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Floral biology, morphology and ecological niche modelling of *Caraipa grandifolia* (Calophyllaceae), an important Amazonian floodplain tree

Biologia floral, morfologia e modelagem ecológica de nicho de *Caraipa grandifolia* (Calophyllaceae), uma árvore importante das planícies inundáveis amazônicas

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Abstract: The present work aims to investigate the floral biology and morphology of one member of the Calophyllaceae (or Clusiaceae sensu lato), *Caraipa grandifolia* in the Caxiuanã National Forest, in the eastern Brazilian Amazon and aims to identify the bioclimatic affinities of this species, providing insights about its environmental requirements and mapping its potential distribution, given the paucity of data about flood-tolerant trees. Nine flowering individuals were located along a 2 km stretch of the Anapu river, and were visited by eleven different species (some of which are potential pollinators) including cockroaches, bees, ants, and wasps. Flower anthesis occurred between 05:00 and 07:30. The flowers are odourous, and osmophores were observed along the petals and also on top of each individual anther. The main reward collected by bees was pollen and an oily resin/scent produced by anther glands, while four different ant species collected nectar in inconspicuous extranuptial nectaries along the flower pedicel, a morphological feature previously unreported for the genus. *Caraipa grandifolia* is considered to be an important tree species in the flooded forest ecosystems given the number of interactions with which it is involved and its potentially wide distribution indicated by our modelling.

Keywords: Clusiaceae. Dictyoptera. Igapó.

Resumo: O presente trabalho tem como objetivo investigar a biologia floral e morfologia de uma espécie de Calophyllaceae (ou Clusiaceae *sensu lato*), *Caraipa grandifolia*, na Floresta Nacional de Caxiuanã, na Amazônia oriental brasileira, e identificar as afinidades bioclimáticas dessa espécie. Fornecemos dados sobre suas exigências ambientais e mapeamos a sua distribuição potencial, dada a escassez de informações sobre árvores tolerantes ao alagamento. Nove indivíduos floridos foram localizados ao longo de um trecho de 2 km do rio Anapu e foram visitados por onze espécies diferentes (algumas são potenciais polinizadores), incluindo baratas, abelhas, formigas e vespas. A antese floral ocorreu entre 05:00 e 07:30 h. As flores são odoríferas, e osmóforos foram observados ao longo das pétalas e também no topo de cada antera. As principais recompensas obtidas pelas abelhas são pólen e resina oleosa com perfume produzida por glândulas nas anteras, enquanto quatro espécies diferentes de formigas coletaram néctar em nectários extranupciais discretos, até então desconhecidos para o gênero, localizados ao longo do pedicelo da flor. *Caraipa grandifolia* é considerada uma importante espécie arbórea nos ecossistemas florestais inundados, dado o número de interações em que está envolvida e a sua distribuição em potencial, indicada na modelagem.

Palavras-chave: Clusiaceae. Dictyoptera. Igapó.

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INTRODUCTION

Despite the important role played by tropical forests in maintaining global climate and carbon cycles (*e.g.* Bonan, 2008), both the biodiversity and underlying ecological processes in the Brazilian Amazonian forest are still poorly understood (*e.g.* Feeley & Silman, 2009; Hopkins, 2007; Hubbell *et al*., 2008; Milliken *et al*., 2010). Knowledge of the biology of the flood-tolerant trees of the Amazonian floodplain is particularly poor (Parolin, 2012). The eastern Brazilian Amazon lies astride the Amazonian Arc of Deforestation (*e.g.* Vieira *et al*., 2008) a region characterised by intense forest degradation from logging and fires (Nepstad *et al*., 1999) and concomitant land-use change due to agriculture (see Adeney *et al*., 2009). Understanding biotic interactions in the remnants of this rapidly disappearing ecosystem is of utmost conservation importance (Gentry, 1988).

Species distribution modelling (SDM) has been used to investigate the environmental suitability for the distribution of discrete species (Hutchinson, 1957; Soberón, 2010; Soberón & Peterson, 2005). Such models may be useful for species which are distributed in remote regions of the globe and for which logistical and cost considerations prohibit fieldwork (Brito *et al*., 2009). The Amazon rainforest has several tree species for which basic information about life cycles, biology or taxonomy is still incompletely known (*e.g.* Kubitzki, 1978; Parolin, 2012). SDMs have been considered a successful tool to predict the distribution of species in cases like this (Brito *et al*., 2009). These techniques combine species occurrence data with environmental variables based on a correlative approach to build a representation of the ecological requirements of a species (Anderson *et al*., 2003). Several algorithms have been applied to create such models, and their computational results can be projected onto a map showing areas that are similar to those where the species is known to occur and that represents the potential areas of occurrence (Stockwell & Peters, 1999; Phillips *et al*., 2006).

The genus *Caraipa* includes 30 species in South America of which 22 occur in Brazil, varying from large trees to shrubs and occurring in many distinct vegetation types (Gustafsson & Nee, 2004; Stevens, 2007; Bittrich *et al*., s. d.). This genus is traditionally positioned within the Clusiaceae or, more recently, within the Calophyllaceae (Ruhfel *et al*., 2011; Souza & Lorenzi, 2012; Stevens, 2001 - onwards). Members of the Calophyllaceae are often considered important timber species (*e.g. Calophyllum brasiliense*, Guanandi) and are currently represented in Brazil by eight genera and nearly 80 species (Souza & Lorenzi, 2012). All species of *Caraipa* are Neotropical and are common forest trees on acidic soils; they have sparse latex, alternate leaves, stellate trichomes and white, contorted petals (Kubitzki, 1978; Gustafsson, 2009).

