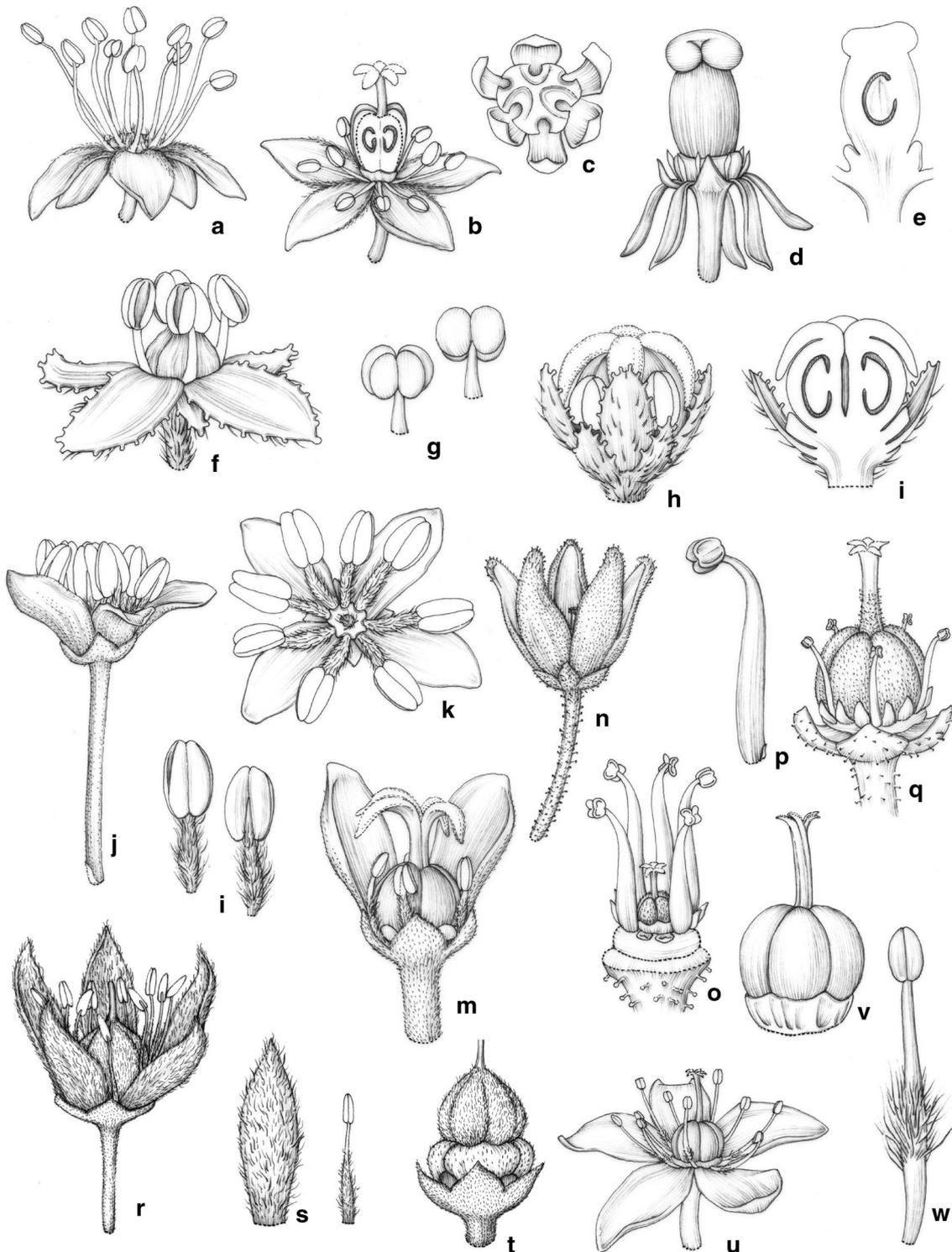
Fig. 2 Illustrations of Simaroubaceae flowers. **a–b** *Ailanthus altissimus*. **c–d** *Amaroria soulameoides*. **f–i** *Brucea javanica*. **j–m** *Castela tweedii*. **n–q** *Eurycoma longifolia*. **r–t** *Gymnostemon zaizou*. **u–w** *Hannoa klaineana*. **a** Male flower. **b** Female flower with a longitudinal view of the ovaries. **c–e** Female flower. **c** Frontal view of the nectariferous disk; pistil removed. **e** Longitudinal view of the gynoecium. **f** Male flower. **g** Stamens. **h** Female flower. **i** Longitudinal view of the gynoecium. **j–k** Male flower. **l** Stamens. **m** Female flower. **n** General view of the flower. **o** Male flower; sepals and petals removed. **p** Stamen. **q** Female flower; perianth removed. **r** General view of the flower. **s** Petal and stamen. **t** Female flower; petals removed, **u** General view of the flower. **v** Gynoecium. **w** Stamen. a,b Modified from Clayton (2011), c–i Modified from Engler (1931a, 1931b, 1931c), **j–m** Modified from Pirani (1987a, 1987b), **n–q, u–w** Modified from Noteboom (1962a), **r–t** Modified from Aubréville (1962)
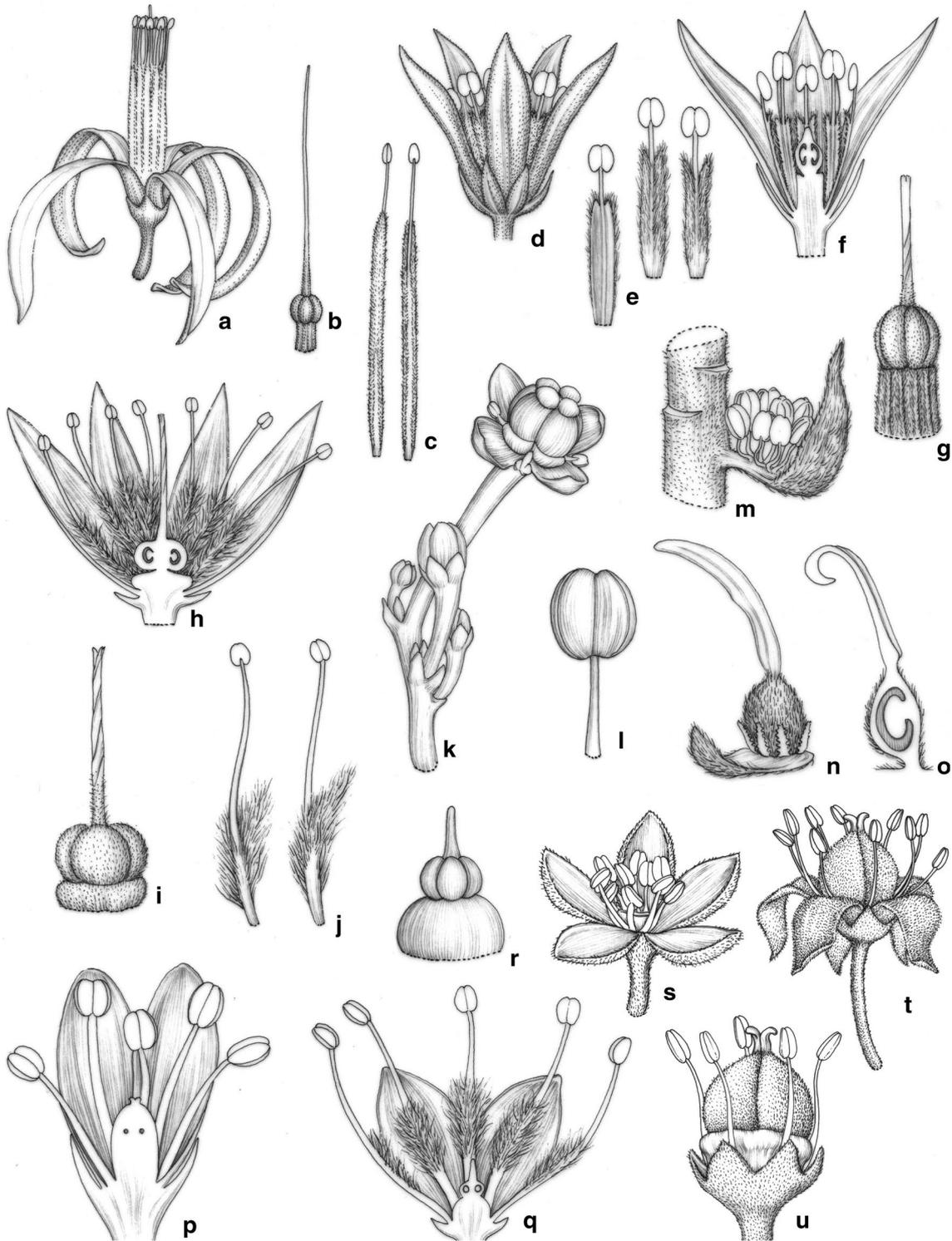
Fig. 3 Illustrations of Simaroubaceae flowers. **a–c** *Homalolepis cedron*. **d–g** *Homalolepis glabra*. **h–j** *Iridosma letestui*. **k–l** *Laumoniera brucea-delpha*. **m–o** *Leitneria floridana*. **p** *Nothospondias staudtii*. **q–r** *Odyendea gabonensis*. **s–u** *Perriera orientalis* Capuron. **a** Bisexual flower. **b** Gynoecium. **c** Stamens. **d** Bisexual flower. **e** Stamens. **f** Longitudinal view of the bisexual flower. **g** Gynoecium. **h** Bisexual flower. **i** Gynoecium. **j** Stamens. **k** Floral buds and female flower. **l** Stamen. **m** Male flowers in detail. **n** Female flower. **o** Longitudinal view of the gynoecium. **p** Longitudinal view of the functionally male flower. **q** General longitudinal view of the flower. **r** Gynoecium. **s** Male flower of *Perriera orientalis* Capuron. **t** Female flower. **u** Female flower; petals removed. **a–g** Modified from Noteboom (1962a, 1962b), **k–l** Modified from Noteboom (1987), **m–o** Modified from Clayton (2011), **s–u** Modified from Capuron (1961)
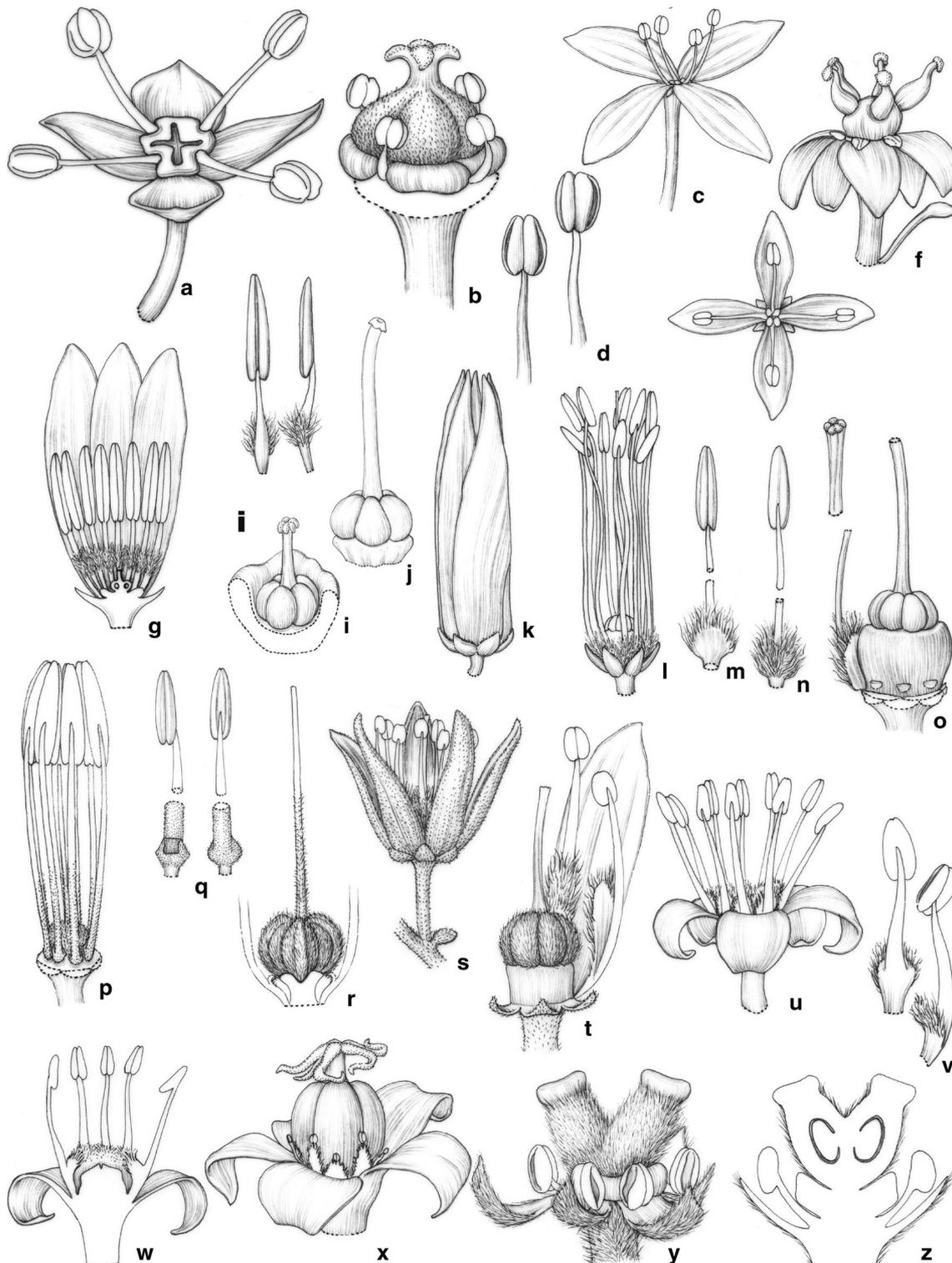
Fig. 4 Illustrations of Simaroubaceae flowers. **a–b** *Picrasma javanica*. **c–f** *Picrolemma sprucei*. **g–j** *Pierreodendron africanum*. **k–o** *Quassia amara*. **p–r** *Samadera indica*. **s–t** *Simaba guinanensis*. **u–x** *Simarouba amara*. **y–z** *Soulamea amara*. **a** Male flower. **b** Female flower; perianth removed. **c** Male flower. **d** Stamens. **e** Top view of male flower. **f** Female flower. **g** Longitudinal view of the male flower. **h** Stamens. **i** Pistillode. **j** Gynoecium. **k** Bisexual flower. **l** Bisexual flower; petals removed. **m** Stamens and appendages. **n** Stigma. **o** Lateral view of the gynophore. **p** Androecium and gynoecium. **q** Stamens and appendages. **r** Gynoecium. **s** Bisexual flower. **t** Lateral view of the gynophore. **u** Male flower. **v** Stamens. **w** Longitudinal. **x** Female flower. **y** Female flower; petals removed. **z** Longitudinal view on the male flower. **a,b** Modified from Noteboom (1962a, 1962b), **l–r** Modified from Engler (1931a), **s,t** Modified from Cavalcante (1983), **u–z** Modified from Engler (1931a) and Clayton (2011)
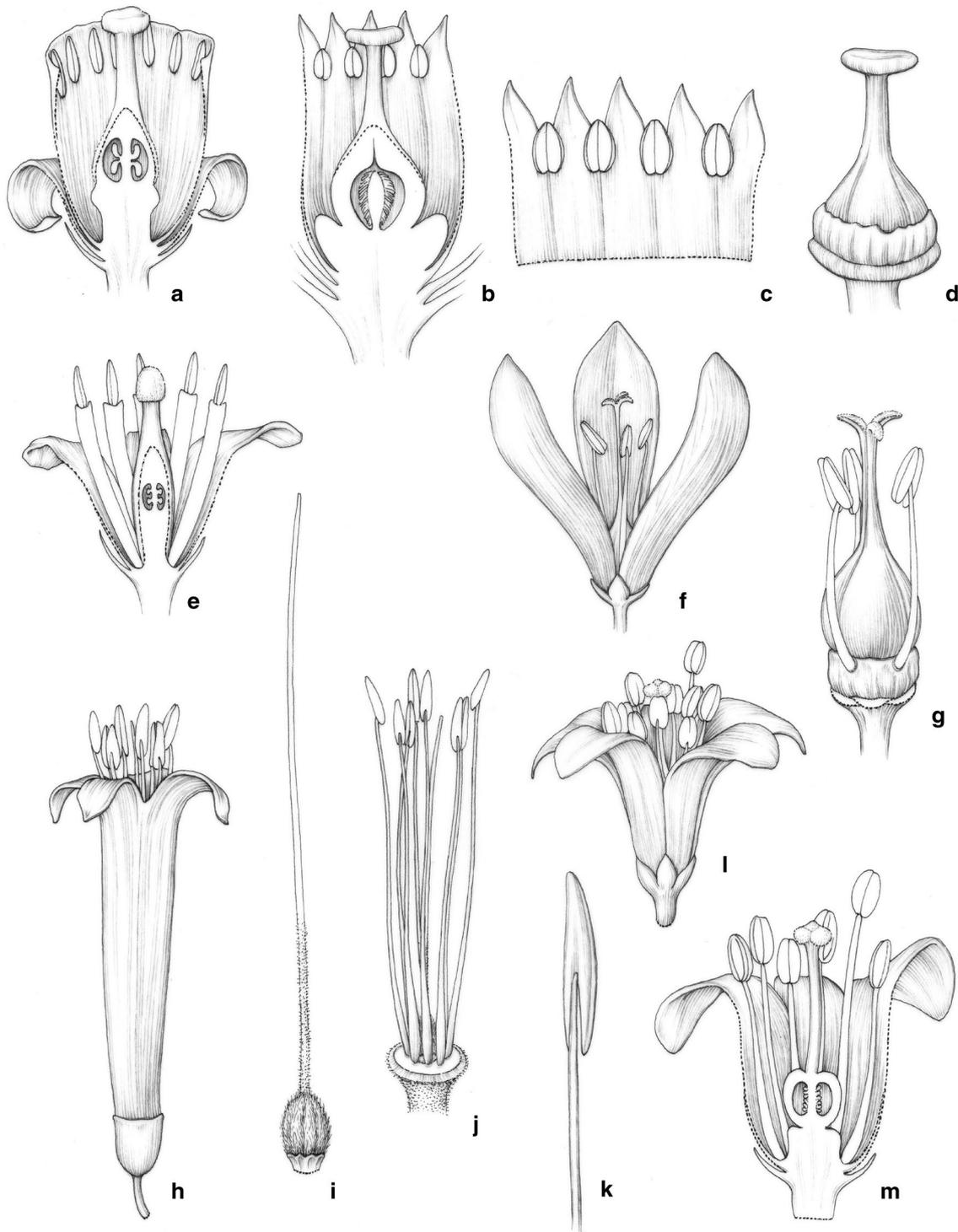
Fig. 5 Illustrations of Meliaceae and Rutaceae flowers. **a–c** *Swietenia mahagoni*. **d–e** *Trichilia claussenii*. **e–f** *Cneorum tricoccum*. **g–j** *Correa speciosa*. **k–l** *Murraya paniculata*. **a** General longitudinal view of the flower. **b** Staminal tube. **c** Gynoecium. **d** General longitudinal view of the flower. **e** General view of the. **f** Androecium and gynoecium. **g** General view of the flower. **h** Androecium and gynoecium. **i** Gynoecium. **j** Stamen. **k** General view of the flower. **l** General longitudinal view of the flower. a–c modified from Harms (1896), d Modified from Gama et al. (2021a), e–l Modified from Engler (1930b)
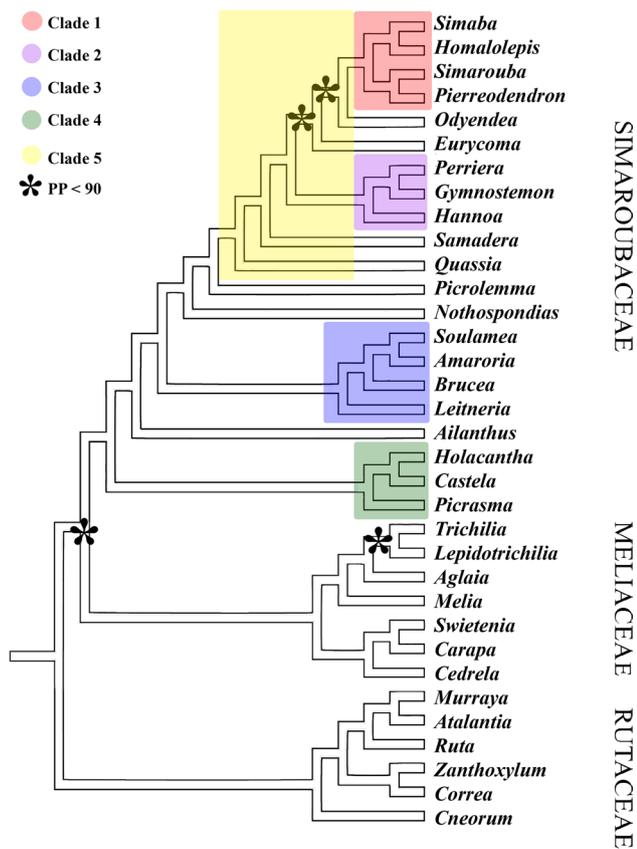
Fig. 6 Consensus phylogenetic tree of Simaroubaceae genera and outgroups. See text for details

