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Chemosystematic Aspects of the Moraceae Family: Phenylpropanoids and Aromatic Polyketides

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Abstract The present study discusses the evolutionary status of Moraceae, from the perspective of chemical features of phenolic micromolecules. A chemosystematics analysis points to affinities between genera and tribe belonging to the Moraceae family, by correlation of the protection parameters of micromolecule hydroxyls resulting from the mixed pathway (acetate/shikimate) and the shikimate pathway. The phenylpropanoid and aromatic polyketide hydroxyl groups are mainly protected by prenylation and methylation mechanisms. A chemometric analysis (grouping and factor analyses) was used to evaluate the evolutionary relationships of the Moraceae genera and tribe, and it was possible to establish taxonomic relationships for the systematic characterization of the Moraceae family through the chemosystematic data. The results of the chemosystematic study suggest evidence that the *Trilepisium* genus is inadequately classified in the Dorstenia tribe and that the *Streblus* genus does not belong to the Moreae tribe. In addition, this chemosystematic study confirms the advanced status of Moraceae and legitimization of intrafamiliar classification.

Keywords Moraceae, Chemotaxonomy, Micromolecules, Mixed pathway, Shikimate pathway

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1. Introduction

The Moraceae family consists in monophyletic taxa [1-3], constituted by a group of cosmopolitan species, comprising about 1500 species [4, 5]. According to APG IV (2016) [6], this family is classified as Rosales. Moraceae species present an impressive range of breeding systems and pollination syndromes, as well as enormous variations in growth [2-3, 7-9]. Furthermore, they present a great diversity of anatomical and morphological characteristics, in addition to floral complexity [4, 10].

The intrinsic diversity of species belonging to the Moraceae family culminates in classification conflicts among its systematics, based on morphological and anatomical characters, as proposed by the researchers Rohwer (1993) and Berg (2001) [11-13] versus systematics based on evolutionary relationships and molecular phylogeny introduced by Dätwyler and Weiblen (2004), Beg (2005) and Clement and Weiblen (2009) [2, 3, 8]. The Moraceae systematic classification is still not resolved, due to suprageneric and infrageneric relationships.

In view of this, we conjecture that the systematic classifications of the Moraceae family based only on morphological and phylogenetic data are insufficient to explain why this taxon “is related to mulberry and bread-fruit” [10]. There are still questions to be answered so that the evolutionary history of Moraceae can be traced. It has been questioned at what point in their history was insect pollination inserted [1-3, 5, 8, 12] and what are the geographical and temporal origins of this family [14-16].

In this context, chemosystematics can contribute to the study of the positioning of Moraceae family genera as a conspicuous and complementary tool. Chemosystematics represents the integration of chemical data and organism morphology dependent on the association of genetic inheritance, and geographic and environmental regulators [17].

The special metabolism stimulated along the angiosperm adaptive process as a defense subterfuge, consisting of micromolecules, is noteworthy, rich in structural diversity and biosynthesized in metabolic pathways derived from the primary metabolism [17, 18]. Chemical evolution in angiosperms is represented in terms of evolutionary channelling [19, 20], in which flavonoid biosynthesis developed prior to lignin biosynthesis [17]. The Moraceae metabolism is conspicuous in the production of metabolites

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from the mixed pathway (acetate/shikimate) compared to the shikimate pathway [21]. The high heterogeneity exerted by flavonoid derivatives corroborates Dalghren (1980) in his classification for Angiosperms [22].

In angiosperms, evolutionary diversity is characterized by molecular protection processes in response to oxidative degradation [17-20, 23]. This occurs in the Moraceae family species concerning phenolic hydroxyls, resulting from the shikimate and the mixed pathway, alongside phenolic hydroxyls produced by methylation, glycosylation and prenylation [21].

Moraceae species present abundant metabolites resulting from the mixed pathway, with unprotected oxylic groups. Prenylated flavonoids, such as flavone, flavonol, flavanone, chalcone, stilbene and diels alder adducts are noteworthy in this context.

In the same way, micromolecules originating from the shikimate pathway, coumarins and lignans, have a majority of unprotected oxylic groups.

