UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
DEPARTAMENTO DE BOTÂNICA
PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

ESTUDOS BIOSSISTEMÁTICOS EM ESPÉCIES DE *HABENARIA* WILLD. (ORCHIDACEAE) NATIVAS NO RIO GRANDE DO SUL

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Colaborador: Dr. João Aguiar Nogueira Batista (UFMG)

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Dissertação apresentada ao Programa de Pós-graduação em Botânica da Universidade Federal do Rio Grande do Sul, como parte dos requisitos para a obtenção do título de Mestre em Botânica.

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RESUMO

Habenaria é um dos maiores gêneros da família Orchidaceae, e estimativas atuais pressupoem a existência de aproximadamente 835 espécies. *Habenaria* seção *Pentadactylae* com 34 espécies é a maior entre as 14 seções do gênero existente no novo mundo e compreende um conjunto de espécies morfologicamente bastante heterogênea. A fim de investigar a monofilia da seção e sua relação com outras seções do gênero, foram executadas análise Bayesiana e de Máxima Parcimônia com o emprego de um marcador nuclear (ITS) e três marcadores plastidiais (*matK*, intron *trnK*, *rps16-trnk*). Os resultados demonstraram que a seção *Pentadactylae* é altamente polifilética. Baseado nas análises filogenéticas e reavaliação de caracteres morfológicos, a seção *Pentadactylae* foi recircunscrita neste trabalho e sete espécies são aceitas: *H. dutraei*, *H. ekmaniana*, *H. exaltata*, *H. henscheniana*, *H. megapotamensis*, *H. montevidensis* e *H. pentadactyla*, enquanto outras 32 espécies foram excluídas. *Habenaria crassipes* é reconhecida como um sinônimo de *H. exaltata*. Lectótipos são designados para *H. crassipes* e *H. recta*. Todas as espécies da seção habitam pântanos ou locais bastante úmidos; com área de distribuição passando pelo norte da Argentina, Uruguai, Paraguai, sul, sudeste e centro do Brasil. O estado do Rio Grande do Sul (sul do Brasil), possivelmente, constitui um centro de diversidade da seção onde todas as espécies podem ser encontradas. A biologia reprodutiva de duas espécies da seção *Pentadactylae*, *H. megapotamensis* e *H. montevidensis*; e duas espécies da seção *Macroceratitae*, *H. johannensis* e *H. macronectar*, foram estudas. Todas as espécies estudadas oferecem néctar como recompensa floral aos polinizadores, produzido no interior de um prolongamento do labelo denominado esporão. *Habenaria montevidensis* é polinizada por borboletas da família Hesperiidae, enquanto as demais espécies são polinizadas por mariposas da família Sphingidae. Todas as espécies estudadas são auto-compatíveis mas dependentes de agentes polinizadores para a produção de frutos. O sucesso reprodutivo é alto (69,48 - 93%). Na área de estudo, todas as quatro espécies estudadas são reprodutivamente isoladas devido a um conjunto de fatores tais como diferenças na morfologia floral e diferentes polinizadores.

Palavras-chave- Análises filogenéticas, análise Bayesiana, Máxima parcimônia, seção *Pentadactylae*, revisão taxonômica, polinização, Sphingidae, Hesperiidae, morfologia floral, sistema reprodutivo.

ABSTRACT

Habenaria is one of the largest genus of Orchidaceae family and current stimates accounts to the existence of 835 species. *Habenaria* section *Pentadactylae* with 34 species is the largest among the 14 New World sections of the genus and comprises a morphologically heterogeneous group of species. To investigate the monophyly of the section and the relation with other sections of the genus, Bayesian and parsimony analyses using one nuclear marker (ITS) and three plastid markers (*matK*, *trnK* intron, *rps16-trnK*) were performed. The results demonstrated that sect. *Pentadactylae* is highly polyphyletic. Based on the phylogenetic analyses and re-evaluation of morphological characters, *Habenaria* sect. *Pentadactylae* is re-circumscribed and seven species are accepted for the section: *H. dutraei*, *H. ekmaniana*, *H. exaltata*, *H. henscheniana*, *H. megapotamensis*, *H. montevidensis* and *H. pentadactyla*, while other 32 species were excluded. *Habenaria crassipes* is included under the synonym of *H. exaltata*. Lectotypes are designated for *H. crassipes* and *H. recta.* All species in the section are from marshes or wet grasslands and range from Northern Argentina, Uruguai, Paraguai and south, southeast and center of Brazil. The Rio Grande do Sul state (south Brazil), possibly constitute a diversity center of the section where every species can be founded. Most are rare, known by few populations, and threatened due to loss of habitat and population decline. The reproductive biology of two species from the section *Pentadactylae, H. megapotamensis* and *H. montevidensis*; and two species from the section *Macroceratitae*, *H. johannensis* and *H. macronectar,* were studied. All studied species offer nectar as floral reward concealed in a labellar process termed spur. *Habenaria montevidensis* is pollinated by Hesperiidae butterflies, while the remaining species are pollinated by Sphingidae moths. All studied species are self-compatible, but pollinator-dependent. The reproductive success is high (69.48 - 93%). At the study site, every four studied species are reproductively isolated by a set of factors that includes differing floral morphologies and different pollinators.

Keywords- Phylogenetic analysis, Bayesian analysis, maximum parcimony, section *Pentadactylae*, taxonomic revision, pollination, Sphingidae, Hesperiidae, floral morphology, breeding system.

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1. INTRODUÇÃO

Orchidaceae, com aproximadamente 22.000 espécies, compreende cerca de 12% de todas as Angiospermas. No Brasil, está representada por cerca de 2.650 espécies, distribuídas em 205 gêneros (GIULIETTI et al., 2005).

Orchidaceae possui representantes em quase todas as regiões do mundo, com exceção da Groenlândia e regiões árticas e antárticas, mas a maior diversidade está concentrada nos trópicos, especialmente em áreas montanhosas (DRESSLER, 1993). A família compreende três formas de vida distintas: espécies terrestres, espécies epífitas e espécies rupícolas. As espécies terrestres nascem sobre o solo e, em geral, são mais comuns em climas mais frios e temperados e estão menos representadas nos trópicos. As espécies epífitas e rupícolas vivem sobre ramos de árvores ou rochas, habitando exclusivamente climas temperados e quentes, e estando ausentes em regiões muito frias (PABST & DUNGS, 1975). A família possui uma série de atributos florais que torna fácil sua identificação. As flores são caracteristicamente zigomorfas e, em geral, hermafroditas. O perianto é composto por dois verticilos trímeros, em geral, vistoso. A pétala mediana é bastante diferenciada das demais sendo, por esse motivo, denominada de labelo. O labelo, frequentemente, apresenta glândulas (nectários, glândulas de óleo, osmóforos, etc.) ou ornamentações (calos) com funções relacionadas ao processo de polinização. Na maioria das orquídeas, o ovário ou o pedicelo sofrem uma torção de 180º no período anterior à antese (flores ressupinadas) permitindo que o labelo, originalmente na porção superior da flor, ocupe a posição inferior podendo, dessa forma, atuar como plataforma de pouso para os polinizadores. O androceu é constituido por 1 antera fértil (mais raramente 2 ou 3) e encontra-se fusionado ao gineceu formando uma estrutura denominada coluna. Na grande maioria das orquídeas, os grãos de pólen encontram-se aglutinados em massas denominadas políneas. As políneas, em geral, são conectadas a uma superfície adesiva denominada viscídio ou retináculo, através de uma projeção, o caudículo. O conjunto formado pela polínea, pelo caudículo e viscídio denomina-se polinário. O ovário é infero, formado por três carpelos fusionados, e abriga numerosos rudimentos seminais. As sementes são adaptadas à dispersão anemófila e carecem de endosperma. A germinação se dá através de um processo simbiótico com um fungo, o qual se prolonga durante toda a vida da planta (JUDD et al., 2007).

A monofilia de Orchidaceae é sustentada por caracteres morfológicos e moleculares (BURNS-BALOGH & FUNK, 1986; DRESSLER, 1981, 1993; DRESSLER & CHASE, 1995; CHASE et al., 2000; FAY et al., 2000; FREUDENSTEIN et al., 2004; JUDD et al., 1993). Análises filogenéticas baseadas em caracteres morfológicos e moleculares sustentam a divisão da família Orchidaceae em cinco subfamílias (BURNS-BALOGH & FUNK, 1986; CAMERON, 2006; CAMERON & CHASE, 2000, CAMERON et al., 1999, DRESSLER, 1986, 1993; DRESSLER & CHASE, 1995; FREUDENSTEIN & RASMUSSEN, 1999; FREUDESTEIN et al., 2000, 2004; JUDD et al., 1993; MOLVRAY et al., 2000; VAN DER BERG et al., 2005), as quais são atualmente aceitas (Fig. 1). Apostasioideae constitui o grupo irmão das demais subfamílias, e retém caracteres ancestrais tais como flores com dois ou três estames ligeiramente adnados ao estilete, e pólen solto. Cypripedioideae se caracteriza pelo labelo saculiforme, pela presença de duas anteras férteis e um estame transformado em estaminódio. Nessa subfamília o pólen é aglutinado mas não ocorre a formação de políneas verdadeiras. Esta última característica citada é compartilhada também pela subfamília Vanilloideae a qual, diferentemente de Cypripedioideae, apresenta somente uma antera fértil. Orchidoideae e Epidendroideae apresentam somente uma antera fértil e as mássulas polínicas estão reunidas em políneas. Orchidoideae difere de Epidendroideae por apresentar polínias quebradiças, cujo conteúdo pode ser espalhado em diferentes flores durante as visitas dos polinizadores. Em contrapartida, em Epidendroideae as políneas são duras e, por esse motivo, todo o conteúdo de mássulas polínicas de um polinário é depositado numa única flor durante a atividade dos polinizadores (JUDD et al., 2007, SINGER, 2004) .

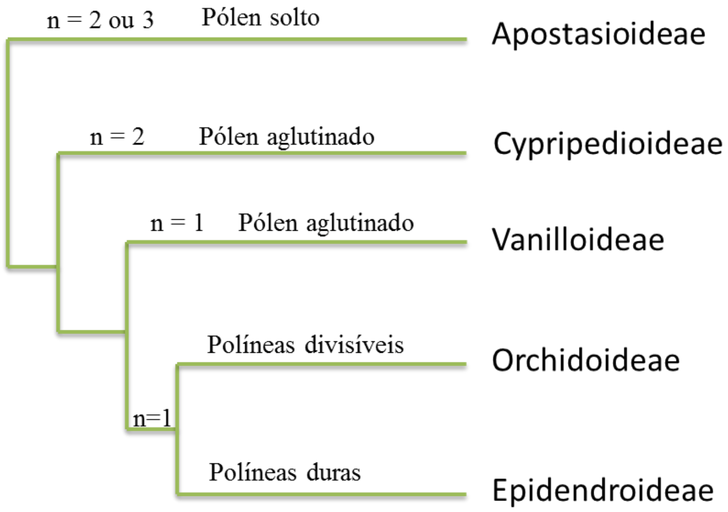

Figura1. Relações filogenéticas entre as subfamílias de Orchidaceae (modificado de CAMERON et al., 1999; SINGER, 2004; SINGER et al., 2008). n = nº de anteras férteis.

O gênero *Habenaria* Willd. é o maior gênero da subfamília Orchidoideae com aproximadamente 835 espécies (GOVAERTS et al., 2010), sendo o maior gênero de orquidáceas terrestres como um todo (BATISTA et al., 2006). O gênero está distribuído pelas diversas regiões temperadas e pantropicais do globo, tendo como principais centros de diversidade, o Brasil, a África central e do sul, e o leste da Ásia (KURZWEIL & WEBER, 1992). No Brasil, o gênero está representado por aproximadamente 170 espécies (HOEHNE, 1940), sendo que o principal centro de diversidade se concentra no bioma cerrado (PABST & DUNGS, 1975; RATTER et al., 1997).

As principais características distintivas de *Habenaria* são as pétalas frequentemente bífidas e não fusionadas a outros órgãos, o labelo geralmente tripartido e desprovido de calosidades; estigmas pedunculados, e lobos do estigma inteiros, que são geralmente livres e não adnados às pétalas (PRIDGEON et al., 2001). Além disso, as flores caracterizam-se pela profunda divisão da antera que, em última instância, resulta na formação de dois polinários separados (SINGER & COCUCCI, 1997).

Kraenzlin (1892) reconheceu 32 seções dentro do gênero *Habenaria* baseado em caracteres morfológicos, especialmente, o grau de divisão das pétalas e labelo, bem como, a estrutura da coluna. *Habenaria* seção *Pentadactylae* foi estabelecida por Kraenzlin (1892) em sua primeira revisão do gênero, o qual atribuiu 24 espécies à seção e caracterizou-a por possuir caule folhoso, folhas basais maiores que as demais, flores com labelo tripartido, pétalas partidas com a parte anterior do mesmo comprimento do labelo, bem como processo estigmático curto. Em linhas gerais, Cogniaux (1893) seguiu a delimitação de seções propostas por Kraenzlin. De acordo com a delimitação proposta por Cogniaux, a seção *Pentadactylae* passou a incluir espécies caracterizadas por apresentarem flores pequenas, glabras, com os segmentos laterais das pétalas e do labelo, em geral, filiformes; e com o segmento anterior das pétalas mais longo do que o segmento posterior. Desse modo, Cogniaux (1893) incluiu mais 16 espécies brasileiras na seção e distribuiu em outras seções algumas das espécies originalmente propostas por Kraenzlin. Em sua última revisão do gênero publicada em 1901, Kraenzlin atribuiu 34 espécies para a seção. No entanto, os tratamentos apresentados por Kraenzlin (1892, 1901) e Cogniaux (1893) para o reconhecimento das seções são claramente artificiais. Consequentemente, a maioria das seções compreende taxa não relacionados, enquanto espécies mais proximamente relacionadas estão dispersas em diferentes seções. Neste sentido, faz-se necessária a determinação das relações filogenéticas das espécies inseridas dentro das seções compreendidas no gênero *Habenaria.*

As características florais da maioria das espécies do gênero *Habenaria*, tais como a coloração branca ou esverdeada, a presença de néctar em um prolongamento do labelo (calcar ou esporão) e a fragrância crepuscular ou noturna; sugerem síndrome de polinização por mariposas (SINGER & COCUCCI, 1997). A maioria dos relatos indica diferentes espécies de Lepdoptera como polinizadores das espécies do gênero (SINGER et al., 2007), embora Singer (2001) demonstrou que *H. parviflora* Lindl. é polinizada por mariposas (Pyralidae) e dípteros (Tipulidae). O polinário pode aderir em diferentes partes do corpo do polinizador. Assim, os polinários de *H. pleiophylla* Hoehne & Schlechter e *H. gourlieana* Gillies ex. Lindl. aderem nos olhos (SINGER et al., 2007; SINGER & COCUCCI, 1997); os de *H. epipactidea* Rchb. f., nas patas dianteiras (PETER et al., 2009); e os de *H. parviflora* aderem na probóscide dos polinizadores (SINGER, 2001). Embora haja alguns estudos visando a determinação da biologia floral e reprodutiva de espécies de *Habenaria* (Singer e Cocucci 1997; Peter et al. 2009; Singer et al. 2007; Singer 2001), nosso conhecimento é ainda incipiente, especialmente em relação as espécies Neotropicais.

2. OBJETIVOS GERAIS

- Redescrever e circunscrever *Habenaria* seção *Pentadactylae* através do emprego de análises filogenéticas moleculares e dados morfológicos.
- Documentar a biologia floral e reprodutiva de *Habenaria johannensis* Barb. Rodr., *Habenaria macronectar* (Vell.) Hoehne, *Habenaria megapotamensis* Hoehne e *Habenaria montevidensis* Spreng.

3. CAPÍTULO 1

Molecular Phylogenetics and Taxonomic Revision of *Habenaria* Section *Pentadactylae* (Orchidaceae)

Artigo a ser submetido ao periódico Systematic Botany. Este capítulo está estruturado seguindo as normas de formatação referentes ao respectivo periódico, exceto o alinhamento.

Molecular Phylogenetics and Taxonomic Revision of *Habenaria* Section *Pentadactylae* (Orchidaceae)

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Abstract—*Habenaria* section *Pentadactylae* with 34 species is the largest among the 14 New World sections of the genus and comprises a morphologically heterogeneous group of species. To test the monophyly of the section and the relation with other Neotropical sections of the genus, Bayesian and parsimony analyses using nucleotide sequences from one nuclear (ITS) and three plastidial (*matK*, *trnK* intron, *rps16-trnK*) DNA regions were performed. Our results show that *H.* sect. *Pentadactylae* is polyphyletic. Based on the phylogenetic analyses and re-evaluation of morphological characters, *H.* sect. *Pentadactylae* is re-circumscribed and seven species are accepted for the section: *H. dutraei*, *H. ekmaniana*, *H. exaltata*, *H. henscheniana*, *H. megapotamensis*, *H. montevidensis* and *H. pentadactyla*, while other 32 species were excluded. There are no unambiguous diagnostic morphological synapomorphies for the section, which is re-circumscribed based on a combination of diagnostic characters. *Habenaria montevidensis* is morphologically distinct from other species in the section and from all other Neotropical species, apparently as a result of pollinator shift, since this species is pollinated by butterflies in contrast to other species that have flower syndromes associated to pollination by nocturnal moths. *Habenaria crassipes* is included under the synonym of *H. exaltata*. Lectotypes are designated for *H. crassipes* and *H. recta.* All species in the section are from marshes or wet grasslands and range from Northern Argentina to central Brazil, but are concentrated in Southern Brazil, particularly in the state of Rio Grande do Sul. Most are rare, known by few populations, and threatened due to loss of habitat and population decline.

Keywords—Phylogeny, ITS, *matK/trnK*, *rps16-trnK*, sectional classification.

INTRODUCTION

Habenaria Willd. (tribe Orchideae, subtribe Habenariinae) is a large genus of terrestrial orchids and current counts estimate that around 835 species are comprised into the genus (Govaerts et al. 2010). Geographical distribution range of *Habenaria* include tropical, subtropical and temperate regions of the Old and New World (Pridgeon et al. 2001; Batista et al. 2011). Its main diversity centers are found in Central and Southern Africa, Eastern Asia and Brazil (Kurzweil and Weber 1992). Brazilian flora is especially rich in *Habenaria* species making about one-fourth of all species known into the genus (Hoehne 1940; Pabst and Dungs 1975; Batista et al. 2011). *Habenaria* species are characterized by often bifid petals, tripartite lip, long rostellar arms, stalked stigmas and the presence of a well-developed nectariferous spur (Dressler 1993; Pridgeon et al. 2001). Furthermore, the flowers are characterized by the deeply division of the anther that, ultimately, results in the formation of two separated pollinaria (Singer and Cocucci 1997).

The integrity of *Habenaria* as a genus has by long been questioned. The genus was first dismembered by Rafinesque (1837), but his work was not well accepted by the scientific community. At one time, several taxa such as *Bonatea* Willd., *Centrostigma* Schltr., *Platycoryne* Rchb. f., *Roeperocharis* Rchb. f. and *Kryptostoma* Geerinck have been either treated as sections of *Habenaria* or segregated to generic level. More recently, Szlachetko recognized three genera, *Bertauxia* Szlach., *Kusibabella* Szlach. (Szlachetko 2004a, 2004b) and *Habenella* Small (Szlachetko and Kras 2006), which were segregated from the New World *Habenaria*. However, this has been undertaken on a piecemeal basis based entirely on floral morphological characters and his genera have not been accepted by most botanists. On the other hand, a phylogenetic analysis of Orchidinae Verm. and selected Habenariinae Benth. using ITS sequence data revealed that *Habenaria* is highly polyphyletic (Bateman et al. 2003), and these authors envisioned an extensive dismantling of *Habenaria* in smaller monophyletic genera. However, only eight species of *Habenaria* were sampled (about 1% of the genus), including only one from the New World, and a more comprehensive sampling is needed before any taxonomic decisions can be made.

The only world-wide revisions of the genus were those presented by Kraenzlin (1892, 1901) in which about 427 species and 32 sections were recognized. Kraenzlin sectional classification was based on morphological characters, especially the degree of dissection of the petals and lip and on gynostemium structure, particularly the length of the stigmas. *Habenaria* sect. *Pentadactylae* was established by Kraenzlin (1892) in his first revision of the genus and characterized by the leafy stem, with the largest leafs concentrated at the base; bipartite petals, with the anterior segment equal in length to the lip segments; tripartite lip; and short or very short stigmatic processes. Kraenzlin (1892) attributed 24 species to sect. *Pentadactylae* (Table 1). Cogniaux (1893), in his review of the genus in *Flora Brasiliensis*, followed, in general lines, the sectional delimitation proposed by Kraenzlin (1892), but added the following characters in the characterization of the section: flowers frequently small, glabrous, lateral segments of the petals and lip filiform, rarely linear, and anterior segment of the petals longer than the posterior segment. Cogniaux (1893) also included more 16 species and excluded eight species from the section (Table 1). Kraenzlin, in his second and last revision of the genus published in 1901, finally attributed 34 species to the section (Table 1). However, an examination of the species attributed to the section *Pentadactylae* by Kraenzlin and Cogniaux reveals it constitutes a highly heterogeneous assemblage of species, raising doubts about the monophyly of the sections and the consistence of the characters used for sectional delimitation of the genus. Here we take advantage of a molecular phylogenetic analysis of the genus that is underway (Batista, pers. comm.), using ITS and *matK* sequence data, and which includes a large sampling of Neotropical species. We have expanded the sampling of species in sect. *Pentadactylae* and used additional DNA regions (*matK/trnK*, *rps16-trnK*) to verify if *Habenaria* sect. *Pentadactylae* is monophyletic. Based on the phylogenetic analyses we evaluate the morphological characters used to characterize the section and present a taxonomic revision of the section.

MATERIALS AND METHODS

Taxonomic Sampling for the Phylogenetic Analyses—A total of 77 terminals in nine genera were sampled. These included 58 samples (46 species) of Neotropical *Habenaria*, seven species of African *Habenaria* and 12 species in the genera *Bonatea*, *Cynorkis* Thouars, *Disa* P.J. Bergius, *Gennaria* Parl., *Orchis* L., *Platanthera* Rich., *Satyrium* L. and *Stenoglottis* Lindl. from the Old World. Of the 40 taxa assigned at one time or another to sect. *Pentadactylae* by Kraenzlin (1892, 1901) and Cogniaux (1893) (Table 1), 27 were sampled. The sampling of Neotropical taxa included representatives of most Neotropical sections, excluding the monotypic sect. *Pycnostachyae*; and taxa from all highly supported terminal clades identified in previous molecular analyses (Batista, pers. comm.). Whenever possible, for the species assigned in this work to sect. *Pentadactylae*, we sampled more than one population in order to evaluate possible intra-specific variation. GenBank sequences from representative species of the tribe Diseae (*Disa*) and Old World species from subtribe Orchidinae (*Orchis*) were used as outgroups.

Molecular Markers—Nucleotide sequences from one nuclear genome region (ITS) and three plastidial (*matK*, *trnK* intron and *rps16*-*trnK*) genome regions were used in the analyses. The nuclear region (nrITS) consists of the internal transcribed spacers (ITS1 and ITS2) and the intervening gene 5.8S of the nuclear ribosomal multigene family. Amplifications were done with the primers 17SE and 26SE (Sun et al. 1994). The plastid DNA regions included the complete sequences of gene *matK* and *trnK* 3’ intron, part of the *trnK* 5’ intron flanking the *matK* gene, and the *rps16*-*trnK* intergenic spacer. The *matK* gene and flanking regions of *trnK* intron were amplified in two reactions: the 3’ end of the *trnK* 5’ intron and the upstream part of *matK* gene with primers -19F (Molvray et al. 2000) and matk356R (5’-AATCGCAACAAATGCAAA-3’), and the remaining part of *matK* gene and complete *trnK* 3’ intron with primers matkF2 (5’-CTAATACCCCATCCCATCCAT-3’) and 2R (Steele and Vilgalys 1994). The *rps16-trnK* intergenic spacer was amplified with primers rpS16x2F2 and trnK(UUU)x1 (Shaw et al. 2007).

DNA Sequences—Genomic DNA was extracted from fresh or silica gel-dried material using the 2× CTAB method adapted from Doyle and Doyle (1987). PCR amplifications were performed in a MJ96G (Biocycler) or Eppendorf Mastercycler Thermal Cycler. The general PCR system consisted of 2–3 μl genomic DNA (about 20–50 ηg of DNA), 1X PCR Buffer (Phoneutria Biotec., Belo Horizonte, Brazil), 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.2 μM of each primer, 1.5 U of Taq DNA Polymerase (Phoneutria Biotec., Belo Horizonte, Brazil) and water in a volume of 30 μl. Cycling conditions were: an initial denaturation at 94 ºC for 4 min, 35 cycles of 94 ºC for 45 s, 58 ºC for 45 s, 72 ºC for 80 s, and a final extension of 5 min at 72 ºC. PCR products were purified by precipitation with polyethylene glycol and sequenced in an Automatic Sequencer 3730XL by Macrogen Inc. (Korea). Sequencing primers were the same used in the amplifications. Bidirectional sequence reads were obtained for all the DNA regions, and the resulting chromatograms were edited and assembled using the Staden Package software (Bonfield et al. 1995). The edited sequences were aligned with MUSCLE (Edgar 2004) and the resulting alignment was manually adjusted using the MEGA4 software (Tamura et al. 2007).

Phylogenetic Analyses—In order to have independent phylogenetic estimates, the data were analyzed with both parsimony and Bayesian inference. Phylogenetic analyses using maximum parsimony (MP) were performed in PAUP version 4 (Swofford 1998) with Fitch parsimony (equal weights, unordered characters; Fitch 1971) as the optimality criterion. Searches were initially performed on each data set separately and the combined plastid sequences. Since no cases of strongly supported incongruence were detected (i.e. no conflicting groups among the two data sets obtaining high internal support were observed), a third search was performed with the combined nuclear and plastid datasets. Each search consisted of 1,000 replicates of random taxon addition with branch swapping using the TBR (tree-bisection and reconnection) algorithm, saving only up to ten trees per replicate to avoid extensive swapping on suboptimal islands. Internal support was evaluated by character bootstrapping (Felsenstein 1985) using 1,000 replicates, simple addition and TBR branch swapping, saving up to ten trees per replicate. For bootstrap support levels, we considered bootstrap percentages (BP) 50–70% as weak, 71–85% as moderate and > 85% as strong (Kress et al. 2002).

Bayesian analysis of individual and combined datasets was implemented in MrBayes v. 3.1.2 (Ronquist and Huelsenbeck 2003), treating each DNA region (ITS, *matK*, *trnK* intron and *rps16-trnK*) as separate partitions. An evolutionary model for each DNA region was selected using the AIC criterion in Modeltest 3.7 (Posada and Crandall 1998). Each analysis consisted of two independent runs with four chains for 3,000,000 generations, sampling one tree every 100 generations. In the combined analysis, in order to improve swapping of chains, the temperature parameter for heating the chains was lowered to 0.05. Convergence between the runs was evaluated by the average standard deviation of split frequencies (<0.01). After discarding the first 25% of the trees as burn-in, the remaining trees were used to assess topology and posterior probabilities (PP) in a majority-rule consensus. Because PP in Bayesian analysis are not equivalent to BP, but are generally much higher (Erixon et al. 2003), we used criteria similar to a standard statistical test, considering groups with PP > 95% as strongly supported, PP ranging from 90–95% as moderately supported and PP < 90% as weakly supported.

Taxonomic Analyses—Descriptions were based on pickled or herbarium material, but live material was also extensively examined in fieldworks by the authors. Habit was examined from herbarium material, perianth from dried and pickled material, and gynostemium details primarily from pickled material. Details of the flowers, particularly the gynostemium, were examined under a stereoscopic microscope and measured with a digital paquimeter. Data about flowering times, habitats, and distribution were based on herbarium labels of collections or on field observations. Dried materials were rehydrated using concentrate ammonium hydroxide (35% (w/v) ammonia solution) as described by Toscano de Brito (1996). A total of 213 materials and images were examined from the following herbaria: AMES, B, BR, BHCB, CESJ, CORD, G, HB, IAC, ICN, K, L, LP, MBM, NY, OXF, P, PACA, PEL, R, RB, S, SI, SP, SPF, U, UPS, UB, UC, UPCB, US, Z and ZT. Descriptive terminology is based in Stearn (1992) and Simpson (2006).

RESULTS

The ITS and *matK* datasets had 77 terminals, while the *trnK* intron and *rps16-trnK* datasets had 58 terminals; of these 9 ITS sequences, 56 *rps16-trnK* sequences and 58 *matK/trnK* sequences were newly generated for this study. The remaining sequences were obtained from GenBank. For the Old World species the *matK/trnK* matrix was included only part of the *matK* gene, corresponding approximately to the same region used for DNA bar-coding of plants. For these taxa the *trnK* introns and the remaining part of *matK* gene were coded as missing data. The *rps16-trnK* matrix included only New World species, since DNA material from Old World taxa was not available for the amplifications and was coded as missing data. Other data-matrix values from the individual or combined data set as well as tree statistics and other parameters of the parsimony analyses are shown in Table 2.

