



Distribution, habitat disturbance and pollination of the endangered orchid *Broughtonia cubensis* (Epidendrae: Laeliinae)

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The geographical distribution, population structure and pollination ecology are key aspects in the conservation and management of rare orchids. Here, we address these aspects and the main threats affecting the endangered Cuban orchid *Broughtonia cubensis*. This rewardless orchid is self-compatible, but pollinator dependent. However, seed production can be negatively affected by insect-mediated selfing. Three species of small bee (genera *Ceratina* and *Lasioglossum*) act as pollinators. As in the case of other nectarless orchids, we detected two species of plant producing large amounts of nectar in the area, the floral morphology of which closely resembles that of *B. cubensis*. The simultaneous flowering of these species could positively affect the reproductive success of *B. cubensis*. Nonetheless, the fitness of this orchid in natural conditions is low, possibly related to strong pollen limitation. To the problems arising from reduced fitness is added the fact that its historical distribution range has been greatly reduced in recent years. Throughout this study, we have detected dramatic reductions in the population sizes, in some cases as a result of human plundering, but also as a consequence of hurricanes. Based on the results of this study, we propose some guidelines to manage and conserve this orchid. © 2013 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2013, **172**, 345–357.

ADDITIONAL KEYWORDS: Caribbean – *Ceratina* – conservation – *Cordia gerascanthus* – Cuba – hurricane – *Lasioglossum* – *Turbina corymbosa* – wild population over-collection.

INTRODUCTION

The Caribbean hotspot has nearly 12 000 spp. of flowering plants, *c.* 60% of them endemic (Myers *et al.*, 2000; Santiago-Valentin & Olmstead, 2004), but, in the Tropics, the Antilles also stand out by having one of the most alarming rates of biological extinction based on habitat destruction rates (Brooks *et al.*, 2002). Particularly critical is the case of the Greater Antilles, with some of the lowest primary forest cover indices in the tropical regions [Food and Agriculture Organization of the United Nations (FAO), 2010; but see Aide *et al.*, 2013]. Orchids are a major component of Caribbean epiphytic communities, both in terms of species diversity and biomass, with Hispaniola and Cuba being the islands with

the highest species richness and endemism (Acevedo-Rodríguez & Strong, 2008). However, about 10% of Cuban orchids are presently endangered (Berazaín *et al.*, 2005). For this reason, it is important that as much information as possible is available about endangered species in order to establish conservation priorities and management plans (Swarts & Dixon, 2009).

One of the main obstacles to the assessment of changes in the distributional range of a species is the lack of historical information available to scientists (Duffy *et al.*, 2009). In the case of orchids, the over-exploitation of some populations and species for commercial purposes makes monitoring more difficult and represents a cause of decline for many species (Sosa & Platas, 1998). In the Caribbean basin, frequent and severe meteorological events are an additional challenge to the conservation of orchids and their habitat

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(Rodríguez-Robles, Ackerman & Meléndez, 1990; Brokaw & Walker, 1991). Hurricanes can be especially harmful for epiphytes, as they are much more exposed to the force of wind and rain than are terrestrial plants (e.g. Rodríguez-Robles *et al.*, 1990; Tremblay, 2008). However, Caribbean orchids have evolved under these circumstances and, in some instances, may have developed adaptations to deal with the effects of hurricanes. In this sense, the resilience of orchids depends on the specific adaptations to survive hurricanes, mainly on the capacity for reproduction and for the recolonization of the new microhabitats available after a hurricane (Tremblay, 2008). Nevertheless, most orchids are not efficient competitors for habitat. They usually require long periods of time after disturbance episodes to occupy appropriate microsites. This could be linked with a frequent dependence on late successional states of vegetation for establishment (Tremblay, 2008), which is mainly caused by the requirements for and abundance of mycorrhizae (Otero, Ackerman & Bayman, 2004; Otero, Bayman & Ackerman, 2005; McCormick *et al.*, 2012). In addition, orchids are usually not able to maintain the attention of their pollinators, as these plants occur in small populations and/or provide scarce (or even no) floral rewards to their pollinators (Tremblay & Ackerman, 2001; Tremblay *et al.*, 2005). Consequently, most orchids are unsuccessful competitors for pollinators (Internicola *et al.*, 2007).

The pollination ecology and breeding system of threatened plants can strongly influence their reproductive success (Lennartsson, 2002; Navarro & Guitián, 2002; Castro, Silveira & Navarro, 2008). These aspects are particularly important in the case of orchids (Tremblay *et al.*, 2005; Phillips *et al.*, 2011), and can affect orchid distribution as a result of the availability of pollinators and co-blooming flowers in new habitats (Micheneau *et al.*, 2008; Pemberton, 2010). Unfortunately, this information is still rare for most tropical orchids (Tremblay *et al.*, 2005).

In Cuba, *Broughtonia cubensis* (Lindl.) Cogn. is one of the most endangered native orchids, as a result of its fragmented distribution, habitat destruction and over-collection of wild individuals. However, despite the degree of threat, there is a lack of information about the status of populations (but see Berazaín *et al.*, 2005; Urquiola *et al.*, 2010), and no information exists regarding the establishment of conservation protocols for action in case of need.

This article reviews the status of the populations and some components of the reproductive cycle of *B. cubensis* that could be of great importance for its management. Specifically, the aims of this survey are as follows: (1) to understand its breeding system; (2) to determine its dependence and interaction with pollinators; (3) to compare some reproductive param-

eters before and after severe environmental disturbance episodes; (4) to compare the factors that could be limiting seed production at different sites; and (5) to update the geographical distribution and population structure of different populations of *B. cubensis*.

