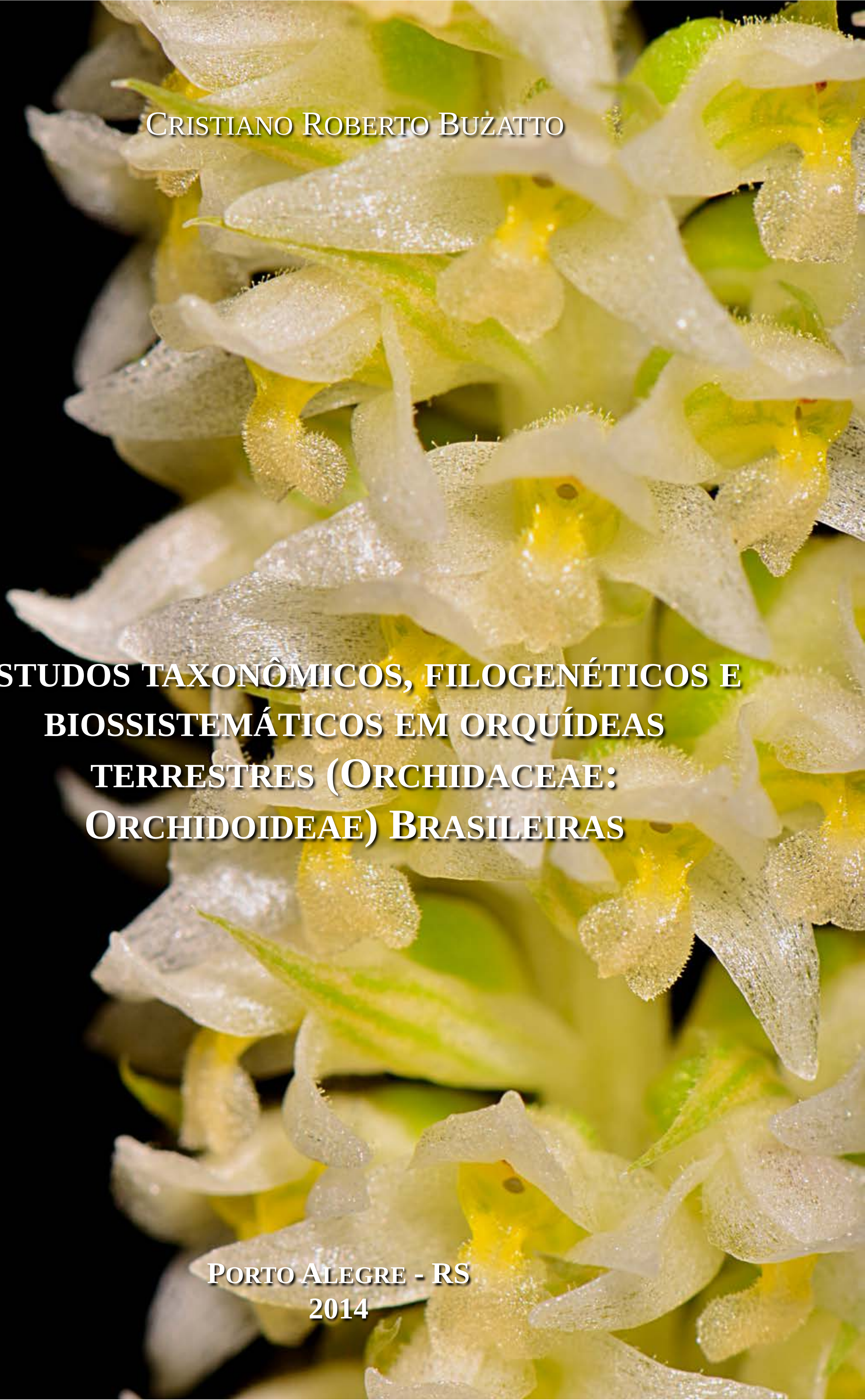

CRISTIANO ROBERTO BUZATTO

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PORTO ALEGRE - RS
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CRISTIANO ROBERTO BUZATTO

Tese 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 Doutor em Botânica.

ORIENTAÇÃO: PROF. DR. RODRIGO BUSTOS SINGER

PORTO ALEGRE – RS
2014

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“*Com grandes poderes vem grandes responsabilidades*”
(Tio Ben)

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RESUMO

Estudos taxonômicos, filogenéticos e biossistemáticos em orquídeas terrestres (Orchidaceae: Orchidoideae) brasileiras

Cristiano Roberto Buzatto
Rodrigo Bustos Singer

Dentre as orquídeas terrícolas brasileiras, dois grupos são particularmente importantes do ponto de vista taxonômico, sistemático e biogeográfico: as orquídeas das subtribo Chloraeinae e Spiranthinae. Na sua atual delimitação, Chloraeinae compreende aproximadamente 70 espécies em três gêneros: *Chloraea*, *Bipinnula* e *Gavilea*. Por outro lado, Spiranthinae compreende cerca de 470 espécies exclusivamente neotropicais, distribuídas em 42 gêneros. As análises filogenéticas mais recentes suportam a existência de quatro grupos: os clados *Stenorhynchos*, *Pelexia*, *Eurystyles+Lankesterella* e *Spiranthes*. Assim, os objetivos deste estudo são: 1) realizar um estudo taxonômico e nomenclatural das orquídeas brasileiras do clado *Pelexia*; 2) documentar a morfologia floral e vegetativa, com ênfase nos caracteres diagnósticos; 3) realizar um estudo taxonômico e nomenclatural das espécies brasileiras de Chloraeinae; 4) construir uma filogenia molecular mais completa e representativa do clado *Pelexia*; 5) estabelecer se os gêneros do clado *Pelexia* são monofiléticos; 6) construir uma filogenia molecular mais completa e representativa de Chloraeinae, com a inclusão de táxons brasileiros; 7) estabelecer um conjunto de caracteres diagnósticos para os gêneros envolvidos neste estudo e, se necessário, propor uma nova delimitação genérica; e 8) propor cenários para a provável evolução de caracteres de importância ecológica. Os resultados de um estudo detalhado, bem como de análises morfológicas e moleculares são: 1) propostas de 48 tipificações, novas sinonímias e notas taxonômicas para as espécies de Goodyerinae e Spiranthinae descritas por José Velloso e Barbosa Rodrigues; 2) novos registros de *Pteroglossa* e *Lyroglossa* para o Rio Grande do Sul; 3) uma correção nomenclatural de *Spiranthes bicolor* e 4) uma sinopse taxonômica das Chloraeinae brasileiras; 5) uma análise filogenética molecular para testar a monofilia dos gêneros atualmente circunscritos no clado *Pelexia*; 6) uma atualização das relações filogenéticas de Chloraeinae, incluindo espécies Pampeanas de *Bipinnula* e a espécie tipo do gênero, *B. biplumata*; 7) um panorama sobre a biologia reprodutiva de duas espécies de *Pachygenium* e dados adicionais sobre *Brachystele* e *Cyclopogon s.l.*; e 8) um estudo detalhado da morfologia floral, biologia da polinização e sistema reprodutivo de *Chloraea membranacea* (Chloraeinae).

Palavras-chave: *Bipinnula*, *Brachystele*, *Chloraea*, Chloraeinae, *Coccineorchis*, *Cyclopogon*, filogenia, *Gavilea*, *matK-trnK*, nomenclatura, nrITS, *Pachygenium*, *Pelexia*, polinização, *rbcL*, *Sarcoglottis*, *Sauroglossum*, sistema reprodutivo, Spiranthinae, *Synassa*, taxonomia, *trnL-trnF*

ABSTRACT

Taxonomic, phylogenetic and biosystematics studies in Brazilian terrestrial orchids (Orchidaceae: Orchidoideae)

Cristiano Roberto Buzatto
Rodrigo Bustos Singer

Among the Brazilian terrestrial orchids, two groups are particularly important from a taxonomic, systematic and biogeographical aspects: orchids of subtribe Chloraeinae and Spiranthinae. In its current delimitation, Chloraeinae comprises approximately 70 species distributed in three genera: *Chloraea*, *Bipinnula* and *Gavilea*. On the other hand, Spiranthinae comprises about 470 exclusively neotropical species distributed in ca. 42 genera. The most recent phylogenetic analyses support the existence of four clades: *Stenorrhynchos*, *Pelexia*, *Eurystyles*+*Lankesterella* and *Spiranthes* clade, respectively. Thus, the aims of this study are: 1) to make a taxonomic and nomenclatural study of Brazilian orchids of *Pelexia* clade; 2) to document the floral and vegetative morphology, with emphasis on diagnostic features; 3) to make a taxonomic and nomenclatural study of the Brazilian species of Chloraeinae; 4) to assemble a more complete and representative molecular phylogeny of the *Pelexia* clade; 5) to establish if the genera of the *Pelexia* clade are monophyletic; 6) to construct a more complete and representative molecular phylogeny of the Chloraeinae, with the inclusion of Brazilian taxa; 7) to establish sets of diagnostic characters for the genera involved in this study and, if necessary, propose a new generic delimitation; and 8) to propose scenarios for the evolution of characters of ecological importance. The main results are: 1) 48 typifications, new synonymies and taxonomic notes for Spiranthinae and Goodyerinae species described by José Velloso and Rodrigues Barbosa; 2) new records of *Pteroglossa* and *Lyroglossa* to Rio Grande do Sul; 3) a nomenclatural correction of *Spiranthes bicolor* and 4) a taxonomic synopsis of Brazilian Chloraeinae; 5) molecular phylogenetic analyses to test the monophyly of the currently circumscribed genera in the *Pelexia* clade; 6) an update of the phylogenetic relationships of the Chloraeinae, including Pampean species of *Bipinnula*, and the type species of the genus, *B. biplumata*; 7) an overview on the reproductive biology of two *Pachygenium* species and additional data on *Brachystele* and *Cyclopogon s.l.*; and 8) a detailed study of the floral morphology, pollination biology and reproductive system of *Chloraea membranacea* (Chloraeinae).

Keywords: *Bipinnula*, *Brachystele*, breeding system, *Chloraea*, Chloraeinae, *Coccineorchis*, *Cyclopogon*, *Gavilea*, *matK-trnK*, nomenclature, nrITS, *Pachygenium*, *Pelexia*, phylogeny, pollination, *rbcL*, *Sarcoglottis*, *Sauroglossum*, Spiranthinae, *Synassa*, taxonomy, *trnL-trnF*

Introdução geral

INTRODUÇÃO GERAL

Orchidaceae Juss. compreende cerca de 24.000 espécies e constitui uma das famílias morfologicamente mais conspícuas, principalmente pelas marcantes adaptações na morfologia floral (Chase *et al.* 2003). Estas espécies estão divididas em cinco subfamílias: Apostasioideae Garay, Vanilloideae Szlach., Cypripedoideae Kostel., Orchidoideae A.A. Eaton e Epidendroideae Kostel. (Pridgeon *et al.* 1999). As orquídeas apresentam um perianto trímero (três sépalas e três pétalas, sendo que a pétala mediana é frequentemente maior e mais colorida e/ou ornamentada) e androceu e gineceu fusionados em uma única estrutura denominada coluna ou ginostêmio (Fig. 1). O pólen da grande maioria das orquídeas encontra-se aglutinado na forma de políneas que são removidas das flores pelos polinizadores com o auxílio de estruturas e/ou secreções adicionais. O conjunto das políneas e estas estruturas e/ou secreções adicionais é denominado de polinário (Fig. 1). O estigma é constituído por três lobos, sendo que parte do lobo mediano torna-se não receptivo e se especializa em funções relacionadas à polinização. Esta região não receptiva do lobo estigmático mediano se denomina rostelo. Por sua vez, uma parte deste rostelo pode tornar-se uma superfície destacável que apresenta uma face adesiva e com as políneas aderidas na face oposta. Esta superfície destacável é denominada viscídio e é a estrutura responsável pela adesão do polinário na superfície corporal do polinizador em grande parte das orquídeas (Dressler 1993, Chase *et al.* 2003). No passado, a estrutura geral, morfologia e mecânica funcional do rostelo/viscídio vinham sendo utilizadas para tentar inferir as relações de parentesco entre gêneros de alguns grupos de Orchidaceae (Dressler 1993).

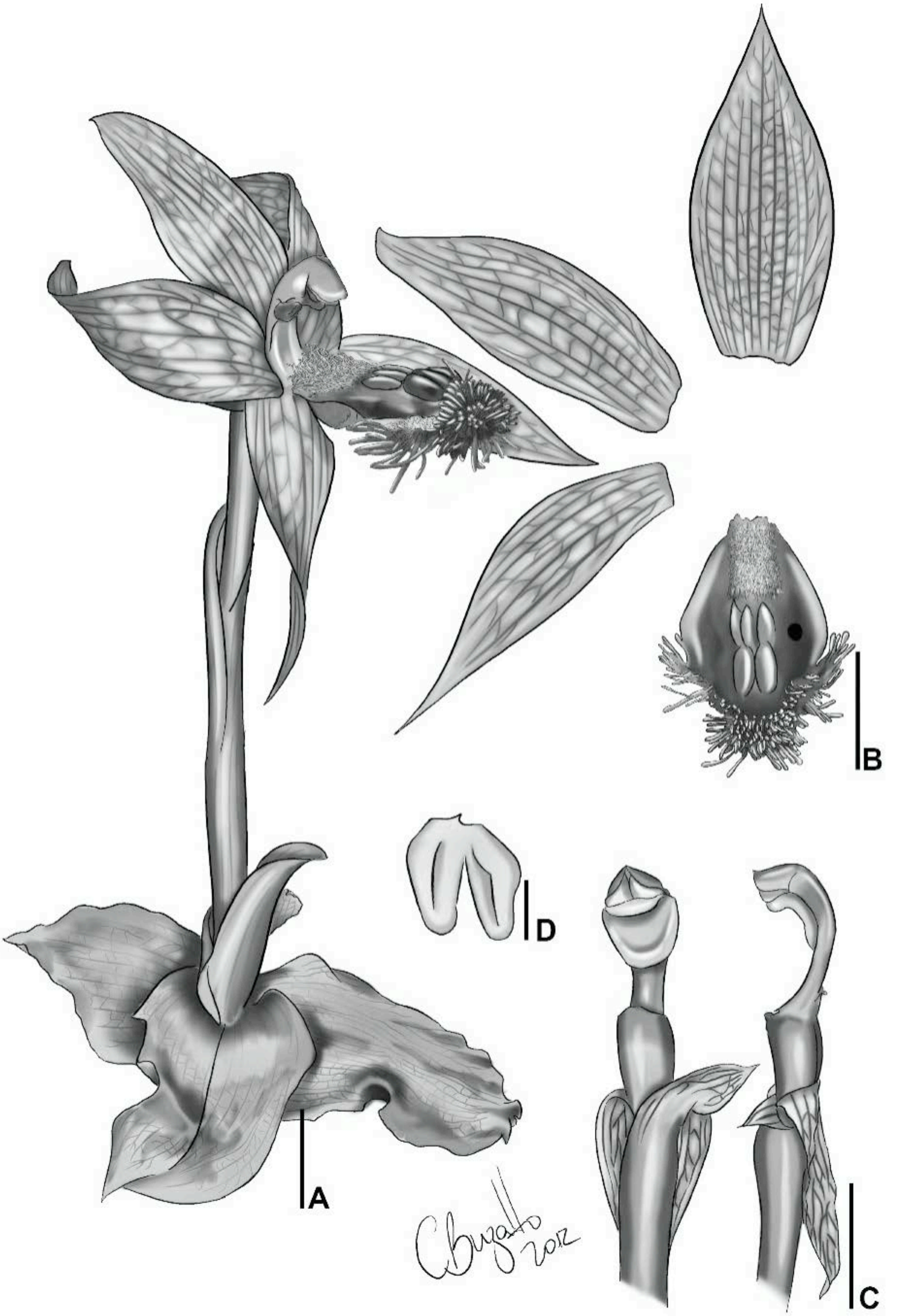

Figura 1. *Bipinnula penicillata* (Rchb.f.) Cisternas & Salazar. A. Hábito; B. Perianto dissecado; C. Coluna, vista dorsal e lateral; D. Polinário. Escalas, 1 cm (A-C); 2 mm (D). Adaptado de Buzatto *et al*. (2014).

ORCHIDOIDEAE EATON

Trata-se de plantas herbáceas, em geral terrícolas, mais raramente rupícolas ou epífitas e de crescimento simpodial (Salazar 2003). As raízes em geral apresentam-se engrossadas e fasciculadas. O caule geralmente é muito curto e as folhas com frequência estão dispostas em uma roseta basal. A inflorescência em geral é uma espiga ou um racemo terminal. As folhas podem ou não estar presentes durante a floração. Em geral as flores são ressupinadas, tubulosas e frequentemente apresentam coloração inconspícua (Salazar 2003). A coluna apresenta-se em posição mais ou menos ereta (paralela ao labelo). O pólen encontra-se aglutinado em políneas de textura farinácea e quebradiça. A coluna em geral apresenta um rostelo bem desenvolvido. Por sua vez, uma parte deste rostelo apresenta uma face adesiva e se torna um viscídio (Salazar 2003). Como um todo, o polinário destas orquídeas é constituído por políneas, caudículas elásticas e um viscídio. A superfície estigmática receptiva é mais ou menos lobada e de posição ventral. O ovário é glabro ou lanuginoso e frequentemente retorcido (Salazar 2003). Até recentemente a taxonomia do grupo se baseava em poucos caracteres florais, com ênfase na estrutura do rostelo na flor intacta e após a remoção do polinário (Salazar 2003). Dentre as orquídeas terrícolas brasileiras, dois grupos são particularmente importantes do ponto de vista taxonômico, sistemático e biogeográfico: as orquídeas da subtribo Chloraeinae Pfitzer, e da subtribo Spiranthinae Lindl. ex Meisn. (ambas tribo Cranichideae Lindl. ex Meisn.).

TRIBO CRANICHIDEAE ENDL.

SUBTRIBO CHLORAEINAE PFITZER

De todas as orquídeas terrestres americanas, as espécies da subtribo Chloraeinae Pfitzer são peculiares por sua complexa morfologia floral. Na sua atual delimitação, Chloraeinae compreende aproximadamente 70 espécies distribuídas em três gêneros (Correa & Sánchez 2003, Chemisquy 2012, Chemisquy & Morrone 2010, 2012, Cisternas *et al.* 2012a, 2012b):

Chloraea Lindl. (45 espécies), *Bipinnula* Comm. ex Juss. (11–12 espécies) e *Gavilea* Poeppig (17 espécies).

Nas últimas décadas, Chloraeinae tem sido interpretada e delimitada de diferentes formas (Tabela 1). Os três gêneros citados acima mais *Geoblasta* Barb. Rodr. e *Codonorchis* Lindl. foram referidos por Dressler (1981, 1993), Szlachetko (1995), Kores *et al*. (2001) e Clements *et al*. (2002) à subtribo Chloraeinae (Tabela 1), com base na presença de muitas folhas, embrião semelhante aos de Spiranthinae e raízes não tuberosas. No entanto, apesar de Dressler (1981, 1993) inserir Chloraeinae na tribo Diurideae Endl., Szlachetko (1995) a inseriu em Geoblasteae Barb. Rodr. (Tabela 1). Mais recentemente, Clements *et al*. (2002) recircunscreveram Chloraeinae, incluindo-a na subtribo Cranichideae Lindl. ex Meisn. e inseriram *Codonorchis* na subfamília Codonorchidoideae Clementes & Jones (Tabela 1). Mais tarde, Correa & Sánchez (2003) reestabeleceram o *status* de tribo à Codonorchideae Cribb baseados nos resultados moleculares de Kores *et al*. (2001). Recentes análises filogenéticas baseadas em sequências nucleares e plastidiais (Chemisquy & Morrone 2010, 2012, Cisternas *et al*. 2012a, 2012b) confirmaram a proposta de Clements *et al*. (2002), que recuperaram Chloraeinae como grupo-irmão de um clado contendo as subtribos Spiranthinae Lindl. ex Meisn., Manniellinae Schltr., Cranichidinae Lindl. ex Meisn. e Goodyerinae Klotz. Barbosa Rodrigues (1891) descreveu *Geoblasta*, baseado em *Geoblasta teixeirana* Barb.Rodr., incluindo-o em Geoblasteae. Mais tarde, Cogniaux (1893) transferiu o nome de Barbosa Rodrigues para *Chloraea*, porém o mesmo voltou a ser aceito por Hoehne (1940) que reestabeleceu para *Geoblasta*.

Chloraeinae é um grupo essencialmente Patagônico (Correa & Sánchez 2003) e apenas nove espécies são encontradas fora desta região, no sul do Brasil, nordeste da Argentina e no Uruguai: *Bipinnula biplumata* (L.f.) Rchb.f., *B. gibertii* Rchb.f., *B. montana* Arechav., *B. penicillata* (Rchb.f.) Cisternas & Salazar, *B. polysyka* Kraenz., *B. ctenopetala* Schltr. (espécie duvidosa), *Chloraea bella* Hauman, *C. membranacea* Lindl. e *Gavilea odoratissima* Poeppig.

Esta última é amplamente distribuída na Patagônia andina, mas tem uma distribuição disjunta com poucas populações isoladas restritas à região da Sierra de la Ventana, no sul da Província de Buenos Aires (Chemisquy 2012, Correa 1956).

Destes gêneros, apenas *Chloraea* (uma espécie: *C. membranacea*) e *Bipinnula* (*B. biplumata* - a espécie *typus* do gênero - *B. montana, B. ctenopetala* e *B. penicillata*) estão representados no Brasil. *Bipinnula* pode ser dividido em dois grupos morfológicos: um grupo de espécies de inflorescência pluriflora (restrito ao Chile) e um grupo de espécies unifloras (restrito ao Pampa *sensu* Bilenca & Miñarro, 2004, incluindo não apenas Brasil, mas também Uruguai e Argentina). Estudos filogenéticos recentes apontam que *Gavilea* (um gênero essencialmente Patagônico) está inserido em *Chloraea*, tornando este último parafilético (Chemisquy *et al*. 2010, Cisternas *et al*. 2012b). Estes mesmos estudos indicam uma grande afinidade filogenética entre *Geoblasta* e *Bipinnula*. Recentemente, Cisternas *et al.* (2012b) mostraram que *Geoblasta penicillata* (Rchb.f) Hoehne estava inserido em *Bipinnula*, no qual parte daqueles autores (Cisternas *et al*. 2012a) propuseram a combinação para *B. penicillata*.

Em anos recentes, populações significativas de todas as Chloraeinae já citadas para a flora Brasileira (Rambo 1965), exceto *B. ctenopetala*, tem sido localizadas no Sul do Brasil. Estes novos registros de populações significativamente grandes tem permitido perceber que estas espécies são morfologicamente mais variáveis do que a literatura sugere, além da existência de problemas de sinonímia entre espécies brasileiras e outras Chloraeinae ocorrentes no Uruguai e Argentina. Em geral estas plantas são pouco coletadas e tem sua morfologia vegetativa pouco informativa. Em alguns casos (*Bipinnula biplumata*, *B. montana* e *B. penicillata*), as espécies não são coletadas há décadas. Uma vez estes táxons são pouco coletados, sua morfologia floral e vegetativa é pouco conhecida. Através da análise das plantas no campo e de flores frescas em laboratório é possível comprovar que numerosos atributos marcantes da morfologia do labelo, coluna e rostelo, não constam na bibliografia mais recente (Pridgeon *et al.* 2003). Ao mesmo tempo, é possível verificar que a morfologia vegetativa das orquídeas brasileiras de Chloraeinae é muito semelhante com a de outros grupos de orquídeas terrícolas. Em particular, as espécies brasileiras de *Bipinnula* vêm se mostrando particularmente variáveis nestes atributos, podendo ou não ter folhas durante a antese e apresentando folhas francamente lineares ou lanceoladas e dispostas em roseta.

SUBTRIBO SPIRANTHINAE LINDL. EX MEISN.

Spiranthinae compreende cerca de 470 espécies distribuídas em aproximadamente 42 gêneros, de distribuição quase que exclusivamente neotropical (Chase *et al*. 2003, Salazar 2003). Esta subtribo é restrita ao Novo Mundo, exceto o gênero circumboreal *Spiranthes* Rich. e a única espécie de *Cyclopogon* C.Presl, *C. obliquus* (J.J. Sm) Szlach., que foi aparentemente introduzida no leste da Ásia (Salazar 2003).

Spiranthinae foi reconhecida formalmente como “Divisão” Spiranthidae da tribo Neottieae na classificação de Lindley (1840). Lindley (1840) incluiu neste grupo aquelas espécies com flores bilabiadas (pela coesão das pétalas, labelo e sépalas em dois conjuntos opostos), coluna terete estendida dentro de um “pé”, labelo canaliculado na face abaxial e um nectário dentro da base das sépalas laterais. Lindley (1840) reconheceu cinco gêneros em Spiranthidae: *Pelexia* Poit. ex Lindl., *Sauroglossum* Lindl., *Spiranthes, Stenorrhynchos* Rich. ex Spreng. e *Cnemidia* Lindl. Este último foi sinonimizado com *Tropidia* Lindl. e removido de Spiranthinae por Bentham (1881). Estudos posteriores confirmaram que estes gêneros não estão filogeneticamente relacionados à Spiranthinae (Stern *et al*. 1993a, 1993b, Clements 1995, Kores *et al*. 1997, 2001, Cameron *et al*. 1999, Chase *et al*. 2001).

Tabela 1. Diferentes propostas de classificação para Chloraeinae, adaptado de Chemisquy & Morrone (2010)

Dressler (1981, 1993)	**Szlachetko (1995)**	**Clements *et al*. (2002)**	**Pridgeon *et al*. (2001, 2003)**	**Chemisquy & Morrone (2010), Cisternas *et al*. (2012a, 2012b)**
Orchidoideae	**Thelymitroideae**	**Orchidoideae**	**Orchidoideae**	**Orchidoideae**
Diurideae	Geoblasteae	Cranichideae	Chloraeeae	Cranichideae
Chloraeinae	Chloraeinae	Chloraeinae	*Bipinnula*	Chloraeinae
Bipinnula	*Bipinnula*	*Bipinnula*	*Chloraea*	*Bipinnula*
Chloraea	*Burnettia*	*Chloraea*	*Gavilea*	*Chloraea*
Codonorchis	*Chloraea*	*Gavilea*	*Geoblasta*	*Gavilea*
Gavilea	*Codonorchis*	*Geoblasta*	Diurideae	Codonorchideae
Geoblasta	*Gavilea*	Diurideae	Megastylidinae	*Codonorchis*
Megastylis	*Geoblasta*	Megastylidinae	*Aporostylis*	
Caladeniinae	*Lyperanthus*	*Aporostylis*	*Burnettia*	
Burnettia	*Megastylis*	*Burnettia*	*Leporella*	
Lyperanthus	*Rimacola*	*Leporella*	*Lyperanthus*	
Rimacola		*Lyperanthus*	*Megastylis*	
		Megastylis	*Pyrochis*	
		Pyrochis	*Rimacola*	
		Rimacola	*Waireia*	
		Waireia	**Codonorchideae**	
		Codonorchidoideae	*Codonorchis*	
		Codonorchis		

Bentham (1881) publicou uma nova classificação de Orchidaceae para ser utilizada no *Genera Plantarum* de Bentham & Hooker (1883). Naquela obra, Bentham (1881) expandiu o conceito de Spiranthidae de Lindley (1840) para incluir também as subtribos Listeridae, Physuridae e Cranichidae. Com exceção de *Listera* e *Neottia* (Listeridae) e *Wullschlaegelia* (Cranichidae), os quais são referidos à subfamília Epidendroideae (Cameron *et al*. 1999, Molvray *et al*. 2000), o conceito de Spiranthidae de Bentham (1881) e equivalente à tribo Cranichideae como posteriormente delimitada por Dressler (1981). Bentham (1881) reconheceu duas séries em Spiranthidae, a primeira incluindo aqueles gêneros com políneas granulares e, a segunda com políneas farináceas. Bentham (1881) reconheceu apenas dois gêneros em a Spiranthinae: *Spiranthes* e *Pelexia*.

