

Gisele Gomes Nogueira Alves

Estrutura e evolução floral de Simaroubaceae

Structure and floral evolution of Simaroubaceae

São Paulo, 2022

Gisele Gomes Nogueira Alves

Estrutura e evolução floral de Simaroubaceae

Structure and floral evolution of Simaroubaceae

Tese apresentada ao Instituto de
Biotecnologia da Universidade de São Paulo
Para obtenção do título de Doutor em Ciências,
Área de concentração em Botânica.

Orientador: Prof. Dr. José Rubens Pirani

Coorientador: Prof. Dr. Diego Demarco

São Paulo

2022

Alves, Gisele Gomes Nogueira

Estrutura e evolução floral de Simaroubaceae

118pp.

Tese (Doutorado) - Instituto de Biociências da Universidade de São Paulo.
Departamento de Botânica.

1. Estrutura floral; 2. Sapindales; 3. Simaroubaceae.

Prof(a). Dr(a).

Prof(a). Dr(a).

Prof(a). Dr(a).

Prof. Dr. José Rubens Pirani

Orientador

Dedico à minha terapeuta, Dra. Marina Araujo Soares.

“Yet each man kills the thing he loves

By each let this be heard

Some do it with a bitter look

Some with a flattering word

The coward does it with a kiss

The brave man with a sword”

Oscar Wilde

Agradecimentos

Gostaria de agradecer meu orientador, Prof. Dr. *José Rubens Pirani* (IB-USP) por ter me recebido como sua aluna, pela fantástica orientação desde a iniciação científica, pelo companheirismo e extrema dedicação ao nosso trabalho, pelo apoio nos momentos mais difíceis, broncas nos necessários, paciência e compreensão por toda esta árdua jornada e principalmente pela confiança em me designar um grupo de estudo tão extraordinário.

Agradeço ao *Instituto de Biociências da Universidade de São Paulo* (IB-USP) que me recebeu e que possibilitou o desenvolvimento deste trabalho. Agradeço também à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior: O presente trabalho foi realizado com apoio da CAPES, por meio da concessão de bolsa de estudos ao longo do doutorado, À Fundação de Amparo à Pesquisa do Estado de São Paulo, pelo o apoio financeiro do projeto Temático (FAPESP proc. nº #2014/18002-2), à International Association for Plant Taxonomy (IAPT), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; proc. #307665/2015-6) que possibilitaram a compra de materiais de laboratório, atividades de coleta em campo (essenciais para o desenvolvimento deste trabalho) e permitiram minha participação no curso de verão “*Oak Spring eFLOWER Summer School*”, sediado por “*Oak Spring Garden Foundation*” em Upperville, Virgínia (18-28/09/2018) e posteriormente a participação de parte deste trabalho na forma de apresentação oral no Simpósio Internacional “*Plant anatomy: traditions and perspectives*”, sediado pela “*Lomonosov State University*” em Moscou, Rússia (16-22/09/2019).

Agradeço meu coorientador, Prof. Dr. *Diego Demarco* (IB-USP) do laboratório de anatomia vegetal do IBUSP, que me acolheu e acompanhou na desafiadora jornada dos estudos anatômicos deste grupo desde o mestrado, sanando todas as minhas dúvidas técnicas e teóricas, além do grande entusiasmo com nosso estudo.

Em especial, agradeço a professora *Juliana Hanna Leite El Ottra*, cuja dedicação e apoio nos estudos deste grupo só foram superados pelo vasto conhecimento e encantamento que ela possui pelos estudos anatômicos florais. Foi um privilégio ter sua coautoria em todos os meus trabalhos e definitivamente o apoio que me deu proporcionou força para superar as dificuldades que acompanham estudos estruturais e anatômicos.

Ao colaborador Dr. *Luiz Henrique Fonseca* cujo magnífico auxílio com as análises, imensa expertise no assunto, parceria e amizade enriqueceram meu trabalho e jornada pessoal no doutorado.

Ao colaborador Dr. *Marcelo Fernando Devecchi*, pela parceria, pelo auxílio constante no laboratório, com as coletas, e claro, por compartilhar comigo a paixão e entusiasmo pelas Simaroubaceae.

Agradeço também à minha grande amiga e colaboradora *Raquel Koch* pelo grande auxílio nos cortes histológicos na reta final deste trabalho. Sem seu impecável trabalho técnico eu não teria conseguido!

À minha família do Laboratório de Sistemática Vegetal (IB-USP):

Aos professores *Renato Mello-Silva* (in memoriam), *Paulo T. Sano* e *Lúcia G. Lohmann* pelos ensinamentos, ótimas conversas e convivência. Aos técnicos deste mesmo laboratório *Adriana Marchiori*, *Viviane Jono*, *Roberta Figueiredo*, *Abel R. Cangussu*, *Eliane Cortazzo* e *José Vitório* e os queridos colegas *Andressa Cabral*, *Annelise Frazão*, *Augusto Giaretta*, *Beatriz Gomes*, *Bruno Bravo*, *Camila Monje*, *Carolina Siniscalchi*, *Caroline Andrino*, *Cíntia Luz*, *Danilo Zavatin*, *Eduardo Leal*, *Eduardo Lozano*, *Erik Kataoka*, *Guilherme Antar*, *Guilherme Paschoalini*, *Herison de Oliveira*, *Ítalo*, *Jenifer Lopes*, *Jéssica Francisco*, *John Lirio*, *Juan Pablo*, *Juliana Lovo*, *Lucas Vilela*, *Luana Sauthier*, *Luisa Lucrecia*, *Luiz Henrique*, *Marcelo Devecchi*, *Marcelo Kubo*, *Marco Pellegrini*, *Maila Beyer*, *Matheus Colli*, *Matheus Matias*, *Mirian Antoniceli*, *Pamela Santana*, *Paulo Baleeiro*, *Paulo Gonella*, *Raquel Pizarro*, *Rebeca Gama*, *Rebeca Viana*, *Renato Magri*, *Renato Ramos*, *Roberto Baptista*, *Sandra Reinales*, *Thais Vasconcelos*.

Aos professores do Laboratório de Anatomia Vegetal (IB-USP): *Veronica Angyalossy*, *Gregório Ceccantini*, *Nanuza Luiza de Menezes* e *Gladys Flávia Melo-de-Pinna*, e os técnicos *Gisele R. O. Costa*, *Tássia C. Santos* pela paciência e auxílio em minha jornada de aprendizado neste laboratório, e também aos queridos colegas *Bruno Edson*, *Caian Souza*, *Elisabeth Tölke*, *Ellenhise Ribeiro*, *Erika Maximo*, *Israel Neto*, *Karina Gagliardi*, *Luíza Teixeira*, *Marcelo Pace*, *Maria Camila Medina*, *Mariana Monteiro*, *Mariana Victório*, *Natalie Capelli*, *Rafael da Silva Cruz*, *Raquel Koch* e *Thália Gama* pela ótima convivência, por me acolherem de forma tão atenciosa, pela amizade e laços incríveis que criamos.

Aos professores de minha banca de qualificação *Gladys Flávia Melo-de-Pinna*, *Gregório Ceccantini* e *Juliana Hanna Leite El Ottra* (IB-USP) pelas correções e sugestões ao projeto, bem como carinho e paciência com meus problemas pessoais na ocasião.

Ao meu namorado *Allan Bruno Sendas*, que embora não entenda a profunda paixão que sentia por este trabalho, sempre me apoiou, trabalhou ao meu lado, contribuiu muito com os resultados desta tese e me segurou em cada queda.

Por último e definitivamente *mais importante* aos meus pais *Luiz Tadeu Alves* e *Sonia GN Alves*; por sempre terem me apoiado, por entenderem minha atípica escolha de carreira e principalmente, por se orgulharem de mim quando nem eu mesma pude fazê-lo. Pelo amor incondicional e maravilhoso exemplo de vida que são. Eu não poderia ter sorte maior em minha vida por terem me escolhido para ser sua filha e agradeço todos os dias por este privilégio fantástico.

Resumo	ii
Abstract	iii
General introduction	04
1. The Order Sapindales	05
2. Simaroubaceae family: a historical overview	06
3. What do we know today about the Simaroubaceae representatives: general descriptions	08
4. Objectives	22
5. Thesis general structure	22
References	24
Chapter I What reproductive traits tell us about the evolution and diversification of the three-of-heaven family, Simaroubaceae	27
Supplementary material	59
Chapter II Floral structure of Simaroubaceae with special reference to variations in androecium and nectaries	68
References	113
Final considerations	117

Simaroubaceae é uma família monofilética da ordem Sapindales, com distribuição pantropical. Embora congregue apenas 23 gêneros e cerca de 117 espécies, seus representantes apresentam uma ampla diversidade morfológica, que levou a muitas controvérsias a sua classificação desde o século XIX. Até hoje as informações sobre representantes de Simaroubaceae são limitadas, com baixa representatividade de vários de seus gêneros em coleções de herbários e flagrante carência de dados fundamentais sobre sua morfologia e anatomia.

Sapindales é uma ordem composta por nove famílias, sendo Rutaceae e Meliaceae os grupos provavelmente mais próximos de Simaroubaceae. Variações relacionadas a caracteres como sexualidade, graus de fusão ou conação entre os órgãos florais, entre outros, são temas comuns nas discussões apresentadas nos estudos estruturais mais aprofundados na ordem. Nesse contexto, os padrões morfológicos já evidenciados em Simaroubaceae na última década têm contribuído para aprimorar as descrições disponíveis sobre seus representantes, e mostram-se muito relevantes para o entendimento da evolução e diversificação das linhagens de Sapindales.

Nesta tese apresentamos primeiramente uma investigação da evolução das características reprodutivas de Simaroubaceae sob uma perspectiva morfológica, combinando dados originais obtidos neste estudo e outros advindos da literatura, na tentativa de elucidar quais caracteres reprodutivos são mais informativos para sistemática do grupo e de sua história evolutiva. Por fim, apresentamos um estudo detalhado sobre a estrutura floral (morfologia, anatomia e histologia) de diversos gêneros da família, com enfoque no androceu e estruturas nectaríferas. Neste estudo, desvendamos novos caracteres para o androceu do grupo, bem como descrevemos de forma inédita as variações dos alongamentos do eixo floral e estruturas nectaríferas associadas. Fornecemos descrições, ilustrações, e diagramas das estruturas descritas, elucidando e atualizando o conhecimento sobre essas flores, bem como discutindo seu provável papel na biologia floral e na evolução do grupo.

Simaroubaceae is a monophyletic family of Sapindales, with pantropical distribution. Although presenting only 23 genera and about 117 species, their representatives present a wide diversity in their morphological characters, which has led to a wide variety of controversies over its classification since the 19th century. To date, information on Simaroubaceae is limited, with low representativity of several of its genera in herbarium collections and a clear lack of fundamental data on its morphology and anatomy.

Sapindales is an order composed by nine families, with Rutaceae and Meliaceae being the closest groups to Simaroubaceae. Variations related to characters such as sexuality, degrees of fusion or conation between floral organs, among others, are common themes in research presenting more in-depth structural studies in the order. In this context, the morphological patterns already evidenced in Simaroubaceae in the last decade have contributed to improve the available descriptions of their representatives, and are very relevant for the understanding of the evolution and diversification of Sapindales lineages.

In this thesis we firstly present an investigation of the evolution of the reproductive features of Simaroubaceae from a morphological perspective, combining original data from this study as well as from the literature, in an attempt to elucidate which reproductive characters are more informative for the systematics and evolutionary history of the group. Finally, we present a detailed study on the floral structure (morphology, anatomy and histology) of several genera of the family, with focus on the androecium and nectariferous structures. In this study novel androecium features were found for the group, as well as variations of the stalk-like elongations of the floral axis and associated nectary were newly described. We provide descriptions, illustrations, and diagrams of the described structures, elucidating and updating the knowledge about these flowers, as well as discussing their putative role in the biology and evolution of the group.

1. The Order Sapindales

Sapindales is a diverse order of Eudicots-Rosids-Malvids clade (APG IV 2016), currently comprising nine monophyletic families (Anacardiaceae, Biebersteiniaceae, Burseraceae, Kirkiaceae, Meliaceae, Nitrariaceae, Rutaceae, Sapindaceae and Simaroubaceae) with approximately 6570 spp. (Stevens, 2001; Muellner-Riehl *et al.* 2016). Sapindales monophyly is supported by macromolecular synapomorphies, as well as by some morphological features as compound pinnate leaves (occasionally palmate, uni or trifoliolate) and flowers with a well-developed nectariferous disk (Judd. *et al.* 2008, Simpson 2010). The members of this order are known for their chemical characteristics and economic potential, in addition to their high morphological diversity (Demarco *et al.* 2022).

The current available phylogeny of the order (Fig. 1) shows Rutaceae, Meliaceae and Simaroubaceae as a clade, although lacking resolution between their relationships. Those three families share limoids and quassoids, uncommon compounds in other Angiosperms (Gadek *et al.* 1996, Kubitzki 2011, Kubitzki & Gottlieb 1984). Burseraceae and Anacardiaceae are sister groups that share a significant number of morphological and anatomical characters, mainly floral (Gadek *et al.* 1996, Kubitzki 2011). Simaroubaceae, Rutaceae and Meliaceae also share some structural characteristics and the literature has great availability of morphological and anatomical data on Rutaceae, while the other two families need more studies.

Floral structural studies, the main focus of the present work, are of great importance for analyses at a specific, generic level, and in higher taxonomic hierarchies, as they can provide a valuable contribution to their circumscriptions, in addition to improve our understanding about the evolution of these groups and their relationships, allowing more robust phylogenetic and diversification hypotheses.

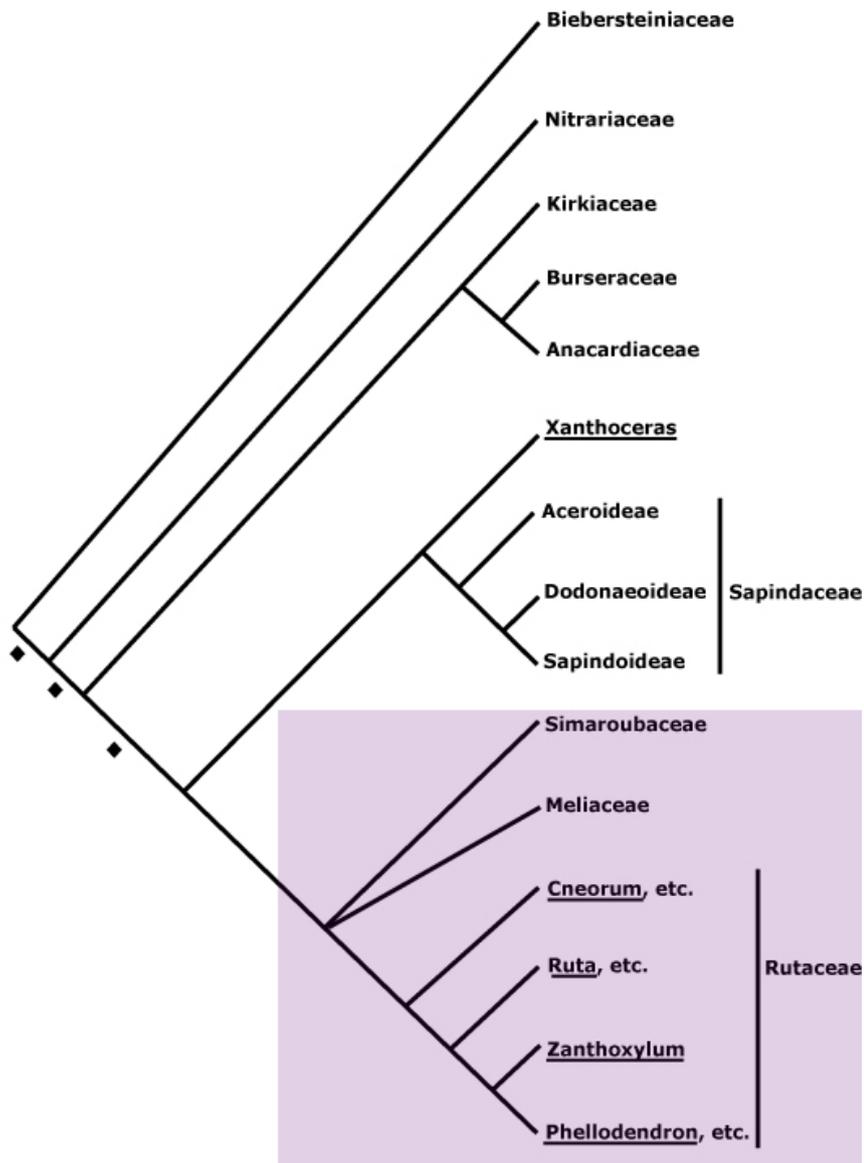


Fig. 1. Phylogenetic relationships between Sapindales representatives. Simaroubaceae+Rutaceae+Meliaceae relationship highlighted (APGweb, 2022).

2. Simaroubaceae: a brief historical overview

Simaroubaceae are a small family belonging to the Sapindales order. Currently composed by around 22-23 genera and 117-120 species (Devecchi & Pirani, 2020, Pirani *et al* 2021), they are distributed across the tropics. The circumscription of the family and the phylogenetic relationships within it were clarified after structural and macromolecular analyzes revealed a monophyletic group, much smaller than that of traditional classifications, essentially including only the members of Engler's subfamily Simarouboideae. The family is

now supported mainly by the occurrence of quassinoids, which are exclusive chemical substances (Fernando *et al.* 1995; Clayton *et al.* 2007), and the remaining Engler's subfamilies were excluded. The main reason that a molecular circumscription was definitive to the taxonomy of this family is basically explained on account of the remarkable morphological diversity of its representatives, which lack a single structural character shared by all its genera that is not present in related families.

After the global level monograph carried by Engler (1931), the most comprehensive treatment of the group was provided by Clayton (2011), including an updated key to the genera and their description. Currently Simaroubaceae can be characterized by bitter substances in the bark (triterpenoids and quassinoids); pinnate to imparipinnate leaves; filaments often with basal appendages, and by free or slightly united carpels (at the base and/or at the styles). Recently, Pirani *et al.* (2021) presented a complete overview of the family with focus on its American taxa, where 22 genera and approximately 120 species are considered, providing a fresh and broad view of the history, taxonomy, phylogeny, morphology and anatomy, floral biology, palynology, chromosome numbers, chemistry, biogeography and ethnobotanical data available for the group.

Even with a high economical and medicinal potential, the representatives of Simaroubaceae have been object of few detailed morphological and anatomical studies. As many other families in Sapindales, Simaroubaceae present varied types of secretory glands, fusion and connation in various degrees on the floral parts, and a very interesting variation in floral merism and cryptic sexual systems. In the last decades floral structure studies improved the knowledge about structure features and provide new information, especially about the sexual systems in this group. Nair & Joshi (1958) discussed the many variations in these flowers from dialisepaly to gamopetaly, bisexual flowers to unisexual, diplostemony to haplostemony, apocarpny to syncarpny and a possible reduction to floral merism. Alves *et al.* (2017) studied the floral structure of the genus *Homalolepis* (still placed within *Simaba* at the time, which was later recircumscribed by Devecchi *et al.* 2018) and showed that its flowers, usually considered as bisexual, are functionally unisexual, with abortion of the ovules. In fact, flowers of several other genera of Simaroubaceae present organ reduction or abortion to vestigial reproductive parts. Since there is general lack of detailed structural studies in the family, we decided to undertake a review of all information available about the general morphology and anatomy of its flowers, aiming to achieve a most possible complete

overview, along with an evolutionary analysis contributing to a better understanding of this underexplored lineage of Sapindales.

3. What do we know today about Simaroubaceae representatives: general descriptions

We initiate our studies with an investigation of the traditional knowledge available for the 22 genera of Simaroubaceae in the literature and summarized it below. The taxonomic circumscriptions and morphological descriptions were updated according to the most recent treatments of the family (Clayton 2011, and Pirani *et al.* 2021).

***Ailanthus* Desf.**

Ailanthus Desf. Mém. Acad. Sci. Paris 1786: 265-271.; Fl. Males. I, 6:215-220 (1962); Fl. Chi. 11:51 100-102.

Dioecious plants. Trees that can reach up to 60 m. With eight species from China, India, SE Asia and North Australia and Turkestan, this genus presents large leaves, imparipinnate or paripinnate, with a long rachis bearing several leaflets that are opposite to subopposite, oblique, petiolulate, entire to coarsely toothed and often with large abaxial glands near the base. The flowers are borne in axillary thyrsoid inflorescences; with sepals 5(6) basally connate, rarely cupuliform; petals 5(6) with induplicate-valvate aestivation; stamens 10, in staminate flowers inserted below the outer margin of the disk; reduced pistillodes in staminate flowers while in pistillate flowers the staminodes can have a relatively normal size (but lacking pollen grains) or reduced/vestigial staminodes; the glandular disk is annular with lobes; styles 2-5 free or connate; stigma elongate and divergent; carpels 2-5 free or slightly connate only at base, each of which develops into a winged mericarp; the mericarps are samaroid with a single, centrally positioned seed surrounded by a narrowly elliptic wing.

Although there are references to unisexual and bisexual flowers for this group, Noteboom (1962) and Hu (1979) comment that the probability of these observations are actually from staminodes very similar to stamens is very high, being the dioecy the most likely sexual state. Additionally, we observed that is very common than the flowers defined as “perfect” (bisexual) have a much smaller gynoeceum when compared to a pistillate flower,

being a pistillode the most probable structure visualized in these flowers. Because of the lack of detailed studies, we consider this genus as dioecious.

Ailanthus is a genus with a very high invasive ability, being considered dangerous and invasive taxa on North America and Europe.

***Amaroria* A.Gray**

Amaroria A.Gray, Bot. U. St. Expl. Exped. 1:356, t. 40 (1854); Fl. Vit. Nova 3:480-487 (1985).