MATERIAL AND METHODS

STUDY SPECIES

The epiphyte *B. cubensis* blooms from November to March, with a peak in February (Díaz, Ferro & Peña, 1998). Each year, the new pseudobulbs bear a single new inflorescence. The species has the smallest flowers of the genus (i.e. flower diameter of 4–5 cm from the dorsal sepal tip to the labellum tip), arranged in the distal part of inflorescences (Fig. 1A). Flowers are white, sometimes pink-spotted towards the central line or the rims of the petals (Fig. 1A–E). The labellum is also white and has a purple base with yellow–orange longitudinally oriented lamellae that do not reach the margin (Fig. 1B, C). The basal portion of the lip forms a tube that hides and surrounds a white to purple column. There are eight yellow pollinia arranged in a sessile pollinarium with two pollinium sizes. Here, as demonstrated previously for the sister species, *B. lindenii* (Lindl.) Dressler, the possible rudimentary nature of the small pollinia is discarded and they were used in the breeding system experiments (Vale *et al.*, 2011a).

Broughtonia cubensis usually occurs in dry and shrubby forests in which two rewarding white-flowered plant species bloom with the orchid: *Turbina corymbosa* (L.) Raf. (Convolvulaceae) (Fig. 1F–H) and *Cordia gerascanthus* L. (Boraginaceae) (Fig. 1I, J). The role of these plants in the reproductive performance of *B. cubensis* is unknown.

SITES AND SEASONS MONITORED

In order to broaden the understanding of the biology of this species and its response to disturbance, we decided to work in three populations: Loma de La Coca (LC), Cabo de San Antonio (CSA) and Cabo Corrientes (CC) (Fig. 2). These sites were chosen because they maintained healthy plant populations and were in protected areas, but were subject to frequent natural disturbances.

The Ecological Reserve of Loma de La Coca is a hill of nearly 150 m a.s.l. in western Cuba, next to a dam, and about 6 km from the north coast. The soil is rocky and ultramafic (derived from serpentine), with low field capacity, and is subject to frequent wildfires. The local xeromorphic vegetation is subject to edaphic drought and persistent chemical stress as a result of the presence of heavy metals. The mean annual temperature is 25.5 °C and the rainfall is about 1400 mm



Figure 1. Guild of white-flowered plants that shared visitors at four localities in western Cuba during the dry seasons of 2002, 2004, 2007 and 2012: A, plant of *Broughtonia cubensis*; B, *B. cubensis* flower detail; C, longitudinal section of a flower of *B. cubensis* showing the orange villose crests; D, *Ceratina cyaniventris* on *B. cubensis*; E, female bee of *Lasioglossum* cf. *gundlachii* wrapping the orange villose crests of the labellum of *B. cubensis*; F, *Turbina corymbosa* (Convolvulaceae); G, cross-section of a flower of *T. corymbosa* showing the orange glandular hairs at the base of the stamens; H, flower of *T. corymbosa* in front view; I, flowering branch of *Cordia gerascanthus* (Boraginaceae); J, flower detail of *C. gerascanthus*.

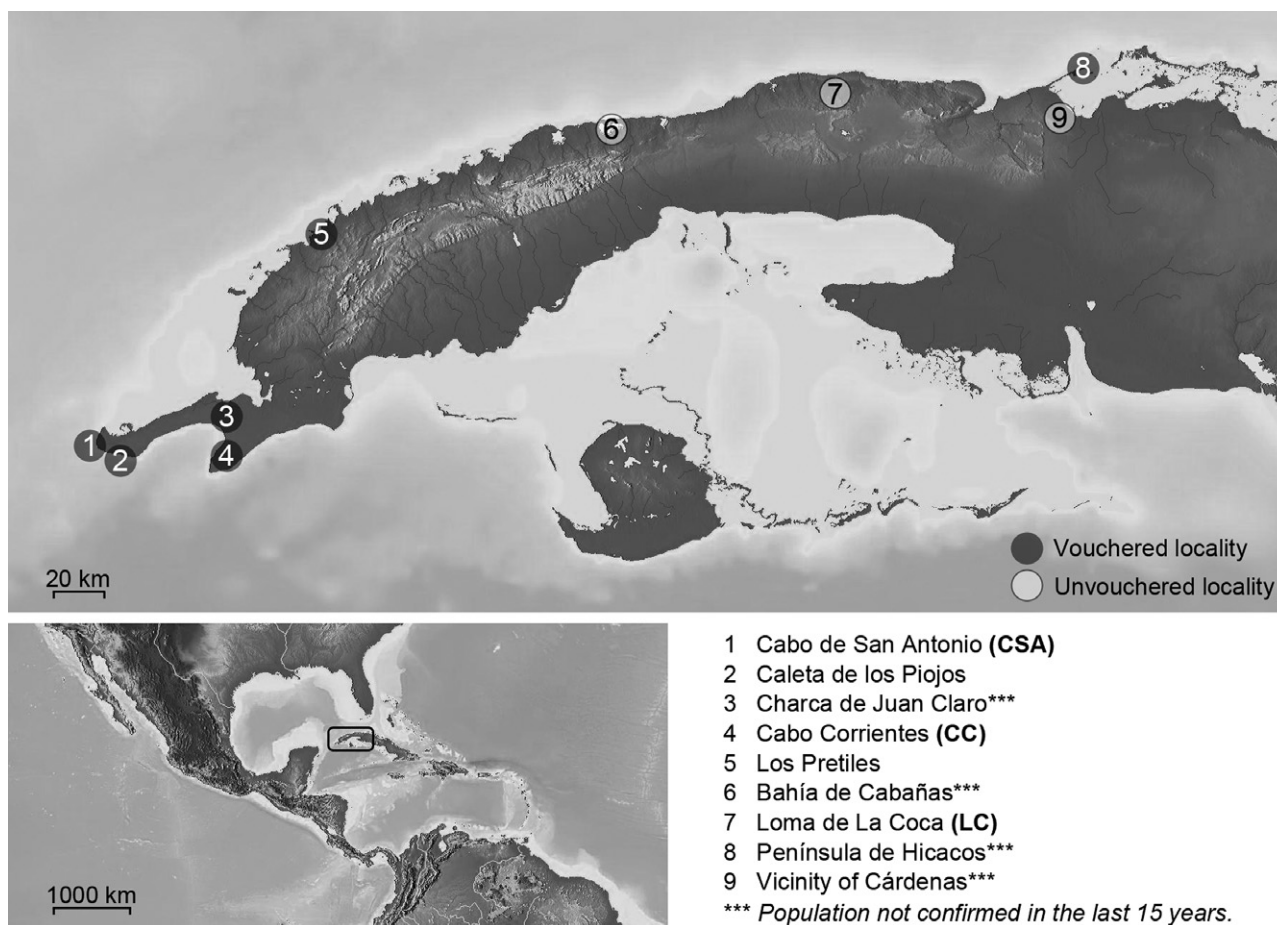


Figure 2. Historical distribution of *Broughtonia cubensis*. Locations correspond to the Appendix where herbaria voucher sheets are cited. Unvouchered locations were reported by specialists in Orchidaceae or are supported by the *in situ* or *ex situ* observation of flowering specimens by the authors AV and JCA. Studied populations are indicated with their corresponding abbreviations (see Material and Methods).

