DARWINIANA
www.ojs.darwin.edu.ar
NUEVA SERIE
ISSN 0011-6793 impresa - ISSN 1850-1699 en línea

DARWINIANA, nueva serie 5(1): 83-92. 2017
Versión final, efectivamente publicada el 31 de julio de 2017
DOI: 10.14522/darwiniana.2017.51.723

FROM ANTHESIS TO DIASPORE DISPERSAL: REPRODUCTIVE MECHANISMS OF RARE HERBACEOUS MORACEAE SPECIES ENDEMIC TO BRAZIL

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Abstract. Araújo, L. M.; A. Valentin-Silva, G. W. Fernandes & M. F. Vieira. 2017. From anthesis to diaspore dispersal: reproductive mechanisms of rare herbaceous Moraceae species endemic to Brazil. *Darwiniana*, nueva serie 5(1): 83-92.

Studies on the reproductive biology of *Dorstenia* species are scarce. We analyzed the sexuality, floral biology, and diaspore dispersal of *Dorstenia arifolia* and *D. bonijesu*, including their breeding systems and pollinators, in natural populations from Minas Gerais state, southeastern Brazil. We report here, for the first time, the occurrence of both unisexual (male) and bisexual coenanthia on the same individuals of *Dorstenia arifolia*, while only bisexual coenanthia were observed in *D. bonijesu*. In both species all coenanthia were long-lived and bisexual coenanthia were protogynous. We observed the occurrence of self-compatibility and myophily in *Dorstenia arifolia*, a species which was pollinated by small Lauxaniidae flies. Those insects not only used pollen as food resource but they also used coenanthia as oviposition sites, which is the first report to the studied species. *Dorstenia bonijesu* produced seeds, even having no floral visitors, suggesting an apomictic mechanism, which is a new breeding system reported to the genus. Both studied species are autochorous, but *D. bonijesu* also exhibited a peculiar type of hydrochory. The novelties reported herein indicate the need of additional studies to improve the understanding of the reproductive attributes of *Dorstenia* species.

Keywords. Apomixis; hydrochory; Lauxaniidae; myophily; oviposition site.

Resumen. Araújo, L. M.; A. Valentin-Silva, G. W. Fernandes & M. F. Vieira. 2017. Desde la antesis hasta la dispersión de diásporas: mecanismos de reproducción de especies herbáceas de Moraceae raras y endémicas de Brasil. *Darwiniana*, nueva serie 5(1): 83-92.

Los estudios sobre la biología reproductiva de las especies de *Dorstenia* son escasos. En este artículo analizamos la sexualidad, la biología floral y dispersión de las diásporas de *Dorstenia arifolia* y *D. bonijesu*, incluyendo sus sistemas reproductivos y polinizadores, en poblaciones naturales localizadas en el estado de Minas Gerais, sudeste de Brasil. Registramos, por primera vez, la presencia de cenantos unisexuales (masculinos) y bisexuales en un mismo individuo de *Dorstenia arifolia*; en cambio, sólo se observaron cenantos bisexuales en *D. bonijesu*. En ambas especies los cenantos fueron de larga duración, y los cenantos bisexuales exhibieron protoginia. Registramos autocompatibilidad y miofilia en *Dorstenia arifolia*, que fue polinizada por pequeñas moscas de la familia Lauxaniidae. Estos insectos no solo usan el polen como alimento sino que utilizan el cenanto como sitio de oviposición, siendo este un hecho registrado por primera vez para para las especies estudiadas. *Dorstenia bonijesu*, aún sin visitantes florales, también produce semillas mediante apomixis, un nuevo sistema reproductivo para el género. Las dos especies estudiadas son autocóricas; sin embargo, *Dorstenia bonijesu* exhibió un tipo peculiar de hidrocoria. Las novedades aquí registradas indican la necesidad de estudios adicionales para mejorar la comprensión de los atributos reproductivos de las especies de *Dorstenia*.

Palabras clave. Apomixis; hidrocoria; Lauxaniidae; miofilia; sitios de oviposición.

Original recibido el 16 de agosto de 2016, aceptado el 7 de julio de 2017
Editor Asociado: Raúl Pozner

INTRODUCTION

Dorstenia L. (Moraceae, tribe Dorstenieae) includes 105 herbaceous species with Pantropical distribution (Berg, 2001; Misiewicz & Zerega, 2012). Neotropical *Dorstenia* (47 species) have two centers of dispersion, based on the distribution of its two most abundant species: *D. brasiliensis* Lam. and *D. contrajerva* L. The former occurs in the Atlantic Forest from eastern Brazil, whereas the latter grows in Central America (Berg, 2001). The other species, according to Berg (2001), form small populations limited to restricted areas, and are therefore considered rare.