Dioecious plants. Trees that can reach up to 20 m. Only one species is known, endemic of Fiji (*Amaroria soulameoides* A.Gray), with leaves alternate, simple, usually congested near apices of branchlets, long-petiolulate. The flowers are borne in axillary thyrses; sepals 4-5 (rarely a sixth partially or completely developed) basally connate; petals 4-5 free, with imbricate aestivation; stamens 8-10 (rarely 9 or 11, partially developed); staminate flowers may present vestigial pistillodes and a intrastaminal semi globose rounded or slightly depressed at the apex disk with conspicuous lobes; filaments terete, carnose, slightly narrowed at apex, the inner filaments surrounded by the deeper disk clefts, the outer filaments affixed in the shallower disk crenations; anthers dorsifixed near base, dehiscent extrorse-laterally; pedicels are longer than the pistillate flowers; pistillate flowers with 8-10 staminodes semi-immersed on the crenations of a cupuliform disk; carpel 1, the ovary sessile within the disk; stigma sessile, carnose, reniform. Fruits laterally-flattened, drupaceous.

Amaroria leaves are almost identical to *Soulamea* Lam. except by the longer petioles in the former and by being strictly dioecious. Although described as a monoecious or dioecious taxon, Smith (1985) commented on Flora Vitensis that with additional material available it would be possible to define a new generic and specific description for this species.

As many Simaroubaceae genera the most detailed studies in *Amaroria* are chemical analyzes, given the medicinal potential of the family representatives, which emphasizes the need for morphological and anatomical studies.

***Brucea* J.F. Mill.**

Brucea J.F. Mill., Icon. t. 25 (1779-1780), nom. cons.; Backer Fl. Batavia 259 (1907); Fl. Vit. Nova 3:486-487 (1985); Fl. Males. I, 6:209-212 (1692); Fl. Chi. 11: 103-104 (2008).

Monoecious or dioecious plants. Shrubs or small trees that can reach up to 12 m. With c. seven species from tropical Africa to tropical and subtropical Asia and northern Australia, the leaves are imparipinnate and leaflets opposite, petiolulate to sessile with obliquely to lanceolate blades entire or crenate-serrate and spot-like glands along the margin situated under the teeth (if present). The flowers are in axillary thyrses; sepals 4 basally connate; petals 4 free with imbricate aestivation; stamens 4 with short filaments inserted between the lobes under the outer margin of the thick lobed disk; anthers cordate or ovate; staminate flowers with rudimentary and lacking styles ovaries; pistillate flowers with short or rudimentary staminodes; disk cupuliform, lobed; carpels 4 free; ovule 1; styles free or basally coherent, recurved, short; stigma elongate and divergent or claviform; fruits drupaceous ellipsoid or ovoids.

Brucea has apparently 4-merous flowers, but Backer (1907) mentioned a rare occurrence of 5-merous flowers in *Brucea javanica*. Although this is possible since the majority of Simaroubaceae genera show a high occurrence of variations in floral merism, the lack of further observations of 5-merous flowers and the anatomical studies (Nair & Joshi, 1958) showing a tetramery without traces of a fifth petal makes us to keep *Brucea* for now consistently 4-merous. The sexuality also has not sufficient information to show whether the unisexual flowers are distributed on the same individual or in distinct individuals, although our observations in herbarium collections and images repositories show the probability that these plants can be monoecious or dioecious.

***Castela* Turpin**

Castela Turpin, Ann.Mus.Natl.Hist.Nat. 7:78 (1860), nom.cons.; J. Arnold Arb. 25:122-128 (1944), rev.; Fl. Illu. Cat. (R. Reitz, ed.) p5-9 (1997).

Dioecious plants. Shrubs or small trees erect or stiff spiny that can reach up to 5 m. With c. 14 species from northeast Argentina, Uruguay, Paraguay, south of Brazil and Bolivia, north Texas to Mexico, Antilles, Colombia, Venezuela, Caribbean and Galapagos islands, the leaves are small, alternate, simple, entire or serrate, occasionally scale-form or deciduous. The flowers are solitary, clustered in leaf axils, or in axillary fascicles; sepals 4(5) basally connate; petals 4(5) free, with imbricate aestivation; stamens 8(10), anthers dorsifixed; staminate flowers with gynoeceum reduced to pistillodes; pistillate flowers with very reduced staminodes; intrastaminal annular disk with conspicuous lobes; carpels 4 (rarely more) free,

sitting on the disk united only by the coherent styles; stigma elongate and divergent; ovule 1; fruits (sub)globose drupaceous.

Holacantha A.Gray, which was considered separated from *Castela* on account of being leafless, is currently placed as a synonym (see Majure *et al.* 2021; Pirani *et al.* 2021).

***Eurycoma* Jack**

Eurycoma Jack, Fl. Males. I, 6:203-206 (1692); Reinwardtia 14(2): 259-263 (2015).

Dioecious plants. Treelets or rarely shrubs that can reach up to 10 m. With three species from tropical SE Asia, Sumatra, Malay Peninsula Borneo and S. Philippines, the leaves are spiral imparipinnate or paripinnate condensed on top; leaflets opposite to subopposite, elliptic to lanceolate with very short petiolule. The flowers are borne in axillary thyrsoids; sepals 5(6) basally connate; petals 5(6) with induplicate-valvate aestivation; stamen 5(6); staminate flowers always with a sterile pistil. Stamens episepalous, filaments narrowing to the top with very small ligulate appendages on the base; occasionally there is a second row of even smaller and entire staminodes outside the stamens; stamens and staminodes can be connate with the base of the petals; disk inconspicuous; carpels 5(6) free, the styles coherent; stigma peltate 5(6) lobed to elongate; ovule 1; fruits ellipsoid ovoid slightly bicarinate drupaceous.

Eurycoma has considerable economic value on Malaya Peninsula given its many purported medicinal uses and local beliefs. Despite its popularity there are few studies approaching a deeper knowledge of your structure. *Eurycoma* flowers, such as in *Ailanthus* and other genera of the family, have records of bisexual flowers and the possibility of monoecious plants. However, it appears to be the same confusion about the staminodes present in female flowers and the scarce analyzed material, giving the imprecision of bisexual flowers and the definition of polygamous in various descriptions of these species.

***Gymnostemon* Aubrév. and Pellegr.**

Gymnostemon Aubrév. and Pellegr., Bull. Soc. Bot. France 84:2, 181-184 (1937).

Polygamous plants. Trees that can reach up to 30 m. With only one specie endemic to Ivory Coast, the leafs are alternate, imparipinnate; leaflets opposite to subopposite, subsessile, oblong with asymmetric base and marked by a few submarginal glands, regularly spaced at

the top. The flowers are in terminal or axillary thyrsoids; sepals 5 united up to three quarter of calyx; petals 5 free, with imbricate aestivation; stamens 10 with filaments enlarged at the base, without appendages; nectariferous disk lobed; carpels 5 free, styles coherent; stigma capitate; ovule 1; fruits drupaceous ovoid.

The only species, *Gymnostemon zaizou* Aubrév. & Pellegr. has very scanty record in herbarium collections, as well as few studies and information available. Its occurrence restricted to western Africa and its use as a locally commercial hardwood plant put this taxa on the IUCN list as an endangered species under the category vulnerable (IUCN, 2022).

***Hannoa* Planch.**

Hannoa Planch. London J. Bot. 5:566 (1846). Hooker's Icon. Pl. 13:44-45 (1877-79); Blumea 11:519 (1962).

Polygamous plants. Large trees that can reach up to 50 m. With seven species in tropical Africa, the leaves are alternate, imparipinnate; leaflets opposite, oblong-obovate with punctate adaxial glands near base or towards the margin and framed glandular apex. The flowers are borne in terminal or axillary thyrsoids; sepals 5 basally connate (often with calyx rupturing into 2-3 irregular lobes); petals 5 free, with imbricate aestivation; stamens 10 with flat filaments and hairy basal appendages and broadly elliptic anthers; disk fleshy (occasionally with gynoeceium sunken within); staminate flowers with rudimentary carpels; pistillate flowers with the pistil only at the base surrounded by the disk or the ovary is sessile on the disk; carpels 5 free, styles coherent; stigma with conspicuous lobes; ovule 1; fruits drupaceous mericarps, ellipsoid or ovoid, slightly bicarinate.

***Homalolepis* Turcz.**

Homalolepis Turcz. Bot. Jour. Linn. Soc. 183: 162-176 (2016); Magnolia Press, Phytotaxa 366: 108 pp. (2018).

Polygamous plants. Trees, treelets or shrubs that can reach up to 30 m. With 28 species distributed in the extra-Amazonian South America, northern Colombia and Venezuela, the leaves are alternate and spirally arranged, imparipinnate or occasional

paripinnate and very variable in size; leaflets opposite to subopposite with laminar glands often on both surfaces and at the apex. The flowers are produced in axillary, terminal or seldom cauliflorous thyrses or thyrsoids inflorescences. Anatomical studies show that the flowers can be bisexual or functionally unisexual (with abortive gynoecium); sepals (4)-5-(6) basally connate or with union up to three quarters; petals (4)-5-(6) free, with contorted aestivation; stamens (8)-10-(12), each filament with an hairy appendage dorsiventrally flattened that vary in degree of coherence to each other by intertwining trichomes being able to form a pseudotube; gynophore entirely nectariferous; carpels (4)-5-(6) free from each other but shortly connate at base and at the apex; ovary lies on top of a conspicuous gynophore with a surface entirely nectariferous; stigma punctiform or lobed; ovule 1. The fruits have drupaceous mericarps, ovoid or subovoid.

Homalolepis is a recently resurrected genus and the largest in the family. The recircumscription of the group include two sections: *Homalolepis* sect. *Homalolepis* and *Homalolepis* sect. *Grandiflorae* (Devecchi *et al.* 2018), the latter with one of the largest flowers buds in the family, up to 3 cm long. Plants of this genus were previously considered as bisexual, but may bear also functionally unisexual flowers (Alves *et al.* 2017) besides the prevailing bisexual ones.

Iridosma Aubrév. & Pellegr.

Iridosma Aubrév. & Pellegr., Bull. Soc. Bot. France 102:7-8, 328-331 (1955); Fl. Gabon 3:47 (1962).

Hermaphroditic plants. Trees. With only one specie endemic from Gabon, the leaves are alternate, imparipinnate; leaflets subsessile with articulate base opposite to subopposite and oblong. The flowers are borne in determinate thyrses; sepals irregularly lobate, hirsute; petals 5(8) with valvate aestivation; stamens 12-13 with long filaments and hairy appendages at the base, small and elliptic anthers; disk with strong indument; carpels 4, free and hairy; style glabrous, united; stigma with 4 conspicuous lobes. Fruit unknown.

Iridosma is rare genus. The only species, *Iridosma letestui* (Pellegr.) Aubrév. & Pellegr. is known only by its type and very few samples. The description with valvate aestivation definitely established doubt since that character would only occur in these flowers in the whole family.

***Laumoniera* Noot.**

Laumoniera Noot. *Blumea* 32:383 (1987); *Rewardia* 10(5): 471 (1988).

Dioecious plants. Small trees that can reach up to 16 m. With only one specie from Malasia, the leaves are paripinnate, petiole shriveled at base; leaflets slightly ovate, acuminate with nearly rounded base and entire margin. The flowers are borne in axillary sparsely pubescent thyrses, dark purple red in vivo; sepals 4, pubescent, connate at base; petals 4, less hairy than sepals; staminodes 4, reduced with hairy anthers, alternating with petals; disk fleshy, slightly hairy; carpels 4, free; stigma 4 connate, slightly 4-lobed and separated by a groove above the partition of the carpels; fruits yellow in vivo, ellipsoids when dried with thin wrinkled mesocarp and hard, smooth thin endocarp.

Laumoniera is a controversial monospecific genus: Noteboom (1962) described one species and pointed that the material was closely related to *Brucea*, differentiating on the paripinnate leaves (*Brucea* has imparipinnate leaves) and the connate stigma covering the top of the ovary, almost discoid. The only species, *Laumoniera bruceadelpha* Noot. was described based on a specimen sent by Dr. Y. Laumonier to Dr. H. P. Noteboom, who described the new genus. In contrast, in 1988 Kostermans A.J.G.H. published a note after analyze samples sent also by Dr. Laumonier, pointing that the characters selected to distinguish the taxa should be discarded, such as fruit size and shape of stigma (not characters of generic value on this author opinion). He also described the proportion of 50-50 of imparipinnate and paripinnate leaves and the presence of tiny glands on the lower surface (not described by Noteboom) that would not be opposed to those of *Brucea*. In this paper the species is placed as *Brucea bruceadelpha* (Noteboom) Kostermans, a new combination to this taxa.

Only female flowers are known, and these species have a scarce collection and studies. Besides the valuable points on the possibility of *Laumoniera* is better placed on *Brucea* genus, we cannot be sure until this specimen is included in phylogenetic studies.

***Leitneria* Chapm.**

Leitneria Chapm. *Bull. Torrey Bot. Club.* 167(3):173-193. (1940). *Acta Phytotax. Geobot.* 63(2):57-62 (2013); *Castanea*, 76(3): 313-338.

Polygamous plants. Shrubs or small trees that can reach up to 6 m. With only one specie distributed in low, wet areas in SE United States, the leaves are unifoliate, entire. The flowers are borne in axillary erect catkin-like inflorescences, which is very distinctive from the others Simaroubaceae inflorescence types, with scale-like bracts spirally arranged; very reduced, the staminate flowers do not have a perianth and this can be vestigial in pistillate flowers; stamens 3-5 per flower with short filaments; pistillate flowers consists in a simple pistil surrounded at the base by very reduced small tepals; carpel 1; stigma is a single branch distally expanded; Fruit is a laterally flattened drupe.

Leitneria has a controversial history; it was previously recognized in its own family Leitneriaceae, based on its quite peculiar morphological features. Molecular data (Fernando & Quinn, 1995; Clayton *et al.* 2007) support its placement within Simaroubaceae.

***Nothospondias* Engl.**

Nothospondias Eng. Beit.Fl.Afr.36:216-217 (1905); Cat. Pl. Oban. Pp23 (1913); Bull. Jard. Bot. Etat Brux. 30:105-109 (1960).

Polygamous plants. Trees that can reach up to 25 m. With only one specie distributed in the tropical west of Africa, the leaves are imparipinnate grouped at the end of the branches; leaflets opposite to alternate, oblong to oblong-ovate. The flowers are borne in axillary thyrsoids; sepals 4 basally united up to three quarters of the calyx; petals 4 with contorted aestivation; stamens 8 with ellipsoid anthers; bisexual flowers with pistil ovoid; functionally males flowers described, with a reduced gynoeceium; disk 4-lobed; carpels 4 free; style simple; stigma claviform in bisexual flowers; fruit drupaceous ovoid-ellipsoid.

Nothospondias was placed by Engler (1876) in Anacardiaceae family, closed to *Spondiopsis* Engl. and *Spondias* L. However, Vand der Veken (1960) show that the gynoeceium was composed by 4 free carpels of which only 1-2 develop, proposing the inclusion of this genera in Simaroubaceae.

There are many inconsistencies on the descriptions of these taxa: Backer, E.G. (1913) mentioned that a short stipe (torus) was observed at the top of which the petals are inserted (what would have gone unnoticed by Engler) and this stipe, certainly not present in all flowers examined would be a inconstant character, such as the 4-lobed disk, which he do not find. In 1960, Van der Vaken, P. published a review of all information available in *Nothospondias*. In this study he mentioned that the 4-cell syncarpic ovary with one style described by Engler is

actually an oblong-obovoid disk over an aborted gynoecium; the aestivation is contorted and not imbricate and a well-developed gynophore are characters supporting the placement of this taxon in Simaroubaceae.

Even that *Nothospondias* is polygamous, pistillate flowers were never observed, bisexual flowers were only observed by Engler and the functionally male flowers are the most reported, so it is very clear that we do not have enough information to establish the dimension of these inconstant characters. In this study we based our decisions on analyses of all information available in the literature and illustrations of these taxa.

Nothospondias staudtii is on IUCN list as an endangered species under the category vulnerable (IUCN, 2022).

***Odyendea* (Pierre) Eng.**

Odyendea (Pierre) Bull. Mens. Soc. Linn. Paris, 2:101-106 (1889-1897); Engl. In Engl. & Prantl, Nat. Pflanzen-fam. III. 4:215 (1896); Aubrév. & Pellegr., Fl. Gabon 3:33-52 (1962);

Hermaphroditic plants. Trees that can reach up to 30 m. With only one species endemic to Cameroon and Gabon, the leaves are imparipinnate; leaflets opposite oblong, entire, with truncated or slightly notched apex, wedge-shaped or rounded at base. The flowers are borne in thyrsoids; sepals 4(5) united up to three quarters of its total length; petals 4(5) free with imbricate aestivation; stamens 8(10) with appendages at the base of the filaments; gynophore in pillow form 8-furrowed; carpels 4 united at base; styles fused in one; stigma punctiform or with elongate and divergent stigmatic branches; fruit is a very large drupaceous mericarp ellipsoid, strongly carinate.

Odyendea has only one species, *Odyendea gabunensis* Engl. and very little information and illustrations. The original description is from Engler (1931) and most of its known data is only from this study and in Flore du Gabon (Aubréville, 1962). Although Engler mentioned the presence of a nectary disk, the illustration of this flower shows a possible gynophore elevating the gynoecium. The stigma type also is mentioned as an elongate and divergent stigmatic branches, but is also capitate (without conspicuous lobes) in this illustration. Unfortunately is not easy to confirm whether both data are correct mostly because of the scarce collected material of this plant.

***Perriera* Courchet**

Perriera Courchet Bull. Soc. Bot. Fr. 52:281-284 (1905); Fl. de Madag. 105:1-4 (1950); Capuron, R. Adansonia 1:84-88 (1961).

Polygamous plants. Trees that can reach up to 30 m. With two species endemic to Madagascar, the leaves are imparipinnate; leaflets with short petiolule, opposite to subopposite, entire, with punctate glands regularly spaced toward apex adaxially. The flowers are borne in axillary thyrsoids; sepals (4)5(6) basally connate; petals (4)5(6) free with induplicate-valvate aestivation; stamen (8)10(12) with filaments larger and hairy at the base; the reduced gynoecium in some samples indicate functionally male flowers; annular disk with conspicuous lobes; carpels (1-)2-3, united only at the base; styles completely free from each other after the flower open; stigma elongate and divergent; fruit ovoid drupaceous.

Picrasma Blume

Picrasma Blume Cronquist, Brittonia 5:138-143 (1944); Fl. China 11: 102-102 (2008); Willdenowia 49: 187-191 (2019).

Polygamous plants. Trees or shrubs that can reach up to 20 m. With nine species distributed in SE Asia, South America and Caribbean Islands the leaves are imparipinnate, base of petiole of petiolule often dilated; leaflets opposite to subopposite, entire or serrate-crenate. The flowers are in axillary thyrsoids; sepals 4(5) free or basally connate; petals 4(5) free, commonly persistent until fruiting with valvate aestivation; stamens 4(5); staminate flowers with vestigial gynoecium; nectary disk with conspicuous lobes; carpels 2-5 free (united only at the apex of the ovary); styles united at base; stigma elongate and divergent; pistillate flowers with 4-5 staminodes sitting on the disk; fruit globose drupaceous.

Picrasma was formerly described as a dioecious genus. In 2019 Monzón *et al.* described a new species from NE coast of Cuba, with bisexual flowers among unisexual ones. Currently, polygamy is the most appropriate definition for the sexuality of this genus, although more studies are needed to confirm the recent information.

Picrolemma Hook.f.

Picrolemma Hook.f. Benth. & Hook., Gen. Pl. 1:312 (1862); Cronquist, Brittonia 5:143-144 (1944).

Dioecious plants. Shrubs that can reach up to 6 m. With two species from Peru and Brazil the leaves are imparipinnate; leaflets opposite to alternate, entire with punctate glands

associated with the secondary venation underneath. The flowers are borne in terminal thyrsoids; sepals (4)5 basally connate; sepals (4)5 free with imbricate aestivation; stamens (4)5 with large anthers and short filaments; staminate flowers with vestigial ovaries and no apparent nectary disk; pistillate flowers with staminodes very reduced and ovaries elevated by a gynophore with several distinct carpels, each bearing a single ovule and style; stigma discoid; Fruit ellipsoid drupaceous.

Cronquist (1944) described the stigma in *Picrolemma* as flattened capitate. We considered it as discoid based on illustrations of pistillate flowers.

Pierreodendron Engl.

Pierreodendron Engl. *Mannia* Hook.f. *Gen.Pl.* 1:309 (1862); *Fl. Gabon* 3:43-44 (1962);

Polygamous plants. Trees that can reach up to 15 m. With two species distributed on Tropical Africa (Ivory Coast, Nigeria to Angola and Congo) the leaves are large and imparipinnate; leaflets opposite to subopposite, oblong-elliptics, rounded and asymmetrical at base, rounded at apex with short and tick abrupt acumen. The flowers are borne in terminal or axillary thyrsoids; sepals 5 united up to three quarters of the calix; petals 5 free with imbricate or contorted aestivation. Stamens 10-18, red, with large oblong anthers as long as or little longer than the filaments, which have hairy appendages; annular disk glabrous, sometimes with gynoeceium sunken within; pistillodes reduced; carpels 5 distinct, style simple; stigma with elongate and divergent stigmatic branches or discoid; fruit a ellipsoid drupaceous.

Pierreodendron is a genus with very few available data. Compiling the bibliography we came to the conclusion that there is a great chance of the flowers analyzed by Engler, Aubréville and Black could be both bisexual and functionally male, once there are mentions about the gynoeceium be sunken in the disk and not, or the style have a shorter size. Noteboom (1962) illustrated two types of gynoeceium forms were a reduced gynoeceium is evident leading us to considerate polygamous flowers as a possible state.

Quassia L.

Quassia L. *Gen.Pl.* 1:308 (1862); Baillon *Adansonia*, 8:89-90 (1867-1868); Engl. In Engl. & Prantl, *Nat. Pflanzenfam.*, 2 edn, 19a:337-379 (1931); Cronquist, *Brittonia* 5:145-146 (1944).