per year. In this locality, *B. cubensis* grows only in a small segment of about 240 m² on the southern hillslope, mainly on *Malpighia horrida* Small (Malpighiaceae), *Pseudocarpidium illicifolium* (A.Rich) Millsp. (Lamiaceae) and *Bucida spinosa* (Northrop) Jennings (Combretaceae), sometimes in contact with the ultramafic substrate.

In the National Park Península de Guanahacabibes, two populations of this species were studied: Cabo de San Antonio (CSA) and Cabo Corrientes (CC) in the western and south-eastern portions of the park, respectively. The sites are separated by > 50 km (Fig. 2) and are located at sea level (4–14 m a.s.l.), with the average temperature fairly uniform across the area (nearly 25 °C) and with an average annual precipitation ranging from c. 1300 mm (CC) to c. 1330 mm (CSA). CSA has lower trees and shrubs and is more sparsely vegetated than CC. The main phorophytes of *B. cubensis* in CC are *Jacaranda coerulea*

Auct. (Bignoniaceae), *Ficus* sp. (Moraceae), *Picrodendron macrocarpum* (A.Rich.) Britton (Picrodendraceae) and *C. gerascanthus* L. (Boraginaceae), whereas, in CSA, *B. cubensis* grows mainly on *Plumeria tuberculata* Lodd. (Apocynaceae) and *Erythroxylum areolatum* L. (Erythroxylaceae) (González *et al.*, 2007). In CSA, plants occupy a sparse coastal scrub on bare karstic limestone with high insolation and large separation between orchid phorophytes (González *et al.*, 2007). The CC population occupies a dense forest about 5 m tall and is also affected by sea spray. There, *B. cubensis* plants are concentrated on a few trees, with > 50% of the population limited to a single host tree of *J. coerulea*.

Although the flowering of this species extends from the end of each year to the beginning of the next (i.e. Cuban dry season), we refer to each period by mentioning only the second year for simplicity (e.g. the dry season of 2003–2004 is referred to as ‘2004’).

During the course of this research, 3–10 February 2001, almost all labelled plants of *B. cubensis* were allegedly removed by unknown collectors at LC. Between 2004 and 2005 three hurricanes affected the localities of CC and CSA: Iván (12–15 September 2004), Katrina (27–28 August 2005) and Wilma (21–24 October 2005).

In LC, we monitored the flowering periods from 2000 to 2004, but data collection after 2001 was after a considerable group of plants had been extracted from the wild, presumably by furtive collectors. Meanwhile, for CC and CSA, data collection was carried out between 2004 and 2007, after the occurrence of the hurricanes in the area.

FLORAL TRAITS AND BREEDING SYSTEM

The average flower longevity was quantified in ten plants with a total of 31 flowers protected from pollinators and herbivores. For each of these flowers, the dates of anthesis and senescence were recorded. In order to evaluate the production of nectar, a total of 30 flowers from ten plants was bagged and, after 24 h, the nectar was extracted and measured with a capillary micropipette. In addition, the 'nectar container' at the end of the floral tube was visually examined with a lens to detect the presence or absence of drops of nectar. To study the breeding system, seven and six plants from CSA and CC, respectively (with at least four flowers for each one), were subjected to the following treatments: spontaneous autogamy, manual geitonogamy, manual outcrossing and open pollination, according to the method described in Vale *et al.* (2011a). In all cases, flowers were inspected 5 and 50 h after manipulation to determine the time of senescence after pollination. Treatments were randomized among the flowers available for each plant and, in all cases (except open-pollinated), were protected with a tulle bag to prevent herbivory. Six to ten weeks later, the mature fruits were cut and stored in paper envelopes in a dry and airy place. The content of each fruit was processed according to Vale *et al.* (2011a), and the proportion of well-developed seeds was the only response variable considered. In this species, the existence of two morphs for the fruit shape invalidated the possibility of using the length and diameter of the fruits as response variables. The comparison of data was performed using a one-way analysis of variance (ANOVA) with the software PAST version 2.14 (Hammer & Harper, 2006). Using the average values for geitonogamy and outcrossing rates, we calculated the inbreeding depression index δ (Charlesworth & Charlesworth, 1987) as $\delta = 1 - (\text{proportion of well-developed seeds after geitonogamous pollination} / \text{proportion of well-developed seeds after allogamous hand-pollination})$.

POLLINATION ECOLOGY

In order to record the floral visitors, periods of continuous observation of 11 h a day (08:00–19:00 h) were performed for 3 days in 2002, 2 days in 2004 and 3 days in 2012, in locations near CSA, CC and LC (Fig. 2). Diurnal visits after 15:00 h did not occur at any location. As a result of the absence of nocturnal fragrance and the occurrence of diurnal anthesis, nocturnal observations were not performed (Faegri & van der Pijl, 1979). All censuses were performed during the flowering peak at each site. The identity of any visitor interacting with *B. cubensis* and co-occurring species with white flowers was recorded. For the orchid, we classified the type of interaction as 'visits' (i.e. the visitor only made contact with the flower) and 'effective visits' (i.e. the visitor removed and received a pollinarium, or deposited one or more pollinia on the stigma). The insects collected were identified and deposited at the National Museum of Natural History in Cuba (MNHN).

