



Original Paper

Phylogeny of *Dorstenia* (Moraceae) reveals the polyphyletic nature of its neotropical sections

Marcelo Dias Machado Vianna-Filho^{1,2,3,8}, Leandro Cardoso Pederneiras^{1,2,5,8}, Vitor Hugo Maia⁴,
Andrea Ferreira da Costa^{2,6} & Vidal de Freitas Mansano^{1,7,9}

Abstract

Dorstenia, the second largest genus of Moraceae, comprises nine sections that are mainly found in Africa and America. Two of them are woody macrospermous, and the other seven are herbaceous microspermous. There are three sections in the Neotropics, all of which are herbaceous and taxonomically complex owing to their great morphological similarity. The most recent molecular phylogenetic studies of *Dorstenia* suggested that the neotropical sections are polyphyletic. These studies also showed that the neotropical species represent a sister group to an African woody macrospermous grade rather than African herbaceous microspermous plants. We have now expanded the number of taxa sampled and included other molecular markers to determine whether the previous phylogeny are to be corroborated or whether new taxonomic interpretations are to be followed. This study inferred the phylogeny of the group based on ITS, ETS, and *trnL-F* regions from 40 of the 58 neotropical species and added a new African taxon, thus including 17 of the 60 known species. Our results reaffirmed the polyphyletic nature of the neotropical sections. *Dorstenia* sect. *Acauloma* emerged within the main clade of *D.* sect. *Kosaria* (both African species), a result that confirms the affinity of these taxa already observed in previous morphological studies. We suggest *Dorstenia* sect. *Dorstenia* as the only neotropical section.

Key words: molecular markers, neotropics, phylogeny, plant evolution, rosids.

Resumo

Dorstenia, o segundo maior gênero de Moraceae, compreende nove seções encontradas principalmente na África e na América. Duas delas são macrospermas lenhosas e os outros sete são microspermas herbáceas. Existem três seções na região Neotropical, todas herbáceas e taxonomicamente complexas devido à sua grande semelhança morfológica. Os estudos filogenéticos moleculares mais recentes sugerem que as seções neotropicais são polifiléticas. Esses estudos também mostraram que as espécies neotropicais representam um grupo irmão de um grupo de macrospermas lenhosos africanos, em vez de microspermas herbáceos. No presente trabalho, expandimos o número de taxa amostrados e incluímos outros marcadores moleculares para determinar se a filogenia anterior deve ser corroborada e se novas interpretações taxonômicas devem ser seguidas. Este estudo inferiu a filogenia do grupo com base nas regiões ITS, ETS e *trnL-F* de 40 das 58 espécies neotropicais e adicionou um novo táxon africano, incluindo 17 das 60 espécies conhecidas. Nossos resultados reafirmaram a natureza polifilética das seções neotropicais. *Dorstenia* sect. *Acauloma*

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¹ Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, DIPEQ, Jardim Botânico, Rio de Janeiro, RJ, Brazil.

² Universidade Federal do Rio de Janeiro, Museu Nacional, Prog. Pós-graduação em Ciências Biológicas (Botânica), Quinta da Boa Vista, São Cristóvão, Rio de Janeiro, RJ, Brazil.

³ Universidade de Coimbra, Instituto do Ambiente, Tecnologia e Vida/ Jardim Botânico da Universidade de Coimbra, Calçada Martim de Freitas, Pólo, Coimbra, Portugal. ORCID:<0000-0002-9806-1455>.

⁴ Pontifícia Universidade Católica do Rio de Janeiro, Centro de Ciências Biológicas e da Saúde, Depto. Biologia, Gávea, Rio de Janeiro, RJ, Brasil. ORCID: <<https://orcid.org/0000-0003-2135-7650>>.

⁵ ORCID: <<https://orcid.org/0000-0003-1822-227X>>. ⁶ ORCID: <<https://orcid.org/0000-0002-9200-4222>>. ⁷ ORCID: <<https://orcid.org/0000-0002-7204-0744>>.

⁸ The first two authors contributed equally to this work.

⁹ Author for correspondence: vidalmansano@gmail.com

surgiu dentro do clado principal de *D.* sect. *Kosaria* (ambas espécies africanas), resultado que confirma a afinidade desses táxons já observada em estudos morfológicos anteriores. Nós sugerimos *Dorstenia* sect. *Dorstenia* com a única seção neotropical, porém maiores estudos moleculares são necessários.

Palavras-chave: marcadores moleculares, Neotrópico, filogenia, evolução das plantas, Rosídeas.

Introduction

The mulberry family, Moraceae, contains 39 genera and approximately 1,100 species that are globally distributed throughout tropical and temperate regions (Berg 2001; Zerega & Gardner 2019). *Dorstenia* L. is the second largest genus of Moraceae, with approximately 115 species (Berg & Hijman 1999; Vianna-Filho *et al.* 2016). Clement & Weiblen (2009), Gardner *et al.* (2017), and Zerega & Gardner (2019) suggested that this genus together with *Brosimum*, *Trymatococcus*, *Helianthostylis* and others comprise the tribe Dorstenieae. The species are distributed in tropical America (ca. 45 species), Africa (ca. 60 species), India, and Sri Lanka (1 species; Carauta 1978; Berg & Hijman 1999; Berg 2001) where they inhabit the undergrowth of lowland rainforests, slopes near streams, and rocky areas (Carauta 1978; Berg 2001).

The genus *Dorstenia* is morphologically characterized by its herbaceous habit, but there are a few succulent and woody species (Fig. 1). Their inflorescences are primarily monoecious, with a flattened, expanded receptacle (the coenanthium) with minute both staminate and pistillate flowers that are often tightly packed together; drupaceous fruits with an explosive dehiscence; and seeds that are either macrospermous or microspermous (Berg 2001; De Granville 1971; Misiewicz & Zerega 2012; Vianna-Filho *et al.* 2016).