Although this taxon does not present a key chemo marker, evolutionary advancement index evaluations provide valuable chemosystematic information.

With this in mind, the chemosystematic aspects of phenylpropanoids and aromatic polyketides biosynthesized by Moraceae species were evaluated herein, according to the evolutionary advancement parameters regarding hydroxyl protection parameters. These data were used for similarity predictions between genera and in the understanding of Moraceae evolutionary chemistry.

2. Materials and Methods

2.1. Chemosystematic Methodology

Chemical data were collected from an extensive literature survey regarding Chemical Abstracts, via *Scifinder*, covering the range from 1907 to 2014. Phenylpropanoids and aromatic polyketides identified in Moraceae species were listed and submitted to the chemosystematic methodology. It is worth noting that genera organization is based on recent phylogenetic studies conducted by taxonomists Dätwyler and Weiblen (2004) both specialists in Moraceae species.

The evolutionary advancement indices for Moraceae genera related to phenylpropanoids and hydroxyl and aromatic polyketides hydroxyl protection mechanisms were determined as proposed by Gottlieb et al. [16] and Emerenciano [24]. These parameters can provide chemical advancements for Moraceae in relation to plant evolution. In addition, they also denote important ecological information regarding adaptive responses and point towards evolutionary trends.

The chemical parameters for micromolecules resulting from the mixed and the shikimate pathways regarding O-glycosyl (AE_G), O-methyl (AE_M), O-prenyl (AE_P), Total O-Protection (AE_{PT}), as well as Total O-unprotection (AE_{UT}), were calculated by Equations 1, 2, 3, 4 and 5, respectively.

$$AE_G = \frac{\sum IG}{NO} \tag{1}$$

$$AE_M = \frac{\sum IM}{NO} \tag{2}$$

$$AE_{Pren} = \frac{\sum IPren}{NO} \tag{3}$$

$$AE_{PT} = \frac{\sum IPT}{NO} \tag{4}$$

$$AE_{UT} = \frac{\sum IUT}{NO} \tag{5}$$

The abbreviations used were as follows:

NO: Number of occurrences
IG: Number of groups O-glycosyl/ Total number of oxylic groups
IM: Number of groups O-methyl/ Total number of oxylic groups
IPren: Number of groups O-prenyl/ Total number of oxylic groups
IPT: Number of groups oxilicos protected/ Total number of oxylic groups
IUT: Number of groups oxilicos unprotected/ Total number of oxylic groups

2.2. Multivariate Analysis

In the present study the following statistical approaches were applied: factorial analysis and cluster analysis to explain observed similarities among Moraceae genera and tribes. The former provides tools to analyse the interrelationships (correlations) of a large number of variables, defining sets of variables which are strongly interrelated, known as factors (representing dimensions which summarize or explain the original set of observed variables). The main purpose of the second approach is to aggregate objects based on their characteristics, by recognizing and indicating relationship patterns [25].

The statistical analyses were conducted with Statistica® 7 for Windows.

3. Results and Discussion

An estimated occurrence frequency of 404 phenylpropanoids and 1827 aromatic polyketides are listed in the Moraceae database. Moraceae metabolism shows a rich bioproduction of phenolic micromolecules preferably by the mixed (acetate/shikimate) pathway, instead of the shikimic acid derivative pathway, supporting the hypothesis that evolutionary channelling occurred in angiosperms. The occurrence frequency of these compounds in Moraceae genera is detailed in Tables 1 and 2. The chemometric exploration of evolutionary specialization and oxidation advancement parameters of phenylpropanoids and aromatic polyketides consisted in the observation of the similarities between genera, through a factorial and a cluster analysis. However, regarding hydroxyl protection, aromatic polyketides are mostly unprotected, and phenylpropanoids show peculiar protection patterns in each of the investigated genera. It must be considered that derivative protection systems must have evolved at the same time with micromolecular diversification. The hydroxyl protection indices of the evaluated genera are summarized in Table 3 and the hydroxyl protection indices of the tribes are displayed in Table 4.