POTENTIAL REPRODUCTIVE LOSS AND COMPARISON OF REPRODUCTIVE PERFORMANCE AMONG POPULATIONS AND YEARS

In LC, a total of 62 plants was monitored every 7 days during the flowering season of 2001, 2002, 2003 and 2004, and 96 plants in CC and 190 plants in CSA were monitored every 15 days in 2004 and 2007. For each labelled plant, the following variables of reproductive investment were recorded: (1) number of new shoots; (2) number of inflorescences recently initiated; (3) length of each inflorescence after the first anthesis; (4) number of flowers per inflorescence; and (5) inflorescence length. For individuals with more than one inflorescence per year, the final average length of the inflorescences was obtained by averaging the length of all inflorescences per plant. The other final variables (i.e. per plant production of shoots, inflorescences and flowers) were calculated as the sum per individual.

To compare the behaviour of these five parameters among populations and years, we used the PERMANOVA statistical package. Given the need for balanced observations, random numbers were assigned to all observations of each combination of levels, and we selected observations at random. As part of PERMANOVA, we conducted 9999 Monte Carlo permutations of raw data on the basis of Bray–Curtis dissimilarities.

In addition, as a measure of reproductive success, the following information was recorded for each inflorescence: number of flowers in anthesis; flowers damaged (by the action of herbivores or mechanical effects); flowers with pollinia removed; flowers polli-

nated; fruit initiated; and dehiscent fruits. In addition, during the survey, we noted the presence of herbivorous agents eating flowers and fruit, and their characteristic marks made on the reproductive tissues of the plant were recorded. Fruit set was calculated for each plant as the total number of fruits produced divided by the total number of flowers produced in that season. Fruit set was compared between populations and years as for the other variables mentioned above.

POPULATION STRUCTURE

The population structure of *B. cubensis* at LC, CC and CSA was characterized and compared before and after the occurrence of strong disturbances. In the last two populations, it was not possible to record the damage immediately after the impact of the hurricanes because field access permission was denied for many months as a means of protecting the devastated environment and to enable repairs to access roads to affected areas. However, given the degree of threat to this species and the importance of this kind of information for future management and protection efforts, we believe that to compare its structure and reproductive performance before and 2 years after the hurricane impact could be useful for setting appropriate management actions adapted to the conditions of each particular site.

At each plot, all individuals of *B. cubensis* were tagged with a clear plastic label. However, after the mentioned disturbances occurred, most labels were lost, requiring the re-labelling of many plants. Plants were classified as juvenile, vegetative or reproductive. The individuals with at least one previous flowering were considered as vegetative; those with inflorescences in progress or recently wilted were considered as reproductive. Other individuals were considered as juveniles. We used Fisher's exact test per population to assess the relationship between the years and the frequency of each reproductive category. All statistical analyses were assessed with $\alpha = 0.05$.

GEOGRAPHICAL DISTRIBUTION

The historic and current distribution of *B. cubensis* was obtained from data on herbarium sheets, specialized publications, personal communications from orchid specialists and an exhaustive search made by the authors in the field and in Cuban institutional and private collections. Reports based on sterile individuals were discarded because young plants of the three Cuban species of *Broughtonia* R.Br. are extremely similar without flowers (Díaz & Pérez, 1986). Once the historical distribution was obtained, it was updated according to the date of most recent

sheets or reports for each locality and by field inspection of localities between 2001 and 2012.

RESULTS

FLORAL TRAITS AND BREEDING SYSTEM

In the three localities, *B. cubensis* had inflorescences with few flowers (mean, 7.0 ± 0.3 ; $N = 172$ plants) and with one or two flowers opening per day. Floral longevity in protected flowers was 21.7 ± 0.5 days ($N = 31$ flowers and ten plants). However, senescence occurred 15 h after the removal or deposition of pollinia. The species is self-compatible, but depends on pollinators for fruit initiation (i.e. protected flowers did not produce fruit, $N = 13$ plants). The percentage of well-developed seeds per fruit differed among the other three pollination treatments ($F_{2,27} = 6.46$, $P = 0.005$). Fruits from allogamous pollinations had a higher proportion of well-developed seeds (mean \pm SD, 0.58 ± 0.16 ; $N = 13$) than did geitonogamous fruits (mean \pm SD, 0.33 ± 0.25 ; $N = 11$) and open-pollinated fruits (mean \pm SD, 0.31 ± 0.25 ; $N = 6$). There were no differences between these last two groups according to Tukey's test ($q_{\text{alo-gei}} = 3.93$, $P = 0.0258$; $q_{\text{alo-op}} = 4.031$, $P = 0.0219$; $q_{\text{gei-op}} = 0.1008$, $P = 0.9973$). The index value of inbreeding depression (δ) was 0.43.

POLLINATION ECOLOGY

Broughtonia cubensis does not produce intrafloral nectar. As a result of the small number of recorded visits at each of the study sites, the results of visitor observation periods were grouped (Table 1). Small-sized bees were the most frequent visitors, accounting for 84.8% of the visits, and were also the only effective pollinators. Among these small bees, species nesting underground (i.e. genus *Lasioglossum*; Halictidae) made 65.8% of all visits, with an effectiveness on pollination of 13.6%. Bees nesting in aerial substrates (i.e. genus *Ceratina*; Apidae) made 19.0% of all visits, but with an effectiveness of 60%. Both the groups of these small bees and other visitors of *B. cubensis* (Table 1) also frequently foraged on the white flowers of the rewarding plants *T. corymbosa* (Fig. 1F–H) and *C. gerascanthus* (Fig. 1I, J).

