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Multivariate Analysis of Vegetative and Reproductive Morphological Characters in *Hyptis* Jacq.

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Abstract: The purpose of this study was to reassess the systematic relationships between 88 species of *Hyptis* Jacq. based on the vegetative and reproductive morphological characters. In order to perform a phenetic analysis, multivariate statistical analysis were used for determination of taxa distribution patterns. The relationships between the examined taxa have been expressed as Ward hierarchical clustering using multiple correspondence analysis based on 50 characters distributed into 168 character states. The perceptual map shows a subdivision of *Hyptis* into two major clusters. Cluster I contained 41 taxa belonging to sections Cyanocephalus, Cyrta, Gymneia, Mesosphaeria, Polydesmia and Tricosphaeria which mainly showed elongated, elliptic, linear bracts, curvature of the calyx's tube upper limb, oblique calyx tube orifice, as well as smooth nutlets. Cluster II contained 47 taxa from sections Apodotes, Eriosphaeria, Hyptis, Induratae, Pachyphyllae, Pusilae and Xylodontes. Their main morphological characteristics included hemispheric or subglobose cymes, the presence of a stylopodium, slightly rough nutlets and nutlets truncate at apex. Cluster analysis focused on six of the morphological characters which were distinguished by discriminant correspondence analysis, revealed similar traits.

Key words: *Hyptis*, Lamiaceae, systematic, numerical taxonomy, multivariate analysis

INTRODUCTION

The Lamiaceae family consists of approximately 258 genera and 7193 species, of which 40% possesses aromatic properties. Genera such as *Salvia* (500 species), *Hyptis* (350), *Scutellaria*, *Coleus*, *Plectranthus* and *Stachys* (200 species each), *Nepeta* (150) and *Teucrium* (100) have a wide and cosmopolitan distribution, although lamiaceous plants are especially abundant in the Mediterranean region (Melendo *et al*., 2003; APG II, 2003). In Brazil and other Cerrado areas of Eastern South America, the Lamiaceae family-mainly represented by the neotropical subtribe Hyptidinae (tribe Ocimeae)-shows strong patterns of floristic and taxonomic variation and yields a large number of new species (Almeida and Albuquerque, 2002). Furthermore, it is characterized by sternotribic flowers whose stamens are held in the lower lip of the corolla which is strongly compressed to form an explosive pollination mechanism (Paton *et al*., 2004).

A total of eight Hyptidinae genera are now recognized: *Hyptis*, *Raphiodon*, *Peltodon*, *Marsypianthes*, *Eriope*, *Hyptidendrum*, *Eriopidion* and *Hypenia*). *Hypenia* (Mart. ex Benth.) R. Harley and *Hyptidendron* R. Harley were separated from *Hyptis* Jacq. based largely on number of chromosomes and morphological grounds. *Hyptis* is a paraphyletic group and is closely related to *Eriope* (Harley *et al*., 2004).

The genus *Hyptis* contains 27 sections and 37 subsections (Epling and Játiva, 1968). In the Brazilian Cerrado region, they exhibit remarkable morphological diversity and are usually found in oligotrophic sandy soils with high aluminum concentrations. Several extremely aromatic species reported in this area present medicinal properties and relevant pharmacological activities (Arrigoni-Blank *et al*., 2008; Coutinho *et al*., 2009).

Recently, the use of classification techniques for numerical taxonomy, including agglomerative and divisive algorithms, suggested subdivision of the four Hyptidinae genera into two major groups (El-Gazzar and Rabei, 2008). This was mainly based on the dichotomous nature of vegetative and floral morphology, as well as stems anatomy, although the distribution of character states

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among groups was not reported. We suggest investigating the subdivision of *Hyptis* into two subgenera through a study with large samples of the species and a wider range of characters.

In this study, we analyzed morphological character variation of *Hyptis* in 12 sections collected in the Brazilian Cerrado region. This was carried out in order to support the taxonomy of its genus as well as El-Gazzar's hypothesis for the two subgenera. Thus, a total of 50 characters from vegetative and floral morphology containing 168 character states were comparatively recorded for 88 *Hyptis* species. *Eriope crassipes* Benth. was used as the outgroup (reference species) in all analyses. Disability data and rare species in Brazil (Harley and França, 2009; Scarano and Martinelli, 2010) were studied.

In order to perform a phenetic techniques analysis, multivariate such as hierarchical clustering, multiple and discriminant correspondence analysis were used for determination of taxa distribution patterns, as well as identification of characters and their character states, which may be distinguished among the groups of species.

MATERIALS AND METHODS

Species examined: The examined taxa were collected during flowering time from central Brazilian Cerrado, Goiás State, Brazil, between 2006 and 2010. The sectional delimitation (Table 1) was arranged according to Epling and Játiva (1968). Taxa were deposited at the Herbarium of Federal University of Goiás (UFG), Goiânia, Goiás State, Brazil.

Morphological analysis: Leaf variations were recorded using a Light Oympus SZ40 Stereomicroscope. Assessment of 50 vegetative and reproductive characters included 22 two-state characters and 28 multistate characters, totalizing 168 character states. All multistate characters were treated as unordered. Table 2 and 3 contain a list of these vegetative and reproductive morphological characters and their character states, respectively.

Statistical analysis: Multiple Correspondence Analysis (MCA) were applied to examine the interrelationships between taxa and taxonomic characters, such as presence/absence verification or multistate characters (89 taxa×50 character/168 character states). These procedures were performed using Portable System Data Analysis software package, version 5.5, International Center for Applied Statistics and Computing, France (2002). Adjustments in eigenvalues and inertia percentages due to artificial inflation in the original data were carried out based on Le Roux and Rouanet (2004) method. Regarding variable selection, above-average character states (100/168 = 0.6% in the three highest eigenvalues) were retained in the data set. Those with contributions below average were effectively eliminated.

