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Plantago australis (Plantaginaceae) produces both chasmogamous and cleistogamous flowers: Field work, herbarium and literature-based evidence

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ABSTRACT

Plantago australis is considered to be obligately cleistogamous. Here we present evidence based on field work, herbarium collections and literature to show that this species produces both chasmogamous and cleistogamous flowers. We also hypothesise that the species is ambophilic.

Plantago australis Lam. belongs to *Plantago* L. subg. *Plantago* sect. *Virginica* Decne. & Steinh. ex Barnéoud, and is one of the most widely distributed and common species in the genus (Rahn, 1974; Hassemer, 2019; Hassemer et al., 2019). The species has a complex nomenclatural and taxonomic history. *Plantago australis* was described by de Lamarck (1792) but remained relatively obscure until Rahn (1964, 1974) lumped under this name a number of species accepted up until then (e.g. Pilger, 1913, 1929, 1937). These species included *P. accrescens* Pilg., *P. asplundii* Pilg., *P. bicallosa* Decne., *P. candollei* Rapin, *P. cumingiana* Fisch. & C.A.Mey., *P. denudata* Pilg., *P. durvillei* Delile ex Fisch. & C.A. Mey., *P. ecuadorensis* Pilg., *P. gigantea* Decne., *P. goudotiana* Decne., *P. hirtella* Kunth, *P. kurtzii* Pilg., *P. lasioneura* Pilg., *P. macropus* Pilg., *P. macrostachya* Decne., *P. oreades* Decne., *P. pflanzii* Pilg., *P. refracta* Pilg., *P. sodiroana* Pilg., *P. stuckertii* Pilg. and *P. valida* Pilg., as well as many other less often used names. The abundance of taxonomic names suggests that *P. australis* is a considerably variable species from a morphological and ecological point of view. More recently, *P. pretoana* (Rahn) Hassemer, a narrow endemic from Serra do Itatiaia in south-eastern Brazil, and *P. cumingiana*, from central and southern Chile and adjacent areas in Argentina, were removed from the circumscription of *P. australis* (Hassemer et al., 2015, 2019, respectively). In the most recent circumscription, *P. australis* is native to the Americas, occurring from the southwestern United States of America (Arizona) to southern Argentina. The species is also naturalized in Chile (Rahn, 1974), Hawaii (Wagner et al., 1990) and New Zealand (Sykes, 1988). All records of *P. australis* from Africa were recently shown to be misidentified *P. tomentosa* Lam. specimens (Hassemer, 2020).

In a recent study Abrahamczyk et al. (2020) studied the reproductive biology of three *Plantago* species, including *P. australis*. They concluded that *P. australis* is obligately cleistogamous, which had already been indicated previously by Primack (1978). Cleistogamy refers to the production of flowers that do not open and undergo self-fertilization, whereas chasmogamy refers to the production of flowers that open to expose the reproductive parts for pollination (Lord, 1981; Cheplick, 2007). In *Plantago*, cleistogamy is recorded for North and South American species (Kuiper and Bos, 1992). It should also be mentioned that most *Plantago* species are cross-pollinated (Sharma et al., 1992), although knowledge of pollination systems is still deficient for many species, especially the rarer ones.

Here we present evidence that *P. australis* produces both chasmogamous and cleistogamous flowers. We base our claim on extensive field work (*in natura*), cultivation experiments and also the study of herbarium specimens from throughout the wide distribution of the species.

We have over 12 years of field experience with *P. australis* (2008–2020), including field work conducted in Argentina, Brazil, Chile, Ecuador, Peru and Uruguay. Photographs of *P. australis* specimens *in natura*, clearly show a combination of chasmogamous and cleistogamous flowers (Fig. 1). The occurrence of chasmogamous flowers does

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not follow a geographically or morphologically coherent pattern, and chasmogamous and cleistogamous flowers are often present in the same population. Furthermore, we observed regular visits to open flowers by a number of species of flies and bees for pollen collecting (Fig. 1B). Considering that *Plantago* species in general are well known for being anemophilous (Rahn, 1974; Primack, 1978; Kuiper and Bos, 1992; Tormo et al., 2001), our observations make us think that *P. australis* is possibly ambophilic. Ambophily is recorded in a few other species in the genus, e.g. *P. media* L. (Leereveld et al., 1976; Meeuse, 1984; Abrahamczyk et al., 2020) and *P. lanceolata* L. (Stelleman, 1978; Sharma et al., 1993). Future studies could focus on the pollination mechanism of *P. australis* and include a test of our hypothesis that the species is ambophilic.

