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Distribution and evolution of secondary metabolites in Eriocaulaceae, Lythraceae and Velloziaceae from “campos rupestres”

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Abstract

Hypotheses are presented on the evolution of structural patterns of secondary metabolites (flavonoids and foliar wax alkanes) and fatty acids of families of “campos rupestres”. The distribution of fatty acids is given for genera of Lythraceae, with emphasis on *Cuphea* (supposedly more advanced) and *Diplusodon*. Compounds with saturated short chains represent a derived condition in Lythraceae although they are probably restricted to *Cuphea*. It is suggested that evolution selected for more complex flavonoid patterns in *Cuphea*, with the inclusion of *C*-glycoflavones and methoxylated flavonols (rhamnetin and isorhamnetin), which are not found in members of *Diplusodon* and *Lafoensia*. The supposedly primitive groups of Eriocaulaceae (e.g., *Paepalanthus*) presented more complex flavonoid patterns characterized by flavones and flavonols, the latter frequently being 6-hydroxylated or methoxylated. More advanced groups of Eriocaulaceae (e.g., *Leiothrix* and *Syngonanthus*) apparently possess only flavones, *C*-glycoflavones are a salient feature of species with smaller habits. In Velloziaceae, members of the primitive subfamily Vellozioideae show distribution of alkanes of foliar epicuticular wax in which C_{27}, C_{29} or C_{31} predominate; members of the derived subfamily Barbacenioideae usually show distributions with a predominance of C_{33} or C_{35}, while species of *Pleurostima* (Barbacenioideae) have C_{31} as the main homologue, thus being intermediate between the two subfamilies. It is suggested that the evolution of alkanes in Velloziaceae follows a trend toward elongation of carbon chains. The condition of advanced or primitive chemical patterns is inferred from the results of cladistic analyses based on morphological characters (Eriocaulaceae and Lythraceae), and morphological and molecular characters (Velloziaceae).

INTRODUCTION

Plant secondary metabolites have been widely used as taxonomic characters for comparisons at all hierarchic levels (Harborne and Turner, 1984), and certain classes of secondary metabolites such as benzylisoquinoline alkaloids, betalaines, glucosinolates, iridoids and polyacetylenes have had a great influence in the establishment of all recent systems of angiosperm classification (e.g., Cronquist, 1981; Dahlgren, 1989; Takhtajan, 1997).

Other classes of secondary metabolites, most importantly flavonoids (Woodland, 1997), have been more frequently used for comparisons at lower hierarchic levels such as genera, species and infraspecific categories. Another class of compounds that has deserved much attention, not only in plant but also in insect taxonomy, are hydrocarbons, in particular alkanes (Hamilton, 1995). Both classes of secondary metabolites combine the advantages of universal occurrence in vascular plants, chemical stability and the availability of rapid isolation and identification methods (Harborne, 1998).

In spite of the widespread use of secondary metabolites in taxonomy they have never achieved the same status as characters for the establishment of phyletic relationships among plant taxa as macromolecules such as proteins and nucleic acids, which have recently received much attention in molecular systematics (Hillis *et al.*, 1996). Inferences regarding the evolution of secondary metabolites depend on comparisons with dendrograms obtained through cladistic techniques based on wide sets of “traditional” morphologic or molecular characters.

This paper discusses the development of secondary metabolite patterns in taxa of three families, Eriocaulaceae, Lythraceae and Velloziaceae, which are widely distributed in the *campos rupestres*, mountainous ecosystems found in the Espinhaço Mountains of the Brazilian States of Minas Gerais and Bahia. We also discuss the evolution of the distribution of secondary metabolites based on recent cladistic analyses, taking into account the distribution of secondary metabolites such as the flavonoids of Eriocaulaceae and Lythraceae, the alkanes of the foliar waxes of Velloziaceae and the fatty acids of Lythraceae.

FLAVONOIDS

Eriocaulaceae

The Eriocaulaceae are pantropical monocotyledons readily characterized by congested capitulla. The family comprises about 1100 species of which 700 occur in the New World, particularly on higher altitude mountains in Minas Gerais and Bahia (Giulietti and Pirani, 1988). Genera particularly well represented in the *campos rupestres* are *Eriocaulon*, *Leiothrix*, *Paepalanthus* and *Syngonanthus*.

Our knowledge about the distribution of flavonoids in

Eriocaulaceae derives from the works of Bate-Smith and Harborne (1969) on six species of *Eriocaulon*, Dokkedal and Salatino (1992) on six species of *Leiothrix*, Mayworm and Salatino (1993) on four species of *Paepalanthus* and Ricci *et al.* (1996) on 22 species of *Syngonanthus*. Figure 1 shows some examples of flavonoid aglycones found in Eriocaulaceae and the genera and some infrageneric categories in which the structural types predominate.

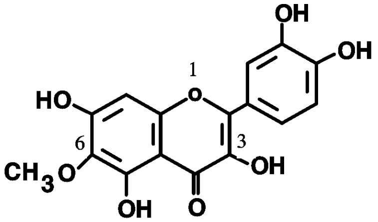
A cladistic analysis based on 49 predominantly morphologic characters (Giulietti *et al.*, 2000) suggests that *Paepalanthus* is polyphyletic and should be divided into smaller monophyletic genera. *Eriocaulon* is closely related to some groups of *Paepalanthus*, while *Leiothrix* and *Syngonanthus* appear as more advanced sister groups (Figure 2). The cladogram shows the evolution in *Syngonanthus* at the sectional level: the *Eulepis* and *Thysanocephalus* sections seem to constitute a more advanced monophyletic group than the *Carpocephalus* and *Syngonanthus* sections (Figure 2).

Some of these relationships are reflected by the amount of phenolic substances in the capitulla of Eriocaulaceae, with specimens of *Paepalanthus* and *Eriocaulon* tending to have higher amounts while the more advanced genera *Leiothrix* and *Syngonanthus* have lower amounts (Salatino *et al.*, 1990) (Figure 2). It seems that evolution has selected for a reduction in the amount of phenolic compounds in capitulla of Eriocaulaceae.

In the case of flavonoid distribution in Eriocaulaceae, there seems to be a relationship between high phenolic content and the predominance of flavonols, and low phenolic content and the predominance of flavones. In fact, in *Paepalanthus* and *Eriocaulon* species, almost always with more than 1% soluble phenols in their capitulla, flavonols are highly predominant, while in *Leiothrix* and *Syngonanthus* species, always with less than 1% phenols, there are apparently only flavones (Figures 1 and 2).