In order to study the similarities among taxa, cluster analysis was also applied based on taxonomic character distribution. Hierarchical clustering was performed at each step of the algorithm to minimize the decrease of chi-square statistics (χ^2). This clustering criterion is equivalent to Ward's variance (inertia) minimizing method (Ward, 1963) where each cluster is weighted by the total mass of its members (Greenacre, 2007).

Data analysis focused on the determination of character states by calculating the following average values: (1) global data sets (GLA); (2) character states within the clusters (CLA) and (3) clustered taxa present in the character state (MOD). The p-values below 0.05 were regarded as significant. Discriminant correspondence analysis was used to differentiate taxa and a priori clusters based on taxonomic characters, which were assessed across the Portable System Data Analysis.

RESULTS AND DISCUSSION

Lamiaceae was among the top 10 plant families with the greatest number of endangered or poorly described species, occupying the 7th and 8th positions, respectively, in the official list of susceptible species of Brazilian flora (Scarano and Martinelli, 2010). Among the Lamiaceae family, *Hyptis* represented 62% of which 42.3% are included in Table 1. In addition, 14 taxa were listed among *Hyptis* species considered rare in Brazil (Giulietti *et al*., 2009). Similar to the four species from section Pachyphyllae which are found in the highest areas of Chapada dos Veadeiros (Harley, 1986; Harley and França, 2009) many of the taxa were endemic to the central Brazilian Cerrado region.

Regarding the vegetative (Table 2) and reproductive morphological (Table 3) data sets, species were characterized according to the presence/absence of character states. For instance, only *H. platanifolia* samples showed attenuate, truncated leaf base; *H. peduncularis* had elongated, elliptic, linear leaves; *H. angustifolia* presented elliptical, obovate-oblanceolate leaves; *H. caprariifolia* showed fasciculate arrangement of branches, although they were deflexed in *H. frondosa*; *H. rugosa* was the only taxon with rounded, slightly tapered leaf base; *H. interrupta* showed linear, setaceous shapes of elliptical, cylindric bracts; *H. pachyphylla* exhibited deltoid, lanceolate leaves on flattened, oval,

Table 1: List of examined taxa from Brazilian Cerrado *Hyptis* species and their sectional delimitation

No.	Taxa	Section	Subsection
1	*H. adpressa* A. St. Hil.	Cyanocephalus Pohl ex Benth.	Cordifoliae Benth.
2	*H. althaeaefolia* Pohl ex Benth.	Polydesmia Benth.	Malvastra Epling
3	*H. alutacea* Pohl ex Benth.	Hyptis R. Harley	Eriodontes Benth.
4	*H. ampelophylla* Epling	Gymneia Benth.	
5[a,c]	*H. angustifolia* Pohl ex Benth.	Eriosphaeria Benth.	Velutinae Benth.
6[b,c]	*H. arenaria* Benth.	Eriosphaeria Benth.	Graciles Epling
7[c]	*H. asteroids* A. St. Hil. ex Benth.	Eriosphaeria Benth.	Sessilifoliae Benth.
8	*H. brevipes* Poit.	Hyptis R. Harley	Marrubiastrae Benth.
9[c]	*H. caduca* Epling	Pusilae Epling	
10	*H. caespitosa* A. St. Hil. ex Benth.	Hyptis R. Harley	Eriodontes Benth.
11[a]	*H. caprariifolia* Pohl ex Benth.	Cyanocephalus Pohl ex Benth.	Lobatae Briq.
12	*H. cardiophylla* Pohl ex Benth.	Cyanocephalus Pohl ex Benth.	Cordifoliae Benth.
13	*H. carpinifolia* Benth.	Polydesmia Benth.	Rigidae Benth.
14[a]	*H. colligata* Epling & Jávita	Xylodontes Benth.	Axilares Benth.
15	*H. conferta* Pohl ex Benth.	Hyptis R. Harley	Hyptis
16	*H. cuneata* Pohl ex Benth.	Cyanocephalus Pohl ex Benth.	Cordifoliae Benth.
17	*H. crenata* Pohl ex Benth.	Eriosphaeria Benth.	Sessilifoliae Benth.
18	*H. crinita* Benth.	Trichosphaeria Benth.	Crinitae Epling
19[a,c]	*H. cruciformis* Epling	Pachyphylla R. Harley	
20	*H. desertorum* Pohl ex Benth.	Cyanocephalus Pohl ex Benth.	Rugosae Epling
21[c]	*H. dictiodea* Pohl ex Benth.	Eriosphaeria Benth.	Heterophyllae Epling
22[a,c]	*H. digitata* R. M. Harley	Cyanocephalus Pohl ex Benth.	Lobatae Briq.
23	*H. dilatata* Benth.	Eriosphaeria Benth.	Sessilifoliae Benth.
24	*H. divaricata* Pohl ex Benth.	Cyrta Benth.	Lavandulaceae Epling
25	*H. duplicato-dentata* Benth.	Polydesmia Benth.	Malvastra Epling
26	*H. eriophylla* Pohl ex Benth.	Trichosphaeria Benth.	Crinitae Epling
27	*H. ferruginosa* Pohl ex Benth.	Xylodontes Benth.	Paniculatae Epling
28	*H. foliosa* A. St. Hil. ex Benth.	Polydesmia Benth.	Glomeratae Benth.
29[b,c]	*H. frondosa* S. Moore	Xylodontes Benth.	Paniculatae Epling
30	*H. goyavensis* A. St. Hil. ex Benth.	Eriosphaeria Benth.	Sessilifoliae Benth.
31	*H. glomerata* Mart. ex Schrank	Polydesmia Benth.	Glomeratae Benth.
32[c]	*H. heterophylla* Benth.	Eriosphaeria Benth.	Heterophyllae Epling
33	*H. hilarii* Benth.	Eriosphaeria Benth.	Velutinae Benth.
34	*H. hirsuta* Kunth.	Xylodontes Benth.	Axilares Benth.
35[c]	*H. humilis* Benth.	Pusilae Epling	
36	*H. imbricata* Pohl ex Benth.	Eriosphaeria Benth.	Gnideaefoliae Benth.
37[b,c]	*H. imbricatiformis* R. Harley	Pachyphyllae R. Harley	
38	*H. interrupta* Pohl ex Benth.	Gymneia Benth.	
39	*H. lanata* Pohl ex Benth.	Cyanocephalus Pohl ex Benth.	Cordifoliae Benth.
40	*H. lanuginosa* Glaziou	Eriosphaeria Benth.	Passerinae Benth.
41	*H. lavandulacea* Pohl ex Benth.	Cyrta Benth.	Lavandulaceae Epling
42	*H. linarioides* Pohl ex Benth.	Hyptis R. Harley	Eriodontes Benth.
43	*H. lippioides* Pohl ex Benth.	Cyanocephalus Pohl ex Benth.	Cordifoliae Benth.
44	*H. lucida* Pohl ex Benth.	Induratae Epling	
45	*H. lutescens* Pohl ex Benth.	Xylodontes Benth.	Paniculatae Epling
46	*H. lythroides* Pohl ex Benth.	Polydesmia Benth.	Glomeratae Benth.
47	*H. malacophylla* Benth.	Gymneia Benth.	
48	*H. marrubioides* Epling	Xylodontes Benth.	Axilares Benth.
49	*H. microphylla* Pohl ex Benth.	Cyrta Benth.	Paludosae Epling
50	*H. multiflora* Pohl ex Benth.	Trichosphaeria Benth.	Crinitae Epling
51	*H. mutabilis* (Rich) Briq.	Polydesmia Benth.	Vulgares Benth.
52	*H. nigrescens* Pohl ex Benth.	Xylodontes Benth.	Paniculatae Epling
53	*H. nitidula* Benth.	Cyanocephalus	Longifoliae Epling
54[c]	*H. nivea* Epling	Polydesmia Benth.	Glomeratae Benth.
55	*H. nudicaulis* Benth.	Apodotes Benth.	
56	*H. obtecta* Benth.	Eriosphaeria Benth.	Obtectae Epling
57	*H. ovalifolia* Benth.	Gymneia Benth.	
58	*H. ovata* Pohl ex Benth.	Eriosphaeria Benth.	Sessilifoliae Benth.
59	*H. orbicular* Pohl ex Benth.	Xylodontes Benth.	Paniculatae Epling
60	*H. origanoides* Pohl ex Benth.	Eriosphaeria Benth.	Velutinae Benth.
61[b,c]	*H. pachyphylla* Epling	Pachyphyllae R. Harley	
62	*H. passerina* Mart. ex Benth.	Eriosphaeria Benth.	Passerinae Benth.
63	*H. pectinata* (L.) Poit.	Mesosphaeria Benth.	Pectinaria Benth.
64	*H. peduncularis* Benth.	Cyanocephalus Pohl ex Benth.	Longifoliae Epling
65[b,c]	*H. penaeoides* Taub.	Pachyphyllae R. Harley	
66	*H. petiolaris* Pohl ex Benth.	Hyptis R. Harley	Hyptis
67	*H. platanifolia* Mart. ex Benth.	Gymneia Benth.	