Pfitzer (1887) propôs uma classificação alternativa para Orchidaceae, a qual se diferencia das classificações anteriores pelo uso de caracteres vegetativos e florais para a delimitação de grupos. Pfitzer (1887) circunscreveu Spiranthinae (como Spirantheae) de um modo mais restrito que a classificação de Bentham (1881), removendo os gêneros com políneas sépteis para Physurideae (atualmente conhecida como Goodyerinae) e alguns gêneros para Cranichideae. A delimitação de Pfitzer (1887) incluiu os quatro gêneros aceitos por Lindley (1840) mais *Listera* R. Br., *Neottia* Guett., *Stenoptera* C.Presl e *Baskervilla* Lindl.

Alguns taxonomistas (e.g. Hoehne 1945, Correa 1955, Brieger 1974-1975, Pabst & Dungs 1975, Garay 1978) geralmente seguiram a circunscrição de Spiranthinae publicada por Schlechter (1926), que apresentou seu sistema de classificação na forma de uma chave dicotômica. Esta chave foi elaborada, basicamente, utilizando estruturas como antera, raízes fasciculadas, folhas basais e margens do labelo adnadas à coluna. Schlechter (1920) também propôs a primeira revisão da classificação genérica de Spiranthinae, na qual delimitou 24 gêneros utilizando caracteres florais tais como presença de um calcar, comprimento e espessura da coluna, presença de um pé da coluna, detalhes do estigma, do rostelo e do viscídio. Os gêneros foram agrupados em quatro grupos ("*Gattungsreihe*", como nomeados por Schlechter

1926, apesar de não prover uma descrição em latim), com base em caracteres do rostelo e do viscídio (Tabela 2).

A classificação genérica de Schlechter (1920) foi recebida com ceticismo, especialmente pelo seu contemporâneo, Oakes Ames. Comentando o trabalho de Schlechter (1920), Ames (1922) encontrou caracteres “desconhecidos” da coluna e outros caracteres florais usados por Schlechter (1920) para delimitar os gêneros e citou uma forma autopolinizada do complexo *Spiranthes sinensis*/*S. australis* (*S. novazelandiae*), que carece de um rostelo bem desenvolvido, como um exemplo de inconsistência dos caracteres do rostelo. No entanto, logo após, Ames (1923) utilizou atributos do rostelo para designar um novo gênero, *Lankesterella* Ames. Como consequência da divergência entre os principais especialistas em orquídeas, não houve consenso quanto à classificação genérica de Spiranthinae. Por outro lado, os pesquisadores da Universidade de Harvard que preparavam floras de Orchidaceae para várias regiões do Novo Mundo em meados do século 20, foram fortemente influenciados por Ames (p. ex. Correll 1950, Williams 1951, Ames & Correll 1952, Schweinfurth 1958) e inseriram muitas espécies de Spiranthinae em um senso amplo de *Spiranthes*, mas outros especialistas preferiram o sistema de Schlechter (p. ex. Hoehne 1945, Correa 1955, Brieger 1974-1975, Pabst & Dungs 1975, Garay 1978).

Em 1982 ocorreram duas novas propostas simultâneas sobre a delimitação genérica de Spiranthinae (Tabela 2), sendo que o número de gêneros e espécies em ambas é muito diferente (Balogh 1982, Garay 1982). Garay (1982), cuja prioridade de publicação foi de dois meses, admitiu que o seu próprio trabalho trazia uma abordagem “*lumping*” dos orquidologistas da escola de Harvard, mas mudou sua visão radicalmente quando teve a chance de estudar a subtribo. Garay (1982) aumentou os 24 gêneros reconhecidos por Schlechter (1920) para 44 (Tabela 2). Garay (1982) apresentou algumas discussões para muitas de suas decisões taxonômicas e justificou os vários gêneros monotípicos propostos como “inevitáveis produtos periféricos da anagênese, isto é, o refinamento evolutivo dentro de um ramo filogenético da família" (Garay 1982: 282). É importante ressaltar que Garay (1982) fez questão de mencionar que não pretendia inferir afinidades filogenéticas entre os *taxa* e que sua intenção era apenas elaborar um sistema prático que permitisse uma rápida separação dos gêneros. Ele distinguiu os gêneros com base na estrutura do rostelo, mas também considerou importantes a fusão das sépalas laterais para formar o tubo floral, bem como a posição terminal ou anterior do estigma.

Balogh (1982) aceitou apenas 17 gêneros e os distribuiu em cinco alianças, apesar de adicionar mais três gêneros posteriormente (Burns-Balogh 1986) e excluir de Spiranthinae *Nothostele* Garay, *Pseudocranichis* Garay e *Manniella* Rchb.f. (Tabela 2). Ainda, Balogh (1982) considerou que cinco gêneros não se enquadravam claramente em nenhuma destas alianças. O trabalho de Balogh (1982) assemelha-se em muitos aspectos ao de Schlechter (1920). Infelizmente, Balogh (1982) não foi clara na metodologia utilizada para efetuar estas delimitações. Balogh (1982) distinguiu os gêneros utilizando caraterísticas do rostelo, polinário e viscídio, posição da entrada do canal estilar e a posição das sépalas laterais. Burns-Balogh (1986) resumiu as diferenças entre sua classificação e a de Garay (1982). Burns-Balogh & Robinson (1983) foram os pioneiros na aplicação de métodos cladísticos para a classificação de orquídeas, utilizando argumentações Hennigianas (Henning 1966) e otimização manual (sem utilizar algoritmos específicos) de caracteres sobre cladogramas para acessar as relações na Aliança *Pelexia*. Esta proposta foi repetida por Dressler (1993), quando apresentou diagramas propondo as relações entre as orquídeas Cranichideae Endl. mostrando os estados de caracteres que sustentariam estas relações. Segundo Dressler (1993), para poder optar objetivamente entre os trabalhos de Garay (1982) e Balogh (1982) seria praticamente necessário refazer todo o trabalho.

Szlachetko (1995) dividiu Spiranthinae em três subtribos (Spiranthinae *sensu stricto*, Cyclopogoninae Szlach. e Stenorrhynchidinae Szlach. – Tabela 2) com base nas estruturas do rostelo e do viscídio. Szlachetko (1995) referiu estes três grupos como “subclados” mas não apresentou qualquer indicação clara sobre quais sinapomorfias os diagnosticariam, deixando de lado a contradição decorrente da sua rejeição declarada de métodos cladísticos em favor da busca de grupos politéticos, “homogêneos” em um contexto taxonômico (Szlachetko 1995). Szlachetko (1995) apresentou gráficos semelhantes a cladogramas onde ele sugeriu a polaridade de determinados estados de caracteres. No entanto, estes gráficos são meros diagramas efetuados sem uma metodologia explícita, com o único propósito de ilustrar os estados de caracteres que, de acordo com este autor, sustentam os grupos por ele propostos. Com poucas exceções (Johnson 2001), nem a classificação nem os gêneros propostos por Szlachetko (1995) têm sido aceitos. Szlachetko e co-autores propuseram diversos novos gêneros de Spiranthinae (Szlachetko 1991a, 1991b, 1993, 1994a, 1994b, González Tamayo & Szlachetko 1995, Szlachetko & González Tamayo 1996a, 1996b, 1996c, Szlachetko *et al*. 2000, 2001), em muitos casos dividindo gêneros que eles considerados “altamente heterogêneos e difíceis de definir” (Szlachetko *et al*. 2001).

Estudos filogenéticos recentes (Salazar 2003, Salazar *et al*. 2003, Figueroa *et al.* 2008, Salazar *et al.* 2009) baseados principalmente em ferramentas moleculares têm sustentado fortemente a monofilia de Spiranthinae como um todo, mas também tem sinalizado relações filogenéticas diferentes das apontadas pelos trabalhos anteriores (Balogh 1982, Dressler 1993, Szlachetko 1995). Todos estes trabalhos (Salazar 2003, Salazar *et al*. 2003, Figueroa *et al.* 2008, Salazar *et al.* 2009) demonstram que têm se dado excessiva ênfase a caracteres florais (em especial ao rostelo e viscídio) e que os subclados internos de Spiranthinae podem ser sustentados por conjuntos de caracteres (muitas vezes vegetativos), mais do que por um único caráter. Estes estudos (Salazar 2003, Salazar *et al*. 2003, Figueroa *et al.* 2008, Salazar *et al.* 2009) claramente indicam que os caracteres tradicionalmente utilizados na delimitação genérica são homoplásicos,

Tabela 2. Classificações dos gêneros que têm sido incluídos em Spiranthinae. *Taxa* e grupos informais diferem significantemente, impossibilitando a comparação linear

Schlechter (1920, 1926, 1929	**Garay (1982)**	**Balogh (1982), Burns Balogh *et al.* (1985), Burns-Balogh (1986)**	**Dressler (1993)**	**Szlachetko (1995), Szlachetko & Rutkowski (2000)**	**Salazar (2003)**
Manniellinae	**Spirathinae**	**Cranichidinae**	**Cranichidinae**	**Cranichidinae**	**Cranichidinae**
Manniella	*Aulosepalum*	*Nothostele*	*Nothostele*	*Nothostele*	*Nothostele*
Spiranthinae	*Beadlea*	*Pseudocranichis*	**Manniellinae**	**Manniellinae**	**Manniellinae**
Incertae sedis	*Beloglottis*	**Manniellinae**	*Manniella*	*Manniella*	*Manniella*
Buchtienia	*Brachystele*	*Manniella*	**Prescottiinae**	**Prescottiinae**	**Prescottinae**
"*Amblyorhyncha*"	*Buchtienia*	**Spiranthinae**	*Pseudocranichis*	*Pseudocranichis*	*Pseudocranichis*
Cyclopogon	*Coccineorchis*	*Incertae sedis*	**Spiranthinae**	**Spiranthinae**	**Spiranthinae**
Deiregyne	*Cotylolabium*	*Buchtienia*	*Aracamunia*	*Aracamunia*	*Incertae sedis*
Gamosepalum	*Cybebus*	*Deiregyne*	*Aulosepalum*	*Beloglottis*	*Aracamunia*
Pelexia	*Cyclopogon*	*Dichromanthus*	*Beloglottis*	*Brachystele*	*Buchtienia*
Sarcoglottis	*Deiregyne*	*Greenwoodia*	*Brachystele*	*Buchtienia*	*Cybebus*
Schiedeella	*Dichromanthus*	*Pseudogoodyera*	*Buchtienia*	*Cybebus*	*Degranvillea*
Trachelosiphon	*Discyphus*	Aliança *Brachystele*	*Coccineorchis*	*Degranvillea*	*Discyphus*
"*Brachyrhyncha*"	*Dithyridanthus*	*Brachystele*	*Cotylolabium*	*Deiregyne*	*Hapalorchis*
Brachystele	*Eltroplectris*	*Odontorrhynchus*	*Cybebus*	*Dichromanthus*	*Helonoma*
Mesadenus	*Eurystyles*	*Sauroglossum*	*Cyclopogon*	*Discyphus*	*Physogyne*
Pseudogoodyera	*Funkiella*	Aliança *Eurystyles*	*Degranvillea*	*Diskyphogyne*	*Pseudogoodyera*
Sauroglossum	*Galeottiella*	*Eurystyles*	*Deiregyne*	*Eurystyles*	Clado *Stenorrhynchos*
Synassa	*Gularia*	*Pseudoeurystyles*	*Dichromanthus*	*Galeottiella*	*Cotylolabium*
"*Raphiorhyncha*"	*Hapalorchis*	*Synanthes*	*Discyphus*	*Gracielanthus*	*Eltroplectris*
Cladobium	*Helonoma*	Aliança *Pelexia*	*Dithyridanthus*	*Greenwoodia*	*Lyroglossa*
Centrogenium	*Kionophyton*	*Cyclopogon*	*Eltroplectris*	*Hapalorchis*	*Mesadenella*
Coccineorchis	*Lankesterella*	*Pelexia*	*Eurystyles*	*Mesadenus*	*Pteroglossa*
Funkiella	*Lyroglossa*	*Sarcoglottis*	*Funkiella*	*Microthelys*	*Sacoila*
Lyroglossa	*Manniella*	Aliança *Spiranthes*	*Galeottiella*	*Odontorrhynchus*	*Skeptrostachys*
Pteroglossa	*Mesadenella*	*Beloglottis*	*Hapalorchis*	*Pseudoeurystyles*	*Stenorrhynchos*
Stenorrhynchos	*Mesadenus*	*Discyphus*	*Helonoma*	*Pseudogoodyera*	*Thelyschista*
"*Schistorhyncha*"	*Microthelys*	*Hapalorchis*	*Kionophyton*	*Sauroglossum*	Clado *Pelexia*
Beloglottis	*Nothostele*	*Spiranthes*	*Lankesterella*	*Schidorhynchos*	*Brachystele*
Discyphus	*Odontorrhynchus*	Aliança *Stenorrhynchos*	*Lyroglossa*	*Spiranthes*	*Coccineorchis*
Galeottiella	*Pelexia*	*Schiedeella*	*Mesadenella*	*Thelyschista*	*Cyclopogon*
Hapalorchis	*Physogyne*	*Stenorrhynchos*	*Mesadenus*	*Triceratostris*	*Odontorrhynchus*
Spiranthes	*Pseudocranichis*		*Odonthorrhynhus*	*Wallnoeferia*	*Pelexia*
	Pseudogoodyera		*Oestlundorchis*	**Cyclopogoninae**	*Sarcoglottis*
	Pteroglossa		*Pelexia*	*Cocleorchis*	*Sauroglossum*

Continuação

Schlechter (1920, 1926, 1929	**Garay (1982)**	**Balogh (1982), Burns Balogh *et al.* (1985), Burns-Balogh (1986)**	**Dressler (1993)**	**Szlachetko (1995), Szlachetko & Rutkowski (2000)**	**Salazar (2003)**
	Spirathinae		**Spiranthinae**	**Cyclopogoninae**	Clado *Pelexia*
	Sacoila		*Pseudogoodyera*	*Cyclopogon*	*Veyretia*
	Sarcoglottis		*Pteroglossa*	*Pelexia*	Clado *Eurystyles*
	Sauroglossum		*Sacoila*	*Sarcoglottis*	*Eurystyles*
	Schiedeella		*Sarcoglottis*	*Stigmatosema*	*Lankesterella*
	Skeptrostachys		*Sauroglossum*	*Veyretia*	Clado *Spiranthes*
	Spiranthes		*Schiedeella*	*Warscaea*	*Aulosepalum*
	Stalkya		*Skeptrostachys*	**Stenorrhynchidinae**	*Beloglottis*
	Stenorrhynchos		*Spiranthes*	*Bunsbaloghia*	*Deiregyne*
	Stigmatosema		*Stalkya*	*Coccineorchis*	*Dichromanthus*
	Thelyschista		*Stenorrhynchos*	*Cotylolabium*	*Funkiella*
			Stigmatosema	*Eltroplectris*	*Kionophuton*
			Thelyschista	*Funkiella*	*Mesadenus*
				Garaya	*Schiedeella*
				Greenwoodia	*Spiranthes*
				Kionophyton	*Stalkya*
				Lankesterella	*Svenkoeltzia*
				Lyroglossa	
				Mesadenella	
				Ochyrella	
				Oestlundorchis	
				Physogyne	
				Pteroglossa	
				Sacoila	
				Schiedeella	
				Skeptrostachys	
				Stalkya	
				Stenorrhynchos	

isto é, sujeitos a reversões e paralelismos. Análises filogenéticas mais recentes sustentam a existência de quatro clados (Tabela 2):

1) O clado *Stenorrhynchos* Rich. ex Spreng. que, além deste gênero, inclui outros tradicionalmente considerados próximos (e.g. *Sacoila* Raf., *Eltroplectris* Raf., *Mesadenella* Pabst & Garay).

2) O clado *Pelexia* Poit. ex Lindl., que contém, além deste gênero, outros filogeneticamente próximos (*Cyclopogon*, *Sarcoglottis* C.Presl, *Veyretia* Szlach.), e também outros gêneros de morfologia floral muito diferente, tais como *Odontorrhynchus* M.N. Correa, *Coccineorchis* Schltr. e *Brachystele* Schltr. Tradicionalmente este clado tem incluído gêneros com viscídio dorsalmente adesivo, como aparece nos gêneros *Cyclopogon*, *Sarcoglottis* e *Pelexia*, mas análises filogenéticas recentes (Salazar com. pess.) sugerem que o grupo inclui gêneros com morfologia rostelar diferente.

3) O clado *Eurystyles* Wawra + *Lankesterella* Ames, que é de fácil diagnóstico, pois inclui plantas que são pequenas epífitas obrigatórias.

4) Um clado de gêneros exclusivos do Hemisfério Norte (*Spiranthes* Rich., *Aulosepalum* Garay, *Deiregyne* Schltr., *Dichromanthus* Garay, etc.).

Todos os estudos filogenéticos (Salazar 2003, Salazar *et al.* 2003, Figueroa *et al.* 2008, Salazar *et al.* 2009) sustentam fortemente estes grupos. No entanto, carecem de uma amostragem mais ampla de gêneros essencialmente brasileiros, de distribuição restrita, e de um maior número de amostras de gêneros morfologicamente diversos ou que já foram objeto de propostas taxonômicas muito díspares. Para quem rotineiramente coleta estas plantas no seu habitat natural resulta difícil, por exemplo, entender quais caracteres realmente separam *Skeptrostachys* Garay de *Sacoila*, ou quais caracteres de fato separam *Stigmatosema* Garay de *Cyclopogon*. Em síntese, embora se tenha um panorama razoavelmente bom do número de clados que integram Spiranthinae, a delimitação genérica atual é bastante insatisfatória e, em geral, tem-se a sensação que o número de gêneros é excessivo. Contudo, só após ter se efetuado uma amostragem mais abrangente será possível tentar uma revisão em nível genérico. Isto é válido para gêneros neotropicais de delimitação atual confusa (e.g.: *Pelexia*). O Brasil é rico em gêneros pouco coletados (e.g.: *Brachystele*, *Pelexia*, *Veyretia*, *Cyclopogon*, *Sacoila*, etc) e pouco representados nas filogenias já publicadas de Spiranthinae (Salazar 2003, Salazar *et al*. 2009). Um amplo programa de coleta destes *taxa* permitiria inserir espécies brasileiras em matrizes filogenéticas pré-existentes, permitindo uma melhor resolução das relações entre os diferentes grupos de Spiranthinae e uma reavaliação dos caracteres morfológicos tradicionalmente utilizados no diagnóstico de gêneros e espécies. Uma vez feitas estas análises filogenéticas mais conclusivas, os caracteres morfológicos tradicionalmente utilizados na diagnose dos gêneros poderiam ser reavaliados e, se necessário ou pertinente, novas propostas nomenclaturais poderiam ser feitas.

Embora todos os estudos recentes confirmem a existência de quatro grandes clados dentro de Spiranthinae (Salazar 2003, Salazar *et al.* 2003, Figueroa *et al.* 2008, Salazar *et al.* 2009), a delimitação e a abrangência genérica continuam um pouco confusas, especialmente para os táxons sul-americanos. As diferenças entre os gêneros hoje aceitos por muitas vezes parecem insignificantes ou até bastante arbitrárias. Ainda, os táxons que ocorrem nas regiões Brasileiras Sul e Sudeste são os que estão menos amostrados nas filogenias já publicadas (Salazar *et al*. 2003, Figueroa *et al*. 2008, Salazar *et al*. 2009, Salazar *et al*. 2011). Uma filogenia mais completa e abrangente do clado *Pelexia* poderia servir como base para uma nova delimitação genérica mais sólida baseada não apenas em caracteres moleculares, mas também em conjuntos de caracteres macromorfológicos (vegetativos e florais).

POLINIZAÇÃO

Entre as espécies neotropicais de Spiranthinae, a estrutura da coluna dos gêneros como *Cyclopogon*, *Pelexia* e *Sarcoglottis* é altamente diagnóstica (Dressler 1993, Singer & Sazima 1999, Singer & Cocucci 1999). Destas orquídeas, o viscídio (a parte destacável e adesiva do rostelo que adere o polinário ao polinizador) é em forma de cunha e a superfície adesiva é na face dorsal (*vs*. superfície adesiva ventral como nas demais espécies de Spiranthinae, ver Singer & Sazima 1999, Catling 1982, Catling 1987). A biomecânica deste tipo de viscídio foi elucidado através dos estudos de biologia da polinização dos gêneros citados acima (Singer & Cocucci 1999, Singer & Sazima 1999). Independentemente das diferenças de tamanho, estas orquídeas são polinizadas exclusivamente por abelhas que se alimentam de néctar e pressionam seu labrum (estrutura em forma de placa que esconde parcialmente as peças bucais do inseto) contra a superfície dorsal e adesiva do viscídio, aderindo o polinário no interior desta estrutura (Singer & Cocucci 1999, Singer & Sazima 1999). O viscídio nestes gêneros de Spiranthinae são tem sido considerado de valor filogenético, suportando as afinidades destes táxons (Dressler 1993, Balogh 1982, Burn-Balogh & Robinson 1983).

Até o momento, há poucos registros sobre o sistema reprodutivo e a biologia da polinização de representantes do clado *Pelexia*. As espécies de *Cyclopogon* são polinizadas por abelhas Halictidae (Galetto *et al*. 1997, Singer & Cocucci 1999, Singer & Sazima 1999). Dressler (1993) mencionou mamangavas polinizando *Pachygenium ekmanii* (Kraenzl.) Szlach., Tamayo & Rutk. [como *Pelexia ekmanii* (Kraenzl.) Schltr.] enquanto que abelhas Euglossini foram capturadas carregando polinários de *Sarcoglottis*. Estas informações foram confirmadas posteriormente, onde *Pachygenium oestriferum* (Rchb.f.) Szlach., Tamayo & Rutk. [como *Pelexia oestrifera* (Kraenzl.) Cogn.] é frequentemente polinizada por mamangavas no sudeste do Brasil e *Sarcoglottis fasciculata* é regularmente polinizada por abelhas Euglossini (Singer & Sazima 1999). Em todos estes casos, as orquídeas secretam néctar e o polinário se adere na parte de baixo do labrum, enquanto as abelhas tentam alcançar o néctar com sua probóscide (Singer & Cocucci 1999, Singer & Sazima 1999). Por outro lado, as flores de *Sauroglossum elatum* Lindl. emite seu perfume durante a noite e tem um pequeno viscídio cuja superfície adesiva é ventral (Singer 2002). De acordo com estes caracteres, estas flores são polinizadas por mariposas que carregam os polinários sobre a superfície da probóscide (Singer 2002). Todas as espécies de orquídeas do clado *Pelexia* cujo sistema reprodutivo já foi estudado, são autocompatíveis de dependentes de polinizadores, isto é, são incapazes de produzir frutos na ausência de polinizadores (Galetto *et al*. 1997, Singer & Sazima 1999, Singer 2002). Notavelmente, desde então (Singer & Cocucci 1999, Singer & Sazima 1999, Singer 2002), nenhuma espécie deste clado tiveram seu sistema reprodutivo e mecanismo de polinização estudados.

A biologia da polinização em espécies da subtribo Chloraeinae tem sido investigada principalmente para um grupo de espécies Patagônicas de *Chloraea*, que focaram no sistema reprodutivo e no sucesso de frutificação, dos quais renderam poucas informações sobre o comportamento dos polinizadores (Clayton & Aizen 1996, Lehnebach & Riveros 2003, Humaña *et al*. 2008). No entanto, estes autores (Clayton & Aizen 1996, Lehnebach & Riveros 2003, Humaña *et al*. 2008) concordaram que todas as espécies de *Chloraea* estudadas até o momento não têm néctar e são auto-compatíveis, mas dependentes de polinizadores (e.g. abelhas, moscas e besouros), ou seja, o polinizador é necessário para a formação de frutos e sementes viáveis (Gumprecht 1975, Clayton & Aizen 1996, Lehnebach & Riveros 2003, Humaña *et al*. 2008).

Com a falta de recursos florais como néctar (Lehnebach & Riveros 2003, Humaña *et al*. 2008), provavelmente todas as espécies de *Chloraea* são polinizadas por engano, no entanto, devido à escassez de dados, esta informação necessita ser provada. Análises detalhadas das características florais, juntamente com observações de polinizadores completos são necessários para determinar a biologia da polinização dessas espécies.

OBJETIVOS

a) Construir uma filogenia molecular mais completa e mais representativa da subtribo Chloraeinae, com a inclusão dos *taxa* brasileiros.

b) Efetuar um estudo taxonômico e nomenclatural das orquídeas brasileiras da subtribo Chloraeinae.

c) Construir uma filogenia molecular mais completa e mais representativa do clado *Pelexia* e estabelecer se os gêneros são monofiléticos.

e) Efetuar um estudo nomenclatural das orquídeas brasileiras do clado *Pelexia*.

f) Documentar amplamente a morfologia floral e vegetativa dos *taxa* acima citados, com ênfase em caracteres diagnósticos.

g) Estabelecer conjuntos de caracteres diagnósticos para os gêneros envolvidos neste estudo e, se necessário, propor novas delimitações genéricas.

h) Com base nos estudos filogenéticos acima citados, propor cenários solidamente sustentados para a evolução de caracteres de importância ecológica (e.g. aparição de uma determinada estrutura rostelar).

APRESENTAÇÃO DA TESE

Com o objetivo de organizar e estruturar diferentes abordagens, esta tese está dividida em três seções, nas quais os capítulos são apresentados em forma de artigos (exceto o capítulo 6), estruturados nos respectivos formatos dos periódicos que foram e/ou que serão submetidos.

A primeira seção trata sobre a taxonomia e nomenclatura de ambas subtribos, abrangendo quatro capítulos. O primeiro capítulo propõe 48 tipificações, novas sinonimizações e notas taxonômicas de espécies de Goodyerinae e Spiranthinae descritas por José Velloso e Barbosa Rodrigues. O segundo apresenta novos registros de *Pteroglossa* e *Lyroglossa* para o Rio Grande do Sul. Já o terceiro capítulo, trata de correções nomenclaturais para o binomial *Spiranthes bicolor*, enquanto o quarto capítulo apresenta uma sinopse taxonômica de Chloraeinae brasileiras.

A segunda seção apresenta hipóteses filogenéticas baseadas em caracteres moleculares e abrange dois capítulos. O primeiro (capítulo de número cinco) testa a monofila dos gêneros atualmente circunscritos no clado *Pelexia* (Spiranthinae) enquanto o sexto capítulo, traz uma atualização das relações filogenéticas dos gêneros de Chloraeinae, quando são incluídas espécies Pampeanas de *Bipinnula*, inclusive a espécie tipo do gênero, *B. biplumata*. Os dois capítulos desta seção devem ser considerados e entendidos apenas como um informe das análises filogenéticas efetuadas até o momento. Os dados ali expressos não serão publicados no formato em que aparecem.

A terceira e última seção abrange dois estudos de biologia da polinização. O capítulo de número sete, apresenta um apanhado geral sobre a biologia reprodutiva de duas espécies de *Pachygenium* e dados complementares sobre os gêneros *Brachystele* e *Cyclopogon s.l.* O oitavo capítulo apresenta um estudo detalhado da morfologia floral, biologia da polinização e sistema reprodutivo de *Chloraea membranacea* (Chloraeinae).