Caraipa grandifolia Mart. is a common tree species along the margins of Anapu river inside the Caxiuanã National Forest, Pará State, Brazil. No information was found in the literature regarding the floral biology and floral visitors of this species, which is popularly known in the Amazon region as tamaquaré and used as an important medicine 'óleo de tamaquaré' to treat different diseases and as a source of wood (Kubitzki, 1978). During our preliminary field observations we noticed that *Caraipa grandifolia* inflorescences were constantly visited by various species, particularly bees and ants, which motivated us to proceed on a detailed observation of the floral biology and morphology of this Amazonian tree as well as to identify environmental factors related to occurrence and other suitable areas for *C. grandifolia*.

MATERIAL AND METHODS

This work was carried out in the area surrounding the Ferreira Penna Scientific Station, in the Caxiuanã National Forest (hereafter CNF) in Melgaço, Pará State, Brazil (1° 42' 30" S, 51° 31' 45" W; Figure 1). The selection of flowering adult individuals and observations were carried out using boats. Nine flowering individuals were marked in the 'igapó' vegetation – flooded portions of the forest

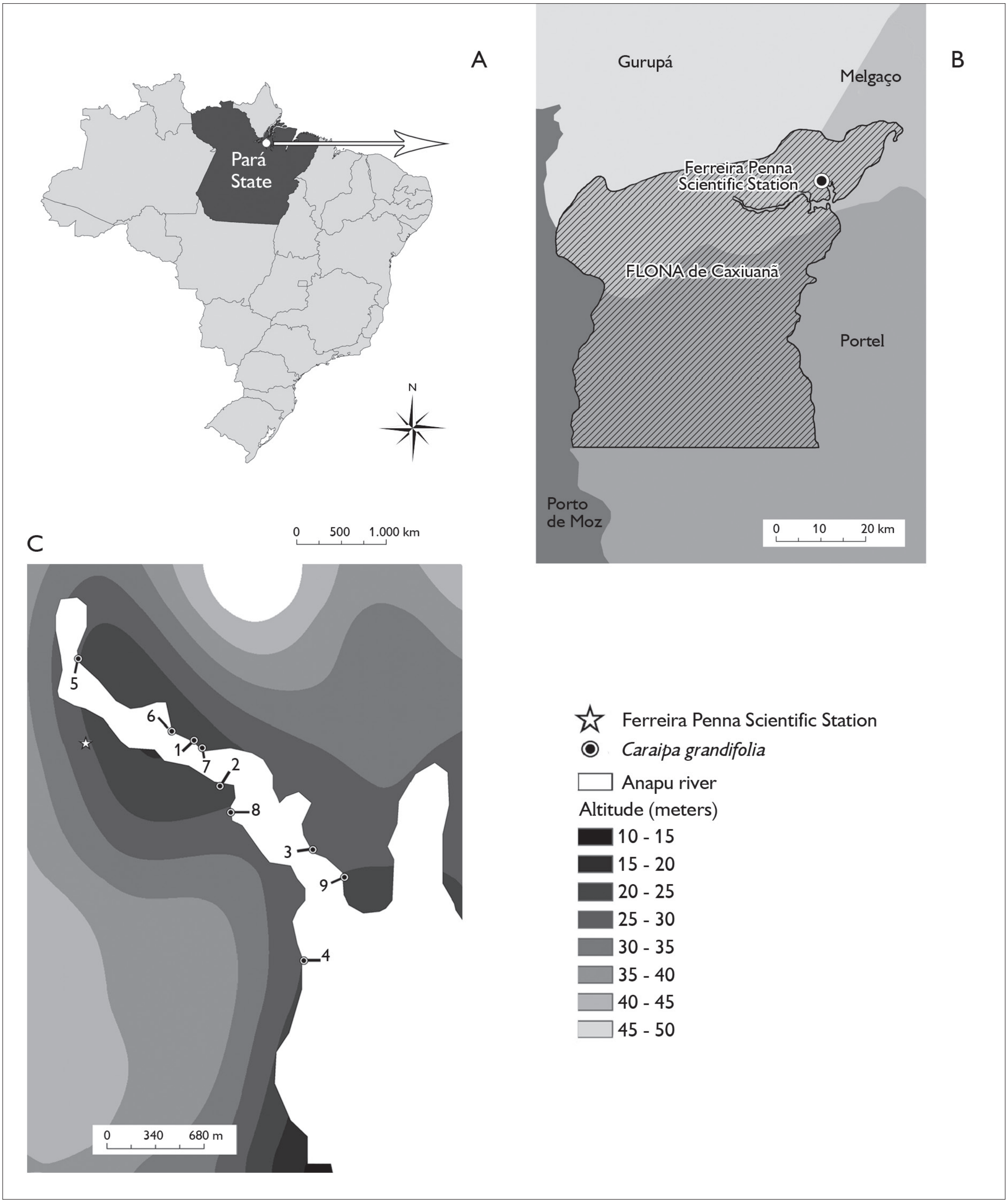

Figure 1. Map of Brazil showing Pará State (A) and the Caxiuanã National Forest (B). Nine blooming individuals of *Caraipa grandifolia* were mapped along the North and South sides of the Anapu river (C).

along the Anapu river (Figure 1C; points 1 to 9). Individuals were monitored in the field from September 17-22 2011. Temperature and humidity were recorded at the time of the observations. Vouchers were deposited in the Herbarium of the Museu Paraense Emílio Goeldi (MPEG) and the Universidade Federal do Rio Grande do Norte (voucher: *L. M. Versieux 514*, UFRN). Ten mature flowers were collected and observed and additional material was preserved in 70% ethanol. These flowers were analysed in the laboratory, using a stereomicroscope and measured using digital callipers. The descriptive terminology follows Stearn (2004).