to basally connate carpels, while a transition to carpels united at the base and the apex by the styles occurred in the clade (*Simaba*, *Homalolepis*). *Amaroria* and *Leitneria* flowers have a single carpel.

Style–stigma proportion (Fig. 10d). A style longer than the stigma(s) (Figs. 2b, 3i, 4t) is the most likely character state in the common ancestor of all three families (PP = 96.7). This state is very conservative and widespread across these families, while a style and stigma of the same length evolved independently in *Perriera*, some species of *Picrasma* (Simaroubaceae), *Swietenia* and *Carapa* (Meliaceae). A transition to a style shorter than the stigma likely took place three times: in the (*Holacantha*, *Castela*) clade (Fig. 2m), in *Simarouba* (Fig. 4x), and in the (*Leitneria*(*Brucea*(*Amaroria*, Soulamea))) clade (Fig. 2h), with further reduction leading to a sessile stigma that likely evolved in the ancestor of *Amaroria* (Fig. 2d) and *Soulamea* (Fig. 4y).

Style union (Fig. 11a). Connate styles emerged as the character state likely present in the common ancestor of all three families (PP = 94), being more conservative in Meliaceae and Rutaceae (only *Zanthoxylum* presents free styles in our sampling) than in Simaroubaceae. Simaroubaceae representatives have free styles that evolved in fully apocarpus

taxa such as in some species of *Ailanthus*, *Nothospondias* and *Picrolemma*. Differently, connate styles are present in all taxa of clade 5 and its ancestor (PP = 99.1). The presence of styles slightly connate (united only at base) is a condition not clearly resolved in our analyses mainly due to missing data, but present in clades 3 and 4.