Berg & Hijman (1999) and Berg (2001) subdivided the genus into nine sections: *Dorstenia* sect. *Nothodorstenia* Engl. (five woody species in Africa), *D.* sect. *Xylodorstenia* Hijman (six woody species in Africa), *D.* sect. *Lecania* Carauta (ca. 25 herbaceous species in the Neotropics and two in Africa), *D.* sect. *Lomatophora* Hijman (26 herbaceous species in Africa), *D.* sect. *Dorstenia* (12 herbaceous species in the Neotropics, *sensu* Carauta 1978), *D.* sect. *Kosaria* (Forssk.) Fisch. & C.A. Mey. (ca. 20 caulescent species in Africa and Asia), *D.* sect. *Bazzemia* Hijman (one acaulescent species in Mozambique), *D.* sect. *Emygdioa* Carauta (ca. 20 acaulescent species in the Neotropics), and *D.* sect. *Acauloma* Hijman (three succulent acaulescent tuberiferous species in

Africa). Carauta (1978), Berg (2001), Vianna-Filho (2012), and Santos *et al.* (2016) pointed out that the circumscription of the three neotropical sections is problematic (*D.* sect. *Dorstenia*, *D.* sect. *Emygdioa*, and *D.* sect. *Lecania*) because there are no clear morphological character states to segregate them.

Although the last taxonomic revisions (Berg & Hijman 1999; Berg 2001) have proposed a sectional classification and discussed the closest phylogenetic relationships between these groups and species, there are few studies based on the molecular phylogeny of *Dorstenia*. The two best-sampled phylogenetic analyses of *Dorstenia* (Misiewicz & Zerega 2012; Zhang *et al.* 2019) considered the genus to be monophyletic and suggested that the previous taxonomical sections were polyphyletic. An interesting finding was the position of *D. elliptica* Bureau, a woody macrospermous plant, as a sister of all neotropical species (Misiewicz & Zerega 2012; Zhang *et al.* 2019).

New possibilities have emerged after fundamental analyses by two molecular studies on *Dorstenia*, which mainly focused on biogeography and character evolution (Misiewicz & Zerega 2012; Zhang *et al.* 2019). In the Neotropics, for example, it was necessary to sample a larger number of taxa in order to determine the degree of monophyletic or polyphyletic sections/species evidenced by the first phylogenies. Misiewicz & Zerega (2012) analyzed 15 neotropical (27% of the total) and 18 African (30% of the total) species, and Zhang *et al.* (2019) analyzed 25 neotropical (55% of the total), 29 African (48% of the total), and one Asian (100% of the total) species. By increasing the set of neotropical taxa analyzed and using three molecular markers for the analysis, we can improve our understanding on the neotropical evolutionary history and make better decisions about the taxonomic subdivisions (sections).

Our objectives were to determine whether the genus *Dorstenia* is monophyletic and if the sections remain polyphyletic after with the increase of taxa sampled in the phylogeny. We also discuss the phylogenetic groupings based on morphological data, and propose further taxonomic changes.

Material & Methods

Taxon sampling

Our sampling included 57 species of ca. 115 *Dorstenia* species currently recognized (e.g.,

Carauta 1978; Berg & Hijman 1999; Berg 2001; Castro & Rappini 2010; McCoy & Massara 2008; Santos & Romaniuc 2012; Santos *et al.* 2013; Machado & Vianna-Filho 2012; Chase *et al.* 2013;

