UNIVERSIDADE TECNOLÓGICA FEDERAL DO PARANÁ

VANESSA TERRA DOS SANTOS

ANÁLISE MORFOMÉTRICA DO COMPLEXO *Senegalia polyphylla* (LEGUMINOSAE)

DOIS VIZINHOS

2023

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Morphometric Analysis of *Senegalia polyphylla* Complex (Leguminosae)

Trabalho de conclusão de curso de Especialização apresentado como requisito para obtenção do título de Especialista em Biologia Molecular- Habilitação Bioinformática da Universidade Tecnológica Federal do Paraná (UTFPR).
Orientadora: Naiana Gabiatti.

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RESUMO

Senegalia é um gênero pantropical que compreende atualmente 235 táxons que ocorrem nas áreas tropicais e subtropicais do Velho e do Novo Mundo. Novas análises estão mostrando novas evidências filogenômicas para a não monofilia do gênero. A delimitação de algumas espécies de *Senegalia* é complexa, pois há sobreposição de caracteres morfológicos utilizados para delimitá-las. Nesse contexto, existem alguns complexos de espécies do gênero *Senegalia* citados na literatura: complexo *Senegalia amazonica*, complexo *S. berlandieri*, complexo *S. burkei*, complexo *S. greggii*, complexo *S. martiusiana*, complexo *S. polyphylla*, complexo *S. riparia*, complexo *S. tamarindifolia* e complexo *S. tenuifolia*. O complexo *Senegalia polyphylla* é composto pelas espécies *S. polyphylla, S. giganticarpa, S. rhytidocarpa, S. loretensis, S. kallunkiae* e *S. klugii*. Análises morfométricas estão sendo utilizadas em complexos de espécies para esclarecer a identidade de alguns táxons em Fabaceae. Neste contexto, são necessários parâmetros morfológicos que segreguem claramente as espécies do complexo *Senegalia polyphylla* ou, caso não estejam presentes, outra análise ou mesmo sinonimizações devem ser realizadas. Portanto, o objetivo deste estudo é encontrar parâmetros morfológicos que auxiliem na delimitação das espécies pertencentes ao complexo *S. polyphylla*, procurando mostrar as principais características vegetativas, florais e frutíferas que auxiliam na separação dessas espécies. Setenta e um espéimes pertencentes ao complexo *S. polyphylla* foram medidos morfologicamente. Quarenta e duas características morfológicas foram analisadas. Uma análise multivariada (PCA) foi realizada. Visando encontrar similaridades entre as variáveis em estudo, realizamos o biplot de distância. Nessa mesma lógica, selecionamos as 10 melhores variáveis nos resultados do PCA para cada estágio. Essas variáveis foram usadas para construir o dendograma das espécies e a análise hierárquica de cluster (Two-way Cluster) foi realizada. Embora nossa análise de PCA tenha encontrado alguns padrões de caracteres promissores na separação dos táxons, eles não foram efetivos para a segregação completa das espécies do complexo estudado. As análises de agrupamento com características vegetativas foram capazes de separar apenas *S. kallunkiae* e *S. kluggii* das demais espécies, bem como mostraram que elas estão relacionadas entre si. Por outro lado, as análises de cluster com características florais e frutíferas indicaram uma mistura de características muito mais complicada do que se pensava anteriormente. Nossas análises morfométricas não foram capazes de separar todas as espécies pertencentes ao complexo *S. polyphylla* ou apontar alguma congruência que possibilitasse estabelecer um padrão para guiar uma sinonimização entre algumas espécies. Como se trata de um complexo de espécies, é de se esperar que a separação clara e distinta dos táxons seja difícil, mesmo considerando que o presente trabalho esgotou as fontes de dados morfológicos que poderiam corroborar a elucidação de caracteres distintivos para essas espécies. Este trabalho aponta para a necessidade de usar outros caracteres e outras fontes de dados que possam ajudar a distinguir esses táxons.

Palavras-chave: Diversidade; Fabaceae; Mimosoideae; Morfometria.

ABSTRACT

Senegalia is a Pantropical genus that currently comprises 235 taxa that occurs in the tropics and subtropics areas of the Old and New Worlds. New analyses are showing new phylogenomic evidence for the non-monophyly of the genus. The delimitation of some *Senegalia* species is complex since there are overlap morphological characters used to delimit them. In this context, there are some complex of species in the *Senegalia* genus mentioned in the literature: *Senegalia amazonica* complex, *S. berlandieri* complex, *S. burkei* complex, *S. greggii* complex, *S. martiusiana* complex, *S. polyphylla* complex, *S. riparia* complex, *S. tamarindifolia* complex, and *S. tenuifolia* complex. The *Senegalia polyphylla* complex is composed of the species *S. polyphylla, S. giganticarpa*, *S. rhytidocarpa*, *S. loretensis*, *S. kallunkiae* e *S. klugii*. Morphometric analyses are being used in species complexes to clarify the identity of some taxa in Fabaceae. In this background, morphological parameters that clearly segregate the species of the *Senegalia polyphylla* complex are necessary or, if they are not present, another analysis or even synonymizations need to be made. Therefore, the aim of this study is to find morphological parameters that help in the delimitation of the species belonging to the *S. polyphylla* complex, trying to show the main vegetative, floral and fruit characteristics that help to separate these species. Seventy-one specimens belonging to the *S. polyphylla* complex were morphologically measured. Forty-two exomorphological traits were analyzed. A multivariate analysis (PCA) was performed. Aiming to find similarities between the variables in study, we performed the distance biplot. In this same logic, we select the firs 10 best variables in PCA results for each stage. Those variables were used to construct the species dendogram and the hierarchical cluster analysis (Two-way Cluster) were performed. Although our PCA analysis could found some promising character patterns in the taxa separation, they were not actually effective for the complete segregation of the species of the studied complex. The cluster analyses with vegetative traits were able to separate only *S. kallunkiae* and *S. kluggii* from the other species as well as showed that they are related to each other. On the other hand, the cluster analyses with floral and fruit characteristics indicated a mix of traits much more complicated than previously thought. Our morphometric analyses were not able to separate all the species belonging to *S. polyphylla* complex or point some congruence that could make possible to stablish a pattern to guide a synonimization between some species. As all species complex, it is to be expected that the clear and distinct separation of the taxa will be difficult, even considering that the present work has exhausted the morphological data sources which could corroborate the elucidation of distinctive characters for these species. This work points to the need to use other characters that can help distinguish these taxa.

Keywords: Diversity; Fabaceae; Mimosoideae; Morphometry.

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LISTA DE ABREVIATURAS E SIGLAS

HUFU	Herbário da Universidade Federal de Uberlândia
NY	The New York Botanical Garden Herbarium

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1 INTRODUCTION

Senegalia Raf. is a Pantropical genus that currently comprises 219 species (235 taxa) that occurs in the tropics and subtropics areas of the Old and New Worlds. There are 99 species of *Senegalia* in the Americas (Brazil is the center of species-richness with 63 species), 68 species in Africa plus Madagascar, 57 species in Asia, and two species occurring in Australia (MASLIN *et al.,* 2019; TERRA *et al.,* 2022). As mentioned by Terra *et al.* (2022), there are some particular hotspots of species richness in Brazil (63 species), Mexico (30 species), East Asia (China, 22 species) and east Africa (e.g. Somalia, 21 species; Mozambique, 20 species).