Stigma type and union (Fig. 11b, c). Evolution of this highly diverse feature is not clearly resolved in the common ancestor of all three families in our analyses. However, the ancestral character state in Simaroubaceae is likely a gynoecium with elongate and divergent stigmas (Fig. 4x) (PP = 79.6). The ancestor of Simaroubaceae, Meliaceae and Rutaceae most likely had united stigmas, with multiple transitions to separate ones in these families.

Stalk-like elongations (Fig. 11d). Our data show that the common ancestor of Simaroubaceae and Meliaceae likely had no gynophore, according to the parsimony analysis (Supplementary Material 7d), while in the Bayesian analysis this is uncertain. The ancestral condition including the Rutaceae ancestor is uncertain in both analyses. Gynophores likely evolved independently in Simaroubaceae and Meliaceae. Androgynophores evolved independently in Simaroubaceae, Meliaceae and Rutaceae. In Simaroubaceae, our data indicate the presence of a gynophore likely arose in the ancestor of the clade formed by (*Nothospondias* (*Picrolemma* (clade 5))) but as a quite unstable feature, with multiple reversals.

Intrastaminal disk (Fig. 12a). Our reconstruction shows that the probable ancestral character state of all three families is not clear in the focal nodes, although intrastaminal disks are present in several genera. The absence of this structure is the most likely ancestral state from (*Nothospondias* (*Picrolemma* (clade 5))) with PP = 92.6.

Fruit type (Fig. 12c). A druparium with drupelets most likely laterally flattened to lenticular in shape is the probable ancestral state of Simaroubaceae fruit (PP = 96.7), also being a highly conservative feature in the family, with further transition to globose drupelets in *Homalolepis* (except in one species, *H. insignis*) and *Nothospondias*. Winged fruits evolved independently in *Ailanthus* and *Soulamea*; in the former, there are peculiar twisted samarids, while the latter is provided with a syncarpous ovary producing a samara. Fruits in Meliaceae and Rutaceae are considerably more diverse, though the Meliaceae common ancestor likely had capsules (PP = 73.5).

4 Discussion

The phylogenetic analysis including all samples recovered a tree topology consistent with Clayton's (2007) and Muellner-Riehl's (2016) results. Our objective here was to

Fig. 7 Reconstruction analysis of ancestral character state based on Bayesian (left) and Parsimony (right) methods. **a** Inflorescence type. **b** Sexual system
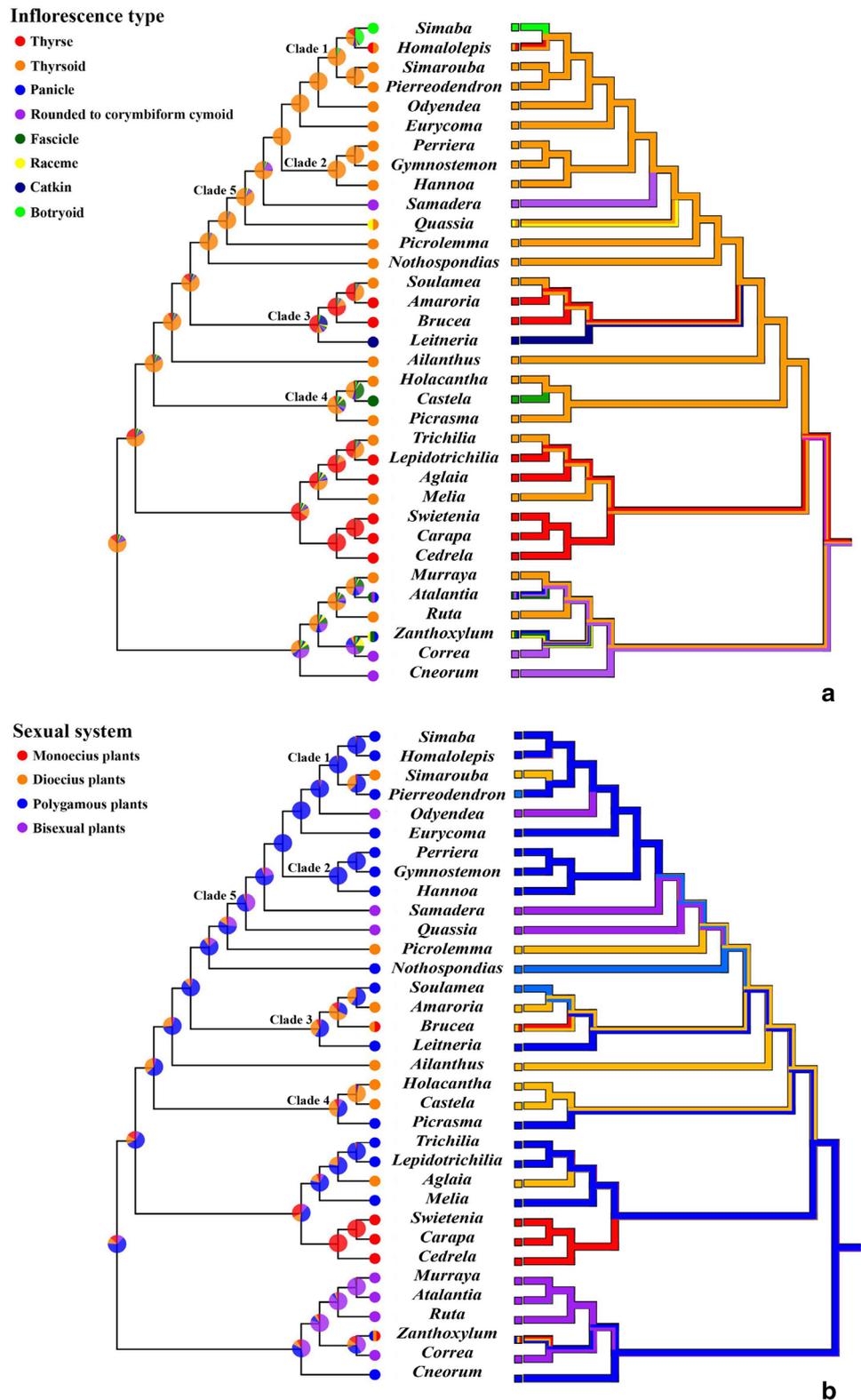
update the tree of Sapindales made available by Muellner-Riehl et al. (2016) with additional sequence data provided by Clayton et al. (2007) and use comprehensive sampling to obtain a robust ultrametric mcc tree and posterior trees.