The Bayesian majority-rule consensus tree was fully congruent with the strict consensus tree of the combined parsimony analyses, but was more resolved and with stronger overall support. Since the Bayesian majority-rule consensus tree of the combined data sets is, overall, better resolved and supported than either of the other analyses, it is the tree that best represents our phylogenetic hypothesis and was chosen for presentation and discussion (Fig. 1). New World *Habenaria* species formed a well-supported monophyletic group (1.00 PP, 93% BS. Fig. 1). The African species *H. tridens* Lindl. is sister to the Neotropical clade (1.00 PP, 100% BS), while the clade formed by *H. dives* Rchb.f., *H. clavata* Rchb.f. and *H. lithophila* Schltr. (1.00 PP, 100% BS), all from Africa, is sister to the *H. tridens*/Neotropical clade (1.00 PP, 100% BS, Fig. 1). *Bonatea* species (all African) formed a clade with other African *Habenaria* (0.99 PP), which was sister to the large clade formed by African and Neotropical species (0.89 PP, Fig. 1). The monospecific genus *Gennaria* (Canarien, west and central Mediterranean) was sister to the previous clades (1.00 PP), while *Cynorkis* was sister to the whole Habenariinae clade (0.99 PP; Fig. 1).

Within the New World clade several well supported terminal sub-clades were formed (Fig. 1). To ease comparison of these sub-clades with the sectional classifications of Kraenzlin (1892, 1901) and Cogniaux (1893), the sectional assignment for each taxa is indicated after the species' name in Fig. 1. In general, morphologically similar species were grouped in the same sub-clades (Fig. 1), but the correspondence between the sub-clades and the sectional classifications of Kraenzlin (1892, 1901) and Cogniaux (1893) is small. The morphologically similar species *H. johannensis* Barb. Rodr. and *H. macronectar* (Vell.) Hoehne formed a well-supported sub-clade (1.00 PP, 99% BS), as did the species pair *H. parviflora* Lindl. and *H. edwalli* Cogn. (1.00 PP, 100% BS), *H. balansae* Cogn. and *H. melanopoda* Hoehne & Schltr. (0.99 PP, 60% BS), and *H. repens* Nutt. and *H. aranifera* Lindl. (1.00 PP, 100% BS). The morphologically similar species *H. paranaensis* Barb. Rodr., *H. achalensis* Kraenzl. and *H. secunda* Lindl. formed a highly supported sub-clade (1.00 PP, 100% BS), but these species were placed in sections *Micranthae*, *Clypeatae* and *Pentadactylae*, respectively, in the sectional classifications of Kraenzlin and Cogniaux. Similarly, the species pairs *H. rupicola* Barb. Rodr. and *H. hexaptera* Lindl., *H. hamata* Barb. Rodr. and *H. leptoceras* Hook., *H. mystacina* Lindl. and *H. setacea* Lindl., *H. crucifera* Barb.Rodr. and *H. pungens* Cogn., formed well-supported sub-clades, but were placed in different sections by Kraenzlin (1892, 1901) and Cogniaux (1893).

Species assigned to sect. *Pentadactylae* by Kraenzlin (1892, 1901) and Cogniaux (1893) are dispersed in several sub-clades of the tree (Fig. 1) and the section is thus highly polyphyletic. However, *H. pentadactyla* Lind., the type of sect. *Pentadactylae*, forms a highly supported subclade (1.00 PP, 53% BS) with *H. ekmaniana* Kraenzl., *H. montevidensis* Spreng. and *H. henscheniana* Barb.Rodr., which is sister, with moderate support (0.91 PP, 72% BS), to a highly supported sub-clade (1.00 PP, 100% BS) formed by *H. megapotamensis* Hoehne, *H. exaltata* Barb.Rodr., *H. dutraei* Schltr. and *H. amambayensis* Schltr. (Fig. 1). This clade containing *H. pentadactyla* is sister with high support in the Bayesian analysis (0.95 PP) to a clade formed by *H. repens* and related species such as *H. aranifera* and *H. warmingii* Rchb. f. & Warm. These clades, together with *H. regnellii* Cogn., *H. johannensis*, *H. macronectar* and *H. macilenta* Rchb. f. form a highly supported clade (1.00 PP, 84% BS), that is sister (0.95 PP) to the clade formed by *H. secunda* and closely related species, *H. parviflora*/*H. edwallii*, and *H. leucosantha* Barb. Rodr. (Fig. 1).

DISCUSSION

In our phylogenetic analysis, the sampled *Habenaria* formed two major clades: one formed by all Neotropical species plus some African species in sections *Dolichostachyae*, *Podandria*, *Bilabrellae*, *Ceratopetalae*, and *Diphyllae*; and other formed by *Bonatea* and species in sections *Chlorinae* and *Multipartitae* (Fig. 1). In the Bayesian analysis of the combined dataset these two clades were sister, but support was low and the relation between them is not resolved (Fig. 1). Determining the exact generic limits of *Habenaria* is beyond the scope of this work, but it is clear that recognition of *Bonatea* at the generic level turns *Habenaria* paraphyletic. In the results of Batemam et al. (2003) Asian species of *Habenaria* grouped with *Pecteilis* Raf. (Asian) and *Herminium* L. (Euro–Asian), while African species and a single Neotropical species were grouped with *Bonatea* (African) and *Gennaria* indicating that, at least for a number of Asian taxa, some taxonomic rearrangements will be necessary in the genus.

On the other hand, in our phylogenetic analyses the Neotropical *Habenaria* species formed a well-supported clade (Fig. 1) and its subdivision in smaller genera does not seem justified. Additionally, the type species of the genus (*H. macroceratitis* Willd. from Jamaica) is from the New World, and the Neotropical clade is the group to which the name is fixed. Despite of the monophyly of New World *Habenaria*, our phylogenetic analyses shows that the currently recognized sections are polyphyletic and will need extensive revision and re-circumscription (Fig. 1). Our results show clearly that sect. *Pentadactylae* is polyphyletic and needs to be re-evaluated (Fig. 1). Here, based on the phylogenetic analyses and a re-evaluation of morphological characters we propose a new circumscription for *Habenaria* sect. *Pentadactylae* including *H. dutraei*, *H. ekmaniana*, *H. exaltata*, *H. henscheniana*, *H. megapotamensis*, *H. montevidensis* and *H. pentadactyla*, and excluding other 32 species or names assigned to the section by previous authors (Kraenzlin 1892, 1901; Cogniaux 1893; Table 1). Of these species only *H. pentadactyla* and *H. exaltata* were previously assigned to the sect. *Pentadactylae* (Table 1). *Habenaria henscheniana* was assigned by Cogniaux (1893) and Kraenzlin (1901) to sect. *Pratenses* Kraenzl., *H. montevidensis* was placed in sect.

Quadratae Kraenzl. by Kraenzlin (1892) and transferred to sect. *Microstylinae* by Cogniaux (1893), *H. ekmaniana* was placed in sect. *Macroceratitae* by Kraenzlin (1911), while *H. megapotamensis* and *H. dutraei* were described after the above mentioned publications of Kraenzlin and Cogniaux and never assigned any formal sectional classification. In the phylogenetic analyses the support values for the clade were not high (0.91 PP, 72% BS; Fig. 1). Nevertheless, six species in the clade, *H. dutraei*, *H. ekmaniana*, *H. exaltata*, *H. henscheniana*, *H. megapotamensis* and *H. pentadactyla* share a series of similar features in morphology, type of habitat and geographic distribution, and form a homogeneous, consistent group.

The inclusion of *H. montevidensis* in the group and the subclade formed with *H. henscheniana* (0.99 PP; Fig. 1) was unexpected, since *H. montevidensis* displays a distinct set of floral features not shared with the others members of *Pentadactylae* section, nor with any other Neotropical species of the genus. The main distinctive characters of *H. montevidensis* are the reflexed, convex dorsal sepal and orbicular, unguiculate petals, which overlap one another and form a hood over the column (Fig. 9B). In all other Neotropical species it is the non-reflexed, concave, dorsal sepal that covers the column (Figs. 4B and D, 7B and D, 9D). While the reflexed dorsal sepal is common in some African species of the genus and one of the main diagnostic characters of *H.* sect. *Replicatae* Kraenzl., the morphology of *H. montevidensis* is unique among Neotropical *Habenaria* and we are unaware of any species in the genus with the above mentioned set of characters. In addition, *H. montevidensis* has completely white petals and lip, simple petals and lacks the projection of the stigmatic processes (Fig. 9B), contrasting with the greenish petals and lip, bipartite petals and projections of the stigmatic processes that partially divide the spur entrance found in other members of sect. *Pentadactylae* (Figs. 4B and D, 7B and D, 9D).

Recently, we studied the pollination mechanism in two species here assigned to sect. *Pentadactylae* (Pedron et al., unpubl. data). *Habenaria megapotamensis* was pollinated by a nocturnal hawkmoth (Lepidoptera) species, consistent with floral features like greenish color, long nectariferous spur, and crepuscular to nocturnal scent emission. *Habenaria dutraei*, *H. ekmaniana*, *H. exaltata*, *H. henscheniana* and *H. pentadactyla* display similar floral features and it is likely that these species are pollinated by a mechanism similar to the one uncovered for *H. megapotamensis*. *Habenaria montevidensis*, however, was pollinated by butterflies, which is consistent with the white petals and a very faint and sweet fragrance, which is best perceived by enclosing the flowers in a vial. In view of these results, we state that the floral divergence observed in *H. montevidensis* when compared with other members of the section can be a consequence of pollinator shift. It is known that floral radiation in plants can involve shift from one mode of pollination to another and adaptation to not related pollinator groups. Ecological mechanisms underlying pollinator shifts are not yet well understood. It has been suggested that strong decline of the main pollinator, expansion of the species range where the initial pollinator is not available and mutation can result in selection to pollinator transition (Ramsey et al. 2003; Campbell 2008; Kay and Sargent 2009). The Columbine genus *Aquilegia* L. (Ranunculaceae) represents an interesting and well-studied case of floral radiation involving different pollination syndromes. It was shown a positive relation between pollinator shift and floral nectar spur into *Aquilegia* and pollinator shift is predicted to be the evolutionary force driving increasingly long nectar spur (Whittall and Hodges 2007). Another well-established example of extensive floral radiation involving pollinator shift is the African genus *Disa* (Orchidaceae) (Johnson et al. 1998). Phylogenetic analysis have shown that pollinator shift to a particular pollinator group into *Aquilegia* and *Disa* genus may have evolved many times in different clades (Johnson et al. 1998; Whittall and Hodges 2007). For example, in *Disa* moth pollinated species have at least three independently origins, and butterfly pollination evolved independently in two distantly related clades (Johnson et al. 1998). In *Habenaria*, moth pollination seems to be shared by most species but mosquitoes and crane-flies can contribute to pollination (Nilsson and Jonsson 1985; Galetto et al. 1997; Singer and Cocucci 1997; Singer 2001; Singer et al. 2007; Peter et al. 2009). Owing to the consistent presence of a set of floral features such as white or yellow-colored and diurnally-scented flowers, we suggest that most species within *Habenaria* sect. *Pratenses* Kraenzl. (sensu Cogniaux 1893) are butterfly pollinated. It is possible that pollinator shift was recurrent into the genus, like in *Aquilegia* and *Disa*, and may account as an evolutionary power to promote divergence inter phylogenetically related species.

TAXONOMIC TREATMENT

HABENARIA sect. PENTADACTYLAE Kraenzl., Bot. Jahrb. Syst., 16: 52. 1892; Cogn., Fl. Bras. (Martius) 3(4): 61. 1893; Kraenzl., Orchid. Gen. Sp. 1: 282. 1901.—TYPE: *Habenaria pentadactyla* Lindl.

Terrestrial to semi-aquatic herbs. Plants 12–149 cm, including the inflorescence. Stem erect or tending to sinuose, 4–128 × 0.1–1.5 cm wide. Leaves 5–18, spreading, largest at the lower half or the center of the stem, linear–lanceolate, ovate-lanceolate or lanceolate, 5–28 × 0.7–4.1 cm. Inflorescence 2.5–38 cm long, few to many flowered, lax to congest, spiral. Bracts ovate or ovate-lanceolate, acuminate, 11–33 × 3–10 mm, generally, shorter than the pedicellate ovary. Flowers 3–110, small to medium size; pedicellate ovary parallel or spreading from the rachis, 13.2–27 mm. ovary arched, 11–19 mm, pedicel 1.5–9.8 mm. Sepals green, mucronate, margins smooth; dorsal sepal concave or convex, when flattened, orbicular, elliptical or ovate, 3.5–8.7 × 2.4–6.6 mm; lateral sepals obliquely elliptical or elliptical, deflexed, reflexed, reclined or turned upward, 4.7–10.4 × 2.3–5.0 mm. Petals bipartite or simple, white or yellowish green, or base whitish turning greenish yellow towards the segment apices; posterior segment oblong-falcate or falcate, 3.2–7.1 × 0.9–2.9 mm, acute, connivent with the dorsal sepal or free from it but not spread; anterior segment, generally, curved laterally and facing upward, linear-filiform, inserted at the base of the posterior segment, 0.9–7.0 mm long, shorter, about the same length or longer than the posterior segment. If simple petals, obliquely orbicular, unguiculate, 7.0–10.0 × 5.0–9.0 mm, obtuse, free from dorsal sepal. Lip tripartite, white, yellowish green or base whitish turning green towards the segment apices; undivided basal part 1.0–2.4 × 1.0–2.7 mm; lateral segments pendent, straight, deflexed or reflexed, linear or slightly lanceolate, 3.0–10.5 mm long, filiform or up to 1.2 mm wide, shorter, about the same length or longer than the median segment; median segment linear or oblanceolate, straight, deflexed or reflexed, 3.5–8.3 × 0.6–2.5 mm; spur deflexed or reflexed, free or partially covered by the bracts, linear or slightly clavate, shorter, about the same size or longer than the pedicellate ovary, 11–78 mm long, base 0.5–1.0 mm wide and apex 0.9-1.6 mm wide, mostly green, if linear 0.7–1.4 mm wide. Gynostemium erect, 2.4–3.1 mm high; connective emarginate or obtuse, greenish or white; auricles fleshy, verrucose, whitish, 1.3–1.7 × 0.7–1.2 mm. Anther loci 1.3–2.5 mm high, spaced 1.4–-2.0 mm; canals short or prominent, 0.5–1.9 mm long; hemipollinaria 2, separated; pollinarium 1.7–3.8 mm long; viscidia exposed, 0.5–0.8 × 0.3–0.6 mm, spaced 0.6–2.5 mm apart; caudicles 0.8–2.4 mm long; pollinia 0.8–1.5 × 0.6–1.5 mm. Stigma lobes 2, closely parallel, 2.3–4.0 mm long; receptive surface whitish or green, concave or convex, turned frontwards, 1.1–2.0 mm long, margins thickened, inner margin with a protruding erect projection placed in front of the rostellum midlobe, which partially divides the spur entrance in two apertures, or absent. Rostellum greenish or white; mid-lobe triangular, fleshy, acute or subacute, erect, completely placed between or partially projected beyond the anther loci, 1.1–

3.0 × 1.7–2.5 mm; side-lobes parallel throughout or convergent towards the apex, 1.1–2.0 mm long.

Distribution and Conservation—One species, *H. ekmaniana*, ranges from central Brazil to northern Argentina, but most species are concentrated in southern Brazil, with some also occurring in Paraguay and Uruguay (Fig. 2). The state of Rio Grande do Sul in southern Brazil, where all seven species occur, is particularly species-rich and constitutes the center of diversity of the section. Despite of the broad distribution of some species, most species of the section are rare, known by few populations, and threatened due to loss of habitat and population decline.

Habitat and Ecology—Species in sect. *Pentadactylae* occur in swamps, wet grasslands, margins of streams or lakes or other types of humid habitats, at altitudes ranging from sea level to 2000 m. Throughout its distribution range, flowering occurs during the rainy season, from December to March. Some species from wet meadows including *H. exaltata, H. henscheniana* and *H. megapotamensis* occur usually associated with *Paepalanthus* sp. (Eriocaulaceae) or *Eryngium* spp. (Apiaceae) growing at the base of the plants or between the leaves inside the dense rosettes (Supplementary material Fig. S1A and B).

Morphology—Excluding *H. montevidensis* the species in sect. *Pentadactylae* form a morphologically uniform group. However, there are no unambiguous morphological sinapomorphies for the section and it is characterized here by a combination of characters. The main features are the leafy and usually robust plants, leaves spreading (patent), flowers with greenish color, bipartite petals, rostellar arms usually convergent toward the apices; and short stigmatic lobes which have a tooth-like process that partially divides the spur entrance in two apertures.

HABIT—Most species in the section have tall, robust stems, reaching up to 1.5 m tall including the inflorescence (Supplementary material Fig. S1A and B), except *H. montevidensis* and *H. pentadactyla* which have, in most cases, short, slender stems 21-79 and 12–45 cm high, respectively. *Habenaria henscheniana* is highly variable in size, with plants ranging from 21 to 118 cm high. The stems are always leafy with the largest leaves concentrated around the center of the stem (Supplementary material Fig. S1B), but smaller specimens of *H. pentadactyla* and *H. henscheniana* may have the leaves concentrated on the lower part of the stem. The leaves are always patent, spread, and usually lanceolate, reaching up to 28 cm long and 4.1 cm wide. In *H. dutraei*, *H. ekmaniana* and *H. megapotamensis* the leaves sheet are wide and form a bowl like structure that retains and accumulates rain water. The well developed leaves and leafy stem differentiate sect. *Pentadactylae* from species in sect. *Nudae*, characterized by the leaves reduced and adpressed to the stem.

INFLORESCENCE—Similarly to most *Habenaria*, development of the inflorescences is directly related to the size and development of the plants. In larger plants the inflorescences are usually long and many flowered, reaching up to about 100 flowers in *H. exaltata* (Fig. 4C). On the other side *H. pentadactyla* has shorter and usually few flowered (3–17) inflorescences (Fig. 9C).

FLOWERS—All species in the group have median-sized flowers, with the lateral sepals ranging from 4.7 to 10.4 mm long (Figs. 4B and D, 7B and D, 9B and D). This character separates the species in sect. *Pentadactylae* from the *H. parviflora* group, which has small flowers (lateral sepals 2–5 mm long), and from the *H. macronectar* group, which has large flowers (lateral sepals 13–30 mm long). However, species in the *H. repens* and *H. secunda* clades have flowers with similar sizes as the ones in sect. *Pentadactylae*. Flowers are mostly green, sometimes with the base of the lip and petals whitish, except *H. montevidensis* which has the petals and lip completely white. Spur size is variable. In most species it has about the same size as the pedicel and ovary, whereas in *H. exaltata* and *H. megapotamensis* it is 2–3 times longer. The pedicel is usually inconspicuous, but sometimes can reach up to 1/3 the length of the ovary.

PERIANTH—Similarly to most *Habenaria*, the sepals have approximately the same size, with the dorsal sepal wider and the lateral sepals longer. However, in *H. dutraei, H. ekmaniana* and *H. henscheniana* the lateral sepals are about twice the size of the dorsal sepal (Figs. 4B, 7B). The reflexed, convex dorsal sepal is a distinctive character for *H. montevidensis* (Fig. 9B), while the other species in the section, similarly to other Neotropical *Habenaria*, have concave not reflexed dorsal sepals, which form a hood over the column (Figs. 4B and D, 7B and D, 9D). In most species the lateral sepals are deflexed, while in *H. pentadactyla* they are only partially opened (ascending; Fig. 9D) and in *H. henscheniana* they are spreading (patent), giving the flower a very typical aspect (Fig. 7B). Petals are usually bipartite and the posterior segment oblong-falcate. The length of the anterior segment and its proportion relative to the posterior segment, which were used by Kraenzlin (1982, 1901) and Cogniaux (1893) for the characterization of the section, are variable. In *H. exaltata* and *H. henscheniana* the anterior segments are shorter and occasionally reduced to a tooth-like process (Figs. 4D, 7B), in *H. ekmaniana*, *H. megapotamensis* and *H. pentadactyla* about the same size (Figs. 7D, 9D), and in *H. dutraei* longer than the posterior segment (Fig. 4B). The exception is *H. montevidensis*, which has simple, unguiculate, orbicular petals (Fig. 9B). These are very distinctive and probably unique characters in the genus, constituting autapormorphies for this species.

GYNOSTEMIUM—Similar features found in most species of the section are the rostellar arms convergent towards the apices, with the viscidia placed closed to each other and the presence of a protruding projection in the inner margin of the stigmatic processes (Fig. 3A-F). This projection is turned upwards and touches or comes close the rostellum midlobe, dividing the entrance to the spur in two openings. Development and position of other features such as connective size, stigmatic processes length and the rostellum midlobe height and position relative to the anther loci are variable. Again the exception is *H. montevidensis* which has the rostellum arms parallel throughout and the viscidia spaced apart (Fig. 3G). However, the above mentioned characters are not unique and distinctive for sect. *Pentadactylae,* since they are also present in the *H. secunda* clade and in at least one distantly related species recently described, *H. psammophila* (Batista et al. 2010). Nevertheless, these characters promptly separate sect. *Pentadactylae* from the species in the sister group of *H. repens*.

Taxonomic Notes—The type of sect. *Pentadactylae* was not indicated by Kraenzlin (1892) when he described the section. According to article 22.6 of the International Code of Botanical Nomenclature (McNeill et al. 2006): "when the epithet in the name of a subdivision of a genus is identical with or derived from the epithet of one of its constituent species, the type of the name of the subdivision of the genus is the same as that of the species name, unless the original author of the subdivisional name designated another type". Therefore the type of sect. *Pentadactylae* is *H. pentadactyla*.

The circumscription of sect. *Pentadactylae* followed here, based on the molecular phylogenetic analysis, is markedly different from previous authors (Kraenzlin 1892, 1901; Cogniaux 1893). However, despite the different approaches, a reanalysis of the morphological characters used to characterize the sections and the morphology of the species reveals a series of mistakes and misplacements. Kraenzlin (1892) placed sect. *Pentadactylae* together with sects. *Meduaseformes, Dolichostachyae, Micranthae* and *Pratenses* in a group characterized by the leafy stems, with the largest leafs concentrated at the base. However, several of the species he assigned to the section do not have these characters. *Habenaria culicina* Rchb. f., *H. lagunae-sanctae* Kraenzl. (= *H. secundiflora* Barb. Rodr.), and *H. setacea,* for example, are characterized by the leaves reduced, adpressed to the stem, while *H. achnantha* Rchb. f., *H. corcovadensis* Kraenzl. (= *H. rodeiensis* Barb. Rodr.), *H. paivaeana* Rchb. f., *H. schomburgkii* Lindl., *H. secunda, H. setifera* Lindl. (= *H. trifida* Kunth), *H. warmingii* and even *H. pentadactyla* have the leaves distributed along the stem with the largest leaves usually at the middle. Among the above mentioned sections the distinctive character for sect. *Pentadactylae* were the lip segments very similar to the petals anterior segment. However, *H. epiphylla* Rchb. f. & Warm. (= *H. glaucophylla* Barb. Rodr.) and *H. leptoceras* have very short petals anterior segments when compared to the lip segments.

Cogniaux (1893) added a few characters in the characterization of sect. *Pentadactylae*, describing the anterior segment of the petals as longer than the posterior segment. However, similarly to Kraenzlin, many of the species he added to the section do not display these characters. *Habenaria alpestris* Cogn., *H. exaltata* and *H. janeirensis* Kraenzl. (= *H. paranaensis*), for example, have the anterior segment of the petals shorter than the posterior segment. Thus most of the discrepancies between our results and the previous sectional treatments are not due to the different data sets used in the analyses (morphology vs. DNA sequences), or to the weight given to a particular character or combination of morphological characters; but to the poor morphological characterization and misplacement of most species in the sectional treatments of Kraenzlin (1892, 1901) and Cogniaux (1893).

Several species previously assigned to sect. *Pentadactylae* by Kraenzlin (1892, 1901) and Cogniaux (1893) and sampled in the phylogenetic analyses do not belong to the same clade with *H. pentadactyla* and are excluded from the section based on the molecular analyses and differences in morphology (Fig. 1; Table 1). These include: *H. alpestris* Cogn., *H. aranifera* Lindl., *H. armata* Rchb. f., *H. caldensis* Kraenzl. *H. confusa* Cogn. (= *H. secundiflora* Barb. Rodr.), *H. corcovadensis* Kraenzl., (= *H. rodeiensis* Barb. Rodr.), *H. epiphylla* Rchb. f. & Warm.(= *H. glaucophylla* Barb. Rodr.), *H. goyazensis* Cogn., *H. graciliscapa* Barb. Rodr. (= *H. imbricata* Barb. Rodr.), *H. humilis* Cogn., *H. imbricata* Lindl., *H. janeirensis* Kraenzl. (= *H. paranaensis* Barb. Rodr.), *H. lagunae-sanctae* Kraenzl. (= *H. secundiflora* Barb. Rodr.), *H. leptoceras* Hook., *H. macilenta* (Lindl.) Rchb. f., *H. moritzii* Ridl. (= *H. armata* Rchb. f., in part), *H. muelleriana* Cogn. (= *H. macilenta* (Lindl.) Rchb. f.), *H. riedelii* Cogn. (= *H. warmingii* Rchb. f. & Warm.), *H. rupicola* Barb. Rodr., *H. schenckii* Cogn., *H. secunda* Lindl., *H. setacea* Lindl., *H. setifera* Lindl. (= *H. trifida* Kunth) and *H. warmingii* Rchb. f. & Warm.

Other species not sampled in the phylogenetic analyses are excluded based on morphological differences when compared to the species in the clade formed by *H. pentadactyla* (Table 1). *Habenaria achnantha* Rchb. f., *H. modestissima* Rchb. f. and *H. taubertiana* Cogn. are similar and related to *H. repens* and *H. aranifera*. *Habenaria ulei* Cogn. is similar and closely related to *H. parviflora*. *Habenaria paivaeana* Rchb. f. is similar to the species in the *H. secunda* clade, and according to Govaerts et al. (2010) *H. achalensis*, which was sampled in the molecular analyses, is a synonym. *Habenaria santensis* Barb. Rodr. is an obscure taxon and similar to *H. rodeiensis*. *Habenaria simillima* Rchb. f. is a synonym of *H. distans* Griseb., which is characterized by the two or three broadly lanceolate or oblong, basal leaves. *Habenaria culicina* Rchb. f. & Warm. is characterized by the leaves linear, reduced and adpressed to the stem. Although not sampled in our analyses this taxon was included in the analyses of Batista et al. (pers. comm.) and groups with *H. crucifera* and *H. pungens*. *Habenaria entomantha* (Lex.) Lindl. and *H. lactiflora* A.Rich. & Galeotti are Mexican taxa. Although not sampled in any of the molecular analyses, in the molecular phylogenetic analyses of Batista et al. (pers. comm.) other Mexican taxa morphologically similar to these species formed a very distinct, highly supported clade, which is related to the clades to which belong *H. leptoceras* and *H. distans*. *Habenaria gracilis* Lindl. is an illegitimate name and its exact identity is unknown. It is morphologically similar to *H. repens* and *H. rupicola* and apparently related to one of these species. The affinities of *H.*

candolleana Cogn. are not clear, but is clearly distinct from the species in sect. *Pentadactylae*. In flower morphology it is similar to *H. juruenensis* Hoehne, which in the analyses of Batista et al. (pers. comm.) forms a highly supported clade with *H. rupicola* and *H. hexaptera*. Lastly, the affinities of *H. schomburgkii* Lindl. also are not clear, but it is morphologically similar and apparently related to *H. repens*.

In his first description of sect. *Pentadactylae*, Kraenzlin (1892) included one species from Africa, *H. conopodes* Ridl., along with the 23 Neotropical species he assigned to the section (Table 1). Sampling of African *Habenaria* in our phylogenetic analysis is low when compared to the number of species and diversity of the genus found in Africa. Nevertheless, none of the African species sampled was internal to the Neotropical clade (Fig. 1), and differences in the DNA sequence between the African and Neotropical species were high. Thus it is not expected that other African taxa will eventually fall inside the Neotropical clade. Additionally, examination of the type material of *H. conopodes* at K Herbarium revealed that the viscidia are spaced apart and the stigma lobes do not have the projection in the inner margin, which are found in most species of sect. *Pentadactylae*. Thus, placement of this species in sect. *Pentadactylae* seems equivocal.