Focal observations were conducted on four different individuals of *Caraipa grandifolia* (Figure 1C; points 1 to 4). During a 24-hour period, observations were conducted for 45 minutes in each hour, for one tree. After this initial observation we noticed that the number of visitors was severely reduced after dusk. After that, focal observations were conducted on three additional trees separated 360-1,400 m from each other along distinct margins of the Anapu river. The number of observers varied from two to four, and each of them continuously and simultaneously monitored one part of the tree crown for 45 minutes per hour, from 05:00 up to 10:00. Floral visitors were collected, identified by specialists (listed in the acknowledgments) and deposited in the entomological collections of the Universidade Federal da Bahia and Museu Paraense Emílio Goeldi. Floral events (time of anthesis, flower life span and senescence, changes in the floral/inflorescence morphology) were also observed and monitored in the field after marking buds and inflorescence and following the development of at least five flowers in each tree.

In order to search for nectar in fresh flowers, microcapillary tubes were placed along the base of the pedicel, sepals, petals, stamens and carpels, and nectar was simply counted as present or absent. Fresh flowers were stained with neutral red for detection of osmophores (Dafni *et al*., 2005). As the neutral red technique has limitations (Dafni *et al*., 2005) we also performed an anatomical study. Petals were conserved in 70% ethanol and were later used to obtain transversal sections using a razor blade. This tissue was bleached by sodium hypochlorite, stained for two minutes in Safrablau or Sudan Black 0.02%, mounted as temporary slides in glycerine and observed/photographed under light microscopy to search for osmophores. One flower was separated into sepals, petals, stamens and pistil. Each part was stained separately following the same procedures as Dafni *et al*. (2005). Petals were also dried at room temperature and directly observed under scanning electron microscopy using a Hitachi Tabletop Microscope TM-3000 in the Laboratório de Engenharia de Materiais, Universidade Federal do Rio Grande do Norte (UFRN).

To qualitatively investigate the floral odour we conducted a 'nose bioassay' (Kearns & Inouye, 1993). One flower was dissected into sepals, petals, stamens and pistil and each part was enclosed in a clean vial for five hours. After that, eight volunteers were questioned if the distinct parts of the flowers had odour, and if so, how they would describe it. Intact stigmas from five flowers of each individual tree (N = 15) were used to assess receptivity using hydrogen peroxide (H_2O_2) (Dafni *et al*., 2005). Unvisited flowers were marked and after the first visit of one potential pollinator their stigmas were removed and brought to the laboratory. The fresh stigmas were stained with Alcian Blue and examined under a light microscope for the presence of pollen grains.

To characterise the species in terms of climate requirements, 77 occurrences of *C. grandifolia* were used, of which 12 were unpublished observations by the authors, seven obtained from literature (Kubitzki, 1978) and 58 were collected from biodiversity databases in the web (14 from the Global Biodiversity Information Facility (GBIF) and 44 from SpeciesLink). The unpublished observations were recorded and georeferenced *in locus* using a Global Positioning System (Garmin GPSmap 60, WGS84). The specimens cited in the databases were filtered to include only those that were revised or identified by specialists or plant collectors with broad experience of the Amazonian flora. The final list of specimens is available from the corresponding author upon request.

To develop the species distribution model (SDM; also referred to as a habitat suitability model), we first obtained a set of 19 topoclimatic variables representing combinations of temperature and precipitation for the past 50 years (Hijmans *et al*., 2005), and one topographical grid (Altitude; USGS, undated), all with a resolution of 2.5 arc-min (approximate cell size: 5 x 5 km). Subsequently, all of the variables were clipped using a polygon according to the following extent: eastern longitudes ranging from -81.848104 (dd) to -40.764196 (dd), about 4,600 km, and latitude ranging from North (12.703218 dd) to South (-12.560172 dd), about 2,800 km.

To reduce co-linearity among predictors, we performed a Pearson's pair-wise correlation in R v.2.15.2 (The R Foundation for Statistical Computing) following the procedures in Aguirre-Gutiérrez *et al*. (2013); only variables with correlation coefficients smaller than 0.75 were selected, totaling eight variables: Mean Temperature Diurnal Range; Maximum Temperature of the Warmest Month; Temperature Annual Range; Precipitation of the Wettest Month; Precipitation of the Driest Month; Precipitation Seasonality; Precipitation of the Warmest Quarter; Precipitation of the Coldest Quarter.

Species distribution modelling provides insights into species-environment relationships (Franklin, 2009). We used the remaining eight climatic variables (with minor colinearity) and the *Caraipa grandifolia* occurrences with the Maxent SDM algorithm, which estimates a target probability distribution by finding the probability distribution with maximum entropy or closest to a uniform distribution (Phillips *et al*., 2006; Phillips & Dudík, 2008). We used the software package version Maxent v.3.3.3k (Princeton University, undated) and its default settings, with 500 iterations. The original database of occurrences was randomly split in calibration (80%) and evaluation subsets (20%; Fielding & Bell, 1997). This modelling technique is especially useful because it can be applied to analyze small datasets and presence-only data sets (Hernandez *et al*., 2006; Wisz *et al*., 2008), a situation that is common in tropical forests (Feeley & Silman, 2011), and consistently performed well in comparison to other modelling methods (Elith *et al*., 2006).

The map output of Maxent represents the occurrence probability for each grid cell used in the model. We classified these probabilities into three categories: maximum probability, corresponding to the cells that show a predicted probability of the presence of more than 75%; high probability, corresponding to a range of probabilities of 50-75%; and low probability, representing probabilities less than 50%. These procedures were performed with ArcGIS 10 (ESRI, 2011).