Table 1: Continued

No.	Taxa	Section	Subsection
68	*H. plectranthoides* Benth.	Mesosphaeria Benth.	Plectranthodon Epling
69	*H. plumosa* Benth.	Trichosphaeria Benth.	Plumosae Epling
70	*H. pulegioides* Pohl ex Benth.	Apodotes Benth.	
71	*H. pycnocephala* Benth.	Eriosphaeria Benth.	Sessilifoliae Benth.
72	*H. recurvata* Poit.	Cyrta Benth.	Tetragonae Benth.
73	*H. remota* Pohl ex Benth.	Xylodontes Benth.	Paniculatae
74	*H. rubicunda* Pohl ex Benth.	Polydesmia Benth.	Vulgares Benth.
75	*H. rubiginosa* Benth.	Xylodontes Benth.	Paniculatae Epling
76	*H. rugosa* Benth.	Cyanocephalus Pohl ex Benth.	Rugosae Epling
77	*H. saxatilis* A. St. Hil.	Eriosphaeria Benth.	Velutinae Benth.
78	*H. sinuata* Pohl ex Benth.	Hyptis R. Harley	Marrubiastrae Benth.
79	*H. spicigera* Lam.	Mesosphaeria Benth.	Spicaria Benth.
80	*H. suaveolens* (L.) Poit.	Mesosphaeria Benth.	Pectinaria Benth.
81	*H. subrotunda* Pohl ex Benth.	Polydesmia Benth.	Glomeratae Benth.
82[b,c]	*H. tagetifolia* R. M. Harley	Cyanocephalus Pohl ex Benth.	Lobatae Briq.
83	*H. tetragona* Pohl ex Benth.	Apodotes Benth.	
84	*H. turnerifolia* Mart. ex Benth.	Eriosphaeria Benth.	Sessilifoliae Benth.
85	*H. velutina* Pohl ex Benth.	Eriosphaeria Benth.	Velutinae Benth.
86	*H. villosa* Pohl ex Benth.	Xylodontes Benth.	Paniculatae Epling
87	*H. violacea* Benth.	Polydesmia Benth.	Rigidae Benth.
88	*H. virgata* Benth.	Gymneia Benth.	
89	*E. crassipes* Benth.		

H: *Hyptis*, *E.*: *Eriope*, [a]Taxa with disability data (Scarano and Martinelli, 2010). [b]Taxa listed as endangered (Scarano and Martinelli, 2010). [c]Taxa listed as rare in Brazil (Giulietti *et al.*, 2009).