There are 39 known species of *Dorstenia* in Brazil (Romaniuc Neto et al., 2015). In the Atlantic Forest, these plants inhabit the understory, occurring preferably in wet, shady places (Carauta, 1978). Such is the case of *Dorstenia arifolia* Lam. and *D. bonijesu* Carauta & Valente (Carauta & Valente, 1983; Berg, 2001), the species studied herein, both of which are endemic to Brazil (Romaniuc Neto et al., 2015). *Dorstenia arifolia* has been reported to Bahia state and southeastern Brazil (Romaniuc Neto et al., 2015), while distribution of *D. bonijesu* is restricted to three Brazilian states: Espírito Santo, Rio de Janeiro (Carauta & Valente, 1983), and Minas Gerais (Araújo et al., 2007). Notwithstanding such scanty geographic distribution of both species, the Atlantic Forest is one of the most threatened biomes on the planet (Myers et al., 2000), and consequently the risk of extinction of its understory species, like those studied herein, is high.

Dorstenia species are mostly monoecious, being rarely dioecious (Datwyler & Weiblen, 2004). Their inflorescences, named coenanthia, consist of fleshy receptacles where pistillate and staminate flowers are inserted (Carauta, 1978; Berg, 2001). The fruit is a drupe, and diaspore (seed enveloped by pericarp) dispersal is autochorous (Granville, 1971; Berg, 2001).

According to Granville (1971), *Dorstenia* species depend on biotic pollinators for the occurrence of fruiting, although the possible agents remain unknown. Berg (2001) stated that some species in that genus are visited by small insects, but the author did not identify them. Other aspects on the reproductive biology of these plants are also poorly known (Berg, 2001; Araújo et al., 2007).

We aimed to analyze the sexuality, floral biology, and diaspore dispersal of *Dorstenia arifolia* and *D. bonijesu*, and investigate the species breeding systems and pollinators. We addressed the following questions: 1) Does any of the species depend on biotic pollinators for fruit production? 2) Should there be such a dependency, what are the species pollinators? 3) Should there not be such a dependency, what are the reproductive mechanisms that maintain the species populations? and 4) Are the studied species autochorous like other *Dorstenia* species?

MATERIAL AND METHODS

Study areas and species

We conducted the study from 2005 to 2008 in two municipalities (Coimbra and Viçosa) from Minas Gerais state, southeastern Brazil, located 20 km apart from one another. The only *Dorstenia* species occurring in them are the two species studied herein. Individuals of *D. arifolia* formed a natural population in a forest fragment of about 7 ha in Coimbra (20º51’24” S, 42º48’10” W). Individuals of *D. bonijesu* formed a natural population at the Mata do Paraíso Station for Research, Environmental Training and Education (20º48’07” S, 42º51’31” W), a forest fragment of 194 ha located in Viçosa. Both areas are located within the limits of the Atlantic Forest (Oliveira-Filho & Fontes, 2000), vegetation in the fragment has been classified as seasonal semi-deciduous forest (Veloso et al., 1991). Plants of *D. arifolia* grew along a watercourse, as did those of *D. bonijesu*. Both had a clustered distribution (Araújo, 2008).

For additional studies, we sampled individuals of both species grown in the greenhouse of the Botanical Garden at the Department of Plant Biology of Federal University of Viçosa. Plants were kept in ceramic pots (4.8 l) under a shade net for 50% light reduction.

Dorstenia arifolia has aromatic rhizomes and short aerial stems (Fig. 1A); leaves have slender petioles and entire or pinnatifid blades; coenanthia are round-shaped or elliptical, being supported by a long peduncle (Carauta, 1978; Fig. 1A). This species reproduces by vegetative propagation; flowering and fruiting may occur throughout the entire year, but predominantly during the rainy season (Araújo, 2008).

Dorstenia bonijesu also has rhizomes but is usually devoid of aerial stems, yet a reduced aerial stem might occasionally be observed (Fig. 1E); leaves are simple, pseudodigitate-trifoliolate-shaped, and sometimes trilobed (Fig. 1E); coenanthia are infundibuliform, being supported by a long peduncle (Carauta & Valente, 1983; Fig. 1E). This species does not reproduce by vegetative propagation; both flowering and fruiting occur during the rainy season (Araújo et al., 2007; Araújo, 2008).