New analyses using 997 single-copy nuclear gene sequence for a representative set of species are showing new phylogenomic evidence for the non-monophyly of the genus (TERRA *et al.,* 2022). These analyses show a grade (Senegalioid grade) that is paraphyletic with the ingoid clade and comprises two well-supported subclades and a third, moderately supported clade, comprising species of *Mariosousa* Seigler & Ebinger, *Pseudosenegalia* Seigler & Ebinger and *Parasenegalia* Seigler & Ebinger. Bouchenak-Khelladi *et al.* (2010); Kyalangalilwa *et al.* (2013); Boatwright *et al.* (2015), consistently recovering those two well-supported clades and the same two clades were recovered by Terra *et al.* (2017) and Koenen *et al.* (2020). On the other hand, Koenen *et al.* (2020), unlike the other author mentioned before, reject the monophyly of Senegalia, revealing that those two clades are not sister to each other.

The delimitation of some *Senegalia* species is challenging since there are overlap morphological characters used to delimit them (TERRA, 2014). In this context, there are some complex of species in the *Senegalia* genus mentioned in the literature: *Senegalia amazonica* complex, *S. berlandieri* complex, *S. burkei* complex, *S. greggii* complex, *S. martiusiana* complex, *S. polyphylla* complex, *S. riparia* complex, *S. tamarindifolia* complex, and *S. tenuifolia* complex (RICO-ARCE, 2007; QUEIROZ, 2009; TERRA, 2014; HAHN 2016; SEIGLER com pes.). This is a large number compared to the total species of the genus (about 220). Morphometric analyses are being used in species complexes to clarify the identity of some taxa in Fabaceae (AGULLÓ *et al.,* 2013; LÔBO; STEFENON 2018; GARCIA-LARA *et al.,* 2015; Pometti *et al.,* 2007; Popoola *et al.,* 2015; RAHMAN; RAHMAN 2012; SOLADOYE, 2010).

The *Senegalia polyphylla* complex is named by the morphological affinities of all species with *Senegalia polyphylla* (DC.) Britton & Rose (RICO-ARCE, 2007;

QUEIROZ, 2009; TERRA, 2014; TERRA *et al.,* 2017). The complex is composed of the species *Senegalia polyphylla, S. giganticarpa* (G.P. Lewis) Seigler & Ebinger, *S. rhytidocarpa* (L. Rico) Seigler & Ebinger, *S. loretensis* (J.F. Macbr.) Seigler & Ebinger, *S. kallunkiae* (Grimes & Barneby) Seigler & Ebinger e *S. klugii* (Standley ex J. F. Macbride) Seigler & Ebinger. *Senegalia giganticarpa* and *S. rhytidocarpa* were described as varieties of *S. polyphylla* in the past (RICO-ARCE, 2006; LEWIS, 1996), showing the morphological similarity between these species and the confusion that can be found within the genus. Furthermore, *S. giganticarpa*, *S. polyphylla, S. rhytidocarpa e S. loretensis* are on the same branch of divergence in the phylogenetic analysis of Terra *et al.,* (2017).

In this background, morphological parameters that could clearly segregate the species of the *Senegalia polyphylla* complex are desirable and necessary or, if they are not present, another analysis should be pointed or even synonymizations need to be made. Therefore, the aim of this study is to find morphological parameters that help in the delimitation of the species belonging to the *Senegalia polyphylla* complex, trying to show the main vegetative, floral and fruit characteristics that help to separate these species.

2 DEVELOPMENT

2.1. Material and methods

The morphometric analyses were based on herbarium specimens of the *Senegalia polyphylla* complex taxa from NY and HUFU herbaria (acronyms according to Thiers, 2022). 71 specimens were morphologically analyzed (table 1 for species and tables 2, 3, and 4 for traits), including eight specimens of *S. giganticarpa*, two specimens of *S. kallunkiae*, 1 specimen of *S. kluggii*, 17 specimens of *S. loretensis*, 21 specimens of *S. polyphylla*, 17 specimens of *S. rhytidocarpa* and five specimens of *S. parviceps* (the outgroup).

Table 1 - Specimens used in the morphometric analyses showing the places where the material was collected, the collector data, the herbaria where is deposited, and the reproduction stage. Legend: fl. flower; fr. fruit.

(to be continued)

Species	Figures legends	Collection place	Colletor data	Herbarium	Stage
Senegalia giganticarpa (G.P. Lewis) Seigler & Ebinger	gig1	Nanuque, MG, Brasil	R.P. Belém 1593	NY	fr.
	gig2	Itapebi, BA, Brasil	R.S. Pinheiro 451 & T.S. Santos 114	NY	fl.
	gig3	Laranja da Terra, ES, Brasil	G. Hatschbach, J.M. Silva & F. Deodato 65279	NY	fr.
	gig4	Araripina, PB, Brasil	G. Eiten & L. Eiten 10854	NY	fl.
	gig5	Santa Marta, Colombia	H.H. Smith 39	NY	fl.
	gig6	Magdalena, Colombia	O. Haught 4357	NY	fl.
	gig7	Magdalena, Colombia	J.H. Kirkbride Jr. 2691	NY	fl.
	gig*	Itabuna	S.A. Mori, L.A. Mattos Silva, T. Soares dos Santos, J.A. Kallunki & T.D. Pennington 9575	NY	fr.
Senegalia kallunkiae (Grimes & Barneby) Seigler & Ebinger	ka1*	Caatiba, Bahia, Brasil	S.A. Mori, L.A. Mattos Silva, T. Soares dos Santos, J.A. Kallunki & T.D. Pennington 9373	NY	fl.
	ka2*	Afonso Cláudio, ES, Brasil	E. Pereira 9854	NY	fl.

(to be continued)

Senegalia klugii (Standley ex J. F. Macbride) Seigler & Ebinger	klu*	San Martín, Juan Jui, Peru	G. Klug 4272	NY	fl.
Senegalia loretensis (J.F. Macbr.) Seigler & Ebinger	lo1	RBO, Acre, Brasil	F. das C.S. Waltier 34	NY	fr.
	lo2	Napo, Equador	F. Hurtado 1166	NY	fr.
	lo3	Maynas, Iquitos, Peru	M. Rimachi 2169	NY	fl.
	lo4	Loreto, Yurimaguas, Peru	E.P. Killip & A.C. Smith 28309	NY	fr.
	lo5	Misericórdia, Rondônia, Brasil	G.T. Prance *et al.,* 6548	NY	fr.
	lo6	Ipacaetá, Bahia, Brasil	L.R. Noblick & C.G. Lôbo 4268	NY	fr.
	lo7	Paraná do Janauacá, Amazonas, Brasil	S. Mori & C. Gracie 22386	NY	fl.
	lo8	Formosa, Goiás, Brasil	B.A.S. Pereira & D. Alvarenga 2963	NY	fl.
	lo9	Beni, Bolívia	T. Killeen, V. García, M. Aguila, J. Strouse & S. Panfield 3314	NY	fr.
	lo10	Vitória do Xingu, PA, Brasil	A.C. Gonçalves sn	HUFU 76347	fl.
	lo11	Vitória do Xingu, PA, Brasil	B.A.S. Pereira sn	HUFU 76357	fl.
	lo12	Aimorés, MG, Brasil	A.A. da Luz 322	HUFU	fl.
	lo13	Juiz de Fora, MG, Brasil	B.B.S. Coelho sn	HUFU 76359	fl.
	lo14	Simão Dias, SE, Brasil	E.M. Carneiro 349	HUFU	fl.
	lo15	Mato Verde, MG, Brasil	V. Terra 475	HUFU	fr.
	lo16	Santa Cruz, Bolívia	M. Saldias, J.C. Cenzano & P. Gil 1518	NY	fr
	lo*	Loreto, Maucallacta, Peru	G. Klug 3956	NY	fl.
Senegalia parviceps (Speg.) Seigler & Ebinger (outgroup)	par1	Teixeira Soares, PR, Brasil	V.Terra e D.M.P. Pena 673	HUFU	fl.