In our analyses, one species uncertainly placed in sect. *Pentadactylae* is *H. amambayensis*. This result was unexpected since in flower morphology this species is more similar to *H. repens*, differs from other species in sect. *Pentadactylae* in the morphology of the gynostemium and has a distinct geographic distribution, ranging from Paraguay to Central and Northern Brazil (Amapá, Mato Grosso do Sul, Mato Grosso, Pará, Rondônia and Roraima States) and Northern South America, reaching French Guiana and Guyana (Batista et al. 2008). In the phylogenetic analysis of the combined datasets this species appeared in a polytomy (0.85 PP) with *H. dutraei* and *H. exaltata*, which was placed with high support in a clade with *H. megapotamensis* (1.00 PP, 100% BS; Fig. 1). However, DNA from *H.*

amambayensis was extracted from a herbarium specimen, and only part of the plastid markers were successfully amplified and sequenced. In flower morphology *H. amambayensis* is similar to *H. repens* and related species. Similarly to *H. repens* and contrary to most species in sect. *Pentadactylae*, *H. amambayensis* lacks the stigmatic projection, and the viscidia are spaced apart. In a similar result, one of the *H. regnellii* samples for which only the plastid marker sequences were available, was placed in the *H. repens* subclade (0.97 PP, 54% BS) and not with the two other samples of the same species, which formed a highly supported subclade (1.00 PP, 98% BS, Fig. 1). Considering that the ITS was phylogenetically more informative than the plastid markers and this sequence was missing for *H. amambayensis*, the morphological similarity of *H. amambayensis* with *H. repens*, and the result that shows that the *H. pentadactyla* and *H. repens* clades are sister, we prefer not to include *H. amambayensis* in sect. *Pentadactylae* until there is more convincing evidence.

KEY TO THE SPECIES OF *HABENARIA* SECT. *PENTADACTYLAE*

1. Dorsal sepal convex, reflexed; petals and lip completely white; petal simple, orbicular, unguiculate, forming a hood-like structure over the column……….. 6. *H. montevidensis*

1. Dorsal sepal concave, not reflexed, forming a hood-like structure over the column; petals and lip mostly green or greenish yellow; petals bipartite, not unguiculate, posterior segment oblong-falcate ……………………………………………………………..…… 2

    2. Spur 11–20 mm long, slightly clavate, apex rounded …….….…….….…….………… 3

        3. Petal anterior segment shorter than the posterior segment; lip lateral segments shorter than the median segment; rostellum midlobe projected beyond the anther loci ……………………………….……..……….…...…… 4. *H. henscheniana*

3. Petal anterior segment and lip lateral segments about the same size or longer than the posterior segment and lip median segment, respectively; rostellum midlobe enclosed between the anther loci …..…………….……......…….…………..….... 4

4. Petal anterior segment and lip lateral segments longer than the posterior segment and lip median segment, respectively ……..………1. *H. dutraei*

4. Petal anterior segment and lip lateral segments about the same size as the posterior segment and lip median segment, respectively ………………..… 5

5. Plants 84–152 cm high; largest leaves ovate-lanceolate, 13–26 × 2.7–5.5 cm; lateral sepals deflexed, 3.5–4.3 mm wide ………………………….….…….………2. *H. ekmaniana*

5. Plants 12–45 cm high; largest leaves linear-lanceolate, 5.0–13.0 × 0.6–1.1 cm; lateral sepals not deflexed or reflexed, only partially opened, 2.4–3.1 mm wide ……………………..… 7. *H. pentadactyla*

2. Spur ≥ 28 mm, linear, not clavate, apex acute ………..………..…………...……….... 6

6. Spur 28–35 mm long; petals anterior segment shorter than the posterior segment; connective emarginate …......................……..… 3. *H. exaltata*

6. Spur 57–78 mm long; petals anterior segment about the same length as the posterior segment; connective obtuse …………..... 5. *H. megapotamensis*

1. HABENARIA DUTRAEI Schltr., Repert. Spec. Nov. Regni Veg. Beih. 35: 19. 1925.—TYPE: BRAZIL. Rio Grande do Sul: São Leopoldo, Fazenda dos Prazeres, Feb. 1904, *J. Dutra 675* (Holotype: B, destroyed; Lectotype, designated by Batista et al. (2011): SP [38372]; Isolectotype: SI [39894]). (Figs. 3A, 4A and B)

Habenaria schnittmeyeri Schltr., Repert. Spec. Nov. Regni Veg. Beih. 35: 23. 1925. *Habenaria schiedmeyeri* Schltr. ex Pabst, Sellowia 10: 127. 1959. *nom. nud.*—TYPE:

BRAZIL Rio Grande do Sul: São Leopoldo, Jan. 1916, *M. Schiedmeyer s.n. ex Herb. Dutra 995* (Holotype: B, destroyed; Lectotype, designated by Batista et al. (2011): SP [40501], fragment; Isotype: HB [1710]).

Semi-aquatic herb. Plants 38–95 cm, including the inflorescence. Stem erect, 28.5–77.0 x 0.4–0.7 cm. Leaves 9–15, spreading, largest at the lower half or center of the stem, ovate-lanceolate, 9–17 × 1.7–4.0 cm. Inflorescence 8–30 cm, many flowered, congest, with flowers disposed in spiral around the inflorescence axis. Bracts ovate-lanceolate, acuminate, 13–22 × 4–7 mm, generally, shorter than the pedicellate ovary. Flowers 10–62; pedicellate ovary parallel to the rachis or spreading from it, 16.4–17.0 mm; ovary arched, 13–14 mm, pedicel 2.4–4.0 mm. Sepals green, mucronate, margins smooth; dorsal sepal slightly concave, when flattened orbicular, 3.5–4.0 × 3.5–4.2 mm, margins revolute; lateral sepals obliquely elliptical, reflexed, 5.9–6.6 × 3.0–4.0 mm. Petals bipartite, base whitish, turning greenish yellow towards the segment apices; posterior segment falcate, 4.0–5.0 × 1.1–1.5 mm, acute, free from the dorsal sepal but not spread; anterior segment curved laterally and facing upward, linear-filiform, inserted at the base of the posterior segment, 5.0–5.9 mm long, about 1.1–1.4 times as long as the posterior segment. Lip tripartite, base whitish, turning green towards the segment apices; undivided basal part 1.0–1.5 × 1.0–1.1 mm; lateral segments pendent, straight or deflexed with apex facing upward, linear to slightly lanceolate, 6.2–7.4 × 0.9 mm, about 1.12–1.21 times as long as the median segment; median segment linear to oblanceolate, straight or deflexed, 5.3–6.6 × 1.0–1.2 mm; spur deflexed or reflexed, free from the bracts, slightly clavate, about the same size as the pedicellate ovary, 14–17 mm long, base 0.5–0.7 mm wide, apex 0.9 mm wide, green. Gynostemium erect, 2.4 mm high; connective emarginate, greenish; auricles fleshy, verrucose, whitish, 1.5 × 1.2 mm. Anther 1.4 mm high; canals short, 1.2 mm long; loci spaced ca. 1.9 mm; hemipollinaria 2, separated; pollinarium

2.6 mm long; viscidia exposed, 0.5 × 0.5 mm, spaced 0.8 mm apart; caudicles 1.4 mm long; pollinia 1.0 × 0.7 mm. Stigma lobes 2, closely parallel, 3.5 mm long; receptive surface whitish, convex, turned frontwards and to the sides, 1.7 mm long, margins thickened, with a protruding, erect projection that partially divides the space between the stigma lobes and the entrance to the spur in two apertures. Rostellum greenish; mid-lobe triangular, fleshy, acute, erect, completely placed between the anther loci, 1.9 × 1.7 mm; side-lobes slightly convergent towards apices, 2 mm long.

Distribution and Conservation Status—Restricted to the Eastern part of the state of Rio Grande do Sul in southern Brazil (Fig. 5). The species is known from only seven collections from four localities. A population recently found in 2011 by one of the authors is the only known extant population of the species, which remained 51 years without records. Based on the World Conservation Union Red List Categories and Criteria (IUCN 2001), *H. dutraei* can be tentatively classified as Endangered (EN: criteria B1ab(iii) + 2ab(iii); D).

Habitat and Ecology—*Habenaria dutraei* occurs in lowlands swamps. Flowering occurs from January to February.

Etymology—Named after João Dutra, collector of the type material.

Illustrations—Hoehne (1940, plate 57, probably based on the type material, *Dutra 675*; plate 48, based on the lectotype of *H. schnittmeyeri*), Pabst and Dungs (1977, pg. 266, fig. 76, as *H. schiedmeyeri*, based on Hoehne 1940, plate 48).

Taxonomic Notes—*Habenaria dutraei* is similar to *H. ekmaniana* in morphology of the flowers. However, in *H. dutraei* the lateral segments of the lip are linear and longer than the median segment and the anterior petal segment longer than the posterior segment. While in *H. ekmaniana* the lip segments are oblanceolate, with the lateral segments about the same length as the median segment, and the anterior segment of the petals is about the same length as the posterior segment.

Additional Specimens Examined—BRAZIL. Rio Grande do Sul: Osório, Fazenda do Arroio, 4 Jan 1950, *Rambo s.n*. (PACA 45101); Porto Alegre, 12 Feb 1960, *Orth 1147* (HB); Porto Alegre, Morro da Gloria, Vila Manresa, 12 or 20 Jan 1933, *Orth 579* (AMES, G, HB, K, PACA, S, SPF, US); Porto Alegre, Morro da Glória, 20 Jan 1933, *Dutra s.n.* (SP 50513); Santo Antônio da Patrulha, 20 Jan 2011, *Pedron 3* (ICN).

2. HABENARIA EKMANIANA Kraenzl., Kongl. Svenska Vetensk. Acad. Handl. 46(10): 15, t. 2, fig. 9. 1911.—TYPE: ARGENTINA. Misiones: near La Granja, 17 Dec. 1907, *E.L. Ekman 432* (Holotype: S [05-3163]; Isotypes: AMES, HBG, S, SI). (Fig. 3B)

Habenaria recta Schltr., Repert. Spec. Nov. Regni Veg. 16: 354. 1920.—TYPE: BRAZIL. Probably from São Paulo, *C. Grossmann s.n.* (Holotype: B, destroyed; no isotype located. Lectotype designated here: Fl. Bras. (Hoehne) 12(1): t. 47. 1940 [based on the holotype]).

Semi-aquatic herb. Plants 84–152 cm, including the inflorescence. Stem erect, 73–127 × 0.4–0.9 cm. Leaves 13–16, spreading, largest at the lower half or center of the stem, ovate-lanceolate, 13–26 × 2.7–5.5 cm. Inflorescence 11–32 cm long, many flowered, congest with flowers disposed in spiral around the inflorescence axis. Bracts ovate-lanceolate, acuminate, 16–22 × 4–5 mm, generally, shorter than the pedicellate ovary. Flowers 11–97; pedicellate ovary mostly parallel to the rachis, 15.8–25.5 mm long; ovary arched, 11.4–20.0 mm, pedicel 3.4–7.0 mm. Sepals mucronate, margins smooth; dorsal sepal slightly concave, when flattened orbicular, 3.0–3.9 × 3.0–4.0 mm; lateral sepals obliquely elliptical, deflexed, 5.1–6.5 × 3.4–4.6 mm. Petals bipartite; posterior segment falcate, 3.6–4.9 × 1.2–1.5 mm, acute, free from the dorsal sepal but not spread; anterior segment curved laterally and facing upward, linear-filiform, inserted at the base of the posterior segment, 3.6–4.0 mm long, about 0.90–1.00 times as long as the posterior segment. Lip tripartite; undivided basal part 1.7–1.8 × 1.4–1.5

mm; lateral segments pendent, linear to lanceolate, 4.4–5.4 × 1.0-1.3 mm, about 0.91–0.94 times as long as the median segment; median segment linear to oblanceolate, 4.7–5.9 × 1.0–1.5 mm; spur deflexed or reflexed, free from the bracts or partially covered for it, clavate, generally, shorter than the pedicellate ovary, 13–17 mm long, base 0.6 mm wide, apex 1.1–1.3 mm wide. Gynostemium erect, 2.3 mm high; connective emarginate, greenish; auricles fleshy, verrucose, whitish, 1.2 × 0.6 mm. Anther 1.8 mm high; canals short, 0.8–0.9 mm long; loci spaced 1.2–1.3 mm; hemipollinaria 2, separated; viscidia exposed, 0.4 × 0.25 mm, spaced 1.1–2.1 mm apart; caudicles 0.6–0.7 mm long. Stigma lobes 2, closely parallel, 2.7 mm long; receptive surface greenish, convex, turned frontwards, 1.6–1.7 mm long, inner margins thickened, apices with a protruding, erect projection that partially divides the space between the stigma lobes in two apertures. Rostellum greenish, 2.7–2.9 mm long; mid-lobe triangular, fleshy, acute, erect, completely placed between the anther loci, 1.3 mm long, 1.8 mm high; side-lobes parallel, 1.6 mm long.

Distribution and Conservation Status— *Habenaria ekmaniana* has the broadest distribution among the species in sect. *Pentadactylae*, ranging from central (Distrito Federal and Mato Grosso do Sul), southeastern (Minas Gerais and São Paulo) and southern Brazil (Rio Grande do Sul), to Northern Argentina (Misiones; Fig. 6). However, throughout its distribution range the species is rare and only known from eight collections from seven localities. There is only one recent record, most of the collections are more than 40 years old and from areas that are now highly modified by human activity, and these populations are probably lost. In the Federal District in central Brazil for example, there is only one record made in 1965. One of the authors has worked on a survey of the Orchidaceae of the Federal District (Batista and Bianchetti 2003), collected intensively there for more than 20 years and never found the species. Based on the World Conservation Union Red List Categories and

Criteria (IUCN 2001), *H. ekmaniana* can be tentatively classified as Endangered (EN: criteria C2a(i)).

Habitat and Ecology—*Habenaria ekmaniana* occurs in low or high-lands swamps. Flowering occurs from December to January.

Etymology—Named after Erik Leonard Ekman, collector of the type material.

Illustrations—Hoehne (1940, plate 56, based on the type material from S; plate 47, probably based on the holotype of *H. recta*), Insaurralde and Radins (2007, pg. 80–81, color photographs), Kraenzlin (1911, table 2, fig. 9, type illustration of *H. ekmaniana*), Pabst and Dungs (1975, pg. 246, fig. 52, based on the type material, *Ekman 432*).

Taxonomic Notes— In general morphology of the flowers *H. ekmaniana*, *H. henscheniana*, *H. dutraei* and *H. pentadactyla* are similar and in some instances, particular in dried herbarium specimens, can be confused with each other. However, they can be distinguished by the differences in the proportion of the petal anterior segment relative to the posterior segment and the dorsal sepal relative to the lateral sepals. In *H. henscheniana* the petals anterior segments are less than half the length of the posterior segments, whereas in *H. dutraei* the petals anterior segments and lip lateral segments are longer than the petal posterior segment and lip median segment, respectively. In *H. ekmaniana* and *H. pentadactyla* the segments are about the same length, but in *H. ekmaniana* the lateral sepals are about twice the size of the dorsal sepal, while in *H. pentadactyla* they are about the same size. In the vegetative parts, a remarkable feature of *H. ekmaniana* and *H. dutraei* are the large broadly lanceolate to elliptical leaves, with the sheets wide at the base, forming a bowl-like structure that retains and accumulates rain water. However, *H. ekmaniana* is, generally, a more robust species with larger leaves than *H. dutraei*.

The holotype of *H. recta* was not indicated, but Grossman types were deposited at B, C and GOET. Schlechter's herbarium and type were mainly at B, and we assume that if the type of *H. recta* was located at B it was destroyed during World War II, since it is not cited in what remains of Schlechter's type collection, in Berlin-Dahlem Herbarium (Butzin, 1978). No other duplicate of the type material has been located, including among the Berlin negatives at the Field Museum. Hoehne (1940, fig. 47) presented an illustration, based on the type material which he examined at the B Herbarium before it was lost. Since this is the only remaining element associated to the type, we designated it here as lectotype. The exact identity of *H. recta* is still unclear. In the protologue, Schlechter compared it with *H. umbraticola* and *H. nemorosa*, but these species have the anterior petal segment absent or reduced to a tooth like projection, while in *H. recta* it has half the size of the posterior segment. Based on the general similarity with *H. ekmaniana*, Batista et al. (2011) considered it a synonym of this species, and we follow here this position. However, there are some differences. The large and wide leaves resemble very much *H. ekmaniana*, but in this species the plants are taller, the inflorescence longer and the number of flowers higher than described for *H. recta*. Another difference is the anterior petal segment about half the size of the posterior segment in *H. recta*, while in *H. ekmaniana* they are about the same size. In this aspect, *H. recta* is more similar to *H. henscheniana*, were the anterior segments are about 0.3–0.7 times as long as the posterior segment. However, the leaves of *H. recta* are described as larger than those of *H. henscheniana*.

Additional Specimens Examined—ARGENTINA. Misiones: Posadas, Dec 2010, *Radins s.n.* (BHCB).

BRAZIL. Distrito Federal: Brasília, Zoobotânico, 20 Dec 1965, *Heringer 10806* (HB, UB). Mato Grosso do Sul: Rio Brilhante, Fazenda Bela Vista, 20 Jan 1971, *Hatschbach 26125* (HB, MBM, S, UC, US). Minas Gerais: Bom Sucesso, 9 Jan 1950, *Krieger 15147* (BHCB, CESJ, HB). Rio Grande do Sul: Porto Alegre, Jan 1942, *Leite s.n.* (NY, SP 46558).

São Paulo: Campinas, Fazenda Campo Grande, 18 Dec 1938, *Guilherme s.n.* (IAC 3225, SP 40965).

3. HABENARIA EXALTATA Barb. Rodr., Gen. Sp. Orchid. 1: 156. 1877.—TYPE: BRAZIL. Minas Gerais: Capivary, 3 Mar. 1870, *A.F. Regnell ser. III 1689* (Holotype: not indicated; Lectotype: designated by Batista et al. (2011): UPS [V-165856]). (Figs. 3C, 4C and D).

Habenaria henscheniana Barb. Rodr. var. *densiflora* Cogn., Fl. Bras. (Martius) 3(4): 85. 1893.—TYPE: BRAZIL. Minas Gerais: Caldas, 5 Mar. 1876, *C.W.H. Mosén 4538* (Holotype: not indicated; Lectotype designated by Batista et al. (2011): S [07-7151]; Isotypes: BR [fragment, mixed with flowers of *H. henscheniana*], UPS [V-165880]).

Habenaria crassipes Schltr., Repert. Spec. Nov. Regni Veg. Beih. 35: 18. 1925, syn. nov.—TYPE: BRAZIL. Rio Grande do Sul: Venâncio Aires, near Tangerinas, 70 m, Jan. 1924, *C. Jürgens 98* (Holotype: not indicated; probably B, destroyed; no isotype located. Lectotype, designated here: Fl. Bras. (Hoehne) 12(1): t. 49. 1940 [based on the holotype]).

Habenaria sceptrum Schltr., Repert. Spec. Nov. Regni Veg. 16: 249. 1919.—TYPE: BRAZIL. Paraná, Pinhaes, 12 Feb. 1914, *P.K.H. Dusen 14498* (Holotype: not indicated; probably B, destroyed; Lectotype, designated by Batista et al. 2011: S [R-2732]).

Semi-aquatic herb. Plants 38–149 cm, including the inflorescence. Stem erect, 31–128 × 0.4–1.5 cm. Leaves 9–17, spreading, largest at the lower half or center of the stem, lanceolate, 8.5–28.0 × 1.5–3.5 cm. Inflorescence 5–23 cm, many flowered, congest, with flowers disposed in spiral around the inflorescence axis. Bracts ovate-lanceolate, acuminate, 12–26 × 3–6 mm, generally, lower longer, and upper with about the same length than the pedicellate ovary. Flowers 17–110; pedicellate ovary spreading from the rachis, 13.2–17.9 mm; ovary arched, 11–15 mm, pedicel 1.5–4.4 mm. Sepals green, mucronate, margins smooth; dorsal sepal concave, when flattened slightly orbicular, 3.7–5.3 × 3.8–5.8 mm; lateral sepals obliquely elliptical, reflexed, 4.7–7.8 × 2.3–4.4 mm. Petals bipartite, base whitish, turning greenish yellow towards the segment apices; posterior segment oblong-falcate, 3.7–5.7 × 1.2–2.3 mm, acute, connivent with the dorsal sepal; anterior segment curved laterally and facing upward, linear-filiform, inserted at the base of the posterior segment, 1.2–4.4 mm long, about 0.22-0.97 times as long as posterior segment. Lip tripartite, base whitish, turning green towards the segment apices; undivided basal part 1.0–2.2 × 1.0–2.2 mm; lateral segments pendent, deflexed or reflexed, linear-filiform, 4.6–8.6 mm long, about 1.02 -1.53 times as longer as the median segment; median segment linear to oblanceolate, deflexed or reflexed, 3.5–6.9 × 0.6–1.3 mm; spur reflexed, slightly or strongly arched, free from the bracts, linear, longer than the pedicellate ovary, 28–35 × 0.7–1.2 mm, green. Gynostemium erect, 2.6 mm high; connective emarginate, greenish; auricles fleshy, verrucose, whitish, 1.6 × 1.0 mm. Anther 1.8 mm high; canals short, 1.4 mm long; loci spaced 1.4 mm; hemipollinaria 2, separated; pollinarium 3.3 mm long; viscidia exposed, 0.8 × 0.5 mm, spaced 0.6 mm apart; caudicles 1.8 mm long; pollinia 1.5 × 1.5 mm. Stigma lobes 2, closely parallel, 3.2 mm long; receptive surface whitish, convex, turned frontwards and to the sides, 1.6 mm long, margins thickened, with a protruding, erect projection that partially divides the space between the stigma lobes and the entrance to the spur in two apertures. Rostellum greenish; mid-lobe triangular, fleshy, subacute, erect, partially projected beyond the anther loci, 1.9 × 2.0 mm; side-lobes convergent towards apices, 1.8 mm long.

Distribution and conservation status—Distribution ranges from the southern part of the state of Minas Gerais, in southeastern Brazil, to São Paulo, Paraná and Rio Grande do Sul, in southern Brazil; and Paraguay (Fig. 6). Despite the broad distribution, *H. exaltata* is an occasional species along all its distribution range. In Minas Gerais it is known only from the vicinity of Poços de Caldas, while there is only one record for São Paulo, two for Paraguay and none for Santa Catarina, which is within the species distribution range. The two populations with recent records from Minas Gerais are reduced to few individuals and threatened due to *Eucalyptus* spp. reforestation and urban expansion. Based on the World Conservation Union Red List Categories and Criteria (IUCN 2001), *H. exaltata* can be tentatively classified as Vulnerable (VU: criteria 2ab(iii); D1).

Habitat and Ecology— *Habenaria exaltata* occurs in lowlands and highlands swamps. Flowering occurs from January to February. In all the collections of the species made by the authors it was found growing in wet meadows associated with *Erygium* sp., usually at the base of the plants or between the leaves inside the dense rosettes.

Etymology—From the Latin *exaltatus* (raised high), probably referring to the size of the plants, that can reach up to 1.5 m high and are usually prominent in relation to the surrounding vegetation.

Illustrations—Cogniaux (1893, plate 18, fig. 2, reproduction of Barbosa Rodrigues' original illustration of *H. exaltata*), Hoehne (1940, plate 49, based on the holotype of *H. crassipes*; plate 58, as *H. sceptrum*), Pabst and Dungs (1975, pg. 246, fig. 50, as *H. crassipes*, based on *Amadeus 37*; fig. 53, based on *Mosen 4538*, type material of *H. henscheniana* var. *densiflora*; fig. 58, as *H. sceptrum*), Sprunger (1996, vol. 1, t. 15B, reproduction of Barbosa Rodrigues' original illustration of *H. exaltata*).

Taxonomic Notes—Barbosa Rodrigues (1877, 1882) described several species based on material from Regnell and other collectors and it is not known which material was used for the descriptions. It is generally accepted that Barbosa Rodrigues herbarium was lost during a flood at his house basement, were the material was assumed to be deposited (Cribb and Toscano de Brito 1996). It is also unclear whether material of other collectors was held by Barbosa Rodrigues. Currently, the herbarium of the Instituto de Botânica de São Paulo (SP) is the only herbarium in Brazil with some duplicates of Regnell Orchidaceae collections. Most of Regnell collections are deposited at S or UPS. The only collection of the type material of *H. exaltata* is found in UPS, and since the holotype was not indicated, this material was selected as lectotype by Batista et al. (2011).

After its description the identity of *H. exaltata* remained obscure for a long time. Cogniaux (1893) in Flora Brasiliensis only cited the type material, while Hoehne (1940) in Flora Brasilica cited an additional collection (*Lindman 2765*), but which is apparently referable to *H. goyazensis* Cogn. Barbosa Rodrigues was a skilled illustrator and his drawings have been essential in clarifying the identity of several of the species he described. However, for *H. exaltata* and some other species such as *H. henscheniana*, only a flower sketch was drawn. This probably explains why the species was re-described as a variety of *H. henscheniana* by Cogniaux and as *H. sceptrum* and *H. crassipes* by Schlechter. Examination of the type material of *H. henscheniana* var. *densiflora* and *H. sceptrum* has clearly shown that they are referable to *H. exaltata*. The holotype of *H. crassipes*, located at Berlin Herbarium (B), was destroyed during World War II, and no isotype has been located. However a critical examination of the species protologue, along with Hoehne' illustration of *H. crassipes* (Hoehne 1940, plate 49), which was based on the type material before it was lost, have shown that it is inseparable from *H. exaltata*. The type material of *H. crassipes* represents a very robust specimen with a densely flowered inflorescence, but which are variable characters in *H. exaltata*, and the flower details agree in all aspects with *H. exaltata*. Since Hoehne' illustration is the only extant material associated to the holotype of *H. crassipes*, we designate it here as lectotype.

Habenaria exaltata original illustration is found in Barbosa Rodrigues' *Iconographie des Orchidées du Brésil*, vol. 1, t. 15B: RB, copy K and reproduced in Sprunger, 1996: 1: 68B. The illustration of *H. exaltata* in Flora Brasilica (Hoehne 1940, plate 68) is probably based on the material *Lindman 2765* from S, since this is the only material of the species

Hoehne cited as examined (Hoehne 1940). We have examined this material (S10-20644) and found that it is not *H. exaltata* and it is apparently referable to *H. goyazensis* Cogn.

Among the species in sect. *Pentadactylae*, distinctive features of *H. exaltata* are the long, linear spur, about twice the length of the pedicel and ovary and the petal anterior segment shorter than the posterior segment (Fig. 4D).

Additional Specimens Examined—BRAZIL. Minas Gerais: Poços de Caldas, MG 877, between Esperança III and São Bento neighborhoods, 21º49’54.7’’S, 46º33’56’’W, 1260 m, 3 Feb 2009, *Batista et al. 2798* (BHCB); Poços de Caldas, Morro do Ferro, 21º54’39.5’’S, 46º31’28.5’’W, 1378 m, 31 Jan 2009, *Batista et al. 2771* (BHCB); Poços de Caldas, Morro do Ferro, 31 Mar 1968, *Emmerich 3174* (HB, R). Paraná: Curitiba, 26 Jan 1904, *Dusen 3272* (R, SP, SPF); Curitiba, Rio Iguaçú, 3 Feb 1967, *Hatschbach 15962* (HB, MBM); Guarapuava, posto agro-pecuário, 18 Jan 1968, *Hatschbach 18323* (MBM); Piraquara, Borda do Campo-Piraquara road, near Borda do Campo, 25º24’10’’S, 49º03’22.8’’W, 905 m, 4 Feb 2008, *Batista et al. 2520* (BHCB); Quatro Barras, 9 Feb 1964, *Hatschbach 10946* (HB, L, MBM, U); São Mateus do Sul, Vila S’ Ana, 8 Feb 1966, *G. Hatschbach et al. 13773* (MBM). Rio Grande do Sul: Muitos Capões, 2 Feb 2011, *Pedron 6* (ICN); Muitos Capões, Estação Ecológica de Aracuri, 13 Jan 1983, *Waechter 1976* (ICN); Muitos Capões, Estação Ecológica de Aracuri, 950 m, 26 Jan 1979, *Arzivenco 521* (ICN); Pelotas, Instituto Agronômico do Sul, 31 Jan 1950, *Amadeu 37* (HB, ICN); between Erval and Pedras Altas, 25 Jan 1966, *Trinta 1204* (HB, K, L, LP); Viamão, Itapuã, 4 Dec 1929, *Dutra 1074* (ICN, SI, SP). São Paulo: 84 km from São Paulo on the road São Paulo-Curitiba, 19 Jan 1952, *Pabst 1318* (B, HB, K, RB, S).

PARAGUAY. Villarrica, Jan 1931, *Jorgensen 4646* (US); Villarrica, 10 Jan 1931, *Jorgensen 4648* (S, SI).

