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Contrasting leaf cuticular wax composition of *Conchocarpus* and *Dryades* species (Rutaceae) from the Atlantic Forest and "Restinga"

Elielson Rodrigo Silveira[1] · Lucas Paradizo Roma[1] · José Rubens Pirani[1] · Déborah Yara Alves Cursino dos Santos[1]

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Abstract
Conchocarpus is the largest genus in the subtribe Galipeinae, tribe Galipeeae, distributed from Nicaragua to northern Bolivia and southern Brazil, with the center of species diversity in the Brazilian Atlantic Rainforest. Five species were recently segregated to a new genus (*Dryades*), thereby restoring the monophyly of *Conchocarpus* sensu stricto. In addition to the wide morphological diversity of *Conchocarpus* species, to date, few chemical aspects have been explored, mainly in regard to wax composition. This study aims to compare the chemical composition of the cuticular wax of selected species of *Conchocarpus* sensu stricto and *Dryades*, in order to determine their similarities and/or differences, as well as discuss possible correlations with habitats, and potential taxonomic implications. The main results showed that in general, *Conchocarpus* and *Dryades* could be distinguished, with species of the former genus exhibiting longer-chain alkanes and those of the latter higher triterpene diversity. Moreover, species inhabiting the Atlantic Rainforest have a thinner wax load, reduced amounts of triterpenes and are rich in alkanes, while those from "Restinga" vegetation exhibit a higher wax content, fewer alkanes, and large amounts of triterpenes.

Keywords *n*-Alkanes · Chemotaxonomy · Sapindales · Terpenoids

1 Introduction

Rutaceae, commonly known as the *Citrus* family, contains approximately 160 genera and 2100 species, distributed in temperate, subtropical, and tropical regions, with greater incidence in Tropical America, Southern Africa, and Australia (Pirani and Groppo 2010). The family is well known for its chemical diversity, especially alkaloids derived from anthranilic acid, coumarins, lignans, flavonoids, and terpenoids (Epifano et al. 2015).

Among rutaceous plants, the tribe Galipeeae comprises two subtribes: Pilocarpinae and Galipeinae. The latter is the most diverse group of Neotropical Rutaceae, with 28 genera and approximately 130 species (Groppo et al. 2008; Bruniera et al. 2015; 2021). Although species belonging to this subtribe can be found from Southern Mexico and the West Indies to Bolivia and Southern Brazil, the Brazilian Atlantic Forest is the center of species richness for Galipeinae, including several endemisms (Pirani and Groppo 2015; Colli-Silva and Pirani 2019).

Conchocarpus J.C.Mikan is the largest genus in Galipeinae (Groppo et al. 2021), distributed from Nicaragua to Northern Bolivia and Southern Brazil, with high diversity in the Brazilian Atlantic Rainforest (Pirani and Groppo 2010; Colli-Silva and Pirani 2019; Pirani and Groppo 2020). A recent phylogenetic study based on morphological and molecular data revealed that *Conchocarpus* was not monophyletic unless two distinct clades were recognized as genera: "the *Conchocarpus* sensu stricto group" comprising around 47 species, and a new proposed genus, *Dryades* Groppo, Kallunki & Pirani, with five species (Groppo et al. 2021). Phylogenetic analysis showed that *Dryades* nested within a lineage consisting of *Andreadoxa, Angostura, Erythrochiton, Galipea,* and *Rauia,* but not including *Conchocarpus s.s.*

Despite the wide diversity of *Conchocarpus*, only six species have been chemically investigated. Quinolinic alkaloids were described for *C. fontanesianus* (A.St.-Hil.)

✉ Déborah Yara Alves Cursino dos Santos
dyacsan@ib.usp.br

[1] Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua Do Matão, 277, São Paulo, SP 05508-090, Brazil

Kallunki & Pirani, *C. gaudichaudianus* (A.St.-Hil.) Kallunki & Pirani [currently = *Dryades gaudichaudiana* (A.St.-Hil.) Groppo, Kallunki & Pirani], *C. heterophyllus* (A.St.-Hil.) Kallunki & Pirani and *C. longifolius* (A.St.-Hil.) Kallunki & Pirani (Mafezoli et al. 2000; Ambrozin et al. 2008; Cortez et al. 2009; Cabral et al. 2012), while acridine alkaloids were identified in *C. inopinatus* Pirani and *C. marginatus* (Rizzini) Kallunki & Pirani (Bellete et al. 2012). In regard to phenolic compounds, flavonoids were found in *C. heterophyllus* (Ambrozin et al. 2008) and coumarins in *C. longifolius* (Mafezoli et al. 2000). However, no study has focused on wax composition in species of this genus.