General remarks and putative ancestral flower features of Simaroubaceae – Most characters studied herein show some degree of homoplasy, which can be very high. Nevertheless, our results indicate that the ancestors of Simaroubaceae
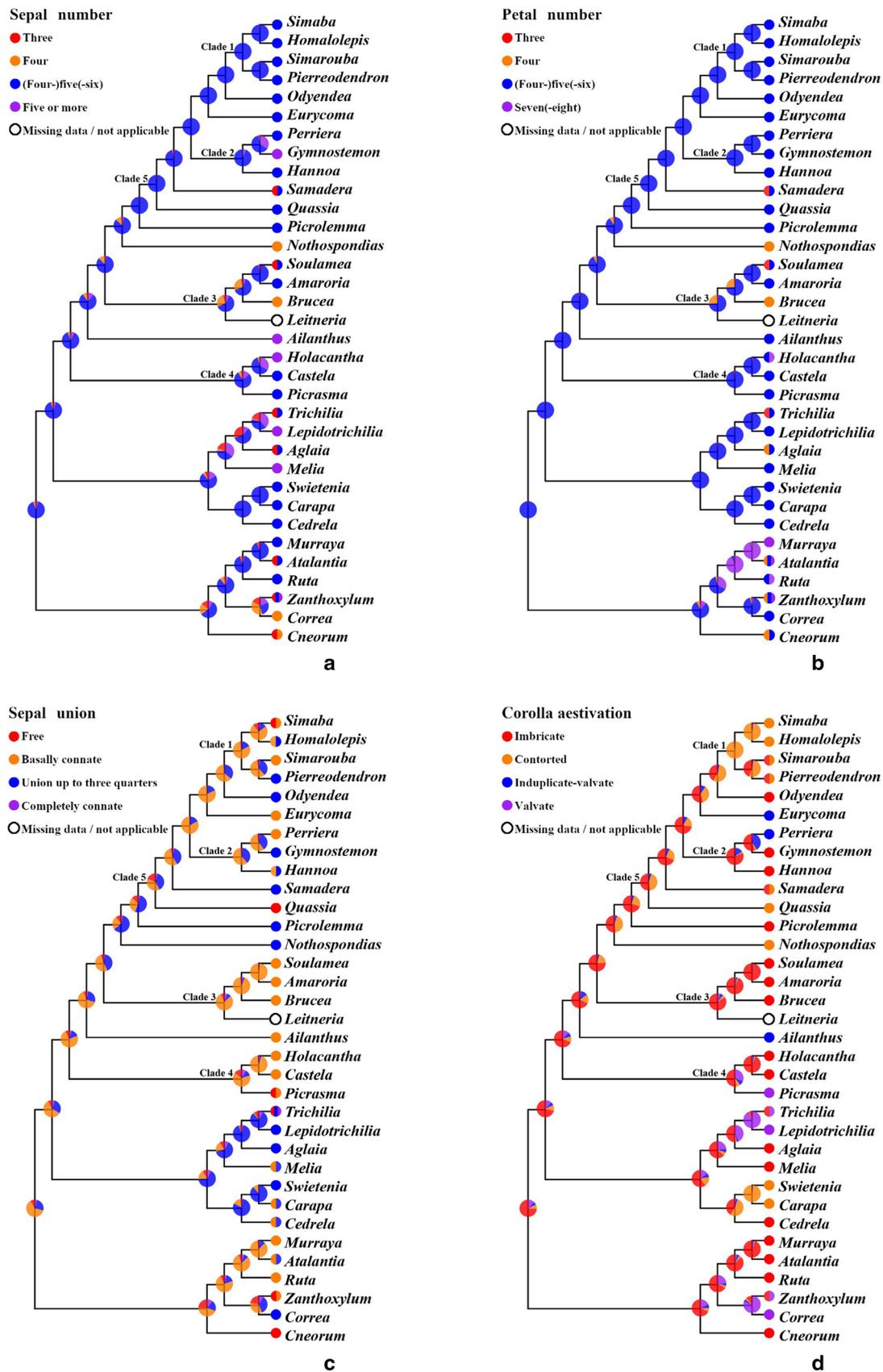
Fig. 8 Reconstruction analysis of ancestral character state based on Bayesian methods. **a** Sepal number. **b** Petal number. **c** Sepal union. **d** Corolla aestivation
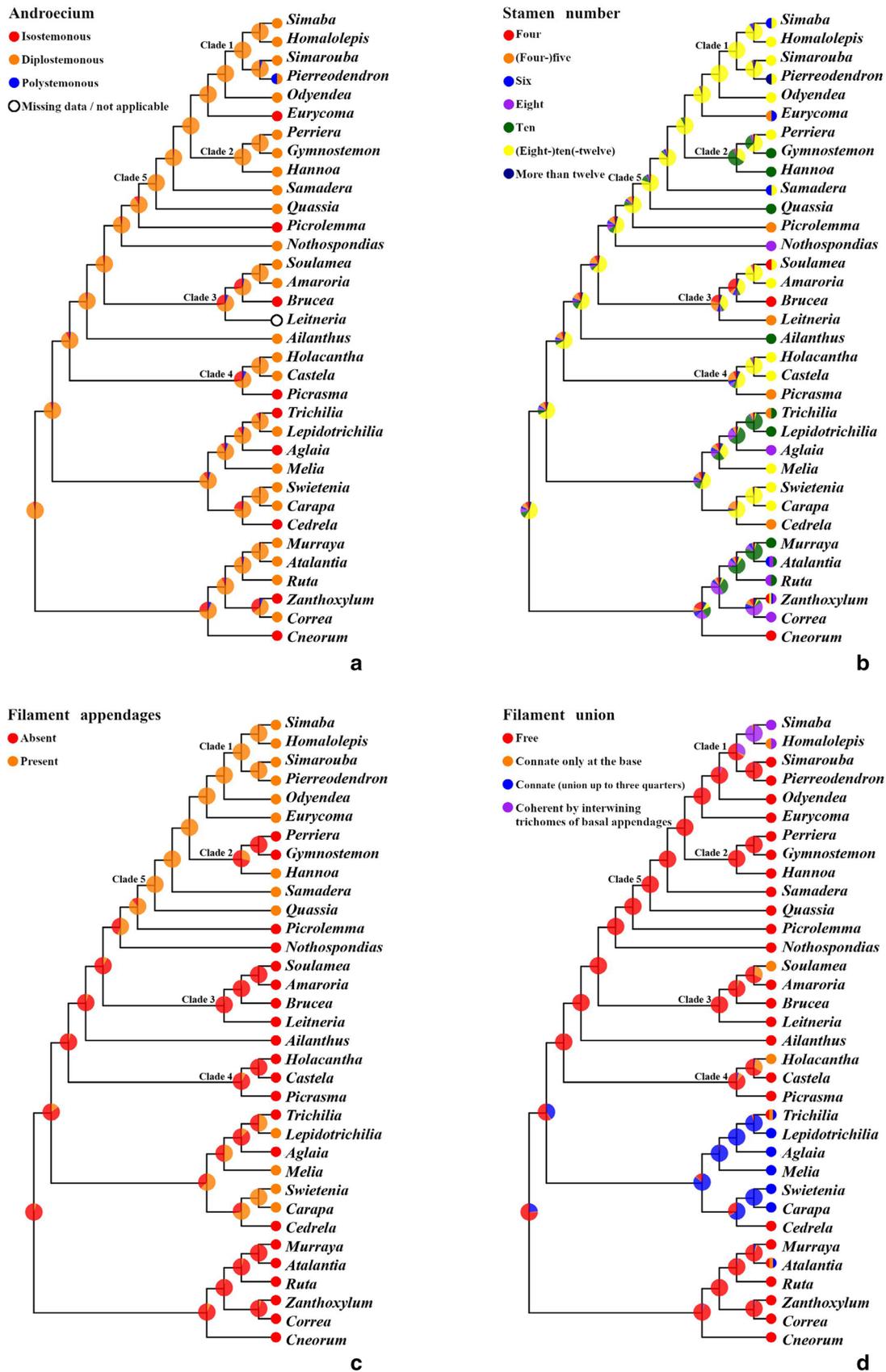
Fig. 9 Reconstruction analysis of ancestral character state based on Bayesian methods. **a** Androecium. **b** Stamen number. **c** Filament appendages. **d** Filament union
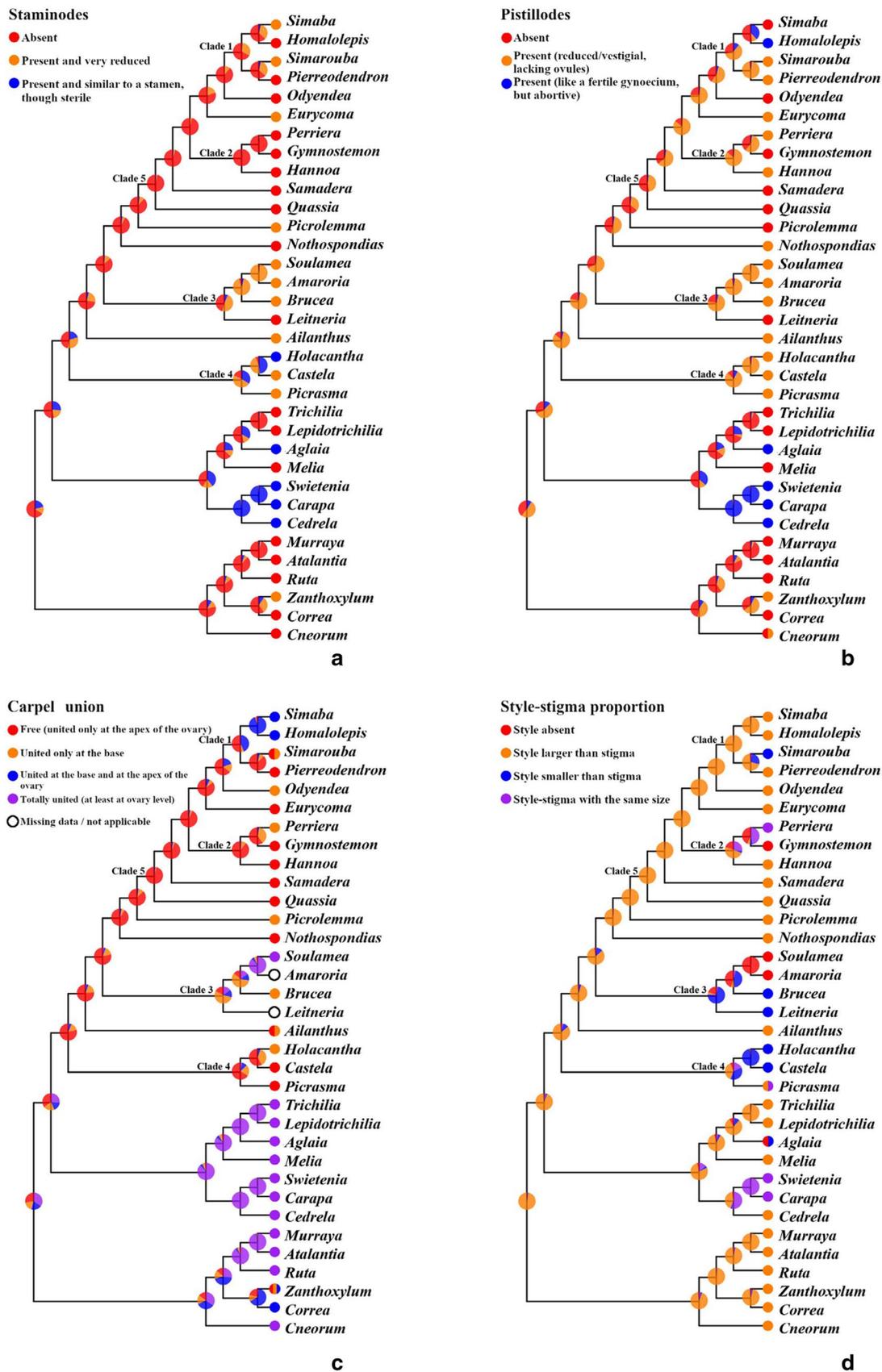
Fig. 10 Reconstruction analysis of ancestral character state based on Bayesian methods. **a** Staminodes. **b** Pistillodes. **c** Carpel union. **d** Style–stigma proportion
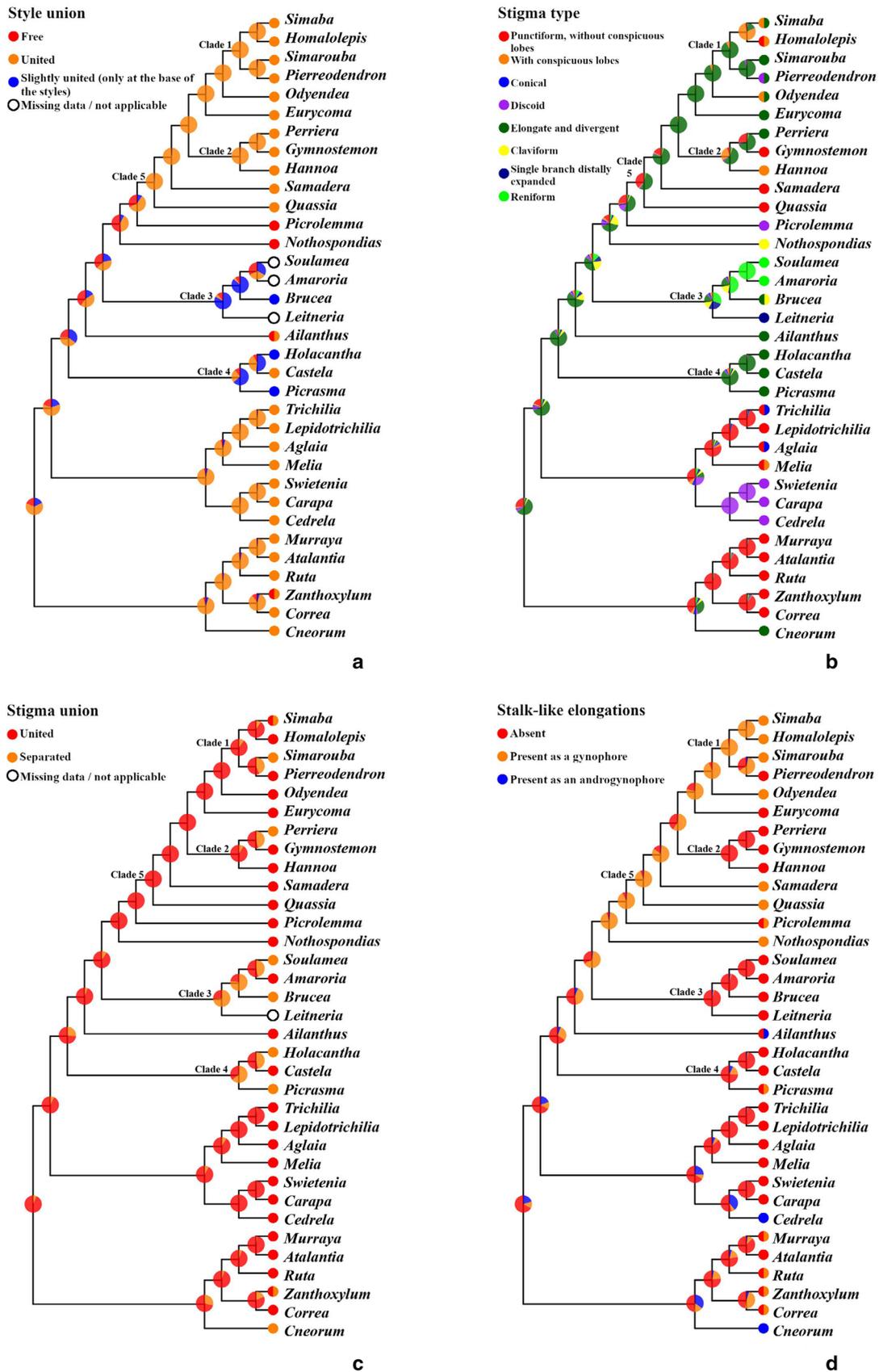
Fig. 11 Reconstruction analysis of ancestral character state based on Bayesian methods. **a** Style union. **b** Stigma type. **c** Stigma union. **d** Stalk-like elongations
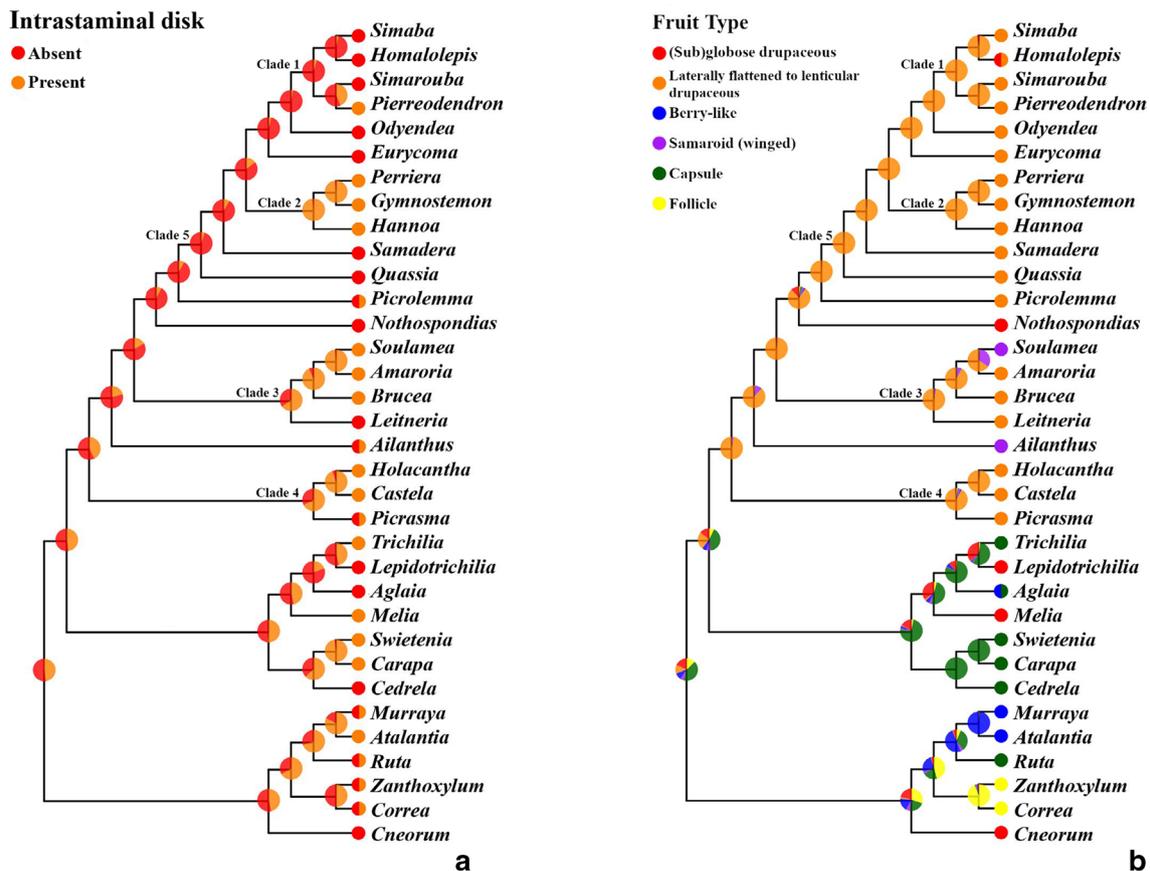
Fig. 12 Reconstruction analysis of ancestral character state based on Bayesian methods. **a** Intrastaminal disk. **b** Fruit type