4. HABENARIA HENSCHENIANA Barb. Rodr., Gen. Sp. Orchid. 1: 157. 1877.—TYPE: BRAZIL. Minas Gerais, Caldas, Rio Verde, 7 Mar. 1868, *A.F. Regnell ser. III 999* (Holotype: not indicated; Lectotype, designated by Batista et al. (2011): S [R-2711]; Isotypes: BR [fragment, mixed with flowers of *H. exaltata*], P, S, US). (Figs. 3E, 7A and B)

Semi-aquatic herb. Plants 21–118 cm, including the inflorescence. Stem erect, 13.3–81 × 0.2–0.8 cm wide. Leaves 5–16, spreading, largest at the lower half or center of the stem, lanceolate, 7–19 × 0.7–2.2 cm. Inflorescence 5–37 cm, few to many flowered, lax to congest, with flowers disposed in spiral around the inflorescence axis. Bracts ovate, acuminate, 12–29 × 3–8 mm, generally, shorter than the pedicellate ovary. Flowers 7–51; pedicellate ovary mostly parallel to the rachis, 16.0–25.8 mm; ovary arched, 11.2–17.0 mm, pedicel 4.8–9.8 mm long. Sepals green, mucronate, margins smooth; dorsal sepal concave, when flattened broadly elliptical, 3.6–4.5 × 2.4–3.6 mm; lateral sepals obliquely elliptical, spreading (patent), 5.5–6.8 × 3.5–4.3 mm. Petals bipartite, base whitish, turning greenish yellow towards the segment apices; posterior lobe oblongo-falcate, 3.3–3.9 × 1.4–2 mm, acute, connivent with the dorsal sepal; anterior segment curved laterally, linear-filiform, inserted at the base of the posterior segment, 0.9–2.4 mm long, about 0.26–0.69 times as long as the posterior segment. Lip tripartite, base whitish green, turning green towards the segment apices; undivided basal part 1.0–2.0 × 1.1–2.0 mm; lateral segments pendent, reflexed, linear, 3.0–4.4 × 1.0–1.2 mm, about 0.71–0.94 times as long as the median segment; median segment linear, reflexed, 3.7–4.8 × 1.2–1.6 mm; spur reflexed, arched downward, totally or partially covered by the bracts, slightly clavate to clavate, shorter than the pedicellate ovary, 15–20 mm long, base 0.5–0.7 mm wide, apex 0.9–1.3 mm wide, green. Gynostemium erect, 2.6 mm high; connective emarginate, greenish; auricles fleshy, verrucose, whitish, 1.3 × 0.7 mm. Anther 1.3 mm high;

canals short, 0.5 mm long; loci spaced 1.4 mm; hemipollinaria 2, separated, 1.7 mm long; viscidia exposed, 0.5 × 0.4 mm, spaced 0.7 mm apart; caudicles 0.8 mm long; pollinia 0.8 × 0.6 mm. Stigma lobes 2, closely parallel, 2.3 mm long; receptive surface green, convex, turned frontwards, 1.1 mm long, margins thickened, with a protruding, erect projection that partially divides the space between the stigma lobes and the entrance to the spur in two apertures. Rostellum greenish; mid-lobe triangular, fleshy, acute, erect, mostly projected beyond the anther loci, 1.1 × 2.5 mm; side-lobes convergent towards apices, 1.1 mm long.

Distribution and Conservation Status—Found only in southeastern and southern Brazil in the states of Minas Gerais, Paraná, Santa Catarina and Rio Grande do Sul (Fig. 5). The species is known only from twelve collections from nine localities. Most of the collections are from the fifties and sixties and there are few recent records of the species. Based on the World Conservation Union Red List Categories and Criteria (IUCN 2001) criteria, *H. henscheniana* can be tentatively classified as Endangered (EN: criteria C2a(i)).

Habitat and Ecology— *Habenaria henscheniana* occurs in highland swamps. Flowering occurs from January to February.

Etymology—Named in honor of Salomon Eberhard Henschen, a Swedish physician who worked with Anders Frederick Regnell and João Barbosa Rodrigues. Henschen, Regnell and other botanists made important contributions to the knowledge of Brazilian flora, especially the flora of Caldas, in the state of Minas Gerais, and their collections contributed with the monumental *Flora Brasiliensis*.

Illustrations—Hoehne (1940, plate 53, probably based on the type material from S), Pabst and Dungs (1975, pg. 246, fig. 59, based on *Becker 286*), Sprunger (1996, vol. 1, t. 15C, reproduction of Barbosa Rodrigues' original illustration of *H. henscheniana*).

Taxonomic Notes—Similarly to *H. exaltata* the holotype of *H. henscheniana* was not indicated, and a lectotype was designated by Batista et al. (2011). The species original illustration is found in Barbosa Rodrigues' *Iconographie des Orchidées du Brésil*, vol. 1, t. 15C: RB, copy K and reproduced in Sprunger, 1996: 1: 68C. There is some confusion on the data label of the type of *H. henscheniana*. The type and all duplicates are from Minas Gerais, Caldas, *Regnell ser. III 999*, but collection date varies between 24 February 1847 (US, S [05-3454], P) and 7 March 1868 (BR, S [R-2711], S [07-7153]). This is probably due to the fact that at that time it was not unusual to use the same collection number for different collections of the same species.

A distinctive feature of *H. henscheniana* among other species in sect. *Pentadactylae* is the position of the lateral sepals (Fig. 7B). The lateral sepals are spreading (patent) and stand wide open in front of the gynostemium. However, while this character is evident in live material, in herbarium specimens the flowers are flattened and the lateral sepal looks as if they were appressed, resembling in this aspect *H. pentadactyla*. Since the petals anterior segments are very short in *H. henscheniana*, it is possible that the spreading lateral sepals may provide the support for pollinators to hold to the flowers.

Additional Specimens Examined—BRAZIL. Minas Gerais: Poços de Caldas, MG 877, between Esperança III and São Bento neighborhoods, 21º49'54.7''S, 46º33'56''W, 1260 m, 3 Feb 2009, *Batista et al. 2802* (BHCB); Poços de Caldas, Morro do Ferro, 5 Mar 1964, *Becker 286* (HB); Poços de Caldas, Morro do Ferro, 31 Mar 1968, *Emmerich 3176* (HB). Paraná: Quatro Barras, Rio Taquari, 850 m, 9 Jan 1969, *Hatschbach 20693* (HB, MBM, UPCB); Quatro Barras, Rio Taquari, 21 Jan 1975, *Hatschbach 35770* (MBM, UC). Rio Grande do Sul: Bom Jesus, Aparados da Serra, 1100 m, 11 Feb 1952, *Pabst 1360* (HB, LP, MBM, PEL); Caxias do sul, Vila Oliva, 3 Jan 1946, *Rambo* s.n. (PACA 30985); Osório, Fazenda do Arroio, 14 Apr 1950, *Rambo s.n.* (PACA 46757); São Francisco de Paula, 21 Jan 2010, *Pedron 2* (ICN). Santa Catarina: Lages, Santa Cecília, 21 Jan 1952, *Pabst 1324* (HB); Urubici, Campo dos Padres, Fazenda Campo dos Padres, 1650 m, 25 Jan 1957, *Smith & Reitz*

10416 (HB, R, US); Urubici, Campo dos Padres, Serra Geral, próximo a nascente do rio Canoas, 27º55’S, 49º18’W, 1500 m, 19 Feb 2008, *Mota et al. 1584* (BHCB).

5. HABENARIA MEGAPOTAMENSIS Hoehne, Arq. Bot. Estado São Paulo n.s., f.m., 1(2): 41, t. 47. 1939.—TYPE: BRAZIL. Rio Grande do Sul: Taquara, Caracol, 13 Jan. 1926, *J. Dutra 873* (Holotype: SP [29635]). (Figs. 3D, 7C and D)

Semi-aquatic herb. Plants 78–122 cm, including the inflorescence. Stem erect, 69–107 × 0.3–1.1 cm. Leaves 11–18, spreading, largest at the lower half or center of the stem, ovate-lanceolate, 14–26 × 2.2–4.1 cm. Inflorescence 8–38 cm long, many flowered, congest with flowers disposed in spiral around the inflorescence axis. Bracts ovate-lanceolate, acuminate, 12–33 × 5–10 mm, generally, shorter than the pedicellate ovary. Flowers 16–74; pedicellate ovary spreading from the rachis, 22.4–27.0 mm long; ovary arched, 16–19 mm, pedicel 6.4–8.0 mm. Sepals green, mucronate, margins smooth; dorsal sepal concave, when flattened slightly orbicular, 5.4–7.0 × 5.5–6.6 mm; lateral sepals obliquely elliptical, reflexed, 7.7–10.0 × 4.0–5.0 mm. Petals bipartite, base whitish, turning greenish yellow towards the segment apices; posterior segment oblong-falcate, 5.5–7.1 × 1.8–2.9 mm, acute, connivent with the dorsal sepal; anterior segment curved laterally and facing upward, linear-filiform, inserted at the base of the posterior segment, 5.2–7.0 mm long, about 0.86–0.98 times as long as the posterior segment. Lip tripartite, base whitish, turning yellowish green towards the segment apices; undivided basal part 1.7–1.8 × 1.8–2.3 mm; lateral segments pendent, deflexed, linear, 8.0–10.5 × 0.7–1.0 mm, about 1.21–1.42 times as long as the median segment; median segment linear to oblanceolate, deflexed, 6.0–8.3 × 1.0–1.6 mm; spur reflexed, slightly or strongly arched, free from the bracts, linear, longer than the pedicellate ovary, 57–78 × 1.0–1.4 mm, green. Gynostemium erect, 3.1 mm high; connective obtuse, greenish; auricles fleshy, verrucose, whitish, 1.7 × 1.2 mm. Anther 2 mm high; canals short, 1.3 mm long; loci spaced 2 mm; hemipollinaria 2, separated, 3.3 mm long; viscidium exposed, 0.8 × 0.6 mm, spaced 0.7 mm apart; caudicles 1.6 mm long; pollinia 1.5 × 1.5 mm. Stigma lobes 2, closely parallel, 4 mm long; receptive surface whitish, convex, turned frontwards and to the sides, 2 mm long, margins thickened, with a protruding, erect projection that partially divides the space between the stigma lobes and the entrance to the spur in two apertures. Rostellum greenish; mid-lobe triangular, fleshy, subacute, erect, partially projected beyond the anther loci, 3.0 × 2.5 mm; side-lobes convergent towards apices, 1.1 mm long.

Distribution and Conservation Status— *Habenaria megapotamensis* is restricted to southern Brazil, in the states of Paraná, Santa Catarina and Rio Grande do Sul (Fig. 8). Most of the collections are from Rio Grande do Sul, where it is a common species and several large populations are known. Based on the World Conservation Union Red List Categories and Criteria (IUCN 2001), *H. megapotamensis* can be tentatively classified as Least Concern (LC).

Habitat and Ecology—*Habenaria megapotamensis* occurs in highland swamps. Flowering occurs from January to March.

Etymology—From the Greek *mega* (large) and *potamos* (river or stream), probably referring to the state of Rio Grande do Sul. In southern Brazil the state is commonly referred as Rio Grande, which means large river.

Illustrations—Hoehne (1939, table 47, type illustration of *H. megapotamensis*), Hoehne (1940, reproduction of Hoehne, 1939), Pabst and Dungs (1975, pg. 247, fig. 71, based on *Richter s.n.*).

Taxonomic Notes—*Habenaria megapotamensis* and *H. exaltata* are remarkably similar, and *H. megapotamensis* looks very much like as larger specimens of *H. exaltata*. Nevertheless, the two species are clearly distinct by the consistently larger flowers and longer spur of *H. megapotamensis* (Fig. 7D). In *H. megapotamensis* the dorsal sepal is 5.4–7.0 mm long and the spur 57–78 mm (Fig. 7D), versus 3.7–5.3 mm long and 28–35 mm in *H. exaltata* (Fig. 4D), respectively. Other differences are found in the petals anterior segment and the column connective. In *H. megapotamensis* the petals anterior segment is 5.2–7.0 mm long and about the same size as the posterior segment (Fig. 7D), whereas in *H. exaltata* it is 1.2–4.4 mm long and usually less than half the length of the posterior segment (Fig. 4D). In the structure of the gynostemium, in *H. megapotamensis* the connective is obtuse, while in *H. exaltata* it is emarginated (Figs. 3C and D). Considering that one usual consequence of polyploidization is growth in size, it is possible that *H. megapotamensis* is a polyploidy derived from *H. exaltata* but, presently, there are no cytogenetic data to test this hypothesis.

Additional Specimens Examined—BRAZIL. Paraná: Piraquara, road toward Borda do Campo, 17 Feb 1953, *Hatschbach 3105* (HB, MBM, SI, Z). Rio Grande do Sul: Bom Jesus, Fazenda Bernardo Velho, 8 Jan 1947, *Rambo s.n.* (S 10-20641); Bom Jesus, Fazenda Caraúna, Feb 1931, *Dutra 1092* (HB, ICN, SP); Bom Jesus, Fazenda do Cilho, 12 Feb 2007, *Setubal et. al. 848* (ICN); Cambará do Sul, 19 Feb 2011, *Pedron 10* (ICN); Canela, Caracol road, 17 Feb 1953, *Richter s.n.* (HB 2119); Canela, Feb 1986, *Sobral & Silva 4984* (ICN, SP); Caxias do Sul: Vila Oliva, 8 Feb 1955, *Rambo* s.n. (PACA 56756, S 10-20643); Jaquirana, 20 Feb 1952, *Rambo s.n.* (HB 1730, PACA 52106, S 10-20642, US 00247310); São Francisco de Paula, 11 Feb 2001, *Wasum 932* (US); São Francisco de Paula, 17 Mar 2001, *Diesel s.n.* (US 00672816); São Francisco de Paula, 8 Feb. 2012, *J. Klein 145* (BHCB); Vacaria, *Dutra 1093* (ICN); Vacaria, Fazenda da Ronda, 5 Jan 1947, *Rambo s.n.* (PACA 34841). Santa Catarina: Bom Retiro, 21 Feb. 2012, *Buzatto & Nervo 760* (ICN); Caçador, 900–1,000 m, 6 Feb 1957, *Smith & Klein s.n.* (US 00247310).

6. HABENARIA MONTEVIDENSIS Spreng., Syst. Veg. (ed. 16), 3: 692. 1826. TYPE: URUGUAY. Montevideo, *F. Sello s.n.* (Holotype: presumably B, probably destroyed; possible isotype: W-R 51316 [not seen]). (Figs. 3G, 9A and B)

Habenaria arechavaletae Kraenzl., Bot. Jahrb. Syst. 16: 185. 1892. TYPE: URUGUAY. Sierra de Minas, Feb. 1874, *J.E. Gibert 1160* (Holotype: B, destroyed; Isotypes: BR [fragment] mounted on the same sheet with *E.H.G. Ule 1904*, HB [fragment], MVM, ZT).

Habenaria arechavaletae Kraenzl. var. *elata* Cogn., Fl. Bras. (Martius) 3(4): 92. 1893. TYPE: BRAZIL. Santa Catarina, Capivare, Serra Geral, Feb. 1891, *E.H.G. Ule 1904* (Holotype: BR [657428]; Isotype: P [408631]).

Habenaria obovatipetala Schltr., Repert. Spec. Nov. Regni Veg. Beih. 35: 21. 1925. *Habenaria arechavaletae* Kraenzl. var. *obovatipetala* (Schltr.) Pabst, Rodriguesia 28–29: 130. 1954. TYPE: BRAZIL. Rio Grande do Sul, Caranna-Bom Jesus, 1000 m, January 1909, *J. Dutra 511* (Holotype: B, destroyed; Lectotype, designated by Batista et al. (2011): ICN [14511]; Isolectotype: SI [39934]).

Semi-aquatic herb. Plants 21–79 cm, including the inflorescence. Stem erect to sinuose, 13–68 × 0.2–0.6 cm. Leaves 7–12, spreading, largest at the lower half or center of the stem, lanceolate, 6–16 × 1.0–2.5 cm. Inflorescence 2.5–12.0 cm, few to many flowered, congest, with flowers disposed in spiral around the inflorescence axis. Bracts ovate, acuminate, 11–25 × 4–7 mm, generally, shorter than the pedicellate ovary. Flowers 3–31; pedicellate ovary mostly parallel to the rachis, 15.0–20.5 mm; ovary arched, 12–15 mm, pedicel 3–7 mm. Sepals green, mucronate, margins smooth; dorsal sepal convex, when flattened ovate, 7.4–8.7 × 4.2–6.6 mm; lateral sepals elliptical, deflexed, 8.6–10.4 × 3.1–5.0 mm. Petals simple, white, obliquely orbicular unguiculate, 7–10 × 5–9 mm, obtuse, free from dorsal sepal. Lip tripartite, white, undivided basal part 1.4–2.4 × 1.3–2.7 mm; lateral segments pendent, deflexed, linear, 7.3–9.4 × 0.8–1.0 mm, about 1.00–1.45 times as long as the median segment; median segment oblanceolate, deflexed, with apex slightly curved forwards, 6.4–7.8 × 1.8–2.5 mm; spur reflexed, arched downward, free or partially covered by the bracts, slightly clavate, about the same size as the pedicellate ovary, 14–18 mm long; base 0.6–1.0 mm wide, whitish; apex 1–1.5 mm wide, light green. Gynostemium erect, 2.8 mm high; connective obtuse, white; auricles fleshy, verrucose, whitish, 1.7 × 1.2 mm. Anthers 2.5 mm high; canals prominent, 1.9 mm long; loci spaced 1.6 mm; hemipollinaria 2, separated, 3.8 mm long; viscidia exposed, 0.4 × 0.3 mm, spaced 2.5 mm apart; caudicles 2.4 mm long; pollinia 1.3 × 1.0 mm. Stigma lobes 2, closely parallel, 3.1 mm long; receptive surface white, concave, turned frontwards, 1.7 mm long, with margins thickened. Space between the stigmas lobes elliptical. Rostellum white; mid-lobe triangular, fleshy, acute, erect, partially projected beyond the anther loci, 2.8 × 2.2 mm; side-lobes parallel throughout, 1.5 mm long.

Distribution and Conservation Status—Southern Brazil (Paraná, Santa Catarina and Rio Grande do Sul) and Uruguay (Fig. 6). There is only one record of the species for the southern part of the state of Paraná, which marks the northern limit of the species distribution. Otherwise, *H. montevidensis* is a common species along its distribution range and populations can be easily found. There is a single collection from the state of Rio de Janeiro (Alto Macaé, Nova Friburgo, 1892, *Glaziou s.n.* (BR). Since this record is far out from the known distribution range of the species, and considering that in many of Glaziou collections the locality details are inaccurate or equivocal (Wurdack, 1970), this record is doubtful. Using the World Conservation Union Red List Categories and Criteria (IUCN 2001), *H. montevidensis* would be classified as Least Concern.

Habitat and Ecology—*Habenaria montevidensis* occurs in low and highland swamps. Flowering occurs from January to March.

Etymology—Named after Montevideo in Uruguay, where the type material was collected.

Illustrations—Cogniaux (1893, table 15, fig. 2, as *H. arechavaletae*), Garay (1976, pg. 117, possibly based on the type material, *Sello s.n.*), Hoehne (1940, plate 32, fig. 2, as *H. arechavaletae*, probably based on the type material, *Gibert 1160*; plate 37, as *H. obovatipetala*), Pabst (1951, as *H. arechavaletae* and *H. obovatipetala*, plates 6–7), Pabst (1954, as *H. arechavaletae* var. *obovatipetala*, plates 1–3), Pabst (1956, reproduction of Pabst 1954, plates 1–3), Pabst and Dungs (1975, pg. 244, fig. 29, as *H. arechavaletae*, based on *Rambo* s.n. PACA 4829; 1977, pg. 266, fig. 42b, as *H. obovatipetala*, based on Hoehne, 1940, tab. 37), Lombardo (1984, table 148, fig. 2).

Taxonomic Notes—We did not locate the type material of *H. montevidensis*. Sello herbarium and types were at B and where mostly destroyed. Garay (1976) recorded a specimen from Reichenbach Herbarium (W-R 51316), consisting of a specimen and a flower sketch drawn by Reichenbach, that could be from Sello, since Reichenbach Herbarium contains a good set of Sello´s collections. Based on this information Batista et al. (2011) recorded the material W-R 51316 as an isotype, but there is no other evidence in this aspect beyond the mentioned above.

For a long time, the identity of *H. montevidensis* Spreng. was obscure. The specific epithet was first used by Sprengel (1826) and latter by Lindley (1835) but are based on different types and species. Cogniaux (1893), in Flora Brasiliensis, confused the two descriptions, using Sprengel’s name with Lindley’s description. Garay (1976) proposed the new name *H. uruguayensis* for *H. montevidensis* Lindl., but the separation of this taxon from the widespread *H. parviflora* Lindl. is not clear. Cogniaux’s mistake was followed by all subsequent authors who used the name *H. montevidensis* Spreng. applying it to *H. parviflora*.

There is some uncertainty regarding the data of the type of *H. arechavaletae*. In the protologue Kraenzlin (1892) recorded the type as: Uruguay, Sierra de Minas, Arechavaleta 1160, while the type at ZT is labeled Montevideo, February 1880. On the other hand, the material at MVM and the fragments at RB and HB, are from Gibert 1160, Uruguay, Pagum, Minas, February 1874. Since Arechavaleta original herbarium and types are at MVM, we follow the data present in this herbarium. It is possible that when duplicates from the Arechavaleta herbarium were distributed, the number 1160 was associated to Arechavaleta instead of Gibert.

As mentioned in the discussion, *H. montevidensis* is distinct from other species in sect. *Pentadactylae* and from all other Neotropical species by the convex, reflexed dorsal sepal, and the unguiculate, orbicular petals, which form a hood over the column (Fig. 9B). Whereas the reflexed dorsal sepal is common and characterizes a large group of African *Habenaria* in sect. *Replicatae*, the last characters are apparently unique in the genus. Pabst (1954, 1956) who collected and examined live material of *H. montevidensis*, was the first to draw the attention to these characters. From the recent investigation of some of the authors (Pedron et al., unpubl. data), who found that *H. montevidensis* is pollinated by butterflies, in contrast to other species in the sections which have flower syndromes associated to nocturnal moth pollination, it seems that the morphological differences are due to pollinator shift. Another major difference of *H. montevidensis*, are the parallel rostellum arms and the viscidia well spaced from one another (Figs. 3G, 9B), while in other species from sect. *Pentadactylae* the rostellum arms are confluent towards the apices and the viscidia are close to one another. In *H. montevidensis* the pollinaria are attached to the pollinator eyes, while in *H. megapotamensis* (Pedron et al., unpubl. data) and probably also the other species in the section, the pollinaria is attached to the pollinator proboscides.

One species with some similarities to *H. montevidensis* is *H. leucosantha*. In this species the dorsal sepal is not reflexed and covers the column, like most other Neotropical species, but the petals and lip are completely white, the lip lateral segments are laterally expanded, and column structure is similar to *H. montevidensis* (Fig. 3G and H). In both species the distance between the viscidia, the relative position between the rostellum arms, stigmas lobes and entrance to the spur are very similar (Fig. 3G and H). These similarities suggest that *H. leucosantha* probably is also pollinated by butterflies.

Additional Specimens Examined—BRAZIL: Paraná: General Carneiro, Rio Iratim, 11 Feb 1966, *Hatchbach 13708* (G, MBM). Santa Catarina: Bituruna, 9 Feb 1948, *Mello-Filho 756* (R); Florianópolis, Trindade, 17 Mar 1945, *Rohr* s.n. (PACA 28898); Santa Cecilia, 100 km de Lages, on the road between Lages and Mafra 1200 m, 21 Jan 1952, *Pabst 1326* (HB, RB); Santa Cecilia, 1000 m, 26 Feb 1962, *Reitz & Klein 12529* (HB); São Joaquim, Altos, 2 Feb 1958, *Mattos 5107* (HB); São Joaquim, Invernadinha, 20 Jan 1958, *Mattos 5003* (HB); São Joaquim, São Franscico Xavier, 1200 m, 4 Feb 1963, *Reitz 6664* (HB, L, MBM, US); Urubici, 20 Feb 2012, *Buzatto & Nervo 758* (ICN). Rio Grande do Sul: Bom Jesus, 20 Jan 1958, *Camargo s.n.* (S 10-20600); Bom Jesus, Caraúna, *Dutra 1084* (ICN, SP); Bom Jesus, Lageadinho, 20 Jan 1958, *Camargo 3111* (PACA); Cambará do Sul, 900 m, Feb 1948, *Rambo s.n.* (B, HB, PACA 36583, S 10-20605, SI); Cambará do Sul, about 20.4 km NE from Cambará, on the road to São José dos Ausentes, 28º56’3.6’’S, 50º02’32.7’’W, 1038 m, 1 Feb 2008, *Batista 2476* (BHCB); Cambará do Sul, about 27.8 km NE from Cambará, on the road to São José dos Ausentes, 28º52’42’’S, 50º01’33.8’’W, 1058 m, 1 Feb 2008, *Batista 2479* (BHCB); Canela, Caracol, Tiririca stream, 27 Jan. 1941, *Rambo s.n.* (PACA 11977); Canela, Passo do Inferno, 10 Feb 1941, *Rambo* s.n. (HB, PACA 4829); Jaquirana, 20 Feb 1952, *Rambo s.n.* (PACA 52057, S 10-20602); Santo Antônio da Patrulha, *Dutra 1183* (ICN); São Francisco de Paula, 1 Feb 1936, *Rambo & Dutra 1541* (SP); São Francisco de Paula, 11 Feb

2011, *Pedron 9* (ICN); São Francisco de Paula, Fazenda Englert, 1 Feb 1936, *Buck s.n.* (B, PACA 1541); São Francisco de Paula, Morrinhos, 7 Feb 1952, *Rambo s.n.* (HB, PACA 52110, S 10-20608, US 00247126); São José dos Ausentes, about 14.6 km NE from São José dos Ausentes, on the road to Silveira, 28º40’17.6’’S, 49º57’58.3’’W, 1185 m, 2 Feb 2008, *Batista 2487* (BHCB); São José dos Ausentes, Jan 2002, *Sobral 9503* (RB); Taquara, Caracol, 30 Jan 1934, *Dutra 1156* (ICN); s.loc, s.d., *Gaudichaud 336* (BR).

URUGUAY. Cerro de Minas, 6 Feb 1952, *Teague s.n.* (HB 1373, 1374, 1375, 1376); Cerro Largo, Cerro de Las Cuentas, 23 Feb 1938, *Rosengurt B2575* (HB, RB).

7. HABENARIA PENTADACTYLA Lindl., Gen. Sp. Orchid. Pl. 307. 1835.—TYPE: URUGUAY. Maldonado, *J. Tweedie s.n.* (Holotype: K [396199], mounted on the same sheet with *Gibert 892*; Isotype: K-L). (Figs. 3F, 9C and D)

Semi-aquatic herb. Plants 12–45 cm, including the inflorescence. Stem erect to sinuose, 4.0–36.0 × 0.1–0.4 cm. Leaves 5–9, spreading, largest at the lower half or center of the stem, linear-lanceolate, 5.0–13.0 × 0.6–1.1 cm. Inflorescence 3.4–13.0 cm long, few to many flowered, lax to congest with flowers disposed in spiral around the inflorescence axis. Bracts ovate-lanceolate, acuminate, 12–25 × 4–5 mm, generally, shorter than the pedicellate ovary. Flowers 3–17; pedicellate ovary mostly parallel to the rachis, 17–21 mm long; ovary arched, 14–16 mm, pedicel 3–6 mm. Sepals green, discreetly mucronate, margins smooth; dorsal sepal concave, when flattened ovate to elliptical, 3.5–4.2 × 2.7–3.2 mm; lateral sepals obliquely elliptical, turned upward, 5.3–6.6 × 2.4–3.1 mm. Petals bipartite, yellowish green; posterior segment falcate, 3.2–4.2 × 1.0–1.2 mm, acute, connivent with the dorsal sepal; anterior segment curved laterally and facing upward, linear-filiform, inserted at the base of the posterior segment, 4.0–4.3 mm long, about 1.00–1.25 times as long as the posterior segment.

Lip tripartite, base whitish green, turning yellowish green towards the segment apices; undivided basal part 1.0–1.2 × 1.1–1.5 mm; lateral segments pendent, straight or slightly deflexed, linear, 4.1–5.5 × 1.1 mm, about 1.02–1.12 times as long as the median segment; median segment linear, straight or deflexed, 3.7–5.4 × 0.8–1.1 mm; spur deflexed or reflexed, arched downward, free or partially covered by the bracts, clavate, about the same size as the pedicellate ovary, 11–15 mm long, base 0.6–0.8 mm wide, apex 1.1–1.6 mm wide, green. Gynostemium erect, 2–2.1 mm high; connective slightly emarginate, whitish; auricles fleshy, verrucose, whitish, 0.85 × 0.6 mm. Anther 1-1.1 mm high; canals very short, 0.3 mm long; loci spaced 1.0–1.3 mm; hemipollinaria 2, separated, 1.7–1.8 mm long; viscidia exposed, 0.3 × 0.2 mm, spaced 0.3–0.4 mm apart; caudicles 0.4–0.5 mm long; pollinia 1.0 × 0.6 mm. Stigma lobes 2, closely parallel, 2.4 mm long; receptive surface greenish, slightly convex, turned upwards, 1.6 mm long, inner margins thickened, with a protruding, erect projection that partially divides the space between the stigma lobes and the entrance to the spur in two apertures. Rostellum whitish, 1.9 mm long; mid-lobe triangular, fleshy, obtuse, erect, completely placed between the anther loci, 1.3–1.4 mm long, 1.3 mm high; side-lobes convergent towards the apices, 0.6–0.7 mm long.