(to be continued)

	par2	Irani, SC, Brasil	V.Terra e D.M.P. Pena 674	HUFU	fl.
	par3	Saldanha Marinho, RS, Brasil	V.Terra e D.M.P. Pena 676	HUFU	fl.
	par4	Panambi, RS, Brasil	V.Terra e D.M.P. Pena 678	HUFU	fl.
	par5	Orleans, SC, Brasil	V.Terra e D.M.P. Pena 681	HUFU	fl.
Senegalia polyphylla (DC.) Britton & Rose	po1	Piauí, Brasil	G. Gardner 1940	NY	fl.
	po2	Tapachula, México	Rosa E. Arcos Vertet 131	NY	fl.
	po3	Maicao, Guajira, Colombia	O. Arboleda, P. Bunch, J. Von Loh 434	NY	fl./fr.
	po4	Vicinity of Sonsonate, Sonsonate, El Salvador	Paul C. Standley 22309	NY	fr.
	po5	Belize, Orange Walk District dos Hombres	D. Lentz, S. Cherpelos, L. Haddad, M. Joo, M. Potter 2893	NY	fr.
	po6	Atiquizaya, Ahuachapán, El Salvador	P. H. Allen 6900	NY	fl.
	po7	Aquila, Coalcoman, Mexico	H. *et al.,* 16110	NY	fl.
	po8	Nayarit, Mexico	D. Seigler & J.T. Miller 16136	NY	fr.
	po9	Reserva no Panga, Uberlândia, MG, Brasil	G.M. Araújo & A.A.A. Barbosa s.n.	HUFU 1426	fl.
	po10	Monte Alegre de Minas, MG, Brasil	P.P. Damaso, J.N. Nakajima & E.K.O. Hattori 140	HUFU	fr.
	po11	Cumari, Goiás, Brasil	G.P.E. Rocha 148	HUFU	fl.
	po12	Cumari, Goiás, Brasil	G.P.E. Rocha 147	HUFU	fl.
	po13	Triângulo Mineiro, Vale do Rio Araguari, MG, Brasil	A.L.P. Mota 617	HUFU	fr.
	po14	Parque Estadual do Pau Furado, MG, Brasil	J.M. Fernandes 910	HUFU	fl.
	po15	Delfinópolis, Parque Nacional da Serra da Canastra, MG, Brasil	J.M. Fernandes 776	HUFU	fr.
	po16	Prados, Serra São João, MG, Brasil	J.M. Fernandes 1095	HUFU	fr.

(to be continued)

	po17	Santana do Riacho, Parque Nacional da Serra do Cipó	J.M. Fernandes 625	HUFU	fl.
	po18	Montes Claros, MG, Brasil	J.M. Fernandes 1186	HUFU	fl.
	po19	Matias Cardoso, MG, Brasil	J.M. Fernandes 1024	HUFU	fl.
	po20	Conceição do Mato Dentro, MG, Brasil	J.M. Fernandes & V.F. Dutra 649	HUFU	fl.
	po21	Corinto, MG, Brasil	G. Hatschbach, M. Hatschbach & E. Barbosa 66181	NY	fl.
Senegalia rhytidocarpa (L. Rico) Seigler & Ebinger	rh1	Equador, Morona, Santiago	David Neill, W. Palacios, J. Zaruma, C. Cerón 7429	NY	fl.
	rh2	La Paz, Bolívia	A. Araújo-M, C. Davidon, S. Davidson, P. Gismondi & T. Miranda 1180	NY	fl.
	rh3	Colombia	W.R. Philipson, J.M. Idrobo, A. Fernández 1657	NY	fl.
	rh4	Napo, Equador	Walter Palacios, 1400	NY	fl.
	rh5	Cruzeiro do Sul, Acre, Brasil	C.A. Cid Ferreira & *et al.,* 10593	NY	fl.
	rh6	Ichilo, Bolívia	I. Vargas, T. Killeen & C. Contreras 1216	NY	fl.
	rh7	La Paz, Bolívia	St. G. Beck 6855	NY	fr.
	rh8	Loreto, Peru	Y. Mexia 6258	NY	fr.
	rh9	Bagua, Peru	J.J. Wurdack 2500	NY	fl.
	rh10	Beni, Bolívia	T. Killeen & J. Krudenky 3589	NY	fr.
	rh11	Peija, Zulia, Venezuela	J. de Bruijn 1184	NY	fl.
	rh12	Napo, Equador	M. Tirano & B. Grefa 1993	NY	fl.
	rh13	Morona Santiago, Equador	L.J. Dorr & L.C. Barnett 5818	NY	fl.
	rh14*	La Paz, Bolívia	R. Seidel, E. Vargas & J. Wiesenmüller 2562	NY	fl.
	rh15*	Santa Cruz, Bolívia	M. Nee 36866	NY	fl.

(conclusion)

	rh16*	Beni, Bolívia	D.N. Smith, M. Buddensiek & V. Garcia 13586	NY	fr.
	rh*/lo	Acre, Brasil	Krukoff´s expedition 5382	NY	fr.

Source: Own authorship (2022).

Forty-two exomorphological traits were analyzed: 29 vegetative (Table 2), 10 florals (Table 3), and three from fruits (Table 4). We divided the traits in those three 3 sets since just one exsiccate had flower and fruits at the same specimen.

For the 29 vegetative traits we were able to score 71 specimens/accessions (Table 1) of all species from the *Senegalia polyphylla* complex (i.e. eight specimens of *Senegalia giganticarpa* of which one is a type material; two specimens both being type materials of *S. kallunkiae*; one specimen being a type of *S. kluggii*; 17 specimens of which 4 are type materials of *S. loretensis*; five specimens of *S. parviceps*; 21 specimens of *S. polyphylla*; and 17 specimens of *S. rhytidocarpa* of which 4 are considered type material for this species).

For the 10 floral traits we were able to score 46 specimens (Table 1) of all species from the *Senegalia polyphylla* complex (i.e. four specimens of *Senegalia giganticarpa*; two specimens both being type materials of *S. kallunkiae*; one specimen being a type of *S. kluggii*; eight specimens of which 1 is a type materials of *S. loretensis*; five specimens of *S. parviceps*; 14 specimens of *S. polyphylla*; and 12 specimens of *S. rhytidocarpa* of which 2 are considered type material for this species).

For the three floral traits we were able to score 24 specimens (Table 1) of some but not all species from the *Senegalia polyphylla* complex (i.e. three specimens of *Senegalia giganticarpa* of which one is a type material; eight specimens of *S. loretensis* and also eight specimens of *S. polyphylla*; and five specimens of *S. rhytidocarpa* of which 2 are considered type material for this species). Unfortunately, all materials from *S. kallunkiae*, *S. kluggii,* and *S. parviceps* were lacking fruits.

Table 2 - Vegetative morphological traits and their states or units used in the morphometric analysis of the *Senegalia polyphylla* complex.