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Seção 1

Taxonomia e nomenclatura de Spiranthinae e Chloraeinae

Capítulo 1

Typifications and taxonomic notes in species of Brazilian Goodyerinae and Spiranthinae (Orchidaceae) described by José Vellozo and Barbosa Rodrigues

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TAXON 62 (3) • June 2013: 609–621 Buzatto & al. • **Typifications in Brazilian *Orchidaceae***

Typifications and taxonomic notes in species of Brazilian *Goodyerinae* and *Spiranthinae* (*Orchidaceae*) described by José Vellozo and Barbosa Rodrigues

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Abstract A total of 46 lectotypes, one neotype and one epitype are designated for species of *Goodyerinae* and *Spiranthinae* originally described by José Mariano da Conceição Vellozo and João Barbosa Rodrigues. Moreover, three new previously accepted names are treated as synonyms.

Keywords Brazil; *Goodyerinae*; nomenclature; *Orchidaceae*; *Spiranthinae*; typification

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INTRODUCTION

Cranichideae (Lindl.) Endl. (*Orchidaceae*) was established by Endlicher in 1842 and is currently circumscribed to comprise six subtribes (*Cranichidinae* Lindl., *Galeottiellinae* Salazar & M.W. Chase, *Goodyerinae* Klotzsch, *Manniellinae* Schltr., *Pterostylidinae* Pfitzer, *Spiranthinae* Lindl.), with over 600 species widely distributed in tropics, subtropics and temperate zones (Dressler, 1993; Cribb, 2003; Salazar, 2003). In Brazil, three subtribes are particularly diverse: *Cranichidinae*, *Goodyerinae*, and *Spiranthinae*.

With 34 currently accepted genera, *Goodyerinae* are widely distributed in both the Old and New Worlds, especially in the tropics and subtropics (Ormerod & Cribb, 2003). The current taxonomic concepts in *Goodyerinae* (Garay, 1977; Ormerod & Cribb, 2003) segregate genera largely based on column features, especially rostellum structure (Garay, 1977; Dressler, 1993). However, inclusive phylogenetic studies in this subtribe are lacking, and the reliability of such characters to delimit monophyletic groups is yet to be tested. Nonetheless, here we follow the subtribal delimitation of *Goodyerinae* of Dressler (1981) and the generic delimitation of Garay (1977) and Ormerod & Cribb (2003).

Spiranthinae include about 42 genera and 470 species of predominantly terrestrial orchids (Chase & al., 2003; Salazar, 2003). This subtribe has two main diversity centers, one in Mexico and Central America and the other in southern and southeastern Brazil (Balogh, 1982; Dressler, 1993).

The high biodiversity found in Brazil has attracted many naturalists in the past four hundred years. The contributions of two early researchers are particularly important: José Vellozo Xavier, also known as Friar José Mariano da Conceição Vellozo (1742–1811), and João Barbosa Rodrigues (1842–1909) (Valle, 1985; Sá, 2001). Vellozo's *Florae Fluminensis* was written in eleven volumes of plates and text, and included 1640 Brazilian species (Carauta, 1973). The tortuous history of this publication has been discussed in depth by Carauta (1969). According to Carauta (1973), the effective date of publication of *Flora Fluminensis* took place in two different moments: (1) the first and incomplete version of the text, printed in 1829 and (2) in 1831, when the plates were published. Lima (1995) comments that a second edition, including a complete version of the text, was published only in 1882. This later edition was published as the fifth volume of the *Arquivos do Museu Nacional*, edited by Ladislau de Souza Mello-Netto, Director of the Museu Nacional do Rio de Janeiro (Mello-Netto, 1882). Problems regarding the species published by Vellozo include the absence of vouchers for all of his taxa (Lima, 1995) and rudimentary descriptions and illustrations, sometimes resulting in the identity of the species being uncertain (see Lima, 1995 for similar observations regarding species described by Vellozo in Leguminosae).

With support from the Brazilian Empire (Mori & Ferreira, 1987; Sprunger & al., 1996; Sá, 2001), Barbosa Rodrigues published a series of botanical, anthropological and natural history works. Following the practice of his time, Barbosa Rodrigues (1877, 1882) cited localities and flowering times but did not designate types for his new orchid species. The watercolors and drawings he prepared to illustrate his work, which he planned to publish as "Iconographie des Orchidées du Brésil" (Barbosa Rodrigues, 1877: 5, footnote) remained unpublished until Cogniaux reproduced some of them as line drawings in his orchid treatment for Martius's *Flora Brasiliensis* (Cogniaux, 1893–1906). Much later, most of the original drawings and watercolors (about 370 drawings) were published in color (Sprunger & al., 1996). The original illustrations prepared by Barbosa Rodrigues are currently housed at the Rio de Janeiro Botanical Garden, Brazil (vols. 1–3, 5–6) and at the Orchid Herbarium of Oakes Ames, Harvard University (vol. 4). It is generally believed that any voucher specimens for the taxa described by Barbosa Rodrigues were destroyed during a tropical deluge (Cribb & Toscano, 1996; Buzatto & al., 2011).

A thorough review of Brazilian and other herbaria has not revealed any original Barbosa Rodrigues collection of terrestrial orchids, with the exception of a pressed specimen deposited at S (see below). Therefore, in many cases we rely on Barbosa Rodrigues's original watercolors of *Goodyerinae* and *Spiranthinae* species for the lectotypification of names published by him (1877, 1882). These illustrations are part of volume 1 of his unpublished work deposited at the Biblioteca Barbosa Rodrigues, part of the Rio de Janeiro Botanical Garden (JBRJ – Jardim Botânico do Rio de Janeiro), Brazil. Conversely, there are good vouchers of terrestrial orchids described by Barbosa Rodrigues (1877, 1882) based on specimens collected by Anders Fredrik Regnell (1807–1884) and Francisco Freire Allemão (1797–1874) and these collections are therefore available for typification purposes

The typifications presented in the following section are summarized in Table 1.

■ TYPIFICATIONS

Goodyerinae Klotzsch

1. ***Aspidogyne aratanhensis*** (Barb. Rodr.) Baptista in Bol. C. A. O. B. 58: 50. 2005 ≡ *Physurus aratanhensis* Barb. Rodr., Gen. Sp. Orchid. 2: 290. 1882 ≡ *Erythrodes aratanhensis* (Barb. Rodr.) Ames, Orchidaceae 7: 66. 1922 – Holotype: BRAZIL. "Hab. dans lês forêts de la serra do Aratanha, près La ville de Fortaleza au Ceará", s.d., *F.F. Allemão 1486* (P no. 331259!).

2. ***Aspidogyne argentea*** (Vell.) Garay in Bradea 2: 203. 1977 ≡ *Ophrys argentea* Vell., Fl. Flumin. Icon. 9: tab. 61. 1831 [Arq. Mus. Nac. Rio de Janeiro 5: 372. 1882] ≡ *Anoectochilus argenteus* (Vell.) Linden, Cat. Pl. Exot. 14: 13. 1859 – **Lectotype (designated here):** Vellozo's original illustration at Manuscript Section of the Biblioteca Nacional of Rio de Janeiro. Later published in Fl. Flumin. Icon. 9: tab. 61. 1831.

3. ***Aspidogyne bicolor*** (Barb. Rodr.) Garay in Bradea 2: 203. 1977 ≡ *Physurus bicolor* Barb. Rodr., Gen. Sp. Orchid. 2: 290. 1882 ≡ *Erythrodes bicolor* (Barb. Rodr.) Ames, Orchidaceae 7: 67. 1922 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 2: tab. 80, cited as tab. 781 (then unpublished) in Barbosa Rodrigues (1882: 290), reproduced in Sprunger & al. (1996: 136).

4. ***Aspidogyne commelinoides*** (Barb. Rodr.) Garay in Bradea 2: 201. 1977 ≡ *Physurus commelinoides* Barb. Rodr., Gen. Sp. Orchid. 1: 193. 1877 ≡ *Erythrodes commelinoides* (Barb. Rodr.) Ames, Orchidaceae 7: 70. 1922 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 2: tab. 78, cited as tab. 478 (then unpublished) in Barbosa Rodrigues (1877: 193), reproduced in Sprunger & al. (1996: 134).

5. ***Aspidogyne metallescens*** (Barb. Rodr.) Garay in Bradea 2: 204. 1977 ≡ *Physurus metallescens* Barb. Rodr., Gen. Sp. Orchid. 2: 291. 1882 ≡ *Erythrodes metallescens* (Barb. Rodr.) Ames, Orchidaceae 7: 72. 1922 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 2: tab. 81, cited as tab. 770 (then unpublished) in Barbosa Rodrigues (1882: 291), reproduced in Sprunger & al. (1996: 135).

6. *Physurus lacteolus* Barb. Rodr., Gen. Sp. Orchid. 2: 292. 1882 ≡ *Erythrodes lacteola* (Barb. Rodr.) Ames, Orchidaceae 7: 71. 1922 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 2: tab. 79, cited as tab. 771 (then unpublished) in Barbosa Rodrigues (1882: 292), reproduced in Sprunger & al. (1996: 135) [= ***Aspidogyne hylibates*** (Rchb. f.) Garay].

Spiranthinae Lindl.

1. *Cyclopogon albopunctatus* Barb. Rodr., Gen. Sp. Orchid. 2: 281. 1882 ("*albo-punctata*") – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 51, cited as tab. 591 (then unpublished) in Barbosa Rodrigues (1882: 281), reproduced in Sprunger & al. (1996: 104) [= ***Mesadenella cuspidata*** (Lindl.) Garay].

2. ***Cyclopogon argyrifolius*** Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: III. 1877 ("*argirifolia*") ≡ *Spiranthes argyrifolia* Barb. Rodr., Gen. Sp. Orchid. 1: 183. 1877 ("*argirifolia*") ≡ *Beadlea argyrifolia* (Barb. Rodr.) Garay in Bot. Mus. Leafl. 28(4): 299. 1982 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 75, cited as tab. 499 (then unpublished) in Barbosa Rodrigues (1877: 183), reproduced in Sprunger & al. (1996: 129).

3. ***Cyclopogon calophyllus*** Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: III. 1877 ≡ *Spiranthes calophylla* Barb. Rodr., Gen. Sp. Orchid. 1: 182. 1877 ≡ *Beadlea calophylla* (Barb. Rodr.)

Garay in Bot. Mus. Leafl. 28(4): 299. 1982 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 76, cited as tab. 496 (then unpublished) in Barbosa Rodrigues (1877: 182), reproduced in Sprunger & al. (1996: 130).

4. ***Cyclopogon cearensis*** Barb. Rodr., Gen. Sp. Orchid. 2: 283. 1882 ≡ *Spiranthes elata* var. *cearensis* (Barb. Rodr.) Cogn. in Martius, Fl. Bras. 3(4): 192. 1895 ≡ *Beadlea cearensis* (Barb. Rodr.) Garay in Bot. Mus. Leafl. 28(4): 300. 1982 – Holotype: BRAZIL. "Hab. dans la Province du Ceará", s.d., *F.F. Allemão & M. Cysneiros 1485* (R no. 35618!).

5. ***Cyclopogon chloroleucus*** Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: III. 1877 ("*chloroleuca*") ["*Cyclopogon chloroleucus* (Barb. Rodr.) Schltr." (in Repert. Spec. Nov. Regni Veg. 16: 322. 1920) based on *Spiranthes chloroleuca* Barb. Rodr. is nonetheless a later isonym; see Art. 6.3, Note 2] ≡ *Spiranthes chloroleuca* Barb. Rodr., Gen. Sp. Orchid. 1: 181. 1877 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 59, cited as tab. 368 (then unpublished) in Barbosa Rodrigues (1877: 181), reproduced in Sprunger & al. (1996: 112 and 127, fig. A).

= *Spiranthes chloroleuca* var. *fontinalis* Barb. Rodr., Gen. Sp. Orchid. 1: 182. 1877 ≡ *Cyclopogon chloroleuca* var. *fontinalis* Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: III. 1877 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 64, cited as tab. 367 (then unpublished) in Barbosa Rodrigues (1877: 182), reproduced in Sprunger & al. (1996: 117), **syn. nov.** [= ***Cyclopogon chloroleucus*** (Barb. Rodr.) Schltr.].

= *Spiranthes chloroleuca* var. *longipetiolata* Barb. Rodr., Gen. Sp. Orchid. 1: 182. 1877 ≡ *Cyclopogon chloroleucus* var. *longipetiolata* Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: III. 1877 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: t. 73, fig. A, cited as t. 495 (then unpublished) in Barbosa Rodrigues (1877: 182), reproduced in Sprunger & al. (1996: 112 and 127, fig. A) [= ***Cyclopogon chloroleucus*** (Barb. Rodr.) Schltr. **syn. nov.**].

We herein treat *Spiranthes chloroleuca* var. *fontinalis* and *S. chloroleuca* var. *longipetiolata* to be synonyms of *Cyclopogon chloroleucus*. In Martius's *Flora Brasiliensis*, Cogniaux (1893–1906) treated *S. chloroleuca* var. *fontinalis* as a synonym of the so called *S. lineata* Lindl. (currently *Hapalorchis lineata* (Lindl.) Schltr.). This circumscription was followed in the reproduction of "Iconographie des Orchidées du Brésil" by Sprunger & al. (1996: 117). However, the features illustrated in Barbosa Rodrigues's original watercolor (1877: tab. 64, cited as tab. 367) and protologue (1877: 182), indicate that this taxon is referable to *Cyclopogon*.

6. ***Cyclopogon congestus*** (Vell.) Hoehne, Fl. Brasilica 12(2): 209. 1945 ≡ *Serapias congesta* Vell., Fl. Flumin. Icon. 9: tab. 54. 1831 [Arq. Mus. Nac. Rio de Janeiro 5: 370. 1882] – **Lectotype (designated here):** Vellozo's original illustration at Manuscript Section of the Biblioteca Nacional of Rio de Janeiro (later published in Fl. Flumin. Icon. 9: t. 54. 1831).

= *Spiranthes alpestris* Barb. Rodr., Gen. Sp. Orchid. 1: 184. 1877 ≡ *Cyclopogon alpestris* Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: III. 1877 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: t. 62, fig. A, cited as tab. 369 (then unpublished) in Barbosa Rodrigues (1877: 184), reproduced in Sprunger & al. (1996: 115).

= *Cyclopogon alpestris* var. *bidentatus* Barb. Rodr., Gen. Sp. Orchid. 2: 283. 1882 ("*bidentata*") ≡ *Cyclopogon bidentatus* Barb. Rodr., Gen. Sp. Orchid. 2 [Index]: III. 1882 ("*bidentata*") ["*Cyclopogon bidentatus* (Barb. Rodr.) Dodson" (in Orquideología 19: 92. 1993) and "*Cyclopogon bidentatus* (Barb. Rodr.) Szlach." (in Candollea 48: 435. 1993) based on *Cyclopogon alpestris* var. *bidentatus* Barb. Rodr. are nonetheless later isonyms; see Art. 6.3, Note 2] – **Lectotype (designated here):** BRAZIL. Minas Gerais, Caldas, "Hab. croissant sur les rochers des montagnes de Caldas, et de la Serra de S. José d'El Rey", 21 Aug. 1877, *A.F. Regnell III1197* (S no. 10-18638!; isolectotypes: S no. 10-18643!, P no. 340963!, P no. 340964!).

The protologue of *Cyclopogon alpinus* var. *bidentatus* indicates the collection *A.F Regnell III1197* as the type. We found that this collection actually consists of eight specimens deposited at S and P. These specimens were collected on three distinct dates: 10 August 1869, 21 August 1877, 16 September 1878 and 31 July 1879. Thus, these specimens are syntypes, and we choose the specimen collected on 21 August 1877 as lectotype.

7. ***Cyclopogon longibracteatus*** (Barb. Rodr.) Schltr. in Beih. Bot. Centralbl. 37: 390. 1920 ≡ *Spiranthes longibracteata* Barb. Rodr., Gen. Sp. Orchid. 1: 185. 1877 ≡ *Beadlea longibracteata* (Barb. Rodr.) Garay in Bot. Mus. Leafl. 28(4): 301. 1982 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 58, cited as tab. 491 (then unpublished) in Barbosa Rodrigues (1877: 185), reproduced in Sprunger & al. (1996: 111).

8. ***Cyclopogon micranthus*** Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: III. 1877 ["*Cyclopogon micranthus* (Barb. Rodr.) Schltr." (in Repert. Spec. Nov. Regni Veg. 16: 322. 1920) based on *Spiranthes micrantha* Barb. Rodr. is nonetheless a later isonym; see Art. 6.3, Note 2] ≡ *Spiranthes micrantha* Barb. Rodr., Gen. Sp. Orchid. 1: 183. 1877 ≡ *Hapalorchis micrantha* (Barb. Rodr.) Hoehne, Fl. Brasilica 12(2): 291. 1945 ("*micranthus*") – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées

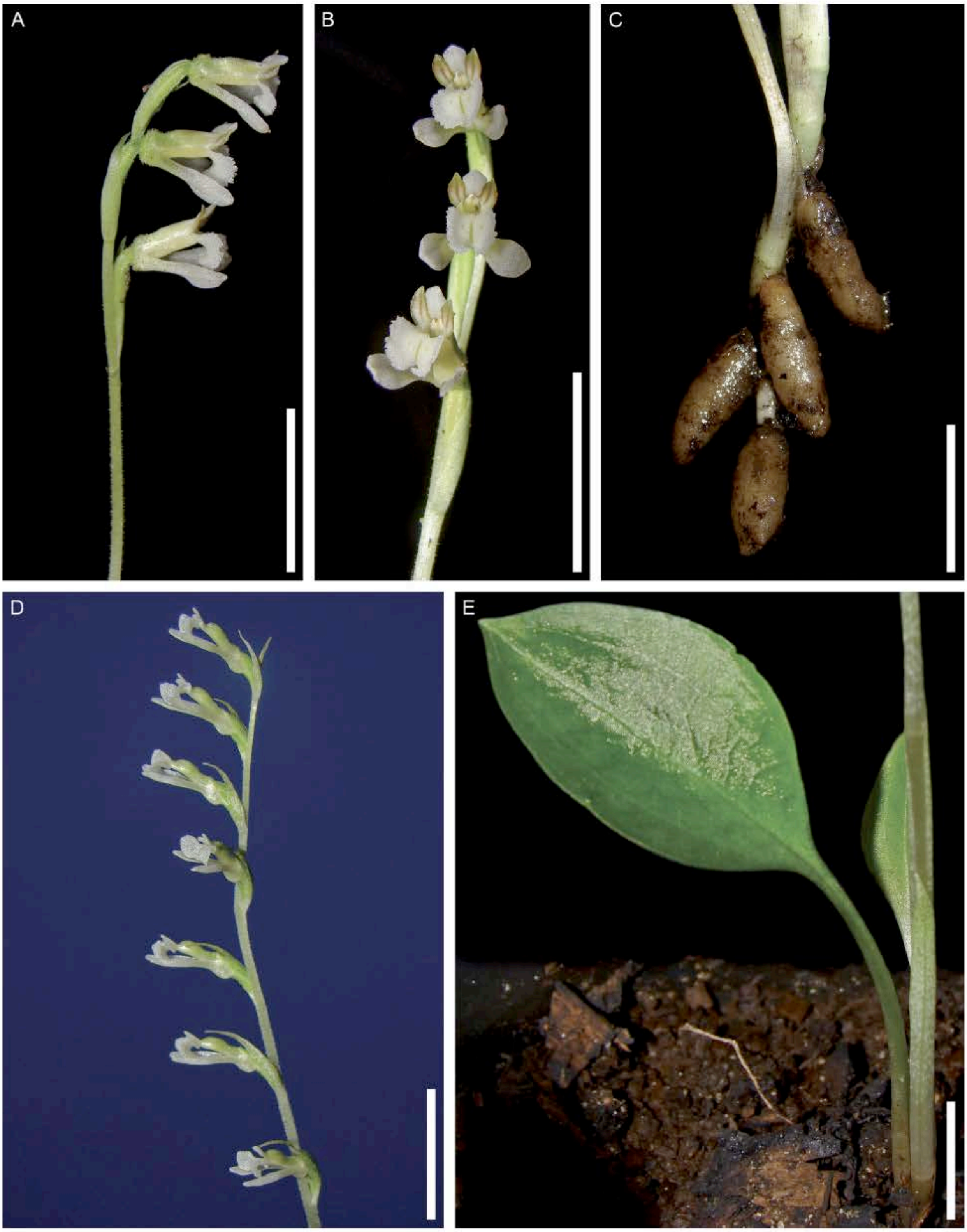

Fig. 1. Comparison of *Hapalorchis* and *Cyclopogon*. **A–C,** *Hapalorchis lineata* (*C.R. Buzatto 554 & R.B. Singer*, ICN). **A,** inflorescence in lateral view; **B,** inflorescence in frontal view; **C,** roots. **D–E,** *Cyclopogon micranthus* (*C.R. Buzatto 739 & R.B. Singer*, ICN). **D,** inflorescence in lateral view; **E,** leave. — Scale bars = 2 cm.

du Brésil" 1: tab. 57, fig. A, cited as tab. 497 (then unpublished) in Barbosa Rodrigues (1877: 183), reproduced in Sprunger & al. (1996: 110, fig. A).

This species was transferred to the genus *Hapalorchis* Schltr. (Hoehne, 1945) apparently based on the very small size of the plant, the petiolated leaves, and the tiny tubular flowers. This taxonomic placement has been accepted and followed by subsequent researchers (e.g., Pabst & Dungs, 1975; Barros & al., 2010). However, we had the opportunity to compare Barbosa Rodrigues's original illustrations to live plants collected in localities of southern (*C.R. Buzatto 739* and *734 & R.B. Singer*, ICN) and south-eastern (*R.B. Singer 9865* and *9871*, UEC) Brazil and we conclude that this species displays the characteristic fasciculate roots (as opposed to one or a few non-fascicled roots, in *Hapalorchis*) (Fig. 1) and rostellar structure of *Cyclopogon*. By examining fresh flowers of this species, we observed (Fig. 2) that they bear the inequivocal dorsal, wedge-like viscidium characteristic of *Cyclopogon* species. The viscidium in *Hapalorchis* is ventral and the rostellum, as a whole, is structured in a different way (Fig. 2). In addition, *Hapalorchis* and *Cyclopogon* are unrelated genera, belonging in different clades (Salazar & Dressler, 2011). Therefore, in our opinion, the name *Cyclopogon micranthus* needs to be reinstated.

9. *Cyclopogon procerus* Regnell & Barb. Rodr. in Barbosa Rodrigues, Gen. Sp. Orchid. 2: 280. 1882 ("*procera*") – **Lectotype (designated here):** [BRAZIL]. Minas Gerais, Caldas, "dans les capões (1) sur les rochers et sur l'humus de la Serra de Caldas croissant à l'ombre des arbres", 10 Aug. 1869, *A.F. Regnell III1724* (S no. 10-18335!; isolectotype: S no. 10-18339!) [= ***Sauroglossum elatum*** Lindl.].

The protologue of *Cyclopogon procerus* indicates the collection *A.F. Regnell III1724* as the type. We found that this collection actually consists of five specimens deposited at S. These specimens were collected on three distinct dates: 10 Aug. 1869, 12 Sep. 1874, and 31 Aug. 1878. Thus, these specimens are syntypes, and we choose the specimen collected on 10 Aug. 1869 as lectotype.

10. ***Cyclopogon taquaremboensis*** (Barb. Rodr.) Schltr. in Bot. Centralbl. 37: 394. 1920 ≡ *Stenorrhynchos taquaremboensis* Barb. Rodr. in Contr. Jard. Bot. Rio de Janeiro 3: 68. 1902 ("*Stenorhynchus*") ≡ *Beadlea taquaremboensis* (Barb. Rodr.) Garay in Bot. Mus. Leafl. 28(4): 301. 1982 – **Lectotype (designated here):** Barbosa Rodrigues's illustration in Contr. Jard. Bot. Rio de Janeiro 3: tab. XI. 1902.

11. ***Cyclopogon trilineatus*** Barb. Rodr., Gen. Sp. Orchid. 2: 284. 1882 ("*trilineata*") – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 74, cited as tab. 819 (then unpublished) in Barbosa Rodrigues (1882: 284), reproduced in Sprunger & al. (1996: 128).

12. ***Cyclopogon variegatus*** Barb. Rodr., Gen. Sp. Orchid. 2: 282. 1882 ("*variegata*") ≡ *Spiranthes variegata* (Barb. Rodr.) Cogn. in Martius, Fl. Bras. 3(4): 193. 1895 ≡ *Beadlea variegata* (Barb. Rodr.) Garay in Bot. Mus. Leafl. 28(4): 301. 1982 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 61, fig. A, cited as tab. 544 (then unpublished) in Barbosa Rodrigues (1882: 282), reproduced in Sprunger & al. (1996: 114, fig. A).

13. ***Cyclopogon venustus*** (Barb. Rodr.) Schltr. in Beih. Bot. Centralbl. 37: 396. 1920 ≡ *Stenorrhynchos venustum* Barb. Rodr. in Contr. Jard. Bot. Rio de Janeiro 1: 49. 1901 ("*Stenorhynchus venustus*") ≡ *Beadlea venusta* (Barb. Rodr.) Garay in Bot. Mus. Leafl. 28(4): 301. 1982 ≡ *Stigmatosema venusta* (Barb. Rodr.) Mytnik & al. in Orchidee (Hamburg) 57: 581. 2006 – Neotype (designated by Szlachetko & Rutkowsky 2008: 198): *A.C. Brade 15175* (K; isoneotype: RB).

When describing *Stenorrhynchos venustus*, Barbosa Rodrigues (1901) cited his son, João Barbosa Rodrigues Júnior, as the collector of the type. In the protologue, Barbosa

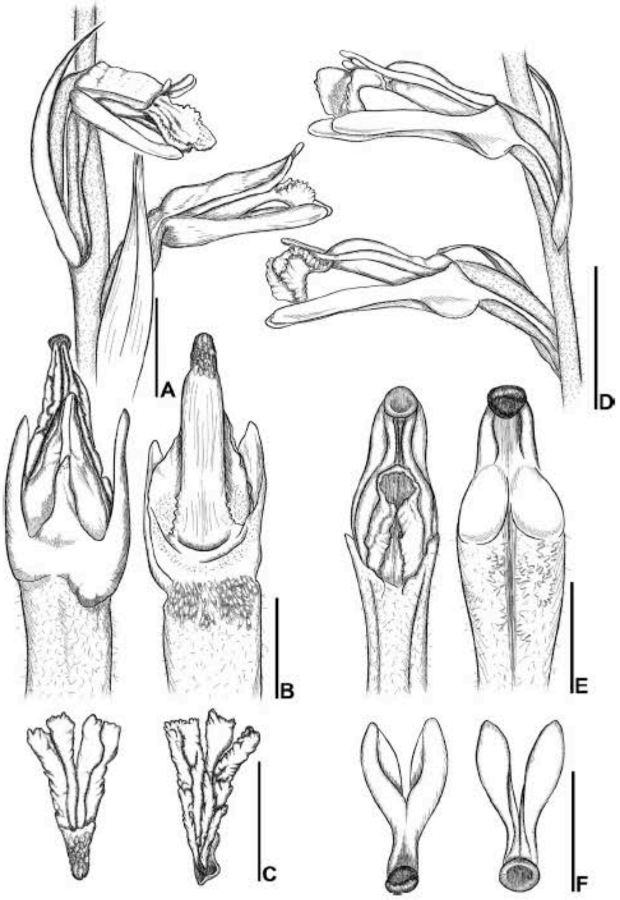

Fig. 2. Floral characters of *Hapalorchis* and *Cyclopogon*. **A–C,** *Hapalorchis* cf. *candida* (*R.B. Singer 9865*, UEC); **D–F,** *Cyclopogon micranthus* (*R.B. Singer 9871*, UEC). **A** and **D,** inflorescence; **B** and **E,** column in dorsal and ventral view; **C** and **F,** pollinaria in ventral and dorsal view. — Scale bars: A, D = 1 cm; B–C, E–F = 5 mm.