likely were polygamous or dioecious plants, with imbricate petals, a diplostemonous androecium, free carpels united only distally at the top of ovary level or only by the styles, stigmas divergent and elongated and producing drupaceous fruitlets that are laterally flattened to lenticular. Imbricate petals and diplostemonous androecium were likely also present in the ancestor of all three families—Simaroubaceae, Meliaceae and Rutaceae.

As Clayton et al. (2007) pointed out, the molecular phylogeny of Simaroubaceae recovered several well-supported clades corresponding to some traditional generic limits based on morphological grounds. Our reconstructions show that several of the features that since Engler (1931a) have been used to circumscribe most genera are not synapomorphies. But they reveal that some of the traits analyzed are potentially useful to improve the characterization of certain clades higher than the genus level, although not fully consistent with Engler's definition of tribes Picrasmeae, Simaroubaceae and Soulameae, as Clayton et al. (2007) previously discussed. We retrieved that the species of clade 5 (Fig. 6) are supported by a quite peculiar putative synapomorphy: appendaged filaments. In these Simaroubaceae taxa, these appendages (Figs. 3c, e, j; 4 h, m) are found at

the adaxial base and flanks of the filaments; clade 5 comprises 12 genera, with the bulk of species of the family, and roughly corresponds to tribe Simaroubaceae in Engler's (1931a) classification, except for two genera lacking those structures, *Gymnostemon* and *Perriera,* which probably lost their appendages. This feature is discussed in detail in Sect. 4.5 Androecium.

Our reconstructions also suggest some interesting events that may have taken place during the structural evolution and diversification of the lineages studied herein. The main reconstructions that help enlighten these events are addressed, with emphasis on morphological evolution.

Inflorescence – The diversity of inflorescence architectures reflects the interplay between development and selection at several levels (Prusinkiewicz et al. 2007), and studies in morphology, function and development are important to clarify the evolutionary path of these traits. Besides some inaccurate descriptions of inflorescence types in Simaroubaceae and other families of Sapindales, with many genera being described as having panicles (Cronquist 1944a, b, 1981), the prevailing type in our taxa sampling is the thyrsoid, which was retrieved as the most likely ancestral type

of inflorescence for Simaroubaceae. Thyrsoids include a terminal flower (determinate inflorescence), and a transition to thyrses (indeterminate inflorescence) probably took place in *Amaroria* and *Brucea* in clade 3 (Fig. 6) and in some species of *Homalolepis,* in clade 1. A former reconstruction elaborated by Devecchi et al. (2018b), with a broader sampling of species of that genus and several related taxa, also recovered the thyrsoid as the most conservative within Simaroubaceae, and also that the thyrse is likely a synapomorphy of a small lineage nested within the clade currently recognized as *Homalolepis* sect. *Grandiflorae* (Engl.) Devecchi and Pirani. Our results indicate another transition from thyrsoids within clade 1 (Figs. 6, 7a): Botryoids emerged as a synapomorphy of *Simaba*, as was also obtained by Devecchi et al. (2018a), who treated the inflorescences in this genus either as botryoids or as depauperate thyrsoids. Likewise, the remaining transitions depicted in Fig. 7a seem to reflect reductions from the thyrsoid ancestral state: a catkin-like inflorescence in *Leitneria* (clade 2), fascicles in *Castela* (clade 4), cymoids in *Samadera*, and racemes in *Quassia*. The fact that the latter genus also has species with thyrsoids (or also botryoids according to Devecchi et al. 2018a) help to support that hypothesis.

Anatomical studies of the peculiar catkin-like male inflorescence of *Leitneria* by Abbe and Earle (1940) and by Tobe (2013) revealed that it is a reduced thyrse bearing many lateral cymules, each of which consists of three flowers (one central and two lateral flowers). Tobe (2013) also observed that "one or more stamens may be lacking in each flower, particularly in the lateral flowers," a fact that expresses further degrees of reduction.

Floral merism variation and synorganization – Although the majority of families in the core eudicots have stable merism with a predominance of pentamerous and trimerous flowers, these variations can fluctuate naturally in many families and some genera and species are more prone to meristic variations (Ronse De Craene and Smets 2016). In our study, flower merism emerged as a quite variable feature in the three families analyzed, but calices and corollas with (4–)5(–6) sepals or petals prevail and were retrieved with high support as the possible ancestral character state in Simaroubaceae, especially in clades 1 and 2 (Figs. 6, 8a, b). Transitions to the presence of flowers either pentamerous or occasionally tetramerous or hexamerous in the same species took place in four genera, most of them not closely related (*Ailanthus, Gymnostemon, Homalolepis* and *Simaba*). Reductions to tetramerous and trimerous conditions became established independently in some species of *Samadera* and *Soulamea*, while petals were lost and sepals became vestigial in female flowers of *Leitneria,* whose male flowers became achlamydeous. In addition, *Leitneria* flowers also lack a nectary disk and are borne in catkins that develop before the leaves emerge; such a strong morphological transition is generally related to wind pollination (Channel and Wood 1962; Clayton 2011; Tobe 2013). Such abiotic pollination is not known elsewhere in Simaroubaceae. This unusual floral structure was responsible for the inclusion of *Leitneria* among the hamamelids in such classification systems as those by Cronquist (1981) and Takhtajan (1997), until molecular data helped clarify its relationships within Simaroubaceae (Clayton et al. 2007). Thus, merism seems to be quite variable in Simaroubaceae with easy transitions among trimery, tetramery, pentamery and hexamery. The lability of floral merism in Simaroubaceae may occur scattered among the inflorescences of an individual, and so descriptions of the variations are somewhat contradictory in the literature of the family. An expressive lability in floral merism has been reported for other families in Sapindales, like Rutaceae (Pirani et al. 2010), and also in other eudicot groups (Ronse De Craene and Smets 2016). These features need to be more carefully analyzed in structural studies to understand if these meristic changes in the family are isomerous—reduction affecting all whorls of a flower, or anisomerous—affecting part of the floral whorls (Ronse De Craene and Smets 2016). In Simaroubaceae, a reduction in floral merism not followed by reduction in vasculature patterns was perceived by Alves et al. (2017). These authors demonstrated that flowers of some species of *Homalolepis* show incomplete tetramery (anisomerous reduction), such as *H. glabra* whose flowers have five sepals and four petals, being the lower petal a result of the fusion of two petals, as evidenced by its double number of vascular traces.

Throughout the course of floral evolution in angiosperms, it has been considered that stability in floral merisms is correlated with occurrence of whorled phyllotaxis (Endress 2010a). Along with whorled phyllotaxis and radial symmetry, a small and stable merism is considered a prerequisite for the evolution of complex synorganizational patterns in flowers (Endress 2010a, 2016; Endress and Doyle 2015). It is important to highlight the possible link between merism lability and meristem size and/or space. In *Inga*, Fabaceae (Paulino et al. 2017), the authors associate the increase in organ numbers with the increase in the floral meristem size through changes in the expression of the CLAVATA-WUSCHEL regulatory pathway, which coordinates the cell proliferation and differentiation in the promeristem. The increase in sepal and petal numbers is common in *Homalolepis* (Simaroubaceae), where a reduction to tetramery is also found. Based on developmental analyses of flowers from two genera of the tribe Rubieae (Rubiaceae), Naghiloo and Classen-Bockhoff (2016) concluded that if a given flower meristem with usually five primordia suffers a reduction in size and space, the initiation of the fifth primordium can be compromised, resulting in a tetramerous merosity. It is plausible to assume that in Simaroubaceae this could be

a motive for the merism lability, since we already have data pointed to a "incomplete reduction" in sepal number (Alves et al. 2017), but ontogenetic studies need to be performed to confirm that.

It is interesting to note that in Simaroubaceae, despite the high level of transitional patterns in merism, there is some degree of floral synorganization, such as some of the ones reported for other Sapindales representatives. Anatomical studies revealed complex patterns of organ synorganization in several species of subtribe Galipeineae (Rutaceae), in which a large floral tube is most often formed by synorganization of petals and filaments (with or without organ fusion), or only by the synorganization of petals (Pirani et al. 2010; El Ottra et al. 2013, 2019). Although the perianth in Simaroubaceae is mostly free to sometimes basally connate, a conspicuous and large tube-shaped anthetic corolla occurs in *Quassia amara*, which is formed by free petals "cohering into a tube" (Clayton 2011). Flowers of this species are deep pink or red, hummingbird-pollinated (Roubik et al. 1985; Clayton 2011). Further studies may reveal whether this tubular corolla involves partial congenital union or only postgenital coherence by petal margins, as described in several anthetic flowers of Galipeineae (Rutaceae, El Ottra et al. 2013). Elsewhere in Sapindales, connected petal margins are reported for Anacardiaceae, Burseraceae and Nitrariaceae but only at the bud stage (Bachelier and Endress 2009; Bachelier et al. 2011). In addition, synorganization of stamens evolved in some Simaroubaceae (see Sect. 4.5 Androecium). The diversity of fusions and connections among petals (and sometimes also among stamens) registered in Sapindales so far indicate, as claimed by Sokoloff et al. (2018), that a continuum exists between loosely contiguous organs and postgenitally fused organs, sometimes without clear boundaries from a free to a postgenitally fused condition (El Ottra et al. 2019).