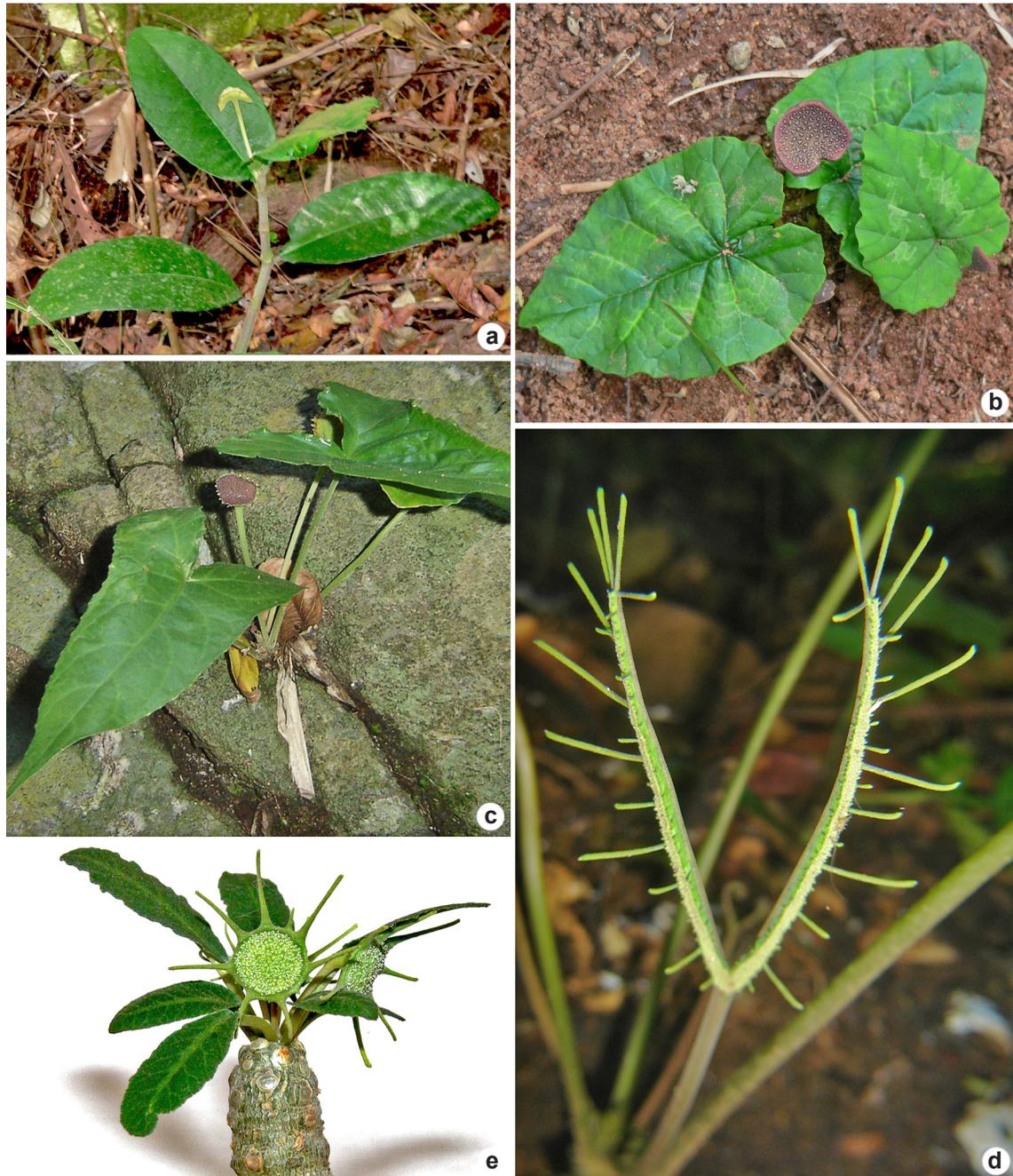


Figure 1 – a-e. Examples of *Dorstenia*'s diversity – a-d. Neotropical species – a. *D. elata*, a well-known *D.* sect. *Lecania* with long stems; b. *D. cayapia*, a geophyte species within *D.* sect. *Emygdioa*; c-d. *D. arifolia* and *D. ramosa*, respectively, two closely related species within *D.* sect. *Dorstenia*, which differ in their inflorescence; e. *D. foetida*, a Paletropical succulent species of *D.* sect. *Kosaria*.

Leal 2014; Machado *et al.* 2014; Rzepecky 2016), encompassing 72% of the neotropical species (40 species out of ca. 55) and 28% (17 species out of ca. 60) of the African species. In some cases, two or more individuals per species were analyzed, a total of 92 terminals (88 *Dorstenia* plus four outgroup terminals; see Table 1 for a list of sequences and their provenance). The phylogeny was rooted in *Helianthostylis sprucei* Baill., *Brosimum guianense* (Aubl.) Huber, *B. alicastrum* Sw., and *Trymatococcus amazonicus* Poepp. & Endl.

The relationships among neotropical *Dorstenia* species were analyzed by sampling taxa from different sections, including samples from the type localities. *Dorstenia* sect. *Dorstenia* was represented by 14 species (including the type species), *D.* sect. *Lecania* was represented by 14 species out of ca. 25 species (including the type species), and *D.* sect. *Emygdioa* was represented by 12 out of 20 species (including the type species).

We did not use the HQ214090, HQ214096, and HQ214105 samples, which represent, according to Misiewicz & Zerega (2012), the African taxa *Dorstenia variifolia* Engl., *D. tayloriana* Rend., and *D. cuspidata* Hochst. ex A. Rich., respectively, despite the fact that they seem to have emerged from within the neotropical clade. However, this was a very doubtful result due to the strong morphological dissimilarity between the African and neotropical species. The study by Zhang *et al.* (2019) confirmed that these taxa were not part of the neotropical clade and, therefore, we have not included them in our data matrix.

DNA extraction and sequencing

Genomic DNA was extracted from leaf material that had been dried in silica gel according to the CTAB protocol of Doyle & Doyle (1987). The plant material was ground in Eppendorf tubes containing metal beads, and the DNA samples were stored in the Brazilian Flora DNA Bank of the Rio de Janeiro Botanical Garden.

The phylogenetic relationships among the neotropical species were inferred using three molecular markers: ITS4/ITS5 for the internal transcribed spacer region (ITS; White *et al.* 1990) of nuclear ribosomal DNA, the *trnL-F* region (including the *trnL* intron and the *trnL-F* spacer; Taberlet *et al.* 1991), and the external transcribed spacer regions (ETS-Hel-1/18S-ETS; Baldwin & Markos 1998) (Tab. 2). These regions can be used to resolve relationships among and/or within the

Moraceae genera (*e.g.*, Weiblen 2000; Datwyler & Weiblen 2004; Weiguo *et al.* 2005; Rønsted *et al.* 2008; Clement & Weiblen 2009; Pederneiras *et al.* 2015).

The polymerase chain reaction (PCR) preparations contained 25 ng of DNA template, 1 × reaction buffer (10 × 10 mM Tris-HCl, pH 8.5, 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin), 0.2 mM dNTPs, 10 pmol of each primer, and 2.5 units of Taq DNA polymerase, which resulted in a final volume of 50 µL. The ITS and ETS primers made up 4% of the DMSO total reaction volume. The BSA was added to a final concentration of 0.5 µg/µL. The following PCR profiles were used: *trnL-F*: 94 °C for 2 min, 35 cycles: 94 °C for 1 min, 48 °C for 1 min, 72 °C for 1 min, and 72 °C for 7 min; ITS: 94 °C for 5 min, 30 cycles: 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, and 72 °C for 7 min; ETS: 94 °C for 1 min, 40 cycles: 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 30 min, and 72 °C for 5 min.

The PCR products were purified and sequenced at Macrogen Inc., Seoul, South Korea. The sequencing was conducted under the BigDye™ Terminator v3.1 cycling conditions. Then, the PCR products were purified using ethanol precipitation and run using an ABI3730XL automatic sequencer. All sequences generated for this study were deposited in GenBank (Tab. 1).

The sequences were assembled and edited with Geneious Pro 5.0.4 software (Biomatters Ltd.). Prior to assembly, the sequences were trimmed based on the quality values of the traces using the Modified-Mott algorithm, which is part of the software. The contig quality was assessed using the confidence mean value, which is the mean of the confidence scores for the contig base calls. Sequence alignments were conducted by Muscle 3.7 (Edgar 2004) using the default parameters, and subsequently checked by visual inspection.

Phylogenetic analyses

Our study included two phylogenetic approaches (maximum likelihood [ML] and Bayesian inference [BI]) for each of the four datasets (ETS, ITS, *trnL-F*, and all together), and there was total of eight phylogenetic trees (strict consensus tree in ML; 50% majority consensus in BI). The best-fit model was estimated for nucleotide substitution by AIC, which is part of the jModeltest (version 0.0.1) package, and the model selected was GTR+G. In the ML analysis, RAxML 8.2.12 (Stamatakis 2014) was used for

Table 1 – Voucher information and *Genbank* accession numbers of the plant material included in this study. GenBank codes in bold are the new molecular sequences generated by this article. The number next to the species name is the laboratory number.