Table 1. Occurrence frequency of aromatic polyketides in Moraceae genera

		Mixed pathway: aromatic polyketides																	
Label	**Moraceae Genera**	**Au**	**Mo**	**Flg**	**Flc**	**Ch**	**Di**	**Fon**	**Fdol**	**Fol**	**Fl**	**Fla**	**Dih**	**Dip**	**I**	**E**	**Na**	**Ca**	**Ada**
A1	*Artocarpus*	9	51	-	1	83	9	549	-	21	58	-	-	-	3	77	1	29	13
A2	*Batocarpus*	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
A3	*Clarissa*	-	-	-	-	-	-	2	-	-	-	-	-	-	-	1	-	-	-
A4	*Hulletia*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A5	*Parartocarpus*	-	-	-	-	9	-	5	-	-	5	-	-	-	-	-	-	-	-
A6	*Prainea*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A7	*Treculia*	-	-	-	2	4	-	1	-	1	-	-	-	-	-	-	-	2	-
C1	*Antiaris*	-	-	-	-	12	-	-	-	3	17	-	-	-	-	-	-	-	-
C2	*Antiaropsis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C3	*Castila*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C4	*Helicostylis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C5	*Maquira*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C6	*Mesogyne*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C7	*Naucleopsis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C8	*Perebea*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C9	*Poulsenia*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C10	*Pseudolmedia*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C11	*Sparratosyce*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D1	*Bosqueiopsis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D2	*Brosimum*	-	1	-	2	6	-	2	-	-	7	14	1	-	7	1	-	1	7
D3	*Dorstenia*	-	-	-	-	52	-	25	-	13	14	1	-	-	-	-	-	-	1
D4	*Helianthostylis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D5	*Scyphosyce*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D6	*Trilepisium*	-	-	-	-	2	1	3	-	-	-	-	-	-	1	-	-	1	-
D7	*Trymatococcus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
D8	*Utsetela*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
F1	*Ficus*	-	-	-	-	3	2	26	1	24	3	-	-	-	17	1	5	16	-
M1	*Bagassa*	-	8	2	-	-	4	-	-	-	1	-	-	-	-	5	-	-	-
M2	*Bleeckrodea*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M3	*Broussonetia*	4	2	-	-	26	2	39	-	51	6	44	-	41	-	1	-	1	1
M4	*Fatoua*	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-
M5	*Maclura*	-	-	-	-	1	7	4	-	10	10	-	-	-	30	3	-	-	-
M6	*Milicia*	-	1	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-
M7	*Morus*	4	39	-	-	1	12	44	-	35	15	1	-	-	-	17	10	-	32
M8	*Sorocea*	-	1	-	-	1	5	5	-	2	-	-	-	-	-	1	-	-	38
M9	*Streblus*	-	-	-	-	-	2	2	-	11	-	-	-	-	5	-	-	2	-
M10	*Trophis*	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Identified aromatic polyketides categories: Fl=Flavanone; Fla=Flavan; DiH= Diarylheptanoid; Dip= Diarylpropanoid; I=Isoflavonoid; E=Stilbene; An= Anthocyanin; Ca= Catechin; Ada= Diels-Alder adducts; Au=Aurone; Mo=Moracine; Flg= Flavolignan; Flc= Flavocoumarin; Ch=Chalcone; Di= Dihydroflavonol; Fon=Flavone; Fdol= Flavanodiol; Fol=Flavonol.

