



A tree nymph of the Brazilian Atlantic Forest: *Dryades* (Galipeinae, Rutaceae), a new neotropical genus segregated from *Conchocarpus*

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ABSTRACT

Subtribe Galipeeae (tribe Galipeeae) is the most diverse group of Rutaceae (the orange family) in the Neotropics, with 27 genera and ca. 130 species. The largest genus in the subtribe is *Conchocarpus*, with ca. 50 species, distributed from Central America to southern Brazil, and is particularly diverse in the Brazilian Atlantic Forest. The circumscription of the genus was recently changed to accommodate the species of *Almeidaa*. However, even with this inclusion, *Conchocarpus* did not appear as monophyletic because the position of *C. concinnus*, which appeared in a clade with the other genera of Galipeeae rather than in the clade with the other species of *Conchocarpus*. The objective of the present study is to investigate the phylogenetic position of four other species of *Conchocarpus* (hereafter called “*C. gauchaudianus* group”) that share morphological traits and geographical distribution with *C. concinnus* suggesting a close phylogenetic affinity. Phylogenetic analyses were based on morphological and molecular data from nuclear regions ITS-1 and ITS-2 as well as plastid regions *trnL-trnF* and *rps-16*, and were conducted with parsimony and Bayesian inference as optimization criteria. Results showed *Conchocarpus* as polyphyletic with its species divided in two clades, one, herein called “the *Conchocarpus sensu stricto* group,” includes the type species *C. macrophyllus*, and the other “the *Conchocarpus gauchaudianus* group” includes *C. concinnus*. The latter group is here recognized as a new genus, *Dryades*, the name given by Carl Friederich von Martius (1794–1868) to the Domain of the Atlantic Forest in Brazil, inspired by the tree nymphs in Greek mythology. Floral structure and leaf morphology provided further support to the findings of phylogenetic analysis. A description of the new genus, new combinations, a key to the species of the new genus, discussions of the affinities of the species are also provided, as well as data on the conservation status of the species of *Dryades*. Additionally, new data on floral structure of *C. heterophyllus*, *C. macrophyllus* and *C. minutiflorus* (all from the *Conchocarpus sensu stricto* group) are provided.

1. Introduction

Rutaceae is a predominantly tropical and subtropical family, consisting of 150–164 genera and 1500–2000 species (Groppo et al., 2008; Kubitzki et al., 2011). The family is best known by the genus *Citrus* because of its edible fruits (oranges, lemons, tangerines, and grapefruits), but the family is also an important source of essential oils, drugs

and as timbers (e.g. Price, 1963; Holmstedt et al., 1979; Moraes et al., 2003; Mandalari et al., 2008; Allevato et al., 2019).

The understanding of the relationships within the family have come a long way since the subfamilial classifications of Engler (1874, 1896, 1931; see Chase et al., 1999; Groppo et al., 2008 for a detailed discussion); and recently new arrangements of the internal groups have become available (see Appelhans et al., 2011; Kubitzki et al., 2011; Groppo et al., 2012;

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Morton and Telmer, 2014). Within the larger subfamily Rutoideae (*sensu* Groppo et al., 2012), the tribe Galipeae comprises two subtribes, the Pilocarpinae and the Galipeinae, the latter being by far the most diverse group of Neotropical Rutaceae, with 28 genera and approximately 130 species (Groppo et al., 2008; Bruniera et al., 2015, see Fig. 1 for some representatives). Although the subtribe ranges from southern Mexico and the West Indies to Bolivia and southern Brazil, the Galipeinae are especially diverse in the Brazilian Atlantic Forest, where 20 genera and 95 species occur (Pirani and Groppo, 2015), with many endemic species to this phylogeographic domain (Colli-Silva and Pirani, 2019). Galipeinae is unique among Neotropical Rutaceae due to its representatives with mostly zygomorphic flowers with a more or less tubular corolla, formed usually by the synorganization of corolla elements and stamens (either by fusion or by postgenital coherence and adherence, El Ottra et al., 2019), a reduction of fertile stamens from five to two, forming filiform staminodes, basally appendage anthers, and plicate cotyledons (Engler, 1931; Kallunki and Pirani, 1998; Groppo et al., 2008; El Ottra et al., 2013; Bruniera et al., 2015). However, exceptions to all these characteristics occur among its representatives (Bruniera et al., 2015).

As currently defined, *Conchocarpus* J.C.Mikan is by far the largest genus of the Galipeinae, with 52 species (Groppo et al., 2019), ranging from Nicaragua to northern Bolivia and southern Brazil. The species are

treelets or shrubs of forest understory, mainly in Brazilian Atlantic Forest. The genus is circumscribed by a combination of character states (and not by exclusive characteristics) as noted by Kallunki and Pirani (1998) who recognized the genus as polymorphic. Therefore, *Conchocarpus* is morphologically variable, including representatives with coherent petals and adherence of filaments to petals by intertwining trichomes, anthers not appendaged, not exerted their whole length from the corolla tube (except in *C. cuneifolius*), and simple trichomes (Kallunki and Pirani, 1998).

Bruniera et al. (2015), in a recent phylogenetic analysis focused on Galipeinae, showed that all five species of *Almeidea* A.St.-Hil. (from the Brazilian Atlantic Forest and Bolivia) were nested within a clade with several species of *Conchocarpus* and the species of the former were therefore transferred to the latter. Despite these transfers, *Conchocarpus* still was not monophyletic because *C. concinnus* Pirani (from the Brazilian Atlantic Forest) was not in the clade with other species of *Conchocarpus* but in a clade with other genera of Galipeinae, as *Andreadoxa* Kallunki, *Angostura* A.St.-Hil., and *Erythrochiton* Nees & Mart. In fact, *C. concinnus* is one of a group of five [(including also *C. hirsutus* Pirani, *C. gaudichaudianus* (A.St.-Hil.) Kallunki & Pirani, *C. cyrtanthus* Kallunki, and *C. insignis* Pirani, none of them sampled in Bruniera et al., 2015)] species restricted to the Brazilian Atlantic Forest that share a set of

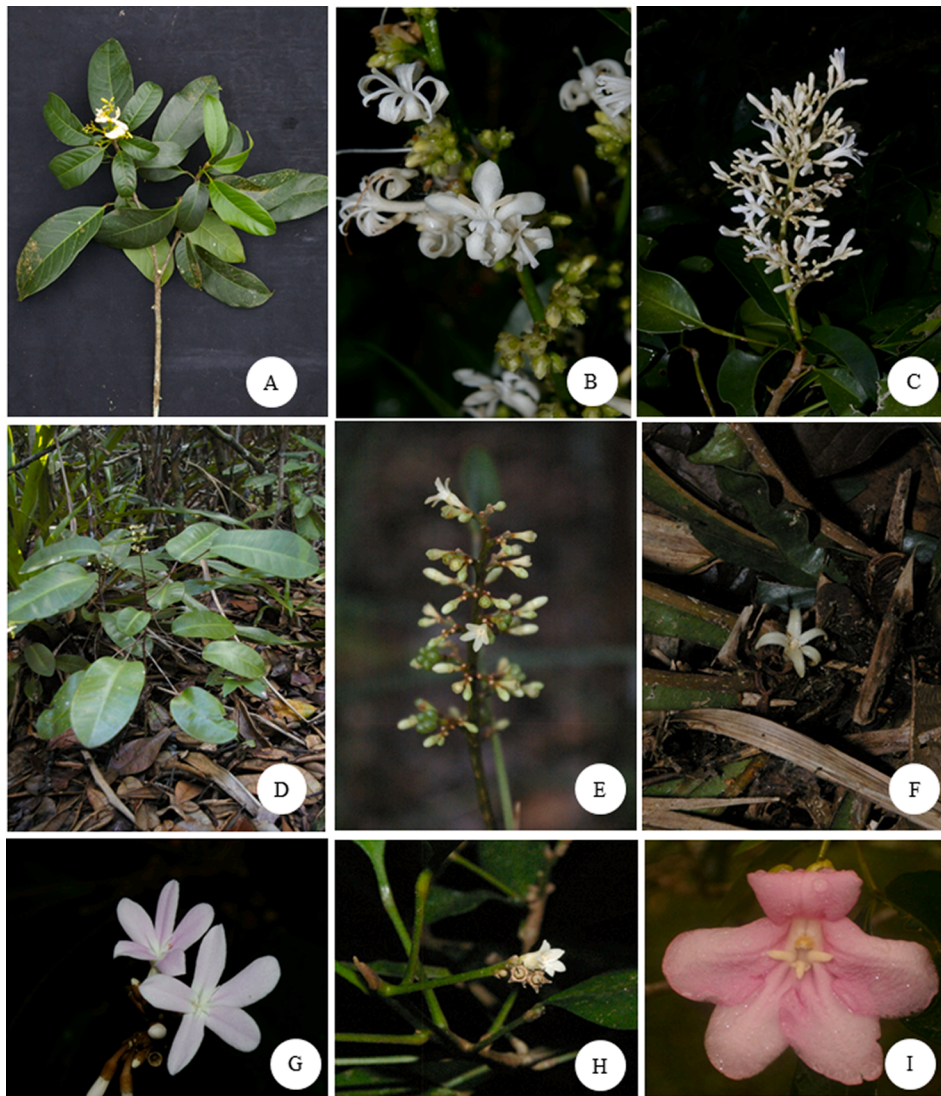


Fig. 1. Some species of Galipeinae used in this study. A. *Andreadoxa flava*. B. *Angostura bracteata*. C. *Conchocarpus albiflorus*. D, E. *C. concinnus* (= *Dryades concinna*). F. *C. gaudichaudianus* subsp. *gaudichaudianus* (= *Dryades gaudichaudiana* subsp. *gaudichaudiana*). G. *C. macrophyllus*. H. *C. minutiflorus*. I. *Ravenia spectabilis*. All photos by Milton Groppo.

characteristics that includes sepals almost free and still overlapping at anthesis (*versus* almost free or connate sepals, in both cases not overlapping at anthesis), anthers broadly (*vs.* narrowly) attached to the filament, and plano-convex (*vs.* conduplicate-plicate) cotyledons. The affinity of these five species had already been noticed by Kallunki and Pirani (1998), and here this group is informally called “the *Conchocarpus gaudichaudianus* group” because “*gaudichaudianus*” is the earliest specific name among them.

The objective of the present study is to investigate the phylogenetic position of four other species of *Conchocarpus* (hereafter called “*C. gauchaudianus* group”) that share morphological traits and geographical distribution with *C. concinnus* suggesting a close phylogenetic affinity. To achieve this goal, we used molecular data from cpDNA (*trnL-trnF* region and *rps16* intron) and two nuclear (nDNA) regions (ITS1 and ITS2) as well as morphological data. Additionally, data from floral structure and leaf anatomy of the species of the *Conchocarpus gaudichaudianus* group as well as other *Conchocarpus* were also investigated and compared. The results obtained here will provide a framework for taxonomic decisions about the genera cited above, being a base to support discussion of putative morphological synapomorphies of the groups and to better understand the phylogeny and evolution of the subtribe Galipeinae, a very diversified group in the Brazilian Atlantic Forest.