Distribution and Conservation Status—Restricted to Rio Grande do Sul, in southern Brazil, Uruguay and part of eastern Argentina, mainly in the coastal region (Fig. 8). *Habenaria pentadactyla* is apparently a rare species, and the most of records date previously to the sixties. Because of the small size and slender plants, and the greenish flower, the species is difficult to localize among the surrounding herbaceous vegetation and this may account, at least in part, for the small number of collections. Many of the collections were made in areas that are now human inhabited and probably the populations of the species are declining, but a more accurate study in necessary to verify this possibility. Based on the

World Conservation Union Red List Categories and Criteria (IUCN 2001), *H. pentadactyla* can be tentatively classified as Endangered (EN: criteria C2a(i)).

Habitat and Ecology— *Habenaria pentadactyla* occurs in sandy low and highlands swamps. Flowering occurs from December to March.

Etymology—From the Greek *penta* (five) and *dactylos* (finger), probably referring to the set formed by the tree segments of the lip and the anterior segments of each petal.

Illustrations—Hoehne (1940, table 121, fig. 2), Pabst and Dungs (1975, pg. 256, fig. 164, based on *Rosengurt B2611*), Lombardo (1984, plate 149, fig. 1).

Taxonomic Notes—Distinctive from other species in sect. *Pentadactylae*, plants of *H. pentadactyla* are smaller, have few flowers, and the lateral sepals are not deflexed (Fig. 9D). Although live material of *H. pentadactyla* is very characteristic, dried specimens can be confused with other species, particularly with *H. henscheniana*. A diagnostic character is the length and proportion of the petals anterior segment relative to the posterior segment. In *H. pentadactyla* the anterior segment is 4–4.3 mm long and about the same size as the posterior segment (Fig. 9D). In *H. henscheniana* the anterior segment is 0.9–2.4 mm long, and about half the length or less of the posterior segment (Fig. 7B). One specimen at ICN (*Sobral* 2103) has intermediate characters between *H. pentadactyla* and *H. henscheniana* and may represent a hybrid between the two species.

In the protologue Lindley recorded the type material from Bonaria, which refers to the city of Buenos Aires in Argentina. However, in Tweedie’s collection the material is recorded from Maldonado, which in fact is located in Uruguay.

Additional Specimens Examined—ARGENTINA. Buenos Ayres, *Tweedie 183* (OXF).

BRAZIL. Rio Grande do Sul: Osório, Fazenda do Arroxo, 6 Mar 1950, *Pabst 561* (HB); Rio Grande, Quinta, 28 Jan 1950, *Bento 4* (HB, ICN); São Francisco de Paula, 19 Feb

2011, *Pedron 11* (ICN); Torres, Faxinal, 31 Mar 1978, *Waechter 781* (ICN); Viamão, Itapuã, 5 Dec 1929, *Dutra 1077* (ICN, SI, SP).

URUGUAY. Canelones, Arroio Sarandy, Rio de la Plata, Costa Azul, 3 Feb 1942, *Augusto s.n.* (ICN 20356); Dep. Maldonado, 28 Jan 1912, *Osten 5775* (CORD, SI); Dep. Maldonado, 3 Feb 1916, *Herter 1198* (SP); Dep. Maldonado, Piriapolis, 29 Mar 1911, *Osten 5515* (SI); Dep. Rocha, Laguna Negra, Angostura, 20 Mar 1938, *Rosengurt B2611* (HB, ICN, RB); Montevideo, Carrasco, 11 Mar. 1932, *Osten 2247* (S); Montevideo, Carrasco, 27 Feb 1914, *Berro 7269* (HB); Montevideo, Carrasco, 28 Feb 1875, *C. Fruchart s.n.* (P 408881, 408882, 408883, SI); Montevideo, Carrasco, Apr 1913, *Berro 6788* (HB); Montevideo, Carrasco, *Legrand 72* (SP); Montevideo, Feb. 1870, *Gibert 892* (BR, K);Canelones, Balneario Guazuvirá Nuevo, 34º46’25’’S, 55º37’23’’W, 11 Feb 2012, *González s.n.* (MVFA); Canelones, Colinas de Solymar, 34º47’77’’S, 55º56’30’’W, 12 Feb 2012, *González s.n.* (MVFA); Canelones, Carrasco, 34º52’01’’S, 56º01’2,83’’W, 4 Mar 2011, *González s.n.* (MVFA).

SPECIES AND NAMES EXCLUDED FROM *HABENARIA* SECT. *PENTADACTYLAE*

Habenaria achnantha Rchb. f., Linnaea. 22: 812. 1849.

Habenaria alpestris Cogn., Fl. Bras. (Martius) 3(4): 74. 1893.

Habenaria aranifera Lindl., Gen. Sp. Orchid. Pl. 313. 1835.

Habenaria armata Rchb. f., Bonplandia 2(2): 10. 1854.

Habenaria caldensis Kraenzl., Bot. Jahrb. Syst. 16: 128. 1892.

Habenaria candolleana Cogn., Fl. Bras. (Martius) 3(4): 73. 1893.

Habenaria confusa Cogn., Fl. Bras. (Martius) 3(4): 65. 1893. = ***H. secundiflora*** Barb. Rodr.

Habenaria corcovadensis Kraenzl., Bot. Jahrb. Syst. 16: 120. 1892. = ***H. rodeiensis*** Barb. Rodr.

Habenaria culicina Rchb. f. & Warm., Otia Bot. Hamburg. 2: 79. 1881.

Habenaria entomantha (Lex.) Lindl., Gen. Sp. Orchid. Pl. 311. 1835.

Habenaria epiphylla Rchb. f. & Warm., Otia Bot. Hamburg. 2: 79. 1881. = ***H. glaucophylla*** Barb. Rodr.

Habenaria goyazensis Cogn., Fl. Bras. (Martius) 3(4): 77. 1893.

Habenaria gracilis Lindl., Gen. Sp. Orchid. Pl. 312. 1835. *nom. illeg*.

Habenaria graciliscapa Barb. Rodr., Gen. Sp. Orchid. 1: 155. 1877. = ***H. imbricata*** Barb. Rodr.

Habenaria humilis Cogn., Fl. Bras. (Martius) 3(4): 67. 1893. *nom. illeg*.

Habenaria imbricata Lindl., Gen. Sp. Orchid. Pl. 313. 1835.

Habenaria janeirensis Kraenzl., Bot. Jahrb. Syst. 16: 127. 1892. = ***H. paranaensis*** Barb. Rodr.

Habenaria lactiflora A. Rich. & Galeotti, Ann. Sci. Nat., Bot. ser 3(3): 28. 1845.

Habenaria lagunae-sanctae Kraenzl., Bot. Jahrb. Syst. 16: 119. 1892. = ***H. secundiflora*** Barb. Rodr.

Habenaria leptoceras Hook., Bot. Mag. 54: t. 2726. 1827.

Habenaria macilenta (Lindl.) Rchb. f., Flora 48: 180. 1865.

Habenaria modestissima Rchb. f., Linnaea 22: 811. 1849 publ. 1850.

Habenaria moritzii Ridl., Trans. Linn. Soc. London, Bot., Ser. 2, 2: 284. 1887. in part = ***H. armata*** Rchb. f., in part = ***H. gollmeri*** Schltr.

Habenaria muelleriana Cogn., Fl. Bras. (Martius) 3(4): 72. 1893. = ***H. macilenta*** (Lindl.) Rchb. f.

Habenaria paivaeana Rchb. f., Xenia Orchid. 3(1): 17. 1878.

Habenaria riedelii Cogn., Fl. Bras. (Martius) 3(4): 80. 1893. = ***H. warmingii*** Rchb. f. & Warm.

Habenaria rupicola Barb. Rodr., Revista Engen. 3: 144. 1881.

Habenaria santensis Barb. Rodr., Gen. Sp. Orchid. 2: 253. 1882.

Habenaria schenckii Cogn., Fl. Bras. (Martius) 3(4): 61. 1893.

Habenaria schomburgkii Lindl., London J. Bot. 2: 673. 1843.

Habenaria secunda Lindl., Gen. Sp. Orchid. Pl. 307. 1835.

Habenaria setacea Lindl., Gen. Sp. Orchid. Pl. 312. 1835.

Habenaria setifera Lindl., Ann. Mag. Nat. Hist. 4: 381. 1840. = ***H. trifida*** Kunth

Habenaria simillima Rchb.f., Xenia Orchid. 3(1): 18. 1878. = ***H. distans*** Griseb.

Habenaria taubertiana Cogn., Fl. Bras. (Martius) 3(4): 69. 1893.

Habenaria ulei Cogn., Fl. Bras. (Martius) 3(4): 74. 1893.

Habenaria warmingii Rchb. f. & Warm., Otia Bot. Hamburg. 2: 80. 1881.

INDEX TO SCIENTIFIC NAMES IN *HABENARIA* SECT. *PENTADACTYLAE* (ACCEPTED NAMES INDICATED BY BOLDFACE LETTERING.)

Habenaria arechavaletae Kraenzl. (6)

Habenaria arechavaletae Kraenzl. var. elata Cogn. (6)

Habenaria arechavaletae Kraenzl. var. *obovatipetala* (Schltr.) Pabst (6)

Habenaria crassipes Schltr. (3)

Habenaria dutraei Schltr. (1)

Habenaria ekmaniana Kraenzl. (2)

Habenaria exaltata Barb. Rodr. (3)

Habenaria henscheniana Barb. Rodr. (4)

Habenaria henscheniana Barb. Rodr. var. *densiflora* Cogn. (3)

Habenaria megapotamensis Hoehne (5)

Habenaria montevidensis Spreng. (6)

Habenaria obovatipetala Schltr. (6)

Habenaria pentadactyla Lindl. (7)

Habenaria recta Schltr. (2)

Habenaria sceptrum Schltr. (3)

Habenaria schiedmeyeri Schltr. ex Pabst (1)

Habenaria schnittmeyeri Schltr. (1)

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TABLE 1. A Comparison of the species assigned to *Habenaria* sect. *Pentadactylae* by different authors. Species sampled in the molecular phylogenetic analyses, including synonyms, are indicated by an asterisk.

Kraenzlin (1892)	Cogniaux (1893)	Kraenzlin (1901)	This work
H. achnantha	*H. achnantha*	*H. achnantha*	*H. dutraei**
*H. aranifera**	*H. alpestris**	*H. alpestris**	*H. ekmaniana**
*H. armata**	*H. aranifera**	*H. aranifera**	*H. exaltata**
H. conopodes	*H. armata**	*H. armata**	*H. henscheniana**
*H. corcovadensis**	*H. caldensis**	*H. caldensis**	*H. megapotamensis**
H. culicina	*H. candolleana*	*H. candolleana*	*H. montevidensis**
H. entomantha	*H. confusa**	*H. confusa**	*H. pentadactyla**
*H. epiphylla**	*H. corcovadensis**	*H. corcovadensis**	
H. gracilis	*H. exaltata**	*H. entomantha*	
*H. imbricata**	*H. goyazensis**	*H. exaltata**	
H. lactiflora	*H. gracilis*	*H. goyazensis**	
H. lagunae-sanctae	*H. graciliscapa**	*H. gracilis*	
*H. leptoceras**	*H. humilis**	*H. graciliscapa**	
*H. macilenta**	*H. imbricata**	*H. humilis**	
H. modestissima	*H. janeirensis**	*H. imbricata**	
*H. moritzii**	*H. lagunae-sanctae**	*H. janeirensis**	
H. paivaeana	*H. macilenta**	*H. lactiflora*	

*H. pentadactyla**	*H. modestissima*	*H. lagunae-sanctae**
H. schomburgkii	*H. moritzii**	*H. macilenta**
*H. secunda**	*H. muelleriana**	*H. modestissima*
*H. setacea**	*H. pentadactyla**	*H. moritzii**
*H. setifera**	*H. riedelii**	*H. paivaeana*
H. similima	*H. rupicola**	*H. pentadactyla**
*H. warmingii**	*H. santensis*	*H. riedelii**
	*H. schenckii**	*H. rupicola**
	H. schomburgkii	*H. santensis*
	*H. secunda**	*H. schenckii**
	*H. setacea**	*H. schomburgkii*
	*H. setifera**	*H. secunda**
	H. taubertiana	*H. setacea**
	H. ulaei	*H. similima*
	*H. warmingii**	*H. taubertiana*
		H. ulaei
		*H. warmingii**

TABLE 2. Data-matrix values and tree statistics for each of the parsimony analyses.

	ITS	*matK*	*trnK* intron	*rps16-trnK*	combined
# terminals	77	77	58	58	77
Aligned length	762	1551	271	957	3541
Percentage of missing data	11.6%			26.5%	25.7%
# variable, non-informative sites	66	128	30	55	346
# parsimony informative sites	72	121	16	67	491
% Informative sites	9.8%	7.8%	5.9%	7%	13.9%
# trees	297	9810	8760	6020	120
Fitch tree length	187	382	57	183	1718
Consistency index (CI)	0.82	0.74	0.86	0.73	0.66
Retention index (RI)	0.87	0.86	0.88	0.87	0.79

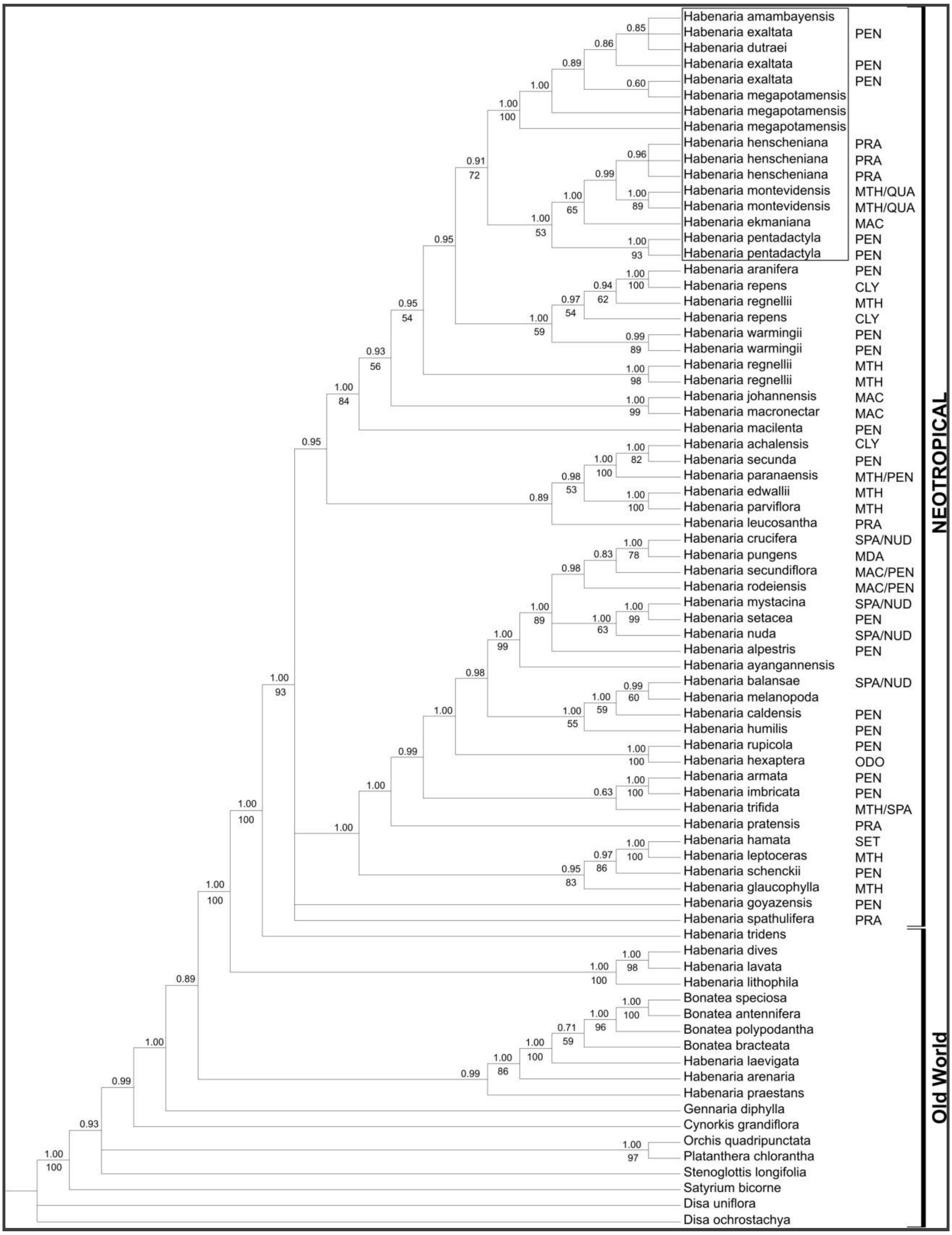


FIG. 1. Bayesian majority-rule consensus tree of the combined plastid and nuclear data sets. Numbers above and below branches represent posterior probabilities (PP) and bootstrap percentages (BP), respectively. The three letter abbreviation at the right of the species name indicates sectional classification. Section *Pentadactylae*, as circunscribed in this work, is boxed. CLY = Sect. *Clypeatae*; MAC = sect. *Macroceratitae*; MTH = sect. *Micranthae*; NUD = sect. *Nudae*; ODO = sect. *Odontopetalae*; PEN = sect. *Pentadactylae*; PRA = sect. *Pratenses*; QUA = sect. *Quadratae*; SET = sect. *Seticaudae*; SPA = sect. *Spathaceae* .

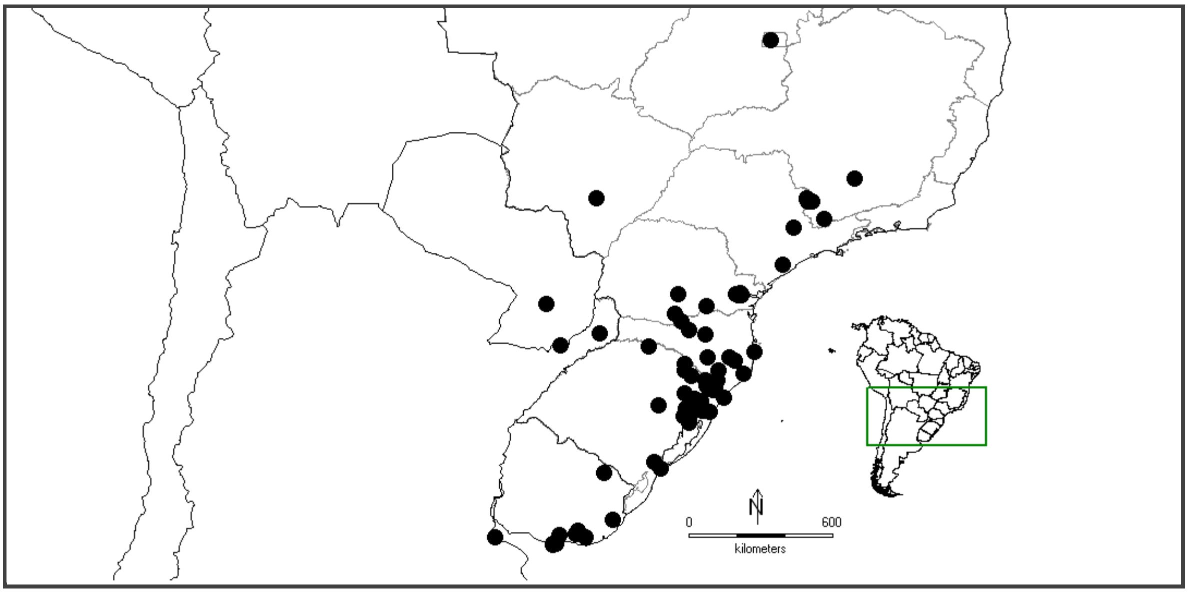


FIG. 2. Distribution of *Habenaria* section *Pentadactylae*.

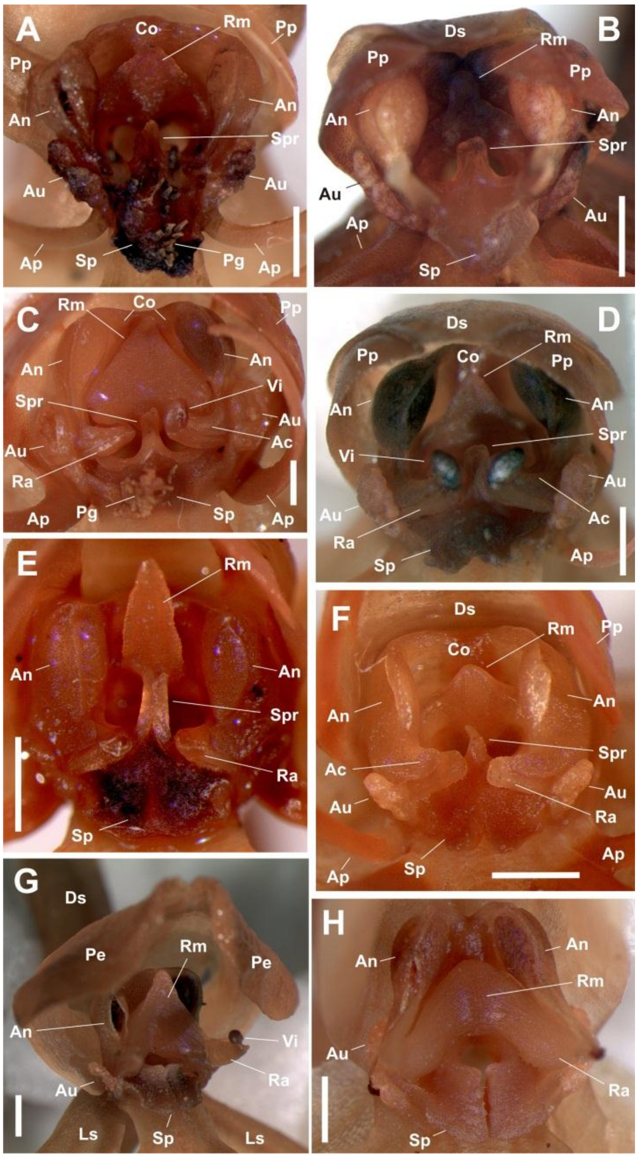


FIG. 3. Gynostemium morphology in *Habenaria* sect. *Pentadactylae*. A. *H. dutraei* (*Pedron 3*, ICN). B. *H. ekmaniana* (*Radins s.n.*, BHCB). C. *H. exaltata* (*Pedron 10*, ICN). D. *H. megapotamensis* (*Pedron 6*, ICN). E. *H. henscheniana* (*Pedron 2*, ICN). F. *H. pentadactyla* (*Pedron 11*, ICN). G. *H. montevidensis* (*Pedron 9*, ICN). H. *H. leucosantha* (*Batista 1604*, BHCB). Scale bars = 2 mm; Ac = anther canals; An = anther; Ap = anterior petal segment; Au = auricules; Co = connective; Ds = dorsal sepal; Ls = lateral sepals; Pe = petal; Pg = pollen grains; Pp = posterior petal segment; Ra = rostellum arms; Rm = rostellum midlobe; Sp = stigmatic processes; Spr = stigmatic projections; Vi = viscidium.

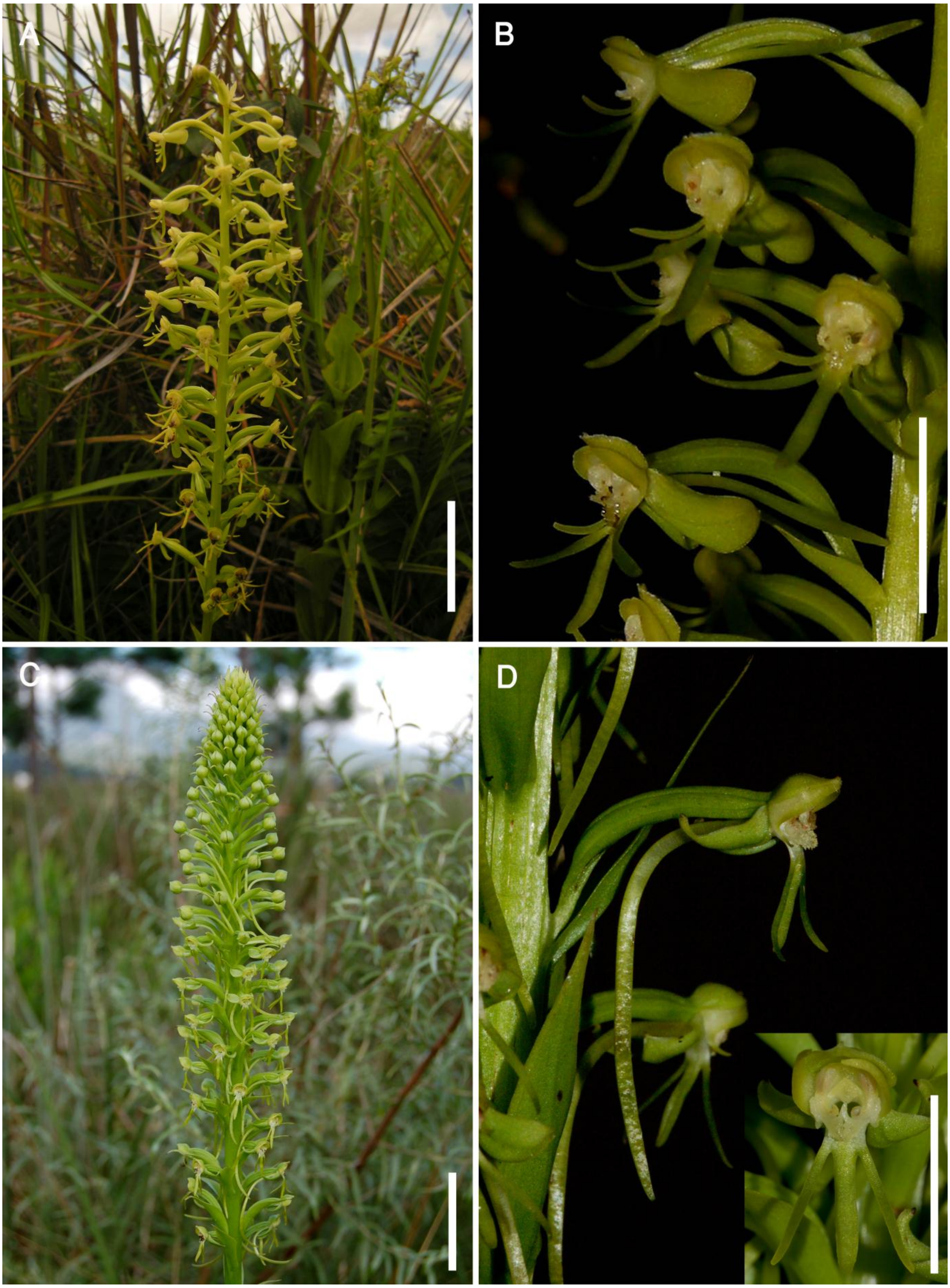


FIG. 4. Inflorescences and flowers from live specimens. A-B. *Habenaria dutraei* from Santo Antônio da Patrulha, Rio Grande do Sul, Brazil. C-D. *Habenaria exaltata* from Piraquara, Paraná, Brazil. Scale bars (A-C) = 3 cm; (B-D) = 1 cm.

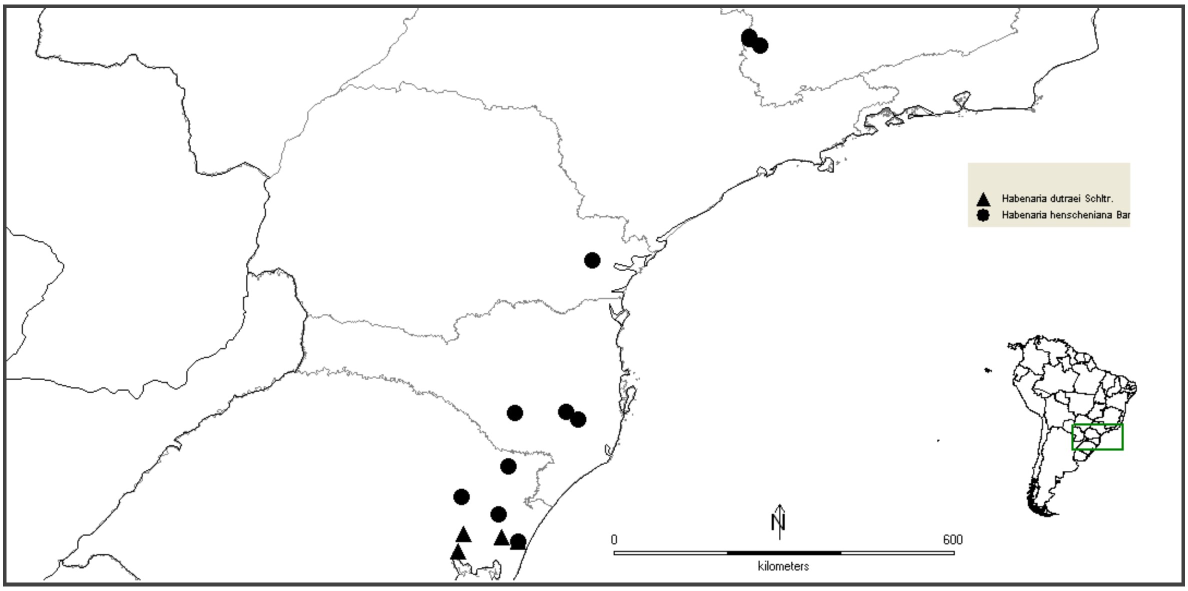


FIG. 5. Distribution of *Habenaria dutraei* (triangle) and *H. henscheniana* (circle).