To assess the occurrence of chasmogamous flowers in the species across its range, we also conducted an extensive review of specimens from the following herbaria (herbarium codes according to Thiers, 2020): ASE, B, BHCB, BO, BRIT, C, CAS, CEN, CGMS, CIIDIR, COL, CONC, DDMS, EAC, EFC, F, FI, FLOR, FT, FURB, GB, GH, HAS, HBR, HRB, HTL, HUFSJ, HUH, HURB, IAC, IBSC, ICN, JBB, JEPS, K, KW, LE, M, MA, MBM, MHA, MO, MVFA, MVJB, MVM, MW, NBG, NY, P, PE, PI,

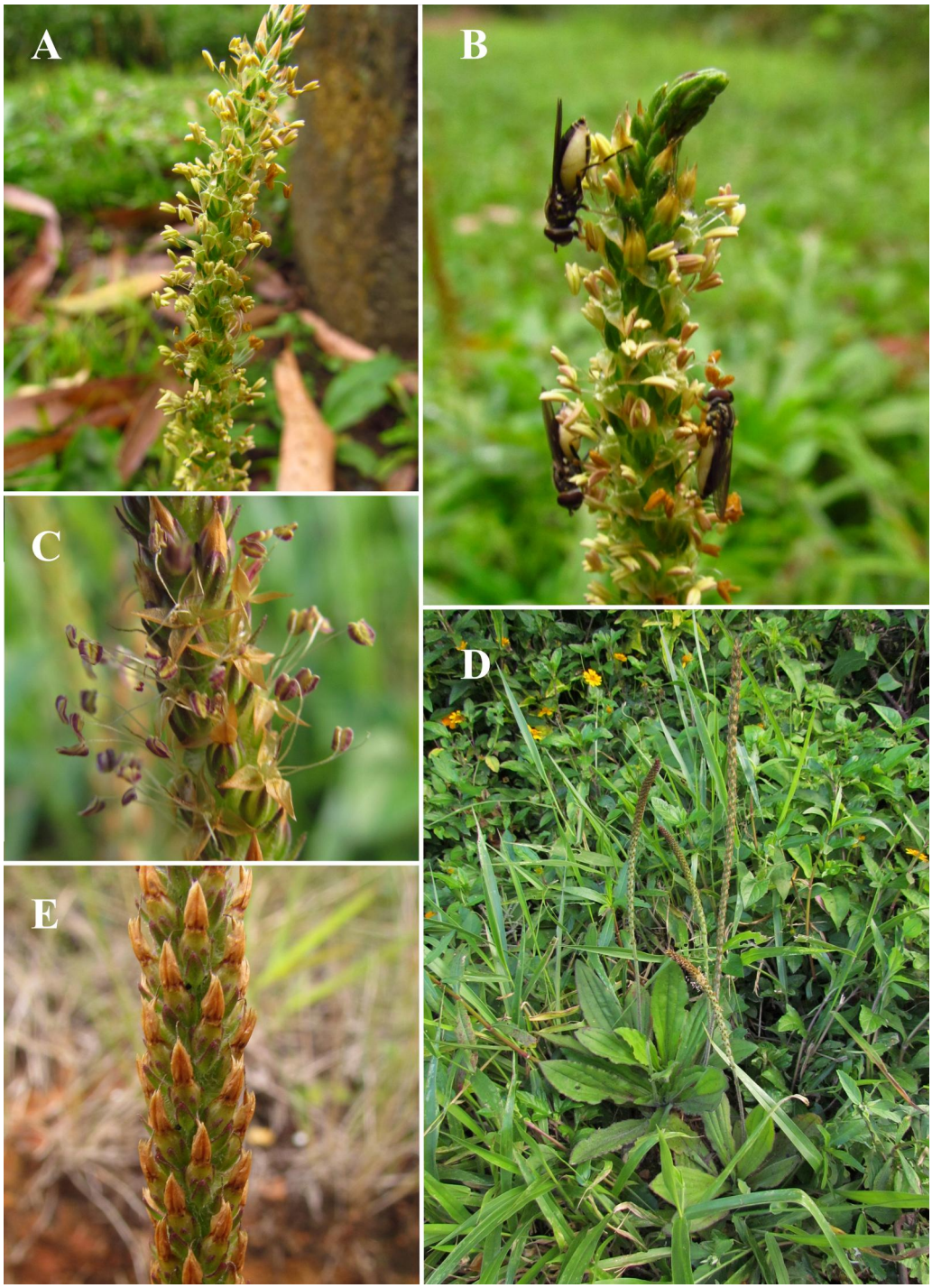

Fig. 1. *Plantago australis in natura*, in its native range. **A**: Chasmogamous flowers, most of the flowers on the spike open simultaneously. **B**: Same as **A**, but with flies visiting the flowers for pollen. **C**: Chasmogamous flowers, but only a few flowers open at a time. **D**: Same as **C**, overview of specimens. **E**: Cleistogamous flowers. Photographs by L.A. Funez, all from Santa Catarina state, southern Brazil.

PRE, RB, SAM, SGO, SP, SPF, TANG, TEPB, TI, TNS, TUB, UB, UC, UESC, UFMT, UPCB, UPS, US and USM. Based on the study of herbarium material, we conclude that specimens of this species can have both cleistogamous and chasmogamous flowers. High-resolution images of 732 specimens of *P. australis* kept at BR, CEN, CESJ, CRI, ECT, ESA, EVB, F, FLOR, FURB, G, GBH, GH, HBG, HUCP, HUEFS, HUEM, HUFABC, HURB, HUTO, INPA, K, L, MBM, MEXU, MO, NY, P, R, RB, RBR, SP, SPF, UC, UEC, US, VIC, VIES and WAG (see the Supplementary Material) were studied for the presence of open flowers with exserted stamens or spikes with consistently open flowers. Such features are indicative of chasmogamy in those specimens. We found 216 (29.51%) specimens featuring chasmogamous flowers, from the following countries: Argentina, Bolivia, Brazil, Colombia, Ecuador, Guatemala, Honduras, Mexico, Panama, Peru, United