Table 2. Occurrence frequency of phenylpropanoids in Moraceae genera

		Shikimate pathway*:* phenylpropanoids				
Label	**Moraceae Genera**	**CA**	**BA**	**Phe**	**Lig**	**Co**
A1	*Artocarpus*	5	8	8	2	2
A2	*Batocarpus*	0	0	0	0	0
A3	*Clarissa*	0	0	0	0	0
A4	*Hulletia*	0	0	0	0	0
A5	*Parartocarpus*	0	0	0	0	0
A6	*Prainea*	0	0	0	0	0
A7	*Treculia*	1	2	0	0	5
C1	*Antiaris*	2	3	1	12	4
C2	*Antiaropsis*	0	0	0	0	0
C3	*Castila*	0	0	0	0	0
C4	*Helicostylis*	0	0	0	0	0
C5	*Maquira*	0	0	0	0	3
C6	*Mesogyne*	0	0	0	0	0
C7	*Naucleopsis*	0	0	0	0	1
C8	*Perebea*	0	0	0	0	0
C9	*Poulsenia*	0	0	0	0	0
C10	*Pseudolmedia*	0	0	0	0	0
C11	*Sparratosyce*	0	0	0	0	0
D1	*Bosqueiopsis*	0	0	0	0	0
D2	*Brosimum*	3	1	1	0	39
D3	*Dorstenia*	1	0	1	0	108
D4	*Helianthostylis*	0	0	0	0	0
D5	*Scyphosyce*	0	0	0	0	0
D6	*Trilepisium*	2	2	0	0	0
D7	*Trymatococcus*	0	0	0	0	0
D8	*Utsetela*	0	0	0	0	0
F1	*Ficus*	7	17	0	7	16
M1	*Bagassa*	0	0	0	0	0
M2	*Bleeckrodea*	0	0	0	0	0
M3	*Broussonetia*	10	2	1	21	10
M4	*Fatoua*	0	0	1	0	19
M5	*Maclura*	0	0	0	0	0
M6	*Milicia*	0	0	0	0	0
M7	*Morus*	10	1	0	0	11
M8	*Sorocea*	0	1	0	0	0
M9	*Streblus*	3	3	0	45	2
M10	*Trophis*	0	0	0	0	0

Identified phenylpropanoid categories: CA= Cinnamic acid; BA= Benzoic acid; Phe= Phenylpropene= Lig= Lignans; Co= Coumarin.

Table 3. Values of the evolutionary protection and unprotection mixed pathway and shikimate pathway advancement parameters of Moraceae genera

		Mixed pathway: Aromatic polyketides					**Shikimate pathway: Phenylpropanoids**				
Tribes	**Genera**	AE_G	AE_M	AE_P	AE_{PT}	AE_{UT}	AE_G	AE_M	AE_P	AE_{PT}	AE_{UT}
Artocarpeae	*Artocarpus*	0.0073	0.0697	0.1440	0.2188	0.7600	0.0192	0.2910	0.0769	0.3038	0.6577
	Batocarpus	0.0000	0.1250	0.2500	0.3750	0.6250	0.0000	0.0000	0.0000	0.0000	0.0000
	Clarisia	0.0000	0.2778	0.0000	0.2778	0.7222	0.0000	0.0000	0.0000	0.0000	0.0000
	Hulletia	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Parartocarpus	0.0000	0.0000	0.2857	0.2857	0.6429	0.0000	0.0000	0.0000	0.0000	0.0000
	Prainea	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Treculia	0.0000	0.0000	0.0000	0.0000	10.000	0.0000	0.3125	0.3125	0.625	0.375
Castilleae	*Antiaris*	0.0141	0.2141	0.0078	0.2359	0.7641	0.0227	0.5341	0.1364	0.7008	0.2992
	Antiaropsis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Castila	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Helicostylis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Maquira	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	1.0000	0.0000
	Mesogyne	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Naucleopsis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	1.0000	0.0000
	Pereba	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Poulsenia	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Pseudolmedia	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Sparratosyce	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dorstenieae	*Bosqueiopsis*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Brosimum	0.0051	0.1075	0.1129	0.2255	0.7745	0.0000	0.2311	0.6023	0.8447	0.1098
	Dorstenia	0.0000	0.0211	0.2025	0.2181	0.7866	0.0045	0.2576	0.6500	0.9273	0.0682
	Helianthostylis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Scyphosyce	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Trilepisium	0.0000	0.1250	0.0000	0.1250	0.8750	0.0000	0.1250	0.0000	0.1250	0.8750
	Trymatococcus	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Utsetela	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Ficeae	*Ficus*	0.0645	0.0954	0.0415	0.1988	0.7986	0.0390	0.2738	0.1596	0.4723	0.3787
Moreae	*Bagassa*	0.0000	0.0500	0.0167	0.0667	0.8333	0.0000	0.0000	0.0000	0.0000	0.0000
	Bleeckrodea	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Broussonetia	0.0398	0.0509	0.0524	0.1434	0.8518	0.0720	0.2955	0.1742	0.5985	0.3845
	Fatoua	0.0000	0.0000	0.0833	0.0833	0.9167	0.0000	0.3458	0.3167	0.7000	0.3125
	Maclura	0.0300	0.0205	0.1372	0.1646	0.8815	0.0000	0.0000	0.0000	0.0000	0.0000
	Milicia	0.0000	0.0000	0.2167	0.2167	0.7833	0.0000	0.0000	0.0000	0.0000	0.0000
	Morus	0.0606	0.0282	0.0821	0.1095	0.8089	0.0606	0.1439	0.0000	0.2045	0.7955
	Sorocea	0.0085	0.0000	0.1805	0.2268	0.7883	0.0000	0.0000	0.0000	1.0000	0.0000
	Streblus	0.1561	0.1568	0.0000	0.3129	0.6871	0.0535	0.1403	0.0000	0.2126	0,7119
	Trophis	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Identified indices: AE_G: O-glycosylation; AE_M: O-methylation, AE_P: O-prenylation; AE_{PT}: Total O-Protection; $AE_{UT:}$ Total O-unprotection.