Name	Voucher	Origin	ETS	ITS	trnL-F
<i>Brosimum alicastrum</i> 70	Moreira (RB 422381)	Brazil, Amazonas	MT726149	MT726054	MT726106
<i>Brosimum guianense</i> 69	Rodarte A.T.A. 163 (R)	Brazil, Rio de Janeiro	MT726150	MT726055	MT726107
<i>Dorstenia acangatara</i> 73	Machado 875 et al. (HUEFS)	Brazil, Espírito Santo	MT726151	MT726056	MT726108
<i>Dorstenia africana</i>	Carvalho 5308			HQ214078	
<i>Dorstenia africana</i>	van der Burgt et al. 527			HQ214077	
<i>Dorstenia albertorum</i> 43	Vianna-Filho 2003 (RB)	Brazil, Espírito Santo	MT726152	MT726057	MT726109
<i>Dorstenia albertorum</i> 52	Vianna-Filho 2004 (RB)	Brazil, Espírito Santo	MT726153	MT726058	MT726110
<i>Dorstenia alta</i>	Ntemi Sallu 638			HQ214081	
<i>Dorstenia alta</i>	Ndangalasi & Rajabu			HQ214082	
<i>Dorstenia arifolia</i>	Carvalho 7169			HQ214101	
<i>Dorstenia arifolia</i> 22	Pederneiras & Vianna-Filho 472 (R)	Brazil, Rio de Janeiro	MT726154	MT726059	MT726111
<i>Dorstenia asaroides</i> 35	Carvalho (RB 488301)	Brazil, Minas Gerais	MT726155	MT726060	MT726112
<i>Dorstenia asaroides</i>	Nee 42307			HQ214107	
<i>Dorstenia bahiensis</i> 5	Vianna-Filho 2510 (RB)	Brazil, Bahia	MT726156	MT726062	MT726113
<i>Dorstenia bahiensis</i> 41	Vianna-Filho 2501 (RB)	Brazil, Bahia		MT726061	
<i>Dorstenia barnimiana</i> 29	Vianna-Filho 2511 (RB)	Ethiopia, cultivated		MT726063	MT726114
<i>Dorstenia benguellensis</i>	Mkeya et al. 1109			HQ214102	
<i>Dorstenia bonijesu</i> 21	Machado (RB 488318)	Brazil, Espírito Santo	MT726157	MT726064	MT726115
<i>Dorstenia bonijesu</i> 30	Fraga 2504 (RB)	Brazil, Espírito Santo		MT726065	
<i>Dorstenia bowmaniana</i> 50	Vianna-Filho 2024 (RB)	Brazil, São Paulo	MT726158	MT726066	MT726116
<i>Dorstenia brasiliensis</i> 38	Hatschbach 11356 (RB)	Brazil, Paraná	MT726159	MT726067	MT726117
<i>Dorstenia brasiliensis</i>	Heringer et al. 735			HQ214110	
<i>Dorstenia brasiliensis</i>	Jansen-Jacobs et al. 4436			HQ214111	
<i>Dorstenia brownii</i>	Mwangoka et al. 3606			HQ214094	
<i>Dorstenia caatingae</i> 65	Melo 8433 (HUEFS)	Brazil, Bahia	MT726160	MT726068	MT726118
<i>Dorstenia caatingae</i> 66	Melo 5921 (HUEFS)	Brazil, Bahia		MT726069	
<i>Dorstenia capricorniana</i> 45	Monteiro et al. 500 (CESJ)	Brazil, Minas Gerais	MT726161	MT726070	MT726119
<i>Dorstenia carautae</i> 10	Silva 1953 (RB)	Brazil, Paraná	MT726162	MT726071	MT726120
<i>Dorstenia cayapia</i> 3	Vianna-Filho (RB 488317)	Brazil, Rio de Janeiro	MT726163	MT726072	MT726121
<i>Dorstenia cayapia</i>	Prance et al. 19219			HQ214109	
<i>Dorstenia choconiana</i>	R. Aguilar 11753 (NY1096054)			FJ916995	