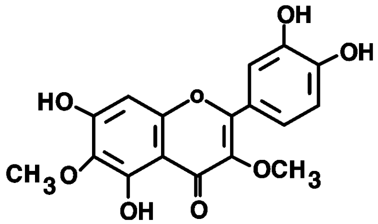
Other structural aspects of the flavonoids seem to have paralleled the evolution of Eriocaulaceae because in the supposedly most primitive groups of the family 6-oxygenated compounds frequently occur, while flavonoids with this characteristic, such as quercetagetin and patuletin (Figure 1), are rarer in angiosperms than flavonoids like quercetin and luteolin, which have no oxygen at position 6 (Figure 1). Evidence suggests that in the Eriocaulaceae 6-oxygenation has been superseded during evolution: in primitive groups (flavonol bearers), like *Paepalanthus* and *Eriocaulon*, 6-oxygenated compounds predominate. In the more advanced groups (flavone bearers), like *Syngonanthus*, the highly advanced sections *Eulepis* and *Thysanocephalus* lack 6-oxygenated derivatives, while in the more primitive sections *Carpocephalus* and *Syngonanthus* 6-hydroxyluteolin predominates (Figures 1 and 2). Surprisingly, the advanced sections *Eulepis* and *Thysanocephalus* show a prevalence of *C*-glycoflavones, a character suggested as an indicator of chemical primitivity in angiosperms (Harborne, 1972).

So far, the available data suggest that flavonoid evolution in Eriocaulaceae has followed the path:



I. Patuletin

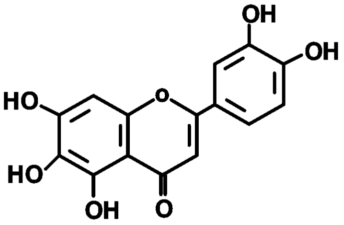
Eriocaulon, Paepalanthus



II. 3-*O*-Methyl patuletin

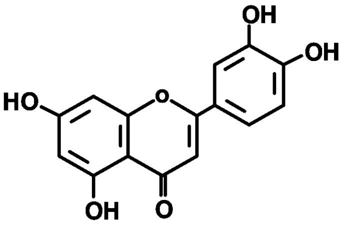
Paepalanthus

Figure 1 - Flavonoids typical of groups of Eriocaulaceae. **I** and **II**, Flavonols; **III**-**V**, flavones; **I**-**III**, 6-oxygenated flavonoids.



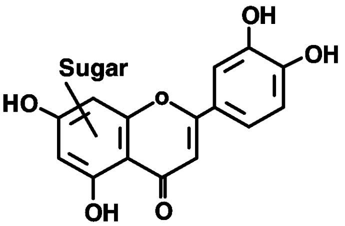
III. 6-Hydroxyluteolin

Paepalanthus, Leiothrix
Syngonanthus sect.
Syngonanthus and *Carpocephalus*



IV. Luteolin

Leiothrix, Syngonanthus



V. Luteolin-*C*-glycoside

Leiothrix, Syngonanthus sect.
Eulepis and *Thysanocephalus*

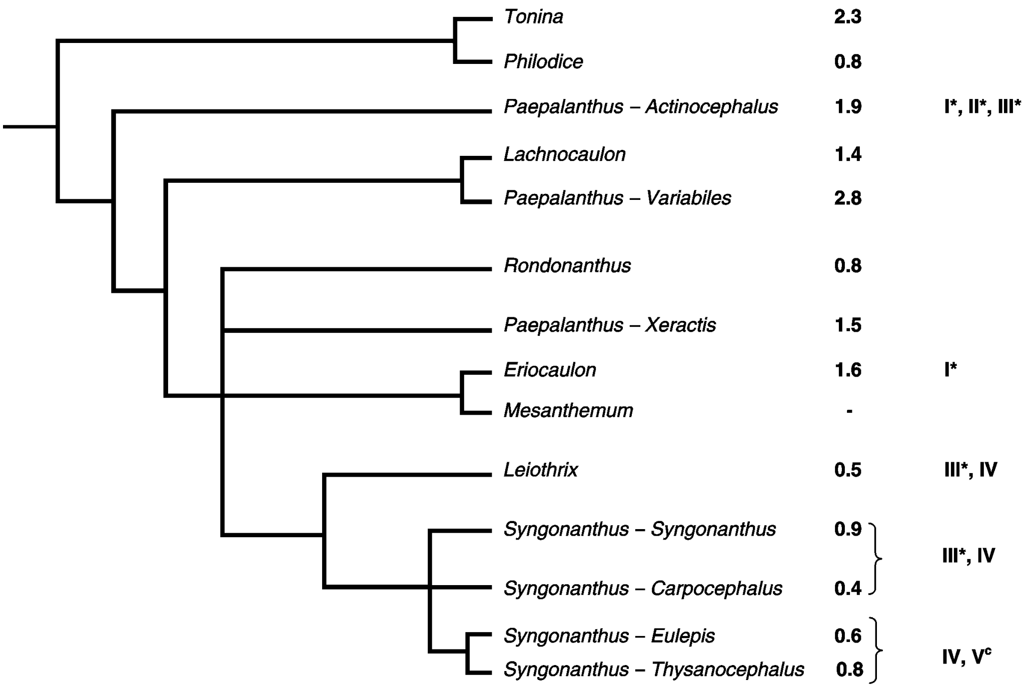


Figure 2 - Phyletic relationships between sections and genera of Eriocaulaceae, based on morphological characters (Giulietti *et al*., 2000). Numerals associated with taxa represent the content of soluble phenols from the capitulla (Salatino *et al*., 1990). Roman numerals refer to flavonoid structural details presented in Figure 1. *: 6-Oxygenated flavonoids; c: *C*-glycoflavones; **I** and **II**: flavonols; **III** and **IV**: flavones.

6-oxygenated flavonols	→ 6-oxygenated flavones	→ *C*-glycoflavones
Paepalanthus, Eriocaulon	*Leiothrix, Syngonanthus* section *Syngonanthus* and *Thysanocephalus*	*Syngonanthus* section *Eulepis* and *Carpocephalus*

Lythraceae

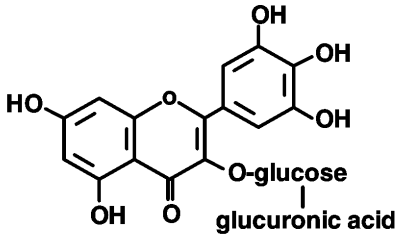
Among the Myrtales the Lythraceae represent a family with 31 genera and about 600 species widely distributed in tropic and subtropic regions of the Old and New World, mainly in mesophytic and humid habitats, with the habits of Lythraceae varying from trees to annual herbs.

Relatively little is known about flavonoid distribution in the family. Graham *et al.* (1980) reported the flavonoids of *Ammania coccinea*; Blatt *et al.* (1994) the flavonoids of 27 species of *Diplusodon*; Santos *et al.* (1995), 16 species of *Cuphea*, and Santos *et al.* (2000), 3 species of *Lafoensia*. Figure 3 shows some examples of flavonoids and the corresponding Lythraceae taxa.

Cuphea is predominantly herbaceous and the largest genus of Lythraceae, comprising 250 species of the New World. *Diplusodon* is the second largest genus in the family, with 74 shrubby or subshrubby species, all endemic to Brazil. *Lafoensia* is a small South American genus, with 9 arboreal or shrubby species.