States of America (Hawaii), Uruguay and Venezuela. We should add that, while the presence of open flowers as described above attests to the specimens in question being chasmogamous, the lack of open flowers does not attest that a specimen is cleistogamous, because it is possible that the flowers of a specimen with chasmogamous flowers had not yet opened or had already closed when the plant was collected. Thus, the proportion of specimens with chasmogamous flowers is admittedly underestimated in this analysis.

Finally, we conducted a literature review aiming at highlighting contributions that recognized that *P. australis* produces chasmogamous flowers. Specifically, we searched for taxonomic studies that explicitly indicated that *P. australis* (including all of its currently-recognized synonyms, as well as all infraspecific taxa) has, or sometimes has, chasmogamous flowers, open flowers, an open corolla, patent corolla lobes, or exserted stamens. This search included studies written in Danish, English, French, German, Italian, Latin, Portuguese, Russian and Spanish. Our search confirmed the presence of chasmogamous flowers for all countries where *P. australis* has been reported, including its native and naturalised distribution (Table 1).

We observed that both chasmogamous and cleistogamous flowers can be present on the same plant. However, it would appear that most often a given plant will only produce flowers of either type. This was likely the case for the lineage of *P. australis* that Abrahamczyk et al. (2020) used for their study, and may explain their finding of obligate cleistogamy in *P. australis*, as they only studied specimens from a single lineage of the species (Abrahamczyk et al., 2020).