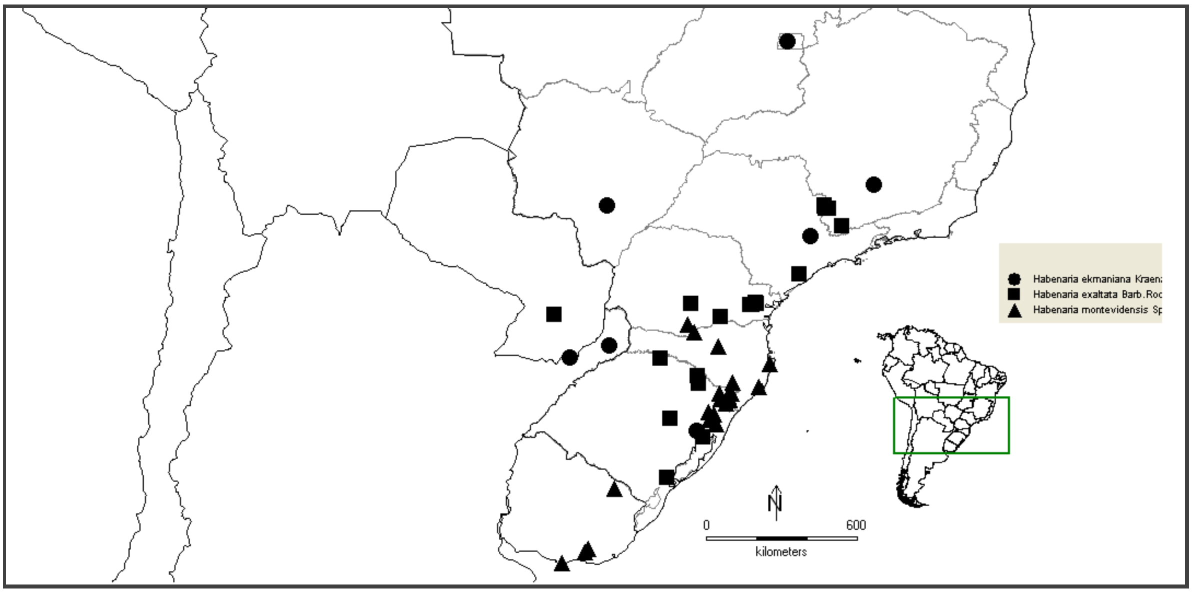


FIG. 6. Distribution of *Habenaria ekmaniana* (circle), *H. exaltata* (square) and *H. montevidensis* (triangle).

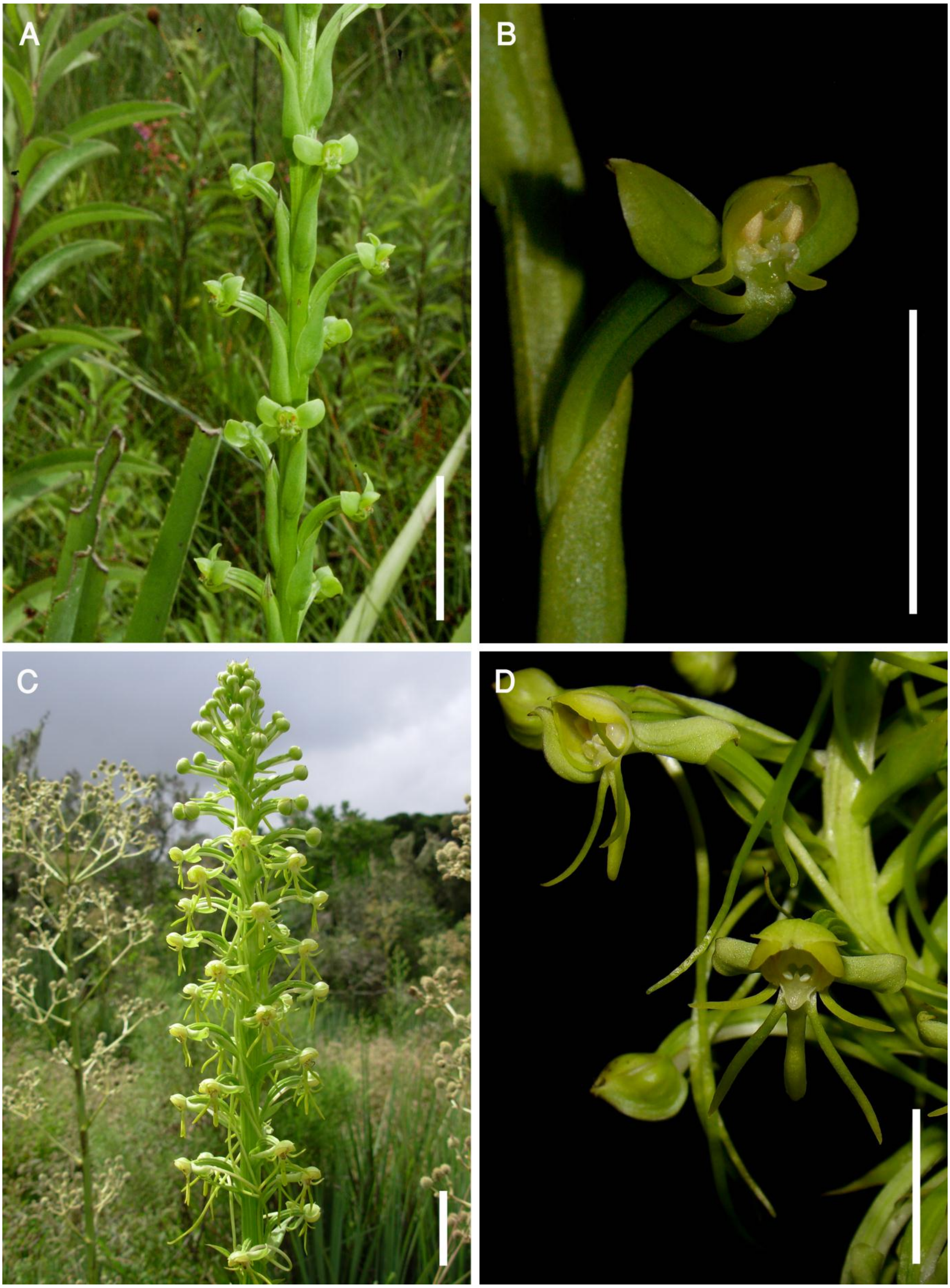


FIG. 7. Inflorescences and flowers from live specimens. A-B. *Habenaria henscheniana* from Poços de Caldas, Minas Gerais, Brazil. C-D. *Habenaria megapotamensis* from Cambará do Sul, Rio Grande do Sul, Brazil. Scale bars (A-C) = 2 cm; (B-D) = 1 cm.

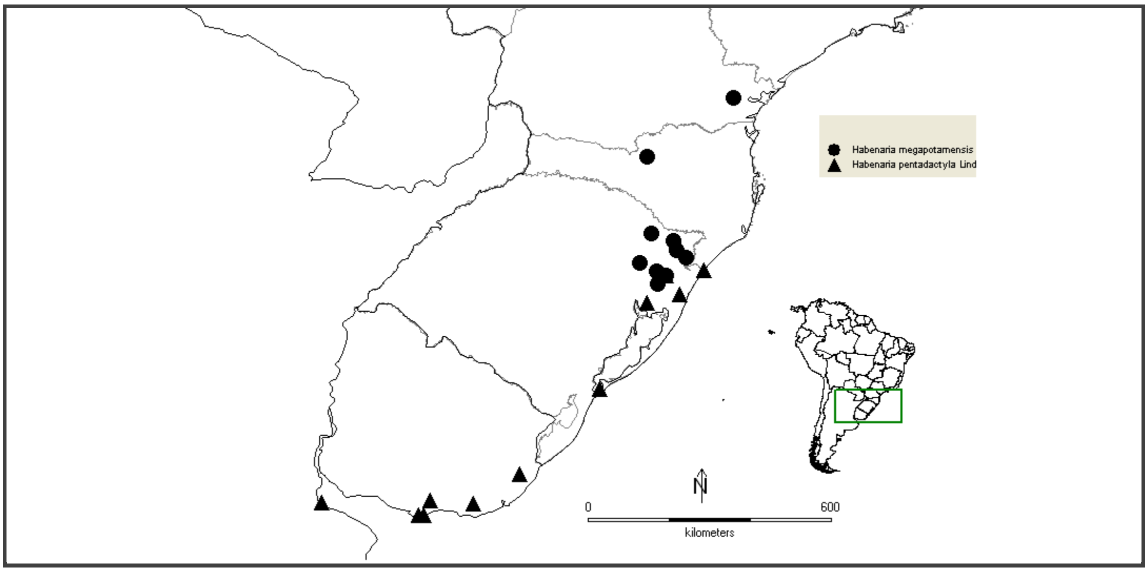


FIG. 8. Distribution of *Habenaria megapotamensis* (circle) and *H. pentadactyla* (triangle).

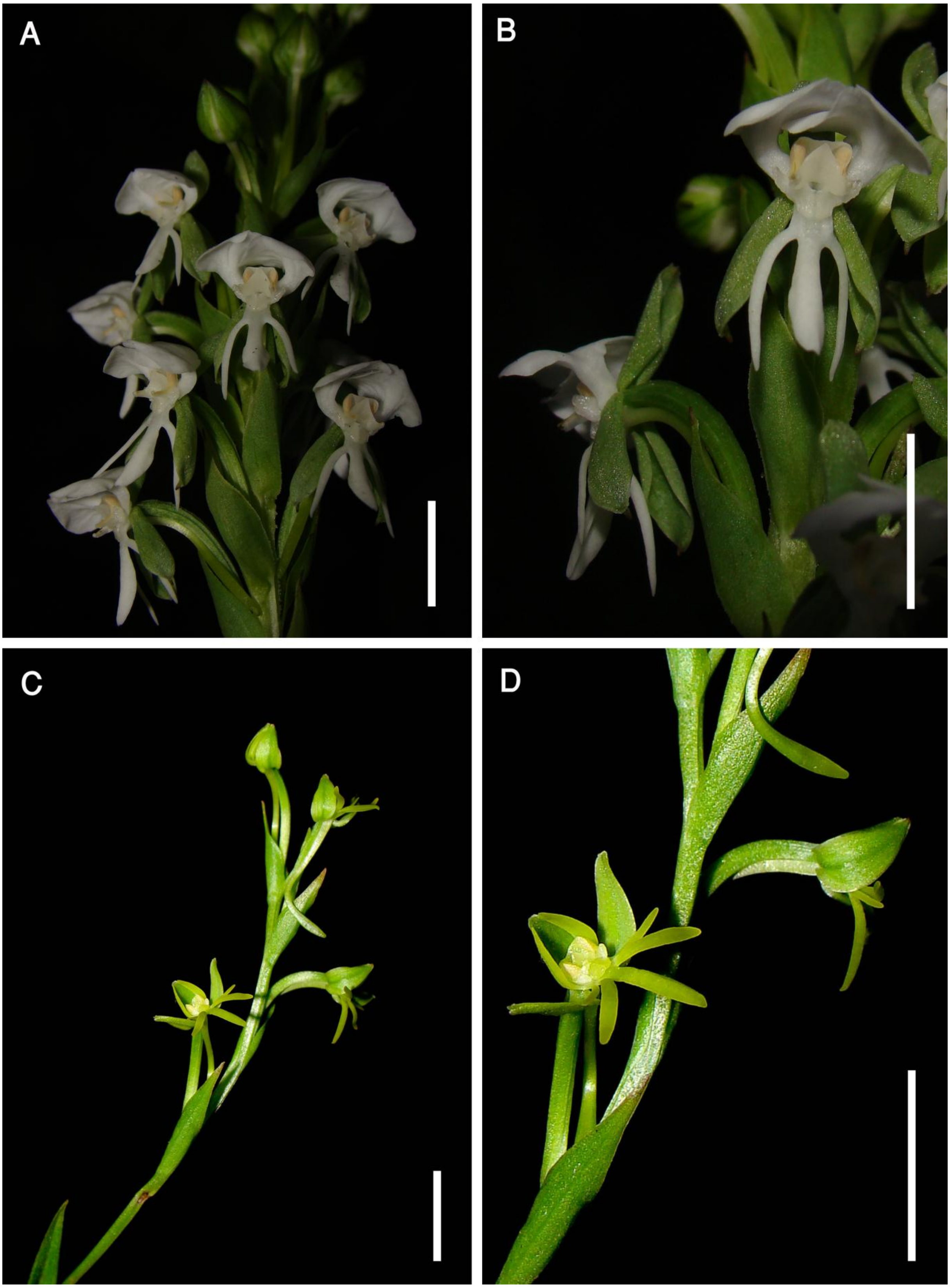
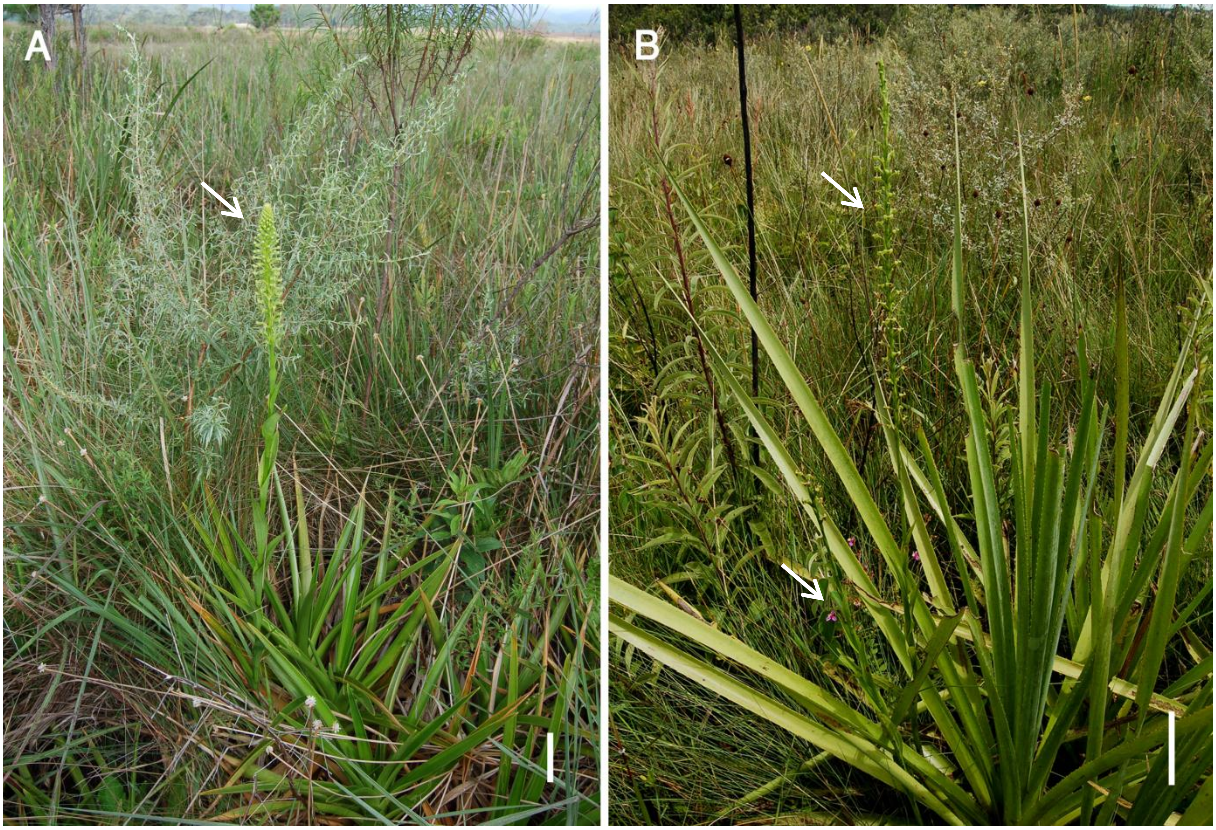


FIG. 9. Inflorescences and flowers from live specimens. A-B. *Habenaria montevidensis* from São Francisco de Paula, Rio Grande do Sul, Brazil. C-D. *Habenaria pentadactyla* from São Francisco de Paula, Rio Grande do Sul, Brazil. Scale bars = 1 cm.

SUPPLEMENTARY MATERIAL



SUPPLEMENTARY FIG. S1. Habit from live specimens. A. *Habenaria exaltata* (arrow) from Piraquara, Paraná, Brazil. B. *Habenaria henscheniana* (arrows) from Poços de Caldas, Minas Gerais, Brazil. Scale bars = 10 cm.

4. CAPÍTULO 2

Pollination biology of four species of *Habenaria* Willd. (Orchidaceae: Orchidinae) in Southern Brazil

Artigo submetido ao periódico Botanical Journal of the Linnean Society. Este capítulo está estruturado seguindo as normas de formatação referentes ao respectivo periódico, exceto para o alinhamento do texto.

Pollination biology of four sympatric species of *Habenaria* Willd. (Orchidaceae: Orchidinae) from Southern Brazil

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ABSTRACT - *Habenaria* is a large genus of terrestrial orchids particularly species-rich in Brazil. However, only a handful species have been studied regarding their reproductive biology. The pollination process and breeding system of the sympatric *Habenaria johannensis, H. macronectar, H. megapotamensis* and *H. montevidensis* was documented for native populations from Rio Grande do Sul, Brazil. All species investigated offer a nectar reward (mean values of total sugars ranging from 18 to 26 %) concealed in a spur. *Habenaria montevidensis* is pollinated by Hesperiidae butterflies (especially of the genus *Urbanus*) that carry pollinaria on their eyes; the other three species are pollinated by Sphingidae moths. *Habenaria johannensis* is pollinated by moths of *Manduca rustica* and *M. sexta* that carry the pollinaria at the base of the proboscis. *Habenaria macronectar* is pollinated by the moths *Eumorpha labrusca* and *M.* cf. *lucetius*, and these bear pollinaria between the palpi. *Habenaria megapotamensis* is pollinated by moths of *M.* cf. *lucetius* that bear the pollinaria on the proboscis. All species studied are self-compatible, but pollinator-dependent. They also displayed high reproductive success (ranging from 69.48 to 97.4 %) and male efficiency factors slightly higher than 1, suggesting that at least one flower was pollinated for each pollinarium removed. At the study sites, the investigated *Habenaria* species are isolated (in terms of pollination) by a set of factors that includes differing floral morphologies, different pollinators and/or different pollinarium placement on the pollinator.

Key words: Orchidaceae, pollination, *Habenaria*, Sphingidae, Hesperiidae, butterflies, floral morphology, breeding system.

INTRODUCTION

Comprising some 835 species (Govaerts *et al.*, 2010) distributed throughout the temperate and tropical regions of the Old and New Worlds, *Habenaria* Willd. is the largest terrestrial orchid genus (Batista *et al.*, 2006). Central and Southern Africa, Eastern Asia and Brazil are its main centres of diversity (Kurzweil & Weber, 1992). About 165-170 species of this genus have been reported for Brazil (Hoehne, 1940; Pabst & Dungs, 1975; Batista *et al.*, 2006), where the *Cerrado* Biome (in essence, a tropical Savanna) seems to be particularly species-rich (Batista *et al.*, 2006). In its current delimitation, *Habenaria* is placed within orchid subtribe Orchidinae (Chase *et al.*, 2003), and its main distinctive features are the often bifid petals that are not fused to the other floral organs, the usually deeply divided median petal (labellum) that lacks a callus, the distinctly stalked stigmas and the entire stigma lobes, which are usually free and not adnate to the petals or lip (Cribb, 2001). In spite of its diversity, the pollination biology and breeding system of very few *Habenaria* species have been studied. Most published reports indicate that *Habenaria* species are mainly pollinated by moths (Nilsson *et al.*, 1985; Galetto *et al.*, 1997; Singer & Cocucci, 1997; Singer, 2001; Singer *et al.*, 2007; Peter *et al.*, 2009) and, to a lesser extent, by crane-flies (Singer, 2001), or diurnal Lepidoptera (Moreira *et al.*, 1996). This is supported by floral features. For example, flowers are usually greenish or pale in colour, the nectar is concealed in a spur and crepuscular/nocturnal emission of fragrance (a feature normally associated with pollination by moths) is evident (Singer, 2001; Singer & Cocucci, 1997; Singer *et al.*, 2007; Peter *et al.*, 2009). Pollinaria of *Habenaria* spp. have been reported to adhere to various, smooth body parts of pollinators, such as the surface of the eye, proboscis and distal parts of the forelegs (Singer, 2001; Singer & Cocucci, 1997; Singer *et al.*, 2007; Peter *et al.*, 2009). Little is known of the breeding system of this orchid genus. The species *H. parviflora* Lindl. is self-compatible, but pollinator-dependent (Singer, 2001); and *H. pleiophylla* Hoehne & Schltr.

appears to be self-compatible, based on the vigorous growth of the pollen tube following manual self-pollination (Singer *et al*., 2007).

In Southern Brazil, several *Habenaria* species grow sympatrically and, during the local summer (December to March), have overlapping flowering periods. Of these species, four are particularly abundant and conspicuous: *H. johannensis* Barb. Rodr., *H. macronectar* (Vell.) Hoehne, *H. megapotamensis* Hoehne and *H. montevidensis* Spreng., and these form the subject of this study. Not only do the flowering periods of these species overlap, but these plants may also be found growing in close proximity, or separated by short distances. For instance, *H. macronectar* and *H. megapotamensis* can often be found growing together. Thus, the aim of this study is to extend our knowledge of the pollination biology and reproductive biology of Neotropical *Habenaria* species. More specifically, we set out to document the pollination process, fruiting success and breeding system of *H. johannensis*, *H. macronectar*, *H. megapotamensis* and *H. montevidensis*.

MATERIALS AND METHODS

Study site - Studies were performed in two neighbouring Municipalities (Cambará do Sul, approx. 29º 10’S, 50º 19’W and São Francisco de Paula, approx. 29º 25’S, 50º 23’W) within the Atlantic Rain Forest domain, in the State of Rio Grande do Sul, Southern Brazil. For conservation reasons, we cite here only the coordinates of both Municipalities and omit the exact location details of the actual populations. These data, however, are available on request. This particular region is locally known as *Campos de Cima da Serra* and consists of a mosaic of native grasslands and forest patches dominated by *Araucaria angustifolia* (Bertol) Kuntze (Araucariaceae), at an altitude of about 900-930 m. Average annual rainfall is about 2468 mm and annual average temperature is approximately 14.5ºC (Moreno, 1961). The climate is characterized by a cool summer, a cold winter, and the lack of a well-defined dry period (Nimer, 1989; Almeida *et al*., 2009). All species studied inhabit swamps dominated by *Eryngium pandanifolium* Cham. & Schltdl. (Apiaceae).

Studied species - *Habenaria johannensis* and *H. macronectar* are currently placed in sect. *Macroceratitae* Kraenzl. (Kränzlin**,** 1892; Cogniaux, 1893), an easily identifiable assemblage of species with proportionally large, greenish-white, long spurred (spur length ≥ 6 cm) flowers.

Habenaria megapotamensis and *H. montevidensis* were never assigned formal sectional classification. According to unpublished phylogenetic analysis based on DNA sequence data, these two species belong in the same clade, which also includes *H. dutraei* Schltr., *H. ekmaniana* Kraenzl., *H. exaltata* Barb. Rodr., *H. henscheniana* Barb. Rodr. and *H. pentadactyla* Lindl. (Pedron *et al.*, unpubl. data).

Of the species studied, *H. johannensis* has the widest geographical distribution, ranging from Bolivia and Paraguay to northeastern, central western, southeastern and southern Brazil. It is typical of wet and marshy areas, but occasionally can occupy human-disturbed habitats, growing on damp roadsides or in ditches. *Habenaria macronectar* is restricted to southeastern and southern Brazil, as well as Uruguay. This species occurs in wet or marshy areas. *Habenaria megapotamensis* is known only from southern Brazil, while *H. montevidensis* also occurs in southern Brazil and Uruguay. These two latter species are typical of marshy areas or wetlands, and most records are derived from the northeastern highlands of Rio Grande do Sul. Throughout this paper, we follow the orchid classification of Chase *et al*. (2003).

Floral features and nectar properties - Ten fresh, intact flowers from each species were used to record and measure flower features (column and pollinarium structure, spur length, nectar-column height, as well as nectar volume and concentration; see Table 1). All these flowers came from five bagged specimens of each species, and nectar parameters were always measured for each of these taxa during the period when their pollinators had been recorded in activity. Nectar volume was measured by means of a microsyringe, and nectar concentration (total sugar) was measured using a manual pocket (0-32) refractometer. Owing to the very small quantity of nectar produced by *H. montevidensis* flowers, nectar volume in this species was measured by means of a P2 micropipette, and nectar concentration measurements were based on the total accumulated nectar of all the flowers analysed. Spur length, nectar volume, height of nectar column and nectar concentration (total sugars) for all studied species were statistically compared by means of an ANOVA test (with permutation) using PAST software (Hammer et al., 2001) (Table 1).

Flower morphology was studied using both, fresh and alcohol-preserved flowers (70 % [v/v] ethanol). Plant vouchers were deposited at the ICN Herbarium of the Universidade Federal do Rio Grande do Sul (UFRGS) under the following accession numbers: *H. johannensis* (*M. Pedron 5*), *H. macronectar* (*M. Pedron 1*); *H. megapotamensis* (*M. Pedron 10*); *H. montevidensis* (*M. Pedron 9*). Throughout this paper, we follow the orchid morphology concepts of Dressler (1993).

Pollination– The pollination biology of *H. megapotamensis* was studied in the field during the flowering seasons of 2010 and 2011 (Table 2). The other three species were studied in 2011 (Table 2). Generally, the observation period for both years began in late January or early February and was completed by mid March, totalling 181 observation hours (Table 2). Both, crepuscular-nocturnal and diurnal observations were made on each species. Here, we define “diurnal observations” as those made between 06:00 and 18:00 h and “crepuscular-nocturnal” observations as those made from 18:00 h onwards. The specific observation period for each species, as well as the specific number of diurnal and crepuscular-nocturnal hours spent observing each species are detailed in Table 2. When our early observations clearly indicated the prevalence of some kind of pollinator activity (e.g., diurnal *vs*. crepuscular-nocturnal), our fieldwork schedule was adapted accordingly. Therefore, most observation hours for *H. johannensis*, *H. macronectar* and *H. megapotamensis* were performed during the nocturnal period (Table 2). Conversely, most observation hours for *H. montevidensis* were made during the day. However, since nocturnal, non-pollinating visitors were also recorded for this species, intact flowers (36 flowers from 21 inflorescences) were marked at the end of the day’s observations and checked at the beginning of the next day, before observations had begun, in order to verify whether they had been pollinated and/or had their pollinaria removed during the night. Pollinator behaviour was documented for all species studied using field notes, photography and, when possible, video. The video record made it possible to gain a better understanding of the pollination process for most of the species studied, especially those visited by crepuscular-nocturnal pollinators. Individuals of both, pollinating and non-pollinating insects were collected and sacrificed for taxonomic identification. These insect vouchers were deposited at the entomological didactic collection of the Insects Ecology Laboratory, Zoology Department, UFRGS.

Breeding system, fruiting success and pollination efficiency–Breeding system experiments were performed *in situ*, by bagging inflorescences in order to exclude natural pollinators. Bags were supported with the help of wooden stakes. Four treatments were applied to these inflorescences: intact flowers (control), emasculation, manual self-pollination, and cross pollination (Table 3). Treatments that set fruit were compared using a χ^2 test ($\alpha = 0.05$). The number of plants per species and flowers used per treatment are detailed in Table 3. Intact flowers of these plants were also used to record flower lifespan for each species.