Based on the contribution of each variable to build the Maxent model (Maxent output), we selected the variables whose combined contributions values (summed) reached at least 75% of the total. These highest contribution variables were intersected with the occurrences of *C. grandifolia* using the function *extract* of the *raster* package in R (Hijmans & Von Etten, 2012). This method consists of capturing the information provided by each selected variable for each individual location of the species in the occurrence data set. Thus, we obtained a set of climatic data able to qualify the requirements of this species in its known distributional range. The range (minimum, quartiles and maximum) of each variable was exhibited in boxplots generated in R.

RESULTS AND DISCUSSION

MORPHOLOGICAL CHARACTERISATION

All individuals of *Caraipa grandifolia* that were observed in CNF were trees growing in the 'igapó' (swamp forest flooded by nutrient poor 'black' waters, usually on acidic soils; Rizzini, 1997) of the Anapu river (Figures 1-2). However, the specimens and the literature consulted also indicated its occurrence in 'várzea' (forest periodically flooded by nutrient rich 'white' waters; Rizzini, 1997) and along 'igarapés' (small rivers that penetrate the várzea forest). These trees tend to have pendulous branches that reach almost to the water surface (Figure 2A). The flowers are shortly pedicellate, and arranged in terminal or axillary cimose inflorescences. The space between flowers is small, so the inflorescences may have a dense and aggregate appearance (Figures 2B, 2R). Flowers are actinomorphic, the perianth is dichlamydeous, presenting a distinct calyx and corolla (Figure 2B) and polystemonous. The calyx is pentamerous (five sepals), green sometimes with ferruginous trichomes (Figure 2M), and has a coriaceous texture. The corolla is rotaceous (dish-shaped) rarely reflexed, with five free petals, pale green to cream colored abaxially, white adaxially, and showed a fleshy texture. The aestivation was slightly contorted, and the flower bud became nearly globose, 1.5-2.0 x 1.5-2.0 cm, and the tips of the petals maintained the shape of the bud by overlapping and attaching inconspicuous hooks on the top (Figures 2B-2C, 2I). The androecium is cyclic, with approximately 200 stamens (N = 5), the stamens are polyadelphous, shortly fused only along the base for approximately 1-3 mm, completely circling the corolla. The outer stamens are shorter (Figures 2D, 2K-2L). All stamens are exserted, and the anthers release pollen in distinct directions. Staminodes are absent. Anthers have a gland in the upper portion where an oily exudate is produced (Figure 2J). The ovary is syncarpous, 3-carpellar, the stigma is 3-lobate, erect, inconspicuously producing an exudate (Figure 2F). The style is terminal and not straight at anthesis (Figure 2E), the ovary is superior and trilocular (Figure 2D). Extranuptial nectaries are present along the base of the flowers pedicels and close to the two prophylls (Figure 2G).

FLOWER STAINING WITH NEUTRAL RED AND ODOUR TEST

Osmophores were detected along the petals (Figures 2H-2J), with the majority of the staining occurring on their adaxial face, and also on the gland on the top of each individual anther (strongly coloured with the neutral red). Odour emission was clearly noticeable particularly at the time of the anthesis. Volatiles may also be produced on edges of the abaxial face of the sepals which also had staining (Figure 2H). The odour of the flowers was referred as 'citric', 'pleasant', 'fruity', 'sweet', or 'somehow resembling fermented fruit' according to interviews from the 'nose bioassay'.

The location of the osmophores suggest that the sweet scent is produced mainly on the adaxial surface of the petals and also on the top of the anthers, which are both the predominant places where insects land, particularly *Trigona* species (Figure 2R). Bittrich & Amaral (1996) observed the production of antheroil in *Symphonia globulifera* L. f., another Clusiaceae s.l. tree. According to these authors, *S. globulifera* pollen grains are intermixed in an oily fluid secreted by the anthers. A pore-like opening structure is observed here in *C. grandifolia* anthers and is described by Kubitzki (1978) as an urceolate gland, which is present in all species of the genus. As the oils produced in the anthers are often strong smelling, they might serve to attract pollinators over longer distances or contribute to aggregate the powdery pollen of other Clusiaceae species into pollenkitt (Bittrich & Amaral, 1996).

The preliminary anatomical analysis conducted on the petals (Figure 3) indicated the presence of stellate hairs, similar to those observed on the leaves of several species of the genus (Kubitzki, 1978). These hairs were strongly stained by Sudan Black dye what may indicate the presence of lipids that may be components of volatile compounds (essential oils) responsible for floral odour (Kearns & Inouye, 1993). The production of different fragrant oils consisting mainly of terpene alcohols along the distinct floral parts of Clusiaceae species has been observed by Nogueira *et al*. (1998). The presence of such indumentum is in accordance with the tomentellous description cited for the petals of *C. grandifolia* by Kubitzki (1978). Additionally, we frequently observed druses of calcium oxalate crystals within the parenchyma (Figure 3E).

Figure 2. *Caraipa grandifolia* floral morphology and floral visitors. A) Habit of an adult tree growing in igapó vegetation; B) close up of an inflorescence; C) flower at anthesis; D) longitudinal section of one flower; E) pistil; F) detail of the stigma; G) region that concentrates the extra-nuptial nectaries; H-J) floral parts stained by neutral red indicating osmophores; K) *Trigona pallens*; L) *Trigona dobzhanskyi*; M) *Cephalotes atratus*; N-P) larvae found within flower buds or post-anthesis flowers. Note the camouflage created using parts of old stamens; Q) a nocturnal Blattellidae visitor; R) several *Trigona pallens* individuals foraging; S) Carpenter bee (*Xylocopa* sp.) approaching a flower. All the photos by the authors and taken at Caxiuanã, except S, courtesy of Leonardo Galetto.