Hermaphroditic plants. Shrubs or small trees that can reach up to 8 m. With two species (one in the Neotropics and the other in West Africa) the leaves are imparipinnate, rachis and petiole conspicuously winged in *Q. amara* L. and narrowly winged or wingless in *Q. africana* Baill., articulate; leaflets opposite, sessile and entire with punctate glands towards leaf apex adaxially. The flowers are borne in terminal or axillary thyrsoids or racemes; sepals 4-6 joined at very base or apparently distinct; petals 4-6 distinct, not spreading at anthesis and with contorted aestivation; stamens 8-12 with long slender filaments with very short and hairy appendages at the base; carpels 5, weakly united above a large gynophore; style simple, long and slender; stigma capitate; fruit ellipsoid carinate drupaceous.

Quassia amara has one of the largest flower in the family and a very peculiar tubular form of the corolla; the flowers can finish anthesis presenting different arrangements of the petals; some keep the petals connivent and both androecium and gynoecium remain united until senescence and others have a spiraled arrangement and the whole androecium and gynoecium are free in the center of the flower. At the time of anthesis all the anthers remain closed (Barata *et al.* 2002).

***Samadera* Gaertn.**

Samadera Gaert., Fruct. 2:352, (1971); *Adansonia* 1:83-86 (1961).

Hermaphroditic plants. Trees that can occasionally reach up to 20 m. With five to six species distributed in Madagascar, Indochinese Union, Se Asia and Australia the leaves are unifoliolate, entire, glabrous with scattered punctate glands. The flowers are borne in axillary rounded to corymbiform cymoid inflorescence; sepals (3)4(5) with union up to three quarters of the calyx with rounded lobes; petals (3)4(5) free with imbricate or contorted aestivation; stamens (6)8(10) filaments with appendages at the base; gynophore cylindrical; carpels 3-5 free, style united; stigma capitate; fruit a ellipsoid flattened drupaceous.

***Simaba* Aub.**

Simaba Aubl. Cronquist, *Lyodia* 7:81-92 (1944); *Mol. Phylogenetics Evol.* 120: 63-83 (2018).

Polygamous or hermaphroditic plants. Trees and shrubs that can reach up to 10 m. With 10 species distributed in the Amazonian area of Tropical South America, the leaves are pinnate; leaflets opposite, ovate to obovate; laminas glands only adaxially at the base and at

the apex, petiolules can be winged or flattened. The flowers are borne in axillary or terminal botryoid inflorescences; the flowers can be bisexual or functionally unisexual; sepals (3)(4)5(6) free or basally connate; petals (3)(4)5(6) distinct with contorted aestivation; stamens (6)(8)10(12) each filament with a hairy appendage dorsiventrally flattened that vary in degree of coherence to each other by intertwining trichomes being able to form a pseudotube; staminodes very reduced at the base of the gynophore, which is usually a terete with a undulate ring; carpels 3-6 united at the base and the apex of the ovary; stigma lobed or elongate and divergent; fruits are drupaceous mericarps, globose, ovoid or subovoid.

Simaba was formerly considered as the largest genus of the family. Devecchi *et al.* (2018) undertook a phylogenetic analysis and reestablished *Homalolepis* as a separate genus, containing all the extra-amazonian species previously treated as *Simaba*.

***Simarouba* Aubl.**

Simarouba Aubl.; Hist. Pl. Guiane: 859 (1775); Gen. Pl. 1:309 (1862); Cronquist, Bull. Torrey Bot. Club, 71:226-234 (1944), rev.

Dioecious plants. Trees or shrubs that can reach up to 35 m. With six species distributed in South and North America and Caribbean Islands, the leaves are paripinnate or imparipinnate; leaflets alternate or subopposite with punctiform glands scattered on upper surface, more so towards apex. The flowers are borne in the flowers are in terminal thyrsoids inflorescences; sepals (4)5(6) basally connate; petals (4)5(6) distinct with imbricate or contorted aestivation; stamens (8)10(12) with short, hairy appendages at the base; staminate flowers with vestigial gynoecium; pistillate flowers with reduced staminodes; annular disk, short; carpels 4-6 free or weakly united; style connate; stigma elongate and divergent; fruit drupaceous obovoid.

Simarouba is a widely known genus having medicinal plants and commercial interest on the quality of their wood.

***Soulamea* Lam.**

Soulamea Lam. Encyc. 1: 449-450 (1783); Gen. Pl. 1:313-314 (1862); Fl. Males. I, 6:221-223 (1962).

Polygamous plants. Shrubs or small trees that can reach up to 15 m. With 13 species being one widespread in SE Asia and Polynesia, one endemic to Seychelles and eleven

4. Objectives

These thesis objectives are:

1. To compile and update the morphological and anatomical data of the representatives of Simaroubaceae, adding new structural, anatomical and histological studies of the reproductive parts of these plants.
2. Investigate the obtained data comparatively and evolutionarily aiming to obtain a deeper understanding on how the floral characters can be interpreted and how can they can be informative for the evolutionary history of the group.

To achieve these objectives two studies with different approaches were carried out during this Doctorate period, with specific objectives and other topics presented below

5. Thesis general structure

This thesis is composed by two chapters (in manuscript format) and a section with final considerations. The first chapter was published at the Brazilian Journal of Botany in January 2022. The second chapter was written to facilitate correction without formal journal formatting, which will be chosen by the author.

❖ Chapter 1. What reproductive traits tell us about the evolution and diversification of the tree-of-heaven family, Simaroubaceae

In this chapter we combined original data and a thorough review of the group literature aiming to investigate if the floral features can contribute to the evolution of the family and closed-related groups. We presented an overview of morphological data in a comparative context, highlighting the variability in sexual systems, patterns of synorganization whorls, nectariferous structures and general patterns of flower and fruit diversity. In this study we analyzed our data using Bayesian and Parsimony optimality criterion to reconstruct ancestral reproductive character states and 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 fattened to lenticular fruits, among other insights.

References

Alves GGN, El Ottra JHL, Devecchi MF, Demarco D, Pirani JR (2017) Structure of the flower of *Simaba* (Simaroubaceae) and its anatomical novelties. *Bot J Linn Soc* 183:162–176

APG 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 181: 1–20.

Barata F.C.A.; Carreira L.M.M.; Maués M.M. 2002. Biologia floral e morfologia polínica de *Q. amara* L. (Simaroubaceae). *Boletim Museu Paraense Emilio Goeldi sér. Bot.* 18 (1):119-156.

Clayton, J.W., Fernando, E.S., Soltis, P.S. & Soltis, D.E. 2007. Molecular phylogeny of the Tree-of-Heaven family (Simaroubaceae) based on chloroplast and nuclear markers. *International Journal of Plant Sciences* 168(9):1325-1339.

Clayton, J.W. 2011. Simaroubaceae. In: Kubitzki, K. (ed.) *The families and genera of vascular plants. Vol. X. Flowering plants. Eudicots: Sapindales, Cucurbitales, Myrtaceae.* Springer. Berlin. p. 408-423.

Cronquist, A. 1944. Studies in the Simaroubaceae. III. The genus *Simaba*. *Lloydia* 7(1):81-92.

Demarco D., Simões, A.O. & Pirani, J.R. (eds.) 2022. Editorial: Diversity and evolution of Neotropical Sapindales. *Brazilian Journal of Botany* 45: 1-4.

Devecchi MF, Pirani JR (2020) Flora do Espírito Santo: Simaroubaceae. *Rodriguésia* 71:e02942018

Devecchi MF, Thomas WW, Pirani JR (2018) Taxonomic revision of the neotropical genus *Homalolepis* Turcz. (Simaroubaceae). *Phytotaxa* 366:1–108

Engler, A. 1931. Simaroubaceae. In: Engler, A. & Prantl, K. (eds.) ed. 2. 19a. *Die natürlichen Pflanzenfamilien.* Engelmann. Leipzig. p. 359–405.

Fernando, E.S., Gadek, P.A. & Quinn, C.J. 1995. Simaroubaceae, an artificial construct: evidence from *rbcL* sequence variation. *American Journal of Botany* 82:92-103.

Fernando, E.S. & Quinn, C.J. 1995. Picramniaceae, a new family, and a recircumscription of Simaroubaceae. *Taxon* 44 (2):177-181.

Gadek, P.A., Fernando E.S., Quinn, C.J., Hoot, S.B., Terrazas, T., Sheahan, M C. & Chase, M.W. 1996. Sapindales: molecular delimitation and infraordinal groups. *American Journal of Botany* 83(6):802-811.

Hu SY (1979) *Ailanthus*. *Arnoldia* 39:29–50

IUCN. 2022. The IUCN Red List of Threatened species. Version 2022-1. <https://www.iucnredlist.org>. Accessed on September 2022.

Judd, W.S., Campbell, C.S., Kellogg, E.A; Stevens, P. F., Donoghue, M. J. 2008. *Plant Systematics. A phylogenetic approach*. Ed. 3. Sinauer Associates. Sunderland.

Kubitzki, K. & Gottlieb, O. 1984 Micromolecular patterns and the evolution and major classification of angiosperms. *Taxon*. 33:375–391.

Kubitzki, K. 2011. Introduction to Sapindales. In: Kubitzki, K. (ed.) *The families and genera of vascular plants. Flowering plants. Eudicots: Sapindales, Cucurbitales, Myrtaceae*. Springer Verlag, Berlin. p. 1–3.

Majure LC, Pham K, Clase T (2021) *Castela senticosa* (Simaroubaceae: Sapindales), a new species of the Greater Antillean clade endemic to Hispaniola. *Syst Bot* 45:237-249

Monzón A.N. & Gutiérrez P.A.G. 2019. *Picrasma pauciflora* (Simaroubaceae), a new specie from the NE coast of Cuba. *Willdenowia*. 49 (2):187-191

Muellner-Riehl NA, Clayton JW, Buerki S, Nauheimer L, Chiang Yu-Chung, Cody S, Pell, SK (2016) Molecular phylogenetics and molecular clock dating of Sapindales based on plastid *rbcL*, *atpB* and *trnL-trnF* DNA sequences. *Taxon* 65:1019–1036

Nair NC, Joshi RK (1958) Floral morphology of some members of the Simaroubaceae. *Bot Gaz* 120:88–99

Nooteboon, H.P. 1962. Generic delimitation in Simaroubaceae tribus Simaroubeae and a conspectus of the genus *Quassia* L. *Blumea* 11:509-528.

Pirani, J.R.; Majure, L.C.; Devecchi, M.F. 2021. Na updated account of Simaroubaceae with emphasis on American taxa. *Brazilian Journal of Botany*. 45, 201-221.

Simpson, M.G. 2010. *Plant systematics*. Ed. 2. Elsevier. Amsterdam.

Smith. A.C., 1985. *Flora Vitensis*. Nova 3:480-487.

Stevens, P.F. 2015. Angiosperm Phylogeny Website. Acesso em 20/01/2015.

Article published at Brazilian Journal of Botany in 04/01/2022. 45:367-397.
<https://doi.org/10.1007/s40415-021-00768-y>; Presented in this thesis with
authorization from the Botany Society of São Paulo.



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

Gisele Gomes Nogueira Alves¹ · Luiz Henrique Martins Fonseca^{1,2} · Marcelo Fernando Devecchi¹ · Juliana Hanna Leite El Ottra¹ · Diego Demarco¹ · José Rubens Pirani¹

Received: 21 July 2021 / Revised: 8 November 2021 / Accepted: 20 November 2021
© The Author(s), under exclusive licence to Botanical Society of Sao Paulo 2021

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 norriterpenoids, 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

✉ Gisele Gomes Nogueira Alves
giselealves@usp.br

¹ Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, São Paulo 05508-090, Brazil

² Department of Biology, Ghent University, Ghent, Belgium

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 dialysepal to gamopetal, bisexual (monoclinous) to unisexual (diclinous) flowers, diplostemony to haplostemony, apocarp 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 apocarp combined with a postgenital fusion of the apex of the carpels. They highlighted the implication of this phenomenon in the evolution of secondary apocarp 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
<i>Ailanthus altissimus</i> (Mill.) Swingle	Alves, G.G.N., 76—XII/2018	SPF
<i>Brucea antidysenterica</i> J.F.Mill.	Hort. Paris L. Netto. Abyssinia. R71159	R
<i>Castela coccinea</i> 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
<i>Castela tweedii</i> Planch.	Estevan, D.A., 238—VII/2003	SPF
	Hatschbach, G. 72,435—IX/2001	SPF
<i>Eurycoma longifolia</i> Jack	Mohd.S., 1515—III/1968	RB
<i>Hannoa klaineana</i> Pierre & Engl.	Dobois, J., 298—05/1958	R
<i>Homalolepis arenaria</i> 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
<i>Homalolepis bahiensis</i> (Morici.) Devecchi & Pirani	Jardim, J.G., 5831—IX/2010	SPF
<i>Homalolepis cedron</i> (Planch.) Devecchi & Pirani	Devecchi, M.F.,238—IX/2013	SPF
<i>Homalolepis cuneata</i> (A.St-Hil. & Tul.) Devecchi & Pirani	Devecchi, M.F.,189—VII/2013	SPF
	Devecchi, M.F.,191—VII/2013	SPF
<i>Homalolepis ferruginea</i> (A.St-Hil. & Tul.) Devecchi & Pirani	Devecchi, M.F.,306—VII/2014	SPF
<i>Homalolepis glabra</i> (Engl.) Devecchi & Pirani	Devecchi, M.F.,322—IX/2014	SPF
<i>Homalolepis intermedia</i> (Mansf.) Devecchi & Pirani	Thomas, W., 4301—X/1985	SPF
	Moresco, M.C., 124—X/2005	SPF
<i>Homalolepis insignis</i> (A.St-Hil. & Tul.) Devecchi & Pirani	Alves, G.G.N. 71, XII/2014	SPF
<i>Homalolepis paraensis</i> (Ducke) Devecchi & Pirani	Van der Werff, H., 18,028—VII/2003	SPF
<i>Homalolepis planaltina</i> Devecchi & Pirani	Devecchi, M.F.,320—IX/2014	SPF
<i>Homalolepis pohliana</i> (Boas) Devecchi & Pirani	Zappi, D.C., 4493—VII/2018	MG
<i>Homalolepis pumila</i> Devecchi & Pirani	Devecchi, M.F.,323—IX/2014	SPF
	Devecchi, M.F., 467—VII/2017	SPF
	Antar, G.M., 232—VIII/2013	SPF
<i>Homalolepis rigida</i> Devecchi	Devecchi,M.F.,308—VII/2014	SPF
<i>Homalolepis salubris</i> (Engl.) Devecchi & Pirani	Devecchi, M.F., 321—IX/2014	SPF
		SPF
<i>Homalolepis suffuticosa</i> (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
<i>Homalolepis trichilioides</i> (A.St-Hil. & Tul.) Devecchi & Pirani		
<i>Homalolepis warmingiana</i> (Engl.) Devecchi & Pirani	Devecchi, M.F.,432—XI/2016	SPF
<i>Picrasma crenata</i> (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
<i>Picrasma excelsa</i> (Sw.) Planch.	Plowman, T., 14,286—VII/1986	SPF
<i>Picrolemma sprucei</i> Hook.f.	Ribeiro, J.E.L.S., 988—VII/1993	SPF
	Cid. C.A.,665—VIII/1979	RB
<i>Quassia amara</i> L.	Frazão, A., 226—IX/2015	SPF
	Devecchi, M.F., 260—XII/2013	SPF

Table 1 (continued)

Taxa	Voucher	Herbarium
<i>Simaba guianensis</i> 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
<i>Simaba obovata</i> Spruce ex Engl.	Devecchi, M.F., 425—IV/2015	SPF
<i>Simaba orinocensis</i> Kunth	Devecchi, M.F., 422—IV/2015	SPF
	Devecchi, M.F., 423—IV/2015	SPF
<i>Simaba polyphylla</i> (Cavalcante)W.W.Thomas	Ribeiro, J.E.L.S.1924—IX/1997	SPF
<i>Simaba</i> new sp.	Assunção, P.A.C., 357—VI/1996	SPF
<i>Simarouba amara</i> 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
	Rizzo, J.A., 10,423—VIII/84	SPF
	Proença, C., 858—VII/1993	SPF
<i>Simarouba versicolor</i> A.St-Hil.	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), Maberley (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:	thyrses (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 *atpB*, *matK*, *rbcL*, and *trnL-F* (Clayton et al. 2007; Muellner-Riehl et al. 2016) and the nuclear region *PhyC* (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				
<i>Ailanthus</i>	1	1	3	1	2	2	1	4	0	0	1	1	0	1	0	4	0	2	0	1	3			
<i>Amaroria</i>	0	1	2	1	0	2	1	5	0	0	1	1	?	0	?	0	7	0	1	1				
<i>Brucea</i>	0	0	1	1	0	1	0	0	0	0	1	1	1	2	2	1	4	5	0	1	1			
<i>Castela</i>	4	1	2	1	0	2	1	5	0	0	1	1	0	2	1	0	4	0	1	1				
<i>Eurycoma</i>	1	2	2	1	2	2	0	1	2	1	1	0	1	1	0	4	0	0	1					
<i>Gymnostemon</i>	1	2	3	2	0	2	1	4	0	0	0	0	0	1	1	0	0	0	1	1				
<i>Hannoa</i>	1	2	2	1	2	1	4	1	0	0	1	0	1	1	0	1	0	1	1	1				
<i>Holacantha</i>	1	1	3	1	0	2	1	5	0	1	2	1	1	2	2	1	4	0	1	1				
<i>Homalolepis</i>	0	1	2	1	2	1	5	1	1	3	0	2	2	1	1	0	0	1	0	0	1			
<i>Leitneria</i>	6	2	?	?	?	?	?	1	0	0	0	0	?	2	?	?	6	0	0	1				
<i>Nothospondias</i>	1	2	1	2	1	1	1	3	0	0	0	1	0	1	0	0	5	1	0	1				
<i>Odyndea</i>	1	3	2	2	0	2	1	5	1	0	0	0	1	1	1	0	1	4	1	0	1			
<i>Perriera</i>	1	2	2	1	2	2	1	5	0	0	0	1	1	3	1	1	4	0	1	1				
<i>Picrasma</i>	1	2	2	0	1	3	2	0	1	0	0	1	1	0	1	3	2	1	4	0	1	1		
<i>Picrolemma</i>	1	1	2	2	0	2	0	1	0	0	1	0	0	1	0	0	3	0	1	0	1			
<i>Pierreodendron</i>	1	2	2	2	0	1	2	1	2	5	6	1	0	0	1	0	1	1	0	3	4	0	1	1
<i>Quassia</i>	1	5	3	2	0	1	2	1	4	1	0	0	0	0	1	1	0	0	1	0	1			
<i>Samadera</i>	3	3	0	2	0	1	0	2	1	2	5	1	0	0	0	0	1	1	0	0	1	0	1	
<i>Simaba</i>	7	2	2	0	1	2	1	2	5	1	3	1	0	2	1	1	0	1	4	1	0	1		
<i>Simarouba</i>	1	1	2	1	0	1	2	1	5	1	0	1	1	0	1	2	1	1	4	1	0	1		
<i>Soulamea</i>	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				
<i>Atalantia</i>	2	3	0	2	1	2	3	1	2	3	4	0	0	1	1	0	0	0	1	2				
<i>Cneorum</i>	3	2	0	1	0	0	1	2	0	0	?	0	0	0	0	1	3	1	1	1	4	2	0	0
<i>Correa</i>	3	3	1	2	3	2	1	3	0	0	0	0	2	1	1	0	0	0	1	0	1	0	1	5
<i>Murraya</i>	1	3	2	1	0	3	1	4	0	0	0	0	3	1	1	0	0	0	1	0	1	0	1	2
<i>Ruta</i>	1	3	2	1	0	2	1	3	4	0	0	0	0	3	1	1	0	0	0	1	0	1	0	4
<i>Zanthoxylum</i>	2	4	0	2	0	3	1	2	3	0	0	1	1	0	1	0	1	0	1	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				
<i>Aglaiia</i>	0	1	0	2	2	0	1	2	0	1	2	2	2	3	0	2	1	0	0	2	0	0	4	2
<i>Carapa</i>	0	0	2	1	2	1	5	1	2	2	2	2	3	3	1	0	3	0	1	4				
<i>Cedrela</i>	0	0	2	1	2	0	1	0	0	2	2	3	1	1	0	3	2	0	4					
<i>Lepidotrichilia</i>	0	2	3	2	3	2	1	4	1	2	0	0	3	1	1	0	0	0	0					
<i>Melia</i>	1	2	3	1	2	1	5	1	2	0	0	3	1	1	0	0	1	0	1	0				
<i>Swietenia</i>	0	0	2	2	1	2	1	5	1	2	2	2	3	3	1	0	3	0	1	4				
<i>Trichilia</i>	1	2	0	2	0	3	0	1	4	0	0	1	3	1	1	0	0	2	0	1	4			

We included 252 sequences available for *atpB*, 77 sequences for *matK*, 71 sequences for *PhyC*, 252 sequences for *rbcL*, and 211 sequences for *trnL-trnF*. 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 *atpB*, *rbcL* and *trnL-F* and Clayton

et al. (2007) for *matK* and *PhyC*. 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