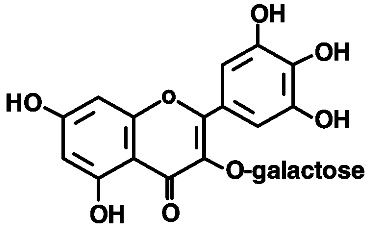
Graham *et al.* (1993) carried out a cladistic analysis embracing all genera of Lythraceae. Unfortunatelly the two genera about which most information on flavonoid chemistry is available (*Cuphea* and *Diplusodon*) are not closely related, occurring in relatively distant clades (Figure 4). The cladistic analysis reveals that the evolution of Lythraceae followed the trend arboreal → shrubby (woody) → herbaceous habits (including herbs of swampy habitats), and it can be seen that in *Cuphea*, an evolutionary dead end in the cladogram (Figure 4), herbaceous habits prevail. By contrast, in *Diplusodon* shrubby and subshrubby plants predominate, suggesting a more primitive condition relative to *Cuphea*. *Diplusodon* emerges from the base of a clade, which has at its ends very advanced aquatic groups of flooded areas, like *Ammania, Nesaea, Peplis* and *Rotala* (Graham *et al*., 1993; Figure 4). As no more information about the flavonoid chemistry of the family is available, we will adopt *Diplusodon* and *Cuphea* as representatives of more primitive and more advanced groups, respectively, of Lythraceae, while *Lafoensia* will be regarded as more primitive and closely related to *Cuphea* (Figure 4).

Chemically, *Diplusodon* is remarkable for the very frequent occurrence of mono- and diglycosides of myricetin (Figures 3 and 4). Harborne (1972) assigned to myricetin a role of primitivity indicator because it is a characteristic compound of woody dicotyledons, including Myrtales (Giannasi, 1988). Perhaps even more remarkable is the frequent occurrence in *Diplusodon* of the glycosidic combination of myricetin and glucuronic acid (Figure 3), the latter figuring as the rarest sugar in flavonoids, such association making the glycosides relatively unstable and readily



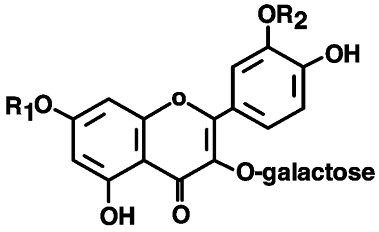
VI. Myricetin-3-*O*-glucosylglucuronide

Diplusodon



VII. Myricetin-3-*O*-galactoside

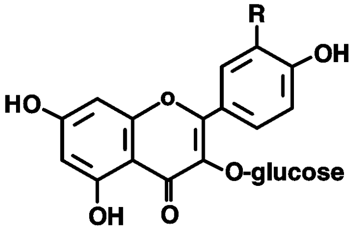
Cuphea, Diplusodon



VIII. R_1=H; R_2=CH_3
Isorhamnetin-3-*O*-galactoside

IX. R_1=CH_3 R_2=H
Rhamnetin-3-*O*-galactoside

Cuphea



X. R=H
Kaempferol-3-*O*-glucoside

XI. R=OH
Quercetin-3-*O*-glucoside

Cuphea, Diplusodon, Lafoensia

Figure 3 - Examples of flavonoids typical of Lythraceae.

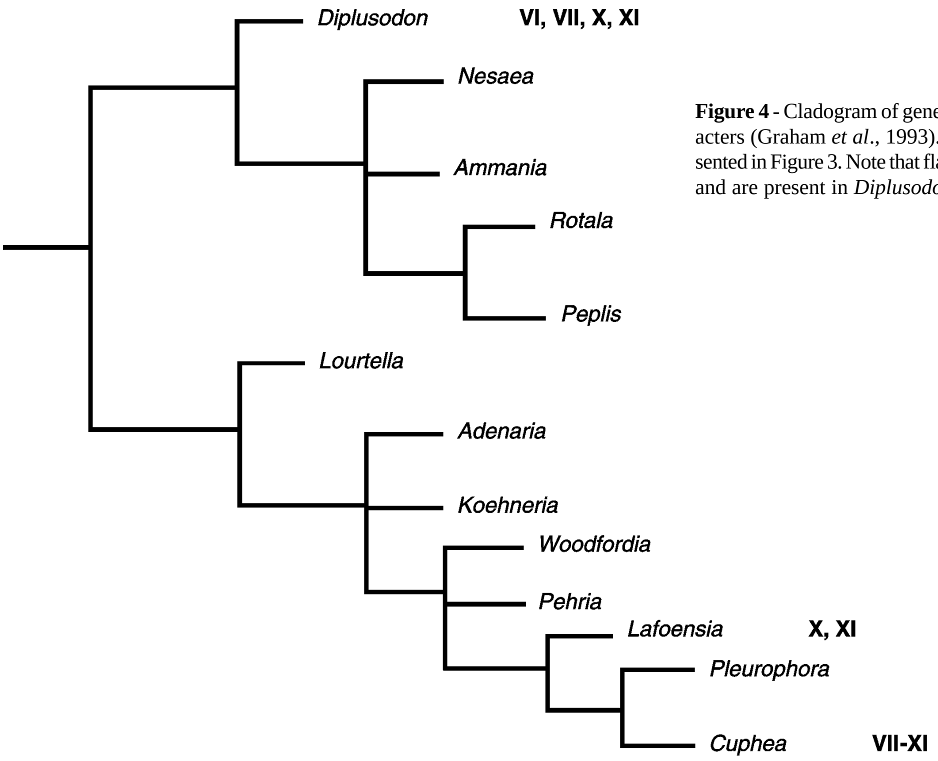


Figure 4 - Cladogram of genera of Lythraceae based on morphological characters (Graham *et al*., 1993). Roman numerals refer to the flavonoids presented in Figure 3. Note that flavonoids **VI** and **VII** are derivatives of myricetin and are present in *Diplusodon* and *Cuphea*, but not in *Lafoensia*.

liable to oxydation. Methylation may be viewed as a protection against oxydation of phenolic hydroxyls (Pugialli *et al.*, 1994), but no methoxylated flavonoids were found in *Diplusodon*.

Assuming *Cuphea* as more advanced than *Diplusodon* the following attributes could be regarded as evolutionary advances of the flavonoid chemistry of the Lythraceae: a) the absence of glycosidic combinations involving myricetin and glucuronic acid; b) the rare occurrence of glucuronic acid; c) the presence of methoxylated flavonoids such as rhamnetin and isorhamnetin and d) the more frequent occurrence of galactose glycosides. However, the present knowledge of the chemistry of the Lythraceae is too sketchy to accept these assumptions as definite proof of either primitivity or chemical advance in the family because the differences observed between the two genera might not be reproduced in other taxa with a similar distance of relative advancement.

An intriguing aspect emerges if one attempts to include the flavonoid pattern of *Lafoensia* (Figure 3) into the cladogram of Figure 4, bearing in mind the flavonoid profiles of *Cuphea* and *Diplusodon* already commented upon. Despite its woody habit (arboreal in several species), *Lafoensia* appears in close association with the predominantly herbaceous *Cuphea* in the cladogram. The flavonoid patterns of *Lafoensia* and *Cuphea* are substantially different (Figure 3) and up to the present, myricetin, *O*-methylated flavonols and flavonoids with glucuronic acid have not been reported for *Lafoensia*. Taking into account the existence of myricetin and glucuronic acid as constituent flavonoids of *Diplusodon* and *Cuphea*, it becomes difficult to accommodate such a simple flavonoid profile as that of *Lafoensia* in the topology of the Figure 4: *Lafoensia* should have some characteristics common to the other two genera. The flavonoid chemistry suggests that *Lafoensia* belongs to an evolutionary line different from *Cuphea*, or is a more advanced group, an alternative, however, which is not compatible with the distribution of fatty acids in *Lafoensia* (see below).