FLOWER LONGEVITY AND PHENOLOGY

Our observations were carried out during only one week in September. The trees produced a large number of flowers daily, in different parts of the tree crown. Anthesis begins at 05:00 and flowers remain opening until 07:30, with a peak close to sunrise (approximately 06:20). The flower buds assume a nearly globose aspect prior to anthesis, with circa 15 x 15 mm, and the tips of the petals are overlapped and hooked one over the other by an inconspicuous asymmetrically hook-shaped mucro on the apex. After opening, the petal

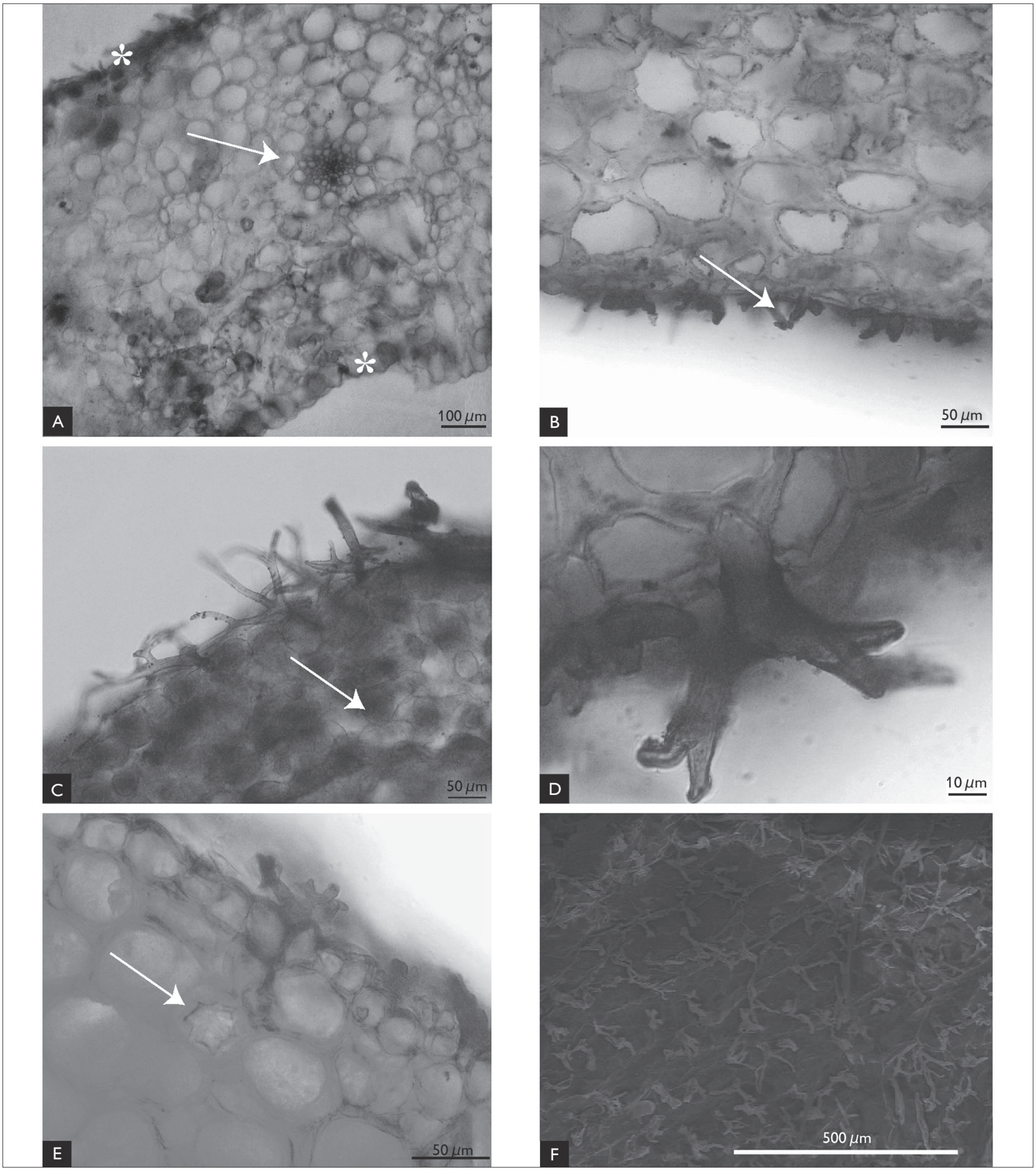

Figure 3. Photomicrographs of transversal sections of the petals of *Caraipa grandifolia*, stained in Sudan Black (A-D), Safrablau (E) or scanning electron microscopy of the petal's epidermis. A) Transversal section of the mid region of the petal, showing both epidermis (asterisks) and vascular bundle (arrow), staining in brownish within the petal's parenchyma suggests essential oil accumulation; B) darker staining of the epidermal stellate hairs (arrow); C) accumulation of oily substance in the parenchymatic cell layers beneath the epidermis; D) detail of one stellate hair; E) calcium oxalate crystals (arrow); F) detail of the epidermis with stellate hairs.

position kept changing until becoming completely reflexed, which occurs after 1-2 hours. This change in petal position may not be seen in flowers that are next to many flower buds, being affected by congestion. Twenty-four hours after anthesis began, the last petal dropped from the flower and the stamens and pistils wither. This life-span observed for each flower here is similar to other studies conducted with the Clusiaceae (*e.g.* Hochwallner *et al*., 2012).

STIGMA RECEPTIVITY

Stigma receptivity was verified immediately after the flowers opened and positive results were seen for all flowers that were tested (n = 15).

NECTAR TEST

No nectar could be detected within the flowers using the microcapillaries, even in recently open flowers. This probably explains the absence of visits of hummingbirds Trochilidae, as observed for other Amazonian Clusiaceae such as *Symphonia globulifera* (Bittrich & Amaral, 1996). However, the flowers still reward visitors with pollen and sweetish fragrance. Additional studies focused on the extranuptial nectaries are necessary to explain if they are responsible for the attraction of ants and the sugar concentration, since such kind of nectaries have not been reported for the genus (Kubitzki, 1978), or for the Calophyllaceae (Stevens, 2001-onwards).