REPRODUCTIVE POTENTIAL LOSS AND COMPARISON OF REPRODUCTIVE PERFORMANCE AMONG POPULATIONS AND YEARS

In general, both locations of Península de Guanahacabibes showed similar values for most of the pre-reproductive and reproductive variables analysed (Fig. 3). However, on average, CC produced more inflorescences per plant than CSA during the 2 years, although CC showed a tendency towards decreasing

Table 1. Composition and effectiveness of floral visitors of *Broughtonia cubensis* (and their co-occurring white-flowered plants) across the range of occurrence of this orchid (data from different localities were pooled). The symbols '+' and '-' represent the incidence of the event enunciated at the column head

Order	Species	Number of visits (percentage of effective visits)	Average number of flowers visited per plant	Removed pollinia	Deposited pollinia	Visitor of <i>Turbina corymbosa</i>	Visitor of <i>Cordia gerascanthus</i>
Hymenoptera	<i>Lasioglossum gundlachii</i>	12 (25.0%)	1.7	+	+	+	+
	<i>Lasioglossum mestrei</i>	38 (10.5%)	1.7	+	+	+	+
	<i>Lasioglossum</i> sp.	2 (0%)	1	-	-	+	-
	<i>Megachile poeyi</i>	1 (0%)	1	-	-	+	+
	<i>Exomalopsis</i> sp.	2 (0%)	1	-	-	+	+
	<i>Apis mellifera</i>	3 (0%)	1.5	-	-	+	+
	<i>Melipona beechii</i>	1 (0%)	1	-	-	+	+
	<i>Ceratina cyaniventris</i>	15 (60.0%)	1.1	+	+	+	+
	Species not identified	1 (0%)	1	-	-	+	+
	Syrphidae not identified	2 (0%)	1	-	-	+	-
Diptera							
Lepidoptera	<i>Cynaenae tripunctus</i>	1 (0%)	1	-	-	+	-
	<i>Polites baracoa</i>	1 (0%)	1	-	-	+	+

in the second year (Fig. 3B). The other variables (except for the length of inflorescences) differed only between years, but not between populations. In both populations, although the production of shoots and inflorescences differed among years (with lower values in the second study year), this reduction was clearer for CC (Fig. 3A, B). Nevertheless, fruit set, the ultimate measure of reproductive success, was drastically reduced only in CSA (Fig. 3E). A similar pattern was also observed for this locality by plotting the percentage of reproductive individuals that produced fruits (Fig. 3F). In contrast, the average production of flowers per plant increased during the second year in CC and was barely affected in CSA (Fig. 3D).

In all three populations, the main factor limiting fruit formation was the low effective activity of pollinators (Fig. 4), although there was some incidence of floral herbivores, e.g. hutia (*Capromys pilorides*; Capromyidae: Rodentia), rats (*Rattus* sp., Muridae: Rodentia) and the leaf-cutting ant *Atta insularis* (Formicidae: Hymenoptera).

POPULATION STRUCTURE

The density of individuals differed among populations and years, and strong disturbances in these populations also affected their structure (Fig. 5). The population of LC suffered a reduction of 90% of individuals in 2002 after a massive specimen extirpation, resulting in a predominance of juveniles and vegetative plants. Two years after being disturbed, CC had less juveniles and a predominance of vegetative over reproductive plants. Meanwhile, in CSA, more than one-third of the marked individuals died or were absent after the disturbance, although the density of juveniles increased and the proportion of reproductive individuals with regard to the vegetative plants was also higher. However, only in CC and CSA were the drastic changes in the number of individuals per reproductive category associated with years (Fig. 5).

GEOGRAPHICAL DISTRIBUTION

Broughtonia cubensis is confirmed as endemic to western Cuba (Fig. 2) and occurs mainly in coastal dry forest. Nine historical populations were found; six are supported by herbarium sheets (Fig. 2; Appendix) and three by references from specialists on Orchidaceae: Bahía de Cabañas (A. Vale, unpubl. data), Loma de La Coca (Díaz & Pérez, 1986; Díaz *et al.*, 1998) and vicinity of Cardenas (M. A. Díaz, Pro-Naturaleza, La Habana, unpubl. data). The species has been allegedly extirpated from at least five of nine localities (Fig. 2) because it has not been validated in the last 15 years or observed during the