2. Material and methods

2.1. Taxonomic sampling

To test the monophyly of the species of the “*Conchocarpus gaudichaudianus* group” and its phylogenetic positioning within the Galipeinae, 28 taxa spanning the morphological variation within the subtribe were included in this study: all five species of the *Conchocarpus gaudichaudianus* group plus 10 additional species of *Conchocarpus* (of c. 47 species) including the type-species of the genus (*C. macrophyllus* J.C. Mikan), and eight species of six other genera – *Andreadoxa* (monotypic, one species sampled) *Angostura* (seven species, one sampled), *Erythrochiton* (six species, one sampled), *Galipea* A.St.-Hil. (14 species, two sampled), *Rauia* Nees & Mart (c. seven species, two sampled), and *Ravenia* Vell. (14 species, one sampled), all morphologically close to *Conchocarpus* and already used by Bruniera et al. (2015) in a first analysis of Galipeinae. Outgroup included species of Pilocarpiinae (the other subtribe of Galipeae), as *Esenbeckia* Kunth, and *Pilocarpus* Vahl. besides *Balfourodendron* (closely related to Pilocarpiinae, see Groppo et al., 2012; Groppo et al., 2017). *Hortia* (assigned traditionally to subfamily Toddalioideae, but now included in Rutoideae and close to Galipeinae, see Groppo et al., 2017) were also included. One species of *Zanthoxylum* (tribe Zanthoxyleae) was used to root the trees. All taxa included in this study belong to subfamily Rutoideae (as circumscribed by Groppo et al., 2012).

2.2. Phylogenetic analysis

2.2.1. Morphological analysis

Morphological analysis was based on anatomical, macro-morphological, and pollen characters. Definition of morphological terms follows those presented in Kallunki and Pirani (1998) and Radford et al. (1974). A list of all 35 characters and their respective states, as well as the morphological matrix are presented in the [Supplementary Material](#) (Appendix 1). Macromorphological characters and their states were determined by direct observation of dry, fixed, fresh samples or from descriptions of the taxa in literature (Emmerich, 1978; Kaastra, 1982; Silva, 1988; Kallunki, 1992; Skorupa, 1996; Pirani, 1998; Kallunki, 1998; Kallunki and Pirani, 1998; Groppo et al., 2005; Kallunki, 2009; Groppo and Pirani, 2012; Pirani et al. (2011)). The matrix of morphological data was built with the software NDE 0.5.0 (Page, 2001).

Pollen characteristics were extracted from literature (Barth, 1982;

Morton and Kallunki, 1993; Groppo et al., 2010; Bruniera et al., 2011, 2015), except for new pollinic data were obtained from *Conchocarpus diadematus*. Pollen grains from this species were taken from mature buds of several collections deposited at SPFR (see Appendix 1). Grains were acetolysed according to the methodology described by Erdtman (1960) and then mounted in glycerine jelly on glass slides. Palynological terminology follows that of Punt et al. (2007) and Hesse et al. (2009).

PAUP* version 4.0b10 (Swofford, 2002) was used for morphological analysis, with parsimony as a criterion to choose the best trees. All characters were unordered and equally weighted (Fitch parsimony, Fitch, 1971). Heuristic searches were performed using the tree-bisection-reconnection (TBR) branch-swapping algorithm with “steepest descent” option and “multrees” options off, 10,000 replicates with random-taxon addition, and 10 trees held in each replicate. All analyses were programmed to retain only 10,000 trees. Robustness of clades was estimated using Bootstrap (Felsenstein, 1985) implemented in PAUP*, using the TBR branch-swapping algorithm with “steepest descent” and “multrees” options off and with 10,000 pseudoreplicates with simple taxon addition.

2.2.2. Molecular analysis

Total genomic DNA was extracted from silica-gel dried leaves (5–10 mg) using the Qiagen DNeasy Plant Mini Kit (Qiagen, California, U.S.A.) according to the manufacturer’s directions. Four regions previously used to infer the phylogenetic relationships in Rutaceae were selected and amplified, two from cpDNA (*trnL-trnF* region and *rps16* intron) and two nDNA (ITS1 and ITS2). The *trnL-trnF* regions were amplified using the universal primers “c” and “f” (Taberlet et al., 1991); *rps-16* intron using primers “rpsF” and “rpsR2” (Oxelman et al., 1997); ITS-1 using primers “18d” and “5.8C”; and ITS-2 using primers “5.8D” and “28CC” (White et al., 1990). PCR reaction mixtures, thermal cycling and purification were carried out after Bruniera et al. (2015). Sequences were assembled and edited using the Geneious, version R7.1.7 (Kearse et al., 2012), followed by manual adjustments with Mesquite version 3.51 (Maddison and Maddison, 2018). A total of 20 sequences were newly generated and deposited in GenBank. *Conchocarpus hirsutus* was not sampled for the molecular analyses, since it is known only by its type collection. Accession numbers of the sequences and their vouchers are listed in [Table 1](#).

Parsimony analysis was performed in PAUP* version 4.0b10 (Swofford, 2002) with the same parameters used in the morphological analysis. Additionally, we performed Bayesian inference analyses in MrBayes version 3.2.6 (Ronquist et al., 2012) using the facility CIPRES Science Gateway (Miller et al., 2010) with the parameters described in Ferreira et al. (2019). Previously to the Bayesian inference, the most appropriate model of sequence evolution for each matrix was selected using the Akaike information criterion (Akaike, 1973) in jModelTest version 2.1.9 (Posada, 2008; Darriba et al., 2012). Selected model was GTR + I + G for all regions. Bayesian and parsimony analyses were performed for each marker alone, and for the combined cpDNA and nDNA sequence data, as well as to all sequences combined into a single matrix.

2.3. Foliar anatomy and flower structure

Besides the morphological and molecular phylogenetic analysis, we investigated the leaf anatomy and the flower structure of the species belonging to the *Conchocarpus gaudichaudianus* group in order to better understand the morphological traits of the species and compare these characteristics with other representatives of *Conchocarpus*. However, the anatomical characteristics of the leaves and the structure of the flowers were not used in the phylogenetic analysis because such data was missing for most taxa in this study.

Leaf anatomy was verified from fully expanded leaves of four species of the *Conchocarpus gaudichaudianus* group, *C. concinnus*, *C. cyrtanthus*, *C. gaudichaudianus* and *C. insignis*, besides the type species of the genus,

Table 1

Voucher information and GenBank accession numbers for sequences produced in this study and those previously published. Herbaria acronyms follow Thiers et al. (2019). Galipeeae, Galipeinae and Pilocarpinae defined according Groppo et al. (2017). * subspecies *gaudichaudianus*.

Species	Herbarium voucher	<i>trnL-trnF</i>	<i>rps16</i>	ITS-1	ITS-2
Tribe Galipeeae – Subtribe Galipeeinae					
<i>Andreadoxa flava</i> Kallunki	Pirani 4973 (SPF)		KP866606	KP866627	
<i>Andreadoxa flava</i> Kallunki	Groppo 1562 (SPFR)	KP866586			KP866649
<i>Conchocarpus albiflorus</i> (Bruniera & Groppo) Bruniera & Groppo	Groppo 1852 (SPFR)	KP866579	KP866600	KP866620	
<i>Conchocarpus albiflorus</i> (Bruniera & Groppo) Bruniera & Groppo	Groppo 1853 (SPFR)				KP866643
<i>Conchocarpus cyrtanthus</i> Kallunki	Groppo 1630 (SPFR)	MK533575	MK533579	MK533596	MK533586
<i>Conchocarpus coerules</i> (Nees & Mart.) Bruniera & Groppo	Bruniera 92 (SPFR)	KP866580	KP866601	KP866621	KP866644
<i>Conchocarpus concinnus</i> Kallunki	Groppo 1610 (SPFR)	KP866587	KP866607		
<i>Conchocarpus diadematus</i> Pirani	Groppo 1634 (SPFR)	MK533576		MK533597	
<i>Conchocarpus gaudichaudianus</i> (A.St.-Hil.) Kallunki & Pirani*	Groppo 1350 (SPFR)	MK533577	MK533580	MK533598	MK533587
<i>Conchocarpus heterophyllus</i> (A.St.-Hil.) Kallunki & Pirani	Groppo 999 (SPF)	KP866588	KP866611	KP866628	KP866653
<i>Conchocarpus insignis</i> Pirani	Groppo 1635 (SPFR)		MK533581	MK533599	MK533588
<i>Conchocarpus macrophyllus</i> J.C.Mikan	Groppo 985 (SPF)	KP866589	KP866612		
<i>Conchocarpus macrophyllus</i> J.C.Mikan	Groppo 1571 (SPFR)			KP866629	
<i>Conchocarpus mastigophorus</i> Kallunki	Groppo 1589 (SPFR)	KP866590	KP866608	KP866630	KP866650
<i>Conchocarpus minutiflorus</i> Groppo & Pirani	Groppo 1617 (SPFR)	KP866591	KP866609	KP866631	KP866654
<i>Conchocarpus odoratissimus</i> (Lindl.) Kallunki & Pirani	Groppo 1540 (SPFR)	KP866592	KP866610	KP866632	KP866651
<i>Conchocarpus pentandrus</i> (A.St.-Hil.) Kallunki & Pirani	Pirani 4996 (SPF)	EU853789	EU853735		
<i>Conchocarpus pentandrus</i> (A.St.-Hil.) Kallunki & Pirani	Groppo 1262 (SPFR)			KP866633	KP866652
<i>Conchocarpus ruber</i> (A.St.-Hil.) Bruniera & Groppo	Bruniera 122 (SPFR)	KP866581	KP866602	KP866622	KP866645
<i>Erythrochiton brasiliensis</i> Nees & Mart.	Groppo 975 (SPF)		KP866613		
<i>Erythrochiton brasiliensis</i> Nees & Mart.	Groppo 1570 (SPFR)	KP866593			KP866655
<i>Galipea jasminiflora</i> (A.St.-Hil.) Engl.	Bruniera 118 (SPFR)	KP866595	KP866616	KP866636	KP866658
<i>Hortia oreadica</i> Groppo, Kallunki & Pirani	Groppo 458 (SPF)	EU853803	EU853750	KP866637	KP866659
<i>Rauia nodosa</i> (Engl.) Kallunki	Pirani 4975 (SPF)	KP866598	KP866619		
<i>Rauia nodosa</i> (Engl.) Kallunki	Groppo 1565 (SPFR)				KP866662
<i>Rauia resinosa</i> (Engl.) Kallunki	Pirani 4932 (SPFR)	MK533578	MK533582	MK533602	MK533591
<i>Ravenia spectabilis</i> (Lindl.) Engl.	Groppo 1514 (SPFR)	KP866618	KP866618	KP866641	
Tribe Galipeeae – Subtribe Pilocarpinae					
<i>Esenbeckia febrifuga</i> (A.St.-Hil.) Juss. ex. Mart.	Groppo 1577 (SPFR)	KP866594	KP866614	KP866634	KP866657
<i>Esenbeckia grandiflora</i> Mart.	Groppo 1149 (SPF)	EU853795	KP866615		
<i>Esenbeckia grandiflora</i> Mart.	Groppo 1827 (SPFR)			KP866635	KP866656
<i>Metrodorea nigra</i> A.St.-Hil.	Groppo 1111 (SPF)	EU853809	EU853757		
<i>Pilocarpus spicatus</i> A.St.-Hil.	Pirani 4995 (SPF)		EU853761		
<i>Pilocarpus spicatus</i> A.St.-Hil.	Groppo 1520 (SPFR)	KP866597		KP866639	KP866661
Tribe Zanthoxyleae					
<i>Zanthoxylum rhoifolium</i> Lam.	Groppo 1145 (SPF)	EU853773	EU853720	KP866642	KP866663