At the infrageneric level, there is an interesting aspect linking flavonoid chemistry and the evolution of *Diplusodon*. This genus is divided into sections according to patterns of foliar venation. The *Penninerves* section is presumably the primitive group, with the more common venation pattern, while the *Palmatinerves* and *Diplusodon* sections (the latter with a single prominent vein in the center of the lamina) can be regarded as derived groups. Flavonols and flavones are mutually exclusive in species of *Diplusodon*; yet flavones have been observed only in representatives of the *Penninerves* section, and in species of this section two flavonoid profiles are observed, exclusively flavonols or exclusively flavones, while in representatives of the other sections only flavonols have been found. Flavonoid evolution in *Diplusodon* can be envisaged according to the scheme depicted in Figure 5, where the primitive condition (*Penninerves* section) is characterized by a more complex profile, with flavonols and flavones, the two groups of phenols probably occurring together in an ancestral group with pennate venation. The evolution that led to derived patterns of venation was paralleled by a blockage of flavone synthesis, leading to the present patterns (Figure 5).

SEED FATTY ACIDS

Lythraceae

In the seed triglycerides of most dicotyledons, unsaturated fatty acids with 18 carbon atoms (C_{18}) predominate,

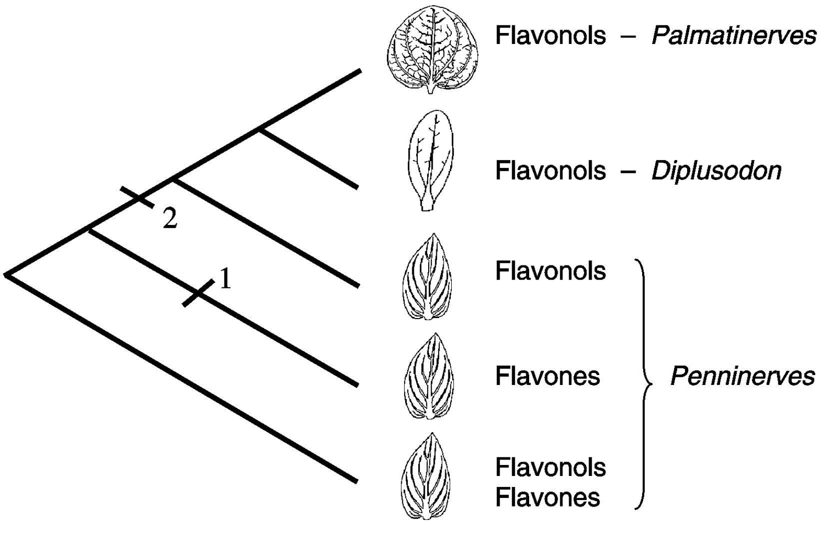


Figure 5 - Hypothetical phyletic relationships between sections of *Diplusodon* (Lythraceae), based on venation patterns and distribution of flavonoids. The basal penninervean group with flavonols and flavones has still not been observed. 1 - Blockage of flavonol synthesis. 2 - Blockage of flavone synthesis.

among which the most common are oleic ($C_{18:1}$, one unsaturation) and linoleic acids ($C_{18:2}$, two unsaturations). More rarely, species can be found with substantial amounts of fatty acids with longer carbon chains such as arachidic ($C_{20:0}$) and behenic ($C_{22:0}$), or shorter chains such as palmitic ($C_{16:0}$), myristic ($C_{14:0}$) and lauric ($C_{12:0}$) acids (Figure 6).

In Arecaceae (palm trees), the seed fatty acids present short and saturated chains (C_{10}-C_{14}). Triglycerides with such fatty acids have high commercial value because of their utility in the production of cosmetics and shampoos. In temperate countries the commercial exploitation of palm trees is not possible, so other vegetable sources of oils with similar characteristics are important, and the discovery of species of *Cuphea* with seed oils resembling coconut and palma oils (Graham *et al.*, 1981; Wolf *et al.*, 1983; Graham and Kleiman, 1987) raised considerable interest. Fatty acid patterns based on palmitic ($C_{16:0}$), oleic and linoleic acids are the rule in dicotyledons and the *Cuphea* pattern of seed fatty acids is regarded as a derived condition (apomorphy). All the evidence thus far available indicates that the patterns of seed fatty acids in *Cuphea* represent an autapomorphy, because similar profiles have not been found in genera of other Lythraceae (Graham and Kleiman, 1987, Lythraceae in general; Santos and Salatino, 1998, *Diplusodon*).

ALKANES OF FOLIAR EPICUTICULAR WAX

Velloziaceae

The Velloziaceae constitute a family of predominantly South American tropical monocotyledons, with approximately 200 species, most of which occur in Brazil, especially in the *campos rupestres*. Other species occur in Venezuela, Bolivia, Argentina and Africa.

The family is divided into two subfamilies, Vellozioideae (supposedly the most primitive) and Barbacenioideae (advanced). The number and delimitation of the genera are controversial subjects, and two systems of classification (Smith and Ayensu, 1976; Menezes, 1980) with several conflicting points have been proposed for the family (see Mello-Silva, 1994, for a detailed discussion). The classification of Menezes (1980) will be adopted in this paper, with the inclusion of the genera *Barbaceniopsis*, *Nanuza* and *Talbotia* from the system of Smith and Ayensu (1976). According to Menezes' system, the Vellozioideae comprise the genera *Vellozia* (South America) and *Xerophyta*, the latter corresponding to South American and African species, plus the genera *Barbaceniopsis* (Venezuela, Bolivia and Argentina), *Talbotia* (Africa, monotypic) and *Nanuza* (Brazil, monotypic) from Smith and Ayensu's (1976) system. The Barbacenioideae comprise the genera *Aylthonia*, *Barbacenia*, *Burlemarxia* and *Pleurostima*.

Cytogenetic analyses (Melo *et al*., 1997) revealed that *Vellozia* and South American *Xerophyta* are diploid, while the Barbacenioideae *Aylthonia*, *Barbacenia*, *Burlemarxia* and *Pleurostima* are tetraploid. Since the African groups *Talbotia* and *Xerophyta* sensu Smith and Ayensu (1976) are hexaploid, Melo *et al*. (1997) suggested that the Velloziaceae originated in South America.

A cladistic analysis based predominantly on morphologic characters (Menezes *et al*., 1994) supports the view (evidenced by morphology and citogenetics) that the Vellozioideae constitute the primitive group of the family, and revealed that they are paraphyletic. The monotypic condition of *Nanuza* and *Talbotia* is reinforced by the cladistic analysis, as well as the inclusion of *Talbotia*, *Barbaceniopsis* and African *Xerophyta* among the Barbacenioideae, which appear in the analysis as a consistently monophyletic group. From the base of the Barbacenioideae, *Burlemarxia* emerges, while *Pleurostima* is the most advanced genus and consistently monophyletic.