Name	Voucher	Origin	ETS	ITS	trnL-F
<i>Dorstenia choconiana</i>	<i>G.Weiblen 1417</i> (MIN)			HQ214085	
<i>Dorstenia conceptionis</i> 19	<i>Carvalho</i> (RB 58223)	Brazil, Minas Gerais	MT726164	MT726073	MT726122
<i>Dorstenia contrajerva</i> 6	<i>Vianna-Filho 2502</i> (RB)	Without provenance, cult.	MT726165	MT726074	MT726123
<i>Dorstenia contrajerva</i>	<i>Martinez et al. 28913</i>			HQ214100	
<i>Dorstenia contrajerva</i>	<i>Chavarria 809</i>			HQ214099	
<i>Dorstenia djettii</i>	<i>Amponsah et al. 1413</i>			HQ214076	
<i>Dorstenia dolichocaula</i> 47	<i>Vianna-Filho 2020</i> (RB)	Brazil, Rio de Janeiro	MT726166	MT726075	MT726124
<i>Dorstenia drakena</i>	<i>Koch et al. 87195</i>			HQ214097	
<i>Dorstenia drakena</i>	<i>Salas et al. 2105</i>			HQ214098	
<i>Dorstenia elata</i> 42	<i>Carrizo 936 et al.</i> (R)	Brazil, Rio de Janeiro	MT726167	MT726076	MT726125
<i>Dorstenia elata</i>	<i>Mello-Silva et al. 1563</i>			HQ214087	
<i>Dorstenia elliptica</i>	<i>Nning 20</i>			HQ214075	
<i>Dorstenia elliptica</i>	<i>Thomas et al. 6990</i>			HQ214074	
<i>Dorstenia excentrica</i>	<i>Nee 22374</i>			HQ214113	
<i>Dorstenia excentrica</i>	<i>Misiewicz & Zerega, unvouchered</i>			HQ214112	
<i>Dorstenia fawcettii</i>	<i>Crosby et al. 770</i>			HQ214114	
<i>Dorstenia fischerii</i> 20	<i>Hottz</i> (RB 488302)	Brazil, Rio de Janeiro	MT726168	MT726077	MT726126
<i>Dorstenia foetida</i> 34	<i>Vianna-Filho</i> (RB 488307)	Ethiopia, cultivated		MT726078	MT726127
<i>Dorstenia foetida</i>	<i>Zerega 312</i>			HQ214104	
<i>Dorstenia gracilis</i> 23	<i>Vianna-Filho</i> (R 216119)	Brazil, Espírito Santo	MT726169	MT726079	MT726128
<i>Dorstenia grazielae</i> 17	<i>Vianna-Filho 2019</i> (RB)	Brazil, Rio de Janeiro	MT726170	MT726080	MT726129
<i>Dorstenia hildebrandtii</i> 26	<i>Vianna-Filho</i> (RB488304)	Without provenance, cult.	MT726171	MT726081	MT726130
<i>Dorstenia hildebrandtii</i>	<i>Zerega 311</i>			HQ214103	
<i>Dorstenia hildegardis</i> 44	<i>Vianna-Filho 2004</i> (RB)	Brazil, Espírito Santo	MT726172	MT726082	MT726131
<i>Dorstenia hirta</i> 11	<i>Vianna-Filho 2015</i> (RB)	Brazil, Rio de Janeiro	MT726173	MT726083	MT726132
<i>Dorstenia hirta</i>	<i>Souza et al. 21</i>			HQ214088	
<i>Dorstenia holstii</i>	<i>Ntemi Sallu 645</i>			HQ214091	
<i>Dorstenia kameruniana</i>	<i>Kibure & Bofu 1045</i>			HQ214079	
<i>Dorstenia lindeniana</i> 55	<i>Gerrit 36958</i> (MO)	Belize, Toledo		MT726084	MT726133
<i>Dorstenia lindeniana</i>	<i>Alvarez 2721</i>			HQ214083	
<i>Dorstenia mannii</i> 56	<i>Walthers 612</i> (MO)			MT726085	
<i>Dorstenia mannii</i>	<i>Gereau 5593</i>			HQ214095	
<i>Dorstenia mariae</i> 31	<i>Fraga 2474</i> (RB)	Brazil, Espírito Santo	MT726174	MT726086	MT726134
<i>Dorstenia maris</i> 53	<i>Vianna-Filho 2014</i> (RB)	Brazil, São Paulo	MT726176	MT726088	MT726136

Name	Voucher	Origin	ETS	ITS	trnL-F
<i>Dorstenia maris</i> 16	Vianna-Filho 2011 (RB)	Brazil, Mangaratiba	MT726175	MT726087	MT726135
<i>Dorstenia milaneziana</i> 46	Machado (R 691404)	Brazil, Espírito Santo	MT726177	MT726089	MT726137
<i>Dorstenia milaneziana</i>	Thomas et al. 11078			HQ214086	
<i>Dorstenia nummularia</i>	Acevedo-Rodriguez et al. 6439			HQ214115	
<i>Dorstenia paraguariensis</i>	Ortiz 218			HQ214108	
<i>Dorstenia peruviana</i> 54	Fuentes 4353 (MO)	Bolivia, without locality		MT726090	MT726138
<i>Dorstenia peruviana</i>	Plowman 5904			HQ214084	
<i>Dorstenia picta</i>	Gereau et al. 5189			HQ214089	
<i>Dorstenia psilurus</i>	W.R.Q.Luke 10351Z (MO)	Congo, Lokutu			
<i>Dorstenia ramosa</i> 12	Vianna-Filho (RB 482511)	Brazil, Rio de Janeiro	MT726178	MT726091	MT726139
<i>Dorstenia roigii</i>	E.L. Eckman 17973 (F)			HQ214116	
<i>Dorstenia romaniucii</i> 67	Machado 893 et al. (HUEFS)	Brazil, Espírito Santo		MT726092	
<i>Dorstenia romaniucii</i> 68	Machado 900 et al. (HUEFS)	Brazil, Espírito Santo	MT726179	MT726093	MT726140
<i>Dorstenia sucrei</i> 51	Carrizo 1359 (R)	Brazil, Espírito Santo	MT726180	MT726094	
<i>Dorstenia tenuis</i> 33	Vianna-Filho (RB 488315)	Brazil, Paraná	MT726181	MT726095	MT726141
<i>Dorstenia tenuis</i> 39	Vianna-Filho 2515 (RB)	Brazil, Paraná		MT726096	
<i>Dorstenia turbinata</i>	Cheek 11086			HQ214080	
<i>Dorstenia turnerifolia</i> 27	Carauta 1687 (RB)	Brazil, Rio de Janeiro	MT726182	MT726097	MT726142
<i>Dorstenia urceolata</i> 8	Andrade (RB 450982)	Brazil, Rio de Janeiro	MT726183	MT726098	MT726143
<i>Dorstenia urceolata</i> 28	Lima 6418 (RB)	Brazil, Rio de Janeiro		MT726099	
<i>Dorstenia vitifolia</i> 18	Carvalho (RB 488319)	Brazil, Minas Gerais		MT726100	
<i>Dorstenia vitifolia</i>	Vieira et al. 554			HQ214106	
<i>Dorstenia vitifolia</i> 2	Vianna-Filho (RB 488316)	Brazil, Minas Gerais	MT726184	MT726101	MT726144
<i>Dorstenia warneckei</i> 37	Vianna-Filho (RB 488308)	Without provenance, cult.		MT726102	MT726145
<i>Dorstenia zanzibarica</i> 59	Vianna-Filho (RB 488303)	Without provenance, cult.		MT726103	MT726146
<i>Helianthostylis sprucei</i> 61	Ribeiro 1531 (INPA)	Brazil, Amazonas	MT726185	MT726104	MT726147
<i>Trymatococcus amazonicum</i> 72	Ribeiro 1931 (INPA)	Brazil, Reserva Florestal Adolpho Ducke, Manaus	MT726186	MT726105	MT726148

Table 2 – Primers and DNA regions analyzed in the present study.