C. macrophyllus. Leaves were collected and fixed in formaldehyde-acetic acid (FAA) 50% for 48 h (Johansen, 1940) and then stored in 70% alcohol. Samples of the leaf midrib, intercostal area, and margin were dehydrated through an ethanol series and infiltrated with hydroxy-ethyl methacrylate resin (Leica Histo-resin). Transversals sections of these samples were then made with a rotary microtome at a thickness of 8 µm and then stained with 0.05% toluidine blue (O'Brien et al., 1964). To analyze the epidermis and stomata, 4 mm² samples were placed in a mixture of 1:1 of glacial acetic acid (96%) and hydrogen peroxide (30%) at 60 °C for about 12 h, then rinsed in distilled water, and stained with 1% safranin (Foster, 1949). Slides were prepared using a semi-permanent method with 50% glycerin (Purvis et al., 1964). Observations and photographic documentation were made with a digital camera connected to a Leica DM1000 light microscope. Images were digitally processed using a Leica Image Management application software (IM50). Vouchers are listed in the Supplementary material (Appendix 1)

Floral structure (i.e., morphology, anatomy and histology) of two species of the *Conchocarpus gaudichaudianus* group (*C. concinnus* and *C. cyrtanthus*), plus other species of *Conchocarpus* not belonging to this group (listed in the Supplementary material, Appendix 1) were comparatively analyzed. For such, floral features were compiled from El Ottra et al. (2013, 2015, 2019). Additionally, new data on floral features are herein described for *C. macrophyllus*, *C. heterophyllus*, and *C. minutiflorus*, in order to better characterize the variation within *Conchocarpus* and to facilitate the comparison with the species of the *Conchocarpus gaudichaudianus* group. Floral buds from these three species at different stages of development and anthetic flowers were collected, fixed in FAA 50% (Johansen, 1940), and stored in 70% ethanol.

Floral structure of *C. heterophyllus*, *C. macrophyllus*, and *C. minutiflorus* was analyzed under stereomicroscope (Leica M125), light microscopy (model Leica DM4000), and scanning electron microscopy (SEM; model Zeiss DMS-940). For SEM studies, the fixed material was dissected, dehydrated in an ethanol series, and critical-point dried. The floral organs were mounted on stubs, sputter-coated with gold, and subsequently analyzed. For light microscopy, the material was dehydrated in an ethanol-butanol series and then infiltrated and embedded in paraffin or paraplast (based on the protocol of Johansen, 1940). The embedded material was sectioned using a rotary microtome Leica RM 2145 and a standard microtome knife D. The sections were stained with astra blue 1% and safranin 1% in ethanol 50% (following the protocol of Bukatsch, 1972), and mounted in Permount. Permanent slides of the microtome sections were deposited at the Instituto de Biociências, Universidade de São Paulo (IB-USP). Descriptions of organs were mostly made from the top of the floral bud, downwards to the floral base. Photomicrographs were taken with a Leica DFC 425 digital camera coupled with the microscope.

3. Results

3.1. Phylogenetic analysis: Morphological characters

Parsimony analysis of the 35 morphological characters produced 98 equally parsimonious trees, with 116 steps (Consistence Index = 0.39, Retention Index = 0.68, Table 2). The strict consensus tree (not shown) resulted in a polytomy with no resolution within the Galipeinae. The majority-rule consensus tree (Fig. 2) showed a better resolution and supported the monophyly of subtribe Galipeinae (Bootstrap Percentage,

Table 2

A summary of the results in the parsimony analyses. PIC = parsimony informative characters; N. trees = Number of equally parsimonious trees; CI = Consistency Index; RI = Retention Index.

	N Characters	N PIC	% PIC	N. trees	N steps	CI	RI
Morphological analyses	35	33	94.2	98	116	0.39	0.68
Molecular analyses							
ITS-1	451	135	29.9	4	507	0.61	0.71
ITS-2	365	131	35.8	9	532	0.57	0.69
rps16	901	129	14.3	270	520	0.72	0.66
trnL-trnF	1117	107	9.5	81	432	0.77	0.67
Combined analyses							
rps16 + trnL-trnF	2018	236	11.7	168	964	0.74	0.65
ITS-1 + ITS-2	816	266	32.5	2	1058	0.58	0.69
rps16 + trnL-trnF + ITS-1 + ITS-2	2834	502	17.7	4	2039	0.65	0.66
Morphology + Molecular	2869	535	18.6	4	2174	0.63	0.66

BP = 74%) and of the five species of the *Conchocarpus gaudichaudianus* group (BP = 61%), of which the latter formed a larger clade with *C. diadematus* and *C. macrophyllus*, but with BP lower than 50%; however, *Conchocarpus* appeared as unresolved. Morphological synapomorphies supporting the *Conchocarpus gaudichaudianus* group as monophyletic include a straight embryo, an umbilicate and glabrous ovary, plano-convex (not conduplicate or plicate) cotyledons, a semi-inferior ovary, and a nectariferous disc thicker toward the base (see Fig. 3).

3.2. Phylogenetic analysis: Combined molecular datasets

Analysis of combined molecular markers retrieved 168 most parsimonious trees (Table 2). Galipeinae (Fig. 4) appear as monophyletic (BP = 78%, Posterior Probabilities, PP = 0.97), with *Hortia* sister to the rest of the subtribe. Three internal clades of Galipeinae emerged in a polytomy, the first (BP = 87%, PP = 1) with all the species of the *Conchocarpus gaudichaudianus* group plus species of *Andreodoxa*, *Angostura*, *Erythrochiton*, *Galipea*, and *Ravenia*, the second with all remaining *Conchocarpus*, i.e., the *C. sensu stricto* group (BP lower than 50%, but PP = 1), and the third with only *Ravenia*. Trees resulting from individual analyses of each marker and of combined plastidial and combined nuclear markers are in the Supplementary Material (Appendix 3). Matrices for all datasets are presented in Appendix 4 (Supplementary Material).

3.3. Phylogenetic analysis: Combined morphological and molecular dataset

Combined molecular and morphological matrices were combined in order to better explore the results, because some authors advocate that combining datasets of different natures (e.g., molecular and morphological) can provide the best explanatory power in phylogenetic analyses (see Nixon, 1996; Smith, 2000).

The topology of the strict consensus tree of the four equally parsimonious trees (Fig. 5) is congruent with that obtained in the combined molecular parsimony analysis (Fig. 4) and in the Bayesian analysis. The monophyly of Galipeinae is strongly supported (BP = 100%, PP = 1), *Hortia* appears as sister to the remaining Galipeinae (BP = 75%, PP = 0.95), and the polyphyly of *Conchocarpus* is again illustrated by two strongly supported clades of species—the *Conchocarpus sensu stricto* group (BP = 99%, PP = 1) and the *Conchocarpus gaudichaudianus* group (BP = 99%, PP = 1). The latter again appears in a larger clade (BP = 92, PP = 1) with the rest of the Galipeinae included in this study, i.e., *Andreodoxa*, *Angostura*, *Erythrochiton*, and the clade *Galipea* + *Rauia*.

3.4. Foliar anatomy

The mesophyll is dorsiventral in all of *Conchocarpus* analyzed, formed by one layer of palisade parenchyma and 10–15 layers of spongy parenchyma. In the margin, the spongy parenchyma cells form a compact tissue (Fig. 6C), and in *C. macrophyllus* it also presents a fiber bundle (Fig. 6D). Xylem and phloem of major bundles are sheathed by fibers, small bundles are sheathed by fibers only beside the phloem, and tiny xylem bundles are surrounded only by endodermis (Fig. 6E).

In transverse sections, the midrib is flat or slightly convex adaxially and strongly convex abaxially in all five species (Fig. 7A and B). Below the epidermis or periderm, three to five layers of angular colenchyma are observed in the abaxial face of the midrib (Fig. 7C), except in *Conchocarpus macrophyllus*, whose cortical parenchyma is formed by smaller cells with slightly thick walls (Fig. 3B). In *C. macrophyllus*, below the epidermis of the adaxial surface, a layer of palisade parenchyma continuous with the mesophyll is observed (Fig. 7B). Vascular system of the midrib is a ring formed by collateral bundles compactly organized (Fig. 7A, B and D). In *C. cyrtanthus*, two concentric vascular bundles face the adaxial surface; in *C. insignis*, 3–4 medullary phloematic bundles (Fig. 7E); and in *C. macrophyllus*, 2–3 medullary collateral bundles (Fig. 7B and F). Idioblasts containing calcium oxalate crystals occur in leaf blade and petiole, in form of druses, raphides (Fig. 6H), and prismatic crystals.

3.5. Floral structure of *Conchocarpus heterophyllus*, *C. macrophyllus* and *C. minutiflorus*

As the three species of *Conchocarpus* analyzed here are not of the *Conchocarpus gaudichaudianus* group, but instead from *Conchocarpus sensu stricto*, we chose to place the descriptions and figures in the Supplementary Material (Appendix 2), which include a detailed description of the floral structure, as well the corresponding figures, for the three species. Those findings are compared with other genera of Galipeinae (mainly with species of *Conchocarpus*) in the Discussion section.

4. Discussion

Phylogenetic trees from the combined data analysis (molecular markers plus morphology) showed a better resolution at all levels. Bayesian analysis of the combined dataset (as a well as in all analyses) recovered more supported clades and is congruent with results obtained from parsimony analysis. Because the combined matrix contains all taxa, including *Conchocarpus hirsutus* (sampled only in the morphological analysis), we choose to discuss our phylogenetic, morphological, and anatomical results on the basis of the Bayesian analysis of the combined morphological plus molecular matrix (Fig. 5).

4.1. Circumscription of *Conchocarpus*

A detailed discussion of the Galipeinae and its putative synapomorphies, as well the occurrence of reversals in some morphological characteristics, were already presented by Bruniera et al. (2015). Results obtained by Bruniera et al. (2015) showing *Conchocarpus* as a polyphyletic group were confirmed in the present study, which is not surprising because the same molecular markers were used in both studies. Nevertheless, an ongoing phylogenetic study of the entire tribe Galipeae, with an even larger sampling of *Conchocarpus* (Groppo et al., 2017), has also shown that *Conchocarpus* is polyphyletic in that it included the same two groups, *Conchocarpus sensu stricto* and the *Conchocarpus gaudichaudianus* group.