Sexual systems – Sexual systems in Simaroubaceae are considerably diverse and complex, and the common occurrence of (sub) dioecy and polygamy illustrates how challenging the morphological classification can be within this group. In fact, our study retrieved polygamous or dioecious plants as likely already present in the Simaroubaceae ancestor. In *Iridosma, Quassia* and *Samadera,* only bisexual flowers are found (Engler 1931a; Clayton 2011), and hence they are described as hermaphrodites (Bawa and Beach 1981). Besides being hermaphroditic, *Quassia amara* has been shown to be self-compatible (Roubik et al. 1985). Contrastingly, *Amaroria, Castela, Holacantha, Laumoniera, Leitneria, Nothospondias, Picrolemma* and *Simarouba* have long been characterized as remarkably dioecious by their distinctive unisexual flowers in separate individuals, and this is a feature traditionally used in floristic and taxonomic works to distinguish them from related genera (Engler

1931a; Cronquist 1944a, b; Pirani 1987a, b; Thomas 1990; Clayton 2011). In those eight genera, male flowers have a much reduced to vestigial pistillode, and female flowers bear very small staminodes. There are many uncertainties about the morphological distinction between unisexual and bisexual flowers, as well as doubts about whether plants are monoecious, dioecious or polygamous. For *Ailanthus, Brucea, Eurycoma,* and *Picrasma,* most authors refer to either monoecious and dioecious species (Nooteboom 1962a; Clayton 2011), or dioecious and polygamous species (Clayton 2011, for *Brucea*), or exclusively polygamous ones (Engler, 1931a). In *Gymnostemon, Hannoa, Homalolepis, Odyendea, Perriera, Pierreodendron, Samadera, Simaba* and *Soulamea,* the flowers are morphologically bisexual, but there is strong evidence that they can be functionally bisexual, male and female, either in separate individuals or in the same plant, which are defined as polygamous, polygamodioecious and polygamomonoecious, respectively. The polygamous condition was previously reported by Engler (1931a) and Clayton (2011) for Simaroubaceae and is supported by recent findings of scattered flowers bearing abortive ovules in some *Homalolepis* and *Simaba* species (Franceschinelli and Thomas 2000; Alves et al. 2017; Devecchi et al. 2018a, b), which were traditionally described as hermaphrodites. These findings reveal that polygamy and subdioecy (or cryptic dioecy) may be widespread in the family, and further floral and reproductive studies on this matter are still necessary.

Flowers that are morphologically bisexual but functionally unisexual are reported as quite common in several genera of Simaroubaceae and found in most other families of Sapindales (except for Bieberesteiniaceae; Bachelier et al. 2011). They are found in *Kirkia* (Kirkiaceae, Bachelier and Endress 2008), *Nitraria* (Nitrariaceae, Bachelier et al. 2011), many Anacardiaceae and Burseraceae (Engler 1931c; Bachelier and Endress 2009), Sapindaceae (e.g., *Acer, Koelreuteria,* Yadav et al. 2016; Avalos et al. 2019), Rutaceae (e.g., *Tetradium, Phellodendron, Skimmia*; Engler 1931b; Ramp 1988; Kubitzki et al. 2011), and most Meliaceae (Styles 1972; Pennington and Styles 1975; Gama et al. 2021a). In the latter family, the distinction between flowers of different sexuality appears to be more subtle than in the remaining groups of the order, a fact that was recently reported also for *Trichilia claussenii* C.DC., in which a peculiar case of male sterility due to tapetum secretion was discovered (Gama et al. 2021b).

It is important to highlight the controversial sexual system definition in the tree-of-heaven genus, *Ailanthus*. Although there are reports of unisexual and bisexual flowers, monoecious, dioecious and polygamous species in this genus, Nooteboom (1962b) and Hu (1979) ponder that some of these descriptions might be misinterpretations of staminodes which are quite similar to stamens, being dioecy the most likely sexual condition of the genus. Nair and Joshi

(1958) have reported that female flowers in *Ailanthus excelsus* Roxb. have anthers bearing sterile pollen grains and which do not dehisce, even though they are provided with a conspicuous fibrous endothecium. Likewise, Clayton (2011) describes only unisexual flowers, in monoecious or dioecious species in this genus. Additionally, our own observations of the *Ailanthus* bibliography indicate that the flowers previously defined as bisexual are in fact female flowers with staminodes quite similar to fertile stamens but smaller when compared to those of male flowers. Hence, we herein considered this genus to be dioecious.

Other divergences may be easily found in the literature of the family. A species recently described as having bisexual flowers, belonging to a genus traditionally considered monoecious or dioecious (*Picrasma pauciflora* A. Noa & P.A. González), was reported by Noa-Monzón et al. (2019). In the original description of *Picrasma*, Blume (1825) characterized the species as hermaphroditic or monoecious by abortion, and Shu (2008) mentioned polygamy in this genus in the Flora of China. We herein treated *Picrasma* as polygamous, but in-depth studies are necessary to elucidate how these reproductive organs are expressed in all species of the genus.

The cases above are just selected examples of the controversy related to sexual systems in Simaroubaceae. In fact, since Darwin (1877) this subject has been addressed and subsequently deepened throughout angiosperm lineages (Bawa and Beach 1981). The interpretation of sexual systems is often doubtful because only morphological features are available for most taxa. When additional observations and functional criteria are applied, a more effective and accurate interpretation of sexuality can be accessed. Hence, the controversial interpretations and current scarcity of accurate data for several taxa of Simaroubaceae lead us to encode as polygamous all genera in which there is evidence of the presence of unisexual and bisexual flowers. In our analysis, the ancestral state for the character "sexual system" was not recovered with certainty (Fig. 7b), but there is a higher probability that the ancestor of the three families studied herein was polygamous, with scattered independent transitions to the other states. This is consistent with the reconstruction made by Devecchi et al. (2018a) for Simaroubaceae, though based on fewer genera. Contrastingly, Gama et al. (2021a) reconstructed the probable ancestral character state for Meliaceae and Simaroubaceae as unisexual flowers in dioecious individuals, followed by transitions to polygamy, monoecy and even hermaphroditism. Our taxon sampling in Simaroubaceae is considerably larger than that of Gama et al. (2021a), allowing for more robust conclusions about this family. Nevertheless, it is very likely that these questions will remain unresolved until detailed studies on sexuality are available for most taxa of Sapindales, ideally including field observations and experiments, as well as structural analyses.

A rise of monomorphic sexual systems (with a single gender class of individuals, such as monoecy or hermaphroditism) from ancestors with dimorphic sexual systems (with two gender classes of individuals, such as polygamy and dioecy), as recovered here and also in Meliaceae by Gama et al. (2021a), has never been discussed in the literature (as previously referred to by Bawa and Beach 1981; Charlesworth and Guttman 1999; Sakai and Weller 1999; Barrett 2002; Barrett and Hough 2013). For instance, dioecy is thought to have evolved not less than 100 times, considering the 160 families in which dioecious species are known (Charlesworth and Guttman 1999). Minelli (2018) estimated that the actual number of transitions to dioecy is likely even higher because this condition evolved multiple times within some families, as in Simaroubaceae. Pannell (2007) states that "separate sexes in plants have ultimately evolved from hermaphroditic ancestors."

Nevertheless, a macroevolutionary study by Goldberg et al. (2017) found that evolution may proceed in the direction of lesser sexual differentiation rather than toward greater sexual differentiation. Likewise, our results point to this possibility. The presence of both reproductive organs in flowers of most Simaroubaceae genera, and the prevailing polygamous or subdioecious condition, suggests that the conservation of fundamental genetic material in a dimorphic sexual ancestor could allow transitions in the sexual expression of phenotypes leading to monomorphic systems.

Furthermore, according to Pannell (2017), plant gender is fundamentally a quantitative trait which may be "measured in terms of the relative allocation to each sexual function." Hence, inconstant gender expression is responsible for the fact that male and female plants may produce some flowers of the other gender in many polygamous and dioecious species (as several Simaroubaceae) (Pannell 2017). Genetic sex determination, which underpins the separation of male and female flowers in dioecious species, is often mediated by genetic polymorphism and sex chromosomes, while in monoecious or hermaphroditic species developmental sex determination takes place at the level of modules, tissues or cells (Pannell 2017). Cases are known in which sex determination involves responses to environmental or hormonal cues (Lloyd and Bawa 1984; Pannell 2017). A cytological investigation in *Simarouba glauca* DC. revealed that both male and female individuals share $2n = 30$ chromosomes and exhibit a symmetrical karyotype, though female plants possess chromosomes which are longer than the male counterparts (Baratake and Patil 2010). Nevertheless, the same authors concluded that "the nature of sex cannot be determined in *S. glauca* at the chromosome level." As information on chromosome data of other Simaroubaceae genera is increasing and diversifying (Romero-da-Cruz et al. 2021), we may expect further progress regarding this subject. In fact, the wide diversity of genetic mechanisms for sex determination

already studied in dioecious plants is likely attributable to the fact that separate sexes have evolved repeatedly in different lineages, often recently (Renner 2014), and that genetic switches involved in sex determination have thus evolved independently many times (Charlesworth and Guttman 1999).

Regarding the sterilized organs in unisexual flowers of Simaroubaceae, our analysis did not retrieve with certainty whether the ancestor of the family had staminodes or not, but there is higher support to the hypothesis that the presence of very reduced to vestigial pistillodes may be the ancestral condition, followed by secondary losses occurring in several clades. Staminodes are relatively uncommon in Simaroubaceae but widely distributed in angiosperms. The phylogenetic distribution of this feature suggests that they typically arise during evolutionary reduction in the number of androecium elements (Walker-Larsen and Harder 2000). In Simaroubaceae, it is interesting to notice that in clade 3 (*Brucea* (*Soulamea*, *Amaroria*)), which is provided with reduced or vestigial staminodes and pistillodes, there are genera with the lowest number of stamens. Finally, it is important to highlight that the dioecious genera in Simaroubaceae are not speciose; instead, they are either monospecific (*Amaroria*, *Laumoniera*, *Leitneria* and *Nothospondias*) or have just a few species: *Castela* (12 spp.)*, Holacantha* (two spp.)*, Picrolemma* (two spp.) and *Simarouba* (six spp.). Even though most of the remaining genera are not speciose, the most diverse genus in the family, *Homalolepis,* with 28 spp., is likely mostly hermaphroditic or partially polygamous (see also Devecchi et al. 2018b). This is somewhat consistent with results from a comparative analysis based on sister-group comparisons of angiosperm taxa with contrasting sexual systems, which showed that dioecious lineages have fewer species than their cosexual (hermaphroditic and monoecious), sister taxa at both the family and genus levels (Heilbuth 2000). The only simaroubaceous sister-group in which the dioecious taxon is larger than its non-dioecious pair is formed by *Pierreodendron* (one sp.) and *Simarouba* (six spp.). It has been suggested that the pattern of lower diversity in dioecious lineages might have resulted from the higher risk of extinction in dioecious species, compared to non-dioecious relatives, or because dioecious clades may have reduced speciation rates (Barrett 2002). Hence, further studies on reproductive biology are needed to help clarify several of the questions above.