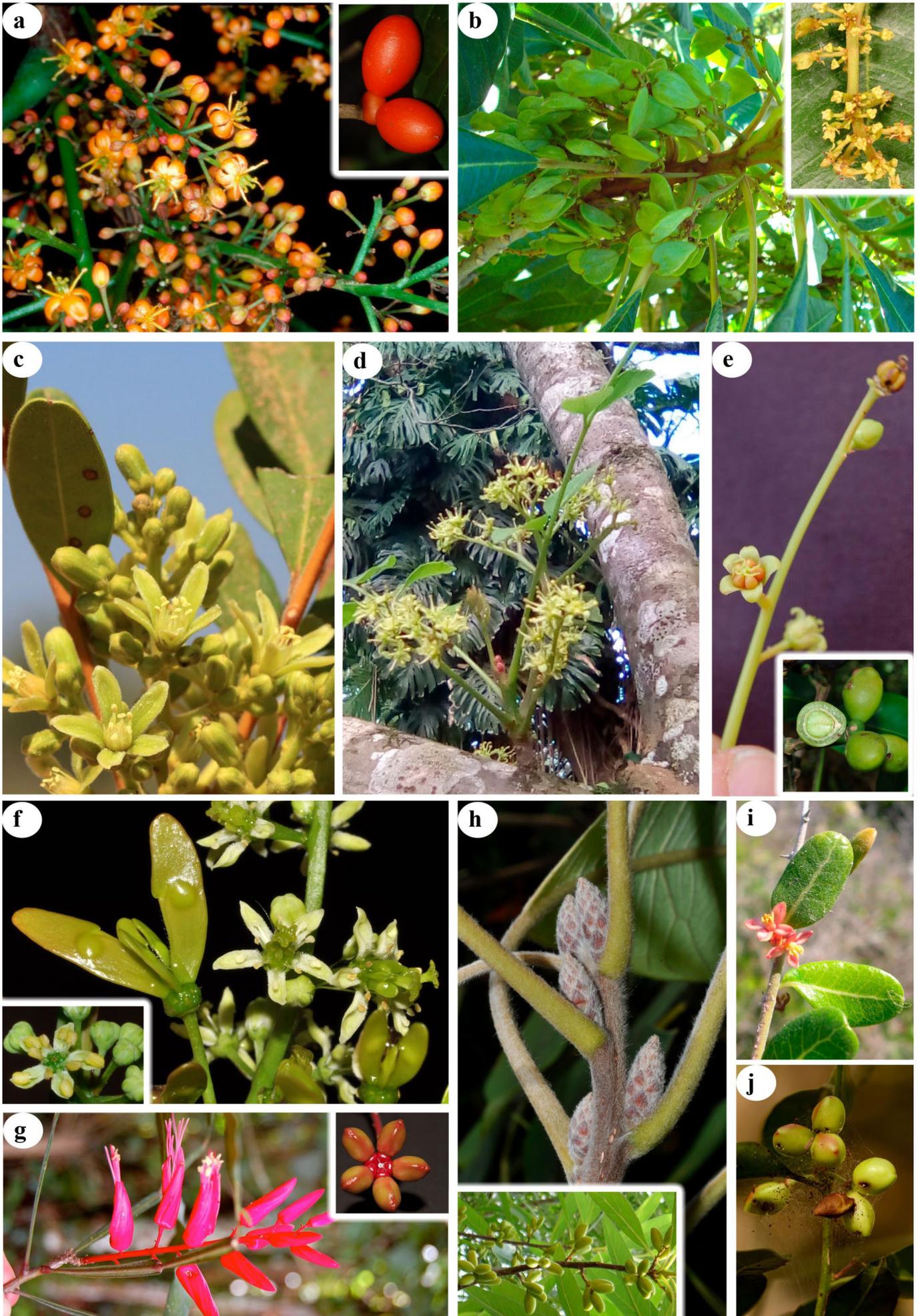


Fig. 1 Morphological diversity in Simaroubaceae. **a** Flowers of *Picrolemma sprucei* in a thyrsoid inflorescence. Laterally flattened drupaceous fruitlets of *Picrolemma* sp. (inset). **b** Samaras of *Soulamea amara*. Flowers of *Soulamea amara* in a thyrsoid inflorescence (inset). **c** Flowers of *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: William 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 *Gymnostemon*. 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 gynoeceum but have abortive ovules.

Carpel union (Fig. 10c). The Simaroubaceae ancestor most likely featured a gynoeceum 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

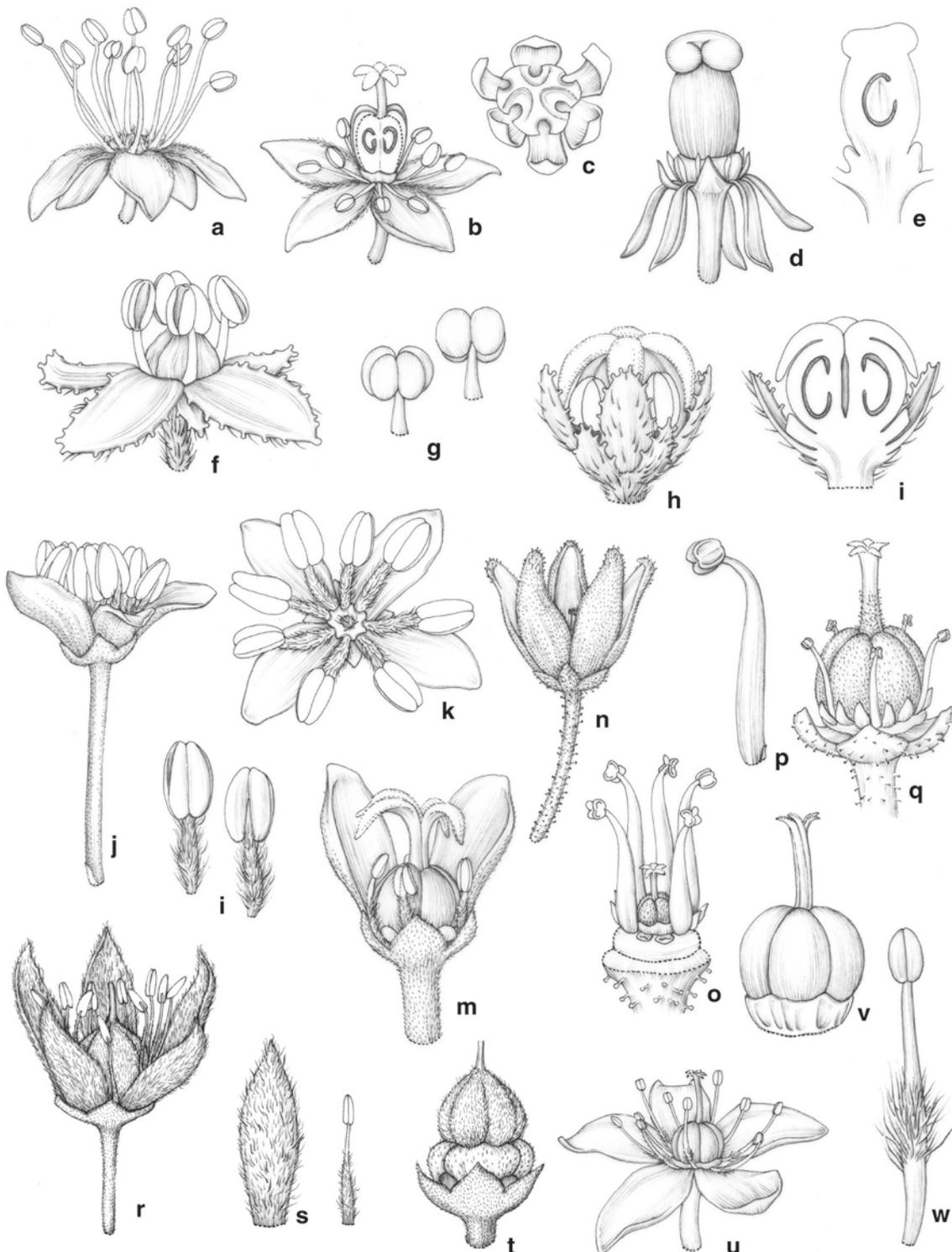


Fig. 2 Illustrations of Simaroubaceae flowers. **a–b** *Ailanthus altissima*. **c–d** *Amaroria soulameoides*. **f–i** *Brucea javanica*. **j–m** *Castela tweedii*. **n–q** *Eurycoma longifolia*. **r–t** *Gymnostemon zaizou*. **u–w** *Hannoa klaineana*. **a** Male flower. **b** Female flower with a longitudinal 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)

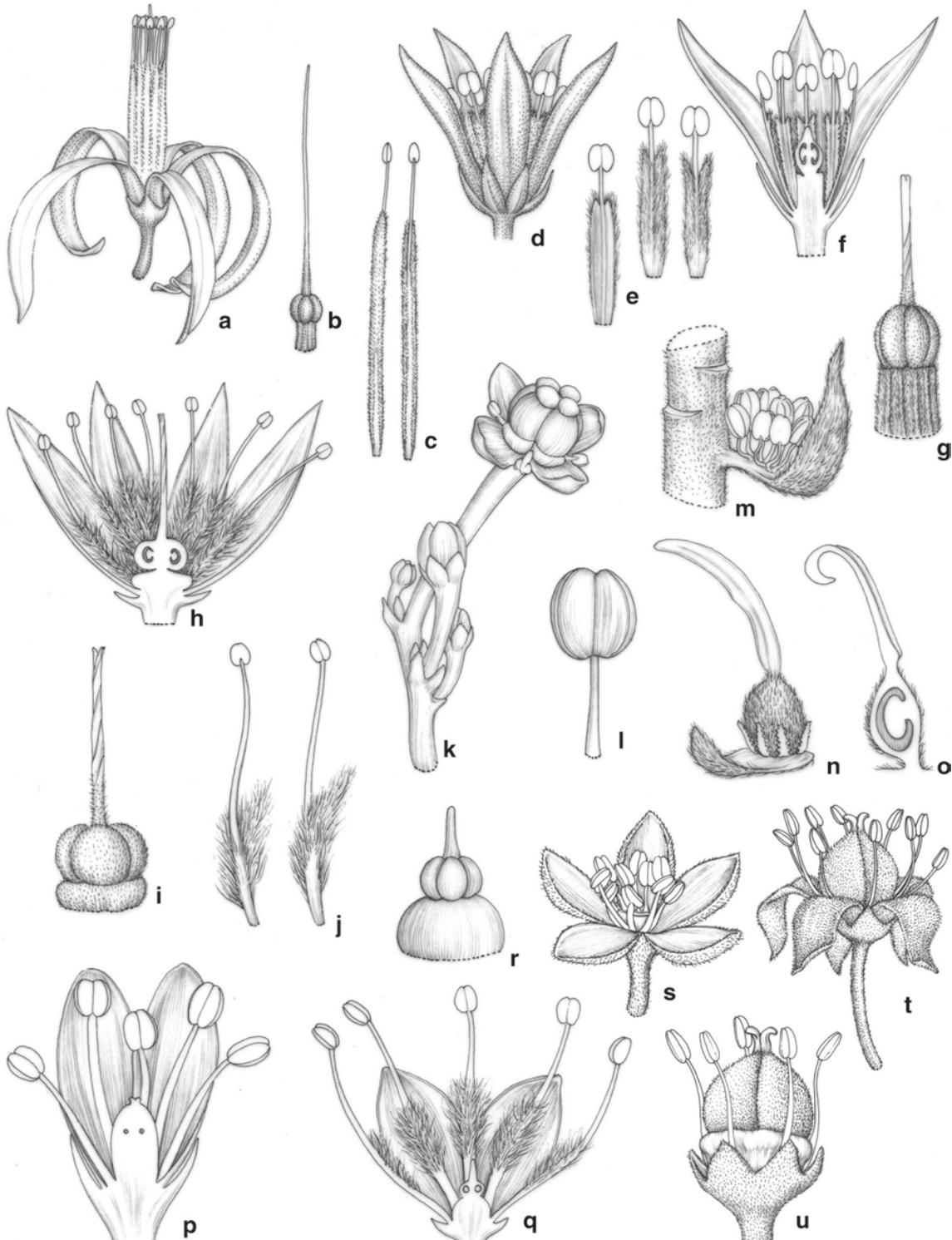


Fig. 3 Illustrations of Simaroubaceae flowers. **a–c** *Homalolepis cedron*. **d–g** *Homalolepis glabra*. **h–j** *Iridosma letestui*. **k–l** *Laumoniera brucea-delpha*. **m–o** *Leitneria floridana*. **p** *Nothospondias staudtii*. **q–r** *Odyendea gabonensis*. **s–u** *Perriera orientalis* Capuron. **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)

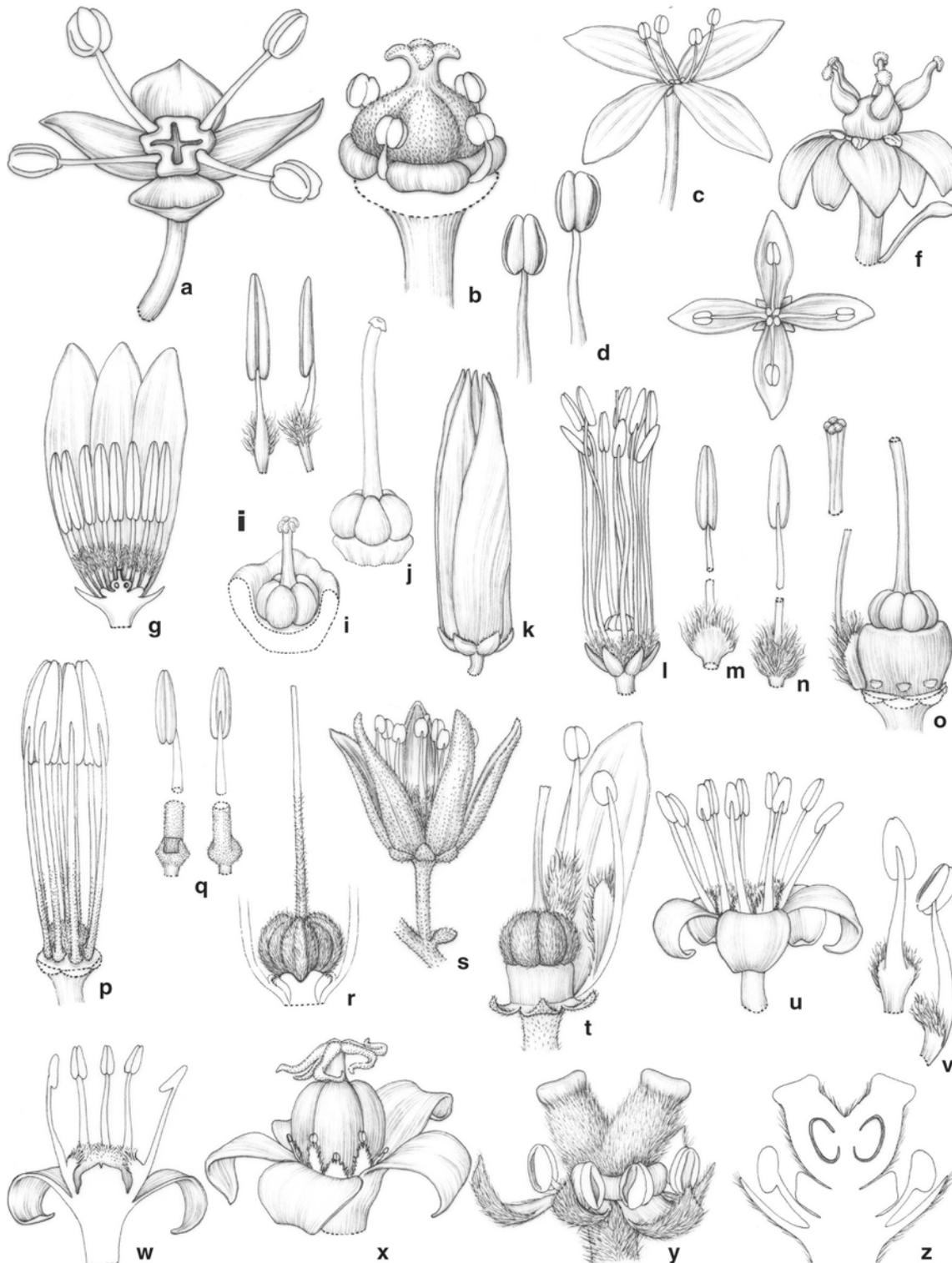


Fig. 4 Illustrations of Simaroubaceae flowers. **a–b** *Picrasma javanica*. **c–f** *Picrolemma sprucei*. **g–j** *Pierreodendron africanum*. **k–o** *Quassia amara*. **p–r** *Samadera indica*. **s–t** *Simaba guinensis*. **u–x** *Simarouba amara*. **y–z** *Soulamea amara*. **a** Male flower. **b** Female 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)

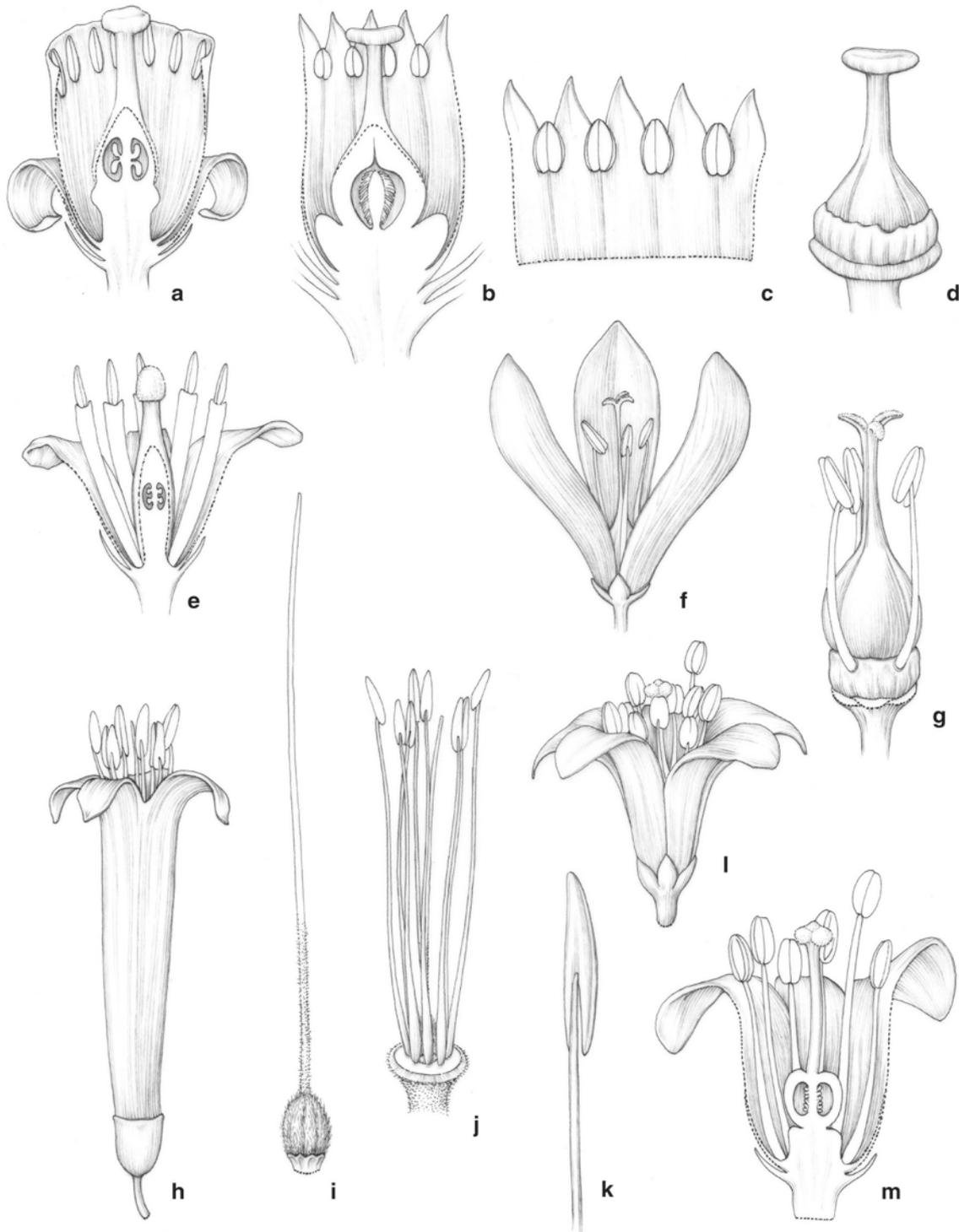


Fig. 5 Illustrations of Meliaceae and Rutaceae flowers. **a–c** *Swietenia mahagoni*. **d–e** *Trichilia clausenii*. **e–f** *Cneorum tricoccon*. **g–j** *Correa speciosa*. **k–l** *Murraya paniculata*. **a** General longitudinal view of the flower. **b** Staminal tube. **c** Gynoecium. **d** General 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)

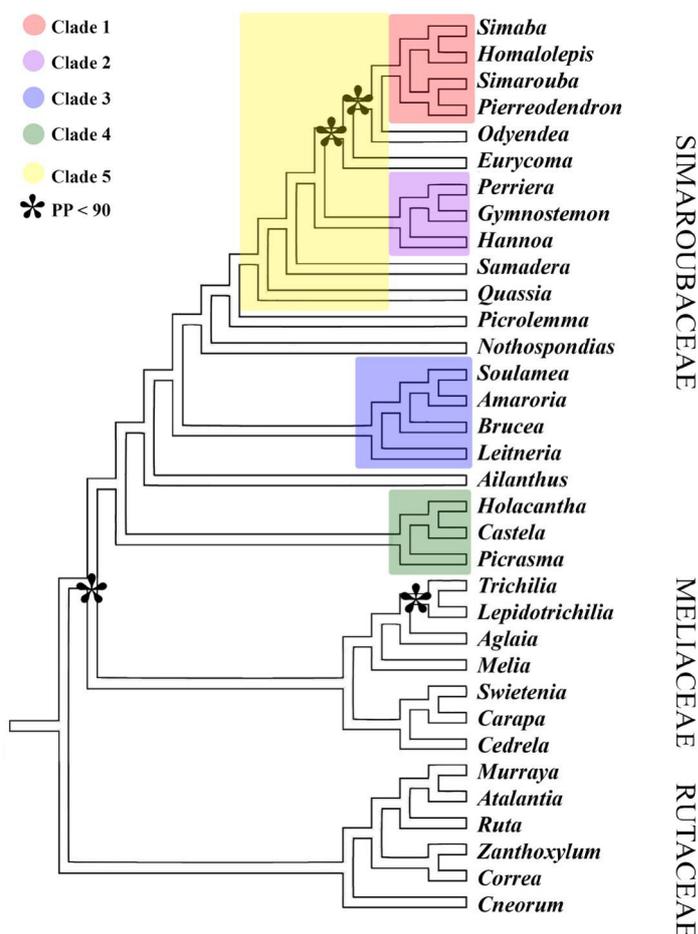


Fig. 6 Consensus phylogenetic tree of Simaroubaceae genera and outgroups. See text for details

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 apocarpous

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 gynoeceum 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.