Rodrigues cited an illustration (“*Tabula VII*”) that did not appear in that publication but only later in the third volume of *Contributions du Jardin Botanique de Rio de Janeiro*, as “*Tab. X. Fig. A.*” (Barbosa Rodrigues, 1902). In the latter work, Barbosa Rodrigues (1902) cited a collection from Gavea’s Hill, near to Rio de Janeiro Botanical Garden. A thorough revision of foreign and Brazilian herbaria has not revealed any original type material of this particular Barbosa Rodrigues species, and the neotype designated by Szlachetko & Rutkowsky (2008: 198) is here accepted.

14. ***Eurystyles actinosophila*** (Barb. Rodr.) Schltr. in Repert. Spec. Nov. Regni Veg. Beih. 35: 39. 1925 ≡ *Stenorrhynchos actinosophila* Barb. Rodr., Gen. Sp. Orchid. 2: 286. 1882 (“*Stenorhynchos*”) ≡ *Spiranthes actinosophila* (Barb. Rodr.) B.D. Jacks., Index Kew. 2: 966. 1895 ≡ *Stenoptera actinosophila* (Barb. Rodr.) Cogn. in Martius, Fl. Bras. 3(4): 255. 1895 ≡ *Trachelosiphon actinosophilum* (Barb. Rodr.) Schltr. in Beih. Bot. Centralbl. 37: 424. 1920 – **Lectotype (designated here):** Barbosa Rodrigues’s original illustration at Biblioteca Barbosa Rodrigues, “Iconographie des Orchidées du Brésil” 1: tab. 56, fig. A, cited as tab. 586 (then unpublished) in Barbosa Rodrigues (1882: 286), reproduced in Sprunger & al. (1996: 109, fig. A).

15. ***Lankesterella ceracifolia*** (Barb. Rodr.) Ames in Notizbl. Bot. Gart. Berlin-Dahlem 15: 217. 1940 ≡ *Stenorrhynchos ceracifolium* Barb. Rodr., Gen. Sp. Orchid. 2: 285. 1882 (“*Stenorhynchus ceracifolia*”) ≡ *Cladobium ceracifolium* (Barb. Rodr.) Schltr. in Beih. Bot. Centralbl. 37: 432. 1920 – **Lectotype (designated here):** Barbosa Rodrigues’s original illustration at Biblioteca Barbosa Rodrigues, “Iconographie des Orchidées du Brésil” 1: tab. 56, fig. B, cited as tab. 670 (then unpublished) in Barbosa Rodrigues (1882: 285), reproduced in Sprunger & al. (1996: 109, fig. B).

= *Stenorrhynchos cornutum* Barb. Rodr. in Rodriguesia 8: 39. 1937 [“1938”] (“*Stenorhynchus cornuta*”) – **Lectotype (designated here):** Barbosa Rodrigues’s illustration in Rodriguesia 8: tab. XXXVI, fig. B in Barbosa Rodrigues (1937: 39).

Barbosa Rodrigues (1937) described *Stenorrhynchos ceracifolium* var. *cornutus* Barb. Rodr., but cited it as “*Stenorhynchus cornutus*” Barb. Rodr. in the figure legend appearing in Rodriguesia 8, tab. XXXVI, fig. B (interpreted here as an illustration with analysis as per *Code*, Art. 38.8 and 38.9). Szlachetko & Rutkowsky (2008: 180) designated the specimen *Zhotsky 1832* (G) as the lectotype of *Stenorrhynchos ceracifolium* but this proposal is untenable, since this specimen is not mentioned in the protologue (not even as supplementary material) and there is no evidence that Barbosa Rodrigues examined this particular specimen. Therefore, we propose Barbosa Rodrigues’s original illustration as the lectotype.

16. ***Mesadenella atroviridis*** (Barb. Rodr.) Garay in Bot. Mus. Leafl. 28(4): 335. 1982 ≡ *Cyclopogon atroviridis* Barb. Rodr., Gen. Sp. Orchid. 2: 284. 1882 ≡ *Spiranthes atroviridis* (Barb. Rodr.) Cogn. in Martius Fl. Bras. 3(4): 206. 1895 ≡ *Garaya atroviridis* (Barb. Rodr.) Szlach. in Polish Bot. Stud. 5: 4. 1993 – **Lectotype (designated here):** Barbosa Rodrigues’s original illustration at Biblioteca Barbosa Rodrigues, “Iconographie des Orchidées du Brésil” 1: tab. 65, fig. B, cited as tab. 824 (then unpublished) in Barbosa Rodrigues (1882: 284), reproduced in Sprunger & al. (1996: 118, fig. B).

17. ***Pelexia hypnophila*** (Barb. Rodr.) Schltr. in Beih. Bot. Centralbl. 37: 409. 1920 ≡ *Spiranthes hypnophila* Barb. Rodr., Gen. Sp. Orchid. 1: 187. 1877 ≡ *Stenorrhynchos hypnophila* Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: X. 1877 – Holotype: BRAZIL. Minas Gerais, Caldas, Pedra Branca, 5 Oct. 1877, *J. Barbosa Rodrigues 1168* (S no. 10-18833!).

18. ***Pelexia hysterantha*** (Barb. Rodr.) Schltr. in Beih. Bot. Centralbl. 37: 402. 1920 ≡ *Spiranthes hysterantha* Barb. Rodr., Gen. Sp. Orchid. 1: 188. 1877 ≡ *Stenorrhynchos hysteranthum* Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: X. 1877 (“*Stenorhynchus hysterantha*”) ≡ *Pachygenium hysteranthum* (Barb. Rodr.) Szlach. & al. in Polish Bot. J. 46: 4. 2001 – **Lectotype (designated here):** Barbosa Rodrigues’s original illustration at Biblioteca Barbosa Rodrigues, “Iconographie des Orchidées du Brésil” 1: tab. 54, cited as tab. 488 (then unpublished) in Barbosa Rodrigues (1877: 188), reproduced in Sprunger & al. (1996: 107).

19. ***Pelexia macropoda*** (Barb. Rodr.) Schltr. in Beih. Bot. Centralbl. 37: 409. 1920 ≡ *Spiranthes macropoda* Barb. Rodr., Gen. Sp. Orchid. 1: 186. 1877 ≡ *Stenorrhynchos macropodum* Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: X. 1877 (“*Stenorhynchus macropoda*”) – **Lectotype (designated here):** Barbosa Rodrigues’s original illustration at Biblioteca Barbosa Rodrigues, “Iconographie des Orchidées du Brésil” 1: tab. 50, cited as tab. 374 (then unpublished) in Barbosa Rodrigues (1877: 186), reproduced in Sprunger & al. (1996: 103).

20. *Sarcoglottis allemanii* Barb. Rodr., Gen. Sp. Orchid. 2: 288. 1882 ≡ *Spiranthes allemanii* (Barb. Rodr.) Cogn. in Martius, Fl. Bras. 3(4): 219. 1895 – **Neotype (designated here):** BRAZIL. “Hab. dans la Province du Ceará”, s.d., *F.F. Allemão & M. Cysneiros 1488* (R no. 2535!) [= ***Sarcoglottis acaulis*** (Sm.) Schltr.].

Szlachetko & Rutkowski (2008: 214) designated as lectotype a specimen of *Allemao 1487* supposedly at B but destroyed, a proposal that is clearly untenable. The specimen cited by Barbosa Rodrigues at the protologue (*Allemao 1487*) is lost and, unlike the other *Orchidaceae* described by Barbosa Rodrigues, the plate cited at the protologue (*t. 738*) was not published later (Cogniaux, 1904; Sprunger & al., 1996) and is likely lost. We have carefully checked the original paintings deposited at Rio de Janeiro and Harvard, as well as the copies of Barbosa Rodrigues’s watercolors made by Mary Thysselton Dyer, deposited at K and we couldn’t find this specific plate. Therefore, we feel confident that this particular plate is either lost or was not concluded. During our review of the orchid collections at R ("Musée de Rio de Janeiro" as cited in Barbosa Rodrigues, 1882: 288) we located a specimen labeled "*Sarcoglottis cearensis* B. Rodr." (R no. 2535) determined by Barbosa Rodrigues in April 1881, which is here proposed as neotype. This specimen is in agreement with the protologue and its provenance is also in agreement with this specified at the original description (Barbosa Rodrigues, 1882).

21. ***Sarcoglottis biflora*** (Vell.) Schltr. in Beih. Bot. Centralbl. 37: 414. 1920 ≡ *Serapias biflora* Vell., Fl. Flumin. Icon. 9: tab. 50. 1831 [Arq. Mus. Nac. Rio de Janeiro 5: 369. 1882] – **Lectotype (designated here):** Vellozo's original illustration at Manuscript Section of the Biblioteca Nacional of Rio de Janeiro (later published in Fl. Flumin. Icon. 9: tab. 50. 1831) – **Epitype (designated here):** BRAZIL. Paraná, Morungava, 26 Apr. 1911, *P.K.H. Dusén 13290* (S no. 10-18394!).

= *Spiranthes lithophila* Barb. Rodr., Gen. Sp. Orchid. 1: 190. 1877 ≡ *Sarcoglottis lithophila* Barb. Rodr., Gen. Spec. Orchid. 1 [Index]: X. 1877 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 70, cited as tab. 440 (then unpublished) in Barbosa Rodrigues (1877: 190), reproduced in Sprunger & al. (1996: 124).

The original illustration of *Serapias biflora* (Fl. Flumin. Icon. 9: tab. 50. 1831) is very rudimentary and not clearly identifiable. Therefore, we designate *Dusén 13290* (S no. 10-18394) as epitype, considering that this material is quite representative of the morphological features of this species.

22. ***Sarcoglottis fasciculata*** (Vell.) Schltr. in Beih. Bot. Centralbl. 37: 415. 1920 ≡ *Serapias fasciculata* Vell., Fl. Flumin. Icon. 9: tab. 53. 1831 [Arq. Mus. Nac. Rio de Janeiro 5: 370. 1882] ≡ *Spiranthes fasciculata* (Vell.) Cogn. in Martius, Fl. Bras. 3(4): 220. 1895 – **Lectotype (designated here):** Vellozo's original illustration at Manuscript Section of the Biblioteca Nacional of Rio de Janeiro (later published in Fl. Flumin. Icon. 9: tab. 53. 1831).

23. *Sarcoglottis multiflora* Barb. Rodr., Gen. Sp. Orchid. 2: 287. 1882 ≡ *Spiranthes multiflora* (Barb. Rodr.) B.D. Jacks., Index Kew. 2: 966. 1895 – **Lectotype (designated here):** BRAZIL. "Hab. dans les fentes des rochers du sommet de la Serra da Pedra Branca à Caldas, Minas Geraes", 18 Mar. 1849, *A.F. Regnell III1688* (S no. 09-16728!; isolectotype: P no. 362975!) [= ***Skeptrostachys balanophorostachya*** (Rchb. f. ex Warm.) Garay].

= *Stenorrhynchos canterae* Barb. Rodr. in Contr. Jard. Bot. Rio de Janeiro 1: 48. 1901 ("*Stenorhynchus*") – **Lectotype (designated here):** Barbosa Rodrigues's illustration in Contr. Jard. Bot. Rio de Janeiro 1: tab. VI, fig. C. 1901 [= ***Skeptrostachys balanophorostachya*** (Rchb. f. ex Warm.) Garay].

= *Stenorrhynchos montevidensis* Barb. Rodr. in Contr. Jard. Bot. Rio de Janeiro 4: 98. 1907 ("*Stenorhynchus*") – **Lectotype (designated here):** Barbosa Rodrigues's illustration in Contr. Jard. Bot. Rio de Janeiro 4: tab. XX, fig. A. 1907 ≡ *Skeptrostachys montevidensis* (Barb. Rodr.) Garay in Bot. Mus. Leafl. 28(4): 360. 1982, **syn. nov.** [= ***Skeptrostachys balanophorostachya*** (Rchb. f. ex Warm.) Garay].

After a careful analysis of Barbosa Rodrigues's (1901, 1907) protologue and illustration we feel compelled to consider *S. montevidensis* a synonym of *Skeptrostachys balanophorostachya*, since we see no characters that unequivocally separate the two species.

24. ***Sarcoglottis ornithocephala*** Barb. Rodr., Gen. Sp. Orchid. 2: 288. 1882 ≡ *Spiranthes ornithocephala* (Barb. Rodr.) B.D. Jacks., Index Kew. 2: 967. 1895 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 71, cited as tab. 541 (then unpublished) in Barbosa Rodrigues (1882: 288), reproduced in Sprunger & al. (1996: 125).

25. ***Sarcoglottis uliginosa*** Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: X. 1877 ≡ *Spiranthes uliginosa* Barb. Rodr., Gen. Sp. Orchid. 1: 190. 1877 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 69, cited as tab. 411 (then unpublished) in Barbosa Rodrigues (1877: 190), reproduced in Sprunger & al. (1996: 123).

Szlachetko & Rutkowsky (2008: 219) indicated the specimen *Regnell III1199* (UPS) as the lectotype and *Weddell 1160* (P, K-Lindl.) as the paratypes. However, this proposal is untenable because this specimen is not mentioned at the protologue and there is no evidence that this specimen was examined by Barbosa Rodrigues.

26. ***Sarcoglottis umbrosa*** (Barb. Rodr.) Schltr. in Beih. Bot. Centralbl. 37: 422. 1920 ≡ *Spiranthes umbrosa* Barb. Rodr., Gen. Sp. Orchid. 1: 188. 1877 ≡ *Stenorrhynchos umbrosa* Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: X. 1877 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 72, cited as tab. 474 (then unpublished) in Barbosa Rodrigues (1877: 188), reproduced in Sprunger & al. (1996: 126).

27. ***Sarcoglottis ventricosa*** (Vell.) Hoehne in Arq. Bot. Estado São Paulo 2: 146. 1952 ≡ *Serapias ventricosa* Vell., Fl. Flumin. Icon. 9: tab. 47. 1831 [Arq. Mus. Nac. Rio de Janeiro 5: 368. 1882] ≡ *Orchis ventricosa* (Vell.) Steud., Nomencl. Bot. 2: 225. 1840 – **Lectotype (designated here):** Vellozo's original illustration at Manuscript Section of the Biblioteca Nacional of Rio de Janeiro (later published in Fl. Flumin. Icon. 9: tab. 47. 1831).

Although lacking detail, Vellozo's illustration (1831: tab. 47) allows the unequivocal identification of this taxon.

28. *Serapias coccinea* Vell., Fl. Flumin. Icon. 9: tab. 48. 1831 [Arq. Mus. Nac. Rio de Janeiro 5: 369. 1882] ≡ *Stenorrhynchos coccineum* (Vell.) Hoehne in Arq. Bot. Estado

São Paulo 2: 146. 1952 – **Lectotype (designated here):** Vellozo's original illustration at Manuscript Section of the Biblioteca Nacional of Rio de Janeiro (later published in Fl. Flumin. Icon. 9: tab. 48. 1831) [= ***Sacoila lanceolata*** (Aubl.) Garay].

= *Serapias tomentosa* Vell., Fl. Flumin. Icon. 9: tab. 51. 1831 [Arq. Mus. Nac. Rio de Janeiro 5: 369. 1882] ≡ *Pelexia tomentosa* (Vell.) Schltr. in Beih. Bot. Centralbl. 37: 406. 1920 – **Lectotype (designated here):** Vellozo's original illustration at Manuscript Section of the Biblioteca Nacional of Rio de Janeiro (later published in Fl. Flumin. Icon. 9: tab. 51. 1831) [= ***Sacoila lanceolata*** (Aubl.) Garay].

29. ***Skeptrostachys arechavaletanii*** (Barb. Rodr.) Garay in Bot. Mus. Leafl. 28(4): 359. 1982 ≡ *Stenorrhynchos arechavaletanii* Barb. Rodr. in Contr. Jard. Bot. Rio de Janeiro 4: 99. 1907 ("*Stenorhynchus*") – **Lectotype (designated here):** Barbosa Rodrigues's illustration in Contr. Jard. Bot. de Rio de Janeiro 4: tab. XXI. 1907.

Szlachetko & Rutkowsky's (2008: 162) indicated the specimen *Warming s.n.* (W-Reichenbach Herbarium) as the lectotype. However, this proposal is untenable because this specimen is not mentioned in the protologue and there is no evidence that this specimen was examined by Barbosa Rodrigues. The specimen collected by Arechavaleta could not be located, and here we designate Barbosa Rodrigues's original illustration as lectotype.

30. *Spiranthes albescens* Barb. Rodr., Gen. Sp. Orchid. 1: 186. 1877 ≡ *Stenorrhynchos albescens* Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: X. 1877 ("*albescentis*") – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 52, cited as tab. 375 (then unpublished) in Barbosa Rodrigues (1877: 186), reproduced in Sprunger & al. (1996: 105) [= ***Pteroglossa macrantha*** (Rchb. f.) Schltr.].

31. *Spiranthes epiphyta* Barb. Rodr., Gen. Sp. Orchid. 1: 189. 1877 ≡ *Stenorrhynchos epiphyta* Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: X. 1877 ≡ *Cladobium epiphytum* (Barb. Rodr.) Schltr. in Beih. Bot. Centralbl. 37: 432. 1920 ≡ *Lankesterella epiphyta* (Barb. Rodr.) Mansf. in Notizbl. Bot. Gart. Berlin-Dahlem 15: 217. 1940 ≡ *Lankesterella epiphyta* (Barb. Rodr.) Hoehne in Arq. Bot. Estado São Paulo 1: 131. 1944 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 55, cited as tab. 407 (then unpublished) in Barbosa Rodrigues (1877: 189), reproduced in Sprunger & al. (1996: 108) [= ***Lankesterella caespitosa*** (Lindl.) Hoehne].

Here, we treat *Spiranthes epiphyta* as a synonym of *Lankesterella caespitosa*, following Garay (1982).

32. *Spiranthes pubescens* Barb. Rodr., Gen. Sp. Orchid. 1: 185. 1877 ≡ *Cyclopogon pubescens* Barb. Rodr., Gen. Sp. Orchid. 1 [Index]: III. 1877 ≡ *Lyroglossa pubescens* (Barb. Rodr.) Schltr. in Beih. Bot. Centralbl. 37: 449. 1920 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 60, cited as tab. 508 (then unpublished) in Barbosa Rodrigues (1877: 185), reproduced in Sprunger & al. (1996: 113) [= ***Cyclopogon bicolor*** (Ker Gawl.) Schltr.].

33. *Stenorrhynchos icmadophila* Barb. Rodr., Gen. Sp. Orchid. 2: 286, [Index]: XV. 1882 ("*Stenorhynchus*" on p. 286) ≡ *Cyclopogon icmadophilus* (Barb. Rodr.) Schltr. in Beih. Bot. Centralbl. 37: 389. 1920 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 61, fig. B, cited as tab. 748 (then unpublished) in Barbosa Rodrigues (1882: 286), reproduced in Sprunger & al. (1996: 114, fig. B) [= ***Brachystele dilatata*** (Lindl.) Schltr.].

34. ***Veyretia cogniauxiana*** (Barb. Rodr. ex Cogn.) Szlach. in Fragm. Florist. Geobot. 3: 116. 1995 ≡ *Spiranthes cogniauxiana* Barb. Rodr. ex Cogn. in Martius, Fl. Bras. 3(4): 214. 1895 ≡ *Sarcoglottis cogniauxiana* (Barb. Rodr. ex Cogn.) Schltr. in Beih. Bot. Centralbl. 37: 415. 1920 – **Lectotype (designated here):** Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, "Iconographie des Orchidées du Brésil" 1: tab. 67, reproduced in Sprunger & al. (1996: 121).

■ NOMEM NUDUM

1. "*Spiranthes macuconensis* Barb. Rodr.", "Iconographie des Orchidées du Brésil" 1: tab. 66. 1877.

Note. – Barbosa Rodrigues illustrated this species but he did not formally describe it in any of his publications. Cogniaux annotated the original drawing as *Pelexia setacea* Lindl. (Barbosa Rodrigues, Icon. Orch. Brésil 1: tab. 66, lower left corner; see Sprunger & al. 1996: 119), which is currently referable to *Eltroplectris calcarata* (Sw.) Garay & H.R. Sweet. Since this watercolor is not an illustration with analysis (as established by the *Code*, Art. 38.9), we consider "*Spiranthes macuconensis*" a nomem nudum.

■ EXCLUDED NAMES

1. *Serapias nitida* Vell., Fl. Flumin. Icon. 9: tab. 52. 1831 [Arq. Mus. Nac. Rio de Janeiro 5: 370. 1882] ≡ *Sauroglossum nitidum* (Vell.) Schltr. in Beih. Bot. Centralbl. 37: 376. 1920 – **Lectotype (designated here):** Vellozo's original illustration at Manuscript Section of the Biblioteca Nacional of Rio de Janeiro (later published in Fl. Flumin. Icon. 9: tab. 52. 1831).

Many Brazilian authors (Hoehne, 1952; Pabst & Dungs, 1975, 1977) have treated this taxon as the basionym of *Sauroglossum nitidum*, a name that, in turn, has very often been

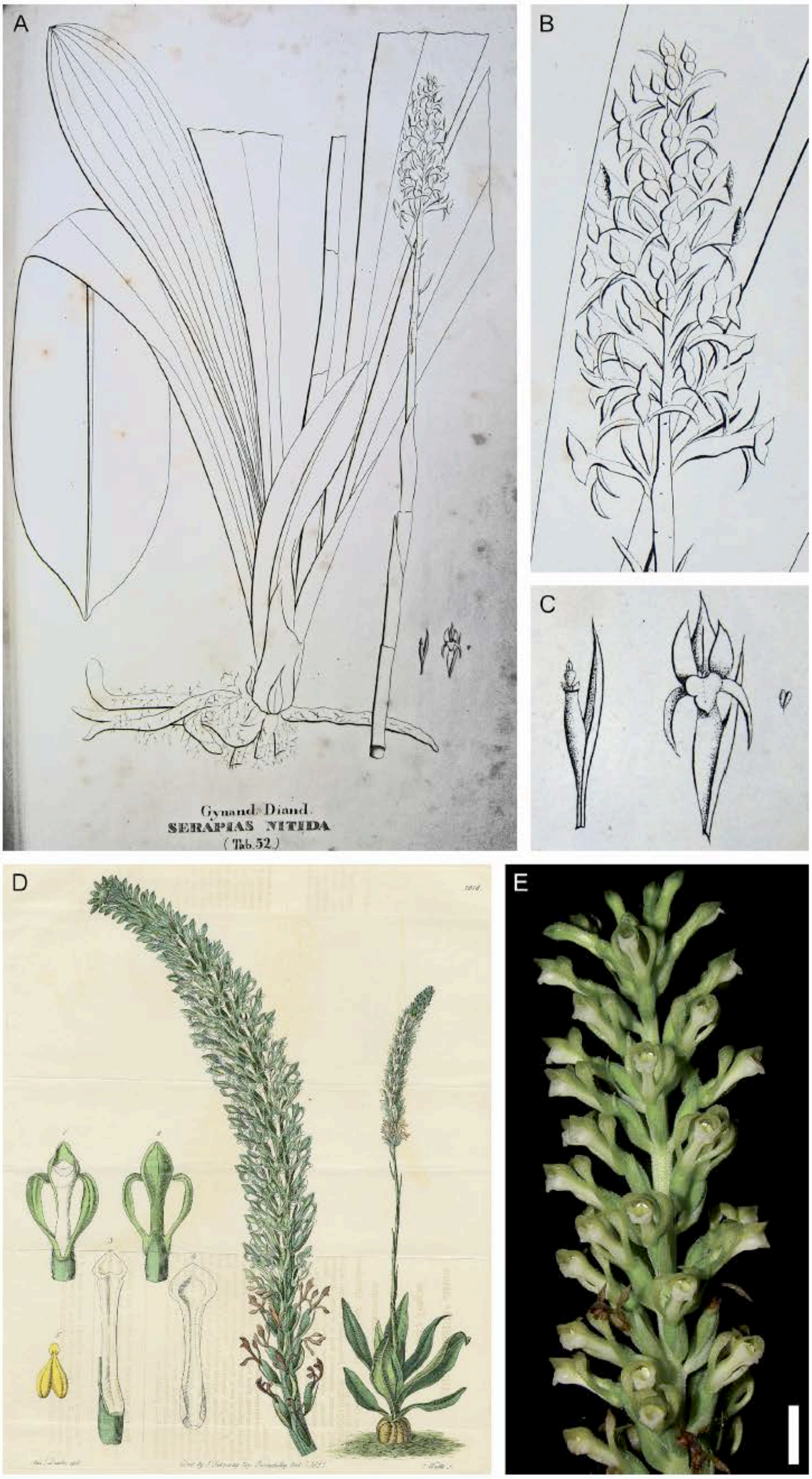

Fig. 3. Original plates of Vellozo's *Serapias nitida* and Lindley's *Sauroglossum elatum*. **A–C,** photo of *Serapias nitida* from Biblioteca Barbosa Rodrigues, Jardim Botânico do Rio de Janeiro, Brazil: **A,** line drawings of habit and floral characters; **B,** detail of inflorescence; **C,** detail of floral characters. **D–E,** *Sauroglossum elatum*: **D,** drawing of type of *S. elatum* from AMES 104122, Harvard University, U.S.A.; **E,** detail of inflorescence. — Scale bar = 1 cm.

Table 1. Binomials typificated of *Goodyerinae* and *Spiranthinae*, published by Vellozo and Barbosa Rodrigues. Names in bold are the currently acepted names. Barb. Rodr., Barbosa Rodrigues; EP, epitype; HT, holotype; LT, lectotype; NT, neotype; Vell., Vellozo.