Pistillate flowers of *Dorstenia arifolia* and *D. bonijesu* are sessile, being immersed in deep alveoli in the coenanthium (Fig. 1D); these flowers are perianthless, consisting of an ovary, style, and two style branches (Fig. 1B); the stigmatic area is located in the ventral region of each branch (Carauta, 1978; Araújo, 2008). There is a single pore at the upper end of the alveoli which enables style emergence. Staminate flowers are located in shallow alveoli, forming small intumescences that are easily visible to the naked eye; each staminate flower is composed of two tepals and two stamens (Fig. 1C-D). Filaments are curved in the floral bud and erect at anthesis; anthers are dithecous (Carauta, 1978).

Voucher material was deposited in the VIC Herbarium. The registration numbers in VIC are: 30136, 30137, and 30138 for *Dorstenia arifolia* and 30133, 30134, and 30135 for *D. bonijesu*.

Sexuality, floral biology, and diaspore dispersal

We collected 42 coenanthia of *Dorstenia arifolia* and 58 of *D. bonijesu* from cultivated plants for a flower count. From all those 100 coenanthia and from 28 other collected in plants from natural populations (15 of *D. arifolia* and 13 of *D. bonijesu*), we examined the sex expression in flowers and individuals, following the terminology of Sakai & Weller (1999). Another 13 coenanthia of individuals from natural populations of each species were tagged and followed from anthesis of the first flowers to diaspore dispersal, to characterized coenanthium longevity, the dynamics of flower opening in the coenanthia, and the changes that occur in the coenanthia prior to diaspore dispersal.

We tested stigmatic receptivity of pistillate flowers in eight coenanthia of each species using Peroxtesmo KO (Machery-Nagel, Düren, Germany), following the method proposed by Dafni & Maués (1998). We evaluated receptivity on a daily-basis from the beginning of stigmatic exposure, using a 20X-magnification hand lens.

Pollen viability was estimated on staminate flowers from seven coenanthia of each species using acetic carmine (Radford et al., 1974). Viable and non-viable grains (Kearns & Inoye, 1993) were counted in samples of 200 grains, taking 1 sample per slide, and two slides per coenanthium.

Breeding system

We performed usual pollination tests (Dafni et al., 2005) on both cultivated and naturally occurring individuals of both species, taking the coenanthium as pollination unit. In cultivated individuals, we tagged 48 coenanthia of *Dorstenia arifolia* and 78 of *D. bonijesu*. The tests performed thereon were: spontaneous self-pollination, hand self-pollination (geitonogamy), apomixis (by cutting the style with a forceps), cross-pollination, and open pollination (control). In natural populations, we tagged 39 coenanthia of *D. arifolia* and 51 of *D. bonijesu*, and on them we performed the spontaneous self-pollination and open pollination tests. Then, we collected the coenanthia and counted the number of senescent pistillate flowers and fruits. We analyzed the data by analysis of variance (ANOVA, for treatments of cultivated individuals) or means test (t test, for treatments of individuals from natural populations). We carried out these analyses using *Excel.*

Floral visitors

Flower visitors were observed throughout the day in natural populations. We captured insects that visited the coenanthia using an adaptation of the method reported by Zerega et al. (2004), by placing sticky traps (insect trap coating; Ladd Research Industries, USA) as close to the coenanthia as possible (between 10 and 15 cm). We also placed traps near plants having no coenanthia or having coenanthia with flower buds (control). Traps were removed after four hours of exposure. In the laboratory, we removed the insects from the traps and inspected them using a stereomicroscope for the presence of pollen on their bodies. Whenever possible, we also collected insect feces and stained them with safranin to verify the presence of pollen.

Previous observations on the natural population of *Dorstenia arifolia* revealed the use of the coenanthium as oviposition site by its main visitor. To confirm this interaction, 60 coenanthia were dissected in the laboratory for evaluation of the presence of larvae.