In order to assess the efficiency of pollination, the fruiting success (number of fruit divided by the number of flowers produced) was calculated for each species, at the end of their respective observation periods. During 2010, 30 inflorescences of *H. johannensis* (totalling 269 flowers), 49 inflorescences of *H. macronectar* (totalling 526 flowers) and 32 inflorescences of *H. megapotamensis* (totalling 1568 flowers) were available. It wasn't possible to study the fruiting success for *H. montevidensis* in 2010, since fruiting inflorescences had been destroyed, probably eaten by unidentified animals. During 2011, 45 inflorescences of *H. johannensis* (totalling 387 flowers), 16 inflorescences of *H. macronectar* (totalling 154 flowers), 17 inflorescences of *H. megapotamensis* (totalling 561 flowers) and 35 inflorescences of *H. montevidensis* (totalling 429 flowers) were available. Fruiting success (mean fruit set per inflorescence) in both years for all species except *H. montevidensis* (see above) was statistically compared using an independent, two-sample t-test (unequal sample sizes, equal variance) by means of PAST software (Hammer et al., 2001) (Table 4).

In 2011, Nilsson's (Nilsson *et al.*, 1992) male efficiency factor (percentage of pollinated flowers divided by the percentage of flowers acting as pollen donors) was also used to calculate pollination effectiveness for all species studied. The total number of flowers used in these calculations, as well as the percentages of flowers that were pollinated and that acted as pollen-donors are detailed in Table 4.

RESULTS

Flower features - Flowers are resupinate and clustered in long, terminal, racemose inflorescences bearing up to 19 flowers in *H. johannensis*, 28 in *H. macronectar*, 87 in *H. megapotamensis* and 31 in *H. montevidensis* (See Supplementary material Fig. S1). The flowers of *H. johannensis*, *H. macronectar* and *H. montevidensis* are greenish-white, and those of *H. megapotamensis* are light green (Fig. 1A and Supplementary material Fig. S1). The lifespan of intact flowers ranged from 12 to 13 days in *H. johannensis*, 14 to 18 days in *H. macronectar* and *H. megapotamensis* and 17 to 21 days in *H. montevidensis*). In all studied species, the dorsal sepal or lateral petals form a hood-like structure that partially hides the column. In *H. montevidensis*, the lateral petals are entire, whereas in the other three species studied, they are bifid. The labellum is trilobed, and its posterior part is prolonged to form a spur that is partially filled with nectar (Fig. 1A). Mean spur length ranged from 1.62 to 13.09 cm, all species showing statistically significant differences ($p<0.0005$; Table 1). The rostellum is trilobed, and the anther canals are adnate to the lateral lobes (rostellar arms) so that a pad-like viscidium is placed at the end of each rostellar arm (Fig 1B-E). In *H. johannensis* the median rostellar lobe is keel-like and anteriorly projecting (Fig. 1B). During floral ontogenesis, the anther undergoes a division that ultimately results in the formation of two separated pollinaria concealed in their respective anther sacs. Each pollinarium is placed alongside each lateral rostellar lobe (Fig. 1B-E). In all studied species the pollinarium consists of massulate pollinia, an arm-like, hyaline caudicle and a pad-like, terminal viscidium. Pollinarium is about 12 mm long in *H. johannensis*, 9 mm in *H. macronectar* and 4 mm in *H. megapotamensis* and *H. montevidensis*). In *H. johannensis* and *H. macronectar*, the rostellar arms are long and arched; with both viscidia almost adjacent and upwardly pointing (Fig. 1B-C). In *H. megapotamensis*, the rostellar arms are short and curved inwards, so that the spur entrance is closely flanked by the viscidium of each pollinarium (Fig. 1D). In *H.*

montevidensis, the rostellar arms are straight and slightly divergent (Fig. 1E), and the distance between the apices of the rostellar arms is about 3 mm. In all studied species, the two stigmatic surfaces are placed below the pollen sacs, surrounding the spur entrance (Fig. 1B-E). In *H. johannensis* and *H. macronectar,* the stigmatic surfaces are long-stalked and involute (Fig. 1B-C). Conversely, those of *H. megapotamensis* and *H. montevidensis* are short and slightly convex (Fig. 1D-E). In *H. megapotamensis*, a tooth-like process partially occludes the spur entrance (Fig. 1D). This process is formed by the protruding, erect projection of the apex of the inner margin of the stigmatic lobes, and this is located in front of the spur entrance, thereby dividing the entrance of the spur into two apertures (Fig. 1D). The flowers of *H. johannensis*, *H. macronectar* and *H. megapotamensis* emit a sweet fragrance, whose secretion is perceptible after 18:00-19:00 h. The flowers of *H. montevidensis* produce a very faint, sweet fragrance throughout the whole day. This fragrance is best perceived by enclosing the flowers in a vial.

Nectar volumes in intact flowers of the same species is subject to remarkable variation and mean values ranged from 1.27 to 35 μL, depending on the species (Table 1). Mean nectar volumes showed significant differences between all studied species (Table 1). In intact flowers of all species the nectar forms a conspicuous column. Except for *H. montevidensis*, the height of this nectar column shows remarkable variation in intact flowers of the same species (Table 1). Mean values of nectar column height ranged from 0.52 to 3.32 cm, depending on the species (Table 1). Nectar column heights showed statistically significant differences, except when comparing *H. macronectar* and *H. johannensis,* and *H. johannensis* and *H. megapotamensis* (Table1). Mean values of nectar concentration ranged from 18.12 to 26.20 % (Table 1). All species showed significant differences ($p<0.0005$) in nectar concentration values, except when comparing *H. macronectar* and *H. megapotamensis* (Table 1).

Pollination mechanism - Pollinarium withdrawal takes place when insects insert the proboscis into the spur, and press the surface of the viscidia with a scale-less, smooth body part (e.g. proboscis, ventral region between the palpi, or the eyes, depending on the species) (Fig. 3A, C, D, and F), thus dislodging the pollinaria on leaving the flower. Pollination takes place when a pollinarium-laden insect visits a flower; the pollinia contact the stigmatic surface and massulae are left behind (Fig. 3B, E, and G). In *H. johannensis*, *H. macronectar* and *H. montevidensis,* the insects insert the proboscis along a straight course. In *H. johannensis*, pollinator movements are restricted by the projected, keel-like median rostellar lobe (Fig 1B), and the pollinators are mechanically guided against the upward-facing viscidia (Fig 3A). In *H. megapotamensis*, two tooth-like stigmatic processes partially block the spur entrance (Fig 1D). Therefore, the insect has to insert its proboscis laterally, thus pressing it against the viscidia that are orientated toward the spur entrance (Fig. 3C).

Pollinators, pollinator features and behaviour - All species investigated are pollinated by Lepidoptera (Figs 2-4), a fact which is consistent with overall floral morphology. Remarkably, most insects recorded bearing five or more pollinaria had proboscises that were shorter (see Table 4) than the mean spur length of the orchids that they pollinated (see Table 1). However, a larger pollinator sample is necessary to address this phenomenon adequately.

During our observations, *H. johannensis*, *H. macronectar* and *H. megapotamensis* were pollinated solely by crepuscular-nocturnal hawkmoths (Sphingidae) that were recorded at the flowers from 19:00 to 21:35 h (Fig. 2A-D, Fig. 4A-B, Table 4). Diurnal visitors or pollinators were never recorded at these three species. Our fieldwork observations, as well as our photographic and film record showed that all Sphingidae displayed similar behavioural patterns. On approaching, the hawk-moth hovers in front the flower and inserts its proboscis into the spur (Fig. 3A and D). In *H. johannensis* and *H. macronectar*, the moths use their forelegs to grasp the perianth (Fig. 2A, C), but this behaviour was not observed for *H. megapotamensis*. During our observations, *H. johannensis* was pollinated by moths of *M. sexta* Linnaeus, 1763 (proboscis length: 10.3 cm, n=1) (Fig. 2A) and *Manduca rustica* (Fabricius, 1775) (proboscis length: 13.5 cm, n=1) (Fig. 2A-B). These moths carried pollinaria of *H. johannensis* (13 and 15, respectively, Table 4) attached to the underside of the base of the proboscis (Fig. 2A-B). Owing to weather conditions the behaviour of the second moth species could not be followed in detail. However, the photographic record suggests that it behaves much like *M. sexta*. The first pollinator was seen moving between two neighbouring inflorescences, spending around 25 seconds at the flowers. Four additional hawkmoth visits were recorded during the observation period, but environmental conditions and the long distance that separated us from the visited inflorescences precluded the unequivocal taxonomic identification of these insects. Also during our observations, moths of *M.* cf. *lucetius* (Cramer, 1780) (Proboscis length: 6.4-7.4 cm, n=3) (Fig. 2C) and *Eumorpha labruscae* (Linnaeus, 1758) (Proboscis length: 5.9 cm, n=1) (Fig. 2D) were recorded as the pollinators of *H. macronectar*. Both moth species carried pollinaria (up to 3 and 12, respectively, see Table 4) attached between the palpi. The second moth species was sighted only once. The first species was sighted 13 times. On five of these occasions, the moths carried pollinaria of *H. macronectar,* and on two of them, were simultaneously bearing pollinaria of *H. megapotamensis* (that was flowering nearby) attached to the base and sides of the proboscis. It was possible to film some individuals of *M.* cf. *lucetius* visiting the flowers of *H. macronectar* (see Supplementary material Video S1), and our video record indicates that moths spent between 2 and 17 s probing individual flowers and between 12 and 140 s at each inflorescence. Remarkably, videos revealed that the same moth inserted its proboscis very differently into flowers on the same inflorescence (inserting or not the full length of the proboscis, see Supplementary material Video S1).The sphingid *Agrius cingulatus* (Fabricius) (proboscis length: 9-12.8 cm, according to Singer and Cocucci, 1997) was photographed at the flowers. This insect never removed pollinaria, possibly because its proboscis is considerably longer than the spur (see Supplementary material Fig. S2). The only pollinators recorded for *H. megapotamensis* were moths of *M.* cf. *lucetius* (Figs 4A-B, see also Supplementary material Video S2). A total of 7 visitation events were recorded and insects were observed carrying pollinaria (up to 15, see Table 4) fixed to the sides and underside of the proboscis base. As stated above, this moth was also observed pollinating flowers of nearby *H. macronectar*. They visit several flowers per inflorescence, usually starting at the base and proceeding towards the top of the inflorescence. During our observations, the pollinators spent 2-9 s at each flower and 30-125 s at each inflorescence. Also, during our observations, *H. montevidensis* was pollinated solely by butterflies of family Hesperiidae (Fig. 4C-D, Table 4). Generally, pollinator activity was observed from 06:00 to 18:00 h. All pollinator species behaved in a very similar manner. Insects landed at the base of the inflorescence and proceeded towards the apex, systematically visiting the flowers. Column structure allows pollinators to place their heads between the rostellar arms (Fig. 3F) and all recorded pollinators bore the pollinaria on their eyes (Fig. 3G, Fig 4C-D). Butterflies of *Cumbre* sp., *Vehilius clavicula* Plötz, 1884 (proboscis length of both: 13 mm, n=1) were sighted only once, bearing 2 and 3 pollinaria, respectively. Butterflies of *Urbanus teleus* (Hübner, 1821) (proboscis length: 11 mm, n=1) and *U. zagorus* (Plotz 1880) (proboscis length: 15 mm, n=1) were more frequent, bore more pollinaria (up to 6 and 14, respectively, see Table 4) and are considered to be the main pollinators of *H. montevidensis* in the study area (Figs 4C-D, see also Supplementary material Video S3). As a whole, butterflies of *Urbanus* spp. were seen ca. 167 times visiting inflorescences. These insects visited 1-4 inflorescences per visit to the population, spending between 1 and 11 s at each flower and between 14 and 80 s at each inflorescence. These skippers were occasionally recorded actively attempting to remove the pollinaria attached to their eyes with their forelegs. Butterflies of *Hesperocharis erota* (P.H. Lucas, 1852) (Pieriidae) and *Phocides pialia* Hewitson 1857 (Hesperiidae) were photographed and filmed visiting inflorescences, but without dislodging pollinaria (see also Supplementary material Fig. S2). Although measurements couldn't be made in these two latter species, the photographic and filmic record clearly indicates that the eyes of both insect species do not enter in contact with the orchid viscidia. Four visits of the nocturnal hawkmoth *Xylophanes fosteri* Rothschild & Jordan, 1906 (Sphingidae, proboscis length: 22 mm, n=1) (see Supplementary material Fig. S2 and Supplementary material Video S3) were recorded. This moth consistently visited flowers without removing pollinaria, as would be expected from an insect with a proboscis significantly longer than the nectariferous spur. Furthermore, intact, marked flowers used to ascertain the possibility of nocturnal pollination (see Material and methods), were still intact the following morning.

Breeding systems - All species studied (Table 3) are self-compatible, but pollinator-dependent. No fruit were formed by emasculated or intact flowers, strongly indicating that all species studied require the agency of a pollinator in order to set fruit. All species showed very high fruiting success, either by self-pollination or cross-pollination, and there was no significant difference between these two treatments (Table 3).

Fruiting success and pollination efficiency - All species investigated displayed high fruiting success for both sampling years, ranging from 69.48 to 97.40 % (Table 4) (fruit set in *H.*

montevidensis could only be determined for 2011, see Material and methods). Mean fruit set per inflorescence was also very high in both years, ranging from 68.62 to 97.38%. When comparing fruit set per inflorescence for 2010 and 2011, *H. megapotamensis* was the only species that did not show a significant difference (Table 4).

All studied species displayed male efficiency factors slightly higher than 1 (Table 4), that is, at least one flower was pollinated for each pollinarium removed. Percentages of pollinated flowers ranged from 72.38 to 86.06 % and the percentages of flowers acting as pollen-donors were also very high, ranging from 63.81 to 83.55% (Table 4). Yet, several flowers used to calculate male efficiency factors contributed one pollinarium, only. In *H. johannensis*, 23.97% of the flowers acting as pollen-donors contributed a single pollinarium. In *H. macronectar*, *H. megapotamensis* and *H. montevidensis*, this value reached 38.80%, 21.76% and 38.17% of the flowers acting as pollen-donors, respectively.

DISCUSSION

On the whole, floral features shown here agree with those already reported for several Neotropical *Habenaria* spp. (Hoehne, 1942; Dressler, 1993; Singer & Cocucci, 1997; Singer, 2001; Singer *et al.*, 2007; Batista *et al.*, 2006). However, some deserve special mention, since they are relevant to the pollination process. Column features such as the close, convergent rostellar arms and upwardly-facing viscidia in *H. johannensis* and *H. macronectar*, the projecting median rostellar lobe in *H. johannensis* and the stigmatic appendices that partly block the spur entrance in *H. megapotamensis* restrict the movements of pollinators and physically guide them against the rostellum, thus precipitating pollinarium withdrawal and pollination. Conversely, most Neotropical *Habenaria* spp. have non-projecting, median rostellar lobes and straight, approximately parallel rostellar arms ending in more or less anteriorly facing viscidia (Hoehne, 1942; Dressler, 1993; Singer & Cocucci, 1997; Singer *et al.*, 2007). The rostellar morphology of *H. montevidensis* follows this latter pattern. Stigmatic appendices similar to those reported here for *H. megapotamensis* are also found in other unrelated Neotropical *Habenaria* species, such as *H. secunda* Lindl., and the recently described *H. psammophila* J.A.N. Bat., Bianch. & Carvalho (Batista *et al*., 2010). Based on the presence of this morphological feature, we suggest that the pollination mechanism of these orchids may be similar to that reported here for *H. megapotamensis*. The viscidia to *H. megapotamensis* flank the spur entrance. A similar condition is found in *H. parviflora,* a species whose pollinaria are also carried on the proboscis of its pollinators (Singer, 2001). However, in *H. parviflora* the viscidium is glove-like and clasps the thin proboscises of its moth and crane-fly pollinators (Singer, 2001).

Pollination by Sphingidae moths is confirmed here for three species (*H. johannensis, H. macronectar* and *H. megapotamensis*) which share important flower features, such as greenish-white or light-green, long-spurred flowers, dilute nectar and crepuscular-nocturnal emission of scent. These features are consistent with those already recorded for other hawkmoth-pollinated orchids of subfamily Orchidoideae (Galetto *et al*., 1997; Johnson & Liltved, 1997; Hapeman & Inoue, 2000; Westwood & Borkowsky, 2004; Peter *et al*., 2009) and Epidendroideae (Nilsson *et al.*, 1985; Nilsson & Rabakonandrianina, 1988; Luyt & Johnson, 2001; Martins & Johnson, 2007). *Habenaria johannensis* and *H. macronectar* belong to *H.* sect. *Macroceratitae* (Kränzlin, 1892; Cogniaux, 1893), an orchid group which is characterized by a distinct set of floral features (proportionally large, greenish-white, nocturnally fragrant, long-spurred flowers, etc.) which is often associated with pollination by hawkmoths (Singer & Cocucci, 1997; Batista *et al*., 2006). In support of this, hawk-moth pollination has already been documented for *H. gourlieana* Gillies (also of *H.* sect. *Macroceratitae*) (Singer & Cocucci, 1997). In Central Argentina, this orchid species was found to be pollinated by the moth *Manduca sexta* (one of two species we recorded pollinating *H. johannensis*) and visited (but not pollinated) by moths of *Agrius cingulatus* (a non-pollinating visitor of *H. macronectar*, according to our observations). On the basis of similar flower features (Batista *et al*., 2006), we expect all other *Habenaria* species within *H.* sect. *Macroceratitae* to be pollinated by Sphingidae moths. Remarkably, pollination solely by Sphingidae moths was also confirmed in this study for *H. megapotamensis*, which does not belong to *H.* sect. *Macroceratitae*. In fact, floral traits often associated with pollination by Sphingidae moths also occur in some *Habenaria* species of the *Laxifloras*, *Leptoceras* and *Seticauda* groups (*sensu* Hoehne, 1940; Hoehne, 1942; Singer & Cocucci, 1997), supporting the view that pollination by hawk-moths is not restricted to the *H.* sect. *Macroceratitae*. The present contribution is the second report on butterfly pollination in the genus *Habenaria*. The first was by Moreira *et al*. (1996), who documented the pollination of *H. pleiophylla* by the butterfly *Heliconius erato phyllis* (Fabricius, 1775) (Nymphalidae) in *Eucalyptus* plantations in Rio Grande do Sul, southern Brazil. Singer *et al*. (2007) subsequently demonstrated pollination of the same orchid by short-tongued Sphingidae, Noctuidae and Arctiidae moths in south-eastern Brazil. The *Habenaria* species of sect. *Pratenses* Kraenzl. (Cogniaux, 1893; Hoehne, 1942; Pabst & Dungs, 1975) consistently display a set of floral features (diurnally-scented, mostly yellow-coloured flowers) indicative of diurnal pollination, and we believe that it may be also pollinated by butterflies (see also Singer & Cocucci, 1997).

Moth and butterfly pollinators have most of their bodies covered with scales. Therefore, few body parts are sufficiently smooth to carry orchid pollinaria. Moth-pollinated orchids deposit their pollinaria on the proboscis, eyes, or more rarely, the forelegs of the visiting insect (Nilsson *et al.*, 1985; Nilsson *et al.*, 1987; Nilsson & Rabakonandrianina, 1988; Johnson & Liltved, 1997; Luyt & Johnson, 2001; Westwood & Borkowsky, 2004; Martins & Johnson, 2007; Peter *et al*., 2009). To our knowledge, this is the first report of attachment of

Habenaria pollinaria to the area between the palpi of its moth pollinators. Earlier reports on *Habenaria* spp. have documented pollinarium attachment to the eyes (Moreira *et al*., 1996; Singer & Cocucci, 1997; Singer *et al*., 2007), the proboscis (Singer, 2001) or the forelegs (Peter *et al*., 2009). Pollinarium attachment to the eyes, as observed here for *H. montevidensis*, has already been documented for *H. gourlieana*, *H. hexaptera* Lindl. (as *H. hieronymi* Kraenzl.) and *H. pleiophylla* (Singer & Cocucci, 1997; Singer *et al*., 2007). All these species have similar column morphology and bear more or less parallel rostellar arms and widely spaced viscidia (Singer & Cocucci, 1997; Singer *et al*., 2007). Since this column structure is widespread among Neotropical *Habenaria* spp., we expect that pollinarium attachment to the eyes will dominate in these taxa. Pollinarium attachment to the proboscis (as reported here for *H. johannensis* and *H. megapotamensis*) was seen in *H. parviflora*, but involved a differently-structured viscidium (see above) (Singer, 2001).

A remarkable finding is that *M.* cf. *lucetius* pollinated both *H. macronectar* and *H. megapotamensis*, with some individuals carrying pollinaria of both species simultaneously. No hybrids between these species are known and this is consistent with the fact that pollinaria of both orchid species become attached to different parts of the insect, and that the respective flowers differ in their morphology. Remarkably, both *Habenaria* species display statistically significant differences in spur length, nectar volume and nectar column length (see Results and Table 1). These species only showed non-statistically significant differences with regard to nectar concentration (see Results and Table 1). Therefore, floral morphological features and pollinarium placement on the pollinator seem particularly important. The short pollinaria of *H. megapotamensis* are very unlikely to make contact with the stalked, long stigmatic surfaces of *H. macronectar*. Conversely, the long, pendulous pollinaria of *H. macronectar* are unlikely to make contact with the short stigmatic surfaces of *H. megapotamensis*, which are placed just below the spur entrance. Thus, a combination of floral morphology and differing pollinarium placement on the pollinator (which is actually also a consequence of floral morphology) are probably sufficient to keep both species separate, even when they share the same pollinator. A similar situation was found by Nilsson *et al*. (1985, 1987) for a community of hawkmoth-pollinated *Angraecum* species (Epidendroideae: Angraecinae) from Madagascar. The hawkmoth *Panogena lingens* (Butler, 1877) was a particularly important pollinator in this community, and was shared by some orchids as pollinator. The pollinaria of the respective species became attached at different points to its proboscis, thus precluding hybridization events (Nilsson *et al*., 1985; Nilsson *et al*., 1987).

As already stated, most insects bearing several pollinaria had proboscises that were shorter than the mean spur length of the orchids that they pollinate (Table 4). This is the case, for example, in *M. sexta* (pollinator of *H. johannensis*), *E. labrusca* (pollinator of *H. macronectar*), *U. teleus* and *U. zagorius* (pollinators of *H. montevidensis*) (see Table 4). A similar situation has already been reported for *H. gourlieana* (Singer & Cocucci, 1997), but in this case, the pollinating moths (*M. sexta*) carried only a few pollinaria on their eyes (2). Although a larger pollinator sample is desirable, the above scenario is not unexpected, since insects with proboscises shorter than the floral spurs are more likely to contact the viscidia while probing flowers and therefore, dislodging pollinaria. The nectar column varied in length according to species (see Table 1), but it is not essential for the insects to reach the very bottom of the spur to take the nectar. As already stated, some individuals of *M.* cf. *Lucetius* were filmed visiting flowers of *H. macronectar*, without pollinating them. This can be partly explained in terms of variation in spur and proboscis length (see Table 1 and Table 4, respectively). Insects with longer proboscises are less likely to disturb the rostellum and dislodge pollinaria. However, videos revealed that the same moth may or may not insert the full proboscis into different flowers on the same inflorescence (see Supplementary material Video S1). In our opinion, this behaviour may be due to nectar columns of different lengths (and thus different volumes of nectar secreted). Insects visiting flowers with longer nectar columns are less likely to disturb the rostellum and dislodge pollinaria, especially if they have proboscises longer than the floral spur. Conversely, insects visiting flowers with shorter nectar columns are more likely to disturb the rostellum and withdraw pollinaria while trying to reach the nectar, especially if they have proboscises shorter than the floral spur. If these observations are correct, even morphologically compatible moths (e.g. with "appropriate" proboscis length) may visit some flowers without effecting pollination. Pollination of these flowers is more likely to commence when their nectar spurs are partially empty. Morphologically compatible pollinators may then need to insert their proboscises fully into the floral tube, thereby disturbing the rostellum and effecting pollination. Indeed, it is important to stress that nectar volume and, consequently, the height of the nectar column are already subject to significant variation in intact flowers of the same species (see Table 1).

All species studied are self-compatible, but pollinator-dependent. The lack of fruit set either in intact or emasculated flowers indicates that no apomixis or autogamy occurs and that the species studied rely on animal pollen-vectors to set fruit. Similar results (self-compatibility coupled with pollinator-dependence) were found in *H. parviflora* (Singer, 2001). All studied species displayed high fruit set (see Results), as already reported for other *Habenaria* species (Singer, 2001; Singer *et al*., 2007). Mean fruit set per inflorescence for 2010 and 2011 showed statistically significant differences for *H. johannensis* and *H. macronectar*. Since all studied species are pollinator-dependent (Table 3), the observed differences may be caused by different visitation rates. The high fruit set observed for all species studied may be partly due to the consistent presence of a nectar reward (Neiland & Wilcock, 1998; Tremblay *et al*., 2005). Indeed, other factors that may be involved include: 1) self-compatibility, 2) pollinators that either are frequent or pollinate several flowers per visit and 3) the presence of massulate pollinia whose pollen content can be spread onto the stigmatic surface of several flowers.

All species studied displayed male efficiency factors of just greater than 1, indicating that slightly more than one flower was pollinated per pollinarium removed. These results are higher than the values previously recorded for *H. gourlieana* (0.38), *H. parviflora* – as *H. montevidensis* - (0.6) and *H. rupicola* Barb. Rodr. (0.7) in Central Argentina (Singer & Cocucci, 1997), but lower or slightly lower than those recovered for populations of *H. pleiophylla* (1.8) in Eastern Brazil and for *H. hexaptera* (1.15) (as *H. hieronymi* Kraenzl.) in Central Argentina (Singer & Cocucci, 1997; Singer *et al*., 2007). Although our data suggest that there is some kind of equilibrium (approximately one flower pollinated per pollinarium removed), we believe that there is some wastage of pollinaria. This is based on the fact that pollinarium-laden skippers of the genus *Urbanus* (the main pollinators of *H. montevidensis* at the study site) were filmed trying to remove the pollinaria attached to their eyes. Similar behaviour has already been recorded for some Noctuidae moth pollinators of *H. pleiophylla* (Singer *et al*., 2007). These findings suggest that some pollinaria may be cleaned by the pollinators and, consequently, lost for pollination purposes. It is important to remember that among the species studied, a proportion of flowers acting as pollen-donors contribute only a single pollinarium (see Results).

Concluding remarks – The present study shows that four sympatric southern Brazilian *Habenaria* spp., whose flowering periods overlap, are isolated (in terms of pollination) by a set of factors that includes differences in floral morphology (especially spur length and column morphology) and different pollinators and/or different pollinarium placement on the pollinator. Species with similar flower features (e.g., nectar concentration in *H. macronectar* and *H. megapotamensis*) can share pollinators, but the latter carry the respective pollinaria on different body parts, thereby physically preventing hybridization.

Long-spurred, hawkmoth-pollinated orchids have captivated the attention of scientists ever since Darwin (1862) made his famous comments on the putative pollinators of *Angraecum sesquipedale* Thouars and proposed a co-evolutionary race between plant and pollinator. Several Neotropical *Habenaria* species rank among the longest-spurred orchids in the Americas, with (for instance) all the species within *H.* sect. *Macroceratitae* having long spurs (≥ 6 cm in length), with that of *H. longicauda* reaching 25 cm (Renz, 1992; Batista *et al*., 2006). Therefore, these orchids parallel the mainly African-Madagascan Angraecinae, at least in terms of floral morphology. Thus, it is tempting to ask whether the long-spurred *Habenaria* species and their Sphingidae pollinators co-evolved. Although the present contribution did not set out to address that question, nevertheless, some of the results presented here suggest that a co-evolutionary relationship (in its strictest sense, see Ridley, 2004) is unlikely since: 1) Pollination by Sphingidae moths occurs in more than one *Habenaria* section/species group, and 2) one studied species (*H. macronectar*) is pollinated by phylogenetically unrelated moths. Well-supported phylogenies are already available for the Sphingidae, and these studies indicate that the observed pollinators of *H. macronectar* are distantly related and belong to different clades (see cladograms in Kawahara *et al*., 2009). A comprehensive molecular phylogeny of Neotropical *Habenaria* is currently being prepared (J.A.N. Batista, pers. comm.). This phylogeny will be the basis for a more robust and reliable infrageneric classification, prompting a re-evaluation of the characters that are normally used to separate species-groups. Furthermore, this phylogeny could be used as a framework to elucidate the evolution of pollination-related features (e.g. long spurs). Only then will it be possible to ascertain whether the long-spurred species *Habenaria* have a co-evolutionary relationship with their Sphingidae pollinators.

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Table 1. Floral and nectar traits of *Habenaria* species.