Phylogenetic analysis of South American Velloziaceae (*Barbaceniopsis* not included), plus *Talbotia elegans* and *Acanthochlamys bracteata* (an Asian species with close affinities with Velloziaceae, based on *rbc*L sequencing; Chase *et al*., 1995), based on sequencing of the *trn*L-F region of the chloroplast DNA (Salatino *et al*., 2001), supports some points raised by the cladistic analysis commented on above but disagrees with other points (Figure 7). The Vellozioideae emerge from the base of the system and are paraphyletic. *Nanuza plicata* does not group with other species, emerging from the base of the system of the Vellozioideae. How-

$CH_3\text{-}(CH_2)_7\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}COOH$

Oleic acid
LYTHRACEAE

$CH_3\text{-}(CH_2)_4\text{-}CH{=}CH\text{-}CH_2\text{-}CH{=}CH\text{-}(CH_2)_7\text{-}COOH$

Linoleic acid
LYTHRACEAE

$CH_3\text{-}(CH_2)_{18}\text{-}COOH$

Arachidic acid
Diplusodon

$CH_3\text{-}(CH_2)_{20}\text{-}COOH$

Behenic acid
Diplusodon

$CH_3\text{-}(CH_2)_{12}\text{-}COOH$

Myristic acid
Cuphea

$CH_3\text{-}(CH_2)_{10}\text{-}COOH$

Lauric acid
Cuphea

Figure 6 - Fatty acids of seed triglycerides and taxa of Lythraceae.

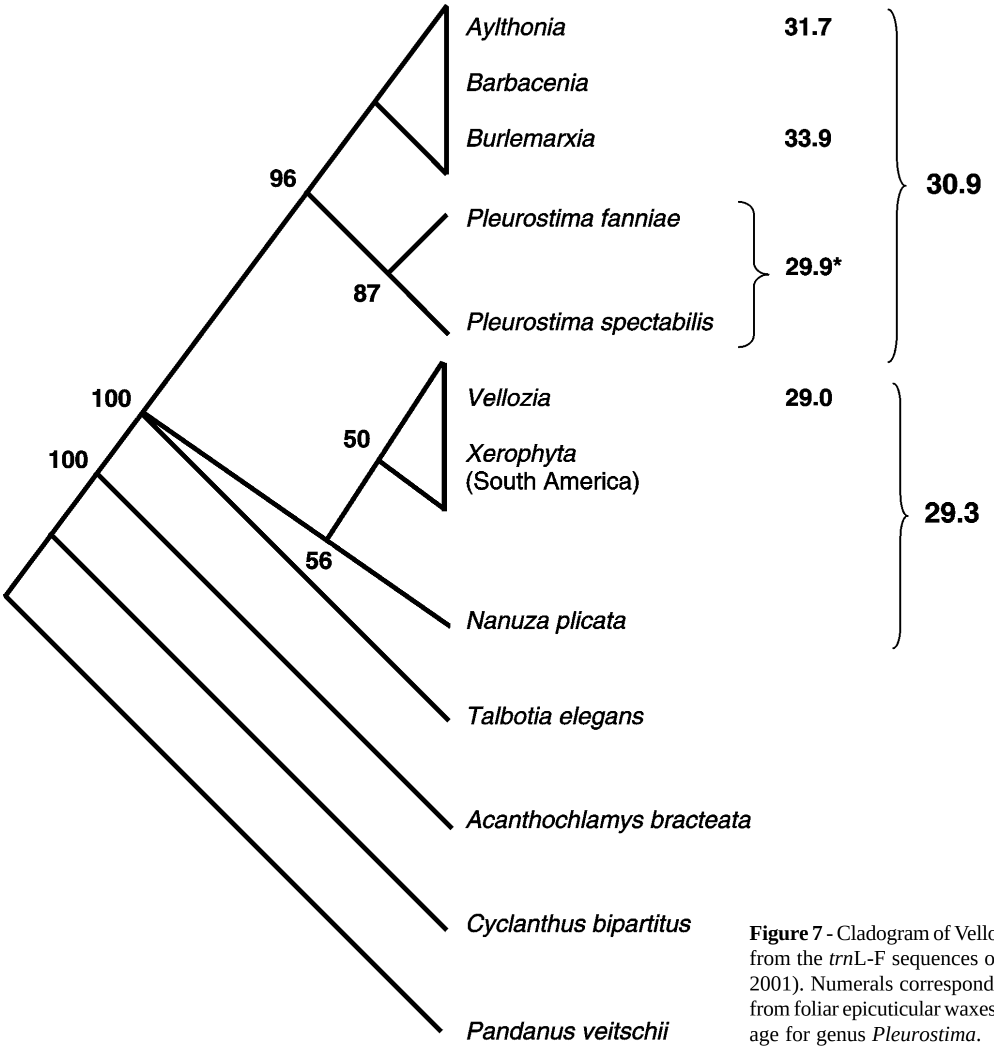


Figure 7 - Cladogram of Velloziaceae taxa and related groups, inferred from the *trn*L-F sequences of the cpDNA (based on Salatino *et al.*, 2001). Numerals correspond to average carbon numbers of alkanes from foliar epicuticular waxes (based on Salatino *et al.*, 1989). *: Average for genus *Pleurostima*.

ever, *Talbotia* figures in a polytomy at the base of the whole system, with no indication as to which subfamily it belongs. The Barbacenioideae are monophyletic, but from its base emerges *Pleurostima* rather than *Burlemarxia* (compare with the results of the morphologic analysis in the previous paragraph). Furthermore, *trn*L-F sequencing suggests that *Burlemarxia* is a very recent group, closely related and genetically indistinguishable from *Barbacenia*.

This section continues with comments on the possible evolutionary trends of the distribution of alkanes in Velloziaceae. No discussion will be made relative to some groups of secondary metabolites relatively well studied in the family, such as flavonoids (see, for example, Williams *et al.*, 1994) and diterpenoids (see, for example, Pinto *et al.*, 1997).

Alkanes of foliar epicuticular wax

Plant cutinized organs present a surface coating formed by amorphous or crystalline deposits of waxes (Baker *et al.*, 1982), which are termed epicuticular, as opposed to the intracuticular waxes which impregnate the cutin lattice of the external cell walls. Epicuticular waxes may have rather complex composition, and different classes of substances such as hydrocarbons, esters, ketones, aldehydes, alcohols, free carboxylic acids, triterpenes and flavonoids may occur in different combinations (Bianchi, 1995). Among these classes of constituents, the alkanes (a class of hydrocarbons) are the most efficient as barriers against water loss, and are seemingly universal in vascular plants.

Alkanes are obtained from epicuticular waxes in general as mixtures of substances with normal carbon chains. Depending on the plant, the alkane distribution can vary from compounds with 15 (C_{15}) to compounds with 37 (C_{37}) carbon atoms, but in most angiosperms the most abundant compounds are C_{27}, C_{29} or C_{31}.