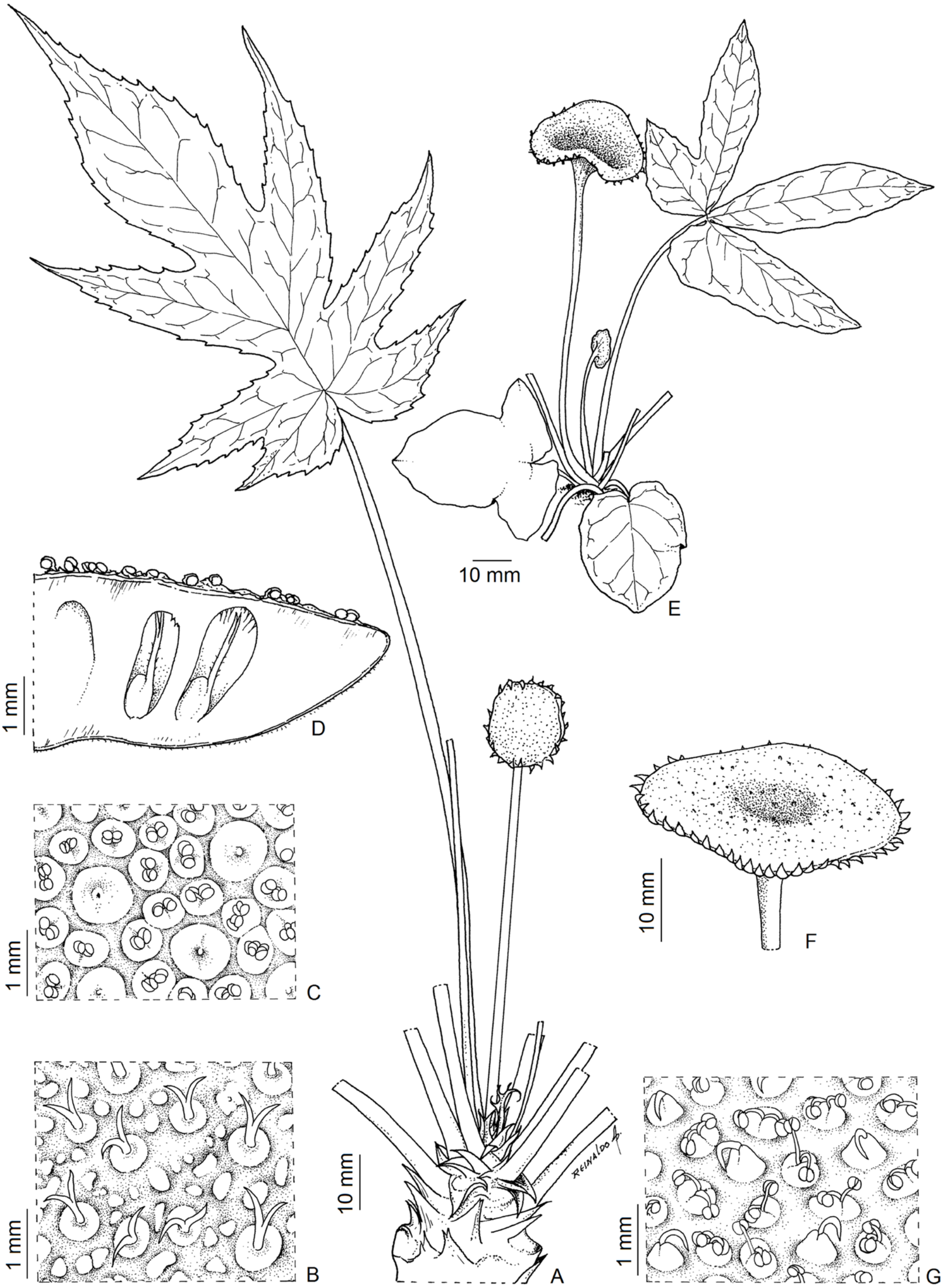

Figure 1. Studied species and details of their respective inflorescences. *Dorstenia arifolia*. **A,** plant habit; note the aerial stem and the coenanthium on a long peduncle. **B,** detail of coenanthium at the female phase; note the bifurcated style branches. **C,** detail of coenanthium at the male phase; the larger circles with a central hole, among staminate flowers, represent pistillate flowers with retracted style. **D,** coenanthium longitudinal section showing pistillate flowers retracted in deep coenanthium alveoli at the male phase; staminate flowers are in shallow alveoli. *Dorstenia bonijesu*. **E,** plant habit; note the absence of an aerial stem and the infundibuliform coenanthium on a long peduncle. **F,** "shallow-cup-shaped" coenanthium. **G,** detail of coenanthium at the male phase; note the pistillate flowers with senescent style.