Table 4. Values of the evolutionary protection and unprotection mixed pathway and shikimate pathway advancement parameters of Moraceae tribes

			Mixed pathway: Aromatic polyketides		
Tribes	**AE_{G}**	**AE_{M}**	**AE_{P}**	**$\mathrm{AE}_{\mathrm{PT}}$**	**$\mathrm{AE}_{\mathrm{UT}}$**
Artocarpeae	0.0073	0.4725	0.6797	1.1573	3.5501
Castilleae	0.0141	0.2141	0.0078	0.2359	0.7641
Dorstenieae	0.0051	0.2536	0.3154	0.5686	2.4361
Ficeae	0.0645	0.0954	0.0415	0.1988	0.7986
Moreae	0.295	0.3064	0.7689	1.3239	6.5509
			Shikimate pathway: Phenylpropanoids		
Artocarpeae	0.0192	0.6035	0.3894	0.9288	1.0327
Castilleae	0.0227	0.5341	2.1364	2.7008	0.2992
Dorstenieae	0.0045	0.6137	1.2523	1.8970	1.0530
Ficeae	0.039	0.2738	0.1596	0.4723	0.3787
Moreae	0.1861	0.9255	0.4909	2.7156	2.2044

Identified indices: AE_{G}: O-glycosylation; AE_{M}: O-methylation, AE_{P}: O-prenylation; $\mathrm{AE}_{\mathrm{PT}}$: Total O-Protection; $\mathrm{AE}_{\mathrm{UT:}}$ Total O-unprotection.

The factorial analysis of the evaluated Moraceae genera combines Factor 1 with O-Glycosylation (mixed pathway), O-Glycosylation (shikimate pathway) and O-Unprotection (shikimate pathway). Factor 2 consists of O-Prenylation (shikimate pathway), Total O-protection (shikimate pathway) and O-Prenylation (shikimate pathway). Factor 3 is composed of O-Prenylation (Mixed pathway), Total O-Protection (Mixed pathway), O-Unprotection (Mixed pathway) and O-Prenylation (Mixed pathway). Finally, Factor 4 is founded on the systematic classification of genera and tribes, based on Dätwyler and Weiblen [2].

The bidimensional diagram displayed in Figure 1 shows the factorial analysis of the 37 Moraceae genera, demonstrating dispersion among them. It is clearly observable that the genera for which there is no number of occurrence of micromolecules in one pathway overlap between -0.5 and -1.0. It is noteworthy that the factorial analysis was useful in reducing the number of data, by grouping the variables (evolutionary advancement parameters) into the factors, thus favoring a chemosystematic analysis of the genera of the Moraceae family.

In addition, corroborating previous analyses [2, 21], the *Trilepisium* genus is unrelated to other Dorstenieae genera. This is probably due to the fact that *Dorstenia* and *Brosimum* widely apply the subterfuge of hydroxyl protection of the shikimate pathway, by methylation and preferably by prenylation, while in *Trilepisium* phenolic micromolecule hydroxyls are usually unprotected. When they display protection, this is due to methylation.