Primers	Sequence (5' → 3')	Region	Reference
ETS-Hel-1	GCTCTTTGCTTGC GCAACA ACT	ETS	Baldwin & Markos 1998
18SETS	ACTTACACATGCATGGCTTAATCT		
ITS4	CATCGATGAAGAACGTAGC	ITS/5.8S	Baldwin 1992
ITS5	GGAAGGAGAAGTCGTAACAAGG		
C	GGTTCAAGTCCCTCTATCCC	<i>trnL-F</i>	Taberlet <i>et al.</i> 1991
F	ATTTGAACTGGTGACACGAG		

phylogeny estimation with the default settings, and the data partitioned by the alignment region. The BI analysis was performed using MrBayes 3.2.7 (Ronquist *et al.* 2012). Two separate runs of four concurrent runs (one cold and three heated) over 30,000,000 generations were employed with sampling at every 3,000 generations. The sampled trees were summarized and those saved prior to the stationarity of the likelihood (burn-in) were excluded. CIPRES Science Gateway (Miller *et al.* 2010) was used for the ML and BI analyses. The ASTRAL (Mirarab *et al.* 2014) package was used to perform an ML analysis of the three independent molecular markers and to analyze the disagreement between the regions.

Results

This study generated 133 new DNA sequences: 52 from ITS, 43 from *trnL-F*, and 38 from ETS. The analysis with the ITS marker produced the same groups as the ETS and *trnL-F* analyses, but there was a larger set of species (92 terminals). The combined analysis of the markers (ETS, ITS, and *trnL-F*) produced a tree that was similar to that obtained using the ITS marker alone, but with better resolution in some terminal branches (*e.g.*, *Dorstenia carautae* C.C. Berg and *D. milaneziana* Carauta, C. Valente & Sucre). The proportion of input quartet trees satisfied by the final ASTRAL species tree was 0.98. According to Mirarab *et al.* (2014), the higher this value, the less disagreement the gene trees have. On this basis, we opted to discuss the phylogenetic hypothesis based on the combined analysis of the markers. We chose to use ML out of the two analyses (ML and BI) because there were no polytomic branches (Fig. 2 and Appendix S1, available on supplementary material <<https://doi.org/10.6084/m9.figshare.16569552.v1>>).

Among the paleotropical taxa, the *Dorstenia* sect. *Xylodorstenia* (BS 100%, PP 1.0) formed a monophyletic group and *D.* sect. *Nothodorstenia* formed a polyphyletic group with a solitary branch (*D. elliptica*) sister to all neotropical species, but this was inconclusive because of low support (BS 50%, PP < 0.5). *Dorstenia* sect. *Kosaria* formed a paraphyletic group because it included *D. barnimiana* Schweinf. (*D.* sect. *Acauloma*; BS 100%, PP 1.0). *Dorstenia* sect. *Lomatophora* formed a monophyletic group sister to *D.* sect. *Kosaria* and *D.* sect. *Acauloma* with moderate to high support (BS 79%; PP 1.0).

The three groups among the predominantly neotropical sections (*Dorstenia* sect. *Dorstenia*, *D.* sect. *Lecania*, and *D.* sect. *Emygdioa*) were polyphyletic with high support (BS = 100%, PP = 1.0) in most of the deep branches. Only *D. picta* Bureau (one of the two African species of *D.* sect. *Lecania*) emerged, along with the paleotropical species, next to the main clade of *D.* sect. *Kosaria* and *D.* sect. *Acauloma*, but had low support (BS 27%; PP 0.56). Within the predominantly neotropical clade, two branches emerged from the deepest node of the tree. One contained the South American species (BS 44%, PP 0.93) and the other contained the Central American species (BS 98%, PP 1.0).

Discussion

The analysis undertaken in this study suggested that *Dorstenia* forms a monophyletic group with both bootstrap and posterior probability values higher than 90% for the first and 0.9 for the second one. According to studies by Carauta (1978) and Berg & Hijman (1999), *Dorstenia* differs from other genera of Moraceae because it mainly consists of herbaceous plants (except for 11 African species), with bisexual inflorescences