FLORAL VISITORS

Eleven species were recorded visiting the flowers of *Caraipa grandifolia* (Table 1, Figure 2). The most frequently-visiting species was *Trigona pallens* (Hymenoptera: Apidae) (Figures 2K, 2R), which visited the flower for a short period of time foraging for pollen grains and resin deposited along the top of the anthers. Ants (Hymenoptera: Formicidae) were also common visiting the inflorescences and flower buds (Figure 2M) and nested within old branches of the trees. Most ants observed were collecting nectar exudates from extrafloral nectaries located along the base of the pair of bracts of the pedicel of each flower. Other visitors observed included members of the Blattellidae (Figure 2Q), and Vespidae.

A wide taxonomic range of pollinators may be associated with the great floral diversity observed in Clusiaceae s.l., including bees, beetles, flies, hummingbirds, parakeets, and cockroaches (*e.g.* Maués & Venturieri, 1996; Bittrich & Amaral, 1997; Gill Jr. *et al*., 1998; Lopes & Machado, 1998; Ribeiro & Bittrich, 1999; Vicentini & Fischer, 1999;

Table 1. List of floral visitors collected in flowers of *Caraipa grandifolia*, showing records of visiting time, duration of visits and reward collected in Caxiuanã National Forest, Melgaço, Pará, Brazil.

Visitor	Time	Duration of visit	Reward
Dictyoptera: Blattellidae	22:00 – 5:00	3 – 10”	Not observed
Formicidae			
Cephalotes atratus	5:00 – 22:00	-	Extranuptial nectar
Camponotus sp. 2	5:00 – 22:00	-	Extranuptial nectar
Camponotus sp. 3	5:00 – 22:00	-	Extranuptial nectar
Dolichoderus quadridenticulatus	5:00 – 22:00	-	Extranuptial nectar
Formicidae 5	5:00 – 22:00	-	Extranuptial nectar
Centris sp.	8:00 – 9:00	4”	Pollen/oily scent (?)
Trigona pallens	6:20 – 12:20	1’	Pollen/oily scent
Trigona dobzhanskyi	8:00 – 12:00	10’	Pollen/oily scent
Xylocopa sp.	7:30 – 9:30	3”	Pollen/oily scent (?)
Vespidae	8:00 – 12:00	12’	Not observed

Kaminski & Absy, 2006; Vlasáková *et al*., 2008; Hochwallner *et al*., 2012). Within the Clusiaceae, *Platonia insignis* have been identified as important source of pollen and nectar used by birds (*e.g.* Psittacidae) and bees (Maués & Venturieri, 1996).

Other reports of visitors and pollinators for other species of Clusiaceae s.l., emphasized the importance of small bees (*e.g.* Bittrich & Amaral, 1997; Lopes & Machado, 1998; Carmo & Franceschinelli, 2002; Kaminski & Absy, 2006; Hochwallner *et al*., 2012). Small bees are possibly important pollinators of *Caraipa grandifolia* due to the frequency of their visits, but this could not be tested in the present work through seed/fruit-set. Additionally, small bees such as *Trigona pallens* and *T. dobzhanskyi* have a limited flight capacity, foraging preferably near their nests (Araújo *et al*., 2004). Nevertheless, the efficiency of large bees such as *Centris* sp. and *Xylocopa* sp. at depositing a large amount of pollen grains on the stigmas of virgin flowers is stronger, although measured here only by staining intensity. A single Vespidae was observed for a long time over one flower; however it behaved more like a passing visitor. During their visits, both *Centris* sp. and *Xylocopa* sp. could have indirectly collected the oily scent produced by the anther gland, despite only visiting the flowers for 3-4 seconds (Table 1). On the other hand, *Trigona* species were observed actively collecting oily scent over the anther gland.

The number of floral visitors observed here for *Caraipa grandifolia* is also comparable to other studies conducted with the Clusiaceae in Amazonian forests as well as other tree species from other plant families (*e.g.* Oliveira *et al*., 2003; Kaminski & Absy, 2006; Jardim & Mota, 2007). However, considering the broad potential distribution of *C. grandifolia* across Amazonian flooded forests, we may consider it important for a wide variety of insects.

A novel finding of the present study is the presence of one species of cockroach in the family Blattellidae that continuously visited the flowers during the night. According to Vlasáková *et al*. (2008), cockroach pollination has been reported in the Neotropics only for *Clusia blattophila*, from French Guiana. More data should be collected in order to confirm pollination of *Caraipa grandifolia* by cockroaches (Dictyoptera), which would be one of few cases anywhere in the world of this form of pollination (Nagamitsu & Inoue, 1997; Corlett, 2004; Vlasáková & Gustafsson, 2011).

INTERACTIONS WITH ANTS

During our field observations we noticed that *Caraipa grandifolia* trees inflorescences were frequently visited by several species of ants. The purpose of ant visits to the flowers was apparently to collect extrafloral nectar, close to the base of the pedicels.

INTRAFLORAL INVERTEBRATE DIVERSITY

Three species of Lepidopteran larvae were observed within the flowers. During the development of the flower bud one species was observed feeding on pollen grains. The second species appeared to have a life cycle associated with the floral senescence. Its larval stages could be related to the sizes of the flower buds. When the flowers are senescent the larvae create a pupa completely covered by anthers and parts of stamens. At this stage the larvae are camouflaged among the older inflorescence parts (Figures 2O-2P). Thysanoptera (thrips) were also frequent in most of the examined flowers but apparently were only feeding on pollen. Similar observations were made for a Curculionidae (Tribe Anthonomini, *Anthonomus* sp.) found frequently inside buds or flowers, feeding on pollen. The role of Thrips and *Anthonomus* sp. as potential pollinators should be addressed in future studies with this genus.