Waxes are a mixture of aliphatic long-chain carbon fatty acid derivatives and small amounts of cyclical compounds such as terpenoid and flavonoid derivatives (Kunst and Samuel 2003; Bernard and Joubès 2013). These compounds, along with cutin, are the main fractions of the plant cuticle, forming a continuous layer that covers the surfaces of leaves, flowers, fruits, and non-woody stems (Jetter et al. 2006). The function of the cuticle is to prevent water loss (Mamrutha et al. 2010) and protect against other environmental stresses, including excess UV radiation, microorganisms, and insects (Lewandowska et al. 2020).

The wax composition of some species of Rutaceae, especially *Citrus*, has already been studied. In this genus, fruit and leaf cuticular waxes are rich in alkanes. *n*-Alkanes ranging from docosane (C_{22}) to hentriacontane (C_{31}) were identified as wax components in fruits of *Citrus sinensis* (L.) Osbeck (Wang et al. 2014), while for leaves of *Citrus aurantium* L., alkanes ranging from tetracosane (C_{24}) to tetracontane (C_{40}) were found (Riederer and Schneider 1990). Alkane composition has also been described for 11 species of *Pilocarpus* Vahl (Skorupa et al. 1998), with the homologue series ranging from heneicosane (C_{21}) to tritriacontane (C_{33}). To the best of our knowledge, the presence of 1-phenyl-5-vinyl-5,9-dimethyl decane in the leaf wax of *P. jaborandi* Holmes is unique among all species analyzed to date.

The distribution of wax components among species, mainly the alkane profiles, has been used in chemotaxonomic investigations since the 1960's. Eglinton et al. (1962) were the first to propose the use of cuticular compounds as taxonomic markers. Since then, several authors have published data supporting the use of these characters in chemotaxonomic studies. Li et al. (2013), for example, were able to distinguish *Sinojackia dolichocarpa* C.J.Qi from other species based on proportions of hentriacontane (C_{31}) and tritriacontane (C_{33}) in the waxes of species of *Sinojackia* (Styracaceae). The distinct occurrence of triterpenes (e.g., α-amyrin) and steroids (e.g., 24-methylenecycloartanol) allowed the recognition of different cultivars of *Lonicera caerulea* L. (Caprifoliaceae) (Becker et al. 2019). However, other authors did not succeed with the use of these characters. Jovanović et al. (2015) found high *n*-alkane variability in the epicuticular waxes of *Sedum* species (Crassulaceae) and suggested that these compounds might not be reliable taxonomic markers for these species.

Wax composition variations related to plant age, environmental conditions, and/or collection sites have been described and contribute to the non-credibility of these data as taxonomic characters. Cerda-Peña et al. (2020) analyzed the *n*-alkyl components of three dominant species from a temperate forest in South America, demonstrating a significant correlation between these compounds and environmental variables. Highly distinct alkane profiles were described for populations of *Juniperus communis* L. var. *saxatilis* Pall. and *J. communis* var. *montana* Aiton collected in different locations of the New and Old World, suggesting a phylogeographic pattern for these species (Dodd and Poveda 2003).

The occurrence of *Conchocarpus* and *Dryades* species in either the moist Atlantic Rainforest or "Restinga", a stressful environment marginal to the former, constitutes a suitable model for a chemical study. Since "Restinga" are among the least-known Brazilian vegetation types in terms of diversity and conservation (Scarano 2002), studies on their plant species are important in improving knowledge about biological and ecological conditions and correlations. In this respect, the main goal of the present study was to compare the chemical composition of the cuticular wax of some species of *Conchocarpus senso stricto* and *Dryades*, from the "Restinga" and Atlantic Rainforest, with a view to determining their similarities and/or differences, identifying potential taxonomic implications, and discussing possible correlations with the contrasting environments in which populations are found.

2 Material and methods

Cuticular wax extraction and content – Mature leaves of adult plants, were collected in two different ecosystems in the states of Bahia and Espírito Santo, Brazil (Table 1): the Atlantic Rainforest (dense ombrophilous forest) and "Restinga" — according to the vegetation classification of Veloso et al. (1991). Voucher specimens are deposited in the Herbarium of the University of São Paulo (SPF), Brazil.

Table 1 List of analyzed species with relevant information about collection site and wax content

Species*	Voucher	Location (State/City)	Vegetation type	Wax content (μg cm^{-2})
Conchocarpus inopinatus Pirani	Kallunki et al. 433	Bahia/Jussari	Atlantic Rainforest	5.53
Conchocarpus macrocarpus (Engl.) Kallunki & Pirani	Kallunki et al. 720	Espírito Santo/Cachoeiro de Itapemirim	Atlantic Rainforest	6.28
Conchocarpus macrophyllus J.C. Mikan	Thomas et al. 9748	Bahia/Uruçuca	Atlantic Rainforest	3.78
Conchocarpus mastigophorus Kallunki	Thomas et al. 9744	Bahia/Uruçuca	Atlantic Rainforest	8.76
Conchocarpus obovatus (Nees & Mart.) Kallunki & Pirani	Kallunki et al. 477	Espírito Santo/Cachoeiro de Itapemirim	Atlantic Rainforest	3.59
Conchocarpus ruber (A.St.-Hil.) Bruniera & Groppo	Pirani et al. 2747	Bahia/Uruçuca	Atlantic Rainforest	6.70
Dryades concinna (Kallunki) Groppo & Kallunki	Pirani et al. 2743	Bahia/Ilhéus	"Restinga" forest	13.17
Dryades cyrtantha (Kallunki) Groppo & Kallunki	Thomas et al. 9747	Bahia/Uruçuca	Atlantic Rainforest	7.83
Dryades cyrtantha (Kallunki) Groppo & Kallunki	Pirani et al. 2748	Bahia/Itacaré	Atlantic Rainforest	6.29
Dryades insignis (Pirani) Groppo & Pirani	Pirani et al. 2762	Bahia/Porto Seguro	"Restinga" forest	9.65
Dryades insignis (Pirani) Groppo & Pirani	Thomas et al. 6023	Bahia/Una	"Restinga" forest	6.88