The positions of the *Conchocarpus gaudichaudianus* group and the *Conchocarpus sensu stricto* group in two separate clades was predicted by the position of *C. concinnus* in the phylogeny of Bruniera et al. (2015). The five species of the *Conchocarpus gaudichaudianus* group, all endemic to the Brazilian Atlantic Forest, share a combination of morphological

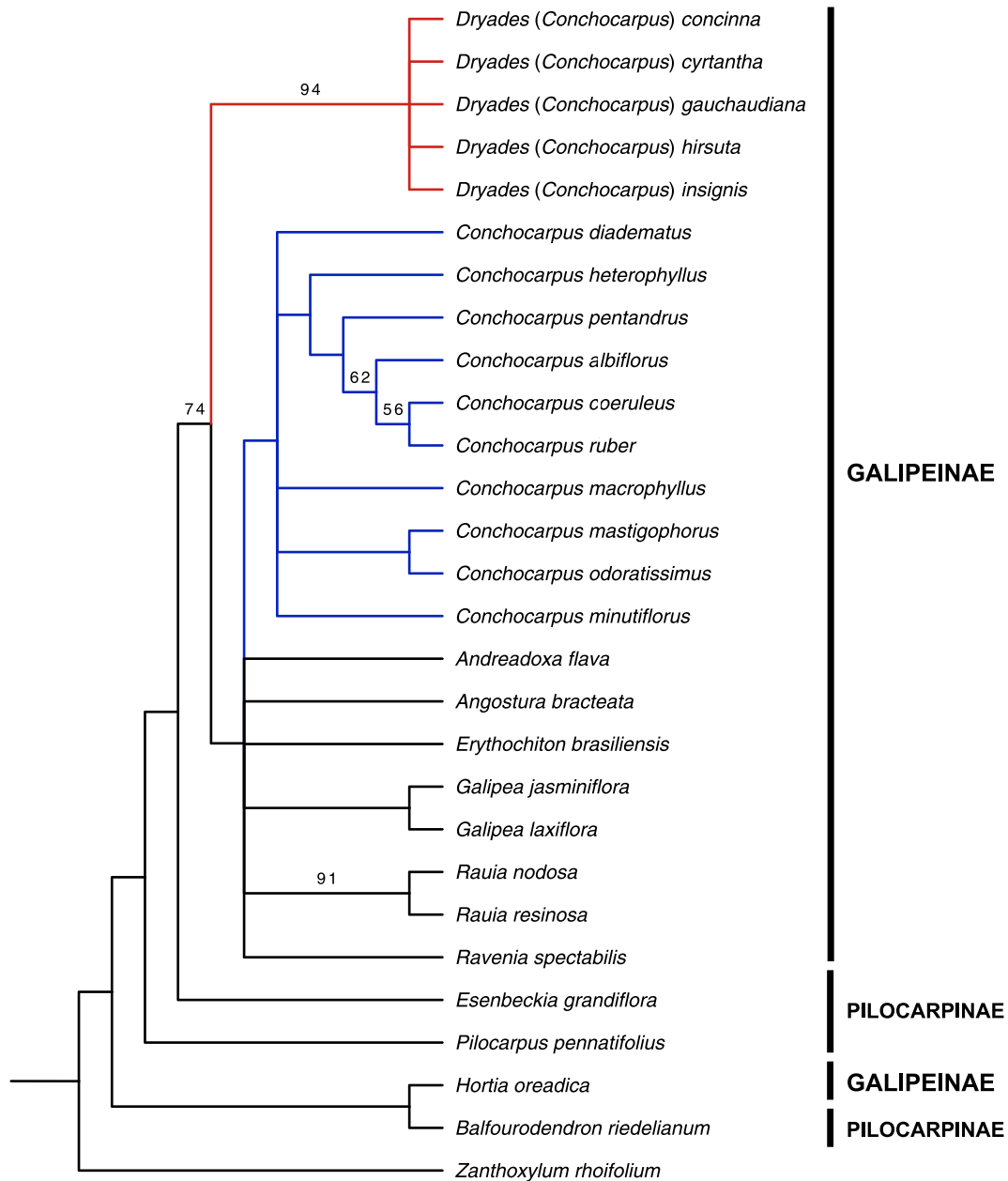


Fig. 2. Majority-rule consensus tree of 98 equally parsimonious trees resulting from analysis of morphological data (35 characters, 116 steps, CI = 0.39, RI = 0.68). Bootstrap percentages ($\geq 50\%$) are given above the branches only when clades in majority-rule consensus agree with bootstrap consensus tree. Branches in the “*Conchocarpus sensu stricto* group” in blue, those from “*Conchocarpus gaudichaudianus* group” (*Dryades*, see text) in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

characteristics that include almost free petals, sepals overlapping at anthesis, a glabrous ovary, and a straight embryo with plano-convex, unequal cotyledons (see Fig. 3). These species also share flowers with an umbilicate ovary, a characteristic also found in some representatives of the *Conchocarpus sensu stricto* group, as in *C. diadematus*, but in this species the ovary is pubescent instead of glabrous (Fig. 5). The possession of a straight embryo without conduplicate-plicate cotyledons is synapomorphic of the *Conchocarpus gaudichaudianus* group clade, as the Galipeinae typically bear curved embryos with conduplicate-plicate cotyledons, a synapomorphic trait of the subtribe (Bruniera et al., 2015). Straight embryos with non-folded cotyledons are present in other groups in the tribe Galipeeae, as the Pilocarpinae (as *Esenbeckia* and *Pilocarpus*, sampled here), in *Hortia* (recognized as Galipeinae by Groppo et al., 2017), and also in more distant rutacean groups, such as *Zanthoxylum*; thus the presence of straight, not folded embryos in the

representatives of the *Conchocarpus gaudichaudianus* group could represent a reversion. However, the embryo in the *Conchocarpus gaudichaudianus* group, with its unequal cotyledons, is different from those found in the Pilocarpinae, *Hortia* and in *Zanthoxylum*, all with equal cotyledons (compare Fig. 3A-C). On the other hand, the appendages at the base of the anthers (see Bruniera et al., 2015) is synapomorphic for the clade formed by the species of *Andreadoxa*, *Erythrochiton*, *Galipea*, *Rauia*, and the *Conchocarpus gaudichaudianus* group, but appendages are lacking in the two latter (perhaps through two independent reversals). Because the present study included only eight of the 27 genera of the Galipeinae and only 10 of about 50 of the *C. sensu stricto* group, analyses of a broader sample of the taxa are required to understand the floral evolution and synapomorphic features of the Galipeinae as a whole, and of *Conchocarpus* in particular.

The relations among the five species of the *Conchocarpus*

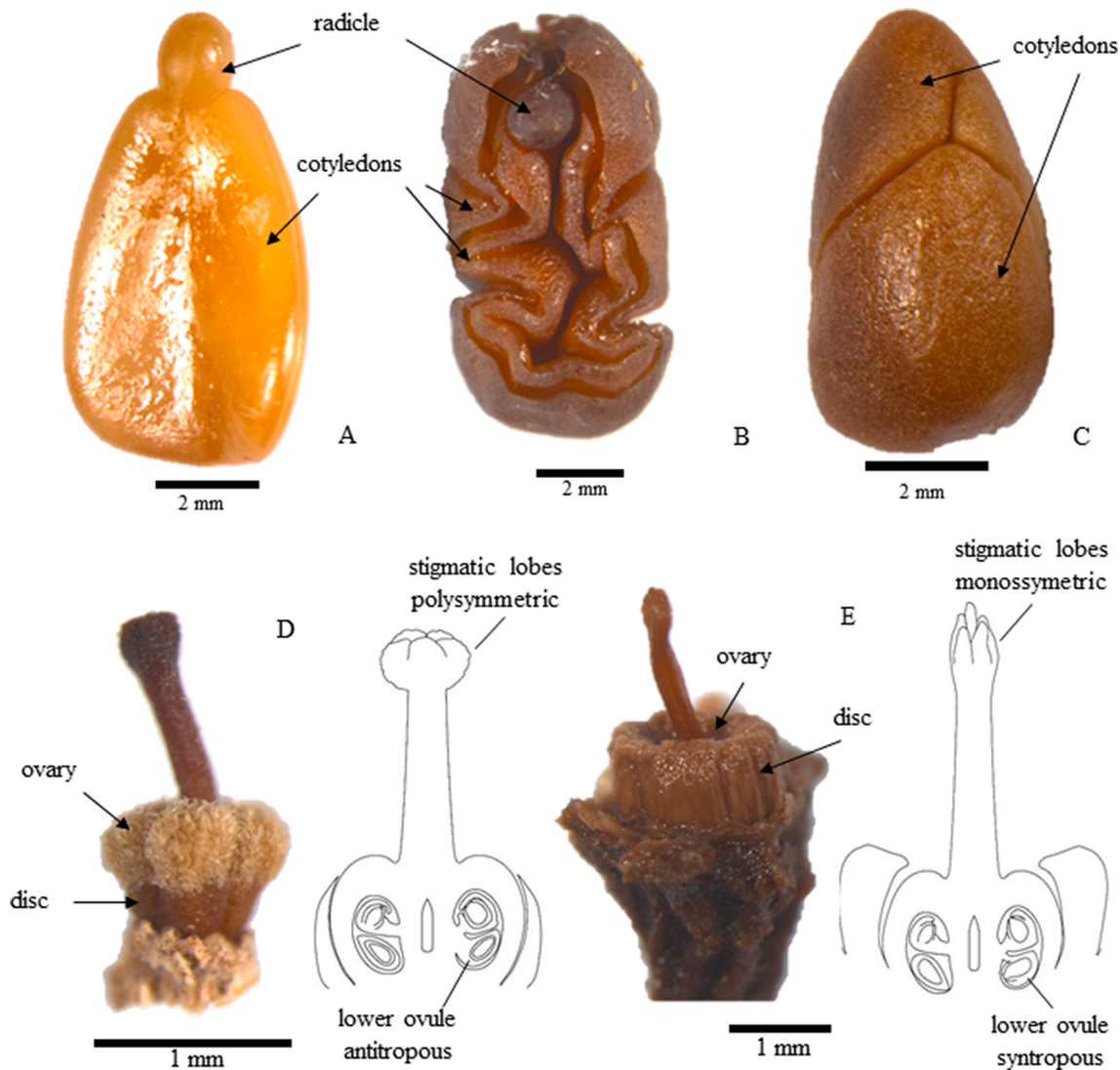


Fig. 3. Some traits of the embryos and flowers used in the morphological analysis and/or discussed in the text. A-C. Morphology of the embryos. A. *Hortia oreadica*, embryo with equal, straight cotyledons. B. *Conchocarpus diadematus* (from *Conchocarpus sensu stricto* group), embryo with curved, conduplicate-plicate (folded) cotyledons, a synapomorphy to most Galipeinae. C. *Dryades (Conchocarpus) concinna* (from *Dryades*, = *Conchocarpus gaudichaudianus* group, see text), embryo with unequal, non-folded, straight cotyledons. D-E. Comparison of some characteristics of the gynoecium, and nectary disc in a representative of the *Conchocarpus sensu stricto* (*Conchocarpus diadematus*) and another from the *Conchocarpus gaudichaudianus* (= *Dryades*, see text) group (*Dryades (Conchocarpus) insignis*). D. *Conchocarpus diadematus*, gynoecium with a superior, pubescent ovary, sketch at right showing polysymmetric stigmatic lobes carpels with upper and lower ovules antitropous, disc thicker toward the base, not inflexed. E. *Dryades (Conchocarpus) insignis*, gynoecium with a glabrous, partially inferior ovary, sketch at right showing monosymmetric stigmatic lobes, carpels with upper ovule antitropous, and lower ovule syntropous and disc thicker toward the base, not inflexed, recovering most part of the ovary.

gaudichaudianus group are still ambiguous because the hypothesized relationships within this clade change depending on the kind of data analyzed, i.e., plastid or nuclear markers or morphological characters. Also, support of the clades, in terms of bootstrap percentages and posterior probabilities, are weak or ambiguous. Analysis of a faster-evolving molecular marker and inclusion of *C. hirsutus* are needed to address the affinities of the species of this clade.