ECOLOGICAL NICHE MODELLING ANALYSES

The floodplain forests of the Brazilian Amazon basin were detected as the centre of the distribution of *C. grandifolia* in our SDM analysis (Figure 4). However, suitable habitats also included areas in Ecuador, French Guiana, Guyana, Colombia, Peru, Suriname and Venezuela. In Brazil, the model predicts high occupancy probability mainly along the Amazon river, notably the horizontal belt covering the three zones along this basin: the high, the middle and the low Amazon (Figure 4). This model is similar to the distributional

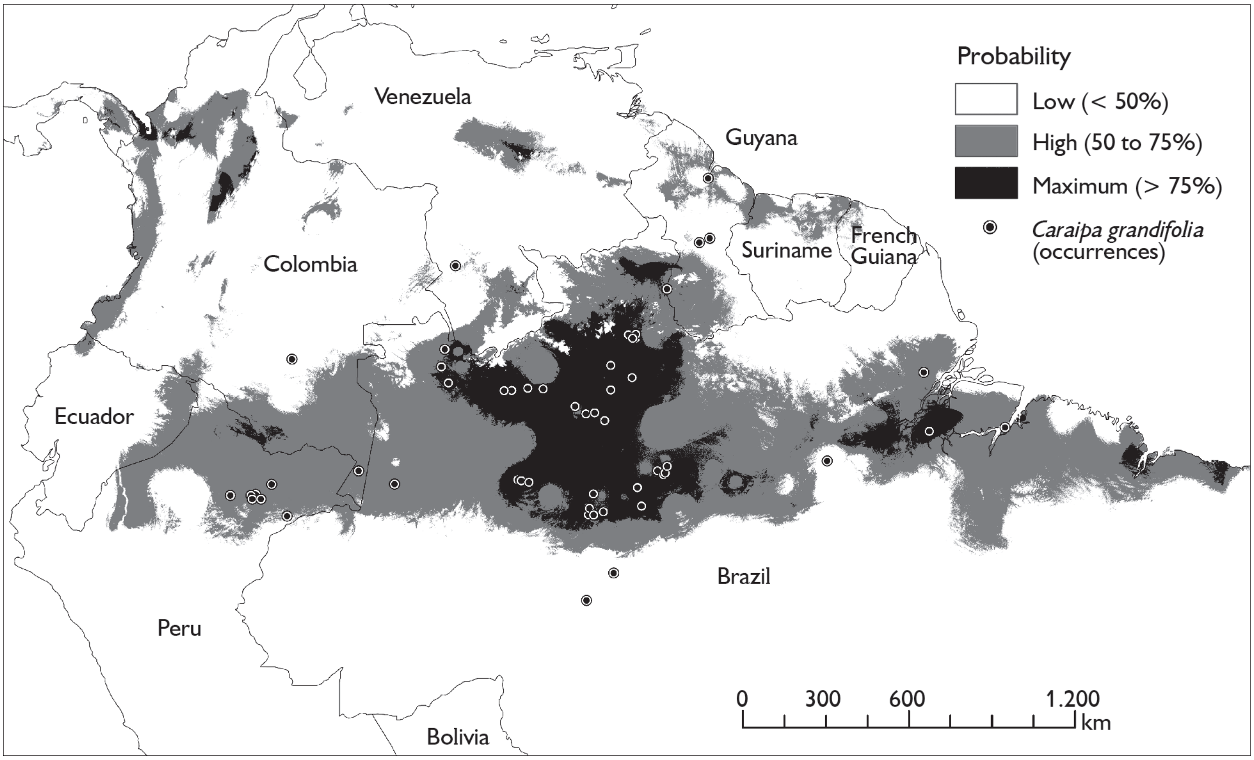

Figure 4. Potential areas of occurrence of *Caraipa grandifolia* in South America.

pattern found for other species of the Amazonian flooded forest such as *Swartzia polyphylla* (Kubitzki, 1989).

The Maxent model achieved an area under curve of receiver operating characteristic (AUC-ROC) value of 0.92, indicating the model to be robust. The climatic variables with highest contribution in explaining the distribution of *C. grandifolia* detected by the Maxent model were: Isothermality (38.2%); Precipitation of Coldest Quarter (33.4%); and Minimum Temperature of Coldest Month (17,7%) (Figure 5). The contribution of these variables together (values summed) reach 89.3%, meaning that these variables are the main determinant of the suitable habitat shape to *C. grandifolia* in terms of the climatic factors considered.

Caraipa grandifolia habitat isothermality extremes range from 73 to 90; the importance of this variable to build the Maxent model denotes that this species is a typical tropical evergreen rainforest species. The range of this variable in worldwide extent (from 8 to 96) is closely related with the global latitudinal gradient, reaching highest values around the equator line and largely covering the global evergreen rainforest belt. Thus, *C. grandifolia* climatic affinities coincide with areas where there are more constant and high temperatures throughout the year, and with minor diurnal temperature fluctuations.

Both the variables precipitation of the coldest quarter and minimum temperature of the coldest month are primarily related to rainy periods, when the temperature are cooler because of reduced insolation. The extremes of precipitation in the coldest quarter for *C. grandifolia* range from 296 to 1,279 mm (Figure 5), suggesting that this species is not strongly limited by rainfall, but even the lowest value is relatively high in global terms.

The values for the minimum temperature of coldest month range from 19.5 to 22.9 °C, a narrow variation of 3.4 °C implying that the species has low tolerance to temperature variation.