RESULTS

Sexuality, floral biology, and diaspore dispersal

Some coenanthia of *Dorstenia arifolia* had pistillate ($\overline{X}$ = 140 ± 27.4) and staminate ($\overline{X}$ = 1,053 ± 135.5) flowers (Fig. 1B-C) at a 1:7.5 proportion, being thus bisexual. Other coenanthia had only staminate flowers ($\overline{X}$ = 941 ± 63.2), being therefore unisexual male. Both types of coenanthia were found on the same individual, characterizing the species as monoecious. Bisexual coenanthia were more or less convex-shaped, having an elliptical to sub-orbicular outline, whereas unisexual male coenanthia were round-shaped. In the latter, we eventually observed pistillate flowers that produced no fruits.

The coenanthia of *Dorstenia bonijesu* had pistilate ($\overline{X}$ = 109 ± 21.1) and staminate ($\overline{X}$ = 563 ± 55.4) flowers at a 1:5 proportion, being thus bisexual and the species being therefore monoecious. Coenanthia either were infundibuliform (Fig. 1E) or had the shape of a "shallow cup" (Fig. 1F); regardless of their shape, all coenanthia had a circular outline.

In coenanthia of both species, pistillate flowers were surrounded by staminate ones (Fig. 1C), and flower anthesis was centrifugal, beginning with the central pistillate flowers. Anthesis started with style emergence and separation of the style branches, thereby exposing stigmatic areas (Fig. 1B); ovaries remained within the alveoli. The female phase of coenanthia lasted on average 15 days in *Dorstenia arifolia* and 14 days in *D. bonijesu*. Pistillate flowers of *D. arifolia*, which were commonly the centermost ones in the coenanthia, exhibited a peculiar feature after pollination, namely the return of the style into the alveolus, which rendered them immersed again (Fig. 1D).

Anthesis of staminate flowers was also centrifugal and subsequent to the one of pistillate flowers, the former being characterized by filaments assuming an erect position and anthers undergoing dehiscence (Fig. 1C, G). Therefore, both species are protogynous. Male phase of coenanthia lasted an average of 38 days in *Dorstenia arifolia* (12 days in unisexual male coenanthia) and 19 days in *D. bonijesu*. In the latter species, there was a change in shape of most coenanthia at early male phase, from infundibuliform (Fig. 1E) to "shallow-cup-shaped" (Fig. 1F); some coenanthia, however, retained their original shape. No change was observed in the shape of coenanthia of *D. arifolia*. Pollen viability was high in both species: 81% and 87.5% of pollen grains were viable in bisexual and unisexual male coenanthia, respectively in *D. arifolia*, while 95% of pollen grains were viable in *D. bonijesu*.

In addition to protogyny, we also observed an overlap of sexual phases in the coenanthia. Such overlap occurred in 10% of *Dorstenia arifolia* coenanthia and 20% of *D. bonijesu* coenanthia.

The fruiting stage lasted on average 26 days in *Dorstenia arifolia* and 28 days in *D. bonijesu*, culminating in diaspore dispersal. At that point, bisexual coenanthia of *D. arifolia* had slightly reflexed edges, favoring release of diaspores, which were dispersed ballistically.

In *Dorstenia bonijesu*, diaspores of "shallow-cup-shaped" coenanthia were also dispersed ballistically. However, the coenanthia that remained infundibuliform got filled with water during heavy rains. Diaspores were then released and remained immersed in that water, until, due to the heavy weigh, the long peduncle with the coenanthium tipped over, pouring the diaspores out. Subsequently, the peduncle and coenanthium returned to their initial position.

Breeding system

Self-compatibility resulting from geitonogamous crosses was observed in *Dorstenia arifolia*, yet no fruit development by apomixis was observed (Table 1). In the natural population, the difference observed between fruit set resulting from spontaneous self-pollination and the one resulting from open pollination (Table 1) reinforced the importance of geitonogamy in that species.

Fruit set was high in all pollination tests performed in *Dorstenia bonijesu* (> 69%; Table 1), including the apomixis one. In the natural population of that species, no difference was observed between the fruit set resulting from spontaneous self-pollination and the one resulting from open pollination (≥ 92.2%; Table 1).

Flower visitors

Flower visitors were observed only at *Dorstenia arifolia*. Coenanthia of that species were visited by insects of two orders, namely Diptera and Hymenoptera, but the sampled species richness was low (Table 2). A higher number of insects were captured on traps deposited along unisexual male coenanthia (60.7%) than on those deposited along bisexual (25.4%) and control-treatment coenanthia (13.9%) (Table 2).

Table 1. Results of pollination tests (means and standard deviations) in coenanthia of *Dorstenia arifolia* and *D. bonijesu*, performed on cultivated individuals (CI) and in natural populations (NP).