4.2. Foliar anatomy

We found some distinctions that differentiate species of the *Conchocarpus gaudichaudianus* group from *C. macrophyllus*, the type species of the genus, which belongs in the *Conchocarpus sensu stricto* group. The most striking differences are the presence in *C. macrophyllus* of a fiber bundle along the margin of the leaf blade and a layer of palisade parenchyma on the adaxial surface of the midrib, two characters that were

not found in the analyzed species of the *Conchocarpus gaudichaudianus* group (Fig. 6). An ongoing study of foliar anatomy of a broader sample of Galipeinae has shown that the presence of a fiber bundle along the foliar margin is common in the *C. sensu stricto* group and that the presence of a layer of palisade parenchyma on the adaxial surface of the midrib is restricted to *C. macrophyllus*. Thus, the presence or absence of this fiber bundle could represent a good trait to distinguish these two groups (Table 3). Further studies in foliar anatomy will show whether these, or other, anatomical characters can be treated as synapomorphies of both the *Conchocarpus sensu stricto* and *Conchocarpus gaudichaudianus* group clades.

4.3. Floral structure

Examination of the floral structure revealed several characteristics that distinguish between the two clades of *Conchocarpus*. Based on the

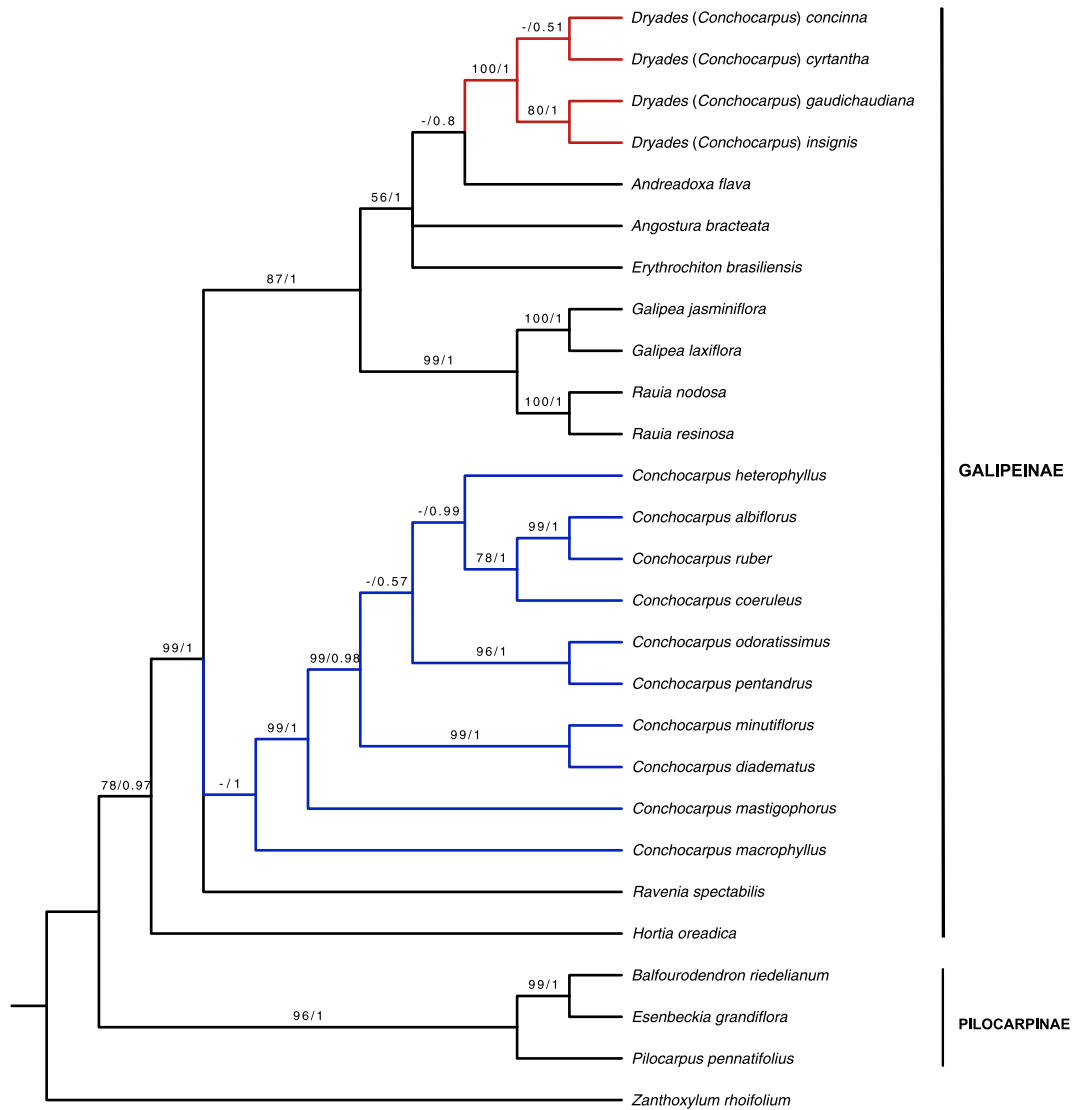


Fig. 4. Majority-rule consensus tree estimated using Bayesian inference resulting from an analysis of the combined data from plastid and nuclear markers (*trnL-trnF*, *rps16*, ITS-1, and ITS-2) of *Conchocarpus* and other Galipeinae. Support for branches is given by Bayesian posterior probabilities and parsimony bootstrap percentages ($\geq 50\%$), those shown only when clade in parsimony analysis agree with the results from Bayesian inference tree, when only a number appears supporting a clade it refers to Bayesian posterior probabilities. Branches in the “*Conchocarpus sensu stricto* group” in blue, those from “*Conchocarpus gaudichaudianus* group” (= *Dryades*, see text) in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

data produced here and in previous comparative work on floral structure of Galipeinae, we have found features that are shared by all (or most) species of *Conchocarpus sensu stricto* while others are particular to species of the *Conchocarpus gaudichaudianus* group. Besides the characteristics used in the phylogenetic analyses, the morphology of the calyx, gynoecium, and disc, as well as some histological characters, further support the monophyletic groups found in phylogenetic analyses and the segregation of part of *Conchocarpus* in another clade (summarized in Table 3). Papillose anthers are likely a synapomorphy of the *Conchocarpus sensu stricto* clade, thought there is some variability in this feature within the genus. In some species, the epidermal cells of anthers do not grow into bullate cells, or co-occur with papillae, while in others, the papillae are well developed and even contribute to the postgenital connection among anthers (*C. ruber*, El Ottra et al., 2016, 2019). Other Galipeinae with similar anther features were found only in *Rauia* and *Ravenia* spp. A disc thicker toward the base and not inflexed is likely a plesiomorphy of *Conchocarpus sensu stricto*, since this feature is shared with most of the Galipeinae, but not with *Conchocarpus gaudichaudianus*, *C. insignis*, *C. cyrtanthus*, and *C. concinnus* (Kallunki and Pirani, 1998; El

Ottra et al., 2013, 2019, see Fig. 5E). The disc features shared among these latter species represent putative synapomorphies of the *Conchocarpus gaudichaudianus* group (Table 3). A partially inferior ovary, with integument reduction (i.e., the outer integument is seen only distally in the convex side of the ovule, or only in part of it) and syntropous lower ovules, is also another putative synapomorphy of this group, since all other Galipeinae have a superior ovary with well-developed bitegmic and antitropous ovules (Supplementary Material, Appendix 2, Fig. 3A, B). Elsewhere in Rutoideae (*sensu* Groppo et al., 2012) an inferior ovary evolved homoplastically in *Hortia oreadica* (formerly in Toddalioidae) and *Coleonema* (Diosmeae of Engler, 1931), while unitegmic ovules were reported only for *Glycosmis* (Aurantieae of Groppo et al., 2008), and syntropous lower ovules only for *Dictamnus* (Diosmae; Engler, 1931; Gut, 1966; Boesewinkel and Bouman, 1978; Jardin, 1984; Ramp, 1988; Souza et al., 2003; Kubitzki et al., 2011; El Ottra et al., 2013; El Ottra, 2014; El Ottra et al., 2019).

Stigmatic features are variable among *Conchocarpus* species and the two predominant types found here occur elsewhere in Galipeinae (Table 3). Capitate polysymmetric stigmas with a rugose surface with

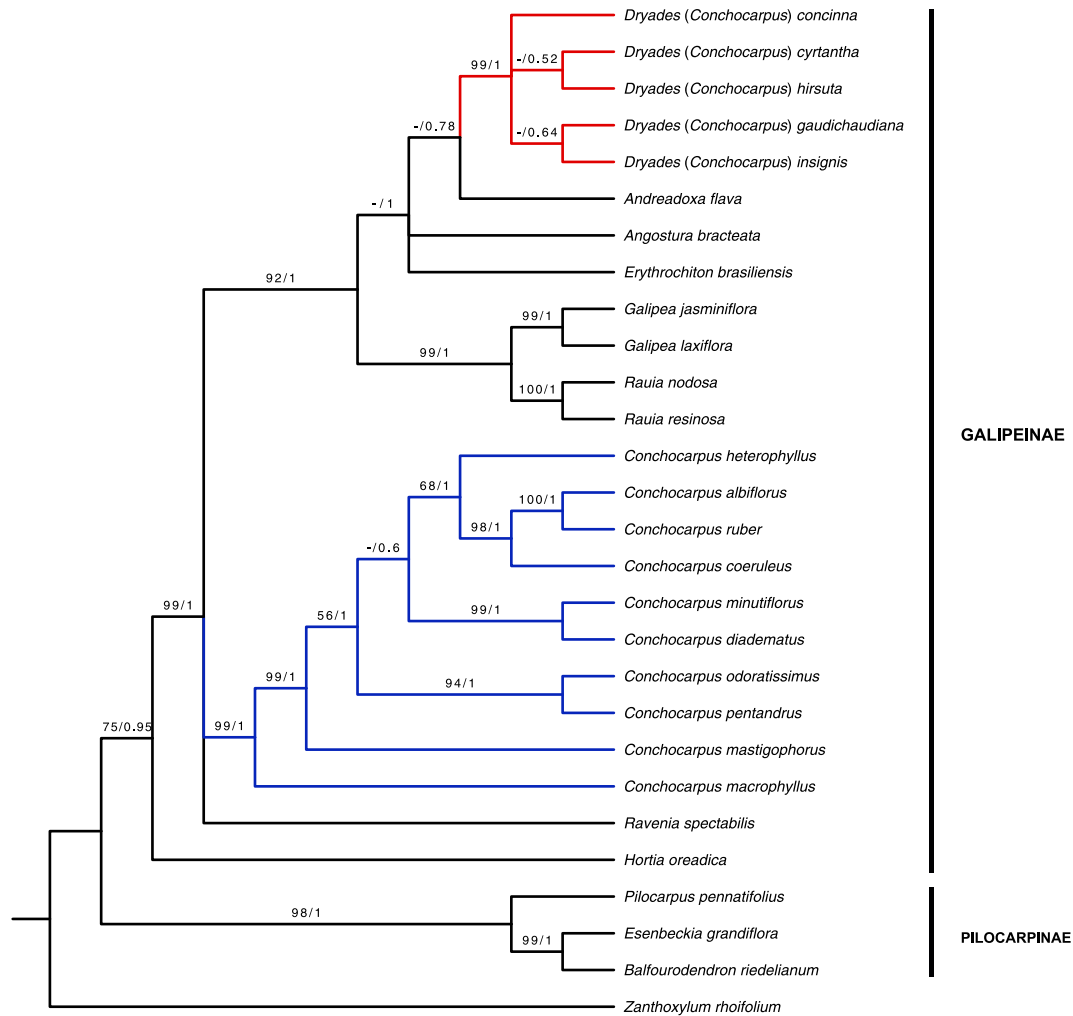


Fig. 5. Majority-rule consensus tree estimated using Bayesian inference resulting from an analysis of the combined data from plastid and nuclear markers (*trnL-trnF*, *rps16*, ITS-1, and ITS-2) plus morphology of *Conchocarpus* and other Galipeinae. Support for branches is given by Bayesian posterior probabilities and parsimony bootstrap percentages ($\geq 50\%$), those shown only when clade in parsimony analysis agree with the results from Bayesian inference tree, when only a number appears supporting a clade it refers to Bayesian posterior probabilities. Branches in the “*Conchocarpus sensu stricto* group” in blue, those from *Conchocarpus gaudichaudianus* (= *Dryades*, see text) group in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