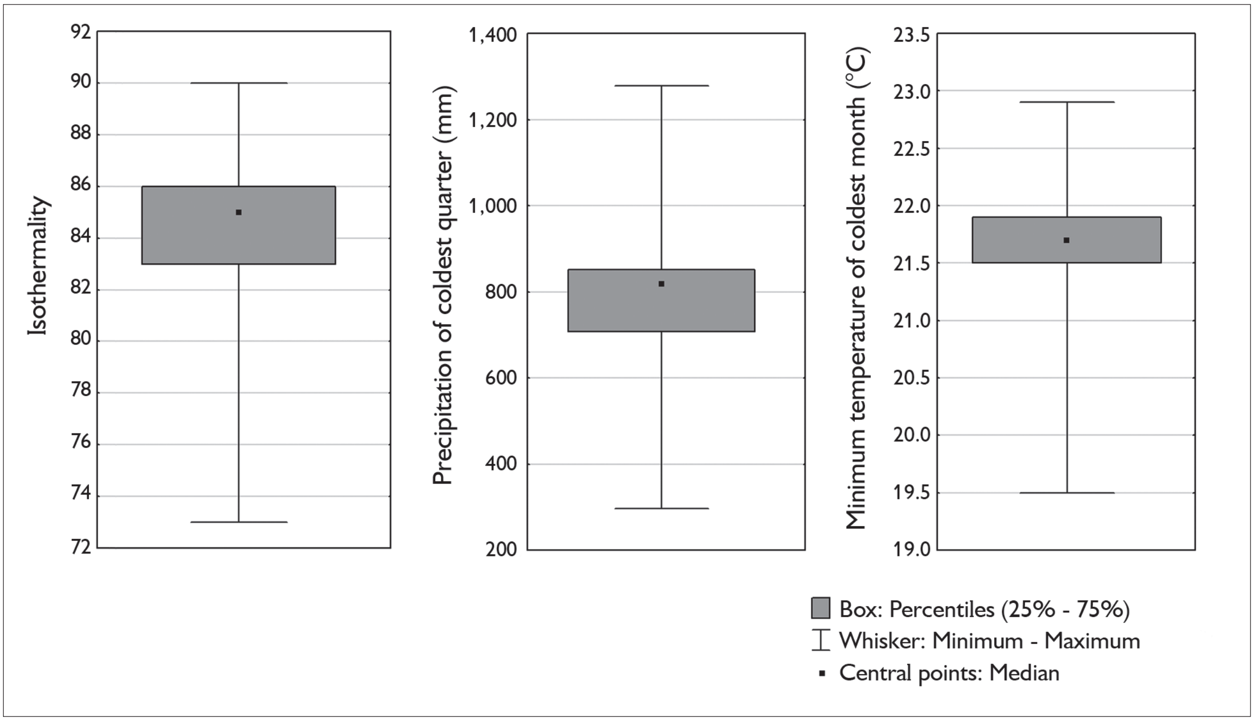

Figure 5. Bioclimatic variables with the highest contribution in the model to explain the distribution of *Caraipa grandifolia*.

We adopted the conservative range between the first (25%) and third quartile (75%) of the total range of each variable (Figure 5) to detect the climatic highest preferences in the environmental space covered by the species habitat (hereinafter referred as *affinity range*). The isothermality affinity range of *C. grandifolia* (from 83 to 86) are also high in relative global terms, supporting the first inference described on the extremes values of this variable. Besides the wide tolerance of species related to the precipitation of coldest quarter variable (from 707 to 852 mm) the affinity range is narrower, with only 145 mm between the first and third quartile. The species affinity range for the minimum temperature of coldest month show the narrowest variation among all variables, only 0.4 °C between the first (21.5 °C) and third (21.9 °C) quartiles. The narrow ranges of this variable (extremes and quartiles) when evaluated together with the isothermality suggest a strong limitation of species to zones with elevated temperatures throughout the year, including in the coldest period of the year, when the minimum value is 19.5 °C, which is high in global relative terms. We conclude that the species' predicted distribution (Figure 4) is driven mainly by variation in temperature.

These models do not qualify the role of each variable in shaping the distribution range of the species; we need to interpret them together and, when possible, combined with prior knowledge about the physiology/ecology of the species. The model results also reflect some possible adaptations or physiological requirements for the species: high humidity and permanent water availability. Another species of this genus (*C. laurifolia* Spruce ex Choisy, synonym of *C. densifolia* Mart.) has floating fruits that are dispersed by fishes (Spruce, 1908 *apud* Gottsberger, 1978), while others are confined to flooded forests (Ferreira & Stohlgren, 1999) or may exhibit clear preference for distinct soil types (Vásquez-Martínez, 1991). All these are potential explanations to be further investigated and that may help to explain our model. Kubitzki (1989) also indicates that the Amazonian flooded forests have developed an intricate biotic relationship with fishes, which are important dispersal agents for several species and must be considered in the interpretation of plant biogeography.

CONCLUSIONS

Caraipa grandifolia is a significant tree species of the 'igapó' flooded ecosystem of CNF, given the abundance of flowers and the number of floral visitors that visit it both diurnally and nocturnally. Further research should focus on ascertaining the ecological role of visiting cockroaches, since this may be a rare pollinator, and on other invertebrates that were seen in the flowers (Curculionidae and thrips), as well as on the extranuptial nectaries, an unreported characteristic for the genus.

Our model predicts a distribution that mostly follows the mainstem Amazon river in ephemerally-flooded ecosystems, and suggests that the suitable climatic envelope is more closely related to temperature than precipitation. However, the suitable area is also restricted to zones with high rainfall throughout the coldest period of the year. Different hypotheses may be created to explain this distribution (edaphic specialisation, dependence on the current or fish dispersal) and ought to be subject to future studies.

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