SUBTRIBE/Binomial	Reference to typification
GOODYERINAE Klotzsch	
1. *Physurus aratanhensis* Barb. Rodr. ≡ ***Aspidogyne aratanhensis*** (Barb. Rodr.) Baptista	HT: *F.F. Allemão 1486* (P no. 331259!)
2. *Ophrys argentea* Vell. ≡ ***Aspidogyne argentea*** (Vell.) Garay	LT: Fl. Flumin. Icon. 9: tab. 61. 1831
3. *Physurus bicolor* Barb. Rodr. ≡ ***Aspidogyne bicolor*** (Barb. Rodr.) Garay	LT: "Iconographie des Orchidées du Brésil" 2: tab. 80
4. *Physurus commelinoides* Barb. Rodr. ≡ ***Aspidogyne commelinoides*** (Barb. Rodr.) Garay	LT: "Iconographie des Orchidées du Brésil" 2: tab. 78
5. *Physurus metallescens* Barb. Rodr. ≡ ***Aspidogyne metallescens*** (Barb. Rodr.) Garay	LT: "Iconographie des Orchidées du Brésil" 2: tab. 79
6. *Physurus lacteolus* Barb. Rodr. = ***Aspidogyne hylibates*** (Rchb. f.) Garay	LT: "Iconographie des Orchidées du Brésil" 2: tab. 79
SPIRANTHINAE Lindl.	
1. *Cyclopogon albopunctatus* Barb. Rodr. = ***Mesadenella cuspidata*** (Lindl.) Garay	LT: "Iconographie des Orchidées du Brésil" 1: tab. 51
2. *Spiranthes argyrifolia* Barb. Rodr. ≡ ***Cyclopogon argyrifolius*** Barb. Rodr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 75
3. *Spiranthes calophylla* Barb. Rodr. ≡ ***Cyclopogon calophyllus*** Barb. Rodr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 76
4. ***Cyclopogon cearensis*** Barb. Rodr.	HT: *F.F. Allemão & M. Cysneiros 1485* (R no. 35618!)
5. *Spiranthes chloroleuca* Barb. Rodr. ≡ ***Cyclopogon chloroleucus*** (Barb. Rodr.) Schltr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 59
Spiranthes chloroleuca var. *fontinalis* Barb. Rodr. ≡ ***Cyclopogon chloroleucus*** (Barb. Rodr.) Schltr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 64
Spiranthes chloroleuca var. *longipetiolata* Barb. Rodr. ≡ ***Cyclopogon chloroleucus*** (Barb. Rodr.) Schltr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 73, fig. A
6. *Serapias congesta* Vell. ≡ ***Cyclopogon congestus*** (Vell.) Hoehne	LT: Fl. Flumin. Icon. 9: tab. 54. 1831
Cyclopogon alpestris Barb. Rodr. = ***Cyclopogon congestus*** (Vell.) Hoehne	LT: "Iconographie des Orchidées du Brésil" 1: tab. 62, fig. A
Spiranthes alpestris Barb. Rodr. = ***Cyclopogon congestus*** (Vell.) Hoehne	LT: "Iconographie des Orchidées du Brésil" 1: tab. 62, fig. A
Cyclopogon alpestris var. *bidentatus* Barb. Rodr. = ***Cyclopogon congestus*** (Vell.) Hoehne	LT: *A.F. Regnell III1197* (S no. 10-18638!; isolectotypes: S no. 10-18643!, P no. 340963!, P no. 340964!)
7. *Spiranthes longibracteata* Barb. Rodr. ≡ ***Cyclopogon longibracteatus*** (Barb. Rodr.) Schltr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 58
8. *Spiranthes micrantha* Barb. Rodr. ≡ ***Cyclopogon micranthus*** Barb. Rodr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 57, fig. A
9. *Cyclopogon procerus* Regnell & Barb. Rodr. = ***Sauroglossum elatum*** Lindl.	LT: *A.F. Regnell III1724* (S no. 10-18335!; isolectotype, S no. 10-18339!)
10. *Stenorrhynchos taquaremboensis* Barb. Rodr. ≡ ***Cyclopogon taquaremboensis*** (Barb. Rodr.) Schltr.	LT: Contr. Jard. Bot. Rio de Janeiro: 3, tab. XI. 1903: 68
11. ***Cyclopogon trilineatus*** Barb. Rodr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 74
12. ***Cyclopogon variegatus*** Barb. Rodr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 61, fig. A
13. *Stenorrhynchos venustum* Barb. Rodr. ≡ ***Cyclopogon venustus*** (Barb. Rodr.) Schltr.	NT, designated by Szlachetko & Rutkowsky (2008: 198): *A.C. Brade 15175* (K; isoneotype: RB)

Table 1. Continued.

SUBTRIBE/Binomial	Reference to typification
14. *Stenorrhynchos actinosophila* Barb. Rodr. ≡ ***Eurystyles actinosophila*** (Barb. Rodr.) Schltr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 56, fig. A
15. *Stenorrhynchos ceracifolium* Barb. Rodr. ≡ ***Lankesterella ceracifolia*** (Barb. Rodr.) Ames	LT: "Iconographie des Orchidées du Brésil" 1: tab. 56, fig. B
Stenorrhynchos cornutum Barb. Rodr. = ***Lankesterella ceracifolia*** (Barb. Rodr.) Ames	LT: Rodriguesia 8: tab. XXXVI, fig. B. 1937
16. *Cyclopogon atroviridis* Barb. Rodr. ≡ ***Mesadenella atroviridis*** (Barb. Rodr.) Garay	LT: "Iconographie des Orchidées du Brésil" 1: tab. 65, fig. B
17. *Spiranthes hypnophila* Barb. Rodr. ≡ ***Pelexia hypnophila*** (Barb. Rodr.) Schltr.	HT: *J. Barbosa Rodrigues 1168* (S no. 10-18833!)
18. *Spiranthes hysterantha* Barb. Rodr. ≡ ***Pelexia hysterantha*** (Barb. Rodr.) Schltr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 54
19. *Spiranthes macropoda* Barb. Rodr. ≡ ***Pelexia macropoda*** (Barb. Rodr.) Schltr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 50
20. *Sarcoglottis allemanii* Barb. Rodr. = ***Sarcoglottis acaulis*** (Sm.) Schltr.	NT: *F.F. Allemão & M. Cysneiros 1488* (R no. 2535!)
21. *Serapias biflora* Vell. ≡ ***Sarcoglottis biflora*** (Vell.) Schltr.	LT: Fl. Flumin. Icon. 9: tab. 50. 1831 EP: *P.K.H. Dusén 13290* (S no. 10-18394!)
Spiranthes lithophila Barb. Rodr. = ***Sarcoglottis biflora*** (Vell.) Schltr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 70
22. *Serapias fasciculata* Vell. ≡ ***Sarcoglottis fasciculata*** (Vell.) Schltr.	LT: Fl. Flumin. Icon. 9: tab. 53. 1831
23. *Sarcoglottis multiflora* Barb. Rodr. = ***Skeptrostachys balanophorostachya*** (Rchb. f. ex Warm.) Garay	LT: *A.F. Regnell III1688* (S no. 09-16728!; isolectotype, P no. 362975!)
Stenorrhynchos canterae Barb. Rodr. = ***Skeptrostachys balanophorostachya*** (Rchb. f. ex Warm.) Garay	LT: Contr. Jard. Bot. Rio de Janeiro 1: tab. VI, fig. C. 1901
Stenorrhynchos montevidensis Barb. Rodr. = ***Skeptrostachys balanophorostachya*** (Rchb. f. ex Warm.) Garay	LT: Contr. Jard. Bot. Rio de Janeiro 4: tab. XX, fig. A. 1907
24. ***Sarcoglottis ornithocephala*** Barb. Rodr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 71
25. *Spiranthes uliginosa* Barb. Rodr. ≡ ***Sarcoglottis uliginosa*** Barb. Rodr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 69
26. *Spiranthes umbrosa* Barb. Rodr. ≡ ***Sarcoglottis umbrosa*** (Barb. Rodr.) Schltr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 72
27. *Serapias ventricosa* Vell. ≡ ***Sarcoglottis ventricosa*** (Vell.) Hoehne	LT: Fl. Flumin. Icon. 9: tab. 47. 1831
28. *Serapias coccinea* Vell. = ***Sacoila lanceolata*** (Aubl.) Garay	LT: Fl. Flumin. Icon. 9: tab. 48. 1831
Serapias tomentosa Vell. = ***Sacoila lanceolata*** (Aubl.) Garay	LT: Fl. Flumin. Icon. 9: tab. 51. 1831
29. *Stenorrhynchos arechavaletanii* Barb. Rodr. ≡ ***Skeptrostachys arechavaletanii*** (Barb. Rodr.) Garay	LT: Contr. Jard. Bot. Rio de Janeiro 4: tab. XXI. 1907
30. *Spiranthes albescens* Barb. Rodr. = ***Pteroglossa macrantha*** (Rchb. f.) Schltr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 52
31. *Spiranthes epiphyta* Barb. Rodr. = ***Lankesterella caespitosa*** (Lindl.) Hoehne	LT: "Iconographie des Orchidées du Brésil" 1: tab. 55
32. *Spiranthes pubescens* Barb. Rodr. = ***Cyclopogon bicolor*** (Ker Gawl.) Schltr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 60
33. *Stenorrhynchos icmadophila* Barb. Rodr. = ***Brachystele dilatata*** (Lindl.) Schltr.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 61, fig. B
34. *Spiranthes cogniauxiana* Barb. Rodr. ex Cogn. ≡ ***Veyretia cogniauxiana*** (Barb. Rodr. ex Cogn.) Szlach.	LT: "Iconographie des Orchidées du Brésil" 1: tab. 67

misapplied to *Sauroglossum elatum* Lindl. However, a revision of Vellozo's protologue and illustration (Mello-Netto, 1882) reveals that this taxon cannot be unequivocally assigned to *Sauroglossum*. Whereas Vellozo's illustration shows lanceolate leaves overall similar to these of *Sauroglossum*, the flowers show acute floral segments wich are absent in this genus. In addition, Vellozo's illustration shows flowers with a clearly pointed, trilobed labellum (obtuse and cochleate in *Sauroglossum*). Overall, the depicted flowers (Fig. 3A–C) are more similar to those of *Pteroglossa* (e.g., *P. glazoviana*) or *Eltroplectris*. By no means can the flowers illustrated by Vellozo be considered to belong to a species of *Sauroglossum* (Fig. 3). Therefore, we currently propose the exclusion of applying this name to any known taxon.

2. *Serapias polyaden* Vell., Fl. Flumin. Icon. 9: tab. 56. 1831 [Arq. Mus. Nac. Rio de Janeiro 5: 371. 1882] ≡ *Stigmatosema polyaden* (Vell.) Garay in Bot. Mus. Leafl. 28(4): 377. 1982 ≡ *Cyclopogon polyaden* (Vell.) F.S. Rocha & Waechter in Acta Bot. Brasil. 20: 78. 2006 – **Lectotype (designated here):** Vellozo's original illustration at Manuscript Section of the Biblioteca Nacional of Rio de Janeiro (later published in Fl. Flumin. Icon. 9: tab. 56. 1831).

Cyclopogon polyaden has been considered by many to be the correct name for the species also known as *C. chloroleucus* Barb. Rodr. However, a thorough examination of Vellozo's protologue and corresponding illustration reveals that this taxon cannot be confidently considered synonymous with *C. chloroleucus*. The illustration of the flowers in the original publication of *Serapias polyaden*, the basionym of *C. polyaden*, is extremely rudimentary and, whereas the leaves are shown to be variegated (as in *C. chloroleucus*), other *Cyclopogon* species (e.g., *C. calophyllus*) display similar leaf shapes and color patterns, making the positive identification of *Serapias polyaden* doubtful. Therefore, we currently propose the exclusion of applying this name to any known taxon.

■ ACKNOWLEDGEMENTS

We thank A.L.V. Toscano de Brito (The Marie Selby Botanical Gardens) for useful suggestions to a previous version of the manuscript; Dr. Gerry Moore and two anonymous reviewers for constructive comments; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq projects 563556/2010-2 and 470353/2011-2). CRB thanks Coordenadoria de Aperfeiçoamento do Pessoal de Nível Superior (CAPES project 1099/2010) for his scholarship and Pró-Reitoria de Pós-graduação (PROPG/UFRGS Edital 001/2011) for financial support. GAR-G acknowledges the generous financial support of the Orchid Society of Arizona and the Massachusetts Orchid Society.

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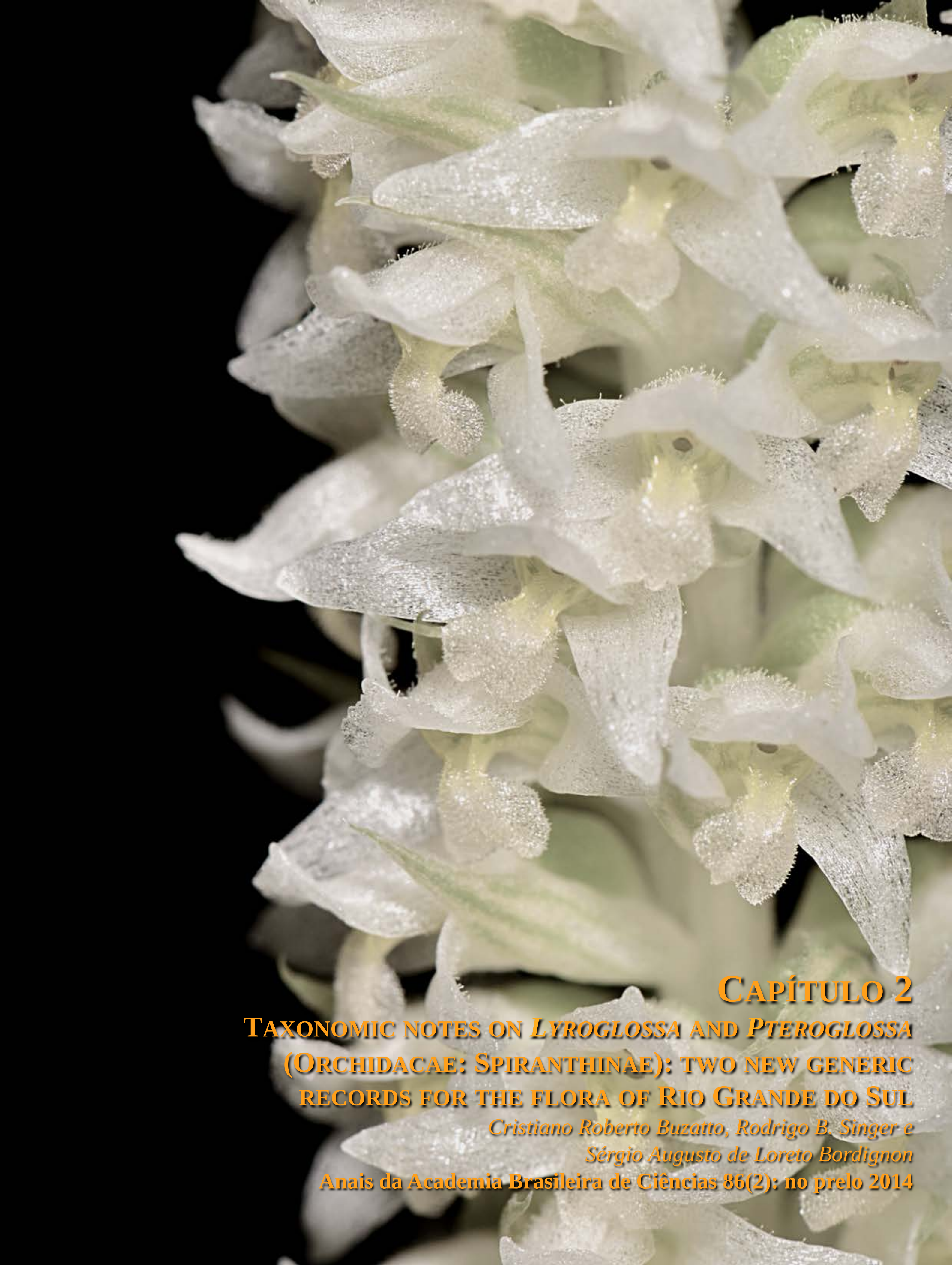

CAPÍTULO 2

TAXONOMIC NOTES ON *LYROGLOSSA* AND *PTEROGLOSSA* (ORCHIDACAE: SPIRANTHINAE): TWO NEW GENERIC RECORDS FOR THE FLORA OF RIO GRANDE DO SUL

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Taxonomic notes on *Lyroglossa* and *Pteroglossa* (Orchidaceae: Spiranthinae): two new generic records for the flora of Rio Grande do Sul

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ABSTRACT

Lyroglossa and *Pteroglossa* (Orchidaceae: Spiranthinae) are first recorded for Rio Grande do Sul. Descriptions, illustrations, taxonomic notes and distribution are presented. We also provide an artificial key to distinguish the genera of the "*Stenorrhynchos* Clade" reported so far in Rio Grande do Sul.

Key words: Brazil, "*Stenorrhynchos* Clade", taxonomy, terrestrial orchids.

INTRODUCTION

Spiranthinae Lindl. ex Meisner comprises about 42 genera and 470 species (Chase et al. 2003, Salazar 2003) of predominantly terrestrial orchids (Salazar et al. 2003). This subtribe has two main biodiversity centers, one in Mexico and Central America and the other in Southern and Southeastern Brazil (Dressler 1993, Balogh 1982).

Spiranthinae has been supported as a monophyletic group in several phylogenetic studies (Salazar 2003, Figueroa et al. 2008, Salazar et al. 2003, 2009, 2011). All these studies support the existence of four clades: "*Stenorrhynchos* Clade", "*Pelexia* Clade", "*Eurystyles* + *Lankesterella* Clade" and "*Spiranthes* Clade".

During fieldwork and the revision of some herbaria we verified the occurrence of two Spiranthinae genera: *Lyroglossa* Schltr. and *Pteroglossa* Schltr., currently included within the so-called "*Stenorrhynchos* Clade", *sensu* Salazar et al. (2003). To date, these taxa have not been formally cited for the orchid flora of Rio Grande do Sul (Rambo 1965, Pabst and Dungs 1975). The purpose of the present contribution is thus to present descriptions, diagnostic features, illustrations, geographical and taxonomic notes for these taxa. In addition, an artificial key of the genera currently placed into the "*Stenorrhynchos* Clade" that are native to Rio Grande do Sul Brazilian State is provided. This paper is part of a series of taxonomic studies on the Orchidaceae native to Rio Grande do Sul (Buzatto et al. 2012, 2014).

MATERIALS AND METHODS

Protologues were examined, as well as the herbaria that hold the type collections (AMES, B, BHCB,

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BM, CEN, CTES, F, G, GH, HAS, HB, HBG, HEPH, HUEFS, IBGE, ICN, INPA, IPA, K, K-L, M, MBM, NY, P, PACA, PEL, R, RB, S, SP, UB, UEC, VIC and W - acronyms according to Thiers 2009) and relevant literature to Orchidaceae (Cogniaux 1895, 1906, Dressler 1993, Salazar 2003). This paper follows the general orchid classification proposed by Chase et al. (2003). Orchid morphology terms were defined as per Dressler (1993) and abbreviations for authorities for plant names follow Brummitt and Powell (1992).

TAXONOMIC TREATMENT

1. *Lyroglossa grisebachii* (Cogn.) Schltr., Anexos Mem. Inst. Butantan, Secc. Bot. 1(2): 27-28. 1921 ≡ *Spiranthes grisebachii* Cogn., Fl. Brasil. 3(4): 207. 1893-1906. Figures 1 and 3E–F.

Type: FRENCH GUIANA. *sine loc.*, 1840, *F.M.R. Leprieur s.n.*, no herbarium cited; lectotype designated by Szlachetko and Rutkowski (2008): G 168827, isolectotypes: P 345573; AMES (photo as G 168827).

Herb to 17.5 cm high, including the inflorescence. *Roots* fleshy, cylindrical, often stipitate, lanuginose. *Leaves* ca. 2.3 × 0.7 cm, 1–2, forming a basal rosette, usually absent during anthesis sometimes withered, sessile, acute, coriaceous, margin slightly translucent, bright to olive green, elliptic-lanceolate, gradually attenuate. *Inflorescence* ca. 3.5 cm long, 6 to 7-flowered, pubescent, scape terete, partially covered by 5 sheathing, acute bracts; raceme congested, most of them opened simultaneously; floral bracts ca. 2.5 cm long, prominent, green, glabrous, loosely concave, ovate, acuminate. *Flowers* resupinate, fleshy, whitish-green. *Sepals* densely pubescent outside. *Dorsal sepal* ca. 6.1 × 2.3 mm, slightly spreading, adherent to the lateral sepals on its proximal part, densely pubescent externally, lanceolate, recurved at the acute apex, the margins entire. *Lateral sepals* ca. 6.4 × 2.8 mm, slightly to strongly patent, shortly connate at base forming a small, conical, rounded spur of ca. 5 mm long, densely pubescent outside, ovate-lanceolate, recurved at apex, acute, margins entire. *Petals* ca. 5.8 × 2.2 mm, glabrous, with 5 conspicuous green veins, ovate, acute, margins entire. *Labellum* ca. 7.2 × 3.5 cm, three-lobed, blade pubescent outside, often papillose near the base, adnate at base with the lateral sepals in the spur, long-clawed and narrowly channeled below the middle; apical lobe deflexed, obtuse to retuse. *Column* ca. 7.2 x 3.1 mm, ca. 2.2 mm height, semi-terete, stout, pubescent, the base extended into a long column-foot, the membranaceous clinandrium margins are rounded and enclose the basal portion of the anther; the anther is thick, massive, ovate, acuminate, with filament adnate; pollinarium ca. 2.6 × 1.1 mm, 0.7 mm height, formed by 2 deeply bipartite, granular pollinia, narrowly clavate, with ventral, apical, oval, grey viscidium; rostellum remnant ca. 0.8 mm long, narrowly triangular, stiff, obscurely tridentate at the portion originally covered by the viscidium; stigma bilobed toward the base. *Ovary* ca. 7.2 mm long, 3.1 mm diameter near the apex, densely pubescent up to pedicel. *Capsule* ellipsoid. *Seeds* minute and fusiform.

Notes: *Lyroglossa grisebachii* is the type-species of the genus (Salazar 2003). *Lyroglossa* shares some floral features with *Pteroglossa*, such as the short, ventrally channelled column, narrowly triangular rostellum, concave anther and marginal, completely adnate nectar glands (Salazar 2003). Based on morphological features, Salazar (2003) tentatively placed it in the so-called "*Stenorrhynchos* Clade". However, its relationships are still not sustained by a molecular phylogeny (Chase et al. 2003, Salazar 2003).

Distribution: *Lyroglossa grisebachii* is currently known in the Atlantic Rain Forest and Cerrado Biomes, in Minas Gerais, Paraná, São Paulo, Goiás, Distrito Federal and Mato Grosso.

In Rio Grande do Sul, this species dwells in wet grassy formations ("*campos*") surrounding

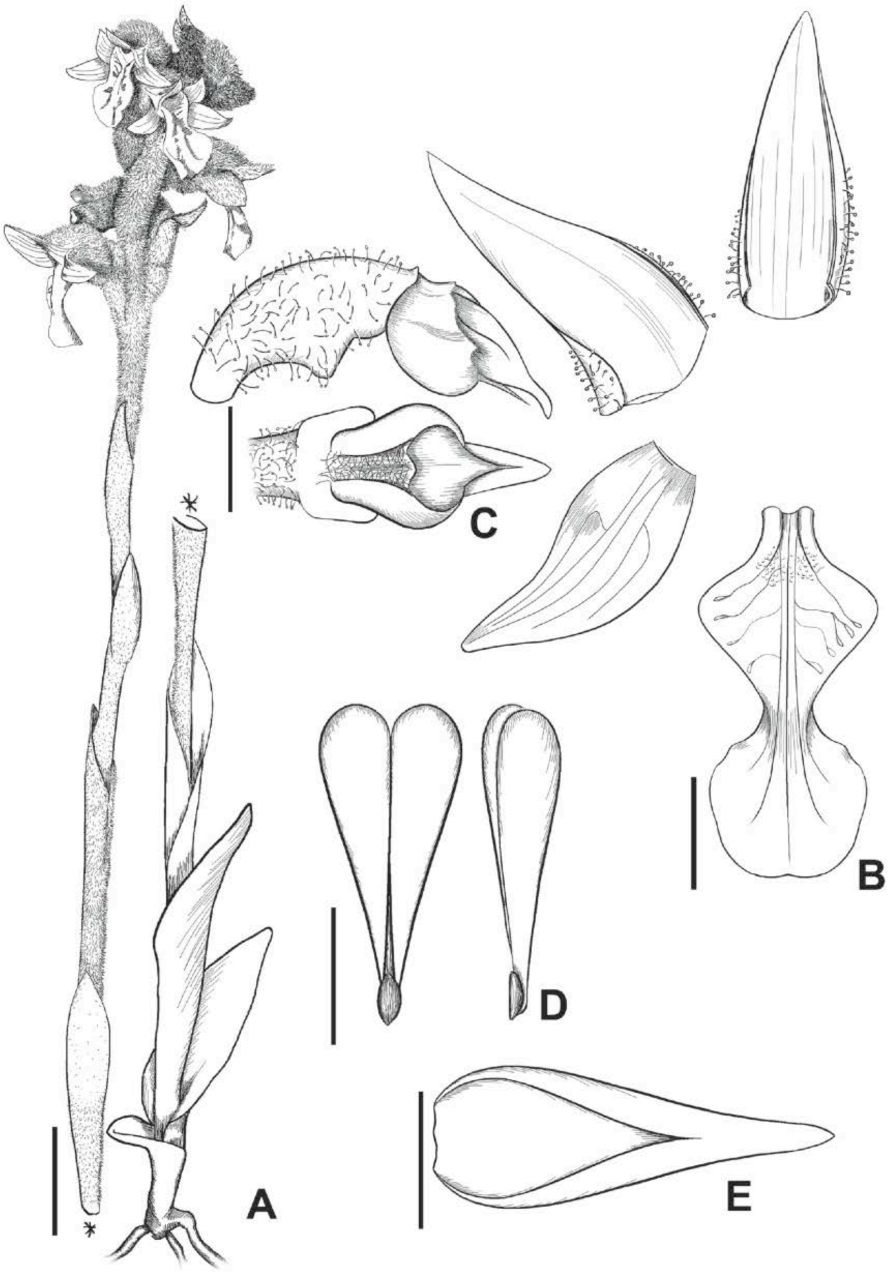


Figure 1 - *Lyroglossa grisebachii* (Cogn.) Schltr.; **A.** Habit; **B.** Floral diagram; **C.** Column in lateral and ventral view; **D.** Pollinarium in ventral and lateral view; **E.** Anther in dorsal view. Scale bars: **A** and **C** = 2 cm; B = 2 mm; **D** and **E** = 1 mm.

Atlantic forests. Additionally, *L. grisebachii* occurs in grasslands within the Pampa Biome, growing in slightly humid soils.

Batista et al. (2012), mentioned the presence of *L. grisebachii* in Rio Grande do Sul without providing a voucher. We herein formally record *L. grisebachii* for the State of Rio Grande do Sul, therefore extending the austral limit of this genus.

Specimens examined: BRAZIL. Rio Grande do Sul: Cachoeira do Sul, December 1986, fl., *M. Sobral et al. 5367* (ICN); São José dos Ausentes, 23 January 2010, fl., *C.R. Buzatto 606 & R.B. Singer* (ICN); 2 February 2008, fl., *J.A.N. Batista 2492* (BHCB).

Additional specimens examined: BRAZIL. Distrito Federal: Brasília, 14 December 1989, fl., *J.A.N. Batista 37* (HUEFS). Goiás: Alto Paraíso de Goiás, 9 February 2004, fl., *C. van den Berg 1226* (HUEFS); Cavalcante, 1 January 1999, fl., *J.A.N. Batista 824* (HUFU, UEC); Pirenópolis, 8 December 1987, fl., *L.C. Bernacci 19970* (UEC); *sine loc.*, 21 December 1968, fl., *Graziela et al. 517* (HB). Mato Grosso: Chapada dos Guimarães, 14 October 1973, fl., *G.T. Prance 18955A* (INPA). Minas Gerais: Minduri, 5 November 1999, fl., *A.O. Simões 952* (UEC); Tiradentes, 6 December 1983, fl., *H.F. Leitão Filho 15266* (UEC). Paraná: Curitiba, 5 January 1965, fl., *L.T. Dombrowski & Y. Saito 1283b* (MBM); Sengés, 19 September 1975, fl., *G. Hatschbach 37112* (MBM). São Paulo: *sine loc.*, 10 October 1917, fl., *A. Gehrt 1041* (NY).

2. *Pteroglossa macrantha* (Rchb. f) Schltr., Beih. Bot. Centralbl. 37(2): 450. 1920 - *Spiranthes macrantha* Rchb. f., Linnaea 19: 378. 1846 ≡ *Gyrostachys macrantha* (Rchb. f.) Kuntze, Revis. Gen. Pl. 2: 664. 1891 ≡ *Stenorrhynchos macranthum* (Rchb. f.) Cogn., Fl. Bras. 3(4): 176. 1895. Figures 2 and 3A–D.

Type: BRAZIL: Minas Gerais, *sine loc.*, 1838, *M. Claussen 391* (P 345647).

Spiranthes albescens Barb. Rodr., Gen. Sp. Orchid. 1: 186. 1877 ≡ *Stenorrhynchos albescentis* (Barb. Rodr.) Barb. Rodr., Gen. Sp. Orchid. 1 (Index): X. 1877.