Character	State/units
Aculeous (Ac)	absent; present
Nectary shape (NS)	cupuliform; pateliform; rouded pateliform; X: flattened pateliform; elongated pateliform; tubular
Nectary insertion	0: sessile; 1: shortly sessile; 2: stipitate
Stipe lenght (SL)	Mm
Nectary dimension (ND)	Mm
Position of the petiolar nectary (PPN)	inferior third; middle; superior third
Raquis nectary (RN)	0: absent; 1: between the last pinna pair; 2: between the last 2 pinna pairs; 3: between the last 3 pinna pairs
Rachiola nectary (RcN)	0: absent; 1: between the last leaflet pair; 2: between the last 2 leaflets pairs; 3: between the last 3 leaflets pairs
Petiole lenght (PL)	Mm
Raquis lenght (RL)	Mm
Distancy between the second and third pinnae (DP)	Mm
Second pinnae lenght (SPL)	Mm
Number of pinnae pairs (NP)	Number
Number of leaflets (NL)	Number
Distance between leaflets (DL)	Mm
Midvein (MV)	subcentric; submarginal; marginal
Leaflet indumentum on the adaxial face (IAdF)	glabrous, glabrescent; sparsely sericeous; sericeous
Leaflet indumentum on the abaxial face (IAbF)	glabrous, glabrescent; sparsely sericeous; sericeous
Trichomes concentrated at the base of the midvein (TB)	0: absent; 1: present
Shape of the median leaflet (SML)	oblong; oblong-falcate; oblong-obovate; elliptic; elliptic-falcate;
Apex of the median leaflet (AML)	acute; apiculate; mucronate
Base of the median leaflet (BML)	oblique; truncate
Length of the median leaflet (LML)	mm
Width of the median leaflet (WML)	mm
Shape of the apical leaflet (SAL)	elliptic; elliptic-falcate; elliptic-obovate; elliptic-ovate; oblong; oblong-obovate; obovate; ovate
Apex of the apical leaflet (AAL)	acute; apiculate; mucronate; rounded; retuse
Base of the apical leaflet (BAL)	oblique; truncate
Length of the apical leaflet (LAL)	mm
Width of the apical leaflet (WAL)	mm

Source: Own authorship (2022).

Table 3 - Floral morphological traits and their states or units used in the morphometric analysis of the *Senegalia polyphylla* complex.

Character	State/units
Peduncle lenght (PL)	mm
Inflorescence diameter (ID)	mm
Calix lenght (KL)	mm
Calix indumentum (KI)	sparsely sericeous; sericeous; X glabrescent
Corolla lenght (CL)	mm
Corolla indumentum (CI)	sparsely sericeous; sericeous; X glabrescent
Stamen lenght (SL)	mm
Stipe lenght (StL)	mm
Ovary lenght (OL)	mm

Source: Own authorship (2022).

Table 4 - Fruit morphological traits and their states or units used in the morphometric analysis of the *Senegalia polyphylla* complex.

Character	States/units
Length (FL)	mm
Width (FW)	mm
Indumentum (FI)	glabrous; sparsely sericeous; sericeous; puberulent

Source: Own authorship (2022).

A multivariate analysis (Principal Component Analysis, PCA) was performed in order to detect whether the morphological characteristics chosen for each stage (Table 2, 3, and 4) explain the delimitation of taxa among the analyzed species. To avoid that variables with larger values contribute more than variables with smaller values, we used the flag scale. = T in prcomp function implemented in R software (R CORE TEAM, 2020).

Aiming to find similarities between the variables in study, we performed the distance biplot (LEGENDRE; LEGENDRE 2012) using *ggbiplot* function implementen in ggbiplot package (VU, 2022). In this same logic, we select the firs 10 best variables in PCA results for each stage. Those variables were used to construct the species dendogram using dendexted package (GALILI, 2015), and the hierarchical cluster analysis (Two-way Cluster) were performed with ward.D2 method.

2.2. Results and discussion

2.2.1. PCA analysis

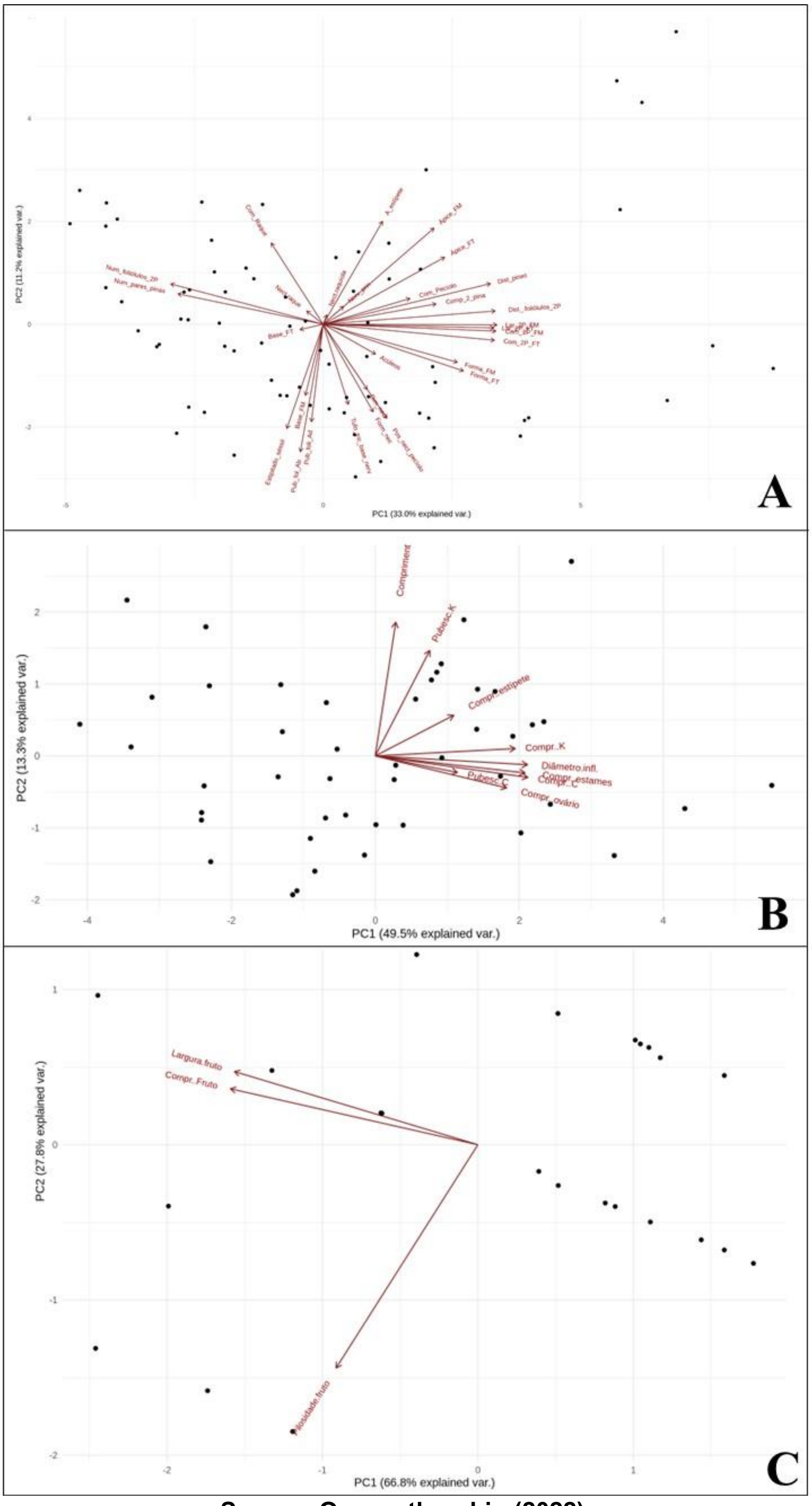
In figure 1 we can see the results of one of the multivariate analyzes (PCA) for our data sets. In Figure 1A, the principal components axes of the two-dimensional analysis were able to express 33.0% of the variations in principal component 1 (PC 1) and 11.2% of the variations in principal component 2 (PC 2). However, they were not sufficient to explain most of the variation, as they accounted for 44.2% of the total variation. The characteristics that were most relevant to explain this variation were the number of pairs of pinnae and the number of leaflets per pinna, demonstrating a strong negative correlation on the PC 1 axis. The distance between leaflets, the width of the terminal leaflet, the width of the median leaflet, the length of the terminal leaflet as well as the length of the median leaflet had a strong positive correlation with the axis, which explained the greater sources of variation for these parameters. As for the PC 2 axis, the variables that had the greatest positive impact on the distribution were the length of the nectary stipe and, negatively, the pubescence of the leaflet on the abaxial face and the sessile nectary. Thus, the vegetative characters did not have their variation represented by the two-dimensional analysis.