*All species are endemic, except *Conchocarpus ruber* which is native

Before extraction, the leaves were washed with water to remove dust and then dried at room temperature. The total leaf area of each sample was calculated using ImageJ software, version 1.49. The cuticular wax was extracted by three consecutive immersions in dichloromethane for 30 s each (modified from Fernandes 1964). The extracts were pooled, filtered, and concentrated in a rotatory evaporator under reduced pressure at 50 °C. The concentrated wax was stored in a desiccator until reaching constant mass. Total wax content was expressed in μg cm^{-2}.

Analysis and identification of wax components – Aliquots of the cuticular waxes, were derivatized by adding 50 μL of *N,O-bis*-(trimethylsilyl)-trifluoroacetamide (BSTFA) + 50 μL of pyridine, for 1 h at 70 °C (adapted from Chu et al. 2017) and analyzed by gas chromatography coupled to mass spectrometry (GC-MS — Agilent 6850/Agilent 5975C), equipped with an Agilent HP5-MS column (30 m × 250 μm × 0.25 μm). The initial column temperature was adjusted to 100 °C for 5 min, followed by heating at 5 °C min^{-1} until a final temperature of 320 °C, maintained for 8 min. The injection volume was 1 μL, and helium was the carrier gas, with a constant flow of 1 mL min^{-1}. Injector, ion source, and quadrupole temperatures were adjusted to 300, 230, and 150 °C, respectively. Mass spectra were detected using electronic ionization (EI) at 70 eV in the full-scan acquisition mode, varying between 50 and 800 *m/z* and 2.66 scans s^{-1}. Wax compounds were identified by comparing mass spectra with their NIST digital library counterparts (version 2.0, 2008).

Data analysis – Statistical analysis was conducted using the R platform (version 4.0.3) and multivariate analysis with FITOPAC software (version 2.1). The graphs were constructed using Graphpad Prism® software (version 8.0.2). Homoscedasticity was assessed with the Bartlett test. Due to the low number of statistically analyzed specimens, normality was not considered, and the Kruskal–Wallis test was applied directly. p values ≤ 0.05 were considered statistically significant.

Principal coordinates analysis (PCoA), based on the Euclidean resemblance matrix, was used as an ordination method to visualize differences between the two genera based on homologue composition.

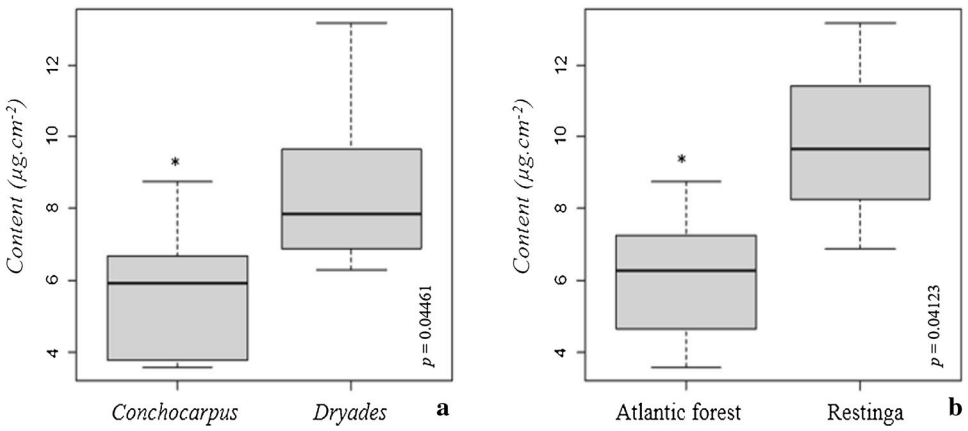

Fig. 1 Mean values of leaf wax content in *Conchocarpus* and *Dryades* species. **a**: *Conchocarpus* and *Dryades* species were considered separately. *Dryades* exhibited higher wax content when compared to *Conchocarpus* ($p = 0.04461$). **b**: The mean values were obtained with specimens of the same ecosystem, without genus separation. "Restinga" species exhibited higher wax content than these from the Atlantic Rainforest ($p = 0.04123$). *significant difference