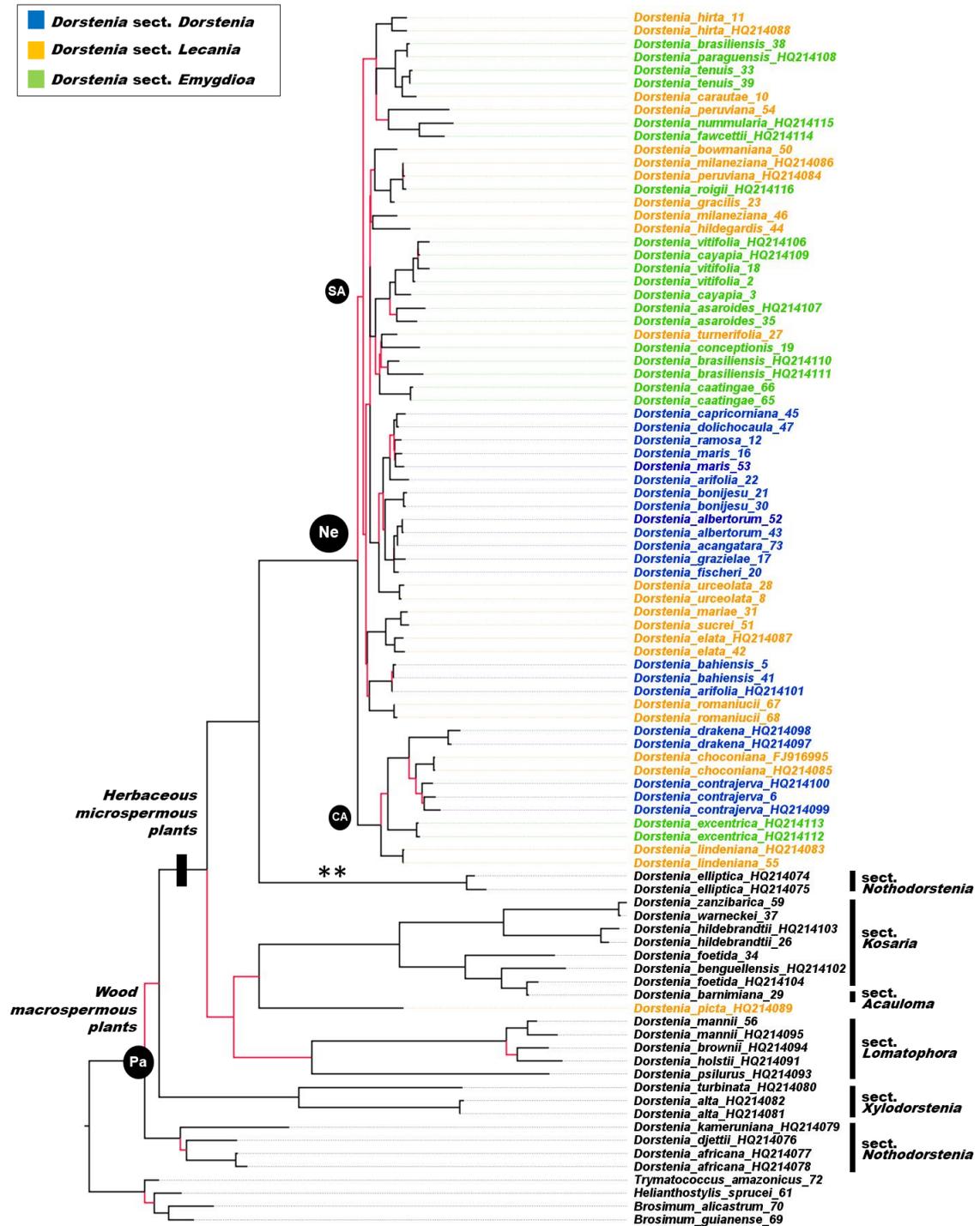


Figure 2 – Maximum Likelihood tree for combined molecular data (ETS + ITS + *trnL-F*) of 57 *Dorstenia* species (+ four outgroup species), representing eight sections. The length of the branches represents the genetic distance of each taxon. Pa = Paleotropical species; Ne = Neotropical species; SA = South American species; CA = Central American species. Branches in red indicate posterior probability < 0.9 or bootstrap < 90% (from the Bayesian Inference analysis, appendix). ** = a single woody macrosperous plant within the clade of herbaceous microsperous plants.

(except *Dorstenia cayapia* Vell.) that are discoid (saucer-shaped to cup-shaped), with pistillate flowers immersed in the receptacle, and bracts on the outer surface. All these synapomorphies are key evolutionary traits in the evolution of the group (Misiewicz & Zerega 2012) that support and identify the species within this clade.

Almost all paleotropical woody macrospermous plants emerged on the deepest nodes of the *Dorstenia* phylogenetic tree. *Dorstenia* sect. *Xylodorstenia* formed a monophyletic group, and *D. sect. Nothodorstenia* was a polyphyletic group. This early divergent position was expected because almost 90% of the Moraceae species are woody plants (only *Dorstenia* and *Fatoua* Gaudich. contain herbaceous species). The polyphyly of *D. sect. Nothodorstenia* section is an intriguing result. This section, together with *D. sect. Xylodorstenia*, only contains woody macrospermous plants. However, according to our results, *D. elliptica* emerges within the set of herbaceous microspermous plants.

The results suggested the validity of the hypothesis that *Dorstenia* sect. *Nothodorstenia* is phylogenetically closer to the neotropical clade (*D. sect. Dorstenia*, *D. sect. Emygdioa*, and *D. sect. Lecania*) rather than to the paleotropical sections. Berg & Hijman (1999) mentioned that retainment of bracts (*Dorstenia* sect. *Nothodorstenia*, *D. sect. Dorstenia*, *D. sect. Emygdioa*, and *D. sect. Lecania*) indicated the existence of a more recent common ancestor for these two groups compared to any other group, including *D. sect. Xylodorstenia*. The results indicated that this theory appears to be at least partially correct since *D. elliptica* (*D. sect. Nothodorstenia*) may be sister to all neotropical plants (Fig. 2).

Dorstenia sect. *Lomatophora* and *D. sect. Kosaria* form a monophyletic group along with the only species of *D. sect. Acauloma* sampled (*D. barnimiana* Schweinf.) and the only African *D. sect. Lecania* sampled (*D. picta*). All these taxa are herbaceous or succulent microspermous plants, paleotropical, and usually have seven or more pistillate flowers per receptacle. According to Berg & Hijman (1999), *D. sect. Lomatophora* and *D. sect. Kosaria* are close for being caulescent plants with scattered leaves and a mostly supraterranean stem.

Dorstenia barnimiana, the only species sampled from *D. sect. Acauloma* (out of three) emerged among the species of *D. sect. Kosaria*, exactly as Berg & Hijman (1999: 120) inferred from

morphological data. These groups are composed of succulent plants and, on the basis of the molecular data presented here, we infer that they should be treated within a single section. This should be better elucidated when the three species of *D. sect. Acauloma* and other species of *D. sect. Kosaria* are sampled.

Dorstenia picta, one of the two species of *D. sect. Lecania* in Africa, emerged from a deep node of the clade *D. sect. Lomatophora* + *D. sect. Kosaria* + *D. sect. Acauloma* and has a very long branch showing high evolutionary divergence. This raises the hypothesis that the African species of *D. sect. Lecania* can be considered a lineage that is parallel to neotropical *D. sect. Lecania*. Previous molecular studies also reported the same results (Misiewicz & Zerega 2012; Zhang *et al.* 2019). Thus, we conclude that these two species (*D. picta* and *D. Subdentata* Hijman & C.C.Berg) should be included in another section. Taxonomic studies that focus on African taxa are needed to confirm this.