Leaf-related morphological parameters such as number of pinna pairs, number of leaflets per pinna pair, leaflet length and width, and inflorescence length/diameter were useful in solving other species complexes in *Senegalia*, such as *Senegalia burkei* complex (HAHN, 2016). The *Acacia Senegal* complex, composed by species related to *Senegalia*, used the traits number of leaflets per pinna, number of pinnae pairs, rachis length and leaflet area to identify differences between species *Acacia senegal* (L.) Willd. and *Acacia dudgeoni* Craib ex Holland (ASSOUMANE *et al.,* 2012).

In figure 1B the main component 1 (PC 1) was responsible for representing 49.5% of the variations, while the main component 2 (PC 2) represented 13.3% of the observed variations, managing to graphically represent 62.8% of the morphological variations found in the flowers. The most relevant characteristics to explain this variation in PC 1 were inflorescence diameter, stamens length and corolla length, which established a positive correlation with the axis. The variable that most influenced PC 2 was the length of the ovary. Due to the high value of the principal components, the graph was effective to represent the existing variations within this set of characteristics.

In figure 1C, the main component 1 (PC 1) was responsible for expressing 66.8% of the variations, while the main component 2 (PC 2) represented 27.8%, totaling 94.68% of the morphological variations found in the fruits. For the PC1 axis, the width and length of the fruits were the factors that most influenced the separation of individuals, with a strong negative correlation in this axis. In the PC 2 axis, the fruit indumentum had the greatest impact to perform the graphic distribution according to the variation. Due to the high percentage of variation explained by the two-dimensional axis, it is possible to infer that it was effective to perform the representation of these characteristics.

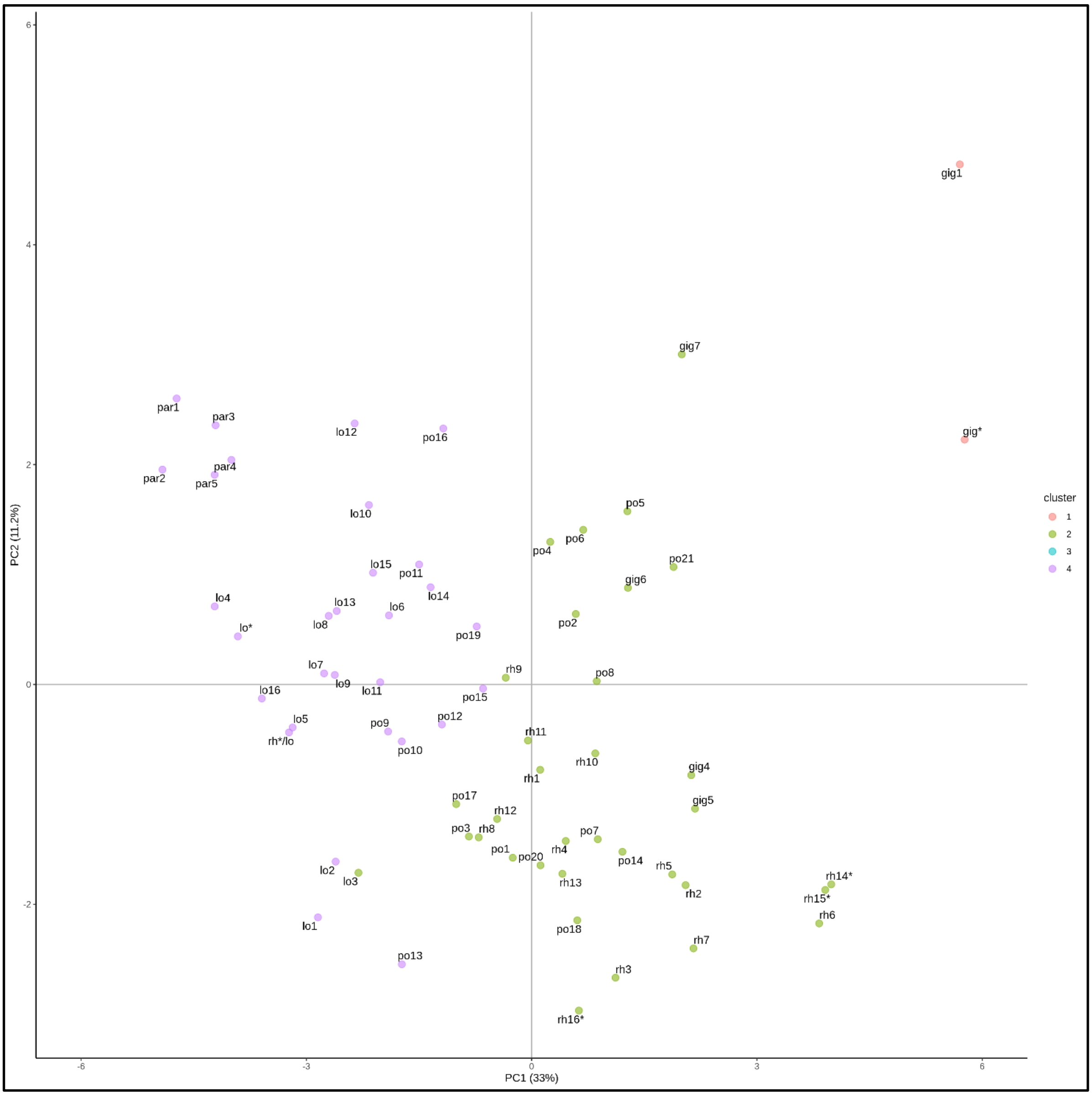
Figure 1 - Two-dimensional analysis of the principal components (PC) of vegetative (A), floral (B) and fruit (C) characters of the *Senegalia polyphylla* complex. The percentage variation corresponding to each PC is represented on the X and Y axes. The vectors indicate how much and how the characteristics are influencing the distribution of the specimens.



Source: Own authorship (2022).

Figure 2 represents the clustering performed using principal component analysis (PCA) of the vegetative characteristics. According to this analysis, it was not possible to make a clear distinction between two of the three groups of individuals, which shows that only with vegetative characteristics it is not feasible to separate these groups. However, it was possible to separate the cluster 1 (in pink), consisting of individuals gig1 and gig*.

Figure 2 - Color grouping performed using the vegetative characteristics on a two-dimensional axis with the two most relevant principal components (PC).