Type: BRAZIL. "*Hab. Dans la Serra de Caldas, Minas Geraes*", s.d., *J. Barbosa Rodrigues s.n.* [lost], lectotype designated by Buzatto et al. (2013).

Herb to 45 cm high, including the inflorescence. *Roots* fleshy, cylindrical, often stipitate, lanuginose. *Leaves* ca. 20–25 × 5–8 cm, 7–8, forming a basal rosette, usually present during anthesis sometimes withered, sessile, acute, coriaceous, margin translucent, light green, lanceolate, external leaves slightly attenuate at base, the inner leaves more attenuate. *Inflorescence* ca. 14 cm long, with ca. 15 flowers, pubescent, scape terete, partially covered by 6–7 tubular, acute bracts; raceme congested, most of them opened simultaneously; floral bracts ca. 4.5–6 cm long, prominent, green, glabrous, loosely concave, ovate, acuminate. *Flowers* resupinate, fleshy, greenish-white with yellow veins. *Sepals* densely pubescent outside. *Dorsal sepal* ca. 26 × 4 mm, slightly spreading, adherent to the lateral sepals on its proximal one-thirds, densely pubescent outside, lanceolate, acuminate, the margins entire. *Lateral sepals* 36 × 6 mm, forming a shortly conical, rounded, prominent spur of ca. 15 mm long, densely pubescent outside, elliptic-lanceolate, falciform, acuminate, margins entire. *Petals* ca. 29 × 5 mm, glabrous, bearing 3 conspicuous veins, fusiform, acuminate, margins entire. *Labellum* ca. 30 × 9 cm, three-lobed, blade pubescent outside, glabrous near the apex, adnate at base with the lateral sepals in the spur, long-clawed and narrowly channelled below the middle; the lateral lobes semi-orbicular, the mid-lobe ovate to obovate-spatulate, acute to rounded. *Column* ca. 15 × 10 mm, 5 mm height, hemi-terete, stout, pubescent, the base extended into a long column foot, the membranaceous clinandrium margins are enclosed with basal portion of the anther; the anther is thick, massive, ovate, long-acute, with filament completely adnate; pollinarium 2 × 3–4 mm, 3 mm height, formed by 2 deeply bipartite, granular pollinia, narrowly clavate, with ventral-apical, oval, grey

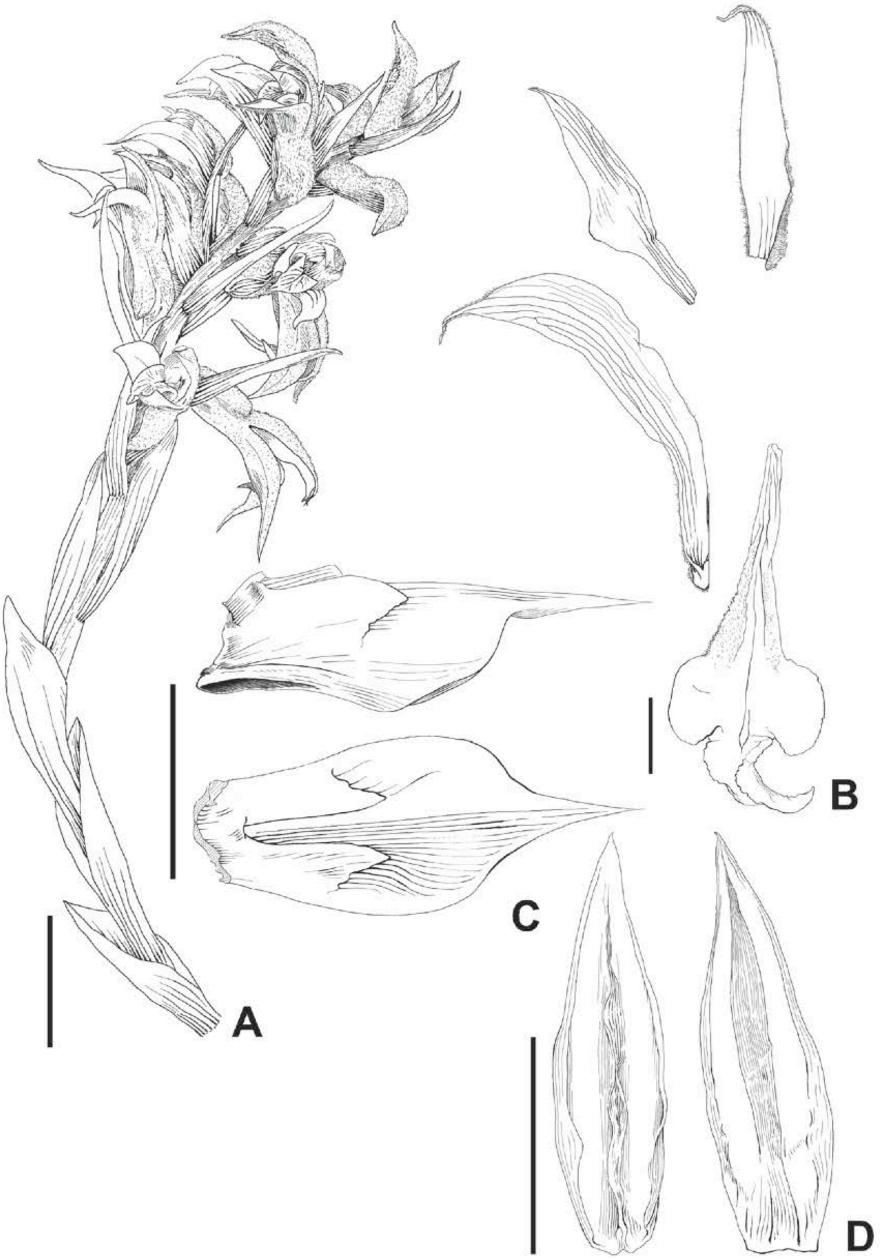


Figure 2 - *Pteroglossa macrantha* (Rchb. f.) Schltr.; **A.** Habit; **B.** Floral diagram; **C.** Column in lateral and dorsal view; **D.** Anther in ventral and dorsal view. Scale bars: **A** and **B** = 1 cm; **C** and **D** = 5 mm.

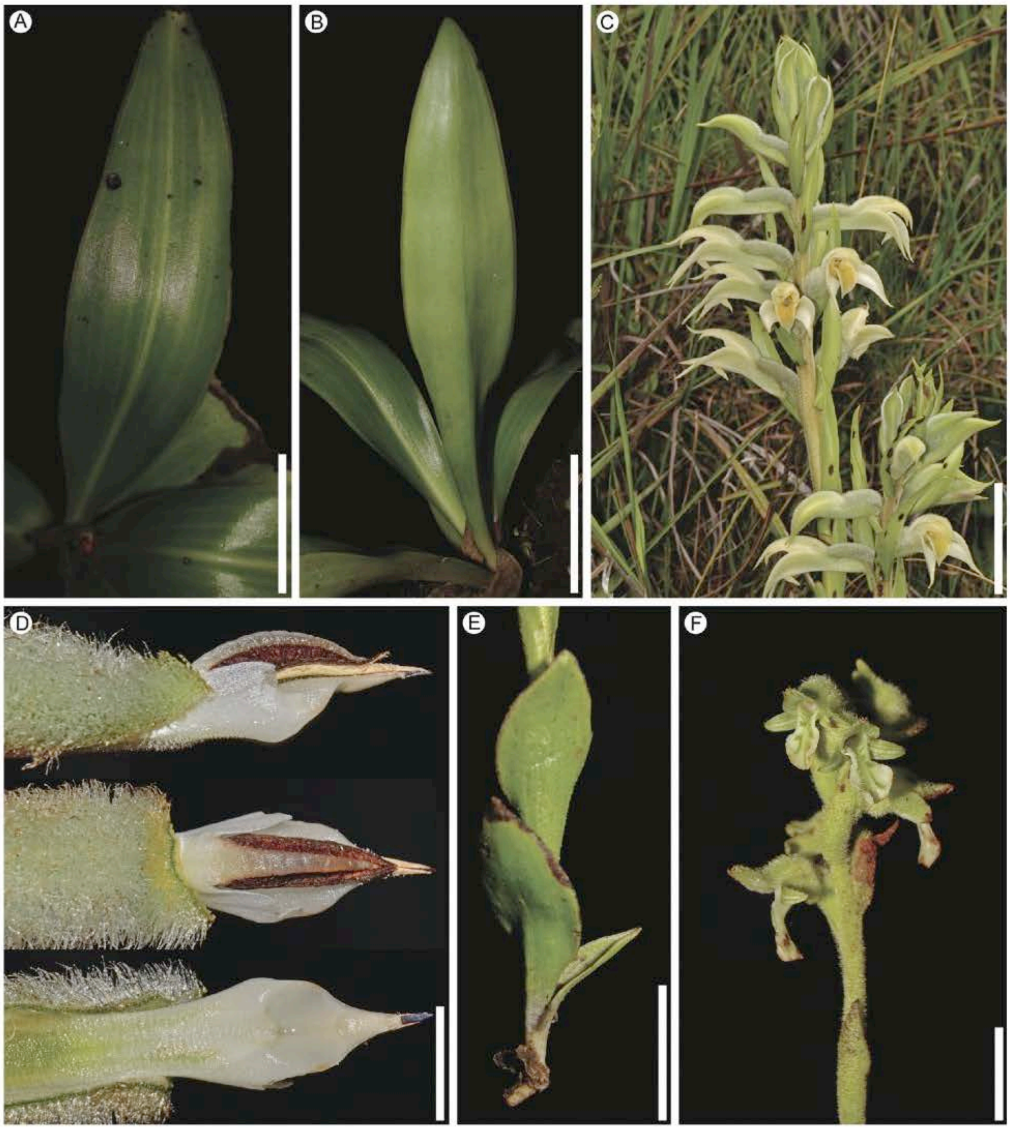


Figure 3 - *Pteroglossa macrantha* (Rchb. f.) Schltr. and *Lyroglossa grisebachii* (Cogn.) Schltr.; **A–D** *Pteroglossa macrantha*; **A.** Adaxial surface of leave; **B.** Abaxial surface of leave; **C.** Detail of inflorescence; **D.** Column in lateral, dorsal and ventral views; **E–F.** *Lyroglossa grisebachii*; **E.** Detail of cauline leaves; **F.** Detail of inflorescence. Scale bars: **A** and **B** = 5 cm; **C** = 1 cm; **D** = 5 mm; E and F = 2 cm.

viscidium; rostellum remnant ca. 3 mm long, narrowly triangular, stiff, obscurely tridentate at the portion originally covered by the viscidium; stigma slightly bilobed toward its base. *Ovary* ca. 20 mm long, 5 mm diameter near the apex, densely pubescent. *Capsule* ellipsoid. *Seeds* minute and fusiform.

Notes: *Pteroglossa macrantha* is the type-species of the genus (Salazar 2003). It is worth noticing here that leaf morphology of the specimens examined do not agree with the description provided by Salazar (2003), who described the leaves of this species as being oblanceolate and long petiolate. The specimens we studied have leaves. Salazar's description of leaves fits very well the description of two other species, namely *P. roseoalba* (Rchb.f) Salazar & M.W.Chase and *P. glazoviana* (Cogn.) Garay. However, the attenuated leaves of *P. macrantha* (Figure 3A–B) are considerably different from this description (see species description) and are remarkably similar to those of robust specimens of *Skeptrostachys* Garay.

Pteroglossa comprises about ten species distributed from Mexico to Costa Rica and from Colombia to Argentina (Salazar 2003). In Brazil, *Pteroglossa* occurs mainly in the Atlantic Rain Forest Biome, in the states of Minas Gerais, Paraná, São Paulo, Rio de Janeiro and Espírito Santo (Salazar 2003). It shares a number of morphological features (e.g. labellum and column shape; Figure 3D) with other members of the "*Stenorrhynchos* Clade", mainly with *Eltroplectris* Raf., and this seems to indicate a closer relationship between these and other genera in this clade. Recent molecular studies (Salazar et al. 2011) support such a relationship and have indicated that *Pteroglossa* is inserted within the "*Stenorrhynchos* Clade", and is closely related to *Sacoila* Raf., *Mesadenella* Pabst & Garay and *Eltroplectris*.

Distribution: *Pteroglossa macrantha* occurs mainly at the Cerrado Biome (especially, Goiás, Distrito Federal and Mato Grosso). After an extensive revision of the literature and the herbarium material available, this species is recorded for the first time to the State of Rio Grande do Sul. This new record makes Rio Grande do Sul the austral limit of this genus.

Specimens examined: BRAZIL. Rio Grande do Sul: Jaquirana, 5 January 2013, *C.R. Buzatto 899 & S.A.L. Bordignon* (ICN); São Francisco de Paula, 23 January 2007, fl., *S.A.L. Bordignon s.n.* (ICN 174065).

Additional specimens examined: BRAZIL. Distrito Federal: Brasília, s.d., fl., *F. Sello s.n.* (HBG 506820). Goiás: Alvorada do Norte, 10 October 1976, fl., *G. Hatschbach 39113* (MBM); *sine loc.*, 26 October 1964, fl., *H.S. Irwin 7371* (NY). Mato Grosso: Coxipó, December 1978, fl., *J.G.A. Lima 860* (HB); Lago São Léo, 11 October 1968, fl., *A. Lima 438–68* (IPA); Xavantina, 12 October 1967, fl., *A. Argent & J. Ramos 6735* (HB); *sine loc.*, 10 October 1964, fl., *H.S. Irwin 6725* (NY); *sine loc.*, s.d., fl., *Willian s.n.* (HB 75708). Minas Gerais: Caldas, 20 October 1866, fl., *A.F. Regnell III1198* (S); Ituiutaba, 10 November 1948, fl., *A. Macedo 1342* (MBM, NY, RB); Paracatú, 21 October 1989, fl., *A.H. Salles et al. 1411* (HEPH); *sine loc.*, 1838, fl., *P. Claussen 391* (P). Rio de Janeiro: Santo Antônio de Imbé, April 1932, fl., *A.C. Brade & S. Lima 11734* (HB). Tocantins: Aparecida do Rio Negro, 1 November 2001, fl., *C.E. Rodrigues Jr. 1514* (HEPH).

Artificial key to the genera of the "*Stenorrhynchos* Clade" native to Rio Grande do Sul

1. Leaves always present during anthesis .... *Mesadenella*

1'. Leaves sometimes absent or
deteriorated during anthesis 2

2. Flowers mostly greenish-white.
Column ventrally channeled 3

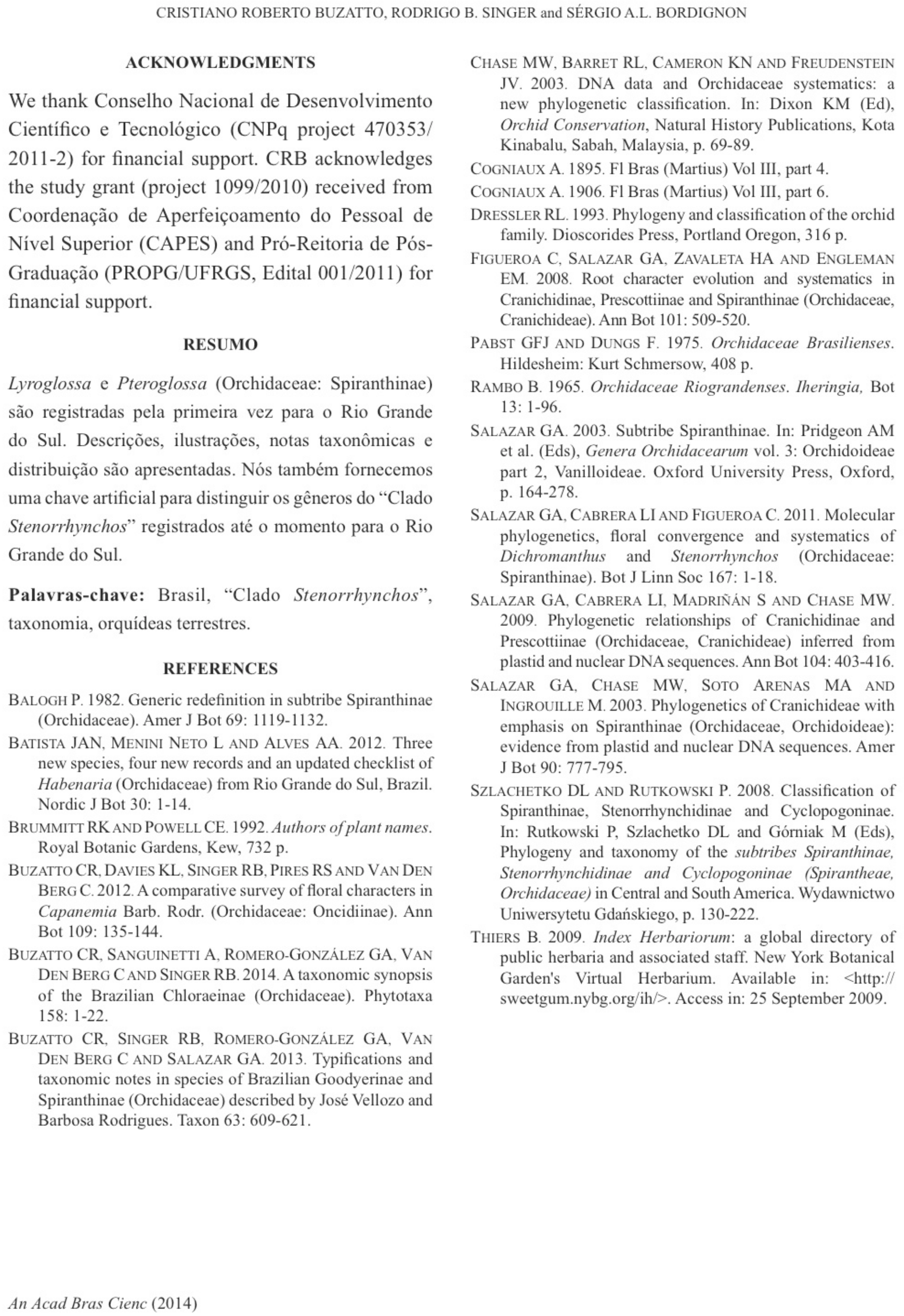
2'. Flowers vivid red, pinkish white or
orange; column not channelled ventrally 4

3. Plants to 17.5 cm high. Labellum whitish
with green veins, longer than sepals .... *Lyroglossa*

3'. Plants to 45 cm high. Labellum
yellowish, as long as the sepals .... *Pteroglossa*

4. Flowers long tubular,
3.2-5 cm, light pinkish or vivid red .... *Sacoila*

4'. Flowers shortly tubular,
2-3 cm, pinkish white or orange ....*Skeptrostachys*



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RESUMO

Lyroglossa e *Pteroglossa* (Orchidaceae: Spiranthinae) são registradas pela primeira vez para o Rio Grande do Sul. Descrições, ilustrações, notas taxonômicas e distribuição são apresentadas. Nós também fornecemos uma chave artificial para distinguir os gêneros do "Clado *Stenorrhynchos*" registrados até o momento para o Rio Grande do Sul.

Palavras-chave: Brasil, "Clado *Stenorrhynchos*", taxonomia, orquídeas terrestres.

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Capítulo 3

Nomenclature and revised typification of *Neottia bicolor* (Orchidaceae: Spiranthinae)

Cristiano Roberto Buzatto, Gustavo A. Romero-González e Rodrigo B. Singer

Botanical Studies (a submeter)

Nomenclature and revised typification of *Neottia bicolor* (Orchidaceae: Spiranthinae)

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Background

The nomenclatural history of *Neottia bicolor* is revised and clarified.

Results

In the absence of any original material, we choose as lectotype of *Neottia bicolor* the plate accompanying the protologue.

Conclusions

The lectotypification of the *Neottia bicolor* is necessary to clarify the true taxonomical history of *Lyroglossa grisebachii*, following the rules of botanical nomenclature.

Keywords
Cyclopogon bicolor, lectotypification, *Lyroglossa grisebachii*, taxonomy

Background

Neottia bicolor was formally described by Ker Gawler (1824: tab. 794), then editor of *The Botanical Register* (Stafleu and Cowan 1979). Lindley (1824) transferred this taxon to *Spiranthes* Rich., proposing the combination *Spiranthes bicolor* (Ker Gawl.) Lindl. All vegetative and floral features illustrated by Ker Gawler (1824) correspond unequivocally to a species of *Cyclopogon* C.Presl. In agreement with this circumscription, Schlechter (1919: 52) proposed the combination *Cyclopogon bicolor* (Ker Gawl.) Schltr., a taxonomic decision that we accept. *Cyclopogon* is characterized by mostly petiolate leaves forming a basal rosette, generally present at flowering time, and flowers for which the pollinaria have a dorsally adhesive viscidium (Salazar 2003). Moreover, this genus is currently included within the so-called "*Pelexia* clade", *sensu* Salazar et al. (2003, 2011).

When describing *Spiranthes grisebachii* Cogn. the author cited three syntypes: Brazil (*Schwacke 7615*, B, destroyed; isosyntype, BHCB), French Guiana (*Leprieur s.n.*, G; isosyntype, P) and Trinidad and Tobago (*Crueger s.n.*, GOET). Cogniaux (1895: 207) also cited "*Spiranthes bicolor* Griseb. *non* Lindl.", indicating that Griesebach had simply misidentified two collections from Trinidad ("Pd. [Purdie], Cr. [Crueger], Savannah de Omara"; Griesebach 1859–1864: 641). Schlechter incorrectly based *Stenorrhynchos bicolor* Schltr. and *Lyroglossa bicolor* Schltr. (Schlechter 1920: 449) on *Spiranthes bicolor* Griseb.: these two combinations are referable to the genus *Lyroglossa* Schltr. but constitute illegitimate superfluous names because Schlechter also listed, in both cases, *Spiranthes grisebachii* in the synonymy, although he chose the epithet "*bicolor*". Later, Schlechter (1921) did propose *Lyroglossa griesebachii* (Cogn.) Schltr. based this time on *Spiranthes griesebachii*.

During a taxonomic study involving *Lyroglossa grisebachii* (Cogn.) Schltr. (Orchidaceae: Spiranthinae; Buzatto et al. 2014), the lectotypification of *Spiranthes bicolor* Griseb. proposed by Szlachetko and Rutkowsky (2008: 179) drew our attention. Here we reconstruct the nomenclatural history of *Lyroglossa grisebachii* and propose a lectotype for *Neottia bicolor* Ker Gawl.

Methods

The lectotype proposed in this paper is made in compliance with the rules and recommendations of the *Code* (McNeill et al. 2012) in particular Article 9.12.

Results and discussion

Typification of *Neottia bicolor* and *Lyroglossa grisebachii*

1. ***Cyclopogon bicolor*** (Ker Gawl.) Schltr. *In*: Repert. Spec. Nov. Regni Veg. Beih. 6: 52 (1919) ≡ *Neottia bicolor* Ker Gawl. *In*: Bot. Reg. 10: 794, t. 794 (1824). TYPE: Jamaica. s. loc., 1823, *Don s.n.*, no herbarium cited. Lectotype, **designated here**: original illustration, Bot. Reg. 10: t. 794. 1824 ≡ *Spiranthes bicolor* (Ker Gawl.) Lindl. *In*: Bot. Reg. 10: sub t. 823

(1824) ≡ *Gyrostachys bicolor* (Ker Gawl.) Kuntze. *In*: Revis. Gen. Pl. 2: 664 (1891) ≡ *Beadlea bicolor* (Ker Gawl.) Garay. *In*: Bot. Mus. Leafl. 28(4): 299 (1982).

In the absence of any other original material, the first choice for the lectotypification of *Neottia bicolor* is the plate accompanying the protologue (Figure 1A). Lindley, when proposing his new combination, *Spiranthes bicolor* (Lindley 1824), and referring to the plate published earlier by Ker Gawler (1824), stated that "… the leaves are inaccurately represented as whole-colored. They are variegated with lighter and darker green, like those of *S. picta*". Lindley, most important of all, later added "It is stated in the Botanical Register, by mistake, that this plant was brought from Trinidad; it was found in Jamaica by Mr. George Don, while there in the service of the Horticultural Society. The leaves are sometimes whole coloured and sometimes mottled, as in *S. picta* Lindl. The base of all the sepals is equally inflated, so as to make the little white flowers protuberant all round above the neck of the ovary" (Lindley 1840: 468).
Szlachetko and Rutkowski (2008: 186) indicated the same "type" to *Cyclopogon argyrifolius* (Barb. Rodr.) Barb. Rodr. (as "*Cyclopogon argyrifolius* (Barb. Rodr.) Szlach. & Rutk., *comb. nov.*") and to *Cyclopogon bicolor*. That incorrect proposal must be corrected, since is in disagreement with the *Code* (McNeill et al. 2012, Art. 9.19).

2. *Lyroglossa grisebachii* (Cogn.) Schltr. *In*: Anexos Mem. Inst. Butantan, Secc. Bot. 1(2): 27 (1921) ≡ *Spiranthes grisebachii* Cogn. *In*: Fl. Bras. 3(4): 207 (1895). TYPE: French Guiana. s.loc., 1840, *Leprieur s.n.*, no herbarium cited. Lectotype designated by Szlachetko and Rutkowski (2008): G 168827, isolectotypes: P 345573; AMES (photo as G 168827).

= *Stenorrhynchos bicolor* Schltr. *In*: Repert. Spec. Nov. Regni Veg. Beih. 6: 53 (1919), *nom. illeg. superfl.* ≡ *Lyroglossa bicolor* Schltr. *In*: Beih. Bot. Centralbl. 37(2): 449 (1920), *nom. illeg. superfl.* ≡ "*Spiranthes bicolor*" *auct non* (Ker Gawl.) Lindl. *In*: Fl. Brit. W. I. 641 (1864).

Szlachetko and Rutkowsky (2008: 179) proposed *Leprieur s.n.* (G) as lectotype. The same authors (Szlachetko and Rutkowsky 2008: 179) erroneously proceeded to propose a lectotype for "*Spiranthes bicolor* Griseb.", which is not a validly published name.

Conclusions

The lectotypification of *Neottia bicolor* is necessary to clarify the true taxonomical history of *Lyroglossa grisebachii*. *Cyclopogon bicolor* and *L. grisebachii*, have a peculiar morphology and are easily distinguishable. In addition, both types were erroneously designated (Szlachetko and Rutkowsky 2008: 179 and 186) and are in conflict with the protologue (McNeill et al. 2012, Art. 9.19).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CRB, RBS and GAR-G designed the study. The authors participated in the research, acquisition of data and drafting of the manuscript. All authors read and approved the submitted manuscript.

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Figure 1. *Spiranthes bicolor* (Ker Gawl.) Lindl. A. Ker Gawler’s original illustration published in *The Botanical Register* (10: t. 794. 1824). B. From the microfilm edition of the Lindley Herbarium (IDC, 1987: L87/19).


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Capítulo 4

A taxonomic synopsis of the Brazilian Chloraeinae (Orchidaceae)

Cristiano Roberto Buzatto, Agustín Sanguinetti, Gustavo A. Romero-González,
Cássio van den Berg e Rodrigo B. Singer

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A taxonomic synopsis of Brazilian Chloraeinae (Orchidaceae: Orchidoideae)

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Abstract

A taxonomic synopsis of Brazilian Chloraeinae (Orchidaceae) is presented. Artificial keys to separate the genera and species are included. Vegetative and floral features of all species are described and illustrated with drawings and photographs, and data on their distribution, phenology and ecology are presented. Two lectotypes and one neotype are proposed. The presence of *Bipinnula biplumata*, *B. montana*, *B. penicillata* and *Chloraea membranacea* in Brazil is confirmed, and *Bipinnula gibertii* is reported for the first time from this country.