An inventory of the alkane distribution of foliar epicuticular waxes and its contribution to the taxonomy of Velloziaceae has been produced by Salatino *et al.* (1989)

and Figure 8 (a, b) shows the distribution of alkanes of two species of Velloziaceae, where a predominance of homologues with odd numbers of carbon atoms is clearly noticed. The distribution relative to *Vellozia fruticosa*, with a peak at C_{29}, is in agreement with the general trend in angiosperms, although the alkane distribution of *Burlemarxia pungens*, which peaks at C_{35}, represents a remarkable deviation from the general tendency. Table I presents the average and confidence interval for alkane distribution for groups of Velloziaceae. Groups within the Vellozioideae have alkanes with numbers of carbon atoms significantly lower than those of Barbacenioideae groups. Among the Vellozioideae there is no significant difference between *Vellozia* and the South American *Xerophyta*. In Barbacenioideae, two groups stand out: *Pleurostima*, with shorter alkane chains than the average (with peaks predominantly at C_{31}), and *Burlemarxia* which has longer chains (two out of three species of the genus presenting peaks at C_{35}).

Taking into account the alkane distribution of Velloziaceae and the evidence on the evolution of the family, there seems to be a tendency of elongation of the carbon chains which parallels the evolution of the group. Among the Barbacenioideae the direction of elongation of the carbon chains - Pleurostima → Barbacenia/Aylthonia → Burlemarxia (Table I) - is more coherent with the phylogeny inferred from chloroplast DNA sequencing (Figure 7) than the phylogeny obtained from morphologic evidence (see above for the conflict between the relative positioning of Barbacenia and Pleurostima between both phylogenies).

In the case of Velloziaceae it is interesting to speculate on the adaptive meaning of longer chains for constituents of foliar waxes. A high proportion of the representatives of the family consists of xerophytic plants, many of them growing on rocky or extremely sandy soil, and in such cases it is reasonable to assume that efficient waxes act as barriers against water loss and are important adaptive attributes, which tend to be strongly fixed genetically, which is probably one of the reasons for the low intraspecific variation of alkane patterns among Velloziaceae (Salatino *et al.*, 1991; Salatino, 1998). It has also been shown that the elongation of carbon chains increases the efficiency of wax constituents as waterproofing agents (Schönherr, 1982), thus alkanes with longer carbon chains could be viewed as a biochemical adaptive advantage related to the reduction of cuticular transpiration, which evolved preferentially in the advanced groups of the family in habitats with limited moisture availability. Perhaps this is the reason for the existence of alkanes with relatively long carbon chains (peaks at C_{33} or higher) in foliar waxes of other obviously xerophytic families, such as Crassulaceae (Eglinton *et al.*, 1962), Portulacaceae (Tuloch, 1974) and Euphorbiaceae (Proksch *et al.*, 1981). It is noteworthy that the leaves of some South American Vellozioideae, like *Nanuza plicata* (syn. *Xerophyta plicata*), dehydrate under

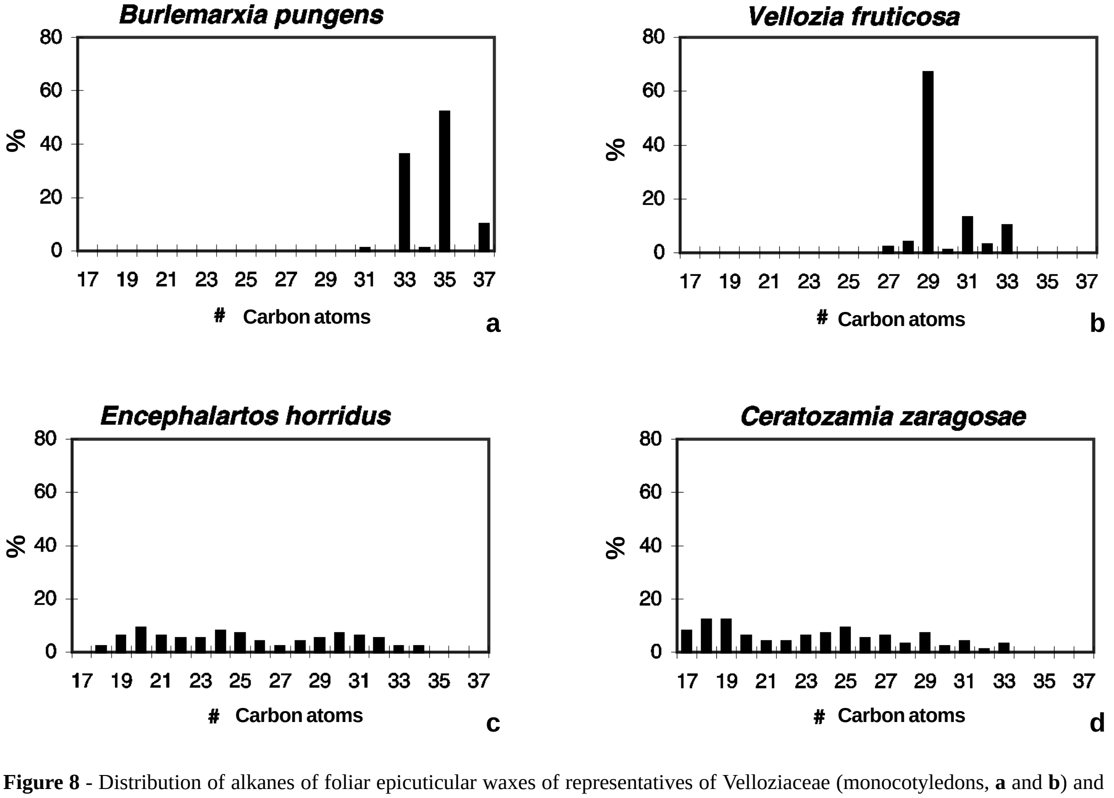


Figure 8 - Distribution of alkanes of foliar epicuticular waxes of representatives of Velloziaceae (monocotyledons, **a** and **b**) and Zamiaceae (Gymnospermae, **c** and **d**).

Table I - Averages of numbers of carbon atoms and confidence intervals (CI) of alkanes of foliar epicuticular waxes of Velloziaceae (based on Salatino *et al*., 1989).

Taxa	Average ± CI
Aylthonia	31.7 ± 1.4
Barbacenia	30.7 ± 1.3
Burlemarxia	33.9 ± 1.7
Pleurostima	29.9 ± 0.9
Xerophyta	29.3 ± 0.3
Vellozia	29.0 ± 1.2
Barbacenioideae	30.9 ± 0.7
Vellozioideae	29.3 ± 0.5

prolonged water stress and remain biologically inactive until they receive water (Meguro *et al*., 1977).

Evolutionarily, the alkanes of Velloziaceae and other xerophytic groups represent the extreme of a biosynthetic development that must have begun with the algae. The latter present alkanes with carbon chains C_{15}-C_{19}, without a clear prevalence between homologues with odd and even numbers of carbon atoms (Blumer *et al*., 1971). In these groups, alkanes are formed from decarboxylation of fatty acids C_{16}-C_{20}. In land plants, the development of the elongation-decarboxylation system ensued (Wettstein-Knowles, 1995), leading to the synthesis of C_{28}-C_{32} fatty acids, which give rise, after decarboxylation, to C_{27}-C_{31} alkanes, with a clear predominance of odd over even homologues, this being the general rule in the angiosperms. The Cycadales form an intermediate step between the more primitive condition of the alkane patterns of the algae and the more advanced pattern of the angiosperms. In these gymnosperms, complex patterns covering the range C_{18}-C_{33} are common, sometimes bimodal (Figure 8c, d), and without a clear predominance of odd over even alkanes (Osborne *et al*., 1989, 1993).