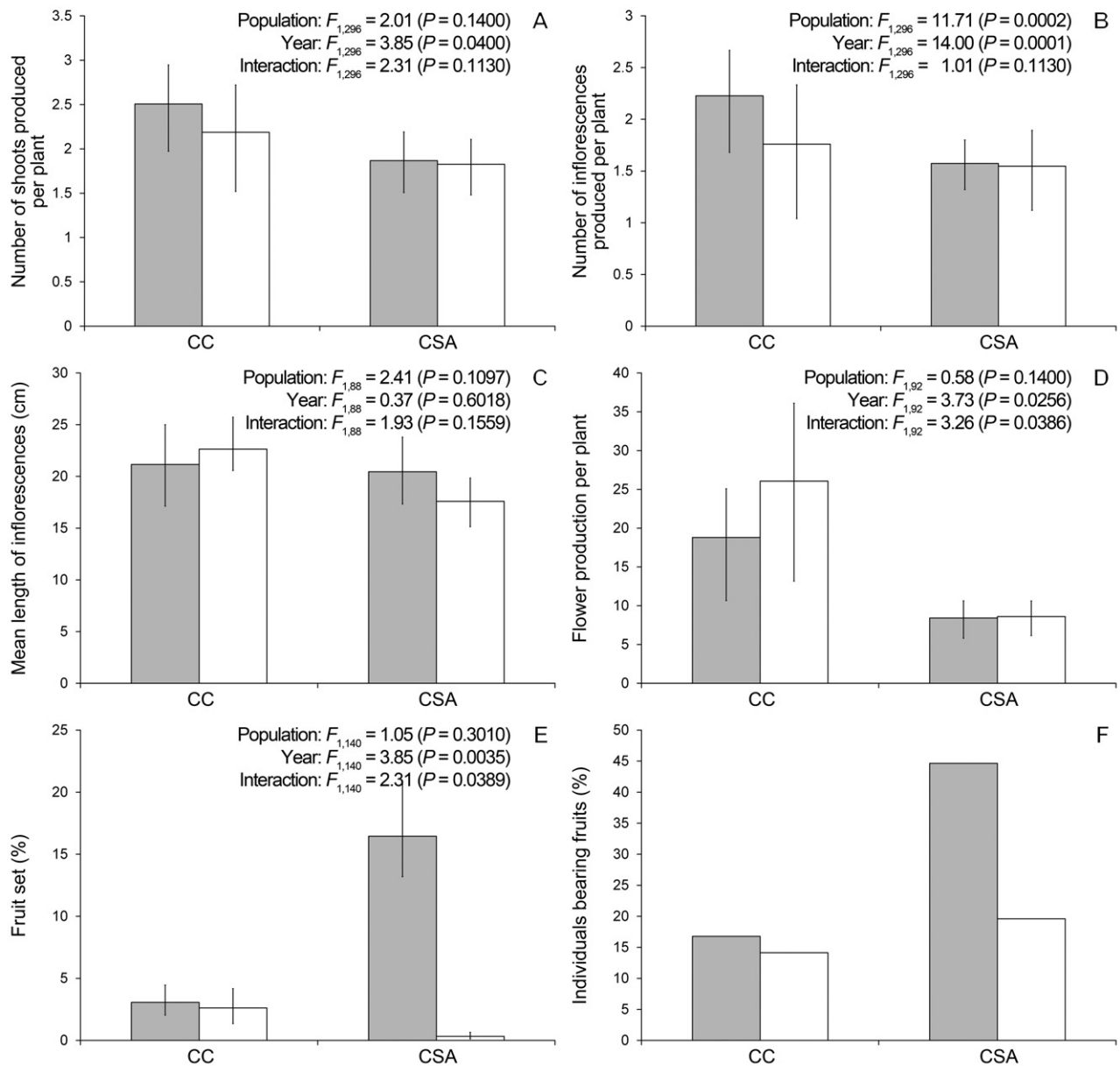


Figure 3. Behaviour of pre-reproductive and reproductive variables in the populations of Cabo Corrientes (CC) and Cabo de San Antonio (CSA). Variables were measured before and 2 years after the hurricanes Iván, Katrina and Wilma at these sites. We present the results of comparisons with PERMANOVA, where P values correspond to the Monte Carlo asymptotic values estimated with 9999 permutations based on Bray–Curtis dissimilarities.

exhaustive field trips of this study. Large populations are more frequent in western Pinar del Río province (e.g. one population in the Ecological Reserve Los Pretiles and the two populations studied here from Península de Guanahacabibes). The fourth largest population, LC, the only one known on serpentine, is currently almost extirpated as a result of the over-collection of plants and recurrent forest fires (Fig. 5).

DISCUSSION

POLLINATION ECOLOGY AND SEED PRODUCTION LIMITATIONS

As reported previously for the congeneric *B. lindenbergii* (Vale *et al.*, 2011a) and many other deceptive orchids (Neiland & Wilcock, 1998; Tremblay *et al.*, 2005), *B. cubensis* is seriously pollen limited (see Fig. 4). The

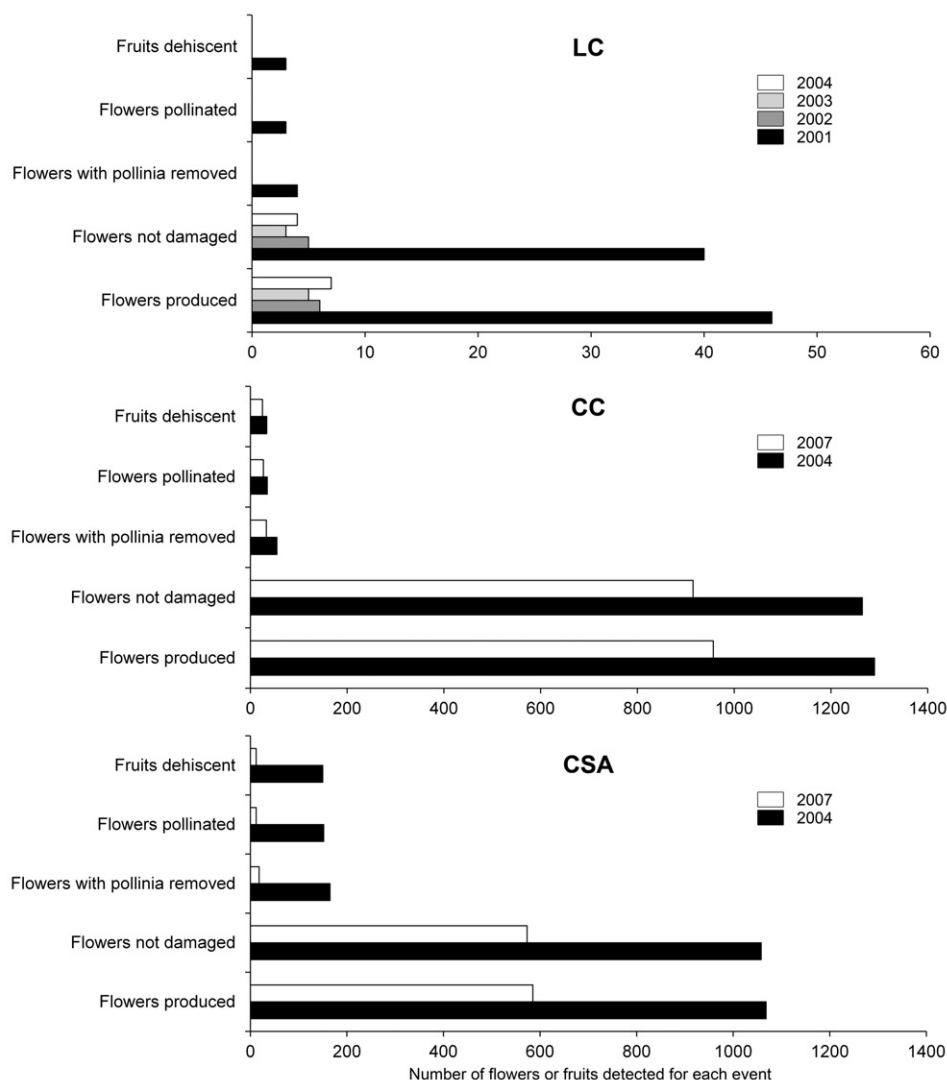


Figure 4. Patterns of reproductive potential loss in populations of *Broughtonia cubensis* before and after periods of intense habitat disturbance. Data for Loma de la Coca (LC) are for 2001 to 2004, before and after the overharvesting of local wild specimens and wildfires at the end of the flowering season of 2001. Data for Cabo Corrientes (CC) and Cabo de San Antonio (CSA) are for 2004 and 2007 before and 2 years after hurricanes Iván, Katrina and Wilma in the National Park Península de Guanahacabibes.