Table 2: Vegetative morphological characters, characters states and their codes for numerical analysis of *Hyptis*

No.	Character	State	Code
1	Habitat	bush	0
		bush and shrub	1
		shrub	2
		shrub and herb	3
		perennial herb	4
		prostrate perennial herb	5
		annual herb	6
2	Leaf blade	not pinnatifid	0
		pinnatifid	1
3	Margin of leaf blade	crenate	0
		crenate-serrate	1
		serrate	2
		entire	3
		dentate	4
		pinnately	5
4	Leaf attachment	petiolate	0
		subsessile	1
		sessile and subsessile	2
		sessile	3
5	Board as curvature	not revolute	0
		revolute	1
6	Leaf arrangement on branches	scattered	0
		extended along the stem	1
		imbricate	2
		basal or near the base	3
		deflexed	4
		fasciculate	5
7	Elliptic-ovate leaves	absent	0
		elliptic	1
		ovate	2
		lanceolate-ovate	3
8	Elongated-elliptical leaves	absent	0
		oblong	1
		linear-elliptic	2
		linear	3
9	Round-elliptical leaves	absent	0
		rounded	1
10	Elliptic-triangular leaves	absent	0
		deltate	1

Table 2: Continued

No.	Character	State	Code
11	Elliptic-rhomboid leaves	absent	0
		rhomboid	1
12	Elliptic-obovate leaves	absent	0
		obovate	1
		oblanceolate	2
13	Leaf apex	obtuse or rounded	0
		obtuse and acute	1
		acute	2
		acute and acuminate	3
		acuminate	4
14	Attenuate-round leaf base	absent	0
		rounded	1
		cordate	2
		auriculiform	3
15	Tapered-attenuate leaf base	absent	0
		acute	1
		cuneate	2
16	Attenuate-truncate leaf base	absent	0
		truncate	1
17	Trichomes	simple	0
		branched	1
18	Ovate-elliptical bracts	absent	0
		elliptic	1
		ovate	2
		lanceolate-ovate	3
		lanceolate	4
		lanceolate-linear	5
19	Elongated-elliptical bracts	absent	0
		oblong	1
		lanceolate-oblong	2
		linear	3
20	Cylindric-elliptical bracts	absent	0
		subulate	1
		subulate-linear	2
		setaceous	3
21	Presence of bracts	multibracteoles in the glomerular base	0
		dibracteoles in the calyx base	1
		unibracteoles in the calyx base	2
22	Bract consistency	firm	0
		membranous	1

Table 3: Reproductive morphological characters and characters states and their codes for numerical analysis of *Hyptis*

No.	Character	State	Code
23	Disposition of cymes	absent	0
		raceme	1
		panicle	2
		tang	3
		glomerular subcorymbose	4
24	Forms of cymes	verticillate	0
		reduced to one flower	1
		spike	2
		capitate	3
		medusoid-head	4
		hemispherical or subglobose	5
25	Number of flowers in cymes	pauciflora	0
		carnations	1
26	Internodes below the inflorescence	waxy-inflated	0
		not waxy-inflated	1
27	Peduncles	presence	0
		subsessile	1
		sessile	2
28	Disposition of calyx at maturity pedicel	not deflexed	0
		deflexed	1
29	Flower pedicel	presence	0
		subsessile or sessile	1
30	Calyx teeth	absent	0
		presence	1

Table 3: Continued

No.	Character	State	Code
31	Flattened triangular-ovate calyx teeth	absent	0
		ovate	1
		lanceolate-deltate	2
		deltate	3
32	Flatttened elongated-ovate calyx teeth	absent	0
		lanceolate	1
		linear	2
33	Flattened cylindric-ovate calyx teeth	absent	0
		subulate	1
		aristate	2
		setaceous	3
		filiform	4
		obsolate	5
		acicular	6
34	Calyx tube curvature	straight	0
		curve near the apex	1
		curve in the upper half	2
		sinuous	3
35	Forms of calyx tubes at maturity	campanulate	0
		turbinated	1
		cylindric	2
36	Pilosity of the calyx faucal area	absent or inconspicuous	0
		conspicuous	1
37	Inner surface of the calyx	glabrous	0
		slightly hirsute	1
		annular-hirsute	2
38	Sinuses between calyx teeth	absent	0
		presence	1
39	Dilation of teeth's apex	absent	0
		presence	1
40	Calyx orifice	straight	0
		oblique	1
41	Tube wall of the cup	entire	0
		split between the anterior sinus near the middle of calyx tube	1
42	Shape of corolla	constricted-campanulate	0
		cylindric	1
		infundibular	2
43	Color of the corolla	purple or lilac	0
		red	1
		cream	2
		white	3
44	Stylopodium	absent	0
		present	1
45	Nutlet forms	flat wings	0
		slightly flat wings	1
		flat	2
		ovate	3
		ovoid or oblong	4
		subglobose	5
		globose	6
46	Nutlet surfaces	smooth	0
		slightly rough	1
		punctuated or alveolate	2
47	Apex pilosity of nutlets	glabrous	0
		hirsute	1
48	Nutlet apex	truncate	0
		obtuse or rounded	1
		acute	2
		acuminate	3
		apiculate	4
49	Length of nutlets	≥ 2 mm	0
		<2 mm	1
50	Xylopodium	absence	0
		presence	1

triangular calyx teeth, whereas *H. duplicato-dentata* was characterized by unique languets on the sinus between the calyx teeth. Some species were identified due to the absence of character status that was found in other species. For example, calyx teeth was only absent from *H. subrotunda* profiles, whereas *H. suaveolens* showed a deflexed pedicel.

Furthermore, some character states were present in only one of the *Hyptis* species and *E. crassipes* (outgroup). For instance, *H. plectranthoides* showed cymes reduced to one flower and *H. suaveolens* had deflexed pedicels at flowering pedicels. Other character states were only present in the outgroup, such as bracts (dibracteoles) on the calyx base, campanulate calyx tube, slightly hirsute inner face of calyx, campanulate, constricted corolla and nutlets with flat wings.