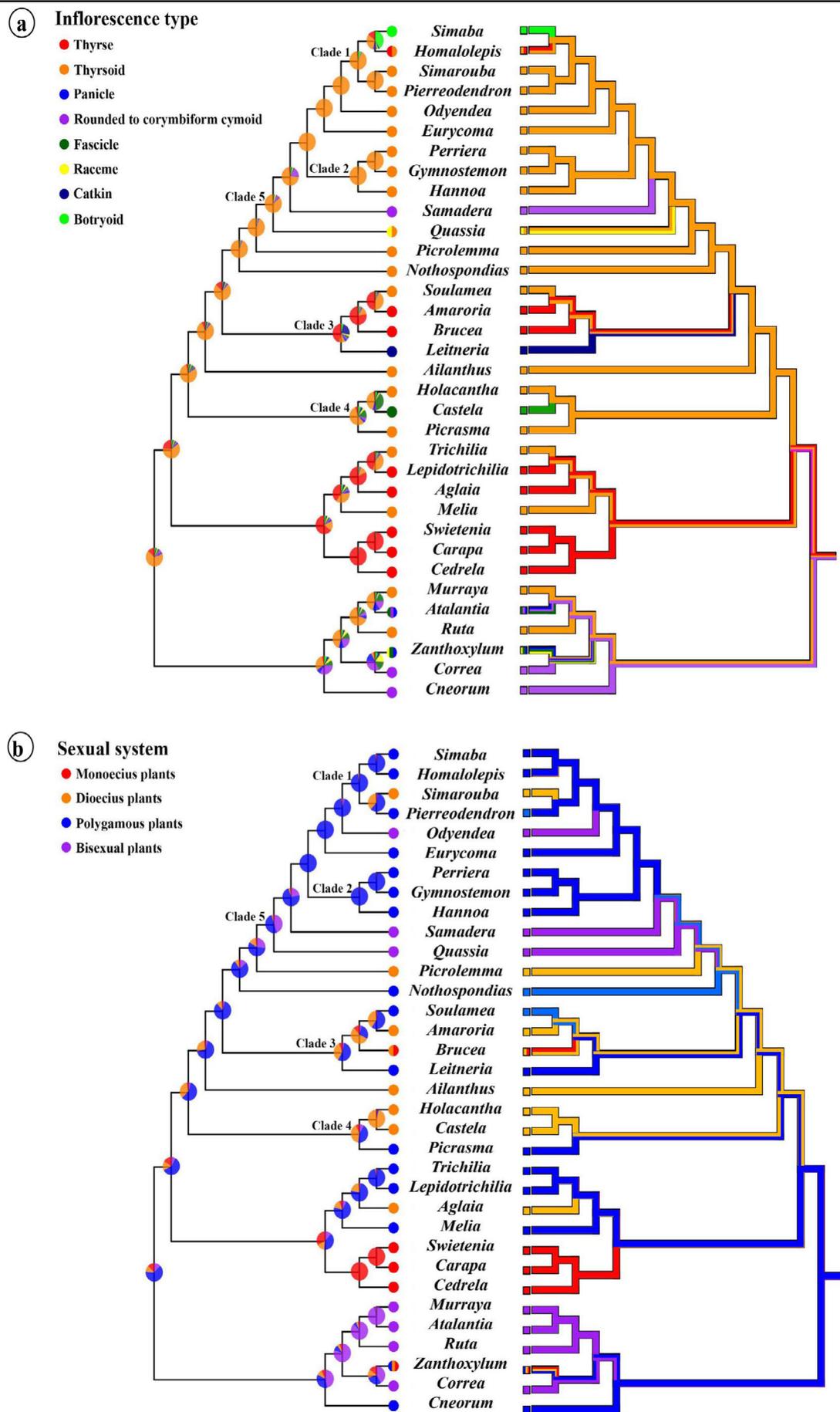
Intrastaminal disk (Fig. 12a). Our reconstruction shows that the probable ancestral character state of all three families is not clear in the focal nodes, although intrastaminal disks are present in several genera. The absence of this structure is the most likely ancestral state from (*Nothospondias*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

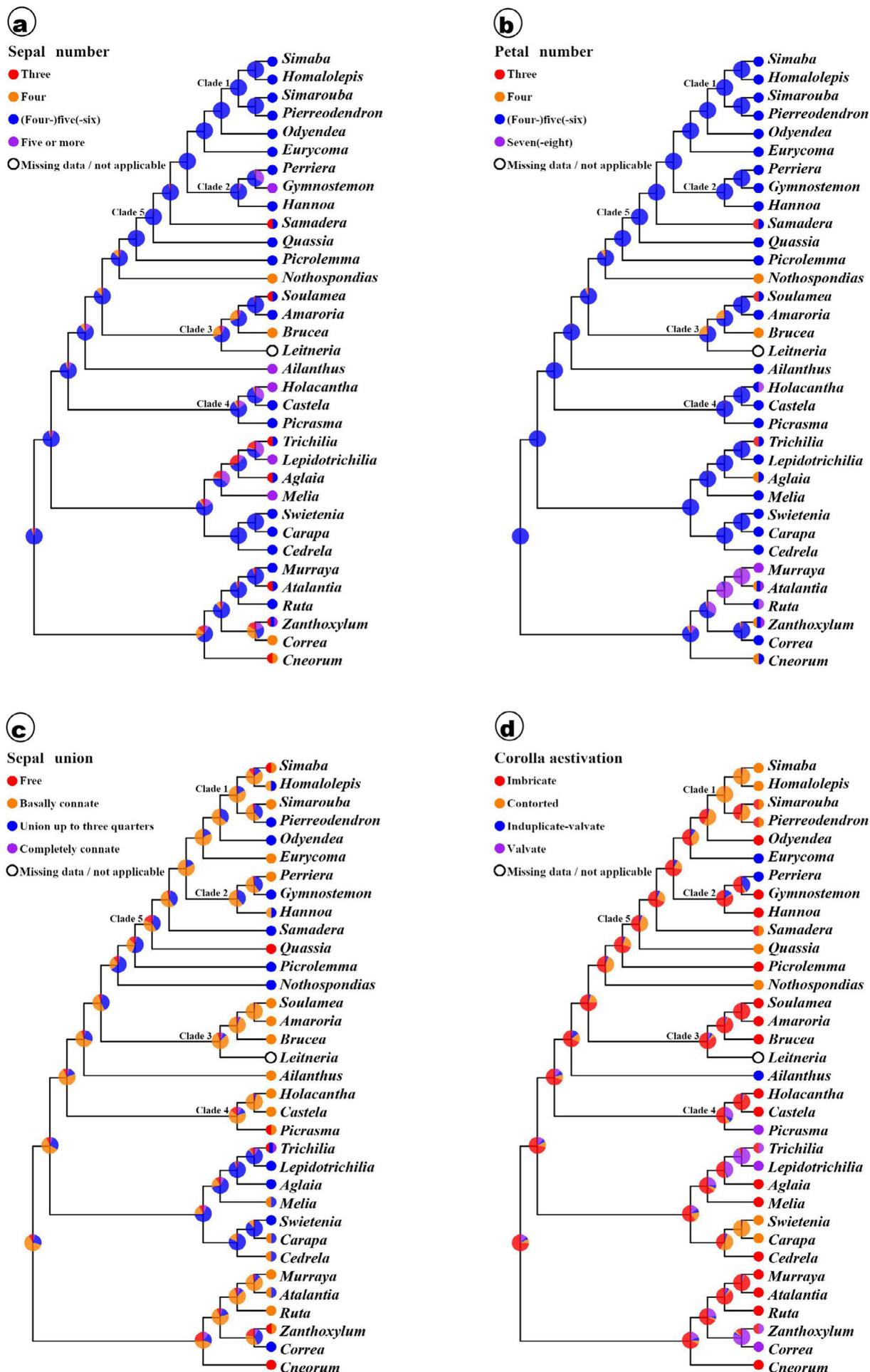


Fig. 8 Reconstruction analysis of ancestral character state based on Bayesian methods. **a** Sepal number. **b** Petal number. **c** Sepal union. **d** Corolla aestivation

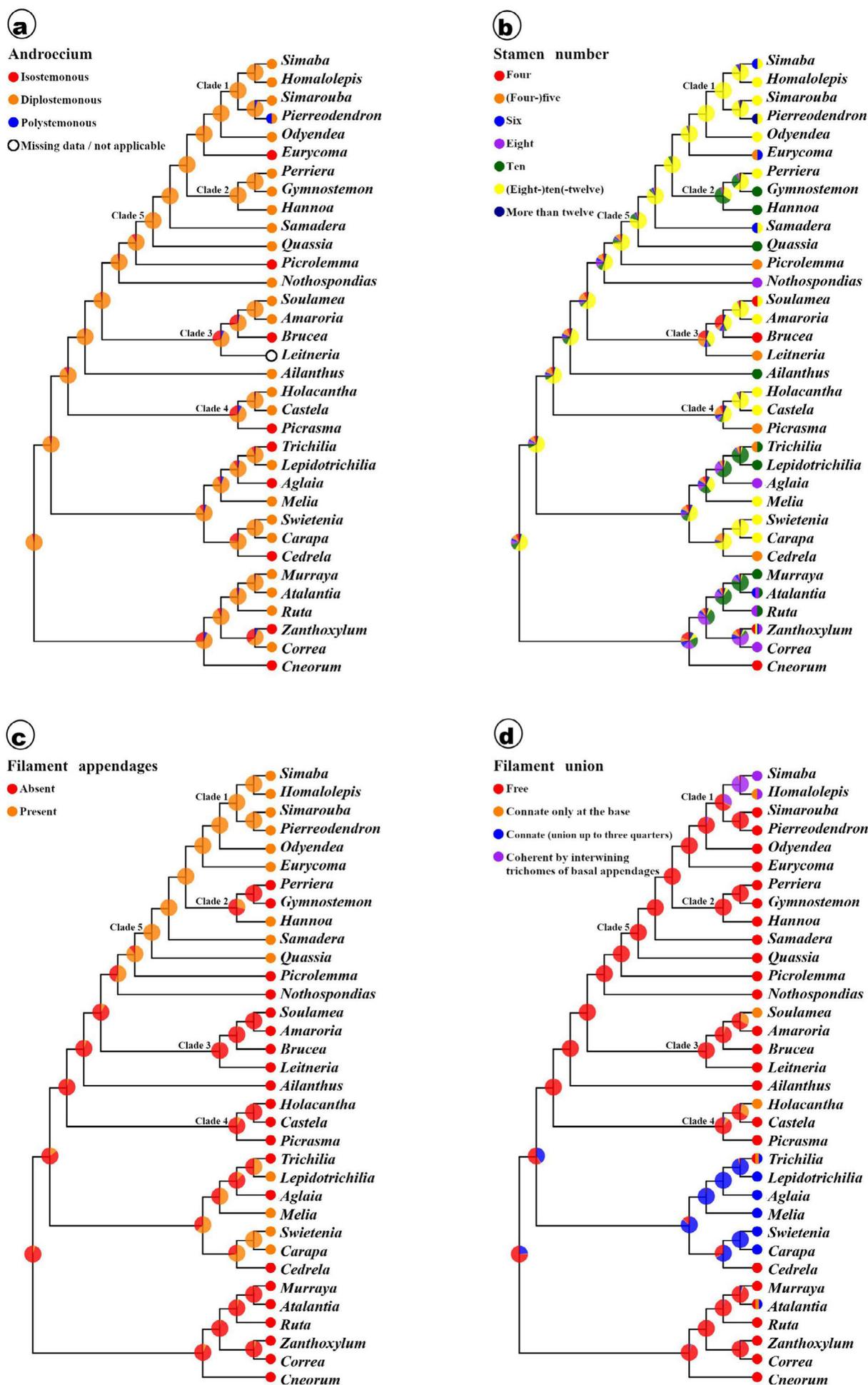


Fig. 9 Reconstruction analysis of ancestral character state based on Bayesian methods. a Androecium. b Stamen number. c Filament appendages. d Filament union

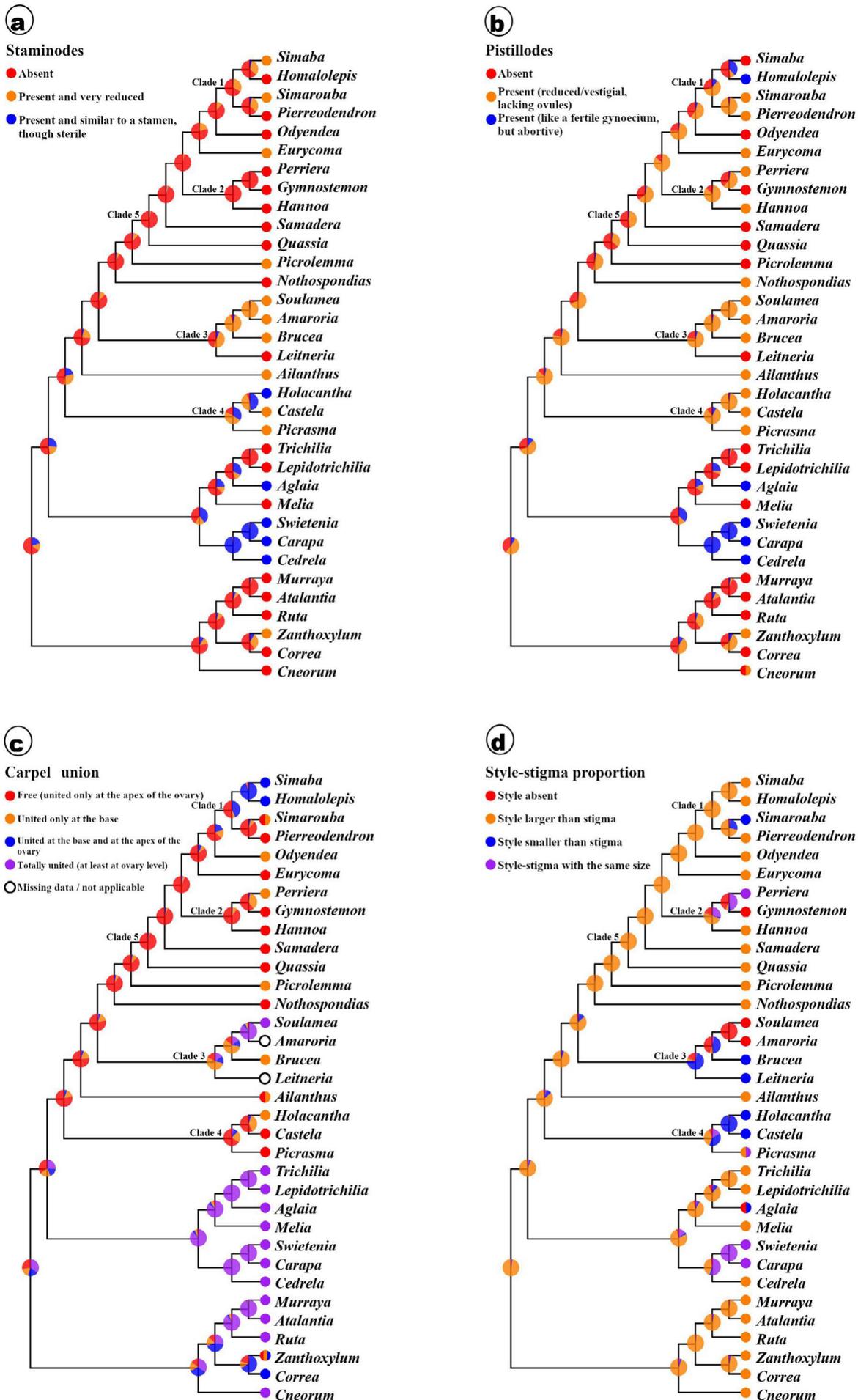


Fig. 10 Reconstruction analysis of ancestral character state based on Bayesian methods. **a** Staminoles. **b** Pistillodes. **c** Carpel union. **d** Style-stigma proportion

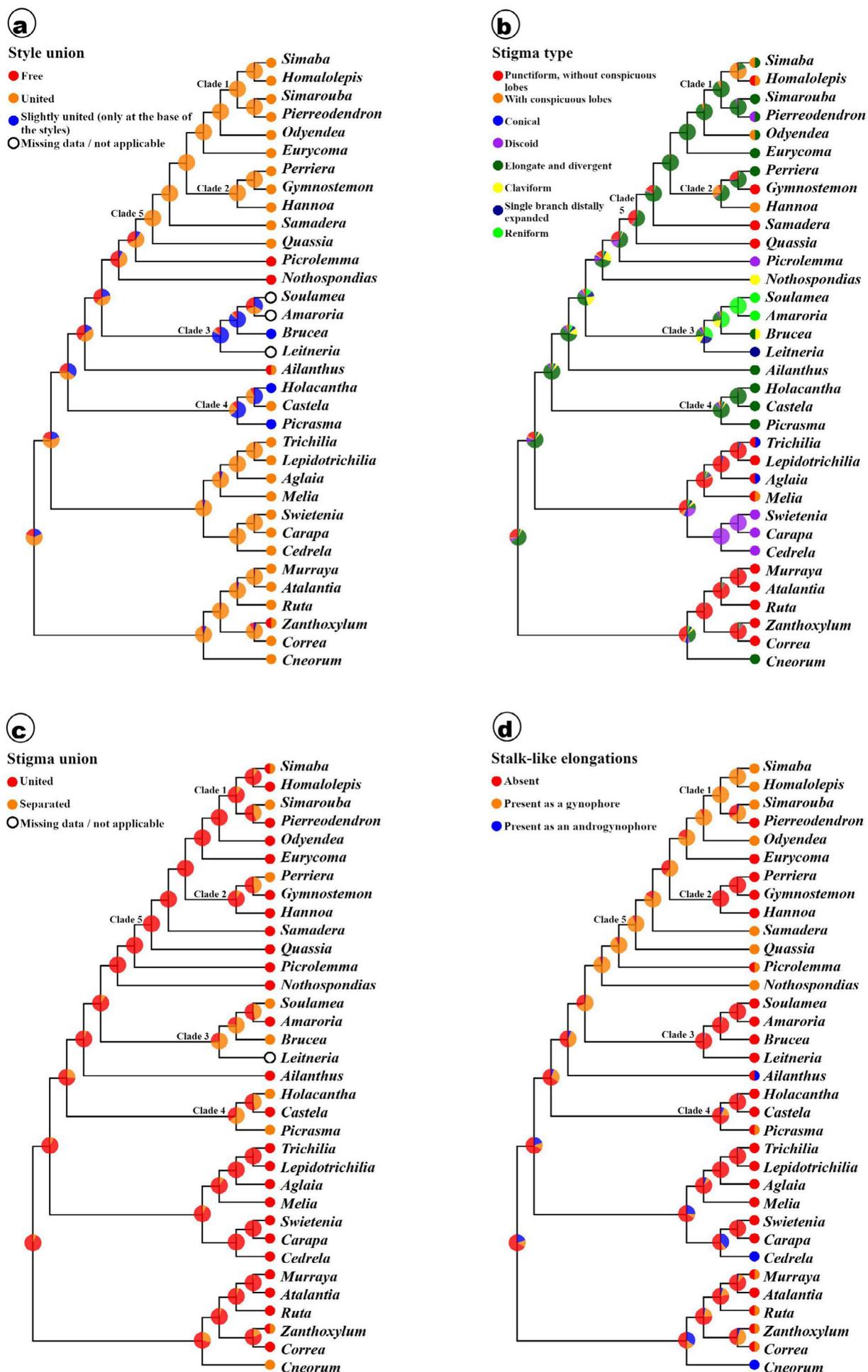


Fig. 11 Reconstruction analysis of ancestral character state based on Bayesian methods. **a** Style union. **b** Stigma type. **c** Stigma union. **d** Stalk-like elongations

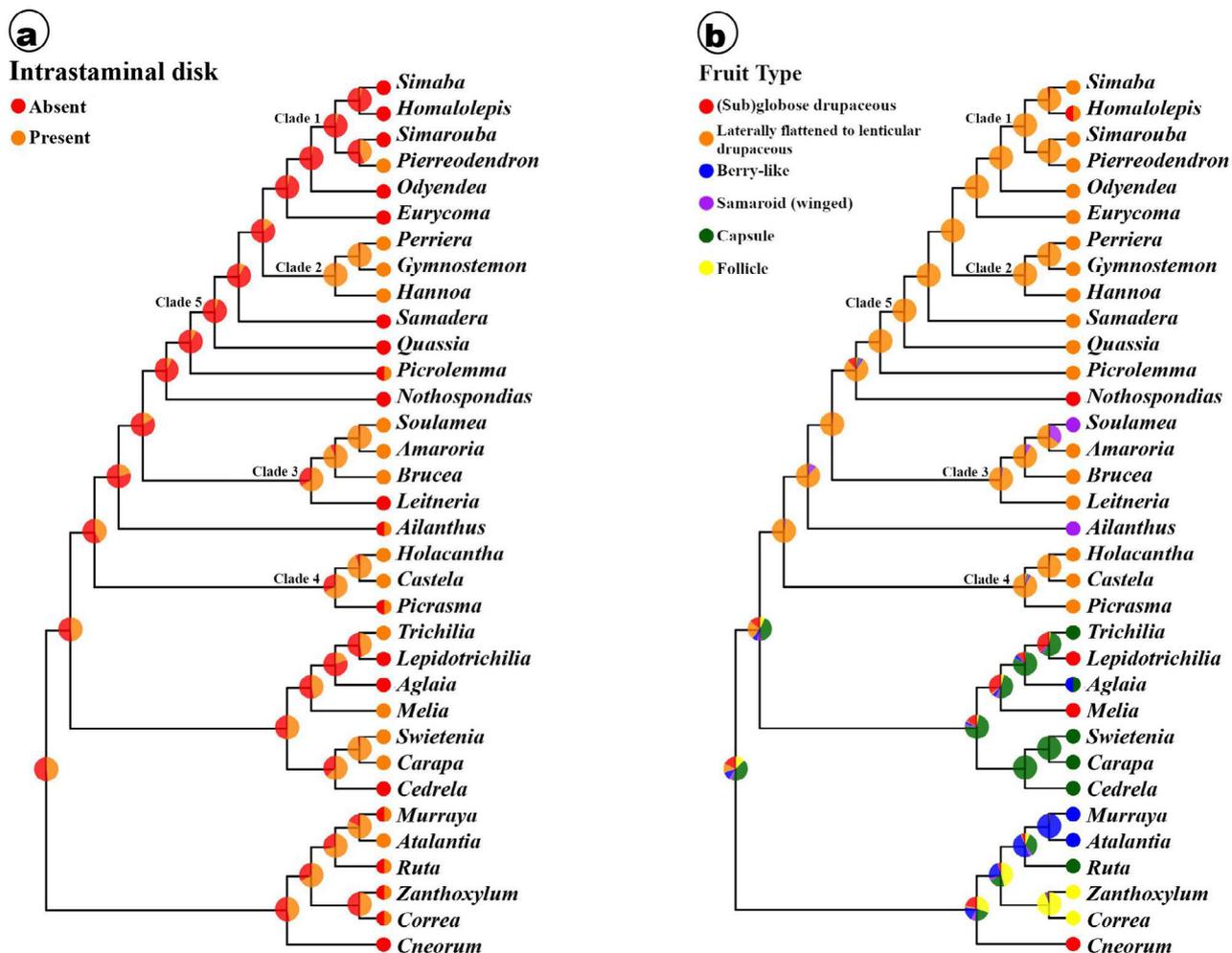


Fig. 12 Reconstruction analysis of ancestral character state based on Bayesian methods. **a** Intrastaminal disk. **b** Fruit type

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 thyrses 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 thyrses 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*, *Odyndea*, *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 polygamomonocious, 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 Biebersteiniaceae; 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*, *Koeleruteria*, 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 clausenii* 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 simaroubaceae 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 Simaroubaceae 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 Galipeae), 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 *Odyndea* 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).