The genera from Moreae tribe are quite scattered throughout the graph displayed in Figure 1, which can be related to their high morphological and floral variety, which allowed for the bioproduction of many metabolites.

However, the proximity of *Ficus* and *Broussonetia* is worth mentioning. Both genera have very close evolutionary advancement parameters referring to the protection of micromolecules resulting from the shikimate pathway: $\mathrm{AE}_{\mathrm{M}\ \mathit{Ficus}:\ 0.2738}$, $\mathrm{AE}_{\mathrm{M}\ \mathit{Broussonetia}:\ 0.2955}$, $\mathrm{AE}_{\mathrm{P}\ \mathit{Ficus}:\ 0.1596}$, $\mathrm{AE}_{\mathrm{P}\ \mathit{Broussonetia}:\ 0.1742}$, $\mathrm{AE}_{\mathrm{UT}\ \mathit{Ficus}:\ 0.3787}$, $\mathrm{AE}_{\mathrm{UT}\ \mathit{Broussonetia}:\ 0.3845\ 0,3845}$.

After a preliminary analysis, the genera with no metabolite records in the literatures were removed. Thus, when comparing Factors 1 and 3, displayed in Figure 2, results were very similar to the previous graph. A high dispersion among genera is also verified.

Thus, the distance of *Trilepisium* to other Dorstenieae genera is clearly noted. The ratification of this evidence is associated with differences in *Trilepisium* protection patterns, both among metabolites arising from the mixed pathway as among metabolites originating from the shikimate pathway.

While hydroxyls of phenolic micromolecules bioproduced in the shikimate pathway of the *Trilepisium genus* are the most unprotected, *Dorstenia* and *Brosimum* possess metabolites displaying high levels of protection, especially by prenylation. Similarly, phenolic metabolites from the mixed pathway are also unprotected, but when protection occurs in *Trilepisium*, it is by methylation, while *Brosimum* shows hydroxyl protection by methylation or prenylation, and *Dorstenia* preferably by prenylation.

Through this analysis, the dispersion among Moreae genera can be observed, which differ from each other basically because they show different patterns of hydroxyl modulations. However, it can be observed that *Milicia* e *Sorocea* are related, due to protection of micromolecules resulting from the mixed pathway, that present high index of unprotection and close O-prenylation: $\mathrm{AE}_{\mathrm{P}\ \mathit{Milicia}:\ 0.2167}$, $\mathrm{AE}_{\mathrm{P}\ \mathit{Sorocea}:\ 0.1805}$, while *Batocarpus* and *Paratocarpus* are close as result of a similar O-prenylation index : $\mathrm{AE}_{\mathrm{P}\ \mathit{Batocarpus}:\ 0.2500}$, $\mathrm{AE}_{\mathrm{P}\ \mathit{Paratocarpus}:\ 0.2857}$. Among Moreae genera, *Clarisia* and *Artocarpus* occur closely, with a very close index of Total O-unprotection: $\mathrm{AE}_{\mathrm{UT}\ \mathit{Clarisia}:\ 0.722}$, $\mathrm{AE}_{\mathrm{UT}\ \mathit{Artocarpus}:\ 0.760}$.

Ficeae presents correlations with Moreae genera, because both have high hydroxyl unprotection values in common and, however tenuous, phenolic hydroxyl protection by glycosylation.

The cluster analysis by Ward's method allowed for the identification of two main groups (Figure 3). The first group is divided into three genera subgroups, namely Artocarpeae and Castilleae. The second group consists of a grouping of the Dorstenieae, Ficeae and Moreae genera.