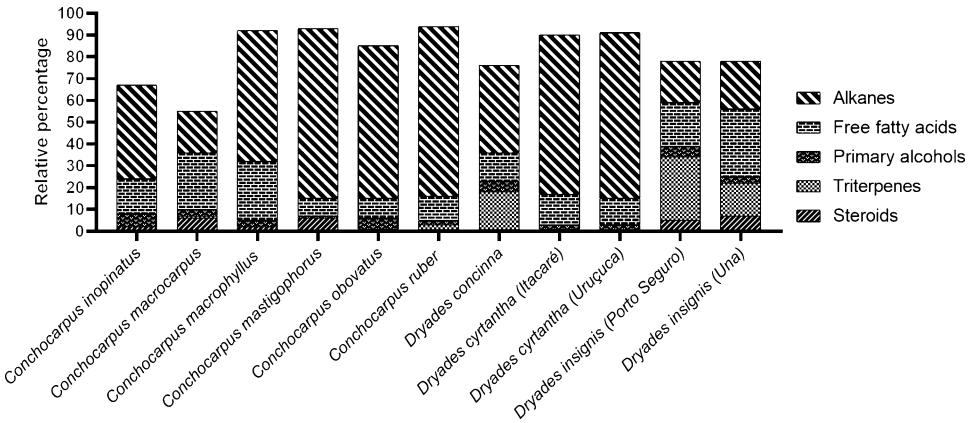

Fig. 2 Relative percentage of the wax classes in species of *Conchocarpus* and *Dryades*

3 Results

The wax loads of the specimens were highly variable, ranging from 3.59 μg cm^{-2} in *Conchocarpus obovatus* to 13.17 μg cm^{-2} in *Dryades concinna* (Kallunki) Groppo & Kallunki (Table 1).

The wax content of *Dryades* species was 52% higher than that of *Conchocarpus*. *Dryades* species from "Restinga" contained higher wax amounts (63%) in relation to the Atlantic Rainforest species. These differences in wax content between *Conchocarpus* and *Dryades* ($p = 0.04461$) (Fig. 1a), and "Restinga" and Atlantic Rainforest species ($p = 0.04123$) (Fig. 1b) were slightly statistically significant (Table 1S).

Wax components included alkanes, free fatty acids, primary alcohols, triterpenes, and steroids (Fig. 2). Alkanes were present in all the species and were the most abundant class in most, reaching 78% of the total wax in *Conchocarpus mastigophorus* Kallunki. Free fatty acids and primary alcohols were also present in all the species. Triterpenes and steroids were less conspicuous, with the former found in higher amounts in species of *Dryades*.

Almost none of the component classes exhibited a significant difference between *Conchocarpus* and *Dryades* or Atlantic Forest and "Restinga" species (Fig. 3, Table 1S). Triterpenes displayed a significant difference ($p = 0.0102$) between "Restinga" and Atlantic Rainforest species and a slightly significant value ($p = 0.055$) between *Conchocarpus* and *Dryades* species. Only alkane content was apparently lower in "Restinga" than Atlantic Rainforest species, differing from all the other classes.

Fifty-seven compounds were identified in the cuticular wax of the *Conchocarpus* and *Dryades* species. The alkane homologue series ranged from docosane (C_{22}) to nonatriacontane (C_{39}), with hentriacontane (C_{31}) being the major compound for most of the species analyzed, except for *C. inopinatus* Pirani and *C. macrophyllus*. Long-chain homologues (C_{34}–C_{39}) were found only in species collected in the Atlantic Rainforest (Table 2). Saturated and unsaturated