Gynoeceum – 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* styler 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 gynoecea 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 apocarp” 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 gynoeceia 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 gynoeceium (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 gynoeceium 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 apocarp” (sensu Endress and Matthews 2006) found in Simaroubaceae seems to function likewise.

Amaroria and *Leitneria* are unique in the family for having monomerous gynoeceia (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 pseudomonomy, 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 gynoeceia are widespread only in Anacardiaceae (especially in Anacardiaceae, 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.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40415-021-00768-y>.

Acknowledgements This study is part of the PhD dissertation of the first author. We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; proc. #2014/18002-2), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; proc. #307655/2015-6) and International Association for Plant Taxonomy (IAPT) for assistance in funding research. We are most grateful to Dr. Alexandra N. Muellner-Riehl for providing the calibrated tree of Sapindales, to Klei Rodrigo Sousa for flower illustrations, Allan Bruno Sendas for enlightenments in figure editing, Dr. J. Richard Abbott, Dr. W. Wayt Thomas and Dr. Pedro Acevedo-Rodriguez for sharing images of Simaroubaceae representatives.

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.

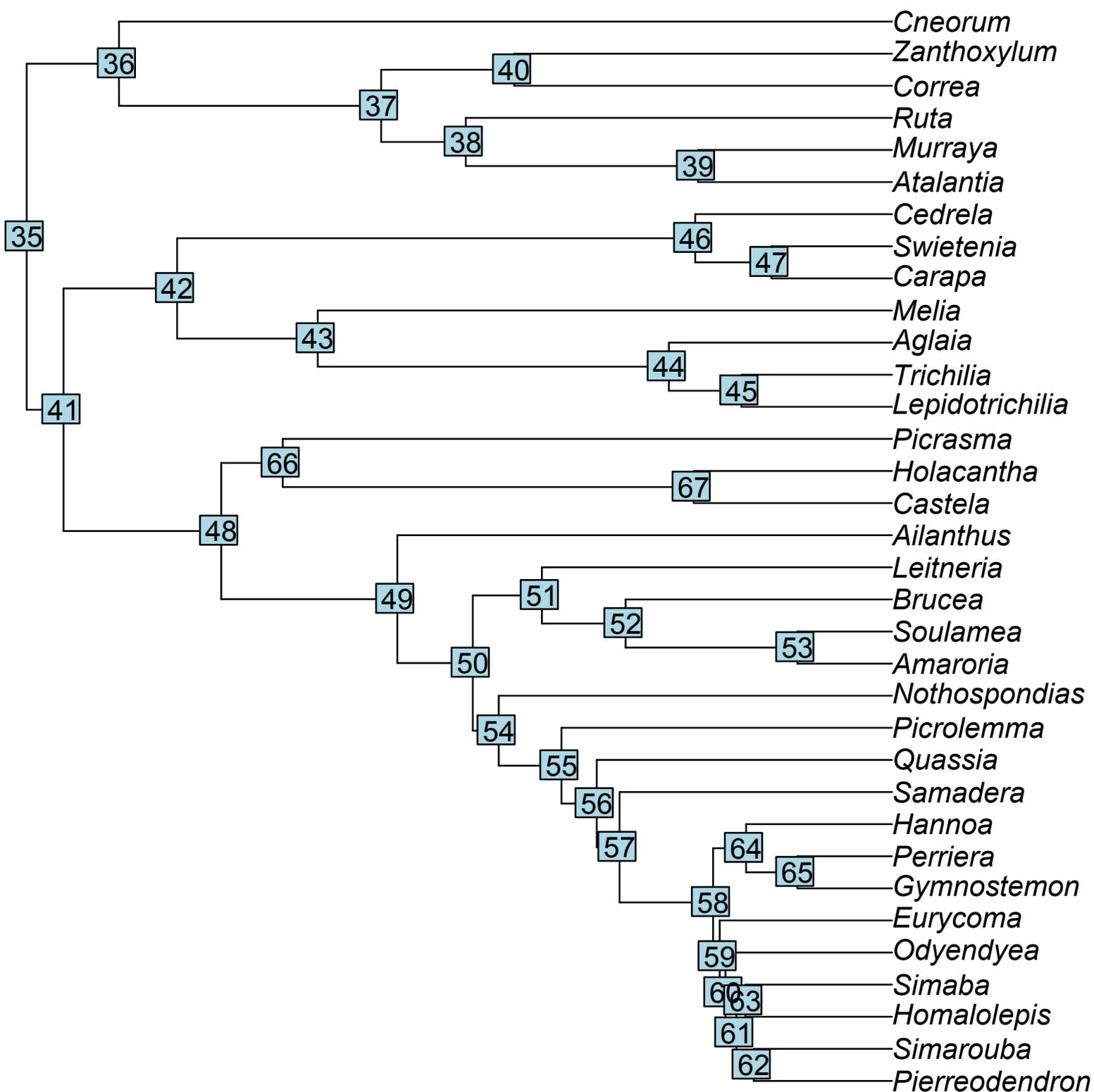
References

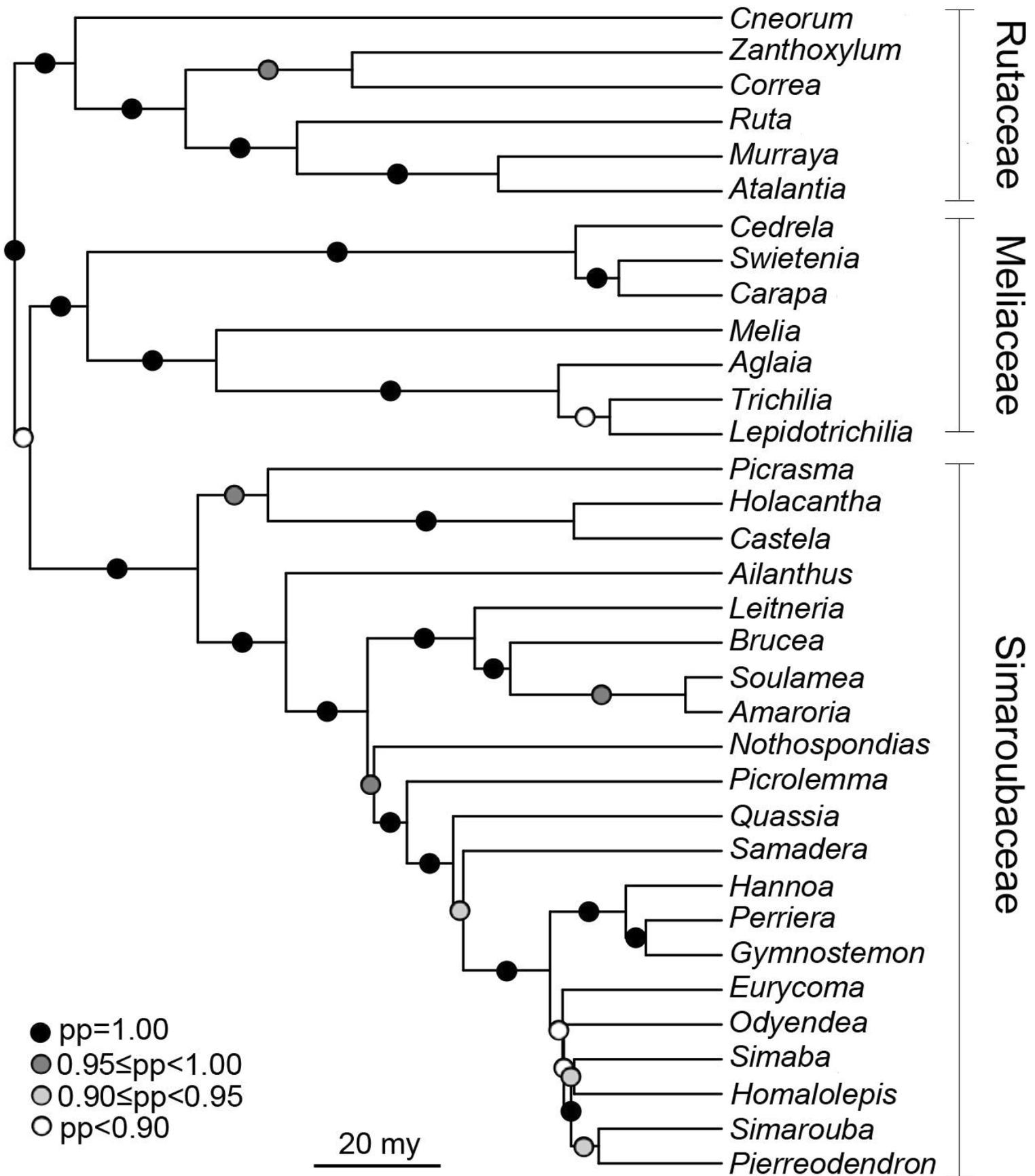
- Abbe EC, Earle TT (1940) Floral anatomy and morphology of *Leitneria floridana*. *J Torrey Bot Soc* 67:173–193
- Alves GGN, El Ottra JHL, Devecchi MF, Demarco D, Pirani JR (2017) Structure of the flower of *Simaba* (Simaroubaceae) and its anatomical novelties. *Bot J Linn Soc* 183:162–176
- Armbruster WS, Debevec EM, Willson MF (2002) Evolution of syncarpy in angiosperms: theoretical and phylogenetic analyses of the effects of carpel fusion on offspring quantity and quality. *Int J Evol Biol* 15:657–672
- Aubréville A (1962) Flora du Gabon Irvingiacées, Simaroubacées, Burséracées. *Mus Natl Hist Nat* 3:3–99
- Avalos A, Zini LM, Ferruci MS, Lattar EC (2019) Anther and gynoecium structure and development of male and female gametophytes of *Koelreuteria elegans* subsp. *formosana* (Sapindaceae): phylogenetic implications. *Flora* 255:98–109
- Bachelier JB, Endress PK (2008) Floral structure of *Kirkia* (Kirkiaceae) and its position in Sapindales. *Ann Bot* 102:539–550
- Bachelier JB, Endress PK (2009) Comparative floral morphology of Anacardiaceae and Burseraceae, with a special emphasis on the gynoecium. *Bot J Linn Soc* 159:499–571
- Bachelier JB, Endress PK, De Craene LPR (2011) Comparative floral structure and development of Nitrariaceae (Sapindales) and systematic implications. In: Wanntorp L, De Craene LPR (eds) *Flowers on the tree of life*. Cambridge University Press, Cambridge, pp 181–217
- Baratake RC, Patil CG (2010) Cytological investigations in polygamo-dioecious tree *Simarouba glauca* DC. *Nucleus* 53:33–36
- Barrett SCH (2002) The evolution of plant sexual diversity. *Nat Rev* 3:274–284
- Barrett SCH, Hough J (2013) Sexual dimorphism in flowering plants. *J Exp Bot* 64:67–82
- Bawa KS, Beach JH (1981) Evolution of sexual systems in flowering plants. *Ann Mo Bot Gard* 68:254–274
- Beurton CH (1994) Gynoecium and perianth in *Zanthoxylum* s.l. (Rutaceae). *Plant Syst Evol* 189:165–191
- Blume CL (1825) *Picrasma*. In: *Bijdragen tot de flora van Nederlandsch Indië*. Ter Lands Drukkerij, Batavia, pp 247–248
- Bolmgren K, Eriksson O (2010) Seed mass and the evolution of fleshy fruits in angiosperms. *Oikos* 119:707–718
- Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A (2019) BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Comput Biol* 15:e1006650
- Bremer B, Eriksson O (1992) Evolution of fruit characters and dispersal modes in the tropical family Rubiaceae. *Biol J Linn Soc* 47:79–95
- Capuron R (1961) Contribution à l'étude de la flore forestière de Madagascar. *Adansonia* 1:65–92
- Caris P, Smets E, De Coster K, Ronse De Craene LP (2006) Floral ontogeny of *Cneorum tricoccon* L. (Rutaceae). *Plant Syst Evol* 257:223–232
- Cavalcante PB (1983) Revisão taxonômica do gênero *Simaba* Aubl. (Simaroubaceae) na América do Sul. *Bol Mus Para Emilio Goeldi* 37:1–85
- Channell RB, Wood CE (1962) The Leitneriaceae in the southeastern United States. *J Arnold Arbor* 18:435–438
- Charlesworth B, Guttman DS (1999) The evolution of dioecy and plant sex chromosome systems. In: Ainsworth CC (ed) *Sex determination in plants*. Bios Scientific, Oxford, pp 25–49
- Clayton JW, Fernando ES, Soltis PS, Soltis DE (2007) Molecular phylogeny of the Tree-of-Heaven family (Simaroubaceae) based on chloroplast and nuclear markers. *Int J Plant Sci* 168:1325–1339
- Clayton JW (2011) Simaroubaceae. In: Kubitzki K (ed) *The families and genera of vascular plants*. Vol X. Flowering plants. Eudicots: Sapindales, Cucurbitales, Myrtaceae. Springer, Berlin, pp 408–423
- Clifford HT, Dettmann ME (2001) Drupe – a term in search of a definition. *Austrobaileya* 6:127–131
- Cronquist A (1944a) Studies in the Simaroubaceae III. The Genus *Simaba*. *Lloydia* 7:81–92
- Cronquist A (1944b) Studies in the Simaroubaceae. IV. Resume of the American Genera. *Brittonia* 5:128–147
- Cronquist A (1981) *An integrated system of classification of flowering plants*. Columbia University Press, New York
- Darwin C (1877) *The different forms of flower plants on plants of the same species*. John Murray, London
- Devecchi MF, Pirani JR (2020) Flora do Espírito Santo: Simaroubaceae. *Rodriguésia* 71:e02942018
- Devecchi MF, Thomas WW, Pluckett GM, Pirani JR (2018a) Testing the monophyly of *Simaba* (Simaroubaceae): Evidence from five molecular regions and morphology. *Mol Phylogenetics Evol* 120:63–82
- Devecchi MF, Thomas WW, Pirani JR (2018b) Taxonomic revision of the neotropical genus *Homalolepis* Turcz. (Simaroubaceae). *Phytotaxa* 366:1–108
- El Ottra JHL, Pirani JR, Endress PK (2013) Fusion within and between whorls of floral organs in Galipeinae (Rutaceae): structural features and evolutionary implications. *Ann Bot* 111:821–837
- El Ottra JHL, Pirani JR, Pansarin ER (2016) Floral biology and pollination of two sympatric species of Galipeinae (Galipeae, Rutaceae) endemic to the Brazilian Atlantic Forest. *Flora* 221:107–116
- El Ottra JHL, Demarco D, Pirani JR (2019) Comparative floral structure and evolution in Galipeinae (Galipeae: Rutaceae) and its implications at different systematic levels. *Bot J Linn Soc* 191:30–101
- Endress PK (1994) *Diversity and evolutionary biology of tropical flowers*. Cambridge University Press, Cambridge
- Endress PK (2010a) Synorganization without organ fusion in the flowers of *Geranium robertianum* (Geraniaceae) and its not so trivial obdiplostemony. *Ann Bot* 106:678–695
- Endress PK (2010b) Flower structure and trends of evolution in eudicots and their major clades. *Ann Mo Bot Gard* 97:541–583
- Endress PK (2016) Development and evolution of extreme synorganization in angiosperm flowers and diversity: a comparison of Apocynaceae and Orchidaceae. *Ann Bot* 117:749–767
- Endress PK, Doyle JA (2015) Ancestral traits and specializations in the flowers of the basal grade of living angiosperms. *Taxon* 64:1093–1116
- Endress PK, Matthews ML (2006) First steps towards a floral structural characterization of the major rosid subclades. *Plant Syst Evol* 260:223–251
- Endress PK, Jenny M, Fallen ME (1983) Convergent elaboration of apocarpous gynoecia in higher advanced dicotyledons (Sapindales, Malvales, Gentianales). *Nord J Bot* 3:293–300
- Engler A (1931a) Simaroubaceae. In: Engler A, Prantl K (eds) *Die natürlichen Pflanzenfamilien*, vol 19a, 2nd edn. Engelmann, Leipzig, pp 359–405
- Engler A (1931b) Rutaceae. In: Engler A, Prantl K (eds) *Die natürlichen Pflanzenfamilien*, vol 19a, 2nd edn. Engelmann, Leipzig, pp 187–359
- Engler A (1931c) Burseraceae. In: Engler A, Prantl K (eds) *Die natürlichen Pflanzenfamilien*, vol 19a, 2nd edn. Engelmann, Leipzig, pp 405–456
- Faegri L, van der Pijl L (1979) *The principles of pollination ecology*, 3rd edn. Pergamon Press, New York