The morphological vegetative and reproductive data matrix (89 species×50 character with 168 character states) was submitted to Multiple Correspondence Analyses (MCA). This is a type of numerical assessment that basically describes the relationships among categories (character states) of discrete variables (characters). Thus, it reveals the structure of a complex ordinal data matrix without excluding any essential information. Furthermore, it has few restrictions and assumptions (Clausen, 1998). The advantages of using MCA are that results may be visually represented as a perceptual map; also, associations among variables may be calculated by analyzing co-ordinate proximity in a two-dimensional space (Abdi and Valentin, 2007). The best set of dimensions is determined by eigenvalues which are in the interval [0, 1] and represent the amount of variation explained (inertia). The sum of all eigenvalues is equivalent to the total inertia (total variance) in the data set (Maraun *et al.*, 2005). Furthermore, each dimension has a corresponding eigenvalue and two dimensions with the largest eigenvalues are chosen in order to explain the maximum amount of inertia (variance).

The MCA analysis yielded a two dimensional solution explaining 43.5% of inertia on the first dimension and 32.7% on the second. The perceptual map (Fig. 1) shows three character groups representing the subdivision of *Hyptis* into two groups and the outgroup (*E. crassipes*). Regarding the first subdivision of *Hyptis* genera, which ranged from 0.0 to -2.04 on the first dimension and -2.48 to 0.72 on the second, taxa were identified in sections Cyanocephalus, Cyrta, Gymneia, Mesosphaeria, Polydesmia and Tricosphaeria. In the second group of clustered *Hyptis* species, characters ranged from 0.0 to 1.50 and -0.76 to 0.36 on the first and second dimensions, respectively. These taxa were related to sections Apodotes, Eriosphaeria, Hyptis, Induratae, Pachyphyllae, Pusilae and Xylodontes. *E. crassipes* was distinguished due to its high negative factor scores. The dendrogram of similarities among species based on inertia minimization (Ward, 1963) may be seen in Fig. 2.

Thus, the perceptual map shows a subdivision of *Hyptis* into two sub-genera which is in agreement with the one proposed by El-Gazzar and Rabei (2008). In this study, species belonging to four genera of subtribe Hyptidinae, including 59 *Hyptis* species, were distributed into two major groups. This was mainly based on dichotomous characters from vegetative and floral morphology, as well as stems anatomy, although none of the characters or character states has been described to distinguish between clustered taxa. Comparing results obtained by El-Gazzar and Rabei (2008) and those from present research, the subdivision of *Hyptis* into two sub-genera revealed a similarity of 62% when restricted to the same taxa. In fact, those authors stated that highly probable subgenera might be expected to emerge regardless of clustering techniques used in the analysis.

We also found that section Gymneia (cluster I) differs significantly ($p<0.0001$) from Polydesmia and sections Mesosphaeria/Trychosphaeria (Fig. 2). This is due to the presence of various traits, mainly (χ^2 = 55.4, CLA = 100%, GLA = 4.44%, MOD = 66.7%) the setaceous form of elliptic-cylindrical bracts (Table 2). Section Polydesmia was significantly ($p<0.0001$; MOD = 100%) characterized by the presence of flowers in pauciflora (χ^2 = 65.2, GLA = 15.6%, CLA = 85.7%) and verticillate cymes (χ^2 = 48.0, GLA = 20.0%, CLA = 66.7%), while sections Mesosphaeria/Trichosphaeria were clustered ($p<0.0001$; CLA = 100%) mainly as a result of the subglobose or globose form of nutlets (χ^2 = 49.5, GLA = 5.56%, MOD = 62.5%) and the medusoid-like (GLA = 4.44%, MOD = 50.0%) or spike-like (GLA = 3.33%, MOD = 37.5%) form of cymes (Table 3).

Section Cyanocephalus was divided into three subgroups. Subsection Lobatae was distinguished from the other sections ($p<0.0001$) due to its unique traits (χ^2 = 87.0), such as pinnatifid leaves, pinnate margins of leaf blades and neither waxy nor inflated internodes below inflorescence (GLA = 3.33%, CLA and MOD = 100%). Subsections Cordifolia/Rugosae were clustered ($p<0.0001$; MOD = 100%) due to dilated apex (χ^2 = 56.0, GLA = 11.1%, CLA = 70.0%) and oblique calyx tube orifice (χ^2 = 20.4, GLA = 25.6%, CLA = 30.4%). Differently, subsection Longifolia presented highly similar characteristics to section Cyrta. This was e specially due to the presence ($p<0.008$) of campanulate form of mature calyx tubes (χ^2 = 10.4, GLA = 14.4%, CLA = 30.8%, MOD = 57.1%) in the latter section (Table 2).

Cluster II revealed that section Eriosphaeria (subsections Gnidiifoliae/Sessilifoliae) was significantly different ($p<0.0001$) from other sections because of the linear-subulate form of elliptic-cylindrical bracts (χ^2 = 56.7, GLA = 6.67%, CLA = 83.3%, MOD = 83.3%),