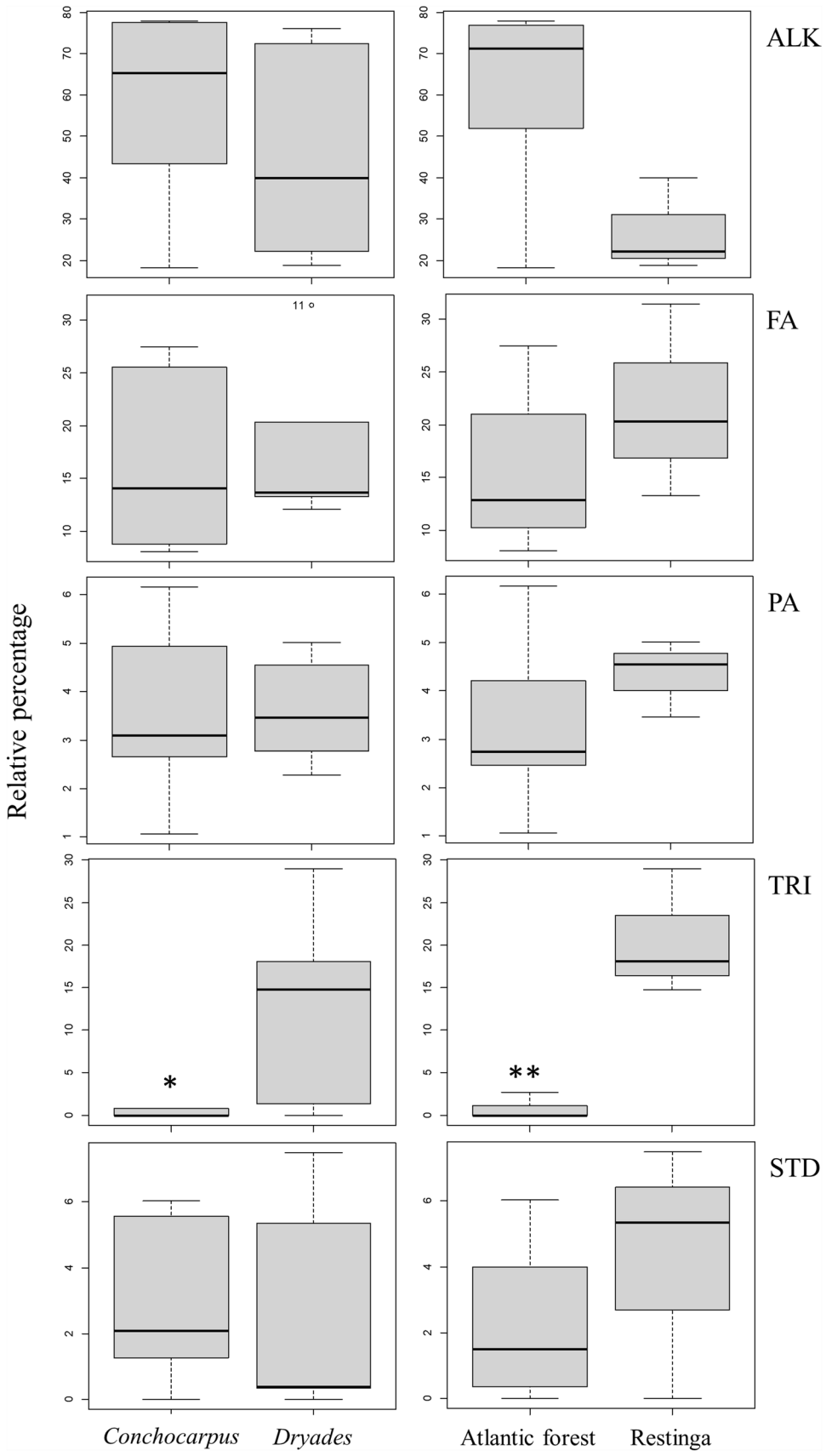

Fig. 3 Mean values of the main classes of wax compounds in *Conchocarpus* and *Dryades* species. On the left side, the species of each genera were considered separately. On the right side, the mean values were obtained with specimens of the same ecosystem, without genus separation. ALK = alkanes, FA = free fatty acids, PA = primary alcohols, TRI = triterpenes, STD = steroids. *slightly significant difference ($p = 0.0555$), **significant difference ($p = 0.0102$)

Table 2 Distribution of the relative abundance of *n*-alkanes of leaf cuticular wax of *Conchocarpus* and *Dryades* species

Species/specimens	Alkanes—Relative abundance (%)																
	C_{22}	C_{23}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}	C_{36}	C_{37}	C_{38}	C_{39}
C. inopinatus	–	–	–	–	–	–	–	–	7.24	1.01	**32**	0.89	2.26	–	–	–	–
C. macrocarpus	–	–	–	–	–	–	0.9	–	**10.06**	0.42	5.24	–	1.28	–	–	–	0.42
C. macrophyllus	–	–	–	–	–	–	0.52	0.55	18.4	3.88	**23.8**	1.62	6.66	0.60	3.73	0.19	0.36
C. mastigophorus	–	–	–	–	–	–	1.42	0.41	**35.6**	1.54	20.6	0.80	8.01	0.46	5.08	–	3.67
C. obovatus	0.17	0.35	0.30	0.28	0.52	0.52	8.97	0.93	**43.1**	1.01	11.6	0.31	0.44	1.11	–	–	0.45
C. ruber	–	–	–	–	0.05	–	24.1	1.91	**35.2**	2.24	12.1	–	1.43	–	0.45	–	0.40
D. concinna	–	–	–	–	0.49	0.60	5.73	2.05	**19.2**	3.00	8.97	–	–	–	–	–	–
D. cyrtantha (Itacaré)	–	0.17	–	–	0.31	0.35	12	2.14	**40.02**	2.80	13.1	1.32	–	–	0.25	–	–
D. cyrtantha (Uruçuca)	–	–	–	–	–	–	7.01	0.96	**35.4**	3.39	26.7	–	2.15	0.45	–	–	–
D. insignis (Porto Seguro)	0.25	0.39	0.26	0.30	0.55	0.58	1.47	–	**7.43**	2.01	5.56	–	–	–	–	–	–
D. insignis (Una)	–	–	–	–	–	–	0.75	–	**11.1**	2.28	8.02	–	–	–	–	–	–