According to the phylogenetic hypothesis presented in this study, the neotropical species (herbaceous microspermous plants) form a clade nested within the paleotropical species and are sisters, in part, to *D. sect. Nothodorstenia* (*D. elliptica*). According to Berg & Hijman (1999), the neotropical species diverged from paleotropical herbaceous plants, event makes by the presence of a bracteate receptacle, being closer to the African *D. sect. Nothodorstenia* (woody macrospermous plants) than to the other herbaceous sections (paleotropical). Therefore, reproductive traits, such as the presence and absence of bracts in the inflorescence, may play a key role in the evolution of the group, and morphological and anatomical studies focusing on this aspect may help to elucidate the systematics of the group.

Within the neotropical sections (*Dorstenia* sect. *Dorstenia*, *D. sect. Emygdioa*, and *D. sect. Lecania*), the present analysis indicated that the three sections are polyphyletic and that the neotropical clade can be subdivided into a Central American group and a South American group. Berg (2001) stated that it was very difficult to precisely delineate the three neotropical sections. Carauta (1978) used habit, leaf, stipules, and inflorescence shape to subdivide the sections, but molecular biology is questioning whether these characters clearly unify the neotropical monophyletic groups. Thus, in view of the lack of a reliable morphological distinction and the polyphyletic relationships among the species of *D. sect. Dorstenia*, *D. sect.*

Emygdioa, and *D.* sect. *Lecania*, we propose the later two as synonymous of *D.* sect. *Dorstenia*.

Taxonomic treatment

Dorstenia sect. ***Dorstenia*** L., Sp. Pl. 121. 1753. Type species. *Dorstenia contrajerva* L. = *Sychinium* Desv., Mém Soc. Linn. Paris 4: 216. 1826. *Dorstenia* sect. *Sychinia* (Desv.) Carauta, Bradea 2(21): 151. 1976. Type species. *Sychinium ramosum* Desvaux, *syn. nov.*

= *Dorstenia* sect. *Lecania* Carauta, Bradea 2(21): 151. 1976. Type species. *Dorstenia turnerifolia* Fisch. & C.A. Mey., *syn. nov.*

= *Dorstenia* sect. *Emygdioa* Carauta, Bradea 2(21): 151. 1976. Carauta, Rodriguésia 29(44): 105. 1978. Type species. *Dorstenia brasiliensis* Lam., *syn. nov.*

Herbs to subshrubs, monoecious; non cactiform; stems supraterranean to entirely subterranean; internodes elongate or short. Leaves spirally alternate, stipules present, subfoliaceous to subulate. Inflorescences usually bisexual and axillary, mostly bracteate, fringe present; flowers connate; interfloral bracts lacking (occasionally rudimentary); staminate flowers among pistillate ones or at the periphery of the flowering face; pedicellate; tepals 2–3(–4), stamens 2–3, inflexed in the bud, pistillode occasionally present; pistillate flowers sessile, perianth tubular, free or sessile, stigma 2, usually unequal. Fruit dehiscent drupelet, exocarp white and fleshy, turgid, ejecting the endocarp when mature (dry); seed small, endosperm present.

The species are distributed in North America (Mexico) to South America (Argentina), with fewer species in the Amazon basin. Fifty six species are listed in *D.* sect. *Dorstenia*. Included species: *D. albertorum* Carauta, *D. appendiculata* Miq., *D. arifolia* Lam., *D. aristeguietae* Cuatrec., *D. asaroides* Gardner, *D. bahiensis* Klotzsch ex Fisch. & C.A. Mey., *D. belizensis* C.C. Berg, *D. bonijesu* Carauta & C. Valente, *D. bowmaniana* Baker, *D. brasiliensis*, *D. brevipetiolata* C.C. Berg, *D. caimitensis* Urb., *D. carautae*, *D. cayapia*, *D. choconiana* S. Watson, *D. colombiana* Cuatrec., *D. conceptionis* Cuatrec., *D. contensis* Carauta & C.C. Berg, *D. contrajerva*, *D. crenulata* C. Wright ex Griseb., *D. dolichocaula* Pilg., *D. drakena* L., *D. elata* Gardner, *D. erythandra* C. Wright ex Griseb., *D. excentrica* Moric., *D. fawcettii* Urb., *D. fischeri* Bureau, *D. flagellifera* Urb. & Ekman, *D. gracilis* Carauta, C. Valente & D.S.D. Araujo, *D. grazielae* Carauta, C. Valente & Sucre, *D. hildegardis* Carauta, C. Valente & O.M. Barth, *D. hirta* Desv.,

D. jamaicensis Britton, *D. lindeniana* Bureau, *D. maris* C. Valente & Carauta, *D. milaneziana*, *D. nummularia* Urb. & Ekman, *D. panamensis* C.C. Berg, *D. peltata* Engl., *D. peruviana* C.C. Berg, *D. petraea* C. Wright ex Griseb., *D. ramosa* (Desv.) Carauta, C. Valente & Sucre, *D. rocana* Britton, *D. roigii* Britton, *D. romaniucii* A.F.P. Machado & M.D.M. Vianna, *D. setosa* Moric., *D. stellaris* Al. Santos & Romaniuc, *D. strangii* Carauta, *D. tentaculata* Fisch. & C.A. Mey., *D. tenuis* Bonpl. ex Bureau, *D. tuberosa* C. Wright ex Griseb., *D. turnerifolia*, *D. umbricola* A.C. Sm., *D. urceolata* Schott, *D. uxpanapana* C.C. Berg & T. Wendt, *D. vitifolia* Gardner.

Excluded taxa: *Dorstenia picta* and *D. subdentata*.

Dorstenia picta (African species) was excluded from the *D.* sect. *Dorstenia* (neotropical taxon) because it does not share the most recent common ancestor with the taxa included in this section. This species emerged from within the clade *D.* sect. *Lomatophora* + *D.* sect. *Kosaria* + *D.* sect. *Acauloma* according to our results. Based on the morphological analysis, we concluded that it is a member of the section *D.* sect. *Lomatophora*, mainly because it has an herbaceous habit with creeping to ascending leafy stems and a partly subterranean stem, which is very similar to *D. psilurus* Welw. *Dorstenia subdentata* was not sampled in the phylogenetic analysis, but after undertaking a morphological study of the species, because it is an ebracteate species, we predicted that it should also be treated as a member of the *D.* sect. *Lomatophora*.

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