Tests	Pistillate flowers	Fruits	
	No.	No.	%*
		Dorstenia arifolia	
Spontaneous self-pollination (CI)	225.3 ± 76.9	86.5 ± 53.9	38.4 ± 18.2 a
Hand self-pollination (CI)	250.3 ± 62.9	88.3 ± 50.8	35.3 ± 18.1 a
Apomixis (CI)	269.0 ± 20.2	1.3 ± 2.5	0.5 ± 0.9 b
Cross pollination (CI)	259.7 ± 56.5	83.8 ± 36.3	32.3 ± 15.6 a
Open pollination (CI)	267.0 ± 28.5	36.4 ± 16.7	13.6 ± 6.1 c
Spontaneous self-pollination (NP)	247.4 ± 45.2	120.3 ± 22.0	48.6 ± 3.7 A
Open pollination (NP)	277.1 ± 17.9	109.3 ± 21.3	39.4 ± 5.8 B
		Dorstenia bonijesu	
Spontaneous self-pollination (CI)	119.7 ± 63.2	83.3 ± 35.6	69.6 ± 16.5 a
Hand self-pollination (CI)	159.8 ± 60.9	138.8 ± 63.7	86.9 ± 23.6 b
Apomixis (CI)	121.7 ± 56.3	100.6 ± 45.3	82.7 ± 17.8 b
Cross pollination (CI)	99.1 ± 48.5	84.9 ± 32.3	85.7 ± 15.7 b
Open pollination (CI)	93.0 ± 49.8	71.6 ± 39.9	77.0 ± 27.2 ab
Spontaneous self-pollination (NP)	105.0 ± 35.8	96.8 ± 47.1	92.2 ± 13.1 A
Open pollination (NP)	129.3 ± 49.2	123.1 ± 46.8	95.2 ± 16.8 A

*Means followed by the same letter do not differ statistically at 5% probability by Tukey test (lowercase letters) or by t test (capital letters).

Flies predominated on all traps (91% of all collected insects), morpho-species 1 (Lauxaniidae, about 3 mm in length) being the most abundant captured insect (48.4% of all insects on bisexual coenanthia and 52.7% of those on unisexual male coenanthia; Table 2). We consider this fly to be the only pollinator among the captured insects due to its abundance and to the presence of pollen not only on its body – primarily on legs (tibia and tarsi) –, but also in its feces.

Visits by morpho-species 1 occurred mainly in the morning, peaking between 9:00 and 10:00 AM, but they also occurred in the afternoon, predominantly between 3:00 and 4:00 PM. The insects remained at the coenanthia for 20 to 30 min, probably feeding on pollen and laying eggs. During movements between coenanthia their flight height was low (< 1 m); the flies visited several plants.

We observed dipteran larvae in coenanthia dissected in the laboratory, thus characterizing these structures as oviposition sites. Larvae were found in bisexual coenanthia ($\overline{X}$ = 56.7 per coenanthium), always during the female phase. These larvae were probably of morpho-species 1, given the abundance of its adults in the study area and on coenanthia (Table 2), and its visiting behavior.

DISCUSSION

Among the five Moraceae tribes, Dorstenieae is characterized by having, among other features, bisexual inflorescences (Datwyler & Weiblen, 2004), as observed in our study. This character is common among monoecious *Ficus* species (tribe Ficeae), unlike tribes Artocarpeae and Castilleae, both of which have species that have unisexual inflorescences and can be either monoecious or dioecious (Datwyler & Weiblen, 2004).

The monoecious sexuality observed in the studied species is considered to be a derived character in the Moraceae family, as cited by Datwyler & Weiblen (2004). According to those authors, dioecious sexuality, which is common among species from tribe Moreae, is an ancestral character.

The occurrence of two distinct types of coenanthia in a same individual of *Dorstenia arifolia*, i.e. bisexual and unisexual male, was an unreported reproductive attribute to the genus. This finding reinforces the need for studies focusing on basic aspects of the reproductive mechanisms in representatives from this plant group, similarly to the approach we present herein.

Table 2. Insects collected on traps close to coenanthia of *Dorstenia arifolia*.