Bold numbers correspond to the main homologue

free fatty acids were identified in all *Conchocarpus* and *Dryades* species, with hexadecanoic acid ($C_{16:0}$) as the major component, except for *Dryades concinna* (Table 3). Tetratriacontanol (C_{34}) was the main primary alcohol in most of the *Conchocarpus* species, while triacontanol (C_{30}) was more prominent in the *Dryades* species. Primary alcohols (PA) ranging from octadecanol (C_{18}) to nonacosanol (C_{29}) were found almost exclusively in *Conchocarpus*, except for a small amount of C_{18} (0.94%) in *Dryades insignis* (Pirani) Groppo & Pirani (Porto Seguro). Tritriacontanol (C_{33}) and hexatriacontanol (C_{36}) were also detected only in species of *Conchocarpus* (Table 4).

Four triterpenes and five steroids were identified in species of *Conchocarpus* and *Dryades*. Triterpenes were more diverse in the latter genus and steroids in the former. Of all the cyclic compounds, only β-sitosterol was widely detected in the species analyzed (Table 5).

A PCoA ordination using the relative percentage of all homologues identified in the cuticular wax reveals species segregation of *Conchocarpus* and *Dryades*, except for one specimen of *D. cyrtantha* (Kallunki) Groppo & Kallunki (Uruçuca) and *C. inopinatus* (Fig. 4). In general, long-chain homologues (alkanes = C_{34}–C_{39}, fatty acids = C_{34}, and primary alcohols = C_{33} and C_{36}), higher steroid diversity, and short-chain primary alcohols (C_{18}–C_{29}) were found almost exclusively in *Conchocarpus*. On the other hands, triterpenes were more abundant and diversified in *Dryades*. The absence of triterpenes in *D. cyrtantha* (Uruçuca) may explain its position in PCoA. In addition to the two genera, segregation between Atlantic Rainforest and "Restinga" species was observed, except for one specimen of *D. cyrtantha* (Itacaré).

4 Discussion

Total wax content was higher in *Dryades* than *Conchocarpus* species, as well as between "Restinga" and Atlantic Rainforest species; in both comparisons, the differences were significant. In addition, the total amount of triterpenes was significantly different between "Restinga" and Atlantic Rainforest species but just slightly significant between *Conchocarpus* and *Dryades* species. Both genera present C_{31} as the main alkane homologue, *Conchocarpus* exhibited longer-chain alkanes and *Dryades* present greater triterpene diversity. These data suggest that wax composition may have potential taxonomic relevance, since they corroborate the difference between the two genera studied.

Table 3 Distribution of the relative abundance of free fatty acids of leaf cuticular wax of *Conchocarpus* and *Dryades* species

Species/specimens	Free fatty acid—Relative abundance (%)																		
	Saturated series															Unsaturated series			
	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{17:0}$	$C_{18:0}$	$C_{20:0}$	$C_{22:0}$	$C_{23:0}$	$C_{24:0}$	$C_{26:0}$	$C_{28:0}$	$C_{29:0}$	$C_{30:0}$	$C_{31:0}$	$C_{32:0}$	$C_{34:0}$	$C_{16:1\ (n-7)}$	$C_{18:1\ (n-9)}$	$C_{18:2\ (n-6)}$	$C_{19:1\ (n-9)}$
C. inopinatus	3.84	–	**9.04**	–	1.69	–	–	–		–	–	–	–	–	–	–	–	1.33	0.52	–
C. macrocarpus	0.63	0.36	**10.9**	0.33	1.88	0.39	0.16	–	0.13	–	–	–	–	–	–	0.51	–	9.59	–	0.62
C. macrophyllus	0.36	0.21	**10.4**	0.21	1.89	0.9	–	–	0.1	–	–	–	0.18	–	0.85	0.67	0.12	6.17	5.29	0.13
C. masligophorus	0.15	0.17	**4.97**	–	1.07	–	–	–	–	–	–	–	–	–	–	–	–	1.17	0.4	0.15
C. obovatus	0.36	0.25	**4.81**	–	1.23	–	–	–	–	–	–	–	–	–	–	–	0.15	1.5	0.5	–
C. ruber	0.19	0.08	**2.86**	0.07	1.23	–	–	–	0.6	–	0.31	0.24	2.31	–	1.37	0.63	0.05	1.14	0.56	0.07
D. concinna	0.39	–	5.07	–	1.87	–	–	–	–	–	–	–	0.63	–	–	–	–	**5.32**	–	–
D. cyrtantha (Itacaré)	0.22	0.2	**5.34**	–	2.44	–	0.14	0.79	–	–	–	–	–	–	1.2	–	0.21	2.77	0.38	–
D. cyrtantha (Uruçuca)	0.14	0.1	**4.49**	0.09	1.76	–	–	–	–	–	–	–	0.42	–	1.44	1	0.12	2.02	0.4	0.07
D. insignis (Porto Seguro)	–	–	**7.75**	–	2.58	–	–	–	–	–	–	–	0.72	–	1.17	–	–	6.76	1.04	0.29
D. insignis (Una)	0.47	–	**11.6**	–	7.58	–	–	–	0.71	0.59	0.6	–	0.99	1.74	–	–	0.46	6.02	0.64	–