- Fernando ES, Gadek PA, Quinn CJ (1995) Simaroubaceae, an artificial construct: evidence from *rbcL* sequence variation. *Am J Bot* 82:92–103
- Forman L, Bridson D (1992) *The Herbarium Handbook*. Royal Botanic Gardens, Kew, London
- Franceschinelli EV, Thomas WW (2000) *Simaba guianensis* subsp. *huberi*, a new Venezuelan taxon of Simaroubaceae. *Brittonia* 52:311–314
- Gadek PA, Fernando ES, Quinn CJ, Hoot SB, Terrazas T, Sheahan MC, Chase MW (1996) Sapindales: molecular delimitation and infraordinal groups. *Am J Bot* 83:802–811
- Gama RL, Muellner-Riehl AN, Demarco D, Pirani JR (2021a) Evolution and reproductive traits in the mahogany family (Meliaceae). *J Syst Evol* 59:21–43
- Gama RL, El Ottra JHL, Pirani JR, Demarco D (2021b) Gynodioecy in *Trichilia* (Meliaceae) and a peculiar case of male sterility due to tapetum secretion. *Braz J Bot*. <https://doi.org/10.1007/s40415-021-00746-4>
- Goldberg EE, Otto SP, Vamosi JC, Mayrose I, Sabath N, Ming R, Ashman TL (2017) Macroevolutionary synthesis of flowering plant sexual systems. *Evolution* 71:898–912
- Gottsberger G (1978) Seed dispersal by fish in the inundated regions of Humaita, Amazonia. *Biotropica* 10:170–183
- Gouvêa CF, Dornelas MC, Rodriguez APM (2008) Floral development in the tribe Cedreleae (Meliaceae sub-family Swietenioideae): *Cedrela* and *Toona*. *Ann Bot* 101:39–48
- Groppe M, Kallunki JA, Pirani JR, Antonelli A (2012) Chilean *Pitavia* more closely related to Oceania and Old World Rutaceae than Neotropical groups: evidence from two cpDNA non-coding regions, with a new subfamilial classification of the family. *PhytoKeys* 19:9219
- Gut BJ (1966) Beiträge zur Morphologie des Gynoeciums und der Blütenachse einiger Rutaceen. *Bot Jahrb Syst* 85:151–247
- Hardesty BD, Dick CW, Kremer A, Hubbell S, Bermingham E (2005) Spatial genetic structure of *Simarouba amara* Aubl. (Simaroubaceae), a dioecious, animal-dispersed Neotropical tree, on Barro Colorado Island. *Panama J Hered* 95:290–297
- Harms H (1896) Meliaceae. In: Engler A, Prantl K (eds) *Die natürlichen Pflanzenfamilien*, vol 19a, 2nd edn. Engelmann, Leipzig, pp 258–308
- Heilbuth J (2000) Lower species richness in dioecious clades. *Am Nat* 156:221–241
- Honda EMS (1974) Contribuição ao conhecimento da biologia de peixes do Amazonas. II – Alimentação de tambaqui, *Colossoma bidens* (Spix). *Acta Amaz* 4:47–53
- Hu SY (1979) *Ailanthus*. *Arnoldia* 39:29–50
- Janzen DH (1979) New horizons in the biology of plant defenses. In: Rosenthal GA, Janzen DH (eds) *Herbivores their interaction with secondary plant metabolites*. Academic Press, Orlando, pp 331–350
- Judd WS, Campbell CS, Kellogg EA, Stevens PF, Donoghue MJ (2008) *Plant systematics. A phylogenetic approach*, 3rd edn. Sinauer Associates, Sunderland
- Kubitzki K (2011) Introduction to Sapindales. In: Kubitzki K (ed) *The families and genera of vascular plants*, vol X. Springer, Heidelberg, pp 1–3
- Kubitzki K, Gottlieb O (1984) Micromolecular patterns and the evolution and major classification of angiosperms. *Taxon* 33:375–391
- Kubitzki K, Kallunki JA, Duretto M, Wilson P (2011) Rutaceae. In: Kubitzki K (ed) *The families and genera of vascular plants*, vol X. Springer, Heidelberg, pp 276–356
- Leins P, Erbar C (2010) *Flower and fruit. Morphology ontogeny phylogeny function and ecology*. Schweizerbart Science Publisher, Stuttgart
- Lin N, Moore MJ, Deng T, Sun H, Yang L, Yan S, Wang H (2018) Complete plastome sequencing from *Toona* (Meliaceae) and phylogenomic analyses within Sapindales. *Appl Plant Sci* 6:1–11
- Lloyd DG, Bawa KS (1984) Modification of the gender of seed plants in varying conditions. *Evol Biol* 17:255–338
- Lorts CM, Briggeman T, Sang T (2008) Evolution of fruit types and seed dispersal: a phylogenetic and ecological snapshot. *J Sys Evol* 46:396–404
- Mabberley DJ (2011) Meliaceae. In: Kubitzki K (ed) *The families and genera of vascular plants*, vol X. Springer, Berlin, pp 185–211
- Maddison WP, Maddison DR (2019) *Mesquite: a modular system for evolutionary analysis*. Version 3.61. Available from <http://mesquiteproject.org>. Accessed in 2019 and 2020
- Majure LC, Clase T, Blankenship A, Noa-Monzón A (2021a) A new species of *Picrasma*, *P. nanophylla* (Simaroubaceae), from the Dominican Republic. *Brittonia*. <https://doi.org/10.1007/s12228-021-09656-x>
- Majure LC, Pham K, Clase T (2021b) *Castela senticosa* (Simaroubaceae: Sapindales), a new species of the Greater Antillean clade endemic to Hispaniola. *Syst Bot* (in press)
- Matthews ML, Amaral MCE, Endress PK (2012) Comparative floral structure and systematics in Ochnaceae s.l. (Ochnaceae, Quiinaceae, Medusagynaceae; Malpighiales). *Bot J Linn Soc* 170:299–392
- Minelli A (2018) *Plant evolutionary developmental biology. The evolvability of the phenotype*. Cambridge University Press, Cambridge
- Moran R, Felger R (1968) *Castela polyandra*, a new species in a new section; union of *Holacantha* with *Castela* (Simaroubaceae). *Trans San Diego Soc Nat Hist* 15:31–40
- Muellner-Riehl NA, Clayton JW, Buerki S, Nauheimer L, Chiang YC, Cody S, Pell SK (2016) Molecular phylogenetics and molecular clock dating of Sapindales based on plastid *rbcL*, *atpB* and *trnL-trnF* DNA sequences. *Taxon* 65:1019–1036
- Naghiloo S, Classen-Bockhoff R (2016) Developmental analysis of merosity and sexual morphs in Rubiaceae: a case study in *Rubia* and *Cruciata*. *Flora* 222:52–59
- Nair NC, Joshi RK (1958) Floral morphology of some members of the Simaroubaceae. *Bot Gaz* 120:88–99
- Noa-Monzón A, Gutiérrez PAG (2019) *Picrasma pauciflora* (Simaroubaceae), a new species from the NE coast of Cuba. *Willdenowia* 49:187–191
- Noteboom HP (1962a) Simaroubaceae. *Flora Malesiana* (ser I) 6:193–226
- Noteboom HP (1962b) Generic delimitation in Simaroubaceae tribe Simaroubae and a conspectus of the genus *Quassia* L. *Blumea* 9:509–528
- Noteboom HP (1987) *Laumoniera*, a new genus of Simaroubaceae from Sumatra. *Blumea* 32:383–384
- Pagel M, Meade A (2006) Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *Am Nat* 167:808–825
- Pannell JR (2017) Plant sex determination. *Curr Biol* 27:191–197
- Paulino JV, Mansano VF, Prenner G, Teixeira SP (2017) High developmental lability in the perianth of *Inga* (Fabales, Fabaceae): a Neotropical woody rosoid with gamopetalous corolla. *Bot J Linn Soc* 183:146–161
- Pennington TD, Styles BT (1975) A generic monograph of the Meliaceae. *Blumea* 22:419–540
- Pirani JR (1987b) Flora da Serra do Cipó, Minas Gerais: Simaroubaceae. *Bol Bot USP* 9:219–226
- Pirani JR (1998) A revision of *Helietta* and *Balfourodendron* (Rutaceae, Pteleinae). *Brittonia* 50:348–380
- Pirani JR, El Ottra JHL, Menezes NL (2010) Morfoanatomia da flor de cinco espécies de *Galipea* Aubl. e seu significado na evolução

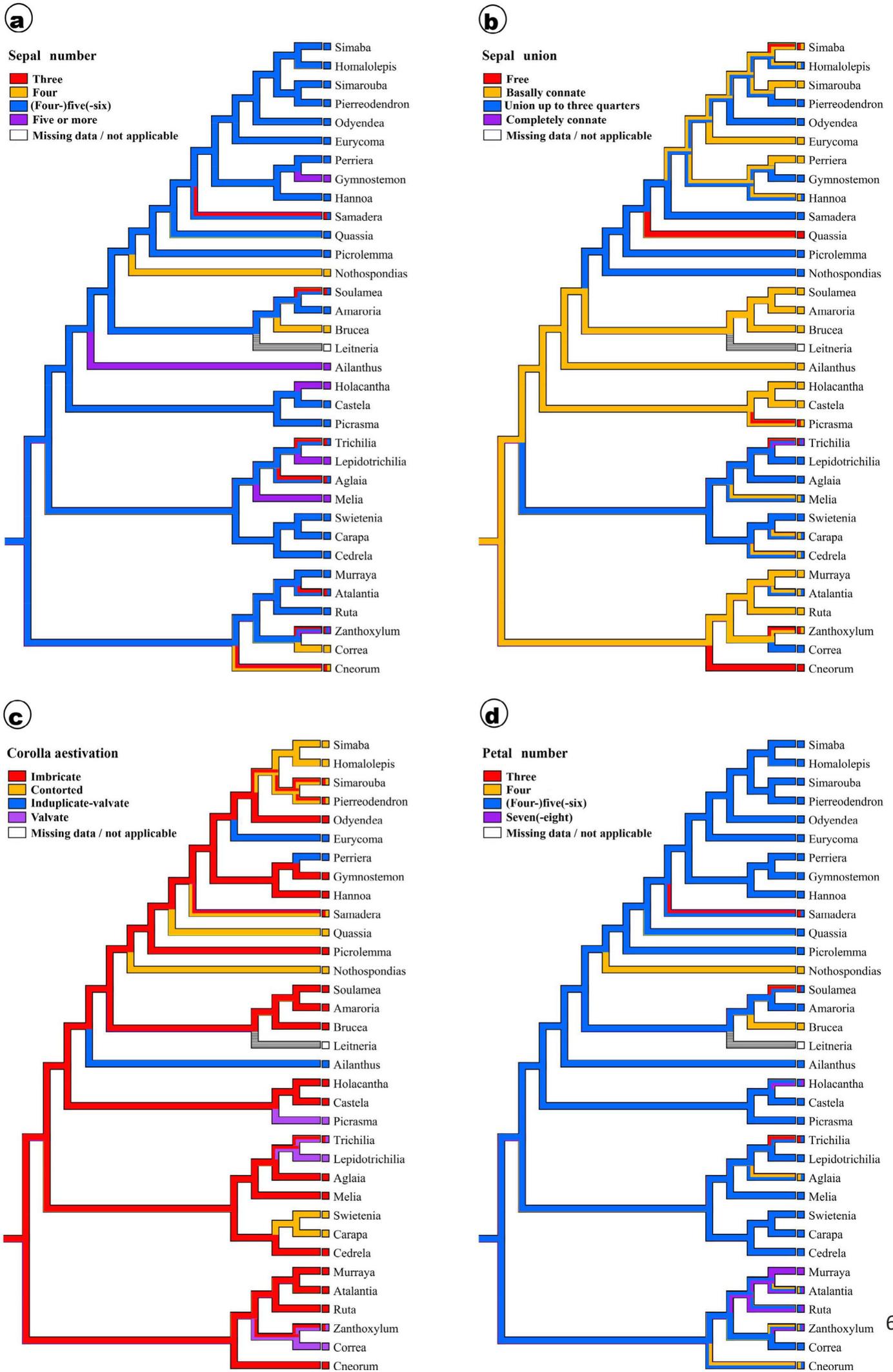
- das flores tubulosas entre as Rutaceae neotropicais. *Rev Bras Bot* 32:301–318
- Pirani JR, Majure LC, Thomas WW, Devecchi MF (2021) An updated account of Simaroubaceae with emphasis on American taxa. *Braz J Bot*. <https://doi.org/10.1007/s40415-021-00731-x>
- Pirani JR (1987a) Simaroubaceae. In: Spichiger R (ed) *Flora del Paraguay*. Conservatoire et Jardin botaniques de la Ville Genève. Missouri Botanical Garden, Saint Louis
- Prusinkiewicz P, Erasmus Y, Lane B, Harder LD, Coen E (2007) Evolution and development of inflorescence architectures. *Science* 316:1452–1456
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst Biol* 67:901–904
- Ramp E (1988) *Struktur, Funktion und systematische Bedeutung des Gynoeciums bei den Rutaceae und Simaroubaceae*. PhD dissertation, University of Zurich, Zurich
- Rendle AB (1952) *The classification of flowering plants. II. Dicotyledons*. Cambridge University Press, Cambridge
- Renner SS (2014) The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. *Am J Bot* 101:1588–1596
- Romero-da-Cruz MV, Guimarães R, Devecchi MF, Pirani JR, Forn-Martins ER (2021) Chromosome numbers in *Homalolepis Turcz* and their significance in Simaroubaceae evolution. *Braz J Bot*. <https://doi.org/10.1007/s40415-021-00729-5>
- Ronse De Craene LP (2010) *Floral diagrams*. Cambridge University Press, Cambridge
- Ronse De Craene LP, Bull-Hereñu K (2016) Obdiplostemony: the occurrence of a transitional stage linking robust flower configurations. *Ann Bot* 117:709–724
- Ronse De Craene LP, Smets E (1995) The distribution and systematic relevance of the androecial character oligomery. *Bot J Linn Soc* 118:193–247
- Ronse De Craene LP, Smets E (2001) Staminoles: their morphological and evolutionary significance. *Bot Rev* 67:351–390
- Ronse De Craene LP, Smets E (2016) Meristic changes in flowering plants: how flowers play with numbers. *Flora* 221:22–37
- Roubik DW, Holbrook NM, Parra GV (1985) Roles of nectar robbers in reproduction of the tropical treelet *Quassia amara* (Simaroubaceae). *Oecologia* 66:161–167
- Sakai AK, Weller SG (1999) Gender and sexual dimorphism in flowering plants: A review of terminology, biogeographic patterns, ecological correlates, and phylogenetic approaches. In: Geber MA, Dawson TE, Delph LF (eds) *Gender and sexual dimorphism in flowering plants*. Springer, Berlin, pp 1–31
- Schilep KP (2011) Phangorn: phylogenetic analysis in R. *Bioinformatics* 27:592–593
- Schrader JA, Graves WR (2011) Taxonomy of *Leitneria* (Simaroubaceae) resolved by ISSR, ITS, and morphometric characterization. *Castanea* 76:313–338
- Shu KM (2008) *Picrasma*. In: *Flora of China*. eFloras. Available from <http://www.efloras.org>. Accessed on 20 August 2019
- Simpson MG (2010) *Plant systematics*, 2nd edn. Elsevier, Amsterdam
- Sokoloff DD, Nuraliev MS, Oskolski AA, Remizowa MV (2018) Gynoecium evolution in angiosperms: monomery, pseudomonomery and mixomery. *Moscow Univ Biol Sci Bull* 72:97–108
- Soltis PS, Brockington SF, Yoo MJ, Piedrahita A, Latvis M, Moore MJ, Chanderbali AS, Soltis DE (2009) Floral variation and floral genetics in basal angiosperms. *Am J Bot* 96:110–128
- Stevens PF (2001) onwards. Angiosperm Phylogeny Website. Version 14, July 2017 [and more or less continuously updated since]. Available from <http://www.mobot.org/MOBOT/research/APweb/>
- Stuessy TF (2009) *Plant taxonomy: the systematic evaluation of comparative data*. Columbia University Press, New York
- Styles BT (1972) The flower biology of the Meliaceae and its bearing on tree breeding. *Silvae Genet* 21:175–182
- Takhtajan A (1997) *Diversity and classification of flowering plants*. Columbia University Press, New York
- Thomas WW (1990) The American genera of Simaroubaceae and their distribution. *Acta Bot Bras* 4:11–18
- Tobe H (2011) Embryological evidence supports the transfer of *Leitneria floridana* to the family Simaroubaceae. *Ann Mo Bot Gard* 98:277–293
- Tobe H (2013) Morphology and structure of staminate inflorescences and flowers of *Leitneria floridana* (Simaroubaceae): revisited. *Acta Phytotaxon Geobot* 63:57–62
- Tölke ED, Demarco D (2020) The development of pseudomonomeric gynoecia: Anacardiaceae (subfamily Anacardioideae) as a case study. In: Demarco D (ed) *Plant ontogeny*. Nova Science, New York, pp 232–262
- Walker-Larsen J, Harder LD (2000) The evolution of staminodes in angiosperms: patterns of stamen reduction, loss, and functional re-invention. *Am J Bot* 87:1367–1384
- Weberling F (1989) *Morphology of flowers and inflorescences*. Cambridge University Press, Cambridge
- Wei L, Xiang XG, Wang YZ, Li ZY (2015) Phylogenetic relationship and evolution of the androecia in Ruteae (Rutaceae). *PLoS ONE* 10:1–14
- Willis JC (1951) *A dictionary of the flowering plants and ferns*. Cambridge University Press, Cambridge
- Yadav N, Pandey A, Bhatnagar A (2016) Cryptic monoecy and floral morph types in *Acer oblongum* (Sapindaceae): An endangered taxon. *Flora* 224:183–190

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

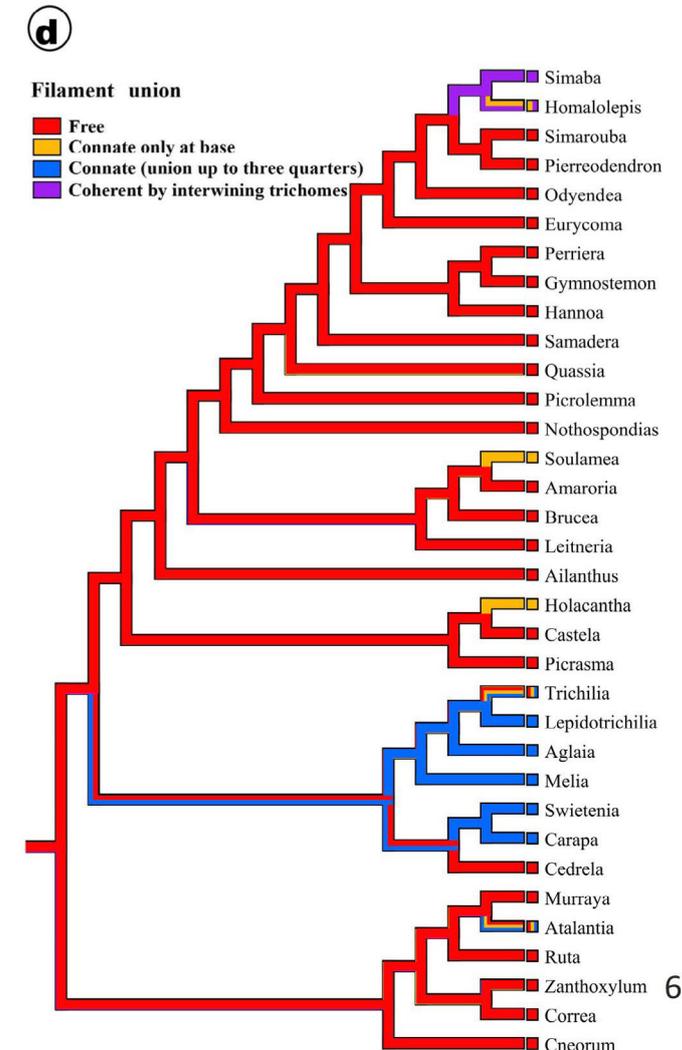
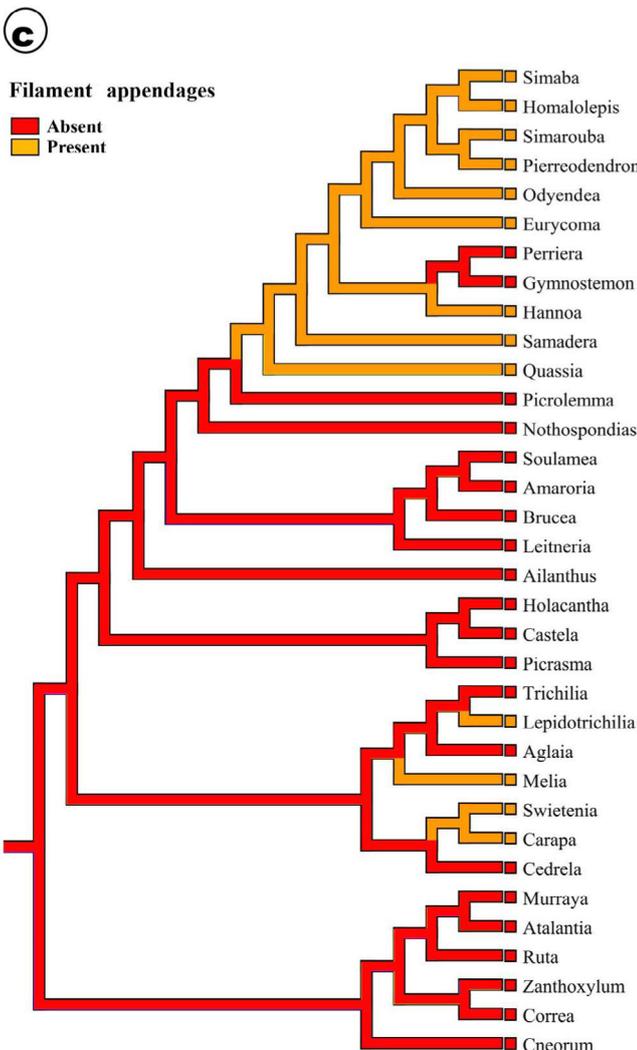
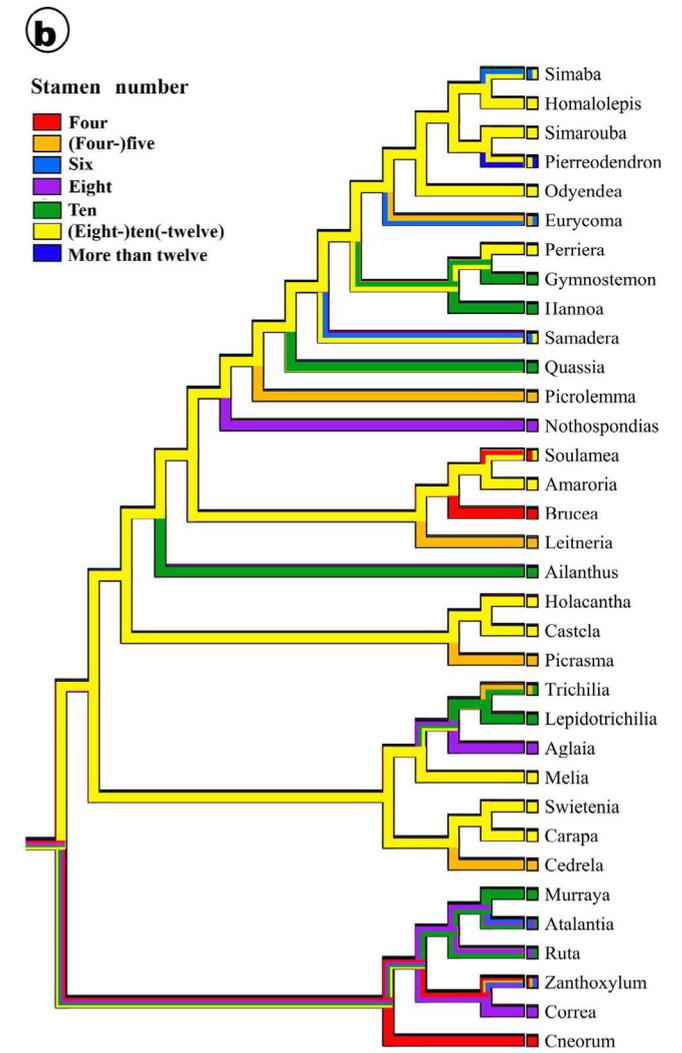
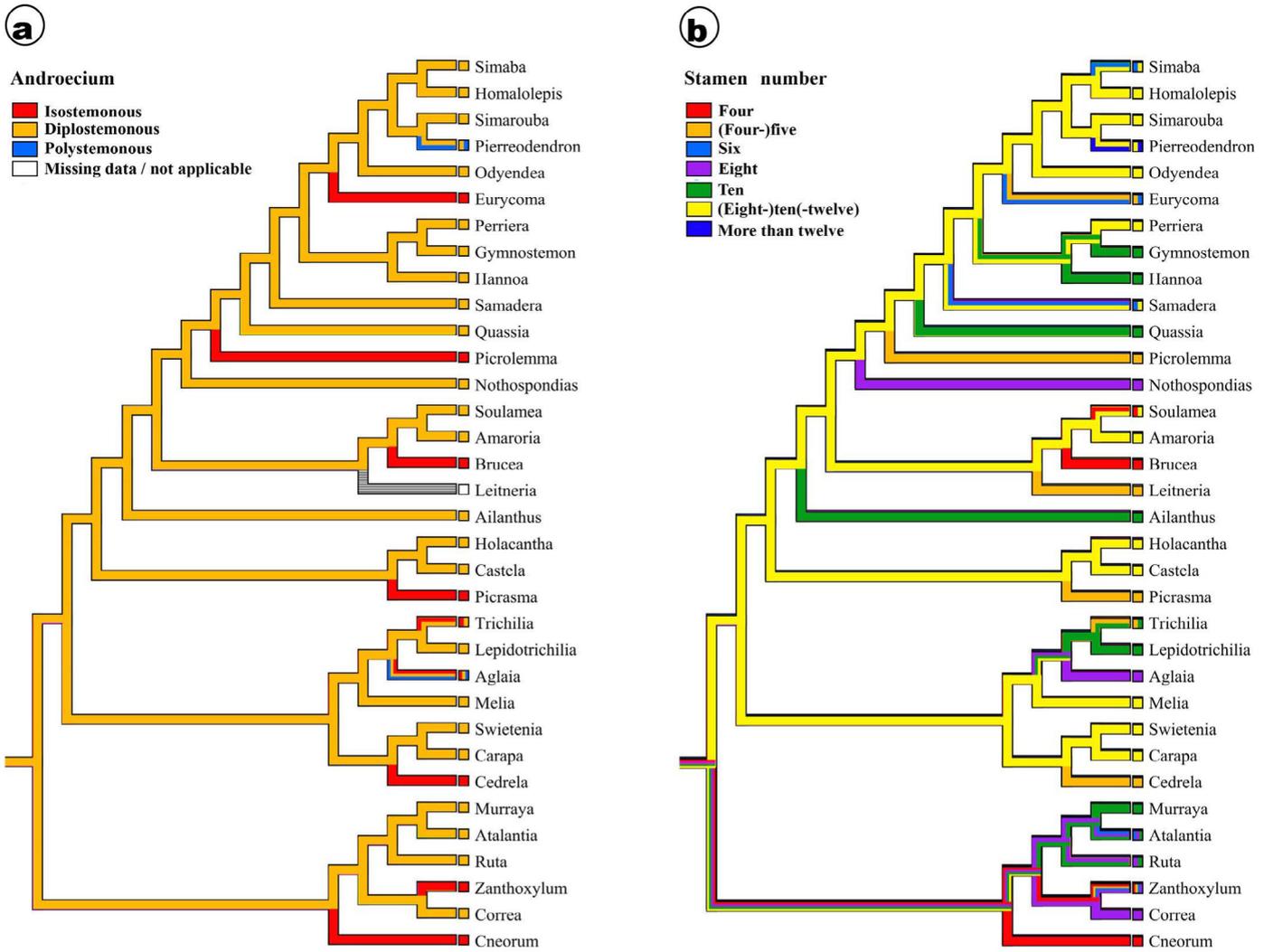