Order	Coenanthia				Control	
	Bisexual		Unisexual			
	No.	%	No.	%	No.	%
Diptera						
Morpho-species 1*	15	48.4	39	52.7	7	41.2
Morpho-species 2	6	19.4	19	25.7	0	0.0
Morpho-species 3	7	22.6	11	14.9	7	41.2
Hymenoptera						
Morpho-species 1	3	9.7	5	6.8	3	17.6
Total	31		74		17	

*Lauxaniidae

The coenanthia of the studied species act as pollination units (pseudanthia), as do the syconia of *Ficus* L., the capitula of Asteraceae and the spadices of Araceae (Endress, 1994; Proctor et al., 1996). All such long-lived units are composed of numerous small, dichogamous flowers (protogyny, in the studied species). The cost of the longevity of those units is the increased likelihood of damage by herbivores and pathogens (Khadari et al., 1995; Kudo, 2006). In *Dorstenia bonijesu*, damage by mollusk herbivory and pathogen attack was commonly observed (pers. obs.).

The longevity of coenanthia is associated not only with protogyny but also with sequential flower anthesis, and long duration of stigmatic receptivity (female phase) and pollen release (male phase). The long duration of stigmatic receptivity may be a trait inherited from an anemophilous ancestor of tribe Moreae (Khadari et al., 1995), since wind-pollinated species usually have long-lived stigmas (Primack, 1985). This tribe has representatives related to Dorstenieae (Datwyler & Weiblen, 2004), which reinforces that hypothesis. The prolonged release of pollen (the floral resource utilized by visitors) maximizes pollen flow (Harder & Aizen, 2004), especially in *Dorstenia arifolia*, a species that, unlike *D. bonijesu*, still maintains interactions with its pollinators.

The interfloral protogynous dichogamy of *Dorstenia arifolia* and *D. bonijesu*, which is also observed in other species of the genus (Carauta, 1978; Berg, 2001), is a floral trait that involves the temporal separation of female and male functions in inflorescences with unisexual flowers, thereby favoring xenogamy (Lloyd & Webb, 1986; Bertin & Newman, 1993). However, the overlap between the female and male phases in coenanthia (intra-coenanthium) of both species indicates that adichogamy may also be occurring on them (Lloyd & Webb, 1986). In that case, should there be self-compatibility, the absence of temporal and mechanical barriers between flowers also favors geitonogamy, as observed by Carauta (1978).

Despite the similarities in their floral biology, habit, and habitat, the two studied species exhibited different breeding systems. *Dorstenia arifolia* has mixed mating systems (xenogamy and geitonogamy), and is therefore self-compatible. The presence of pollinators increased fruit set in that species, as shown by the open pollination test results, of 13.6% in the greenhouse (pollinators absent) versus 39.4% in the natural population (pollinators present). Biotic pollination has also been suggested for *D. embergeri* Mangenot and *D. contrajerva*, by Granville (1971), but the author did not identify the pollinators. *Dorstenia bonijesu*, on the other hand, seems to be independent on pollinators. This species reproduces almost exclusively by apomixis, given the high fruit set obtained in all pollination tests and the absence of pollinators. The occurrence of apomixis differs from what has been observed in other *Dorstenia* species (Granville, 1971).

The restricted distribution of *Dorstenia bonijesu* and the absence of pollinators may be the selective factors leading to apomixis, which should act as a mechanism of reproductive assurance in this species. A similar fact has been reported to the apomictic species *Coccoloba cereifera* Schwacke (Polygonaceae), which is endemic to the rocky outcrops of Serra do Cipó, southeastern Brazil (Silva et al., 2008). Nevertheless, apomixis in *D. bonijesu* seems to be facultative, since pollen viability in that species is high, a similar result to that obtained with *D. arifolia*, which has sexual reproduction. Male sterility generally occurs in obligatory apomictic species, such as those in Melastomataceae (Goldenberg & Shepherd, 1998) and Asteraceae (e.g., Montgomery & Fairbrothers, 1970; Bertasso-Borges & Coleman, 1998, 2005). Facultative apomictic species with viable pollen are able to maintain efficient gene flow over long distances in the presence of pollinators (Asker & Jerling, 1992), which is not the case of *D. bonijesu* individuals from the studied population. Studies in other populations of this species may offer new perspectives to this subject.

The other reproductive mechanism found in *Dorstenia arifolia*, i.e. vegetative propagation, is absent in *D. bonijesu* (Araújo, 2008). This reproductive alternative is similar to the apomixis of *D. bonijesu*, since both mechanisms result in the formation of clones. However, clonal reproduction by seeds (apomixis) also combines all the advantages associated with the seed, such as facilitated dispersal and the dormancy (Richards, 1997). In *D. arifolia*, the loss of pollinators would result in loss or reduction of these advantages.