Bold numbers correspond to the main homologue

Wax components such as chemotaxonomic characters are controversial. While Li et al. (2013) were able to segregate species of *Sinojackia* based on alkane profiles, Jovanović et al. (2015) were unsuccessful with species of *Sedum*. Despite the low number of species investigated, the data from the present study support the proposal of *Dryades* and *Conchocarpus* segregation made by Groppo et al. (2021). On one hand, *Conchocarpus* species have a thinner wax layer, long-chain alkane homologues (e.g., alkanes C_{34}–C_{39}), short-chain primary alcohols (C_{18}–C_{29}), and greater steroid diversity, while on the other, *Dryades* have a thicker wax layer and more triterpenes. However, this needs further investigation, including a larger number of species from both genera. Nordby and Nagy (1977) reported the potential of leaf hydrocarbons for chemotaxonomic studies in *Citrus*, the most studied genus in Rutaceae. An unusual alkane found in the leaf wax of *Pilocarpus jaborandi* makes this species unique among other vascular species (Skorupa et al. 1998).

One of the main reasons that makes wax profile unreliable as a chemotaxonomy marker is its possible variability in response to different environmental conditions, plant age, or geographic location (Dodd and Poveda 2003; Cerda-Peña et al. 2020). In the present study, species collected in the Atlantic Rainforest exhibit a thinner wax load and low amounts of triterpenes and exhibited greater alkanes richness, especially long-chain ones, while "Restinga" species have a higher wax content, with fewer alkanes, and large amounts of triterpenes. "Restinga" are the prevailing vegetation type found in sandy quaternary substrates restricted to coastal areas (Flexor et al. 1984). Compared to plants inhabiting the neighboring rainforest, "Restinga" plants cope with the harsh environmental conditions, caused mostly by full (or almost full) exposure to sunlight, high temperatures, and low water retention in the sandy soil (Scarano 2002; Voltolini and Santos 2011). Light reflection is one of the roles of the cuticular layer, mainly due to waxes (Barnes and Cardoso-Vilhena 1996; Koch et al. 2009). The thicker wax load in "Restinga" species may be correlated with the environmental conditions. Large amounts of alkanes and long-chain compounds were expected in the wax of "Restinga" species, since in addition to high solar radiation, this environment is also characterized by low soil water retention (Mantovani and Iglesias 2008; Cooper et al. 2017). Oliveira et al. (2003) compared the efficiency of the wax layer in preventing water loss, demonstrating that the waxes of "Caatinga" species were more efficient than those of "Cerrado" species. Isolated alkanes and alcoholic triterpenes were also more efficient than ketone and acid triterpenes. The large proportion of alcoholic triterpenes in "Restinga" species might contribute to the water loss barrier. Further investigations on more species of Galipeinae as well as other groups of angiosperms inhabiting the "Restinga" and Atlantic Rainforest may help elucidate the possible role of leaf wax content in stressful habitats on the Brazilian coast.

Table 4 Distribution of the relative abundance of primary alcohols of leaf cuticular wax of *Conchocarpus* and *Dryades* species

Species/specimens	Primary alcohols—Relative abundance (%)										
	C_{18}	C_{20}	C_{22}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}	C_{36}
C. inopinatus	0.30	–	–	–	–	–	–	0.29	**3.84**	0.62	1.11
C. macrocarpus	0.10	0.13	0.11	–	–	–	–	0.58	**2.55**	–	–
C. macrophyllus	0.21	0.16	–	–	0.14	–	0.74	–	**0.96**	0.21	0.23
C. mastigophorus	–	–	–	–	–	–	–	–	**0.78**	–	0.29
C. obovatus	0.31	0.31	**2.83**	–	–	–	–	–	1.49	–	–
C. ruber	0.7	0.09	–	0.15	0.28	–	0.51	–	**0.86**	–	0.12
D. concinna	–	–	–	–	**1.66**	–	1.39	–	1.49	–	–
D. cyrtantha (Itacaré)	–	–	–	–	**1.32**	–	0.56	–	0.40	–	–
D. cyrtantha (Uruçuca)	–	–	–	–	0.98	–	0.62	–	**1.18**	–	–
D. insignis (Porto Seguro)	0.94	–	–	–	**1.68**	0.82	–	–	0.73	0.84	–
D. insignis (Una)	–	–	–	–	0.89	–	–	–	1.13	**1.44**	–

Bold numbers correspond to the main homologue

Table 5 Distribution of the relative abundance of cyclic compounds (triterpenes and steroids) of leaf cuticular wax of *Conchocarpus* and *Dryades* species