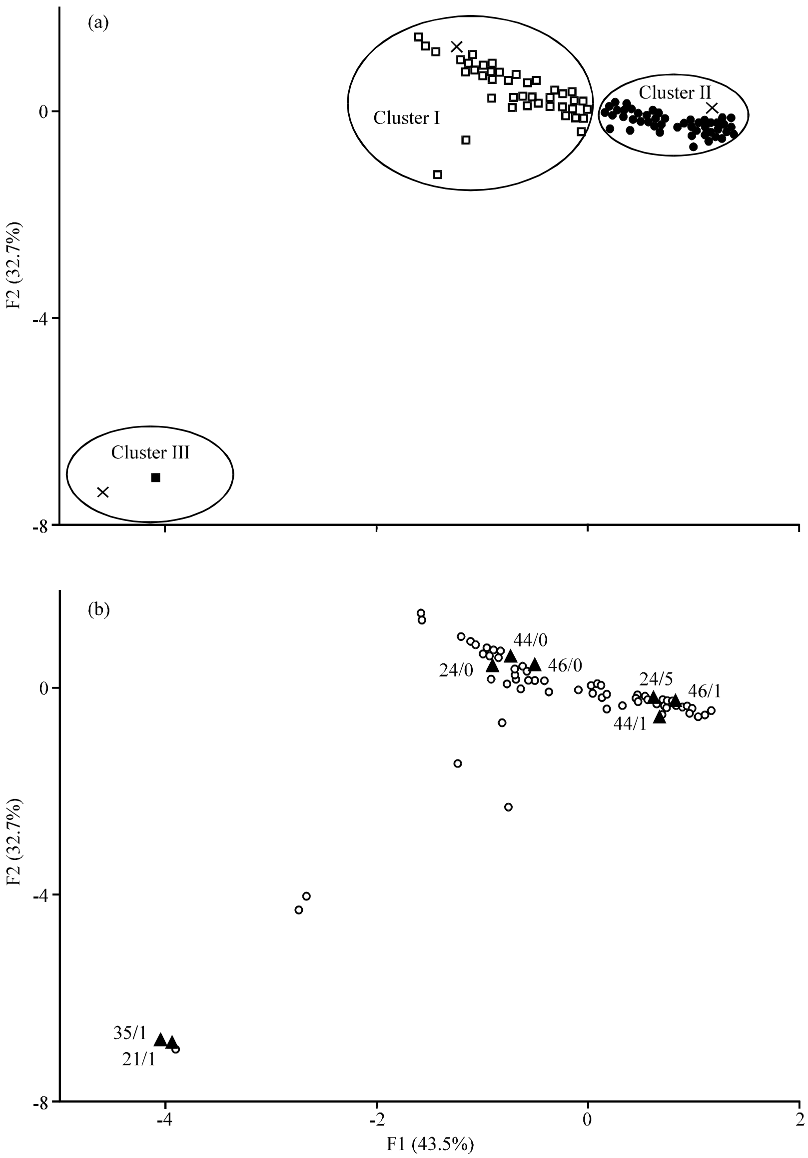

Fig. 1: MCA scatter plots of 88 *Hyptis* species and the outgroup *E. crassipes* (a) belonging to clusters I (▫), II (●) and III (■) based on morphological character (○) distribution, (b) Its discriminant characters/character states (▲) are encoded according to Tables 2 and 3. Crosses represent cluster centroids. Values expressed between parentheses refer to the explained inertia of each factorial axis

glomerular-subcorimbous cymes (χ^2 = 54.0, GLA = 10.0%, CLA = 66.7%, MOD = 100.0%), as well as the presence (χ^2 = 35.5, GLA = 14.4%, CLA = 46.2%, MOD = 100%) of crenate margins of leaf blades. Most taxa from section Pachyphyllae and *H. imbricata* were grouped with subsections Passerina/Obtectae (Eriosphaeria). The formers might be mainly distinguished ($p<0.001$, MOD = 100%) on the presence of revolute curvature of boards (χ^2 = 68.0, GLA = 5.56%, CLA = 80.0%) and entire margin of the leaf blade (χ^2 = 23.7, GLA = 14.4%, CLA = 30.8%) (Table 2). On the other hand, subsections Passerina/Obtectae were characterized by leaf blades extended along the stem in leaf arrangement on branches (χ^2 = 42.0, GLA = 6.67%, CLA = 50.0%, MOD = 100%) and showed ovate calyx teeth in flattened, oval-triangular shapes (χ^2 = 21.4, GLA = 5.56%, CLA = 40.0%, MOD = 66.7%). Despite the possibility of hybridization between *H. cruciformes* and other Pachyphyllae taxa (Harley and França, 2009), the latter species was not grouped with Pachyphyllae (Fig. 2).