short bullate cells occur in most species of *Conchocarpus sensu stricto* and in the monotypic *Sigmatanthus*. In contrast, stigmas with smooth surface with columnar cells occur not only in species from the *Conchocarpus gaudichaudianus* group, but also in *Angostura bracteata* (Nees & Mart.) Kallunki, *Neoraputia alba* (Nees & Mart.) Emmerich ex Kallunki, *N. trifoliata* (Engl.) Emmerich ex Kallunki, and in the monotypic *Raputiarana*. Except for *Raputiarana*, the above-mentioned taxa, including *Sigmatanthus*, also share monosymmetric stigmas due to stigmatic lobes of unequal size of (Fig. 3, El Ottra, 2014, El Ottra et al., 2016, 2019).

Tanniferous cells are commonly found dispersed in the epidermis and/or mesophyll of vegetative and floral organs in representatives of Galipeinae, as usually found in other Rutaceae (Ramp, 1988; El Ottra, 2014; El Ottra et al., 2019). However, these cells form an extensive tissue only in some parts of the flowers and not in all taxa of the subtribe. Specifically, in *Conchocarpus* an extensive tanniferous tissue was found at the upper thickened dorsal walls of the ovary only in *C. concinnus* and *C. cyrtanthus* (El Ottra et al., 2019) and, thus, is likely another synapomorphy for the *Conchocarpus gaudichaudianus* group. Elsewhere in Galipeinae, a similar feature occurs in *Rauia* and *Raputiarana* species. The occurrence of larger amounts of tanniferous tissue in this pair of *Conchocarpus* species could be functionally related to the absence of indumentum covering the ovary in these taxa. Both trichomes and tannins are considered protective to plants in relation to the activity of

herbivores (Rosenthal and Kotanen, 1994; Wagner et al., 2004) and could be considered equivalent in function. In fact, in all other analyzed species of *Conchocarpus sensu stricto* ovaries with indumentum have lesser amounts of tannins (El Ottra et al., 2013, 2019; El Ottra, 2014).

The type species of *Conchocarpus*, *C. macrophyllus*, has some floral features that differ from those of all other *Conchocarpus*. Particularly, a thick cover of glandular multicellular peltate trichomes on the calyx and ovary, a long papillate clavate stigma, and large intercellular spaces in the mesophyll of the tip of petals occur exclusively in the type species and not in other species of *Conchocarpus*. The long branch length of *C. macrophyllus* in previous phylogenies (see Bruniera et al., 2015) may indicate that these differences are the results of a larger morphological and molecular divergence in evolutionary time in relation to other *Conchocarpus* species. Additionally, a floral tube formed by the congenital union of filament to petals at their lower half makes the type species more similar to the *Conchocarpus gaudichaudianus* group than to any known species of *C. sensu stricto* (Table 3). This resemblance is likely caused by a homoplastic pattern of evolution in the type of synorganization of stamens with petals within the Galipeinae, given the variability of this character and its distribution among different taxa of the subtribe. For instance, a floral tube formed exclusively by the coherence of petals and adherence of filaments to petals throughout its length is likely a synapomorphy of the clade comprising all species of *Conchocarpus sensu*

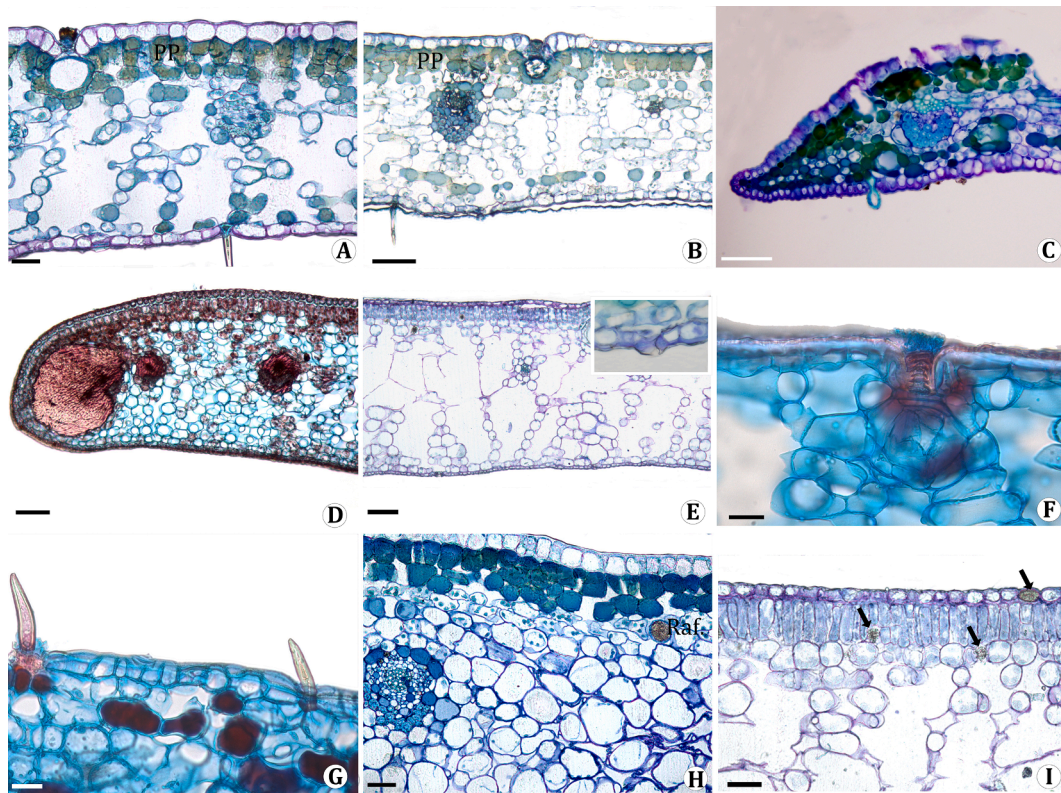


Fig. 6. Transverse sections of the intercostal area and margin of leaf blade of three species of *Conchocarpus gaudichaudianus* group (=Dryades, see text) and *C. macrophyllus* (*Conchocarpus sensu stricto* group). A: dorsiventral mesophyll of *Dryades* (*Conchocarpus*) *concinna*. B: dorsiventral mesophyll of *Dryades* (*Conchocarpus*) *cyrtantha*. C: margin of *Dryades* (*Conchocarpus*) *insignis*. D-I: *C. macrophyllus*. D: leaf margin. E: dorsiventral mesophyll, with a detail of stoma. F: glandular trichome. G: unicellular non glandular trichome. H: raphides in the mesophyll. I: mesophyll. Scale bars: 30 μm (F), 50 μm (A,C,H,I), 100 μm (B,D,E,G). Arrows calcium oxalate crystals, PP palisade parenchyma, Raf. raphides.

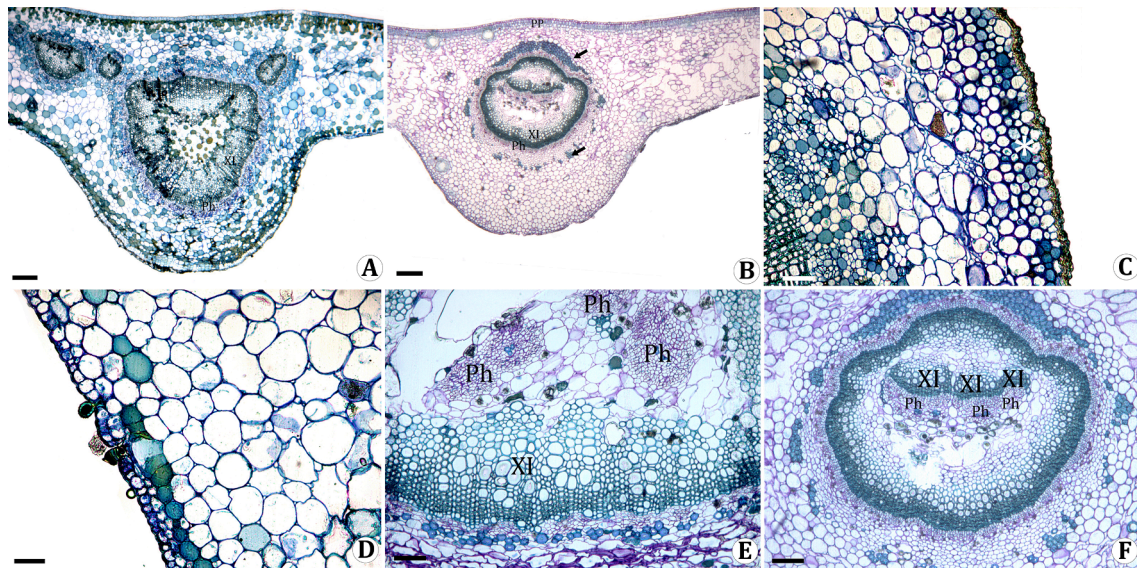


Fig. 7. Leaf midrib transverse sections of three species of *Conchocarpus gaudichaudianus* group (=Dryades, see text) and *C. macrophyllus* (*Conchocarpus sensu stricto* group). A: Midrib section of *Dryades* (*Conchocarpus*) *cyrtantha*. B: Midrib section of *C. macrophyllus*. C: Midrib cortical region of *Dryades* (*Conchocarpus*) *gaudichaudiana* subsp. *gaudichaudiana*. D: Trichomes. E: Medullary phloem bundles in *C. insignis*. F: Medullary collateral bundles in *C. macrophyllus*. Scale bars: 50 μm (C,D), 100 μm (E,F), 200 μm (A,B). Arrows fibers, asterisk collenchyma layers, XI xylem, Ph phloem.

stricto except for *C. macrophyllus*, but it also occurs in the clade of *Rauia* spp. and in *Neoraputia*. In contrast, the type of synorganization found in the corolla and stamens of *Conchocarpus gaudichaudianus* group also occur in *Galipea* spp., *Ravenia spectabilis* and *Sigmatanthus* (Pirani et al.,

2010; El Ottra et al., 2013, 2019; El Ottra, 2014). A study on floral evolution of Galipeinae is necessary to corroborate all the above-mentioned hypotheses.

Table 3

Leaf and floral features shared by most species of *Conchocarpus sensu stricto* compared with the *Conchocarpus gaudichaudianus* group (= *Dryades*, see text for a detailed discussion).