In Velloziaceae and some other groups, the elongation system added some additional steps, leading to the synthesis of fatty acids up to C_{38}, which consequently gave rise to alkanes of up to C_{37} (Figure 8b). Taking all the evidence into consideration, it seems reasonable to assume that, in Velloziaceae, alkanes with longer carbon chains in the foliar waxes represent an evolutionarily advanced state.

CONCLUDING REMARKS

Although phylogeny reconstruction has not been attainable through analyses of the distribution of secondary metabolites, it is scientifically exciting and taxonomically useful to know the evolutionary trends of these plant substances. In addition to plant taxonomy, other areas can benefit from the progress in studies about the evolution of secondary metabolites. Evolutionary ecology is one of these fields, since it takes into account aspects of the evolution of the organisms in connection with the processes of their adaptation to the environment. A particularly fruitful area in evolutionary ecology refers to plant-insect relationships, and studies in this field frequently reveal that secondary metabolites play a key role as intermediaries in mutualistic and parasitic interactions. In this way the understanding of the evolution of secondary metabolites in plant groups has the potential to help achieve a better understanding of the evolution of such interactions and, consequently the evolution of herbivorous insects.

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RESUMO

Hipóteses são apresentadas sobre a evolução de padrões estruturais de metabólitos secundários (flavonóides e alcanos de ceras foliares) e ácidos graxos, em famílias bem representadas nos campos rupestres. A distribuição de ácidos graxos é comentada no confronto entre os gêneros de Lythraceae, com maior ênfase em *Cuphea* (supostamente mais avançado) e *Diplusodon*, com a proposta de que compostos saturados com cadeias curtas representam uma condição derivada em Lythraceae, embora restrita provavelmente a *Cuphea*. A evolução deve ter gerado perfis flavonoídicos mais complexos em *Cuphea*, com a inclusão de *C*-glicoflavonas e flavonóis metoxilados (ramnetina e isoramnetina), ausentes em representantes de *Diplusodon* e *Lafoensia*. Ao contrário, grupos supostamente primitivos de Eriocaulaceae (*Paepalanthus*, por exemplo) apresentam perfis flavonoídicos mais complexos, caracterizados por flavonas e flavonóis, estes últimos freqüentemente com hidroxilação e metoxilação no carbono 6; alguns grupos mais avançados da mesma família (*Leiothrix* e *Syngonanthus*) aparentemente possuem apenas flavonas, com uma tendência ao maior acúmulo de *C*-glicoflavonas nas espécies de menor porte. Em Velloziaceae, os representantes da subfamília supostamente primitiva (Vellozioideae) apresentam preferencialmente distribuições de alcanos da cera foliar epicuticular com modas em C_{27}, C_{29} ou C_{31}; os representantes da subfamília derivada (Barbacenioideae) apresentam modas preferencialmente em C_{33} ou C_{35}. No entanto, no gênero *Pleurostima* (Barbacenioideae) as modas recaem geralmente em C_{31}. As condições de avanço ou primitividade dos perfis de metabólitos secundários são comparadas com resultados de análises cladísticas baseadas em caracteres morfológicos (Eriocaulaceae e Lythraceae) e morfológicos e moleculares (Velloziaceae).

REFERENCES

Baker, E.A., Bukovac, M.J. and **Hunt, G.M.** (1982). Composition of tomato fruit cuticle as related to fruit growth and development. In: *The Plant Cuticle* (Cutler, D.F., Alvin, K.L. and Price, C.E., eds). Academic Press, London, pp. 33-44.

Bate-Smith, E.C. and **Harborne, J.B.** (1969). Quercetagetin and patuletin in *Eriocaulon*. *Phytochemistry 8*: 1035.

Bianchi, G. (1995). Plant waxes. In: *Waxes: Chemistry, Molecular Biology* *and Functions* (Hamilton, R.J., ed.). The Oily Press, Dundee, pp. 175-222.

Blatt, C.T.T., Salatino, A., Salatino, M.L.F., del Pero Martinez, M.A. and **Cavalcanti, T.B.** (1994). Flavonoids of *Diplusodon* (Lythraceae). *Biochem. Syst. Ecol. 22*: 101-107.

Blumer, M., Guillard, R.R.L. and **Chase, T.** (1971). Hydrocarbons of marine phytoplankton. *Mar. Biol. 8*: 183-189.

Chase, M.W., Stevenson, D.W., Wilkin, P. and **Rudall, P.J.** (1995). Monocot systematics: a combined analysis. In: *Monocotyledons: Systematics and Evolution* (Rudall, P.J., Cribb, D.F., Cutler, D.F. and Humphries, C.J., eds). Whitstable Litho Printers Ltd., London, pp. 685-730.

Cronquist, A. (1981). *An Integrated System of Classification of Flowering Plants*. Columbia University Press, New York.

Dahlgren, G. (1989). An updated angiosperm classification. *Bot. J. Linn. Soc. 100*: 197-203.

Dokkedal, A.L. and **Salatino, A.** (1992). Flavonoids of Brazilian species of *Leiothrix*. *Biochem. Syst. Ecol. 20*: 31-32.

Eglinton, G., Hamilton, R.J., Raphael, R.A. and **Gonzalez, A.G.** (1962). Hydrocarbon constituents of the wax coatings of plant leaves: a taxonomic survey. *Phytochemistry 1*: 89-102.

Giannasi, D.E. (1988). Flavonoids and evolution in the dicotyledons. In: *The Flavonoids - Advances in Research Since 1980* (Harborne, J.B., ed.). Chapman and Hall, London, pp. 479-504.

Giulietti, A.M. and **Pirani, J.R.** (1988). Patterns of geographic distribution of some plant species from the Espinhaço Range, MG - BA, Brazil. In: *Proceedings of the Workshop on Neotropical Distribution Patterns* (Vanzolini, P.E. and Heyer, W.R., eds.). Academia Brasileira de Ciências, Rio de Janeiro, pp. 39-69.

Giulietti, A.M., Scatena, V.L., Sano, P.T., Parra, L.R., Queiroz, L.P., Harley, R.M., Menezes, N.L., Isepon, A.M.B., Salatino, A., Salatino, M.L.F., Vilegas, W., Santos, L.C., Ricci, C.V., Bomfim-Patrício, M.C. and **Miranda, E.B.** (2000). Multidisciplinary studies on Neotropical Eriocaulaceae. In: *Monocots: Systematics and Evolution* (Wilson, K.L. and Morrison, D.A., eds.). CSIRO, Melbourne, pp. 580-589.

Graham, S.A. and **Kleiman, R.** (1987). Seed lipids of the Lythraceae. *Biochem. Syst. Ecol. 15*: 433-439.

Graham, S.A., Timmermann, B.N. and **Mabry, T.J.** (1980). Flavonoids glycosides in *Ammania coccinea* (Lythraceae). *J. Nat. Prod. 34*: 644-645.