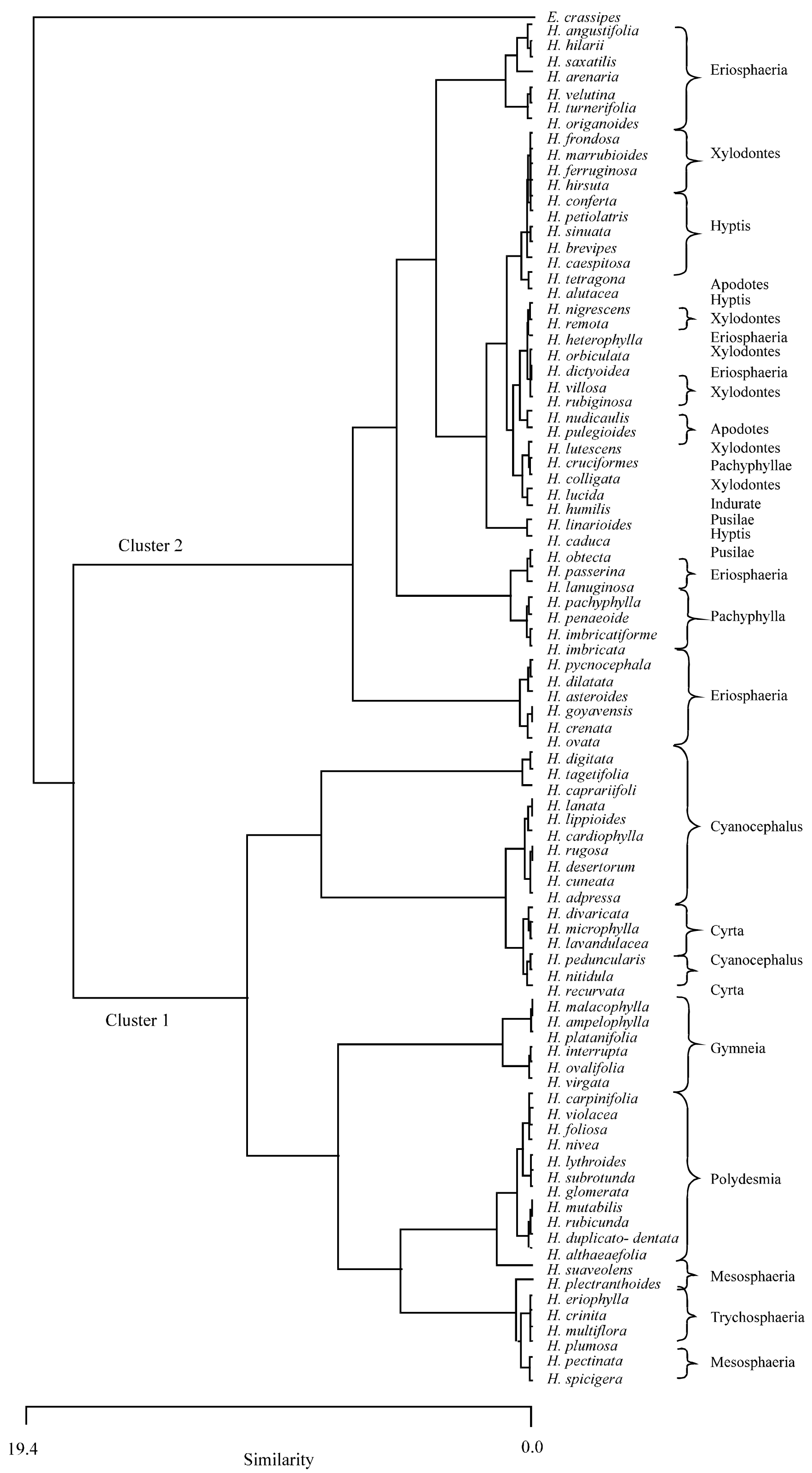

Fig. 2: Dendrogram of similarities between *Hyptis* taxa and *E. crassipes* (outgroup) based on vegetative and reproductive morphological characters to whose cluster it belongs: I, II and III

Clustered subsections Velutinae/Graciles (section Eriosphaeria) were characterized ($p<0.003$) mainly due to nutlets truncate at apex (χ^2 = 21.6, GLA = 24.4%, CLA = 31.8%, MOD = 100%), slightly rough nutlets (χ^2 = 13.1, GLA = 26.7%, CLA = 25.0%, MOD = 85.7%) and linear-lanceolate to elliptic-ovate bracts (χ^2 = 24.4, GLA = 5.56%, CLA = 60.0%, MOD = 42.9%). The dendrogram shows that section Pusilae was significantly different ($p<0.002$) due to linear, elongated-elliptic leaves (χ^2 = 37.4, GLA = 3.33%, CLA = 66.7%, MOD = 66.7%) and entire margin of leaf blades (χ^2 = 17.8, GLA = 14.4%, CLA = 23.1%, MOD = 100%), whereas sections Hyptis, Induratae, Apodotes, Eriosphaeria (subsection Heterophyllaea) and Xylodontes presented many similarities. Section Xylodontes showed the most intense fragmentation and was clustered with *H. cruciformes* (section Pachyphyllae; Fig. 2).

These results show that the delimitation of some species in the dendrogram does not agree with their subsectional delimitation, especially regarding taxa in cluster 2. This tree indicated higher heterogeneity among some sections (such as Eriosphaeria, Xylodontes and Cyanocephalus) than among those described by Epling and Játiva (1968). However, other Epling sections (such as Apodotes, Polydesmida, Mesosphaeria and Trychosphaeria) were more heterogeneous than the ones observed in the dendrogram of similarity, revealing the complex taxonomy of *Hyptis* species.

In order to identify character states that may be distinguishable among groups, the data set was submitted to Discriminant Correspondence Analysis (DCA) which summarizes variations among predefined classes for the classification of variables. The first DCA indicated that *Hyptis* subdivision may be differentiated ($p<0.0001$) based on three morphological traits: (1) verticillate or hemispherical-subglobose (χ^2 = 118) form of cymes; (2) smooth or slightly rough (χ^2 = 61.2) nutlet surface and (3) presence or absence of a stylopodium (χ^2 = 55.6). Furthermore, the second DCA revealed significant differences (χ^2 = 89.0; $p<0.0001$) in the outgroup due to the turbinated aspect of mature calyx tubes and also the presence of dibracteole bracts on the calyx base (Table 3).

Therefore, two main *Hyptis* groups were identified according to the taxon's original section: cluster I (41 taxa) included species from sections Cyanocephalus, Cyrta, Gymneia, Mesosphaeria, Polydesmia and Tricosphaeria (Fig. 2). This cluster was mainly characterized ($p< 0.0001$, χ^2 = 27.4) by: (1) elliptical-elongated linear bracts (GLA = 32.6%, CLA = 93.1%, MOD = 64.3%); (2) curvature of the calyx tube's upper limbs (GLA = 24.7%, CLA = 100%, MOD = 50.0%); (3) oblique calyx tube orifice (GLA = 25.8%, CLA = 91.3%, MOD = 50.0%) (4) smooth nutlets (GLA = 55.1%, CLA = 83.7%, MOD = 97.6%) and (5) absence of a stylopodium (GLA = 56.2%, CLA = 82.0%, MOD = 97.6%). On the other hand, cluster II (47 taxa) included all *Hyptis* species from sections Apodotes, Eriosphaeria, Hyptis, Induratae, Pachyphyllae, Pusilae and Xylodontes, which contained: (1) hemispherical-subglobose cymes (GLA = 51.7%, CLA = 95.7%, MOD = 95.7%); (2) presence of a stylopodium (GLA = 43.8%, CLA = 94.9%, MOD = 80.4%); (3) slightly rough nutlets (GLA = 27.0%, CLA = 100%, MOD = 52.2%) and finally (4) nutlets truncate at apex (GLA = 24.7%, CLA = 95.5%, MOD = 45.7%), which was the main ($p<0.0001$, χ^2 = 35.6) morphological trait (Table 3).