The production of both chasmogamous and cleistogamous flowers is a relatively widespread condition among flowering plants, and most species that produce cleistogamous flowers are also able to produce open, potentially cross-pollinated chasmogamous flowers (Lord, 1981; Culley, 2002; Cheplick, 2007). Showy chasmogamous flowers are energetically more costly to produce and are often assumed to be outcrossing, typically appearing when pollinators are present (Schemske, 1978; Waller, 1979; Culley, 2002). On the other hand, cleistogamous flowers are comparatively reduced and require less energy to produce, being structurally modified for self-pollination, and usually appear when pollinators are absent or under limiting resource conditions (Culley, 2002). The differential cost in production of the two types of flowers is believed to lead to chasmogamous flowers being developmentally favoured in optimal growth conditions, when a surplus of resources is available (Jain, 1976; Waller, 1984; Sternberger et al., 2020).

In our opinion, the issue raised here illustrates the perils of systematic studies based on limited sampling, particularly if samples originate from *ex situ* sources. In addition to sampling artefacts, several additional problems can occur, such as misidentifications or *ex situ* hybridization or mutations. We consider these issues to be underestimated and underappreciated in the literature, despite their considerable impact on studies based on such specimens. This case also illustrates the importance taxonomic expertise for non-taxonomic biological studies (de Carvalho et al., 2008; Sluys, 2013; Goulding and Dayrat, 2016; Ely et al., 2017; Hassemer et al., 2020). *Ex situ* conservation techniques and efforts have considerable potential to prevent the irreversible loss of biodiversity (Guerrant et al., 2004; Guerrant and Kaye, 2007; Abeli et al., 2019), but should always be associated with appropriate taxonomic expertise, lest the conserved specimens lose their usefulness to both science and biodiversity conservation.

Table 1
Result of the literature survey of the taxonomic studies that indicated references to chasmogamous flowers for *Plantago australis* (including all of its currently-recognized synonyms, as well as infraspecific taxa), organised by country. For each country, we include which species-rank synonyms of *P. australis* are reported to produce chasmogamous flowers. An asterisk after a country name indicates that *P. australis* is not native there.

Country	Works	Synonyms of *P. australis*
Argentina	Pilger (1912, 1913, 1929, 1937), Pontiroli (1965), Rahn (1974, 1979, 1999), Cabrera (1993), Tolaba and Fabbroni (1998)	*P. accrescens, P. denudata, P. hirtella, P. kurtzii, P. macropus, P. macrostachya, P. refracta, P. stuckertii*
Bolivia	Pilger (1912, 1913, 1937), Rahn (1974)	*P. hirtella, P. pflanzii*
Brazil	Pilger (1913, 1937), Rahn (1966, 1974), de Paula-Souza and Souza (2002), Hefler et al. (2011)	*P. bicallosa, P. gigantea, P. hirtella, P. macrostachya*
Chile*	Rahn (1974)	
Colombia	Pilger (1913, 1937), Rahn (1964, 1974)	*P. goudotiana, P. hirtella, P. oreades*
Costa Rica	Pilger (1913, 1937), Rahn (1974), Burger (1986)	*P. hirtella*
Ecuador	Pilger (1913, 1937), Rahn (1974, 1975)	*P. ecuadorensis, P. hirtella*
El Salvador	Rahn (1974)	
Guatemala	Pilger (1913, 1937), Rahn (1974)	*P. hirtella*
Honduras	Rahn (1974)	
Mexico	Pilger (1913, 1937), Rahn (1964, 1974)	*P. hirtella*
New Zealand*	Sykes (1988)	
Nicaragua	Rahn (2001)	
Paraguay	Pilger (1913, 1937), Rahn (1974)	*P. hirtella*
Peru	Pilger (1913, 1937), Rahn (1974), Gonzáles (2012)	*P. hirtella, P. oreades, P. pflanzii*
United States of America	Pilger (1937), Rahn (1974), Huisinga and Ayers (1999)	*P. hirtella*
Uruguay	Pilger (1913, 1937), Rahn (1974)	*P. hirtella, P. macrostachya*
Venezuela	Pilger (1913, 1937), Rahn (1974)	*P. hirtella*

Author statement

GH conceptualised this work and wrote the text with the help of APS, ABS and LAF. GH and ABS did the literature review, LAF took the field photographs, and all authors analysed herbarium specimens.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.flora.2020.151724.

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