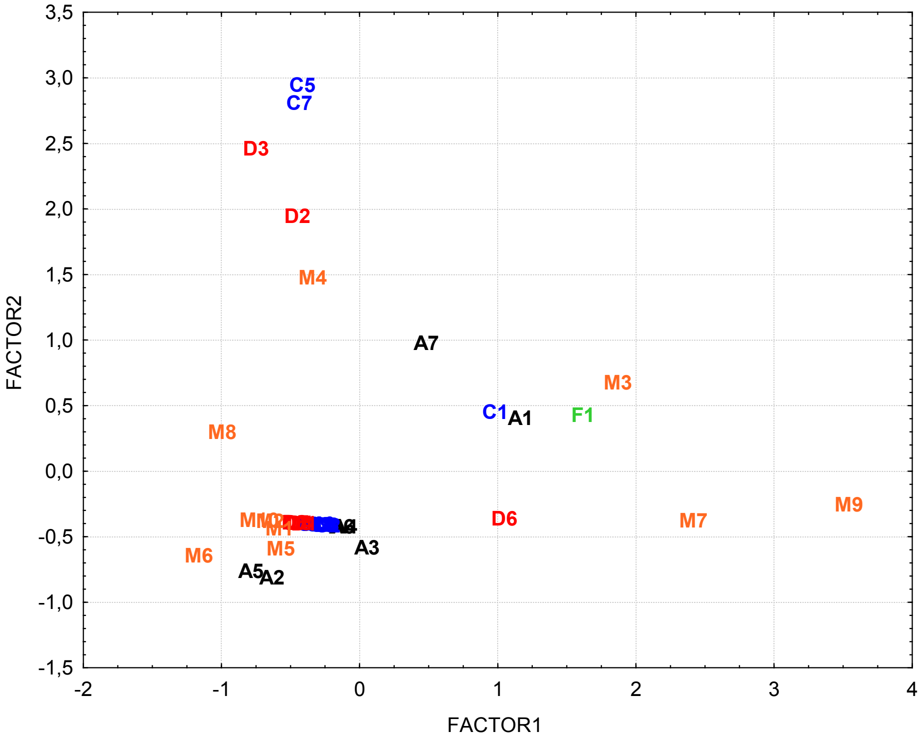


Figure 1. Bidimensional diagram (Factor 1 x Factor 2) displaying the interrelationships between the 37 Moraceae family genera analyzed in the present study

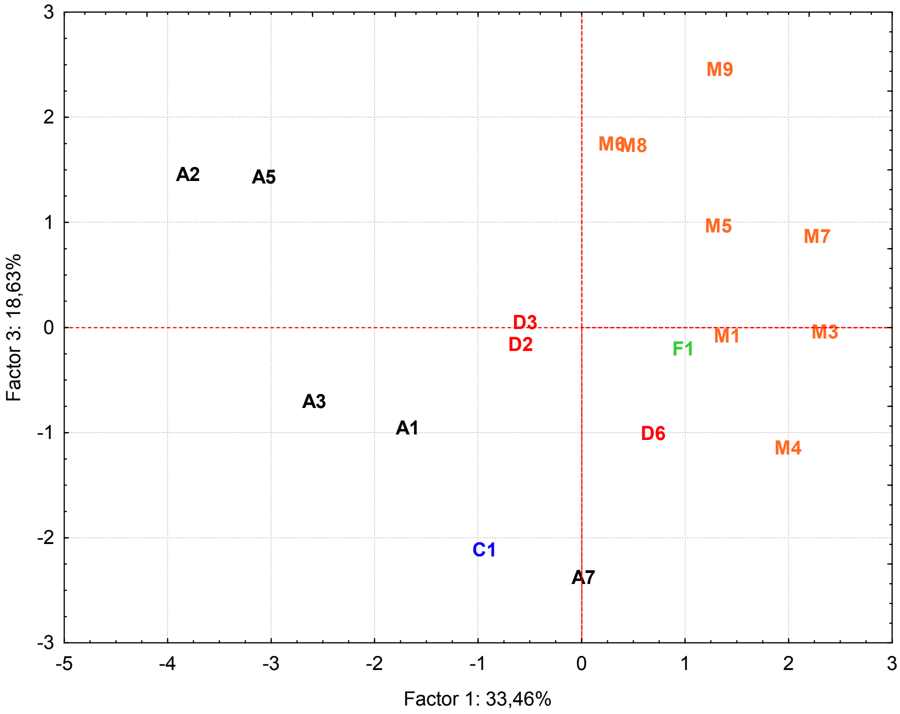
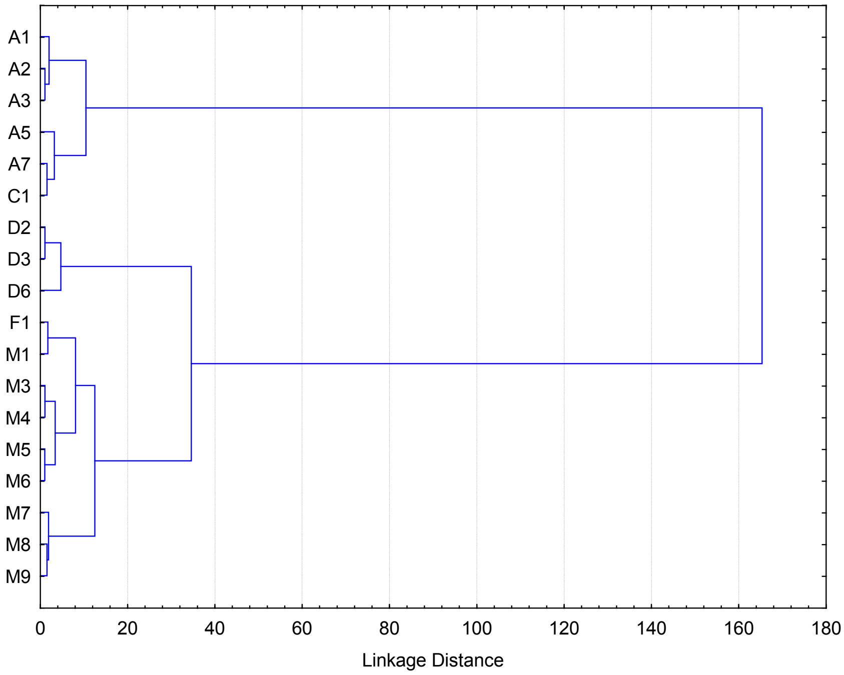