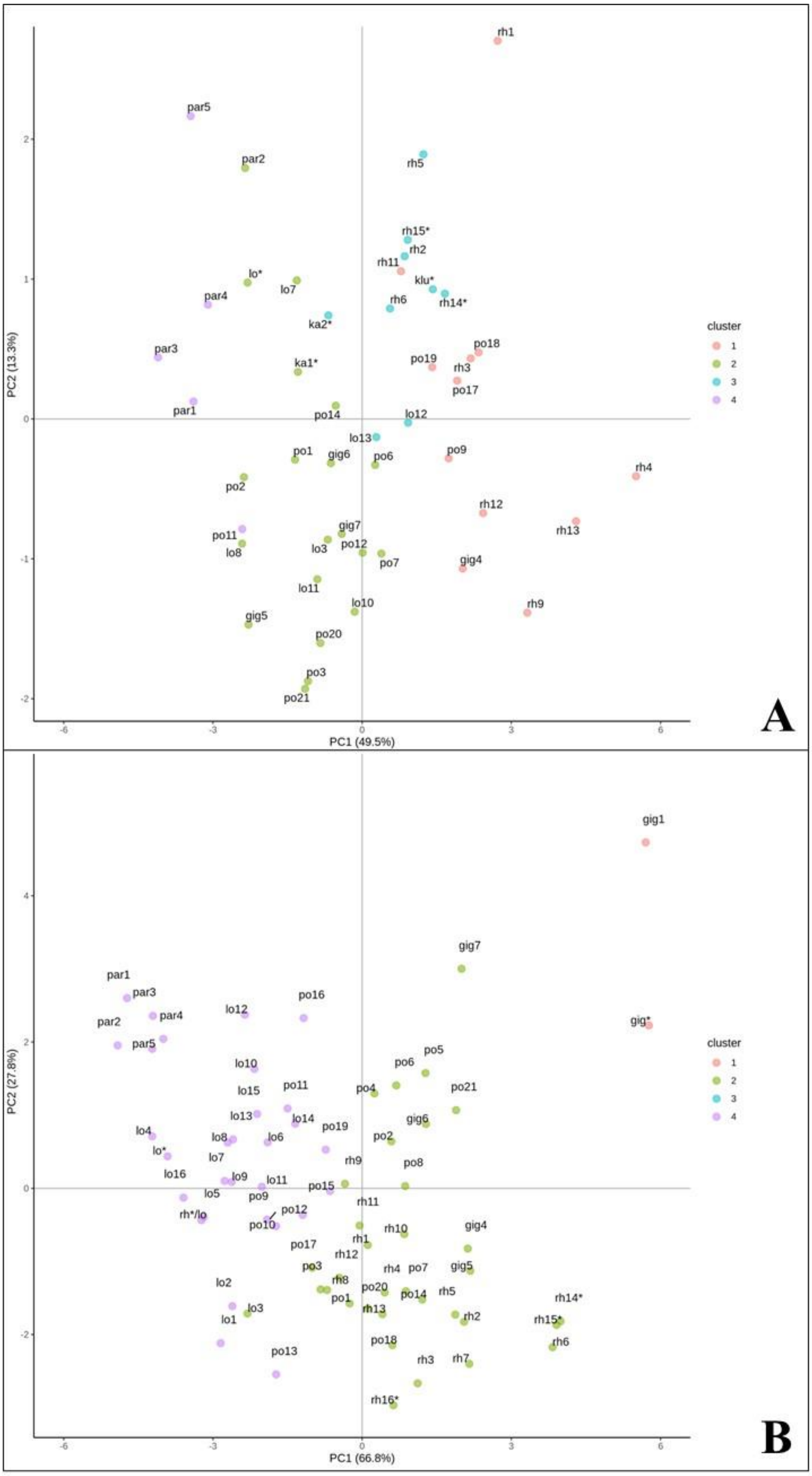
Source: Own authorship (2022).

In Figure 3A we can observe overlap between the four groups formed using the analysis of principal components for the floral characteristics, having a group with greater variation of characteristics (cluster 1) and another with less variation of characteristics (consisting of the individuals that belong to the cluster 3).

In figure 3B (set of fruit traits) there was a separation of three groups. Two of those groups were overlapping and the third one was clustered separately (in pink), consisting of individuals gig1 and gig*.

Although the characteristics evaluated were not enough to graphically represent the vegetative variations, when grouping the individuals, these characteristics had a result similar to the characteristics of the fruits, indicating that the two individuals (gig1 and gig*) probably form a separate cluster.

Figure 3 - Color grouping performed using the floral (A) and fruit (B) characteristics in a two-dimensional axis with the two most relevant principal components (PC).



Source: Own authorship (2022).

2.2.2. Cluster analysis

Figures 4, 5, and 6 for are showing dendrograms generated from the mean values of the vegetative, floral and fruit traits, respectively.

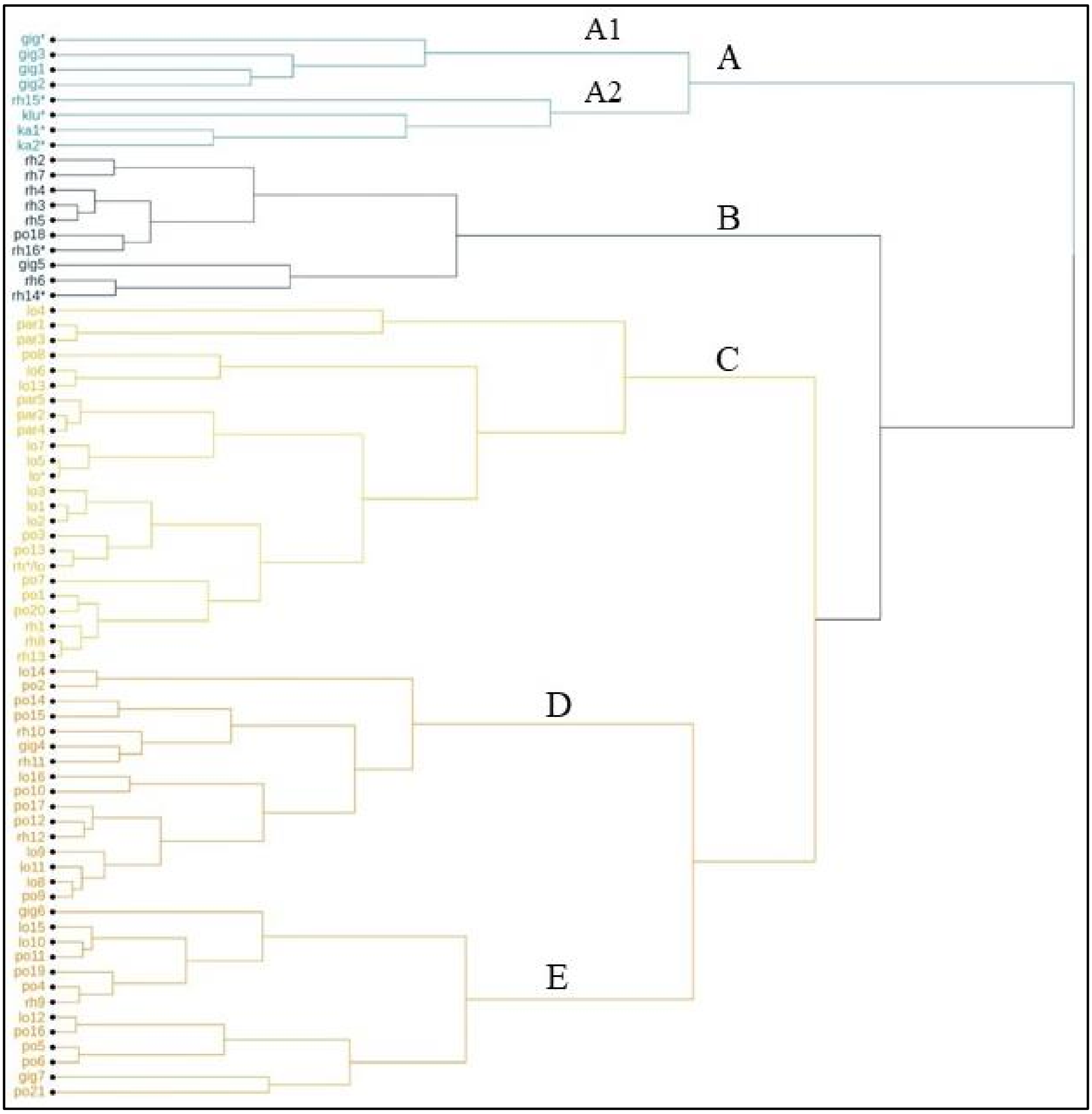
Figure 4 was based on the mean values of the 29 vegetative traits (Table 2) of the 71 accessions (Table 1) of the species of *Senegalia polyphylla* complex and outgroup. In this figure we can see five different clusters: A, B, C, D, and E.