Flowers of contrasting morphology are likely related to different pollination strategies, as are the cases of reduced flowers of the dioecious genus *Leitneria*, which are wind-pollinated, taxa with larger flowers, such as the tubular corolla of *Quassia* (which are bird-pollinated) and species bearing a long staminal pseudotube, as found in *Homalolepis* (see Androecium section), whose pollination system remains to be investigated. Entomophily seems to be the predominant pollination system in the family, since the flowers are relatively small, actinomorphic, open, mostly fragrant and attract a wide range of generalist insects, including bees and moths (Aubréville 1962; Hardesty et al. 2005; Devecchi et al. 2018b).

Androecium – The androecium can be characterized by the number of stamens in relation to sepals and petals basically as isostemonous, diplostemonous or polystemonous, but considering their position in relation to the perianth parts, the following conditions are usually recognized in more detail: haplostemonous, obhaplostemonous, diplostemonous and obdiplostemonous. However, these configurations have been described so far based mostly on mature flowers, compromising the characterization since these classifications are mostly dependent on structural and developmental interpretations (Endress 2010a, b; Ronse De Craene and Bull-Hereñu 2016). Despite traditional taxonomic works such as Willis (1951) and Rendle (1952) describing Simaroubaceae as an obdiplostemonous group, the lack of detailed structural and developmental studies prevented us from accurately defining these more detailed types for any taxa. Contrastingly, regarding the isomerous genera it is easy to characterize *Brucea*, *Eurycoma* and *Picrasma* as haplostemonous, and *Picrolemma* as obhaplostemonous. The latter condition is a relatively rare feature in angiosperms, often correlated with a delay in petal development (Ronse De Craene 2010).

Although variation in androecium characters was used by Engler (1931a) to delimit subtribes, Clayton et al. (2007) has already pointed out that "the phylogeny reveals lability in the nature of the androecium, with typically diplostemonous flowers in the family," but also with polystemonous and isostemonous flowers, this latter condition having evolved multiple times within the group.

Likewise, our reconstruction shows that the common ancestor of Simaroubaceae, Meliaceae and Rutaceae was most likely diplostemonous. This feature is conserved in most Simaroubaceae lineages with four independent transitions to isostemony, a condition that evolved independently within the two other families. An extreme reduction to only (1–)4 stamens occurred in *Leitneria,* which have also lost their perianth, and a transition to polystemony occurred in *Pierreodendron,* in which the outer whorl of stamens is doubled (Clayton 2011), and also in *Aglaia* (Meliaceae), a result consistent with Gama et al. (2021a). However, Wei et al. (2015), in a study of androecium ontogeny and evolution in tribe Ruteae, found the haplostemonous state as likely plesiomorphic for Rutaceae, with further evolution to obdiplostemony, as in the ancestor of the tribe Ruteae, and to polystemony, as found in the "Citrus" group. Diplostemony probably represents the ancestral androecial configuration in core eudicots, as it is both widespread and considered

plesiomorphic in most major clades (Ronse De Craene and Bull-Hereñu 2016).

Obdiplostemony is still a controversial topic of morphological research. This concept can be interpreted as a simple derivative of diplostemony or as an important transitional character state in flower evolution (Ronse De Craene and Bull-Hereñu 2016). Alves et al. (2017) showed that *Homalolepis* in Simaroubaceae present secondary obdiplostemony, following the definition of Ronse De Craene and Smets (1995). Ronse De Craene and Bull-Hereñu (2016) revised the concept and occurrence of obdiplostemony using an evolutionary approach, expanding the definition previously considered "rigid" without consideration of the diversity in apetalous flowers, anisomerous gynoecium and flowers with sterile or incomplete whorls. According to the authors, an obdiplostemonous androecium is linked to the evolution of at least three major shifts in flower Bauplan occurring mainly in the rosids. Considering that different developmental pathways can lead to obdiplostemony, and that this can be seen as a transitional state for the development of diplostemony, haplostemony or obhaplostemony (Ronse De Craene and Bull-Hereñu 2016), the development of the androecium in Simaroubaceae should be studied in more detail in the future, as all these types of androecium are present in the family. In this way, an accurate discussion on the evolutionary pathways of the androecium in Simaroubaceae, as well as in most Sapindales representatives, will be possible.

Staminodes are present in female flowers of several Simaroubaceae genera, but only three genera also have staminodes in male flowers, *Eurycoma, Picrolemma* and *Simaba.* Thus, our reconstruction (Fig. 10a) indicates that staminodes evolved independently within the family, as was previously discussed by Clayton et al. (2007). Accordingly, each of the three genera displays quite distinct staminode positions, which indicate putative different origins regarding the sterilization between different whorls, or in part of each whorl of the diplostemonous androecium. In *Picrolemma,* the staminodes alternate with petals and fertile stamens are opposite the petals (the flower is obhaplostemonous, as discussed above), while in *Eurycoma* there are staminodes alternating with fertile stamens, as already pointed out by Clayton (2011). Contrastingly, *Simaba* has rudimentary staminodes forming a partial whorl between the base of the petals and stamens (Devecchi et al. 2018b), but the lack of detailed studies obscures whether this is a partial whorl of antepetalous or antesepalous staminodes.

Filaments are free in most genera lacking staminal appendages in Simaroubaceae, and a connation at their bases probably evolved independently only in *Holacantha* and *Soulamea.*

Another especially relevant feature of the androecium in several Simaroubaceae is the presence of laminar appendages at the adaxial base and flanks of the filaments. The staminal appendages are partially free from the filament along its extension, and in some species of *Homalolepis* they are basally connate and slightly postgenitally coherent to one another as a result of intertwining trichomes, especially in species of *H.* sect. *Grandiflorae,* as shown by Devecchi et al. (2018b), where they form a structure termed a "pseudotube" by Alves et al. (2017). We retrieved staminal appendages as a putative synapomorphy of clade 5 (Figs. 6, 9c), but the peculiar arrangement of filament union by intertwining trichomes is a possible unique synapomorphy of the clade (*Simaba*, *Homalolepis*) (Fig. 9d). However, this hypothesis would not be supported in the topology elaborated by Devecchi et al. (2018a, b), in which a distinct sister-group relationship was found: (*Simaba* (*Simarouba, Homalolepis*)). As flowers of *Simarouba* have free stamens bearing reduced, curved appendages, this may be seen as a reversion regarding the presence of the pseudotube, or this structure appeared independently in each of the other two genera.

Tubular structures in flowers (corolla tubes, stamen–petal tubes, staminal tubes, among others) likely have the function to protect the gynoecium or nectary, influencing the availability of floral rewards to pollinators (Faegri and van der Pijl 1979; Endress 1994). This was seen in pollination biology studies undertaken in some Galipeinae, Rutaceae, by El Ottra et al. (2016). Functional studies on the pseudotube of Simaroubaceae are still lacking. Our reconstruction analysis retrieved that the common ancestor of Simaroubaceae, Meliaceae and Rutaceae probably lacked staminal appendages. These structures likely appeared in the common ancestor of the lineage containing *Quassia* and 11 other genera (clade 5, Figs. 6, 9c), but *Gymnostemon* and *Perriera* subsequently lost the appendages. It is interesting to notice that this large lineage with *Quassia* holds the greatest number of species in Simaroubaceae, and that is why authors such as Engler (1931a) attributed a relative importance to this character, as also highlighted in the family description presented by Clayton (2011). In fact, Engler (1931a) defined tribe Simaroubeae essentially on the basis of appendaged stamens, including in the tribe all genera of clade 5 except the two genera lacking those structures, *Gymnostemon* and *Perriera.* On the other hand, appendaged stamens have evolved independently in some Meliaceae (Pennington and Styles 1975; Gama et al. 2021a; Fig. 9c), and also in some genera of Rutaceae not included in our sampling but well known in the literature. For instance, *Dictyoloma, Spathelia* and *Sohnreyia* (Rutaceae, Engler 1931b) bear hairy appendages on the filaments that look like those of most Simaroubaceae taxa. Additionally, in some rutaceous genera from the subtribe Galipeinae (tribe Galipeeae), macromorphological and anatomical studies have shown that filaments may form partial or complete tubes with various degrees of fusion or postgenital coherence between each other or adherence to

petals by the intertwining of their trichomes (Pirani et al. 2010; El Ottra et al. 2013, 2019). In Meliaceae, flowers of *Cabralea, Carapa, Guarea, Swietenia* and *Trichilia* have staminal appendages that are either free from one another or partially to completely fused, forming a tube (Pennington and Styles 1975; Gama et al. 2021a). Even though detailed comparative studies of staminal tubes and pseudotubes in taxa of these three families are still scarce, these structures may have developed under similar selective pressures related to flower biology—and thus likely represent a convergent trait.

Stalk-like elongations and intrastaminal disk – Conspicuous nectariferous disks are widespread in Sapindales (Judd et al. 2008; Kubitzki 2011) and are indicated as a putative synapomorphy of the order (Gadek et al. 1996), generally intrastaminal in position, with the exception of Sapindaceae (Cronquist 1981; Judd et al. 2008). Although our reconstruction demonstrates uncertainty in the ancestral character state of flowers of the three families studied herein, many Simaroubaceae genera are provided with intrastaminal disks, whose presence is a possible ancestral character state, as discussed by Muellner-Riehl et al. (2016) for Sapindales, and as also obtained in the reconstruction by Devecchi et al. (2018a), which focused on *Simaba* and *Homalolepis*. A disk is evident in most Simaroubaceae taxa and can be macromorphologically inconspicuous, including the cases in which nectariferous tissue is placed on the entire surface of the gynophores, so far detected in *Quassia* and in three genera from clade 1: *Simarouba, Simaba* and *Homalolepis* (Fig. 11d). The conspicuous gynophore of *Picrolemma* is likely nectariferous as well (pers. obs.). Our analyses show that a flower without stalk-like elongations (i.e., without gynophores or androgynophores; Leins and Erbar 2010) is most likely the ancestral character state in Simaroubaceae, consistent with the reconstruction by Devecchi et al. (2018a). These authors retrieved gynophores as a putative synapomorphy for the clade including *Quassia* (similar to clade 5 in our Fig. 6), which is more or less equivalent to Engler's (1931a) tribe Simaroubaceae; however, a reversion to an indistinct nectary probably took place in the common ancestor of *Odyendea* and clade 1. Additionally, Devecchi et al. (2018a) explored variations in shape of the distal part of the gynophore and of the form of the disk (urceolate, annular), which are not addressed herein. Finally, gynophores likely evolved independently also in Meliaceae and Rutaceae (Fig. 11d). In far fewer taxa of the three families, an androgynophore evolved independently, such as in *Cedrela* and *Toona* (Meliaceae, Gouvêa et al. 2008; Gama et al. 2021a), *Cneorum* (Rutaceae, Caris et al. 2006) and *Ailanthus glandulosus* Desf. (Simaroubaceae, Ramp 1988).