<i>Conchocarpus sensu stricto</i>	<i>Conchocarpus gaudichaudianus</i> group
Fiber bundle along the margin of the leaf blade	Fiber bundle absent
Triangular sepal lobes, not overlapped at anthesis	Rounded or obtuse sepal lobes, overlapped at anthesis
Floral tube formed by the coherence of petals and adherence to filaments to petals throughout its length	Floral tube formed by the adnation of staminal filaments to the petals, up to their upper half or up to their lower half
Papillose anthers	Non-papillose anthers
Disc thicker toward the base, not inflexed	Disc thicker toward the apex, inflexed
Superior ovary	Partially inferior ovary
Stigmatic lobes polysymmetric	Stigmatic lobes monosymmetric
Stigma with a rugose surface and bullate cells	Stigma with smooth surface with columnar cells
Ovules with two distinct integuments	Ovules with integument reduction
Carpels with upper and lower ovules antitropous	Carpels with upper ovule antitropous, and lower ovule syntropous
Tanniferous cells disperse in the dorsal region of the ovary	Extensive tanniferous tissue in the dorsal region of the ovary
Druses as cristal idioblasts, raphides absent	Raphides as cristal idioblasts, rarely druses

4.4. Biogeography and dispersal

The recognition of the *Conchocarpus gaudichaudianus* group as a new genus *Dryades* (see “taxonomic implications” below) establishes yet another taxon that is endemic to the Atlantic Forest, a domain rich in endemisms and recognized as a biodiversity hotspot (according to the definition of Myers et al. (2000)). *Dryades* is the first genus with more than one species (*Andreodoxa*, also endemic to this domain, is monotypic) in the subtribe Galipeinae that occurs exclusively in the Atlantic Forest. Several genera of Galipeinae that also occur in the Brazilian Atlantic Forest but are more common elsewhere, such as Amazonia, include *Angostura* (seven species, one endemic to the Atlantic Forest), *Erythochiton* (six species, one endemic), *Ravenia* (14 species, one endemic), and *Conchocarpus sensu stricto* (34 species), the most diverse group of Galipeinae in this domain.

Dryades as a genus is widely but irregularly distributed in the Atlantic Forest (see Fig. 8). All five species occur in the Southern Bahian Moist Forest where *D. concinna*, *D. hirsuta*, and *D. gaudichaudiana* var. *bahiensis* are endemic but poorly represented elsewhere in the Atlantic Forest, especially at its northern and southern limits. Southern Bahia was proposed as center of plant endemism in the 1980s (e.g. Prance 1982) and has been identified since as an area of endemism of many groups of both animals and plants (e.g. Silva et al. 2004; Sigrist and Carvalho 2009; DaSilva et al. 2017, see Martini et al. 2007 for a general view). More recently, Colli and Pirani (2019) hypothesized that this region was a center of endemism of Galipeinae (the subtribe to which *Dryades* belongs). Based mostly on the distributions of species they identified two minor areas of endemism nested in the Southern Bahian Moist Forest, mostly represented by species of *Conchocarpus* (including also the species of *Dryades*), and suggested that this region is a putative center of origin of some lineages of that genus, and discussed vicarious events that may have influenced speciation processes. Additional biogeographic and new phylogeographic studies focused on the Galipeinae as a whole may suggest answers to questions about the center of origin and historical distribution of *Dryades* and other lineages in the subtribe.

The fruits of *Dryades* are formed by 1–5 follicular mericarps with a papery endocarp which dettachs from the rest of the pericarp, expelling the seeds (1 per carpel) as it opens. This kind of autochoric dispersal is similar to that in the castor bean (*Ricinus* spp., Euphorbiaceae) and is found throughout the subtribe Galipeinae as well as in the tribe Pilocarpinae (e.g. *Esenbeckia*, *Metrodoea*, and *Pilocarpus*) in in other

Rutaceae such as those of the tribe Boronieae (Australasia). This dispersal mode is abiotic, and the distance to which a seed can be ejected is limited. This limitation in the reach of the seeds may be greater for species of shrubs or treelets, like *Dryades* (and in other Galipeinae), that occur in the understory of forested areas and is likely to be one of the factors that explain the frequently gregarious distribution of individuals of these species in forests. Studies on the morphology and ontogeny of the fruit in the subtribe Galipeae (Alonso et al., in preparation) will help to clarify the evolution of this type of dispersal in *Dryades* and other Galipeinae.

5. Conclusions

The complexity in the internal relationships in the subtribe Galipeinae is being addressed and partially solved step by step by recent phylogenetic studies. The present analysis focused on a group of five species endemic to the Brazilian Atlantic Forest each previously assigned to *Conchocarpus*—the *Conchocarpus gaudichaudianus* group—and described it as a new genus *Dryades* (see below) as monophyletic and separate from *Conchocarpus*, by far the largest genus in the subtribe. Additionally, morphological data, including novel features of floral anatomy, indicate that some of the characters shared by all five species of *Dryades* could be synapomorphic for the newly recognized genus. The ongoing investigative phylogenetic studies of Galipeinae conducted by the authors shall shed light on the genealogical history of the group, lead to a better circumscription of its internal taxa, contribute hypotheses about the evolution of structural characters and biogeographic history, especially in the Brazilian Atlantic Forest, where the subtribe is more diversified.

Implications for nomenclature

The phylogenetic position of the species *Conchocarpus gaudichaudianus* group, separated from the *Conchocarpus sensu stricto* group, requires recognition of the former as a new genus. Because none of the species of the *Conchocarpus gaudichaudianus* group has been previously assigned to a distinct genus, a new generic name, *Dryades*, is proposed here. This name is a reference to the Brazilian Phytogeographic Province Dryades (after the tree nymphs of Greek mythology), a term coined by Martius (1840–1869) to designate the phytogeographic region roughly corresponding to the current circumscription of the Brazilian Atlantic Forest, to which all species of the new genus are endemic. This name is also a celebration of the 200 years of the end of the travel of Friedrich Philipp von Martius (1794–1868, German Botanist) and Johann Baptist von Spix (1781–1826, German Biologist) in Brazil. These bold naturalists came with the Austrian Expedition to Brazil, a great expedition of scientific inquiry which explored the country, with prominence in the areas of Botany, Zoology and Ethnography, and lasted from 1817 to 1835, while Martius and Spix returned to Europe in 1820. Thousands of plants and animals were collected then and sent to institutions in Europe, as well as the collection of ethnographic and Brazilian music data of the period (see Spix and Martius 1824). New transfers of names from *Conchocarpus* to *Dryades*, a description and diagnosis of the new genus, citation of nomenclatural types of each species, statements of geographical distribution, and a key to the species are presented below.

To subsume the species of the *Conchocarpus gaudichaudianus* group into other genera to which they appear phylogenetically related (e.g., such as *Andreodoxa*, *Angostura*, or *Erythochiton*) would only obfuscate the limits of these genera. As mentioned above, species of these genera have embryos with conduplicate-plicate cotyledons (a synapomorphy of the Galipeinae), while all species of the *Conchocarpus gaudichaudianus* group have straight embryos with unfolded cotyledons (a synapomorphy of this group). In addition, these three genera have terminal styles, but the *Conchocarpus gaudichaudianus* group have gynobasic styles and an umbilicate ovary. Besides, appendages at the base of the anthers are present in all these three genera, but are lacking in the representatives of

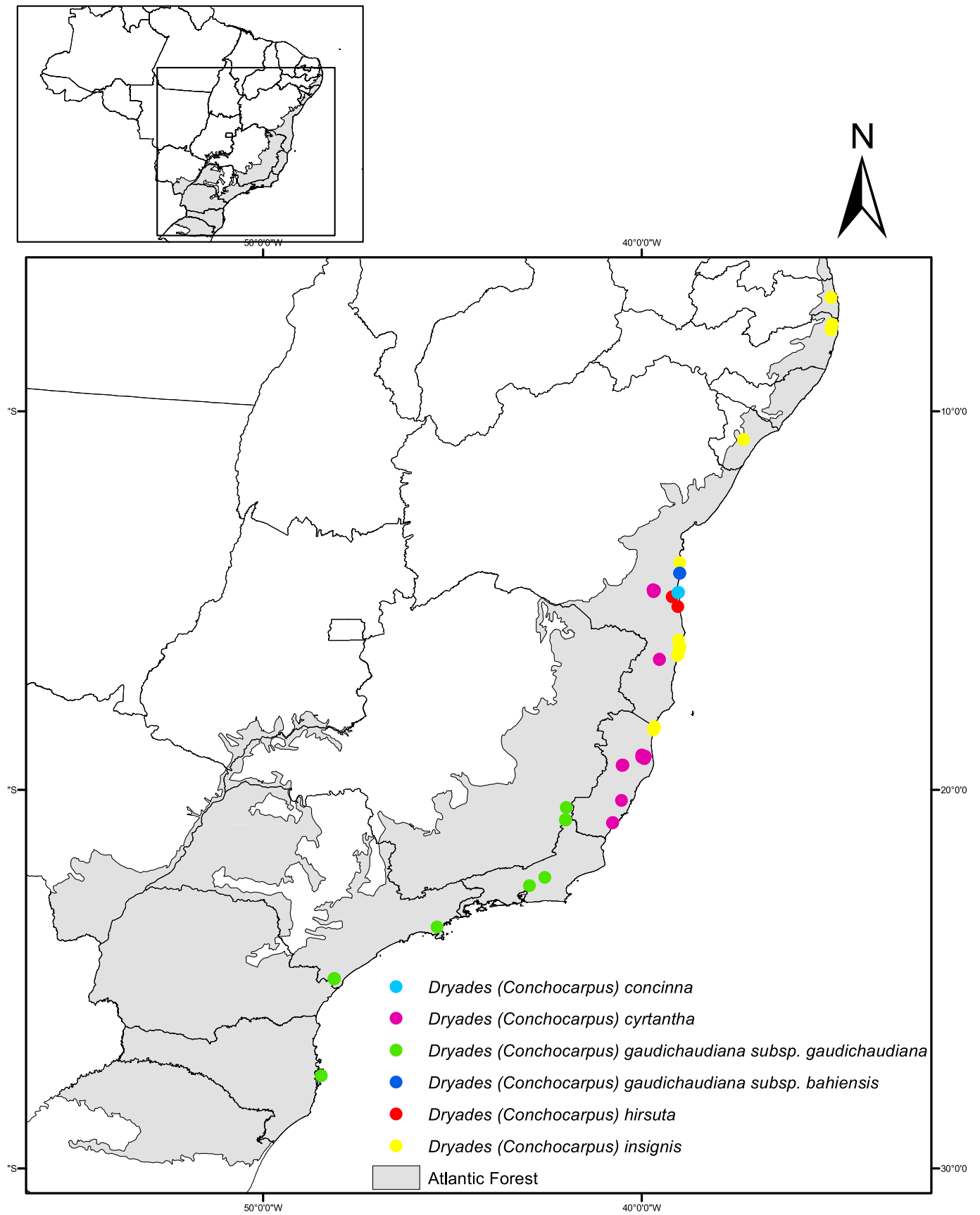


Fig. 8. Known distribution of the species of *Dryades* (former *Conchocarpus gaudichaudianus* group, see text) in Eastern Brazil. Approximate limits of the Brazilian Atlantic forest shaded gray.

the *C. gaudichaudianus* group. Other traits unique in these genera are absent from the *Conchocarpus gaudichaudianus* group: stellate hairs in all seven species of *Angostura* (present, in Galipeinae, only in *Euxylophora*, a monotypic genus from Amazonia) and the showy (red, green or white), campanulate to tubular calyx in the six species of *Erythrochiton* (an autpomorphy of this genus). Thus, the option for a new generic name (*Dryades*) appears to be appropriate.