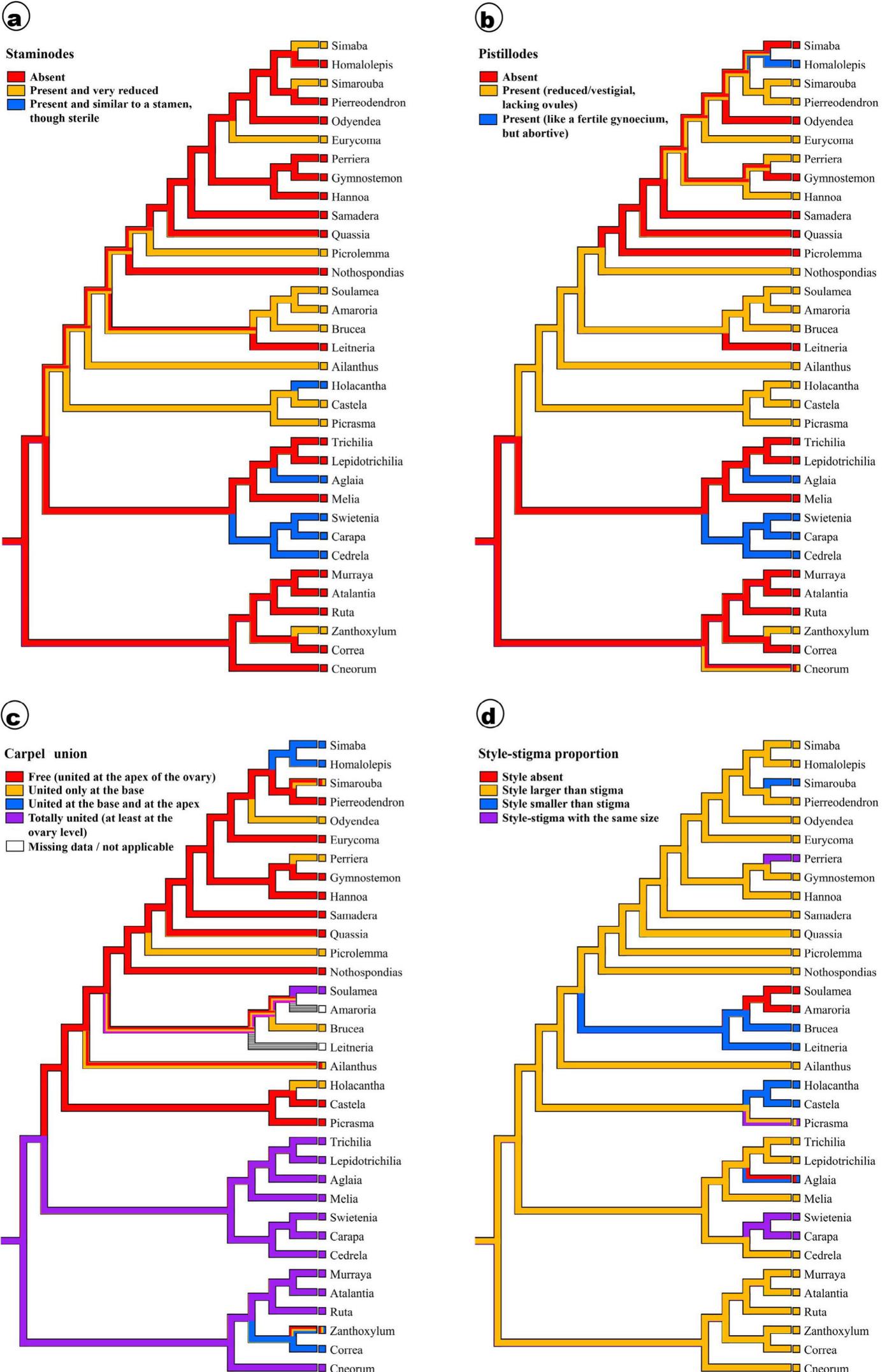
SM. 4 Reconstruction analysis of ancestral character state based on Parsimony criterion. a Sepal number. b Sepal union. c Corolla aestivation. d Petal number

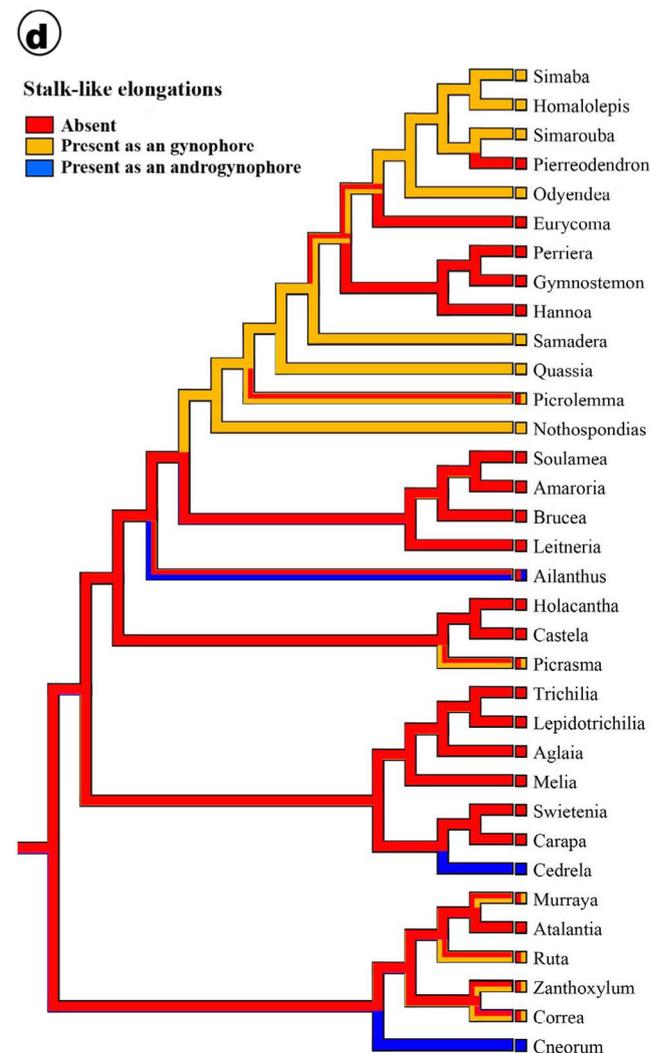
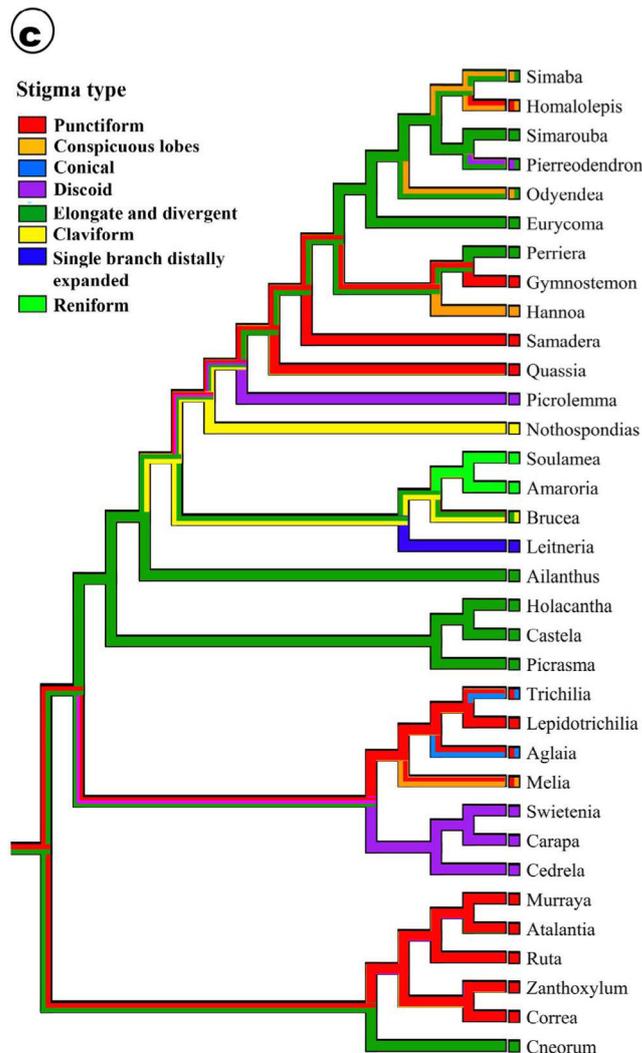
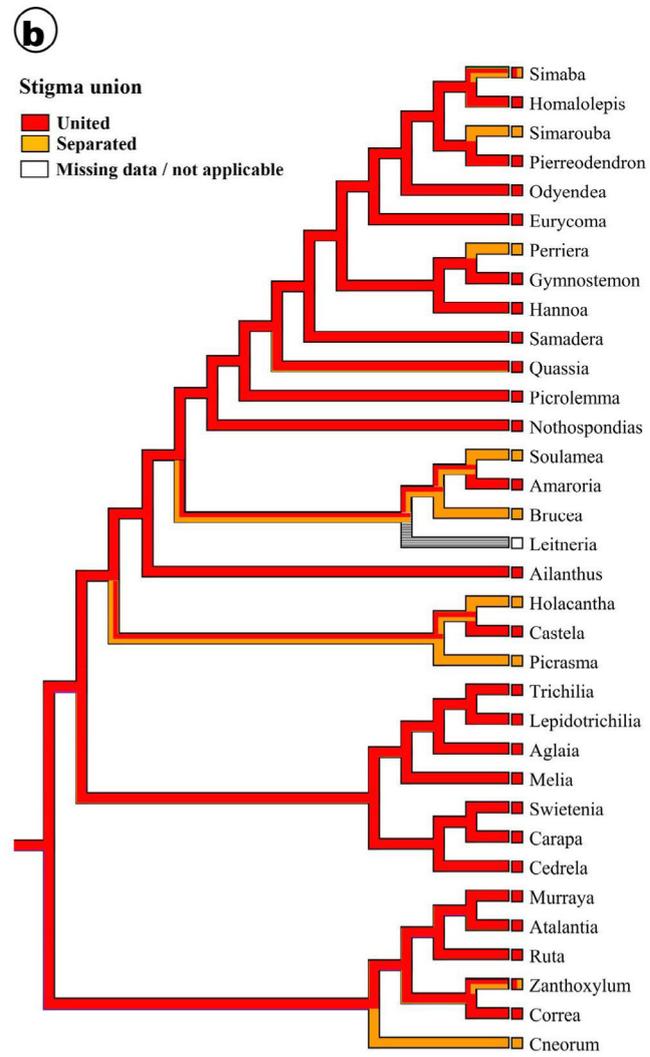
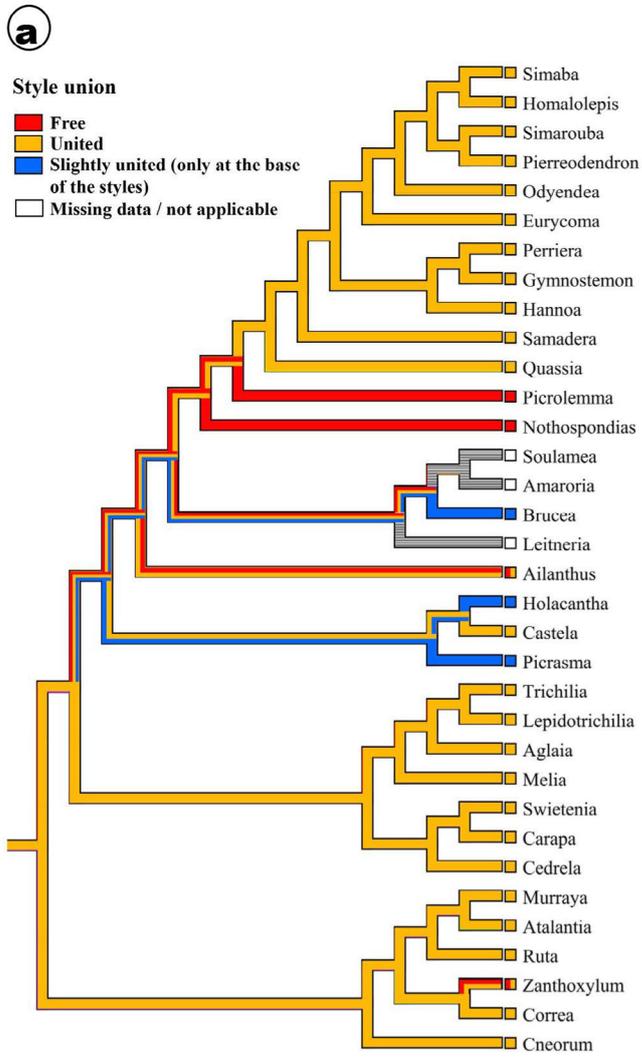


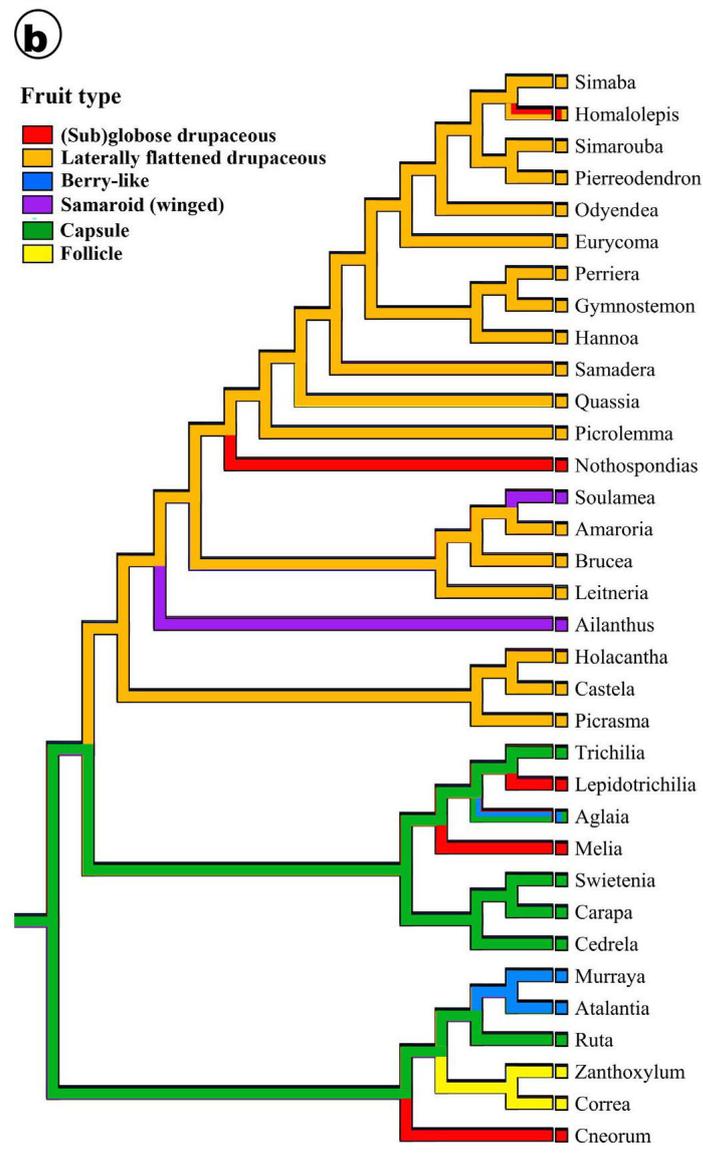
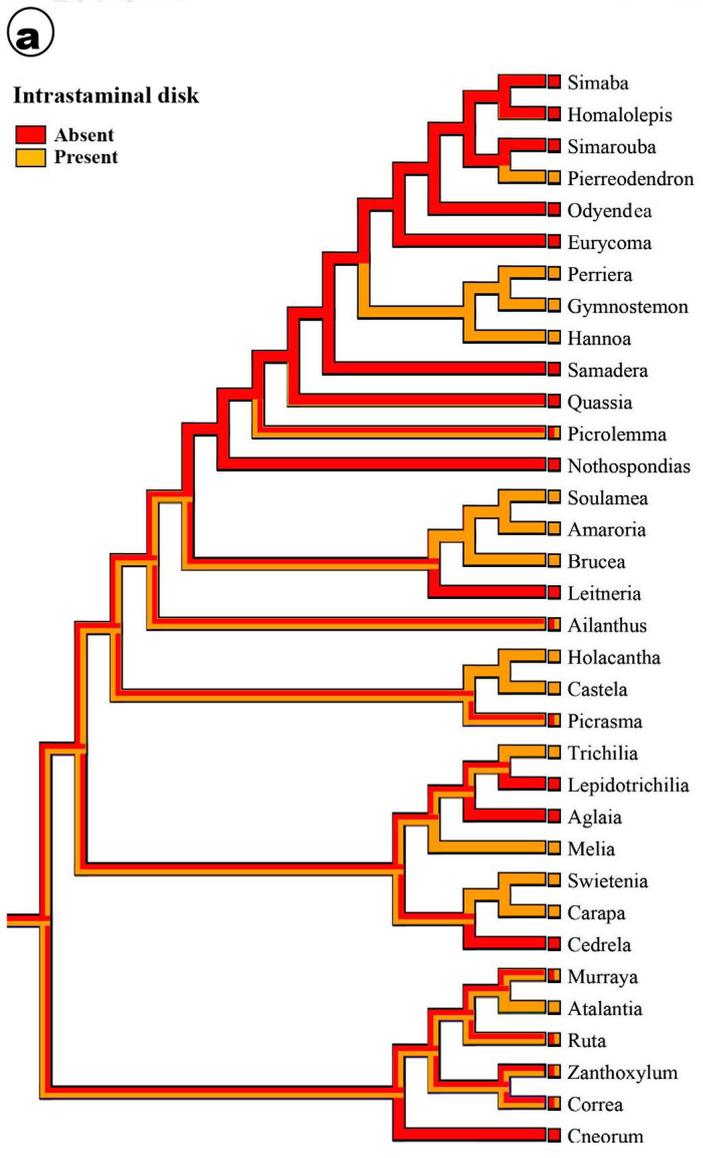
SM. 5 Reconstruction analysis of ancestral character state based on Parsimony criterion. a Androecium. b Stamen number. c Filament appendages. d Filament union



SM. 6 Reconstruction analysis of ancestral character state based on Parsimony criterion. a Staminodes. b Pistillodes. c Carpel union. d Style-stigma proportion







**Chapter 2 not fully available until
publication.**