The cluster A holds the subcluster A1 and A2. The subcluster A1 is composed by some but not all specimens of *S. giganticarpa* (including the type material) and the subcluster A2 is composed by one accession of *S. rhytidocarpa* (one of the type materials sampled) and all accessions sampled of *S. kluggii* and *S. kallunkiae* (all type materials). After measuring all characteristics to perform these analyses we could see clearly that some species in the *Senegalia polyphylla* complex have big leaflets that can make easier to distinguish them from the other species belonging to the complex. In this context we can cite *S. kallunkiae* and *S. kluggii.* It was expected to see both appearing as individual branches at the dendrogram. Those two species were the only ones that are completely separated by vegetative traits. The leaves of *S. kallunkiae* and *S. kluggii* have a shape and size that resemble the leaflets of *Senegalia altiscandens* (Ducke) Seigler & Ebinger and these three species belong to the same clade in the phylogenetic analyses performed by Terra *et al.,* (2017). Those three species are located together in a basal portion of the cladogram, suggesting that perhaps this feature is ancestral to the genus. The species of *S. rhytidocarpa* related to this cluster composed by *S. kallunkiae* and *S. kluggii* are the species with the biggest leaflets if compared with the others *S. rhytidocarpa* samples, what can explain the position of this accession very close to the species with big leaflets as mentioned before.

The cluster B is composed by species of *S. rhytidocarpa* (eight specimens, 2 being type materials), one accession of *S. polyphylla* and one of *S. giganticarpa* accessions. The clusters C is composed by *S. loretensis, S. parviceps, S. polyphylla,* and *S. rhytidocarpa* specimens. The clusters D and E are composed by species of *S. loretensis, S. giganticarpa, S. polyphylla,* and *S. rhytidocarpa*. All those clusters (B, C, D, and E) are indicating a mix of traits much more complicated than previously thought.

That said, our morphometric analyses using vegetative characteristic were able to separate *S. kluggii* and *S. kallunkiae* from the other species of *S. polyphylla* complex but were not able to separate the other species belonging to this complex or even showing some congruence that could make possible to stablish a pattern to guide a synonimization between some species.

Figure 4 - Dendrogram generated from the mean values of the 29 vegetative traits (Table 2) of the 71 accessions (Table 1) of the species of *Senegalia polyphylla* complex and outgroup. Legend: *Senegalia giganticarpa* (gi); *S. kallunkiae* (ka); *S. kluggii* (klu); *S. loretensis* (lo); *S. parviceps* (par); *S. polyphylla* (po); *S. rhytidocarpa* (rh). * type material.



Source: Own authorship (2022).

Figure 5 was based on the mean values of the 10 floral traits (Table 3) of the 46 accessions (Table 1) of the species of *Senegalia polyphylla* complex and outgroup. In this figure we can see three different clusters: A, B, and C and also an accession of *S. rhytidocarpa* isolated from the clusters at the top of the dendrogram.

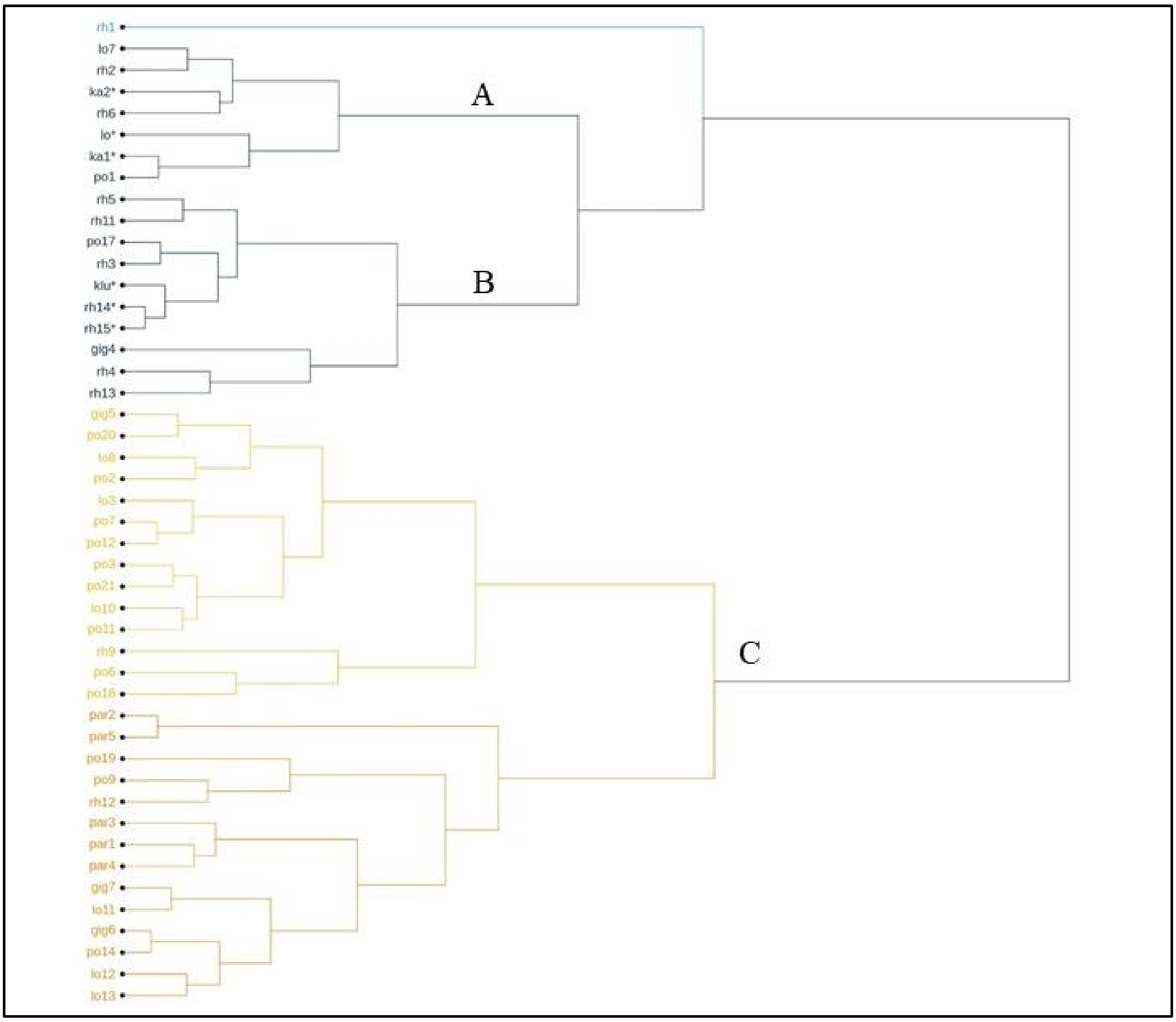
The cluster A is composed by both specimens (and type material) of *S. kallunkiae,* specimens of *S. loretensis* (one being a type material)*, S. polyphylla* and *S. rhytidocarpa.* Both specimens of *S. kallunkiae* are in the clade A but differently from the vegetative cluster, they are not directly related to each other and are presented in different subclusters.