Taxonomy

Accepted names are in bold-faced and italic type, synonyms in italics only.

Dryades Groppo, Kallunki & Pirani, *gen. nov.* Type: ***Dryades gaudichaudiana*** (A.St.-Hil.) Groppo, Kallunki & Pirani (designated here).

Diagnosis: *Dryades* differs from *Conchocarpus*, from which it is segregated, by a set of characters that includes a glabrous, umbilicate ovary (vs. a pubescent ovary, this either umbilicate or with a terminal style in *Conchocarpus*), by its almost free sepals with imbricate lobes overlapping even after anthesis (vs. sepals free or, if connate, not overlapping at anthesis), and by its straight (vs. curved) embryo with plano-convex (vs. conduplicate-plicate) cotyledons.

Shrubs or treelets up to 6 m tall, the stems usually single or with few, orthotropic, relatively thick branches and often bearing leaves congested at apex of the stem or branch or less often diffusely branched and leaved; indumentum of simple trichomes, usually pale and inconspicuous, partially caducous (but persistent and brown-hirsute in *D. hirsuta*). Leaves ascending or patent, alternate, 1-foliolate, petiolate, a pulvinus present at junction of petiole and blade; petiole subterete, pulvinate at its blade; blade entire, chartaceous to coriaceous, not pellucid-punctate (volatile oils present, but glands not visible) to inconspicuously pellucid-punctate, the venation brochidodromous. Inflorescence a thyrses, terminal or axillary, erect, borne on leafy stems, the partial inflorescences alternately attached along an elongate rachis, stalked, the secondary axis developed, dichasial at first node, monochasial at distal nodes. Flowers bisexual, 5-merous, pedicellate. Sepals quincuncial, coriaceous, shortly connate at base, the lobes rounded or obtuse, overlapping at anthesis, persistent in fruit. Corolla tubular, zygomorphic or (sub) actinomorphic due to unequal divergence of lobes and presence of only two fertile stamens; petals creamy-white, connate into a tube or coherent by pubescence (and then still forming a tube), imbricate, the innermost in upper position, the inner surface tomentulose (curled trichomes), especially at throat, the outer surface of the lobes usually glabrous (partially pubescent in *D. insignis*). Androecium of two fertile stamens flanking the innermost (and upper) petal; filaments free from each other, adnate to the corolla or free from it at base, but adherent to it at the throat by the intertwining of their abaxial tomentulose pubescence with that of the corolla; filaments of the fertile stamens flattened and villous at apex, not exerted from the corolla tube; staminodes flattened, adaxially glabrous at base for the length of the corolla tube, villous at throat, the exerted apical portion attenuate, subterete, pubescent; anthers free, rimose, ovate to lanceolate, glabrous, lacking a basal appendage, basifixed and broadly attached to the filament, not exerted from corolla tube; pollen grains 3–4-colporate, exine reticulate. Disc cupular, carnos, glabrous, slightly shorter than or surpassing the ovary (in this case the margins inflexed). Ovary of 5 free carpels, united only in the single style, umbilicate, glabrous; stigma 5-lobed or 5-sulcate, included in the corolla throat at anthesis; locules 5, the ovules 2 per carpel, superposed on axial placentae. Fruit of 1–5 follicular mericarps, rounded, beaked, or carinate apically, glabrous or sparsely to densely pubescent and sometimes mealy; endocarp papery, detaching from the rest of the pericarp and expelling the seed as it opens. Seed 1 per carpel, smooth to (sub)rugulose, glabrous, the testa papery. Embryo straight, the radicle adaxial, the cotyledons plano-convex, subequal, thick and stiff.

Distribution: a genus with five species endemic to the Atlantic Forest domain in Eastern Brazil, ranging from from the states of Pernambuco and Bahia to Santa Catarina; three species inhabit the understory of moist forests, and two species inhabit the *restinga* forest, a coastal

formation on Quaternary sandy soils (Fig. 8, map of distribution).

Iconography: Kallunki and Pirani (1998).

Key to the species of *Dryades*

1. Twigs, petioles, and abaxial surface of leaves hirsute with erect, brownish trichomes (Bahia) *D. hirsuta*
1. Twigs, petioles, and abaxial surface of leaves glabrous, if pubescent, then with short, appressed, pale trichomes.
 2. Inflorescence, fruit, and calyx densely pubescent with yellowishgrey trichomes (Bahia, Espírito Santo) *D. insignis*
 2. Inflorescence and fruit sparsely pubescent and calyx glabrous or (not yellowish-grey) pubescent, trichomes translucent when fresh, brownish when dried
 3. Leaf-blade usually wide-oblong or elliptic, broadly rounded at base (and often at apex); corolla tube formed by coherent petals (Bahia) *D. concinna*
 3. Leaf-blade oblanceolate, usually cuneate; corolla tube formed by connate petals.
 4. Inflorescence terminal, only 1 per branch; internodes of twig evident, not obscured by leaves (Bahia, Espírito Santo) *D. cyrtantha*
 4. Inflorescence apparently axillary, often > 1 per branch; internodes of twig obscured by densely clustered leaves.
 5. Inflorescence axis 1–1.5 cm long; corolla 10.4–18 mm long; mericarp 18–19 × 11 mm (Rio de Janeiro, Minas Gerais, São Paulo, Paraná, and Santa Catarina) *D. gaudichaudiana* subsp. *gaudichaudiana*
 - Inflorescence axis 5.7–8 cm long; corolla c. 6.8 mm long; mericarp 14 × 7 mm (Bahia) *D. gaudichaudiana* subsp. *bahiensis*

Dryades concinna (Kallunki) Groppo & Kallunki, *comb. nov.*

≡ *Conchocarpus concinnus* Kallunki, Kew Bull. 53(2): 278. 1998.

Type: Brazil, Bahia, Olivença, c. 14°57'S 39°00'W, 6 Feb 1993, J.R. Pirani & J.A. Kallunki 2743 (holotype SPF; isotypes CAS, CEPEC, K, MBM, MO, RB, NY, U).

Distribution: Brazil, Bahia. Understory of *restinga* (coastal formation on sandy soils) forest, in the Atlantic Forest domain (Kallunki and Pirani 1998; Pirani and Groppo 2015); known only from the vicinity of the type locality. See Fig. 1D–E.

Dryades cyrtantha (Kallunki) Groppo & Kallunki, *comb. nov.*

≡ *Conchocarpus cyrtanthus* Kallunki, Kew Bull. 53(2): 285. 1998.

Type: Brazil, Bahia, Itacaré, c. 14°20'S 39°00'W, 6 Feb 1993, J.R. Pirani & J. A. Kallunki 2748 (holotype SPF; isotypes MO, NY, SPF).

Distribution: Brazil, states of Bahia and Espírito Santo, in understory of moist forest in Atlantic Forest domain (Kallunki and Pirani, 1998; Pirani and Groppo, 2010).

Dryades gaudichaudiana (A.St.-Hil.) Groppo, Kallunki & Pirani, *comb. nov.*

≡ *Galipea gaudichaudiana* A.St.-Hil., Fl. Bras. Merid. 1: 89. 1825.

Type: Brazil, provincia Rio de Janeiro, pr. Mandioca, s.d., Gaudichaud s.n. (holotype P; possible isotype G—*Gaudichaud* 1830).

≡ *Cusparia gaudichaudiana* (A.St.-Hil.) Engl. in Mart & Eichler, Fl. Bras. 12(2): 116. 1874.

≡ *Angostura gaudichaudiana* (A.St.-Hil.) Albuquerque, Acta Amazon. 11: 850. 1981.

≡ *Conchocarpus gaudichaudianus* (A.St.-Hil.) Kallunki & Pirani, Kew Bull. 53(2): 295. 1998.

Notes on typification: in the original description of *Galipea gaudichaudiana*, Saint-Hilaire et al. (1825) stated that he based his new species on a collection made by Gaudichaud in “*sylvis primaevus praedii Mandioca prope Sebastianopolim*” (Rio de Janeiro). In the P Herbarium (where Saint-Hilaire used to work) there is a single specimen with Gaudichaud's original label with the precise locality. This single sheet corresponds to the holotype of the species. Another specimen identified as *Galipea gaudichaudiana*, collected by Gaudichaud (marked with a number 1830), and deposited at G was cited by Kallunki and Pirani (1998) as a possible isotype, but it is not a duplicate of the Gaudichaud collection at P, having probably been collected by Gaudichaud at a later date.

Distribution: Brazil, in states of Bahia, Minas Gerais, Rio de Janeiro, São Paulo, Paraná and Santa Catarina, in the understory of moist forest in the Atlantic Forest domain (Kallunki and Pirani, 1998). *Dryades gaudichaudiana* comprises two subspecies, distinguished by the characters in the key above.

Dryades gaudichaudiana (A.St.-Hil.) Groppo, Kallunki & Pirani subsp. *gaudichaudiana*, *comb. nov.*

≡ *Conchocarpus gaudichaudianus* (A.St.-Hil.) Kallunki & Pirani subsp. *gaudichaudianus*. Type: same as *Galipea gaudichaudiana*.

Distribution: Brazil, in the states of Rio de Janeiro, Minas Gerais, São Paulo, Paraná and Santa Catarina, in the understory of moist forest in the Atlantic Forest domain (Kallunki and Pirani, 1998; Pirani and Groppo, 2010). See Fig. 1F.

Dryades gaudichaudiana subsp. *bahiensis* (Kallunki) Groppo & Kallunki, *comb. nov.*

≡ *Conchocarpus gaudichaudianus* subsp. *bahiensis* Kallunki. Kew Bull. 53(2): 295. 1998. Type: Brazil, Bahia, mun. Itacaré, T.S. dos Santos et al. 3729 (holotype CEPEC; isotype NY).

Distribution: Brazil, state of Bahia, in the understory of moist forest in Atlantic Forest domain (Kallunki and Pirani, 1998; Pirani and Groppo, 2015).

Dryades hirsuta (Pirani) Groppo & Pirani, *comb. nov.*

≡ *Conchocarpus hirsutus* Pirani, Kew Bull. 53(2): 301. Type: Brazil, Bahia, Mun. Ilhéus, 5 Feb 1982, L.A. Mattos Silva et al. 1423 (holotype CEPEC, isotypes NY, SPF).

Distribution: Brazil, state of Bahia, in the understory of moist forest in Atlantic Forest domain (Kallunki and Pirani, 1998; Pirani and Groppo, 2010); known only by the type collection.

Dryades insignis (Pirani) Groppo & Pirani, *comb. nov.*

≡ *Conchocarpus insignis* Pirani, Kew Bull. 53(2): 305. 1998. Type: Brazil, Espírito Santo, Conceição da Barra, Praia da Costa Dourada, 19 Feb 1994, J.R. Pirani et al. 3052 (holotype SPF, isotypes CAS, CEPEC, K, MBM, MO, NY, RB, SP, U, US).

Distribution: Brazil, in states of Alagoas, Bahia, Espírito Santo, Pernambuco, and Sergipe, in the understory of *restinga* forest at Atlantic Forest domain (Kallunki and Pirani, 1998; Pirani and Groppo, 2010).

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2020.106971>.

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