Graham, S.A., Hirsinger, F. and **Röbbelen, G.** (1981). *Cuphea* seed lipids and their systematic significance. *BioScience 31*: 244-246.

Graham, S.A., Crisci, J.V. and **Hoch, P.C.** (1993). Cladistic analysis of the Lythraceae *sensu lato* based on morphological characters. *Bot. J. Linn. Soc. 133*: 1-33.

Hamilton, R.J. (1995). *Waxes: Chemistry, Molecular Biology and Functions.* The Oily Press Ltd., Dundee.

Harborne, J.B. (1972). Evolution and function of flavonoids in plants. *Recent Adv. Phytochem. 4*: 107-141.

Harborne, J.B. (1998). *Phytochemical Methods.* Chapman & Hall, London.

Harborne, J.B. and **Turner, B.L.** (1984). *Plant Chemosystematics*. Academic Press, London.

Hillis, D.M., Moritz, G. and **Mable, B.K.** (1996). *Molecular Systematics*. Sinauer Associates, Inc., Sunderland.

Mayworm, M.A.S. and **Salatino, A.** (1993). Flavonoids de quatro espécies de *Paepalanthus* Ruhl. (Eriocaulaceae). *Acta Bot. Bras. 7*: 129-133.

Meguro, M., Joly, C.A. and **Bittencourt, M.M.** (1977). “Stress” hídrico e alguns aspectos do comportamento fisiológico em *Xerophyta plicata* Spreng. - Velloziaceae. *Bolm Bot. Univ. S. Paulo 5*: 27-42.

Mello-Silva, R. (1994). A new species, new synonyms, and a new combination in Brazilian Velloziaceae. *Novon 4*: 271-275.

Melo, N.F., Guerra, M., Benko-Iseppon, A.M. and **Menezes, N.L.** (1997). Cytogenetics and cytotaxonomy of Velloziaceae. *Plant Syst. Evol. 204*: 257-273.

Menezes, N.L. (1980). Evolution in Velloziaceae, with special reference to androecial characters. In: *Petaloid Monocotyledons: Horticultural and Botanical Research* (Brickell, C.D., Cutler, D.F. and Gregory, M., eds). Academic Press, London, pp. 117-137.

Menezes, N.L., Mello-Silva, R. and **Mayo, S.J.** (1994). A cladistic analysis of the Velloziaceae. *Kew Bull. 49*: 71-92.

Osborne, R., Salatino, M.L.F. and **Salatino, A.** (1989). Alkanes of foliar epicuticular waxes of the genus *Encephalartos*. *Phytochemistry 11*: 3027-3030.

Osborne, R., Salatino, M.L.F., Sekiya, C.M. and **Vazquez-Torres, M.** (1993). Alkanes of foliar epicuticular waxes from five cycad genera in the Zamiaceae. *Phytochemistry 32*: 607-608.

Pinto, A.C., Pizzolatti, M.G. and **Epifanio, R.D.A.** (1997). The isolation of novel diterpenoids, including a C-40 bis-diterpenoid, from the Brazilian plant *Vellozia magdalenae* (Velloziaceae). *Tetrahedron 53*: 2005-2012.

Proksch, P., Sternburg, C. and **Rodriguez, E.** (1981). Epicuticular alkanes from desert plants of Baja California. *Biochem. Syst. Ecol. 9*: 205-206.

Pugialli, H.R.L., Kaplan, M.A. and **Gottlieb, O.R.** (1994). Evolução flavonoídica em Zingiberales. *An. Acad. Bras. Cienc. 66*: 491-494.

Ricci, C.V., Patrício, M.C.B., Salatino, M.L.F., Salatino, A. and **Giulietti, A.M.** (1996). Flavonoids of *Syngonanthus* Ruhl. (Eriocaulaceae): Taxonomic implications. *Biochem. Syst. Ecol. 24*: 577-583.

Salatino, A. (1998). Importância ecológica e taxonômica dos constituintes das ceras vegetais. In: *Proceedings of the VI Congreso Latinoamericano de Bótanica* (Fortunato, R. and Bacigalupo, N., eds). Missouri Botanical Garden Press, St. Louis, pp. 505-514.

Salatino, M.L.F., Salatino, A., Menezes, N.L. and **Mello-Silva, R.** (1989). Alkanes of foliar epicuticular waxes of Velloziaceae. *Phytochemistry 28*: 1105-1114.

Salatino, A., Salatino, M.L.F. and **Giulietti, A.M.** (1990). Contents of soluble phenolic compounds of capitula of Eriocaulaceae. *Química Nova 13*: 289-292.

Salatino, A., Salatino, M.L.F., Mello-Silva, R. and **Duerholt-Oliveira, I.** (1991). An appraisal of the plasticity of alkane profiles of some species of Velloziaceae. *Biochem. Syst. Ecol. 19*: 241-248.

Salatino, A., Salatino, M.L.F., Mello-Silva, R., van Sluys, M.-A., Giannasi, D.E. and **Price, R.A.** (2001). Phylogenetic inference in Velloziaceae using chloroplast *trn*L-F sequences. *Syst. Bot.* (in press).

Santos, D.Y.A.C. and **Salatino, A.** (1998). Fatty acids of seed oils of species of *Diplusodon* Pohl (Lythraceae). *Biochem. Syst. Ecol. 26*: 109-115.

Santos, D.Y.A.C., Salatino, M.L.F. and **Salatino, A.** (1995). Flavonoids of species of *Cuphea* (Lythraceae) from Brazil. *Biochem. Syst. Ecol. 23*: 99-103.

Santos, D.Y.A.C., Salatino, M.L.F. and **Salatino, A.** (2000). Flavonoids of *Lafoensia* (Lythraceae). *Biochem. Syst. Ecol. 28*: 487-488.

Schönherr, J. (1982). Resistance of plant surfaces to water loss: transport properties of cutin, suberin, associated lipids. In: *Physiological Plant Ecology II. Encyclopedia of Plant Physiology* (Lange, O.L., Nobel, P.S., Osmond, C.B. and Ziegler, H., eds). New Series, Vol. 12B. Springer-Verlag, Berlin, pp. 154-179.

Smith, L.B. and **Ayensu, E.S.** (1976). A revision of American Velloziaceae. *Smithson. Contr. Bot. 30*: 1-172.

Takhtajan, A. (1997). *Diversity and Classification of Flowering Plants*. Columbia University Press, New York.

Tulloch, A.P. (1974). Leaf wax of *Portulaca oleracea. Lipids 9*: 664-668.

Wettstein-Knowles, P. von (1995). Biosynthesis and genetics of waxes. In: *Waxes: Chemistry, Molecular Biology and Functions* (Hamilton, R.J., ed.). The Oily Press, Dundee, pp. 131-174.

Williams, C.A., Harborne, J.B., Greenham, J. and **Eagles, J.** (1994). Differences in flavonoid patterns between genera within the Velloziaceae. *Phytochemistry 36*: 931-940.

Wolf, R.B., Graham, S.A. and **Kleiman, R.** (1983). Fatty acid composition of *Cuphea* seed oils. *J. Am. Oil Chem. Soc. 60*: 103-104.

Woodland, D.W. (1997). *Contemporary Plant Systematics*. Andrews University Press, Michigan.