flowers are long-lasting and wilt after any kind of pollination event. These two features have been reported as adaptations to enhance reproductive success under scarce pollinator activity (Huda & Wilcock, 2011 and references cited therein; Jin *et al.*, 2012) and a mode of favouring cross-pollinations over selfing (Vale *et al.*, 2011a). In addition, the breeding system of *B. cubensis* showed some level of inbreeding depression at seed level and full pollinator dependence for seed production. All these elements add new challenges for the management of this orchid. Consequently, if human-mediated pollen flow is required to increase seed release in the field or for cultivation

in vitro, the origin and quantity of pollen to be used should be chosen carefully. Finally, the percentage of well-developed seeds produced by open pollination in this study was similar to that produced by geitonogamous pollinations. This suggests that: (1) pollinators probably deposit only one or two pollinia per stigma (as observed in the field), which was experimentally probed as a cause of the low percentage of well-developed seeds in *B. lindenii* (Vale *et al.*, 2011a); and/or (2) pollinators frequently perform geitonogamous or short-distance inter-individual pollinations, which could cause a putative loss of seed quality. Consistent with the second hypothesis, the small-

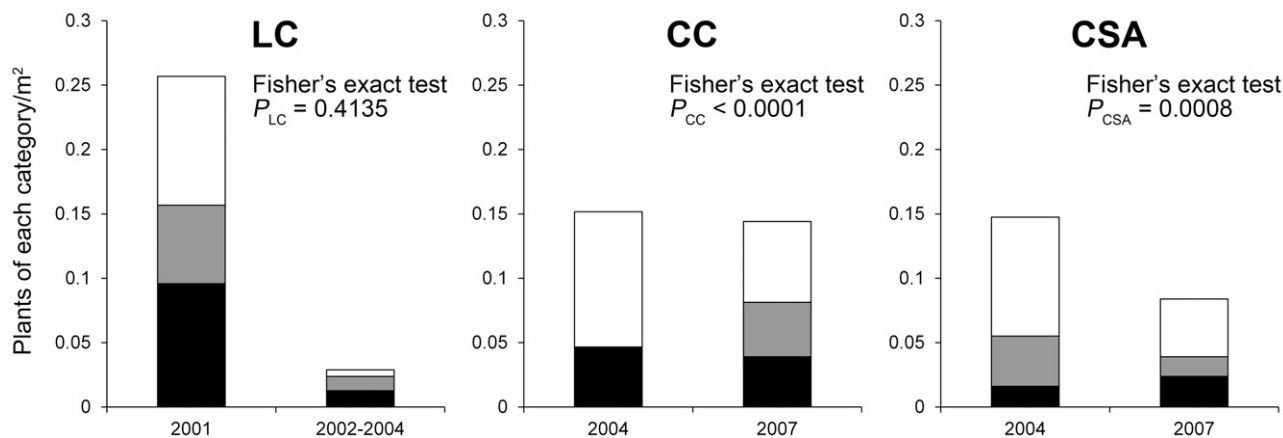


Figure 5. Distribution and density of individuals of *Broughtonia cubensis* for each reproductive category at Loma de La Coca (LC), Cabo Corrientes (CC) and Cabo de San Antonio (CSA). Values for two flowering seasons per locality are provided. Black areas represent juveniles, grey areas vegetative plants and white areas reproductive individuals. Note the death or removal of individuals in the second census of each locality.

sized bees reported here as pollinators (*Lasioglossum* and *Ceratina*) are characterized by short foraging home ranges (Westrich, 2006; Beil, Horn & Schwabe, 2008), which may limit the pollen-mediated interconnection of isolated individuals with genetically more diverse core populations (Lennartsson, 2002). Unfortunately, we lack detailed information about the efficiency and local abundance of both groups of bees pollinating *B. cubensis*, and about their particular susceptibility to hurricane damage. Nevertheless, ground-nesting bee species (i.e. *Lasioglossum* spp.) are usually more threatened by environmental disasters than those using cavities in wood or stems (Williams *et al.*, 2010). Consequently, as *B. cubensis* is a generalist (i.e. it is pollinated by two small pollinators with quite different nesting requirements), it may be able to survive local extirpation of one or more pollinator species.

Deceptive orchids usually rely on the abundance of floral rewards of other plant species (Internicola *et al.*, 2007; Carmona-Díaz & García-Franco, 2009; Vale *et al.*, 2011b). In the case of *B. cubensis*, the role of *T. corymbosa* and *C. gerascanthus* as possible model plants *sensu* Dafni (1984), magnet plants *sensu* Johnson *et al.* (2003) or simply as co-occurring nectariferous species *sensu* Internicola *et al.* (2007) and Peter & Johnson (2008) could be a key element in the management of this orchid *in situ*. In particular, *T. corymbosa*, one of the most melliferous plants in the Neotropics (Ordetx, 1978), could be crucial for the maintenance of the community of small bees pollinating *B. cubensis*. In addition, certain characteristics of *T. corymbosa* support a putative Batesian mimicry with the orchid. In the field, *Lasioglossum* and *Ceratina* bees were the main native insects that visited and pollinated *T. corymbosa* (see Table 1). The

presence of orange epidermal structures with similar appearance and location at the flowers of both species (Fig. 1C, G) reinforces the resemblance between these flowers. The structures are similarly manipulated by small bees to reach the nectar in *T. corymbosa* (see Fig. 1G) and by pollen-collecting (Fig. 1E) or nectar-seeking (see Fig. 1D) bees in *B. cubensis*.