The site of occurrence of the study species (i.e., the understory), their herbaceous habit, monoecy, long-lived coenanthia with numerous small flowers, protogyny, and pollen as floral resource are all traits that indicate the occurrence of interactions between these plants and small pollinators from the inner forest. This, however, was true only for *Dorstenia arifolia*. This species interacted with small flies, and the myophily is reinforced by the following findings: 1) flies were predominant on all traps, representing 91% of all collected insects; 2) pollen was found not only adhered to the body of morpho-species 1 but also in its feces; 3) coenanthia were used as oviposition sites, probably by morpho-species 1; and 4) the results of open pollination tests indicated that the presence of pollinators in natural populations increased fruit set. The myophily syndrome has been suggested to occur in Neotropical *Dorstenia* species by Berg (2001), based on inflorescence features such as shape, color, and odor. Our study reports, for the first time, the occurrence of interactions between small flies and a *Dorstenia* species, confirming the supposition of Berg (2001).

Representatives of the Lauxaniidae family, which includes the pollinator of *Dorstenia arifolia*, are visitors to a number of flowers (Proctor et al., 1996). However, the role of these flies as well as of those from other acalyptrate families as pollinators is rarely investigated (Larson et al., 2001). Flies feed on pollen, which is an important protein source needed for their testicular maturation and normal ovarian development (Woodcock et al., 2014). Among the Moraceae tribes, pollen is an important resource for Castilleae pollinators (thrips) (Sakai, 2001; Zerega et al., 2004). The increased availability of pollen grains in *D. arifolia* due to the presence of unisexual male coenanthia, in addition to the presence of bisexual coenanthia in that species, might be related to a higher attractiveness to pollinators (Primack & Lloyd, 1980; O'Brien, 1994). In fact, we collected most insects on traps close to unisexual male coenanthia.

Another probable resource obtained by the pollinators of *Dorstenia arifolia* was the use of the species coenanthia as nesting site. The coenanthia were used as a source of food for larvae of morpho-species 1, and thus phytophagy by Lauxaniidae individuals is reported here for the first time. Offering of an oviposition site, a common trait among some Moraceae tribes such as Artocarpeae and Castilleae (Sakai et al., 2000; Berg, 2001; Zerega et al., 2004), has been suggested to occur among members of Dorstenieae that have unisexual male inflorescences (Berg, 2001). Our study seems to confirm this supposition of Berg (2001). However, nesting occurred on bisexual coenanthia, only. Further studies are needed on this probable interdependency between *D. arifolia* and its pollinator.

Fruit dispersal in both studied species is autochorous, as reported to other *Dorstenia* species (Granville, 1971; Carauta, 1978). According to Granville (1971), autochory allows for a maximum dispersal distance of five to seven meters and is related to variations in the osmotic pressure within mesocarp cells. In *D. bonijesu*, the coenanthia that retain an infundibuliform shape formed a "water reservoir" where fruits were released, thus preventing the usual dispersal process reported to *Dorstenia* species. In that case, the distance of fruit dispersal is lower than that achieved through ballistic autochory. Future studies should evaluate the contribution of this dispersal mode, especially in unfavorable weather conditions (rainy days) to autochory, and verify whether it makes hydrochory a secondary and complementary dispersal mechanism in *D. bonijesu*.

In this study, we found that the analyzed *Dorstenia* species not only have the same habitats and habits, but also exhibit similarities in their sexuality (monoecy), floral biology (protogyny, stigmatic receptivity and pollen release for long periods), and dispersal (autochory). Their breeding systems, however, are distinct, being directly associated with the presence (*D. arifolia*) or absence (*D. bonijesu*) of pollinators. *Dorstenia arifolia* has unisexual male coenanthia in addition to bisexual ones, a combination reported here for the first time to the genus, and is self-compatible. However, the presence of pollinators, small Lauxaniidae flies, increases its fruit set. The flies utilize pollen as food and the coenanthia as oviposition sites; this latter finding is another unpublished report to the studied plant group. *Dorstenia bonijesu* is apomictic, which is also unreported to the genus, apomixis being the reproductive mechanism responsible for maintaining the studied population. Furthermore, this species has a peculiar type of hydrochory in addition to autochory.

ACKNOWLEDGMENTS

We thank Pedro Jorge Carauta for plant identification. The third author thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support. The last author thanks CNPq for a research productivity grant. This study is part of the doctoral thesis of the first author, by the Graduate Program in Botany of Federal University of Viçosa.

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