Key words: *Bipinnula*, *Chloraea*, orchid nomenclature, orchid taxonomy

Introduction

Orchidaceae Jussieu (1789: 64) comprise about 24,000 species and constitute one of the more conspicuous angiosperm families because of their remarkable floral and vegetative morphological adaptations. Among American terrestrial orchids, species of subtribe Chloraeinae Pfitzer (1887: 98, as Chloraeeae) are particularly appealing due to their showy and morphologically complex flowers. Chloraeinae are essentially an Andean orchid group (Correa & Sánchez 2003), and only nine species are found outside that region, in southern Brazil, northeastern Argentina and Uruguay: *Bipinnula biplumata* (Linnaeus 1781: 405) Reichenbach (1883: 62), *B. gibertii* Reichenbach (1877: 51), *B. montana* Arechavaleta (1899: 282), *B. penicillata* (Reichenbach 1878: 51) Cisternas & Salazar in Cisternas *et al*. (2012a: 10), *B. polysyka* Kraenzlin (1888: 317), *B. ctenopetala* Schlechter (1925: 24) (a dubious species; see below), *Chloraea bella* Hauman (1920: 99), *C. membranacea* Lindley (1840: 401) and *Gavilea odoratissima* Poeppig (1833: 19). The last is common and widely distributed in the Patagonian Andes, but has a disjunct distribution, with some small and isolated populations restricted to Sierra de la Ventana system in South Buenos Aires Province (Chemisquy 2012, Correa 1956).

In their current delimitation, Chloraeinae comprise about 70 species distributed in three genera (Correa & Sánchez 2003, Chemisquy 2012, Chemisquy & Morrone 2010, 2012 Cisternas *et al*. 2012a, Cisternas *et al*. 2012b): *Chloraea* Lindley (1827: 47; 45 species), *Bipinnula* Commerson ex Jussieu (1789: 65; 11–12 species) and *Gavilea* Poeppig (1833: 18; 17 species). In the last decades, Chloraeinae have been interpreted and delimited in different ways. The three genera cited above plus *Geoblasta* Barbosa Rodrigues (1891: 132; included in *Bipinnula*, see below) and *Codonorchis* Lindley (1840: 410) were referred by Dressler (1981, 1993), Szlachetko (1995), Kores *et al*. (2001) and Clements *et al*. (2002) to subtribe Chloraeinae based on multiple leaves, spiranthoid embryos, and non-tuberoid roots. However, although Dressler (1981, 1993) placed Chloraeinae in tribe Diurideae Endlicher (1842: 21), Szlachetko (1995) placed them in Geoblasteae Barbosa Rodrigues (1891: 132). More recently, Clements *et al.* (2002) recircumscribed Chloraeinae, including them in tribe Cranichideae Lindley ex Meisner

(1843: 384) and placing *Codonorchis* in subfamily Codonorchidoideae (Cribb 2000: 169) Clements & Jones (2002: 439). Later, Correa & Sánchez (2003) reinstated the tribal status of tribe Codonorchideae Cribb (2000: 169) based on the molecular results of Kores *et al.* (2001). Recent phylogenetic analyses based on plastid and nuclear DNA sequence data (Chemisquy & Morrone 2010, 2012, Cisternas *et al*. 2012a, Cisternas *et al*. 2012b) confirm Clements *et al*.'s (2002) inclusion of Chloraeinae within Cranichideae, as the sister-group of a clade containing subtribes Spiranthinae Lindley ex Meisner (1843: 385), Manniellinae Schlechter (1926: 572), Cranichidinae Lindley ex Meisner (1843: 384) and Goodyerinae Klotzsch (1846: tab. 24, as Goodyereae). Barbosa Rodrigues (1891:132) established *Geoblasta*, based on *Geoblasta teixeirana* Barbosa Rodrigues (1891:133), referring it to Geoblasteae. Later, Cogniaux (1893) transferred the name proposed by Barbosa Rodrigues to *Chloraea*, but Hoehne (1940) argued for re-establishment of *Geoblasta* (Correa 1968).

Brazilian Chloraeinae have been rarely collected or illustrated and generally have received little attention. These plants are, at best, cited as part of floristic inventories, but they have some nomenclatural problems (Hoehne 1940, Rambo 1965, Pabst & Dungs 1975). After years of fieldwork in southern Brazil and study of specimens housed in several Brazilian and foreign herbaria (see list below), we detected major important gaps in knowledge of these remarkable terrestrial orchids. These gaps range from basic data (i.e. lack of information on root, leaf, floral and fruit morphology) to important nomenclatural/taxonomical issues (typifications, synonym, etc.) as well as unrecorded species. In this study, we present a detailed account of Chloraeinae native to Brazil, including nomenclature, detailed morphological descriptions and illustrations and ecological data, based on our own field observations as well as study of major Brazilian and foreign herbaria.

Material and methods

The following herbaria were consulted: AMES, B, BA, BM, CEN, CTES, F, G, GH, GOET, HAS, HB, HEPH, IBGE, ICN, K, K-L, LINN, LP, M, MBM, MPU, MVFA, NY, P, PACA, PEL, R, RB, S, SI, SP, UB, UEC, VIC, and W (acronyms according to Thiers 2012). This paper follows the general orchid classification proposed by Chase *et al*. (2003) and the descriptive terminology of Dressler (1993). Abbreviations of authorities for plant names follow Brummitt & Powell (1992). Generic descriptions are adapted from Correa & Sánchez (2003) and Izaguirre de Artucio (1973). For colour terms, we follow Kornerup & Wanscher (1978).

Data on distribution, habitat, and phenology were obtained from herbarium labels complemented by personal observations made during field trips. Fieldwork extended from 2007 to 2013. A total of 67 field trips were made in southern Brazil (states of Rio Grande do Sul, Santa Catarina and Paraná). Vegetative and floral features were recorded by mean of photographs. In agreement with Brazilian environmental laws (decree nº 42099 of Dec 31, 2002; Assembléia Legislativa 2013), only leaves and inflorescences were pressed to avoid destruction of entire plant. Some studied species (see taxonomic treatments) lack leaves or have degenerating leaves at flowering time. In these cases, populations were marked with GPS and visited at a different time of the year so that leaf features could be properly documented. Voucher specimens were deposited at ICN, with duplicates at AMES and K. For conservation purposes, we here cite only municipalities and omit precise data (coordinates) for populations. These data, however, are available on request. For the sake of practicality, species descriptions were primarily based on general, easily recognizable vegetative and floral morphological features.

Taxonomic treatment

Artificial key to the Brazilian genera of Chloraeinae

1. Inflorescence one-flowered (exceptionally 2-flowered); labellum darkly coloured, pubescent, as a rule with appendices on sides and apex..1. *Bipinnula*

\- Inflorescence 8–15-flowered; labellum white with numerous, clavate projections with greyish-white stalks and black tips ....2. *Chloraea*

1. ***Bipinnula*** **Commerson** ex Jussieu (1789: 65)

Type:—*Bipinnula biplumata* (Linnaeus 1781: 405) Reichenbach (1883: 62). Basionym: *Arethusa biplumata* L.f., lectotype designated by Kraenzlin (1904)

Geoblasta Barbosa Rodrigues (1891: 132). Type:—*Geoblasta teixeirana* Barbosa Rodrigues (1891: 133)

Jouyella Szlachetko in Szlachetko & Margonska (2001: 124). Type:—*Jouyella fimbriata* (Poepp.) Szlachetko & Margonska (2001: 125); basionym: *Chloraea fimbriata* Poeppig (1833: 15)

Terrestrial herbs. Roots fascicled, cylindrical and tuberous with conspicuous annular thickenings. Leaves 2–6, green with light-green veins, erect or prostrate, oblong to linear-lanceolate, apex acute to cuspidate, margin entire; withered or sometimes absent or deteriorating at anthesis. Inflorescence terminal, 1-flowered or exceptionally 2-flowered (in Brazilian species), scape slender, bearing several sheathing, acute bracts; floral bracts lanceolate, acute, seldom hooded and partially covering the flower, as long as or longer than ovary. Flowers predominantly greenish, with reticulate green veining and labellum differently coloured with respect to other perianth segments. Dorsal sepal much broader than lateral sepals, lanceolate, concave, acute, margin entire, acuminate or acute. Lateral sepals basally connate, longer and much narrower than dorsal sepal, entire (in *Bipinnula penicillata* and the Chilean *B. apinnula* Nieuwenhuizen, 1993: 11) or fimbriate-pectinate in the apical third or fourth, the marginal projections simple or forked. Petals as long as or shorter than dorsal sepal, ovate, ovate-lanceolate, unequal, entire or with margin sinuate, lobed or crenate, apex acute, connivent with the dorsal sepal, membranous. Labellum clawed or sessile, dark-coloured, articulated basally, entire to trilobed, ecalcarate; lamina fleshy, glabrous to pubescent, as a rule with appendices on sides and apex. Column elongate, incurved; column wings absent or vestigial. Anther terminal or dorsal, erect, persistent, with reflexed apex, bilocular; pollinia two, oblong, imperfectly bipartite, yellow, granular, without viscidium. Rostellum short, triangular and fleshy. Stigma ventral, entire, concave, prominent, ovate or oblong. Ovary clavate. Capsule erect, ellipsoid, dehiscent via two dorsal sutures. Seeds pale brown, club-shaped to kidney-shaped.

***Notes*:—**Recent phylogenetic studies (Cisternas *et al*. 2012b) have clearly shown that *Geoblasta penicillata* (Reichenbach 1878: 11) Hoehne (1940: 98) is nested within *Bipinnula*. The combination *Bipinnula penicillata* (Rchb.f.) Cisternas & Salazar in Cisternas *et al*. (2012a: 10) is adopted here. Monophyly of *Bipinnula* has strong support in molecular phylogenetic studies (Chemisquy & Morrone 2010, 2012, Cisternas *et al*. 2012b), contradicting Szlachetko & Margonska (2001) who argued that *Bipinnula* is not monophyletic and transferred its multiple-flowered Chilean species to their newly proposed genus *Jouyella.* In light of recent phylogenetic studies of Chloraeinae (Cisternas *et al*. 2012b), these nomenclatural transfers are unjustified. As a whole, *Bipinnula* can be divided in two groups: multiple-flowered species from Chile and 1-flowered (rarely 2-flowered) species almost restricted to the pampas *sensu* Bilenca & Miñarro (2004), which includes grasslands in Rio Grande do Sul (Brazil), northeastern Argentina and Uruguay (Correa & Sánchez 2003). Nieuwenhuizen (1993) proposed three sections in the genus: *Bipinnula* sect. *Bipinnula*, accommodating the single-flowered species; *B*. sect. *Trilobatae* Nieuwenhuizen (1993: 13), to include the Chilean *B. volkmanii* Kraenzlin, 1904: 22 and *B. apinnula*; and *B.* sect. *Multiflorae* Nieuwenhuizen (1993: 15), to encompass all remaining multiflowered species. However, the results of recent phylogenetic analyses (Cisternas *et al*. 2012b) cast doubt on the monophyly of these sections, since the phylogenetic trees showed that the single-flowered species (*B.* sect. *Bipinnula*) and *B. apinnula* (*B.* sect. *Trilobatae*) are nested within the species of *B*. sect. *Multiflorae*.

After examining several living specimens, we realized that some *Bipinnula* species are morphologically more variable than suggested in literature (Kraenzlin 1904, Izaguirre de Artucio 1973, Correa & Sánchez 2003, Cisternas *et al*. 2012b) and that some vegetative and floral features were only partially known or even unknown for some species. In general, vegetative features of *Bipinnula* have been overlooked in traditional taxonomic studies. A distinctive vegetative feature of all the Brazilian species is a root with conspicuous basal annular thickenings (Fig. 1A–B); these are usually associated with being contractile (Fahn 1982). However, this feature is not exclusive to *Bipinnula*, since similar roots were recently found in a species of *Chloraea* from Patagonia and the pampas. Remarkably, the roots of multiflorous Chilean species of *Bipinnula* are apparently devoid of annular thickenings (Fig. 1 of Cisternas *et al*. 2012b). Leaves can vary from light to dark green and may not be present during anthesis, which apparently varies with environmental conditions. Shaded and well-watered plants can have intact leaves during anthesis, whereas plants under strong sunlight in dry conditions do not. Leaves of *B. biplumata* and *B. montana* are erect and linear, whereas those of *B. gibertii* and *B. penicillata* are oblong and prostrate. As noted above, the fruit is an erect capsule that dehisces through two dorsal sutures.

To our knowledge, there are neither confirmed herbarium specimens of *Bipinnula* from the state of Santa Catarina, nor have we found species of this genus during fieldwork in this Brazilian state. In addition, this species apparently has not been collected in Paraná State in the last 50 years. However, significant populations of some *Bipinnula* species recently were found in the Brazilian state of Rio Grande do Sul. Prior to this, most species had not been collected for decades there. Thus, we suggest the possibility of a continuous distribution of *Bipinnula* species from Rio Grande do Sul through Santa Catarina to Paraná. These taxa likely are part of temperate Austral-Antarctic and Andean lineages (Rambo 1953).

With the exception of *B. montana*, no other *Bipinnula* species are included in national or regional lists of threatened flora (Ministério do Meio Ambiente 2008, Assembléia Legislativa 2013). However, inclusion of *Bipinnula* species in these lists should be suggested in future studies until we have a better understanding of their conservation status, using the appropriate criteria.

Key to the Brazilian *Bipinnula* species

1. Leaves 2–4, ovate, prostrate 2
- Leaves 5–6, linear-lanceolate, erect 3
2. Lateral sepals fimbriate-pectinate at the apical third or fourth .... 1.2. *B. gibertii*
- Lateral sepals entire .... 1.4. *B. penicillata*
3. Labellum disk densely strigose-scaly; apical part puberulent .... 1.1. *B. biplumata*
- Labellum disk densely tomentose; apical part with (3–)4(–5) linear glabrous crests or carinae .... 1.3. *B. montana*

Bipinnula biplumata (L.f.) Reichenbach (1883: 62); basionym: *Arethusa biplumata* Linnaeus (1781: 405). Type:—ARGENTINA. "*in Freto Magellanico*", 1767, *Commerson s.n.* (holotype: LINN HS1394–5 [image!]; isotypes: MPU 017561! & 017562 [image!]). Figs. 1A, 2, 3.

Homotypic synonyms: *Bipinnula bonariensis* Sprengel (1826: 745), *nom. illeg.*; *Bipinnula commersonii* Lindley (1827: 52), *nom. illeg.*

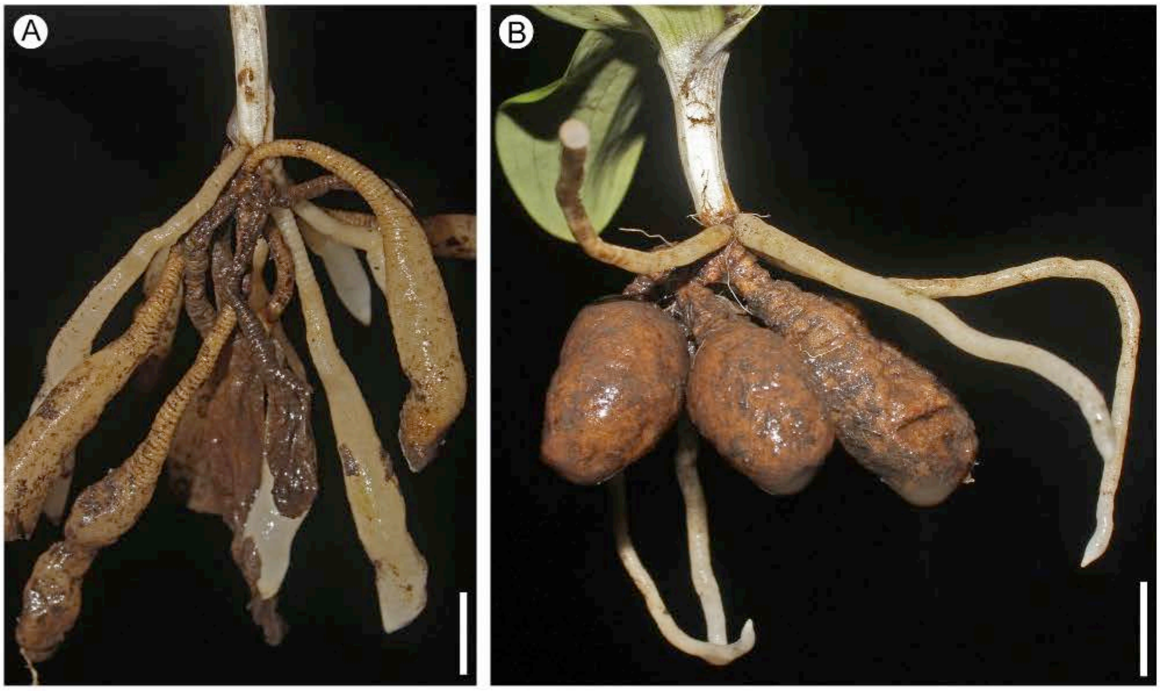

FIGURE 1. A. Roots of *Bipinnula biplumata* (L.f.) Rchb.f.; B. Roots of *Bipinnula penicillata* (Rchb.f.) Cisternas & Salazar. Scale bars, 1 cm (A–B).

Herbs up to 50 cm tall, including inflorescence. Roots approx. 15, 4–7 cm long, asymmetric, oblanceolate, apex conical. Leaves 5–6, deep green, erect, linear-lanceolate, 14.0–20.0 × 1.8–2.3 cm, apex acute. Flowers mostly greenish-grey, with conspicuous dark green to greyish-brown veins and dark green to reddish labellum. Dorsal sepal 2.5 × 1.3 cm. Lateral sepals 4.8 × 0.4–0.6 cm, flat, slightly recurved, fimbriate-pectinate in the apical third or fourth. Petals 2.5 × 1.4–1.8 cm, ovate, asymmetric, entire. Labellum 1.2 × 0.8 cm, sessile, white, involute, slightly trilobed, lateral lobes with dark minute cristae; the basal part densely strigose-scaly; central disk densely strigose-scaly; apical part puberulent. Column 1.7 × 0.4–0.5 cm; pollinia 0.7 × 0.4–0.5 cm. Ovary slightly pear-shaped. Capsule 2.0–4.0 × 0.9–1.5 cm.

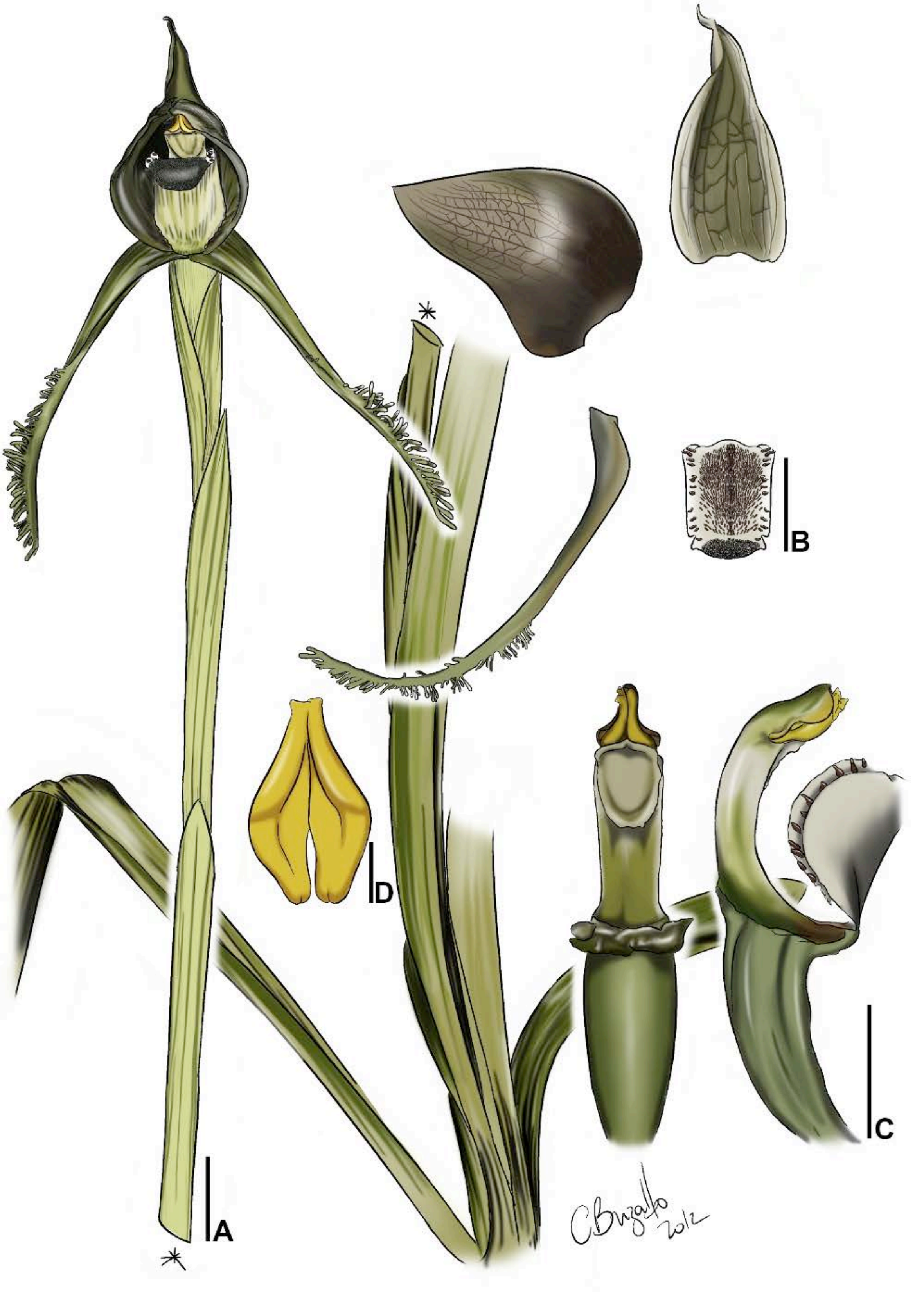

FIGURE 2. *Bipinnula biplumata* (L.f.) Rchb.f. A. Habit; B. Dissected perianth; C. Column, dorsal and lateral views; D. Pollinarium. Scale bars, 1 cm (A–C); 2 mm (D). Drawn by C.R. Buzatto from *Buzatto 561* (ICN).

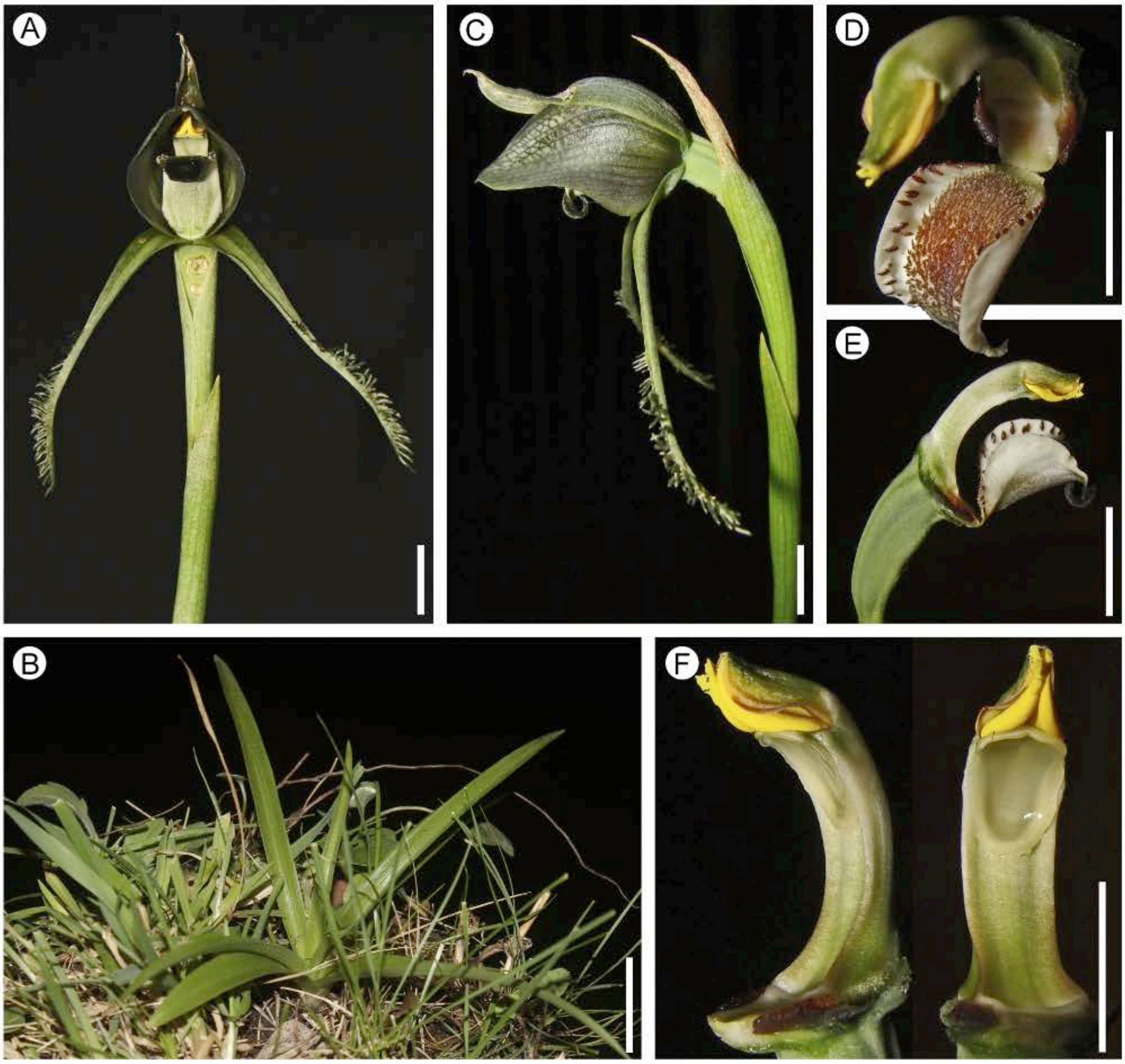

FIGURE 3. *Bipinnula biplumata* (L.f.) Rchb.f. A. Flowers, front view; B. Leaves; C. Flower, side view; D. Detail of labellum; E. Column articulated with labellum; F. Column, lateral and dorsal views. Scale bars, 1 cm (A, C–F); 2 cm (B).

***Brazilian specimens examined*:**—Paraná: Lapa, 17 October 1948, *Hatschbach 1030* (MBM). Rio Grande do Sul: Porto Alegre, 27 October 2009, *Buzatto 561* (ICN).

***Additional specimens examined*:**—ARGENTINA. Buenos Aires: Azul, 15 November 2012, *Sanguinetti 63* (BA); Buenos Aires, 1896, *Desvaux s.n.* (P 372108); Sierra Chica, Olavarria, 20 November 1906, *Spegazzini 1989* (LP). URUGUAY. Montevideo: Cerro, 1838, *Arsene Isabelle 100* (W); Cerro, 12 October 1889, *Fruchard s.n.* (P 372106); Montevideo, 1847, *Commerson s.n.* (P 372104); Montevideo, September 1763, *Commerson s.n.* (P 372105); Montevideo, October 1867, *Fruchard 817* (P); Montevideo, November 1877, *Arechavaleta 2628* (MVFA); Montevideo, s.d., *Commerson s.n.* (P 372103); Montevideo, December 1867, *Gibert 817* (MVFA); Montevideo, 21 December 1977, *Brescia 15131* (MVFA).

***Distribution*:**—This species occurs in Uruguay, Argentina, and Brazil (Rio Grande do Sul and Paraná). In Brazil, this species probably occurs also in Santa Catarina, situated between Rio Grande do Sul and Paraná, but its presence there has not yet been confirmed.