DISTRIBUTION AND POPULATION STRUCTURE

Broughtonia cubensis is not well represented in herbaria and is currently restricted to half of its known historical range (see Fig. 2, Appendix). This may be a consequence of reproductive problems and also of habitat loss and specimen overharvesting (Díaz *et al.*, 1998). In this sense, to search for unreported populations in sites that match the geographical and ecological requirements of the species could be helpful for interconnecting extant populations by artificial genetic flow (Zettler & McInnis, 1992). Particularly, although Dietrich (1980) reported *B. cubensis* in the central mountains of Cuba, we did not include this locality in the distribution of the species. This xerophytic epiphyte only occurs in rocky and broad shrubby forest at sea level. In this sense, the Loma del Burro locality (at c. 500 m. a.s.l.) does not match the locations of the other populations of *B. cubensis* in having a much more humid environment at higher altitude. Moreover, the report obtained in this locality was not vouchered and was based on a sterile plant observed outside the blooming period (Dietrich, 1980), when *B. cubensis* could be easily confused with juveniles of *B. lindenii* or *B. ortgiesiana* (Rchb. f.) Dressler (Díaz & Pérez, 1986). In consequence, we consider the report as dubious.

Here, we report the drastic loss of individuals, density declines and reproductive inactivity of adult

plants between years (Fig. 5). *Broughtonia cubensis* has shown an ability to recover spatial and population structure after disturbance (Raventós *et al.*, 2011). This study and our data reveal a strong short-time vulnerability to environmental change (see, for example, the decrease in fruit set and percentage of fruiting individuals after the hurricanes in CSA; Fig. 3E, F). Thus, different levels of dependence on biotic interactors (i.e. pollinators, phorophytes, co-blooming plants and mycorrhizae) could strongly affect resilience during the life cycle of this orchid throughout its range (e.g. Phillips *et al.*, 2011; McCormick *et al.*, 2012). This suggests the need to monitor disturbance levels and the environmental health of its habitat to ensure appropriate microsites for the reproductive performance of this orchid (Jersáková & Malinova, 2007). In recent decades, the frequency and destructiveness of hurricanes in the Atlantic Ocean have increased significantly (Goldenberg *et al.*, 2001; Emanuel, 2004). This tendency could modify the tree composition of coastal habitats and reduce suitable substrates for the germination of *B. cubensis* (Raventós *et al.*, 2011). In this sense, because of the small effective sizes of *B. cubensis* populations, it becomes desirable to consider a program of *in vitro* germination in order to reinforce impoverished or extirpated populations. In addition, this could also help to diminish the collecting pressure over wild populations by satisfying the demand for plants among amateurs (Díaz & Pérez, 1986; Fay, 1992). The breeding system data offered here provide new insights into the management of populations and the sexual reproduction of the species under *in situ* and *ex situ* conditions (see above).

The population structure of *B. cubensis* is similar to that reported for other Neotropical epiphytic orchids (i.e. with a preponderance of reproductive and vegetative individuals over juveniles and seedlings; Tremblay *et al.*, 2005; Winkler, Hülber & Hietz, 2009). Nevertheless, the population structures varied among populations and years, especially before and after the disturbance episodes (see Fig. 5). Thus, considering the critical levels of recruitment in epiphytic orchids in general (germinated seeds range from 0.006 to 0.400% of the seed produced; Winkler *et al.*, 2009) and the extreme slowness of growth and maturation in these plants (Jersáková & Malinova, 2007; Winkler *et al.*, 2009), the effects of local and punctual disturbances on the population dynamics of *B. cubensis* could have serious conservation and evolutionary consequences for the species in its current range (e.g. Tremblay & Ackerman, 2001).

CONCLUSIONS AND GUIDELINES FOR CONSERVATION

The fact that *B. cubensis* occurs at only a few locations in Cuba highlights the need for the monitoring

and conservation of its currently available populations. Meanwhile, the possibility and urgency to re-establish extirpated populations must be evaluated. To perform these tasks, a basic knowledge of the spatial ecology and phorophyte preferences of this orchid is available (González *et al.*, 2007; Raventós *et al.*, 2011), which could be improved by studying more locations across the range of the species. We also advise that new efforts could be initiated to enable the evaluation of local abundance, the activity of pollinators and the density of the co-occurring nectariferous plants, before the initiation of any management. For populations that are reproductively depressed, it may be helpful to institute artificial pollen flow, to create nesting facilities for both groups of small bees and to plant *T. corymbosa* and *C. gerascanthus* to support bee communities and improve orchid visitation rates. Research on mycorrhizal and germination ecology is more than desirable, it is a priority. Moreover, to counteract the over-collection from wild populations, it is crucial to satisfy at the same time the *in situ* and *ex situ* conservation of this plant and to encourage exchange among orchid growers, botanical gardens, amateurs and scientists based only on *in vitro* propagated plants. The results obtained in this study and the guidelines must not be limited to the management of this species of orchid. The focus of this study and its results should be taken into account in the management plans of other endangered deceptive orchid species.

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APPENDIX

Localities known for *Broughtonia cubensis*, estimated population sizes (by *in situ* counting of adult-sized plants) and herbarium specimens consulted. The geographical coordinates of the populations are available from the authors on request. Bold text indicates localities as shown in Figure 2

Locality (Province)	Estimated population size (individuals)	In a protected area?	Collector/observer	Herbarium number	Herbarium
Barra de la Sorda, Cabo de San Antonio , Sandino, Península de Guanahacabibes (Pinar del Río)	>1000	Yes	J. Ferro & colleagues	4310 4510	HPPR HAC
Península de Guanahacabibes, 2–3 miles E of Caleta de los Piojos , near Sawmill. (Pinar del Río)	200–1000	Yes	W. Osment	s.n.	USF
Charca de Juan Claro , La Bajada (Pinar del Río)	Extirpated	Yes	Lastra	20795	AMES
Jaimanitas y Valle de San Juan, Cabo Corrientes , Sandino, P. de Guanahacabibes (Pinar del Río)	200–1000	Yes	R. Pérez and A. Hernández	58307	HAJB.
Los Pretils (Pinar del Río)	>1000	Yes	J. A. Bosmenier	s.n.	HAC
Bahía de Cabañas (Artemisa)	Extirpated?	No	A. Vale	NA	NA
Loma de la Coca (La Habana)	<10	Yes	M. A. Díaz, A. Vale	NA	NA
Península de Hicacos , Varadero (Matanzas)	Extirpated?	Yes	L. C. Scaramuza	22401	HAC.
Vicinity of Cárdenas (Matanzas)	Extirpated?	No	M. A. Díaz & J. D. Ackerman	NA	NA