Gynoecium – Simaroubaceae representatives have been described as mostly apocarpous, with uniovulate locules (Ramp 1988; Fernando et al. 1995; Clayton 2011). In most genera, carpels are at least partially united by the styles and/or stigmas. Available ontogenetic studies have shown that such carpels are postgenitally fused, usually in the distal part of the ovary, also forming a single style (Ramp 1988). When such fusion also reaches the most distal parts of the style, it forms a single stigma, but in taxa such as *Brucea, Castela, Perriera,* and *Simarouba* stylar lobes may be separate and divergent in several genera (Nair and Joshi 1958; Endress et al. 1983; Ramp 1988; Kubitzki et al. 2011; Alves et al. 2017). In fact, similar apocarpous gynoecia with postgenitally fused carpel apices are widespread also in many Rutoideae of Rutaceae, not broadly sampled in this study (only *Correa* and *Zanthoxylum* were included), and elsewhere in the rosids–malvids clade this has also been described for Malvales (Gut 1966; Endress et al. 1983; Ramp 1988; Bachelier and Endress 2008, 2009; Matthews et al. 2012; El Ottra et al. 2013, 2019). The postgenitally fused apices of carpels form a dilated structure called a stigmatic head in several families of Sapindales, thus being a putative synapomorphy of the order (Bachelier and Endress 2008, 2009).

Although the condition of united carpels for the common ancestor of Simaroubaceae, Meliaceae and Rutaceae is not highly supported in our Bayesian analysis, the ancestor of Meliaceae most likely was syncarpous in both analyses, while free carpels (united only distally) emerge as a putative synapomorphy of the lineage formed by the three families (Fig. 10c). However, our finding should be reevaluated in future evolutionary studies, considering that molecular analyses have retrieved different sister-group relationships among the three focal families (Gadek et al. 1996; Stevens 2001; Lin et al. 2018) and that many taxa of Rutoideae of Rutaceae have similarly free carpels (with postgenitally united apices, Engler 1931a; Gut 1966; Endress et al. 1983; Ramp 1988; Kubitzki et al. 2011; El Ottra et al. 2013, 2019). Alternative topologies could shift this last feature in a shared condition among rutaceous and simaroubaceous ancestors. Future evolutionary studies should clarify this issue.

In Simaroubaceae, at least five transitions occurred to basally united carpels, and one transition to carpels united at the base and the apex (by the styles) in the ancestor of the clade (*Simaba, Homalolepis*). Completely free carpels evolved only in a few genera, among which the most remarkable case is *Picrolemma* in which carpels are conspicuously spread away from each other. Convergent evolution of partially or completely free carpels from syncarpous ancestors has been reported for other families (Endress et al. 1983), and a broad evolutionary developmental perspective is required for a better understanding of the underlying developmental processes leading to such a homoplastic change. Minelli (2018) stated that "morphology has more or less

direct and more or less important functional correlates that may have played a strong role in the fixation of a convergent trait." In this sense, testable hypotheses linking general patterns of morphological diversity with gene expression are essential.

Endress and Matthews (2006) define as an "elaborated apocarpy" the situation in which the free carpels have their upper portion postgenitally united at anthesis, which allow the formation of a compitum. The compitum in such gynoecia is formed by the union of the pollen tube transmitting tissue of each carpel, in the postgenitally fused apical region of the ovary and style, allowing pollen tubes to cross between carpels and fertilize ovules that would otherwise be more prone to remain unfertilized. It has been suggested that this arrangement provides the advantages of a syncarpous in relation to a mostly apocarpous gynoecium (Endress et al. 1983). Hence, in Simaroubaceae the united styles provide for centralized pollination, and later in development the carpels separate from each other and form fruitlets. Each ripe fruitlet bears a viable seed, and this may constitute a clear advantage over a regular syncarpous gynoecium forming a single fruit composed of all of the carpels, bearing viable seeds or not, as pointed out by Endress et al. (1983). The potential advantages of syncarpy have been related to the centralized selection of pollen tubes (Endress et al. 1983), or also to a high proportion of evolutionary transitions promoting pollen competition and pollen tube access to all carpels increasing offspring quality and quantity (Armbruster et al. 2002). The "elaborated apocarpy" (sensu Endress and Matthews 2006) found in Simaroubaceae seems to function likewise.

Amaroria and *Leitneria* are unique in the family for having monomerous gynoecia (one carpel, uniovulate), and both probably are derived from a reduction in an ancestor having free carpels (Fig. 10c). Reductions in carpel number are reported in many Sapindales, but in some cases resulting in pseudomonomery, a feature involving the presence of at least one fertile carpel along with one or more aborted carpels (as defined by Sokoloff et al. 2017). Pseudomonomerous gynoecia are widespread only in Anacardiaceae (especially in Anacardioideae, Tölke and Demarco 2020), being a putative synapomorphy of the family (Bachelier and Endress 2008, 2009).

Our hypothesis on evolution of stigma types is that stigmas formed by long, divergent, spreading branches evolved early in the history of Simaroubaceae, although with low probability in the Bayesian analysis (79.6%. PP). This is consistent with findings by Devecchi et al. (2018a). Later transitions among different lineages probably led to a remarkable array of forms in the family, with high levels of homoplasy. Furthermore, stigma shape has been long used as a very useful diagnostic character in infrafamilial taxonomy of Simaroubaceae, including infrageneric levels in the largest genus, *Homalolepis* (Engler 1931a; Cronquist 1944a, b;

Noteboom 1962b; Clayton 2011; Devecchi et al. 2018a, b). Remarkable variations in stigma shape also provide valuable taxonomic characters for infrafamilial classification in Meliaceae (Pennington and Styles 1975), though with high levels of homoplastic evolution (Gama et al. 2021a). In Rutaceae, a diversity of stigma types has also already been reported (Ramp 1988; El Ottra et al. 2019), but its evolution has not been evaluated so far.

Fruit – Fruits separating into fruitlets (drupaceous fruitlets), most commonly laterally flattened to lenticular in shape, emerged as the probable ancestral state of Simaroubaceae and are the prevailing type among the genera. Transitions to (sub)globose drupelets evolved independently in *Nothospondias* and *Homalolepis* [except *Homalolepis insignis* (A. St. Hil. and Tul.) Devecchi and Pirani], showing that the drupaceous condition is a conservative feature in Simaroubaceae, varying only in shape throughout most lineages. Some apparent conflicts with reconstructions inferred by Devecchi et al. (2018a) are just a matter of codification of states and distinct taxon sampling. These authors suggested that acquisition of strongly laterally flattened fruitlets in *Simaba obovata* Spruce ex Engl. and *S. orinocensis* Kunth seems to be associated with the occupation of seasonally flooded areas along river margins in the Amazon basin. The flattened shape enables these drupelets to float on water, and the fleshy and edible mesocarp promotes dispersal by fish (Honda 1974; Gottsberger 1978). Contrastingly, subglobose drupelets of most species in *Homalolepis* can be very large, especially those of the widespread *H. cedron*. Janzen (1979) pointed out that the restricted occurrence of trees of this species (then treated as *Simaba cedron*) growing in forests of tropical Central America could be related to the extinction of mastodons in the last 10,000 years. As the fruit wall is very hard in this species and some related ones (e.g., *H. trichilioides*, *H. arenaria*, *H. rigida*), only a few animals can crack and eat them. Unlike most drupaceous fruits, the endocarp in these species is relatively thin and the hard portion of the pericarp is formed mainly by a thick fibrous mesocarp (Devecchi et al. 2018b).

The independent transitions to samaroid fruits in *Ailanthus* (samarium) and *Soulamea* (samara) probably appeared as modifications from an ancestral druparium consisting of laterally flattened drupelets. In fact, structural studies have shown that carpels of *Ailanthus* are already laterally flattened since bud stages (Ramp 1988). Thus, the shape of carpels might be conserved even in carpels with different dispersal modes.

Fruits are considerably more diverse in Meliaceae (Pennington and Styles 1975; Gama et al. 2021a) and Rutaceae (Engler 1931b; Kubitzki et al. 2011). Meliaceae have syncarpous fruits, such as subglobose drupes, berrylike, and even capsules. The latter fruit type is widespread in the

family—and likely present in the ancestor of the group (as seen in Bayesian analyses), which is in accordance with the finding of Gama et al. (2021a), where "septifragal capsules with a rudimentary columella" were found to be the ancestral state for the family. Considering the fruit types in Rutaceae, they are much more diverse, presenting fruits separating into fruitlets, such as follicles (e.g., *Zanthoxylum*), follicaria (e.g., *Erythrochiton*), druparia (subglobose, e.g., *Cneorum*) and samarium (e.g., *Helietta*), or syncarpous fruits, such as berries (e.g., *Hortia* and the "Citrus" group), samaras (e.g., *Balfourodendron*) and capsules (e.g., *Metrodorea*) (Engler 1931a; Pirani 1998; Groppo et al. 2012; Paschoalini et al. in prep.).

Lorts et al. (2008) studied fruit evolution and dispersal in angiosperms and showed that there is a lack of phylogenetic constraint across major lineages, resulting in the same type of fruit evolving independently within the families. Fleshy fruits dispersed by birds and mammals, which likely is the case of some *Homalolepis* as mentioned above, are considered an important adaptation molded by the selective force of the dispersal agents (Bremer and Eriksson 1992), and likely evolved many times throughout angiosperm history with a consistent association with higher seed mass. Bolmgren and Eriksson (2010) suggested that the changes in fruit type are not the main driver of changes in seed mass, and there is a necessity for studies of seed dispersal effectiveness where gape width, fruit size and seed mass are examined in relation to seed fall patterns and recruitment success. We also cannot ignore the role of frugivory and vegetation changes possibly affecting those transitions, evidencing the need for more integrated studies that can elucidate the drivers of macroevolutionary patterns of fruits.

5 Final remarks

Recent advances in phylogeny include explicit character state reconstructions using available molecular-based trees, contrasting with historical intuitive interpretations of evolutionary trends. However, we are aware that the macroevolutionary hypotheses generated in our study will remain mainly speculative as long as fundamental anatomical and developmental studies are scarce for most taxa of Simaroubaceae. As floral genetics "has set the stage for new investigations of the origin and diversification of the flower" (Soltis et al. 2009), increasing information related to genes known to affect floral features in model systems will certainly allow one to accurately explore floral development and evolution in non-model plants in the future. Studies gradually emerging can help to identify candidate genes for testing specific hypotheses like the ones presented herein.

This overview of morphological data of Simaroubaceae flowers in a comparative context constitutes an improvement to the knowledge of the family and related groups. Several of the homoplastic features identified here in Simaroubaceae are also widespread elsewhere in the remaining Sapindalean groups and in other core eudicots as well, probably related to similar reproductive functions. Among these, we highlight the various sexual systems, variable patterns of floral merism, synorganization of whorls including tubular corollas and androecia, occurrence of stamens with filament appendages of variable length and, at times, with partial connation and coherence forming a pseudotube, nectariferous disks and gynophores. A drupaceous, laterally flattened to lenticular fruits and free carpels are putative synapomorphies of the family retrieved in this study. Moreover, the general patterns of flower and fruit diversity in Simaroubaceae, and related evolutionary hypotheses generated herein, clearly indicate several aspects demanding further detailed structural, biological and functional investigation. Additionally, results from studies like these will certainly provide valuable subsidies to the macrosystematics and macroevolution of Sapindales.

Author contributions GGNA, LHMF, MFD, JHLO, DD and JRP conceived the study and performed writing—review and editing; GGNA, LHMF, DD and JRP contributed to methodology; GGNA, LHMF and JRP were involved in formal analysis and investigation; GGNA and JRP performed writing—original draft preparation and funding acquisition; DD and JRP supervised the study. All authors have read and approved the final version of the manuscript.

Declarations

Conflict of interest Diego Demarco is the editor-in-chief of the Brazilian Journal of Botany, and this article was entirely handled by an Associate Editor.

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