Figure 2. Bidimensional diagram (Factor 1 x Factor 3) displaying the interrelationships between Moraceae family genera analysed in the present study



A1 – *Artocarpus*; A2 – *Batocarpus*; A3 – *Clarissa*; A5 – *Parartocarpus*; A7 – *Treculia* Representatives of the Castileae tribe: C1 – *Antiaris*; D2 – *Brosimum*; D3 – *Dorstenia*; D6 – *Trilepisium*; F1 - *Ficus*; M1 – *Bagassa*; M3 – *Broussonetia*; M4 – *Fatoua*; M5 – *Maclura*; M6 – *Milicia*; M7 – *Morus*; M8 – *Sorocea*; M9 – *Streblus*; M10 – *Trophis*.

Figure 3. Dendrogram of 37 Moraceae family genera analysed in the present study (Ward's method based on Euclidean Distances)

4. Conclusions

This chemosystematic study greatly contributes to knowledge on the Moraceae family and ratifies that the evolutionary and oxidative advance parameters of Moraceae have systematic values.

Analyses of the chemosystematic evolutionary advancement parameters of this family showed a high incidence of aromatic polyketides, with most hydroxyls unprotected, characterizing the primitive positioning of the Moraceae family. Phenylpropanoids have a high variety of hydroxyl protection and each genus showed a particular hydroxyl protection pattern.

In sum, the chemosystematic data of phenolic micromolecules presented both similarities and dissimilarities between Moraceae genera and tribes. The main conclusions obtained herein were the consolidation that the *Trilepisium* genus forms a clade unlinked to the other genera of the Dorstenieae tribe, and that the *Streblus* genus is a discrepancy of the Moreae tribe and consist in a polyphyletic group.

Chemosystematic studies, through the chemometric analysis of chemical evolutionary parameters can, thus, contribute greatly to the infrageneric classification of the Moraceae family. Even if the inventory of the Moraceae family is complemented and new data are aggregated, the evolutionary tendencies pointed out in this study will not be altered, due to the biosynthesis characteristics of special metabolites of each genus.

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