Species/specimens	Relative abundance (%)								
	Triterpenes				Steroids				
	α-Amyrin	β-Amyrin	Ursolic acid	Lupeol	β-sitosterol	Stigmasterol	Sitostenone	Cholesterol	Campesterol
C. inopinatus	–	–	–	–	**2.11**	–	0.31	**–**	–
C. macrocarpus	–	**0.80**	–	–	**4.70**	–	–	0.28	1.05
C. macrophyllus	–	–	–	–	**1.47**	–	0.13	0.14	–
C. mastigophorus	–	–	–	–	0.96	**4.59**	–	**–**	–
C. obovatus	–	–	**–**	–	**1.10**	–	–	0.16	–
C. ruber	0.95	–	–	**1.77**	–	–	–	**–**	–
D. concinna	5.06	3.23	–	**9.76**	–	–	–	–	–
D. cyrtantha (Itacaré)	0.54	–	**0.88**	–	**0.37**	–	–	–	–
D. cyrtantha (Uruçuca)	–	–	–	–	**0.33**	–	–	**–**	–
D. insignis (Porto Seguro)	**10.4**	9.93	–	8.61	**4.84**	–	–	–	0.50
D. insignis (Una)	**6.02**	3.31	–	5.42	**5.61**	–	1.88	–	**–**

Bold numbers correspond to the main compound

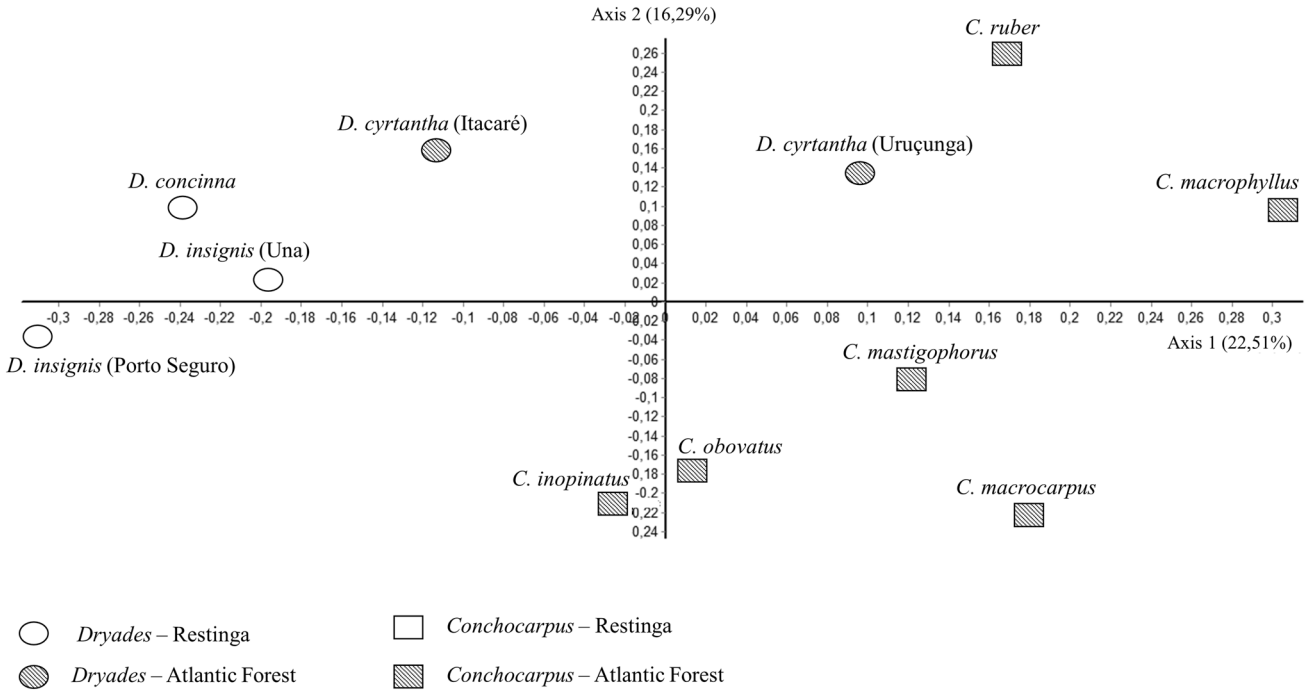

Fig. 4 Principal coordinates analysis (PCoA) based on homologue distribution of waxes from *Conchocarpus* and *Dryades* species collected in the "Restinga" and Atlantic Rainforest. Homologue relative abundance was used to build the Euclidian distance matrix

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Authors' contributions ERS, LPR, and DYACS designed the study and revised the manuscript; JRP collected and identified the plant material and collaborated in the discussion of the data; ERS prepared the extract and wrote the manuscript; LPR performed statistical analyses; ERS and LPR conducted the experiments and identified the wax compounds. All the authors approved the final version of the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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