In the present report, the results of the phenetic analysis suggest that the morphological characters and states for discrimination between sections (and subsections) are most effectively made when combined with a multivariate method of numerical analysis. For example, in present study the Gymneia section seems to split into two hitherto unrecognized subsections (Fig. 1). Moreover, some sections (such as Polydesmida and Mesosphaeria) showed a high homogeneity with a slight tendency for the formation of subsections. In contrast, these same sections showed a high heterogeneity with each appearing in different places in the botanical keys (Epling and Játiva, 1968).

These variations could be due to the limited range of floral features used for keys while the whole plant morphological characters were considered in the present study. In addition, the botanical keys were based on small taxonomic sample, for example *H. tagetifolia* were collected in only three field trips, while *H. caduca*, *H. dictyodea* and *H. humilis* were known only from the type species (Harley and França, 2009).

On the other hand, the relationships between central Brazilian Cerrado *Hyptis* species, which were based on vegetative and reproductive morphological characters, agree with their delimitation in the two sub-genera proposed by El-Gazzar and Rabei (2008).

CONCLUSION

Although, we reported a method for the analysis of morphological variations, limitations exist for its use in proposing evolutionary relationships. Ward's clustering algorithm may have limitations for constructing branching patterns that reflect these aspects. The method presented here is superior, however, at assessing high levels of variation, as cladistic algorithms are not well suited to studies at these levels (Scotland *et al.*, 1995).

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REFERENCES

APG II, 2003. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. Bot. J. Linn. Soc., 141: 339-436.

Abdi, H. and D. Valentin, 2007. Multiple Correspondence Analysis. In: Encyclopedia of Measurement and Statistics, Salkind, N.J. (Ed.). Sage, Thousand Oaks, California, pp: 651-657.

Almeida, C.F.C.B.R. and U.P. Albuquerque, 2002. Check-list of the family Lamiaceae in Pernambuco, Brazil. Braz. Arch. Biol. Technol., 45: 343-353.

Arrigoni-Blank, M.F., A.R. Antoniolli, L.C. Caetano, D.A. Campos, A.F. Blank and P.B. Alves, 2008. Antinociceptive activity of the volatile oils of *Hyptis pectinata* L. Poit. (Lamiaceae) genotypes. Phytomedicine, 15: 334-339.

Clausen, S.E., 1998. Applied Correspondence Analysis: An Introduction. Sage Publication Inc., Oakes, CA.

Coutinho, H.D.M., J.G.M. Costa, E.O. Lima and J.P. Siqueira-Junior, 2009. *In vitro* phototoxic activity of *Eugenia jambolana* L. and *Hyptis martiusii* Benth. J. Photochem. Photobiol. B, 96: 63-65.

El-Gazzar, A., and S. Rabei, 2008. Taxonomic assessment of five numerical methods and its implications on the classification of *Hyptis* s.l. (Labiatae). Int. J. Bot., 4: 85-92.

Epling, C. and C. Játiva, 1968. Supplementary notes on American labiatae. Brittonia, 20: 295-313.

Giulietti, A.M., A. Rapini, M.J.G. Andrade, L.P. Queiroz and J.M.C. Silva, 2009. Rare Plants of Brazil. International Conservation/State University of Feira de Santana, Belo Horizonte, ISBN: 9788598830124.

Greenacre, M.J., 2007. Correspondence Analysis in Practice. 2nd Edn., Chapman and Hall/CRC, Boca Raton, Florida, ISBN-13: 9781584886167.

Harley, R.M., 1986. *Hyptis* sect. *Pachyphyllae* in Brazil: Notes on new world Labiatae: IX. Kew Bull., 41: 995-1005.

Harley, R.M., S. Atkins, A.L. Budantsev, P.D. Cantino and B.J. Conn *et al.*, 2004. Labiatae. In: The Families and Genera of Vascular Plants, Kubitzki, K. and J.W. Kadereit (Eds.). Vol. 7, Springer-Verlag, Berlin, ISBN-13: 978-3540405931, pp: 167-275.

Harley, R.M. and F. França, 2009. Lamiaceae. In: Rare Plants of Brazil, Giulietti, A.M., A. Rapini, M.J.G. Andrade, L.P. Queiroz and J.M.C. Silva (Eds.). International Conservation/State University of Feira de Santana, Belo Horizonte, ISBN: 9788598830124, pp: 192-200.

Le Roux, B. and H. Rouanet, 2004. Geometric Data Analysis. From Correspondence Analysis to Structured Data Analysis. Kluwer, Dordrecht, pp: 475.

Maraun, M.D., K. Slaney and J. Jalava, 2005. Dual scaling for the analysis of categorical data. J. Pers. Assess., 85: 209-217.

Melendo, M., E. Gimenez, E. Cano, F. Gomez-Mercado and F. Valle, 2003. The endemic flora in the South of the Iberian Peninsula: Taxonomic composition, biological spectrum, pollination, reproductive mode and dispersal. Flora Morphol. Distrib. Funct. Ecol. Plants, 198: 260-276.

Paton, A.J., D. Springate, S. Suddee, D. Otieno and R.J. Grayer *et al.*, 2004. Phylogeny and evolution of basils and allies (Ocimeae, Labiatae) based on three plastid DNA regions. Mol. Phylogenet. Evol., 31: 277-299.

Scarano, F.R. and G. Martinelli, 2010. Brazilian list of threatened plant species: Reconciling scientific uncertainty and political decision-making. Braz. J. Nat. Conservat., 8: 13-18.

Scotland, R.W., D.J. Siebert and D.M. Williams, 1995. Models in Phylogeny Construction. Oxford University Press, Oxford, ISBN-13: 978-0198548249.

Ward, J.H., 1963. Hierarchical grouping to optimize an objective function. J. Am. Stat. Assoc., 56: 236-244.