The cluster B is composed by one specimen of *S. giganticarpa* and *S. kluggii* (type material) and also some specimens of *S. polyphylla* and *S. rhytidocarpa.*

The cluster C is the biggest cluster to the flower set of traits and is composed by specimens of *S. giganticarpa, S. loretensis, S. parviceps, S. polyphylla* and *S. rhytidocarpa*, all appearing messed up with each other with no smaller clusters that could make able see some pattern for some of the species sampled. As mentioned for the vegetative analyses (Figure 2), all floral clusters (A, B and C) are indicating a mix of traits much more complicated than previously thought.

Once again, our morphometric analyses using floral characteristic were not able to separate the other species belonging to this complex or even showing some congruence that could make possible to stablish a pattern to guide a synonimization between some species.

Figure 5 - Dendrogram generated from the mean values of the 10 floral traits (Table 3) of the 46 accessions (Table 1) of the species of *Senegalia polyphylla* complex and outgroup. Legend: *Senegalia giganticarpa* (gi); *S. kallunkiae* (ka); *S. kluggii* (klu); *S. loretensis* (lo); *S. parviceps* (par); *S. polyphylla* (po); *S. rhytidocarpa* (rh). * type material.



Source: Own authorship (2022).

The figure 6 was based on the mean values of the three fruit traits (Table 4) of the 24 accessions (Table 1) of the species of *Senegalia polyphylla* complex. Unfortunately, the outgroup material was lacking fruits and were not included in this set. In this figure we can see two different clusters: A and B.

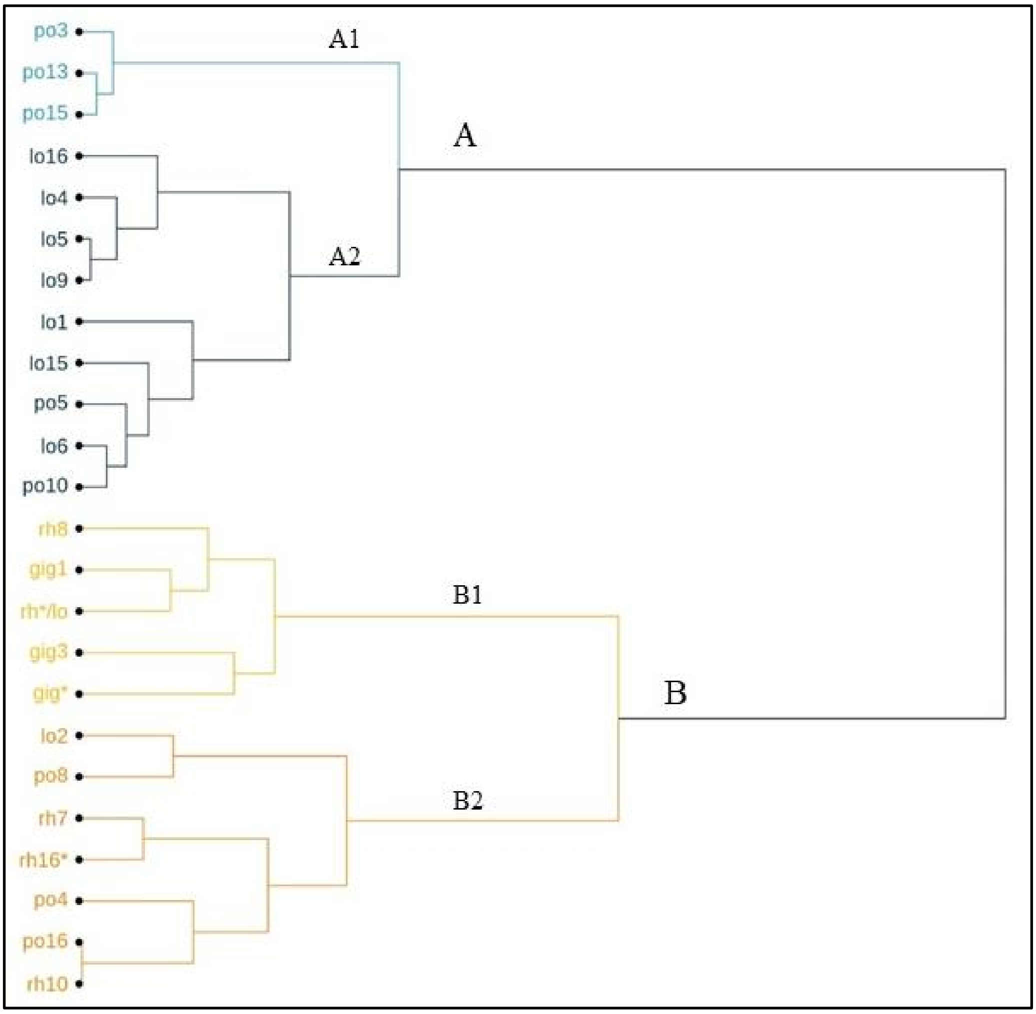
The cluster A holds the subcluster A1 and A2. The subcluster A1 is composed by some but not all specimens of *S. polyphylla* sampled. The subcluster A2 is composed by most of *S. loretensis* samples and one specimen of *S. polyphylla.*

The cluster B also holds two smaller clusters, the subcluster B1 and B2. The subcluster B1 is composed by the three *S. giganticarpa* specimens sampled for these set of characteristics (one being a type material) and two accessions of *S. rhytidocarpa*, one of those being considered a type material so far. The subcluster B2 is composed by one specimen of *S. loretensis*, two accessions of *S. polyphylla*, and three *S. rhytidocarpa* specimens (one being a type material).

We could not find any pattern for the fruit dataset. As mentioned for the vegetative (Figure 3) and floral analyses (Figure 4), all fruit clusters are indicating a mix of traits much more complicated than previously thought.

Our morphometric analyses using fruit characteristics were not able to separate the other species belonging to this complex or even showing some congruence that could make possible to stablish a pattern to guide a synonimization between some species.

Figure 6 - Dendrogram generated from the mean values of the three fruit traits (Table 4) of the 24 accessions (Table 1) of the species of *Senegalia polyphylla* complex. Legend: *Senegalia giganticarpa* (gi); *S. loretensis* (lo); *S. polyphylla* (po); *S.rhytidocarpa* (rh). * type material.



Source: Own authorship (2022).

As mentioned before, *S. giganticarpa* and *S. rhytidocarpa* were described as varieties of *S. polyphylla* in the past (LEWIS, 1996; RICO-ARCE, 2006) and treated by their authors as closely related to *S. polyphylla* as well as to *S. loretensis*. These species were located into the same clade as a polytomy in the plastid analyzes performed by Terra *et al.* (2017), showing that in fact they are indeed a complex of difficult resolution.

Within Leguminosae, anatomical characters have shown great importance as an auxiliary tool in the distinction of varieties within some genera, as is the case of *Chamaecrista* Moench. (COUTINHO *et al.,* 2016, 2013; FRANCINO *et al.,* 2015), *Crotalaria* L. (DEVECCHI *et al.,* 2014), *Rhynchosia* Lour. (VARGAS *et al.,* 2015) and many others.

An important feature in species segregation is the type of extrafloral nectary. Within the *Senegalia polyphylla* complex, this feature was not promising, however, a broad study of the anatomy and histochemistry of the secreted nectar could contribute to the separation of the species, as demonstrated for other genera of Leguminosae (COUTINHO *et al.,* 2012; COUTINHO; MEIRA 2015; MARAZZI *et al.,* 2019; MELO *et al.,* 2010; SILVA *et al.,* 2017).

3 CONCLUSIONS

As all species complex, it is to be expected that the clear and distinct separation of the taxa will be difficult, even considering that the present work has exhausted the morphological data sources which could corroborate the elucidation of distinctive characters for these species. This work points to the need to use other characters that can help distinguish these taxa. This is necessary, as the separation of species is very important in decision-making regarding many different fields in botany, as the delimitation of conservation areas, for example.

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