Section/Species	**Spur length (cm)**	**Nectar volume (μL)**	**Nectar column height (cm)**	**Total nectar sugar concentration (%)**
H. sect. *Macroceratitae*				
H. johannensis	12.5 – 14 (13.09 ± 0.51)^{a}	20 – 40 (35 ± 7.07)^{a}	1.7 – 3.9 (2.73 ± 0.84)$^{a, b}$	20.6 – 26.6 (24.46 ± 2.01)^{a}
H. macronectar	6.3 – 7.6 (6.66 ± 0.41)^{b}	10 – 30 (18.8 ± 5.67)$^{b, e**}$	0.9 – 4.3 (2.2 ± 0.98)$^{a, d**}$	12 – 23.6 (18.12 ± 4.04)$^{b, c}$
section not defined				
H. megapotamensis	6.8 – 7.8 (7.51 ± 0.37)^{c}	10 – 20 (11 ± 3.16)$^{c, f**}$	2.4 – 4 (3.32 ± 0.58)$^{b, e**}$	15 – 23.6 (19.04 ± 3.13)^{c}
H. montevidensis	1.6 – 1.7 (1.62 ± 0.04)^{d}	0.9 – 1.7 (1.27 ± 0.28)^{d}	0.4 – 0.6 (0.52 ± 0.08)^{c}	26.2*

* Owing to the small volumes of nectar available, nectar concentration in this species was measured by pooling the nectar of ten flowers. In all cases, values outside parentheses represent the range (minimum and maximum) observed; and values in parentheses represent the mean value ± standard deviation. Different letters indicate significant differences (ANOVA; $p<0.0005$ and $p<0.01$**).

Table 2. Observation period and numbers of hours spent in recording the pollination biology of each *Habenaria* species studied.

		Diurnal pollination		**Nocturnal pollination**		
Species/Locality	**Period**	**Observation Period**	**Observation hours**	**Observation Period**	**Observation hours**	**Total**
H. johannensis						
Brazil, Rio Grande do Sul, São Francisco de Paula	24 Jan. and 18 to 20 Feb. 2011	10:00 to 13:00 h	10 h	18:00 to 22:30 h	34 h	44 h
H. macronectar						
Brazil, Rio Grande do Sul, Cambará do Sul	19 Feb. to 12 Mar. 2011	15:00 to 18:00 h	9 h	18:00 to 23:00 h	55 h	64 h
H. megapotamensis						
Brazil, Rio Grande do Sul, Cambará do Sul (two populations)	10 Feb. 2010; 19 to 21 Feb. 2011	10:20 to 11:20 h; 15:00 to 18:00 h	5 h	18:00 to 23:00 h	30 h	35 h
H. montevidensis						
Brazil, Rio Grande do Sul, São Francisco de Paula	08 to 19 Feb. 2011	06:00 to 18:00 h	24 h	18:00 to 22:00 h	14 h	38 h
					Total for all spp.:	**181 h**

Table 3. Breeding system experiments. Fruiting success (%) in *Habenaria* species for intact flowers (Control), emasculated flowers (Emasculation), hand self-pollinated (Self-pollination) and cross-pollinated (Cross-pollination) treatments.

Section/Species	**N**	**Control**	**Emasculation**	**Self-pollination**	**Cross-pollination**	**χ^2 (self x cross pollination) values, P<0.2)**
H. sect. *Macroceratitae*						
H. johannensis	15	0 (0/37)	0 (0/33)	100 (35/35)	100 (34/34)	0 (NS)
H. macronectar	17	0 (0/31)	0 (0/29)	85.29 (29/34)	87.5 (28/32)	0.0093(NS)
section not defined						
H. megapotamensis	12	0 (0/49)	0 (0/45)	100 (45/45)	100 (45/45)	0 (NS)
H. montevidensis	12	0 (0/32)	0 (0/33)	100 (30/30)	100 (30/30)	0 (NS)

Numbers in parentheses represent the number of fruit obtained over the number of flowers used in each treatment. N represents the number of individuals used in the experiments. NS = non-significant.

Table 4. Pollinators, pollinator features, fruiting success and male efficiency factor in *Habenaria* spp.

Species/Locality	**Pollinators (proboscis length in mm)**	**Site of pollinarium attachment (maximum number of pollinaria observed per taxon)**	**Fruiting success (%) ****		**Male efficiency factor (2011) *****
			2010	**2011**	
H. johannensis	*Manduca rustica* (Sphingidae: proboscis length: 13.5 cm)	Underside of the base of the proboscis (15)	97.40 (97.38 ± 8.13)^{a}	92.51 (89 ± 16.41)^{b}	1.04 (N: 208, 86.06/82.21)
	M. sexta (Sphingidae: proboscis length: 10.3 cm)	Underside of the base of the proboscis (13)			
H. macronectar	*Eumorpha labruscae* (Sphingidae: proboscis length: 5.9 cm)	Between the palpi (12)	86.12 (84.39 ± 16.35)^{a}	69.48 (68.62 ± 20.32)^{b}	1.13 (N: 105, 72.38/63.81)
	M. cf. *lucetius* (Sphingidae: proboscis length: 6.4-7.4 cm)	Between the palpi (3)			
H. megapotamensis	*M.* cf. *lucetius* (Sphingidae: proboscis length: 6.4-7.4 cm)	Underside and sides of the base of the proboscis (15)	84.69 (85.07 ± 12.2)^{a}	92.87 (90.84 ± 11.98)^{a}	1.01 (N: 231, 84.42/83.55)
H. montevidensis	*Cumbre* sp. (Hesperiidae: proboscis length: 13 mm)	On the eyes (2)	*	78.32 (77.94 ± 28.12)	1.12 (N: 367, 80.11/71.39)
	Vehilius clavicula (Hesperiidae: proboscis length: 13 mm)	On the eyes (3)			
	Urbanus teleus (Hesperiidae: proboscis length: 11 mm)	On the eyes (6)			
	Urbanus zagorius (Hesperiidae: proboscis length: 15 mm)	On the eyes (14)			

Different letters indicate significant differences according to independent two-sample t-test ($p<0.0005$).

* In 2010, the fruiting inflorescences of *H. montevidensis* were destroyed, presumably by herbivores.

****** Numbers in parentheses represent mean fructification per inflorescence.

******* Numbers in parenthesis represent: N= total number of flowers used to calculate Nilsson’s male efficiency factor, % of pollinated flowers/% of flowers acting as pollen donors.

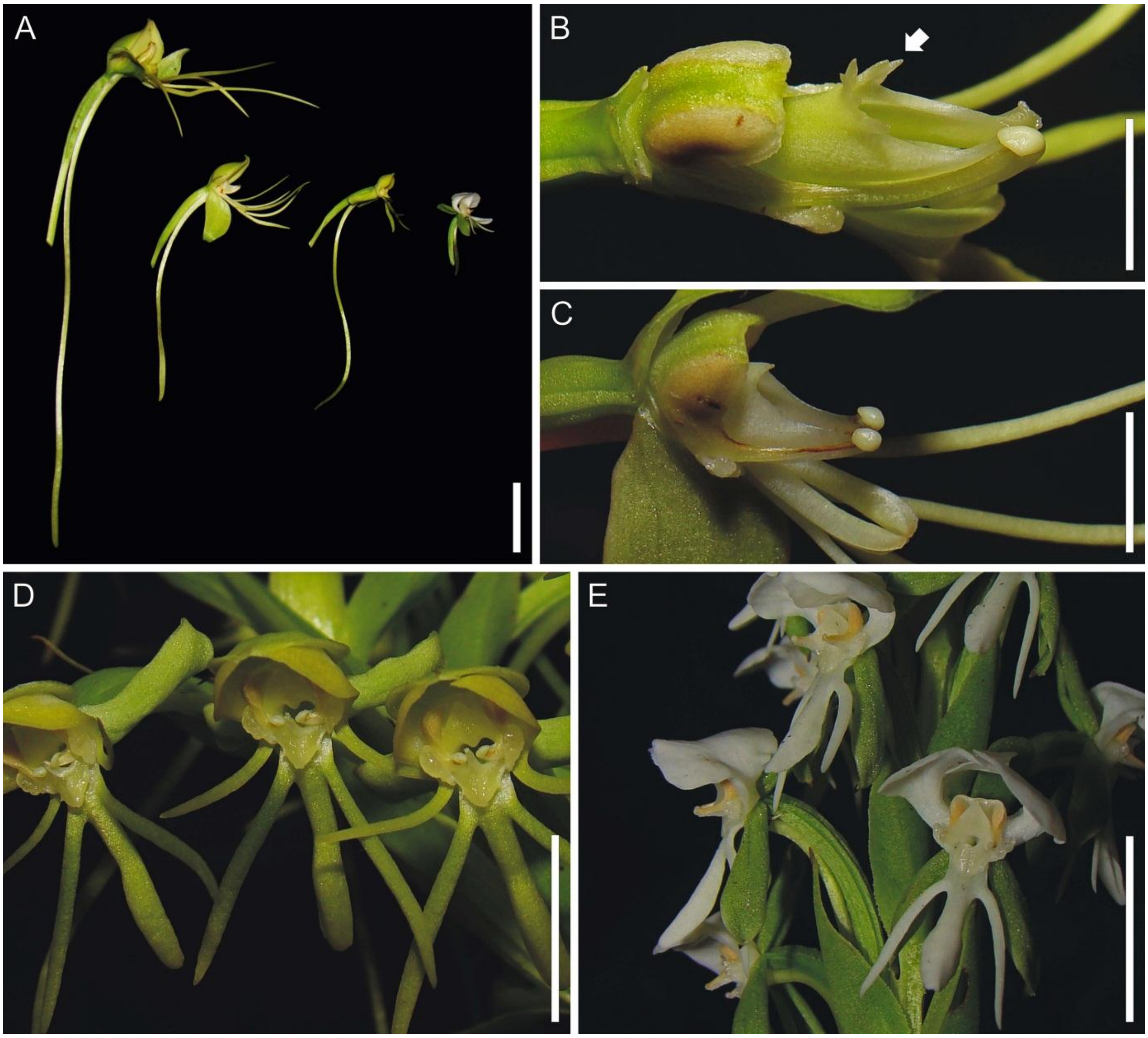


Fig. 1. Key floral features of *Habenaria* species. (A) Comparative floral morphology. (From left to right: *H. johannensis*, *H. macronectar*, *H. megapotamensis* and *H. montevidensis*). Notice the very different spur lengths. (B) *H. johannensis*: dorsal view of rostellar arms. Notice the upward-facing viscidia. The arrow points to the projected median rostellar lobe. (C) *H. macronectar*: dorsal view of rostellar arms. Notice the upward-facing viscidia. (D) Close-up of *H. megapotamensis* flowers. The spur entrance is partially occluded by two, tooth-like stigmatic projections, and the viscidia are directed towards the spur entrance. (E) Close-up of *H. montevidensis* flowers. Rostellar arms are straight and slightly divergent. Scale bars (A and D-E) = 1 cm; (B and C) = 5 mm.

Fig. 2. (A, B) *H. johannensis* and its pollinators. (A) *Manduca sexta* (Sphingidae) pollinating *H. johannensis* flower. Notice the pollinaria attached below the proboscis. (B) *Manduca rustica* (Sphingidae) with *H. johannensis* pollinaria attached below the proboscis. (C, D) *H. macronectar* and its pollinators. (C) *Manduca* cf. *lucetius* (Sphingidae) pollinating *H. macronectar* flowers. Notice that the pollinaria are already attached. (D) *Eumorpha labruscae* (Sphingidae) with *H. macronectar* pollinaria attached between the palps. Scale bars (A and C) = 2 cm; (B and D) = 1 cm.

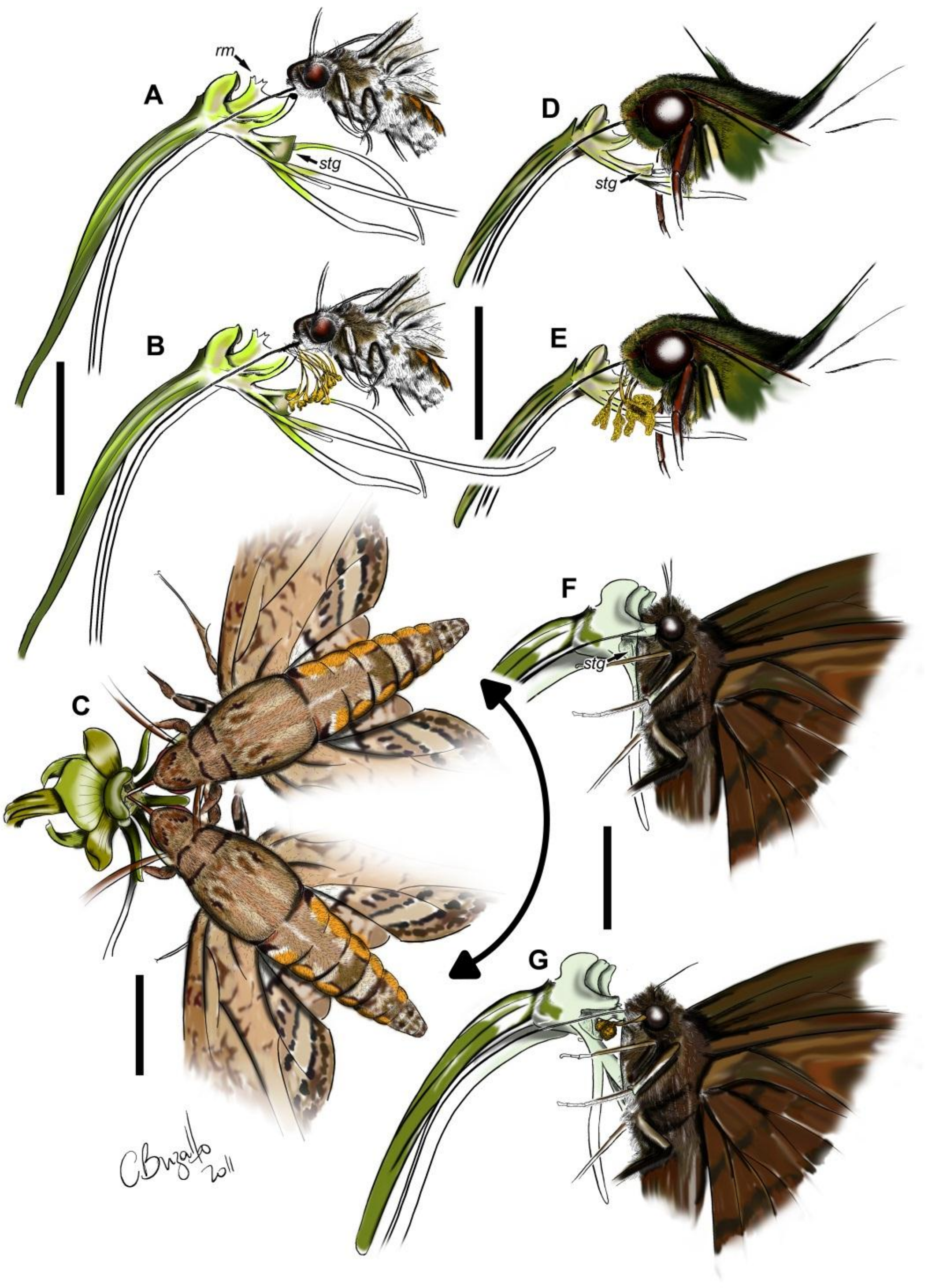
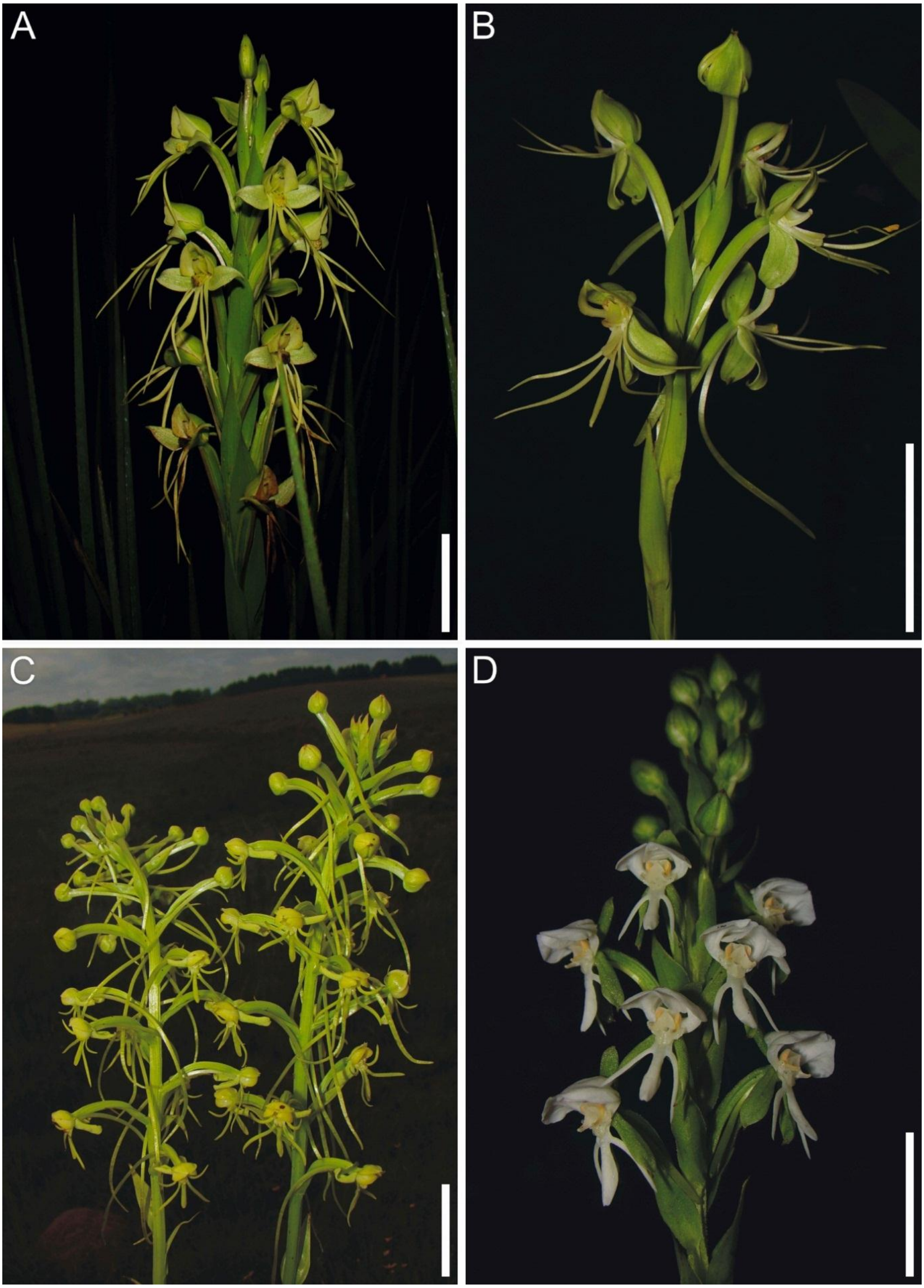


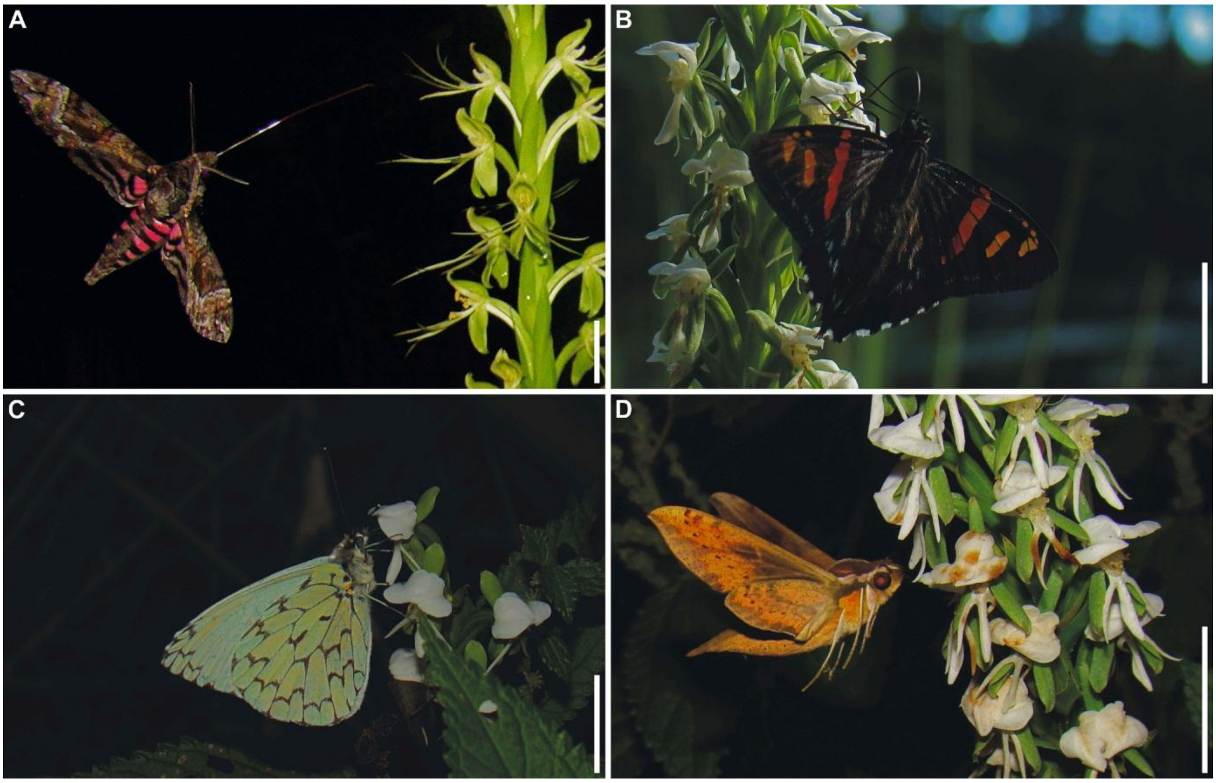
Fig. 3. Pollination mechanism of *Habenaria* species. (A-B) *H. johannensis.* (A) A hawkmoth inserts its proboscis into the spur. The keel-like, median rostellar lobe restricts its movement and the insect is physically guided against the rostellum. (B) A pollinarium-laden insect visits another flower and brushes the stigmatic surface with the pollinia. (C) *H. megapotamensis*. The spur entrance is partially obstructed by tooth-like projections of the stigma. Therefore, the insect has to insert its proboscis laterally, thus pressing it against the viscidia that are orientated towards the spur entrance. (D-E) *H. macronectar*. (D) A hawkmoth inserts its proboscis into the spur and the viscidia come into contact with the region between the palpi. (E) A pollinarium-laden insect visits another flower brushing the pollinia against the stigmatic surfaces. (F-G) *H. montevidensis*. (F) A butterfly inserts its proboscis into the spur, placing its head between the rostellar arms, and the pollinaria become attached to its eyes. (G) Pollination takes place when a pollinaria-laden insect visits another flower and brushes the pollinia against the stigmatic surfaces. Scale bars (A-B; D-E) = 2 cm; (C) = 1 cm; (F-G) = 5 mm.

Fig. 4. (A, B) Pollination of *H. megapotamensis*. (A) *Manduca* cf. *lucetius* (Sphingidae) inserting its proboscis into the spur. Notice that this individual already carries several pollinaria attached to its proboscis (B) A pollinaria-laden moth visits a flower, brushing the pollinia against the stigmatic surfaces. (C, D) Pollination of *H. montevidensis*. (C) *Urbanus teleus* (Hesperiidae) inserting its proboscis into the floral spur. Notice that this specimen already carries pollinaria that are attached to its eyes. (D) A pollinaria-laden insect inserts its proboscis into a floral spur, brushing the pollinia against the stigmatic surfaces. Scale bars (all figures) = 1 cm.

SUPPLEMENTARY MATERIAL



Supplementary material Fig. S1. Habit of the four species studied. (A) *Habenaria johannensis*. (B) *H. macronectar*. (C) *H. megapotamensis*. (D). *H. montevidensis*. Scale bars (A-C) = 2 cm; (D) = 1 cm.



Supplementary material Fig. S2. Non-pollinating flower visitors. (A) *Agrius cingulatus* (Sphingidae) visiting *Habenaria macronectar*. (B-D) Visitors of *H. montevidensis*. (B) *Phocides pialia* (Hesperiidae). (C) *Hesperocharis erota* (Pieriidae). (D) *Xylophanes fosteri* (Sphingidae). Scale bars (all figures) = 1 cm.

Supplementary material Video S1. Pollination in *H. macronectar*. *Manduca* cf. *lucetius* (Sphingidae) visiting an inflorescence and an individual of *Eumorpha labrusca* (Sphingidae) captured with several pollinaria of this orchid between the palpi.

Supplementary material Video S2. Pollination in *H. megapotamensis*. *Manduca* cf. *lucetius* (Sphingidae) visiting an inflorescence. Notice that the moth bears a pollinarium attached to its proboscis.

Supplementary material Video S3. Pollination in *H. montevidensis*. *Urbanus zagorius* (Hesperiidae) pollinating flowers and *Xylophanes fosteri* (Sphingidae) visiting an inflorescence without precipitating pollination.

5. CONCLUSÃO

Nossas análises filogenéticas demonstram que *Habenaria* seção *Pentadactylae* (sensu Kraenzlin 1892, 1901; Cogniaux 1893) é altamente polifilética. Dessa forma, a seção *Pentadactylae* foi recircunscrita onde 32 espécies foram excluídas e passou a ser constituída pelas espécies *H. dutraei*, *H. ekmaniana*, *H. exaltata*, *H. henscheniana*, *H. megapotamensis*, *H. montevidensis* e *H. pentadactyla*. Embora esse clado seja somente moderadamente suportado (0.91 PP, 72% BS) a maioria das espécies compartilham uma série de cararacterísticas morfológicas, tipo de hábitat e distribuição geográfica. A inclusão de *H. montevidensis* para o grupo e o subclado formado por *H. henscheniana* (0.99 PP) foi inesperada, uma vez que esta espécie exibe um conjunto de características não compartilhadas com os outros membros da seção. Em *H. montevidensis* a sépala dorsal das flores é reflexa e convexa e as pétalas são simples, unguiculadas, de coloração branca e cobrem a coluna. Nas demais espécies da seção a sépala dorsal é côncava e cobre parcialmente a coluna; e as pétalas laterais são bipartidas, não unguiculadas, de coloração verde-amarelada. Diferenças marcantes podem ser percebidas também na estrutura da coluna. Enquanto nas demais espécies da seção os lobos laterais do rostelo convergem em direção ao ápice, e os lobos estigmáticos possuem uma projeção que parcialmente divide a abertura do esporão; em *H. montevidensis* esta estrutura está ausente e os rostelos são levemente divergentes.

Neste trabalho estudamos a biologia reprodutiva de duas espécies pertencentes à seção *Pentadactylae*. *Habenaria montevidensis* foi polinizada por borboletas da família Hesperidae na área de estudo. Este modo de polinização é esperado para esta espécie, uma vez que as flores apresentam as pétalas brancas e emitem um fraco perfume que pode ser percebido durante o dia. *H. megapotamensis* foi polinizada por mariposas da família Sphingidae e isto é consistente com as características florais associadas a esta espécie, tais como a presença de longos esporões parcialmente preenchidos com néctar, crepuscular e noturna emissão de fragrâncias, e coloração verde-amarelada. Como as demais espécies da seção possuem características associadas com polinização noturna, nós sugerimos que a divergência floral em *H. montevidensis* pode ser consequência de substituição de polinizadores. Em adição, nós estudamos a polinização de duas espécies pertencentes a seção *Macroceratitae* (sensu Kraenzlin 1892), *H. johannensis* e *H. macronectar*. Assim como *H. megapotamensis*, ambas espécies são polinizadas por mariposas Sphingidae, consistentemente com a morfologia floral.

Habenaria macronectar e *H. megapotamensis* compartilham pelo menos 1 espécie de polinizador na área de estudo, mas possíveis híbridos entre estas espécies não foram detectados. As diferenças morfológicas entre as duas espécies sugerem uma grande distância genética (que pôde ser revelada em nossas análises filogenéticas) e a formação de híbridos entre estas espécies é muito improvável. Além disso, a diferença na posição dos polinários no corpo do polinizador (na probóscide em *H. megapotamensis* e entre os palpos em *H. macronectar*) e na morfologia floral dificultam a polinização heteroespecífica. Apesar de *H. johannensis* e *H. macronectar* serem filogeneticamente relacionadas, diferem no tamanho dos esporões nectaríferos. Enquanto esporões de *H. macronectar* medem entre 6.3-7.6 cm, em *H. johannensis* são mais longos e medem entre 12.5 e 14.0 cm. Dessa forma, polinizadores de *H. macronectar* dificilmente alcançariam a coluna de néctar de *H. johannensis*. De maneira inversa, polinizadores de *H. johannensis* alcançariam a coluna de néctar em *H. macronectar* sem contactar os polinários. Todas espécies estudadas são autocompatíveis e dependem de agentes polinizadores para frutificação. Além disso, apresentam boa frutificação em condições naturais (acima de 69%). Esta característica pode ser atribuída, principalmente, à autocompatibilidade e à abundância e comportamento dos agentes polinizadores.

Nossos resultados demonstram que espécies de *Habenaria* filogeneticamente relacionadas podem ser polinizadas por grupos não relacionados de polinizadores. Inversamente, espécies de *Habenaria* mais distantemente relacionadas (diferentes seções) podem compartilhar polinizadores. Estudos envolvendo a biologia reprodutiva de maior quantidade de espécies de *Habenaria* contribuirão para a compreensão da evolução entre estas espécies e seus agentes polinizadores.

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