***Phenology*:**—Flowering October to December.

***Habitat and ecology*:**—*Bipinnula biplumata* is a rare species in Brazil, known only from two collections. According to our collection (*Buzatto 561*), this species grows in granite hills of the pampas. In Argentina and Uruguay, this species dwells in similar conditions in scrubland and grasslands associated with rocky outcrops at an elevation of *c.* 115–220 m.

Notes*:—*Bipinnula biplumata is easily identified in the field by its wide petals that hide almost completely the column and sides of the labellum (Figs. 2A, 3A,C). In all other species of *Bipinnula* occurring in Brazil, the column and labellum are more exposed. In habit, fresh material of *B. biplumata* is similar to *B. montana*, both species having long linear leaves (Fig. 3B) that may be present during anthesis.

Linnaeus (1781: 405), when describing this species, indicated that the plant was collected in the Straits of Magellan (*in Freto Magellanico*), Argentina, and that it had been communicated by Thouin ("*Communicavit D. Thouin*"*)*. Smith (1789) later published an illustration of *Arethusa biplumata* presumably based on Commerson's material, citing the same locality and Commerson as collector, also mentioning Thouin ("*In Americâ Meridionali ad Fretum Magellanicum legit Commerson, Thouin*").

The same year, Jussieu (1789), who had been given Commerson's herbarium in 1776 (Stafleu & Cowan 1976), added, in an entry dedicated to *Bipinnula* and most likely referring to *B. biplumata*, that the species had been collected in Buenos Aires and not in the Strait of Magellan (*Herba Bonariensis, non Magellanica*). A year later, Smith (1790), in the *Addenda et corrigenda*… published in the second fascicle of his work, amended the text of his plate quoting Jussieu (*Habitat in agro Bonariensi, nec ad Fretum Magallanicum*). The holotype at LINN appears to be the specimen illustrated in Smith (1789).

Sprengel (1826) and Lindley (1827) proposed *Bipinnula bonariensis* and *Bipinnula commersonii*, respectively, explicitly basing both combinations on *Arethusa biplumata*. Their names are illegitimate because they used an epithet different from that of the basionym.

1.2. ***Bipinnula gibertii*** Reichenbach (1877: 51). Type:—URUGUAY. Montevideo, "*in campis*", October 1867, *Gibert 487* (holotype W!; isotypes K!, MVFA!, P [image!]). Figs. 4, 5.

Herbs 10–12 cm in height, including inflorescence. Roots 2–5, 1–3 cm long, symmetric, globose tuberoid, apex rounded. Leaves 2–4, green, prostrate, oblong, 2.5–4.0 × 1.0–1.5 cm, apex acute. Flowers mostly light greenish, with darker greenish vein and a greyish labellum, darker towards the apex. Dorsal sepal 1.9 × 0.8 cm. Lateral sepals 3.3 × 0.3 cm, plane, slightly revolute, fimbriate-pectinate in their apical third or fourth. Petals 1.4 × 0.8 cm, ovate, asymmetric, with a crenate margin. Labellum 1.2 × 0.5 cm, clawed, green, revolute, unlobed; the basal part densely puberulent; central disk densely puberulent, black clavate to pilate appendices gradually protruding from base to apex; apex darker. Column 0.6 × 0.3 cm; pollinia 0.4 × 0.3 cm. Ovary slightly pear-shaped. Capsule 1.5–2.0 × 0.3–0.6 cm.

***Brazilian specimens examined*:**—Rio Grande do Sul: Cambará do Sul, 29 October 2010, *Buzatto 652 & Singer* (ICN); Cambará do Sul, 3 September 2011, *Buzatto 726 & Nervo* (ICN); Cambará do Sul, 11 October 2013, *Buzatto 915 & Sanguinetti* (ICN, AMES, K); Jaquirana, 5 October 2011, *Buzatto 737 & Singer* (ICN).

***Additional specimens examined*:**—URUGUAY. Banda Oriental: [Las Piedras], 1816–1821, *Saint-Hilaire C2 2092* (P); Maldonado: s.loc., s.d., *Tweedie s.n.* (K 364475); Minas: Cerro Verdún, 3 December 1899, *Berro* 2317 (MVFA); Montevideo: Cerro, September 1828, *Gay s.n.* (P 372259); Cerro, 23 December 1852, *Courbon 486* (P); Cerro, October 1926, *Herter 442* (B); Cerro, 13 October 1971, *Izaguirre, Laguardia & Marchesi s.n.* (MVFA 10744); Parque Lecoq, 18 October 1949, *Rosengurt B-5724* (MVFA); Punta Brava, 31 August 1874, *Fruchard s.n.* (P 372255); Punta Brava, July 1876, *Fruchard s.n.* (P 372256); Punta Gorda, 23 October 1910, *Osten 5289* (SI); Rincón, 1826–1830, *sin col.* (K 364472); Rincón, 11 August 1871, *sin col.* (P 372257); Rivera: quebrada entre Masoller y Rivera, 1 September 1969, *del Puerto & Marchesi s.n.* (MVFA 2748). Without locality data: s.d., *Arechavaleta 309* (MVFA); s.d., *Nadeaud s.n.* (P 2079836, P 2079837, P 2079838, P 2079839); s.d., s.col. (K 364473; W 12683, 43178, 43179).

***Distribution*:**—Previously, *B. gibertii* had been considered endemic to Uruguay (Izaguirre de Artucio 1973, Correa *et al.* 2009). After extensive fieldwork, we have found populations of this species in the state of Rio Grande do Sul, which are the first records of this species for Brazil.

***Phenology*:**—Flowering September to December, fruiting October to January.

***Habitat and ecology*:**—In Brazil, this species occurs at higher elevations (approx. 950 m), in natural grassy vegetation ("campos") surrounding forests of *Araucaria angustifolia* (Bertoloni 1819: 411–412) Kuntze (1898: 375), within the Atlantic Rain Forest Biome (Overbeck *et al*. 2007). In Uruguay, *Bipinnula gibertii* dwells in mountain environments (approx. 200 m) on rocky hillsides and ravines but also in open grasslands (Izaguirre de Artucio 1973, Correa *et al*. 2009).

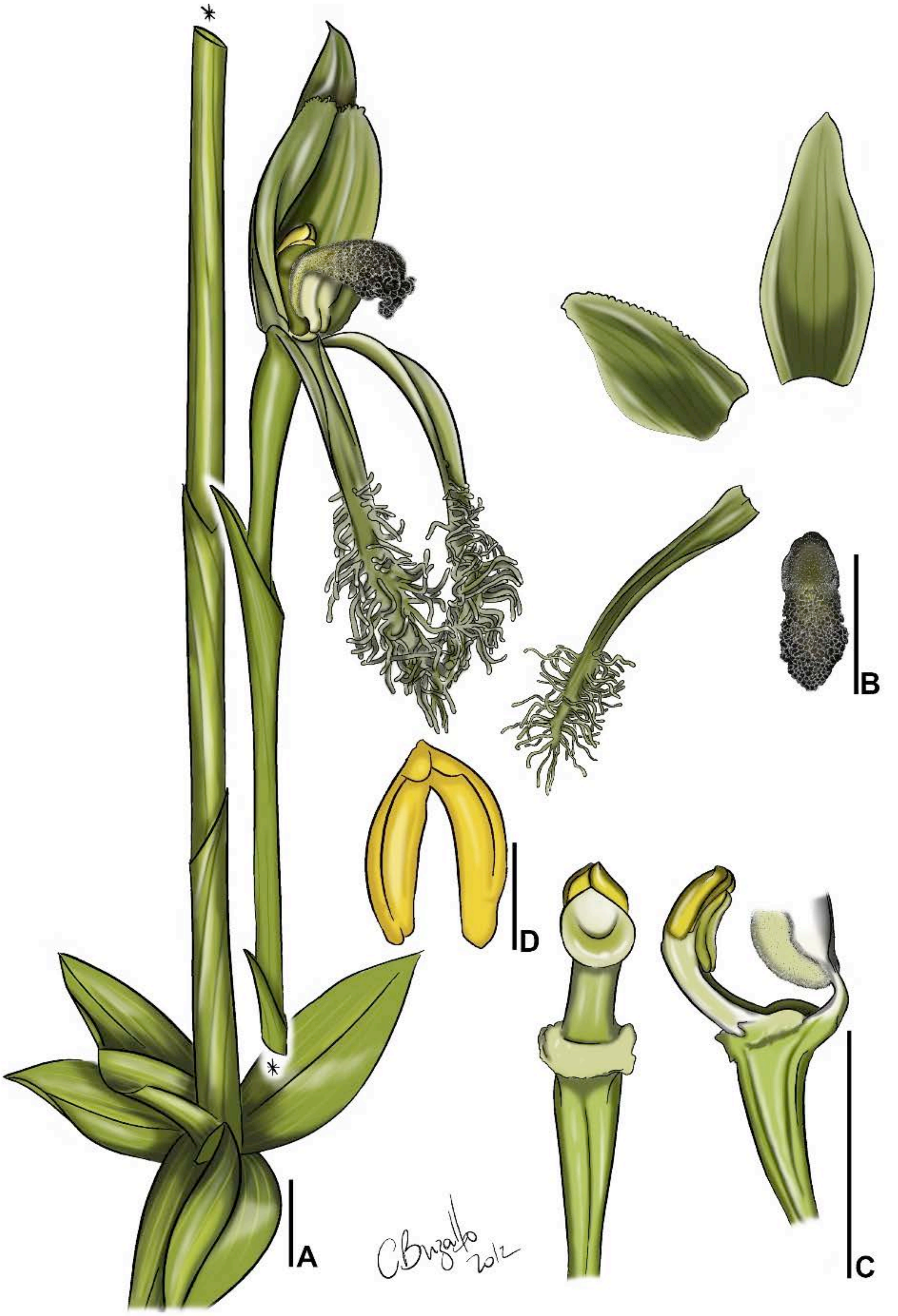

FIGURE 4. *Bipinnula gibertii* Rchb.f. A. Habit; B. Dissected perianth; C. Column, dorsal and lateral views; D. Pollinarium. Scale bars, 1 cm (A–C); 2 mm (D). Drawn by C.R. Buzatto from *Buzatto 726 & Nervo* (ICN).

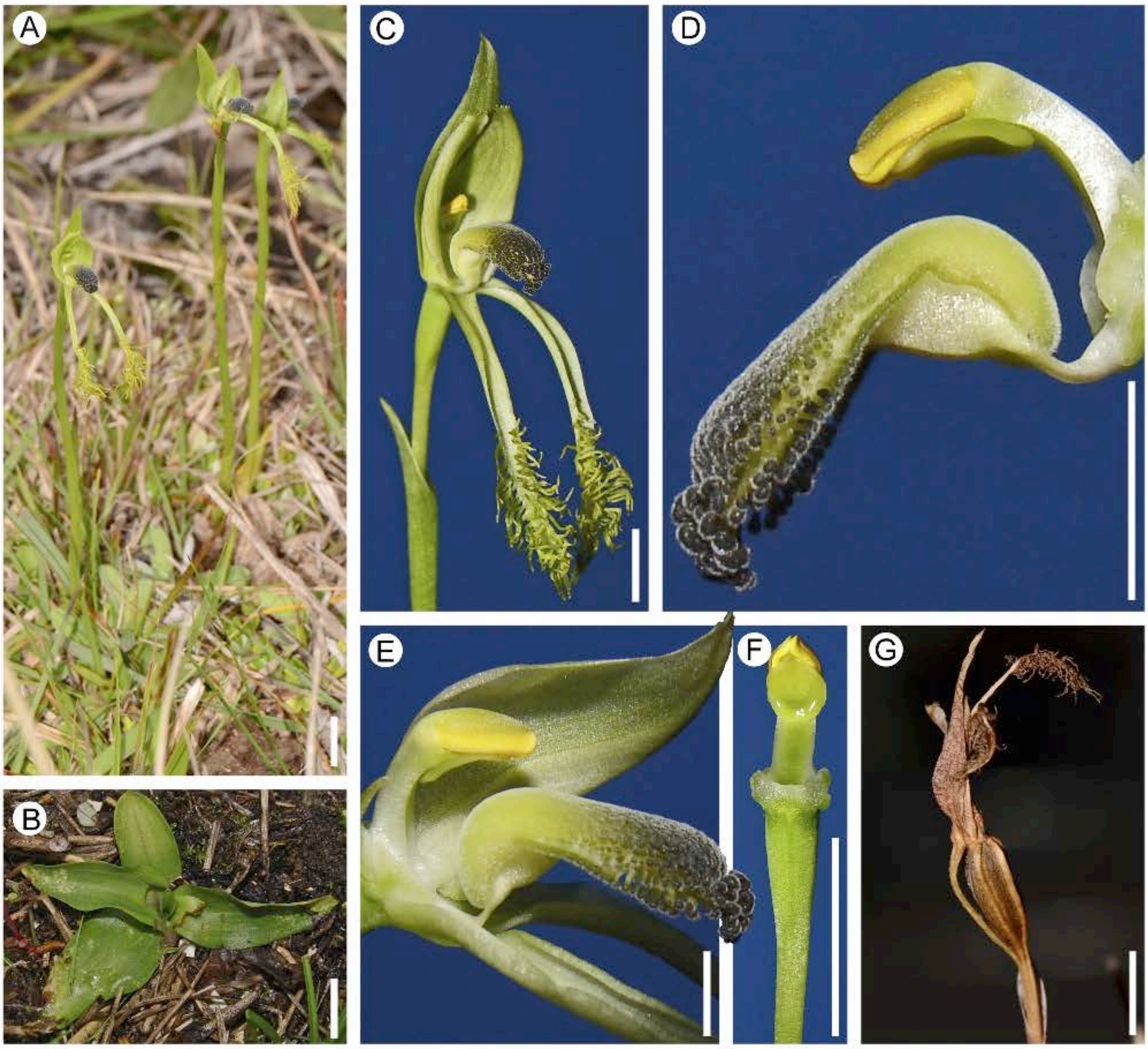

FIGURE 5. *Bipinnula gibertii* Rchb.f. A. Habit; B. Leaves; C. Flower; D. Column articulated with labellum; E. Detail of labellum; F. Column, dorsal view; G. Fruit. Scale bars, 2 cm (A); 1 cm (B–C, F–G); 5 mm (D–E).

***Notes*:—**Among Brazilian Chloraeinae, *B. gibertii* is easily recognized in the field by its delicate greenish flowers with a dark labellum bearing an insect-like, greyish apex (Fig. 5). Vegetatively, *B. gibertii* (Fig. 5B) is similar to *B. penicillata*, but usually has more leaves than the latter, and its leaves are more acute. As in *B. penicillata*, this species completely lacks leaves or they are wilted during anthesis.

When Reichenbach (1877) described this taxon, he simply cited "Em. Gibert!". Later he specifically cited, in his usual convoluted way (Reichenbach 1883), *Gibert 487* from Montevideo, Uruguay (W!). Duplicates of this collection are housed at K (000364475), the Rossengurtt's herbarium at the University of Uruguay (MVFA) and Paris (P 372111, indicated as "isotype"), available too in microfilm and catalogued under the number 260/12 (Burns-Balogh 1987). The specimen deposited at K is actually a compound sheet comprising four specimens (barcoded from K000364472 to K000364475). However, only K000354475 is part of *B. gibertii*'s type material. After a detailed analysis of the protologue and all these specimens, we conclude that the specimen deposited at W (W 43180) is the holotype, and the specimens at MVFA, K (000364475) and P (372111) are isotypes.

1.3. ***Bipinnula montana*** Arechavaleta (1899: 282). Type:—URUGUAY. Maldonado, "*Cerro de los Gigantes*", s.d., *Arechavaleta s.n.* (not located). Lectotype, designated here: plate 1 in Arechavaleta (1899: 283).

Heterotypic synonym: *Bipinnula canisii* Dutra ex Pabst (1959: 109). Holotype:—BRAZIL. Rio Grande do Sul, Porto Alegre, "*Morro da Polícia*", September 1933, *Orth 1180* (ICN!). Figs. 6, 7.

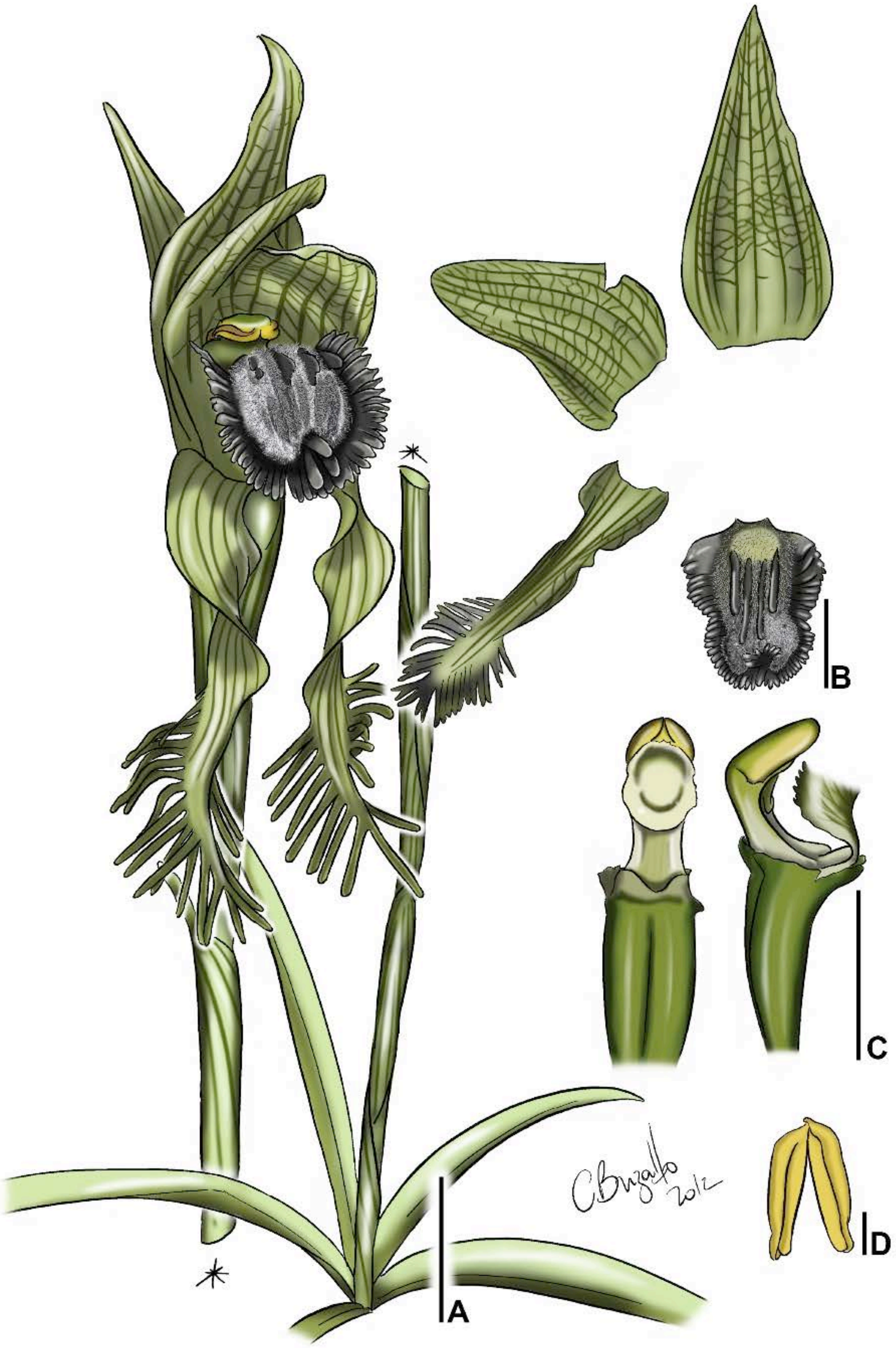

FIGURE 6. *Bipinnula montana* Arechav. A. Habit; B. Dissected perianth; C. Column, dorsal and lateral views; D. Pollinarium. Scale bars, 1 cm (A–C); 2 mm (D). Drawn by C.R. Buzatto from *Buzatto 645 & Singer* (ICN).

Herbs 15–40 cm tall, including inflorescence. Roots 4–8, 4–12 cm long, symmetric, oblanceolate, apex conical. Leaves 5–6, green, erect, linear-lanceolate, 10.0–15.0 × 2.0–2.5 cm, apex acute. Flowers light green, with conspicuous dark-green veins and labellum coloured in black and white. Dorsal sepal 3.7 × 1.7 cm. Lateral sepals 3.7 × 1.8 cm, twisted, fimbriate-pectinate in the apical third. Petals 2.7 × 1.7 cm, ovate, asymmetric, entire. Labellum 2.0 × 1.7 cm, clawed, dark grey, involute, slightly trilobed; the basal part densely tomentose; central disk white-colored, densely tomentose, with dark grey, glabrous, coriaceous, terete to conical, sometimes bifid, thickenings along its margin; apical part with (3–)4(–5) linear glabrous crests or carinae. Column 1.0 × 0.3 cm; pollinia 0.35 × 0.17 cm. Ovary slightly pear-shaped. Capsule 3–5 × 2–3 cm.

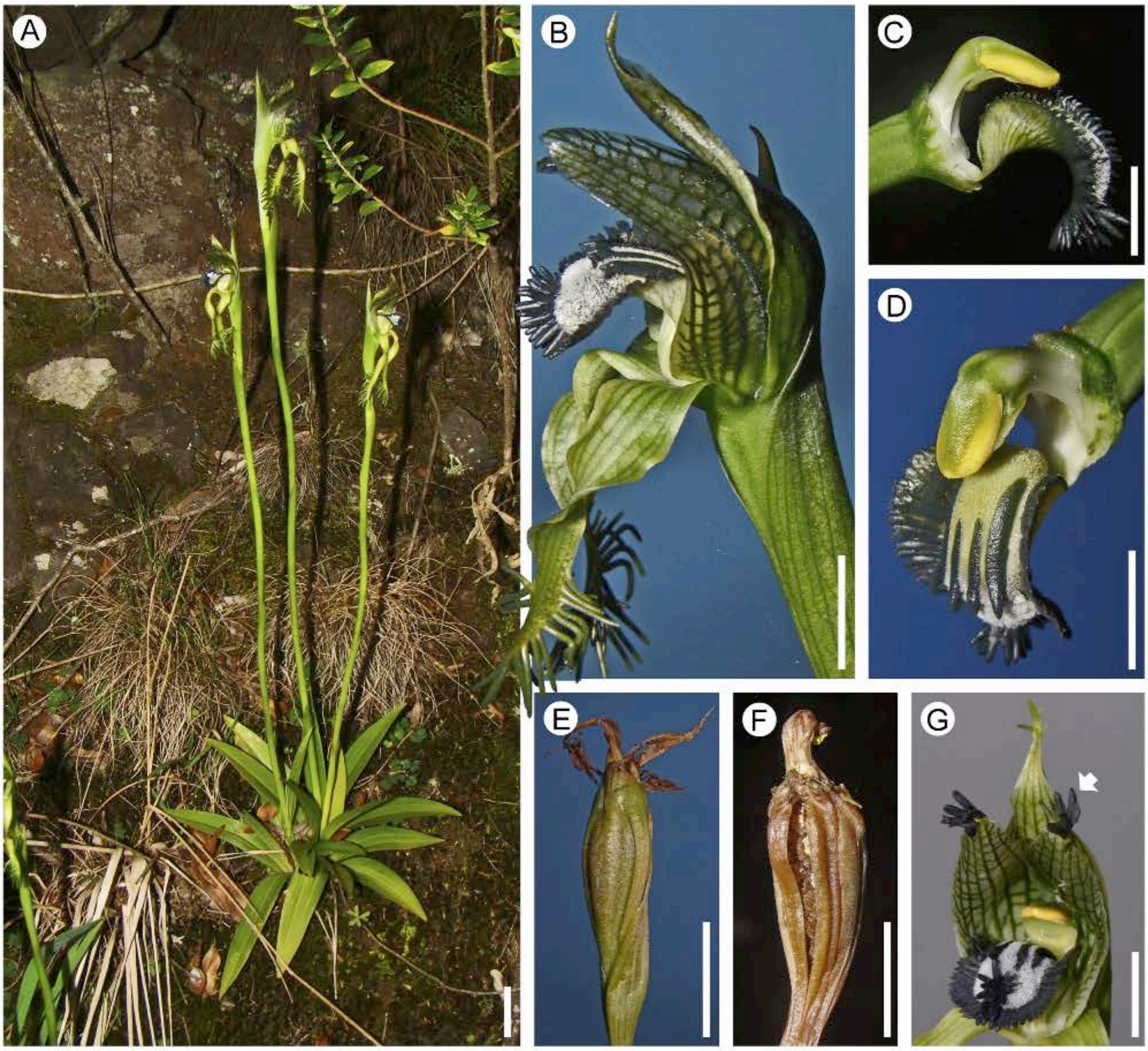

FIGURE 7. *Bipinnula montana* Arechav. A. Habit; B. Flower, lateral view; C. Column articulated with labellum; D. Detail of labellum; E–F. Fruit; G. Extremities of petals with clavate thickenings (arrow). Scale bars, 2 cm (A); 1 cm (B and D); 5 mm (C and G).

***Brazilian specimens examined*:**—Rio Grande do Sul: Bagé, 23 September 2013, *Buzatto 911 & Sanguinetti* (ICN, AMES, K); Caçapava do Sul, 9 October 2010, *Buzatto 645 & Singer* (ICN, AMES); Pinheiro Machado, 27 September 2013, *Torchelsen s.n.* (ICN); Porto Alegre, 21 August 1948, *Rambo 37555* (PACA); Porto Alegre, 9 September 1949, *Rambo 43342* (PACA); s.loc. 1816–1821, *Saint-Hilaire 2037* (P).

***Additional specimens examined*:**—URUGUAY. Banda Oriental: [Las Piedras], 1816–1821, *Saint-Hilaire C2 2037* (P); Lavalleja: Cerro Verdún, 16 October 2010, *Morrone 6316 & Giussani* (SI); Sierra de Polanco, 24 September 1993, *Bayce, Spreoni & Grela s.n.* (MVFA 22278); Maldonado: Cerro San Antonio, 25 November 1971, *Izaguirre 2791* (MVFA); Loreley, September 1932, *Herter 442* (GOET); Ruta 12 de Pan de Azúcar a Minas, 10 October 1963, *Arrillaga 1620, Izaguirre & Brescia* (MVFA); Sierra de las Ánimas, 21 September 1972, *del Puerto & Marchesi s.n.* (MVFA 11277); Sierras de Pan de Azúcar, 10 October 2012, *Sanguinetti 54* (BA); Rivera: Quebrada entre Masoller y Rivera, 1 September 1969, *del Puerto & Marchesi s.n.* (MVFA 2749).

***Distribution*:**—In Brazil, *Bipinnula montana* has only been recorded in the State of Rio Grande do Sul. This species is also known from Uruguay, restricted to the pampas (*sensu* Bilenca & Miñarro 2004).

***Phenology*:**—Flowering August to November; fruiting September to December.

***Habitat and ecology*:**—*Bipinnula montana* grows mainly in dry grasslands in granitic mountains at *c*. 140–280 m (Fig. 7A) and less frequently under shrubs. In Uruguay, it is found in mountainous environments (*c*. 115–220 m), in sunny areas and under trees in ravines (Izaguirre de Artucio 1973, Correa *et al*. 2009). *Bipinnula montana* is reported in the Rio Grande do Sul Threatened Flora List, under Endangered (EN) category (Assembléia Legislativa 2013). However, a detailed analysis is necessary to ascertain the conservation status of this species. All populations of *B. montana* observed by us in the field consisted of approximately 200 individuals scattered over extended dry grasslands.

***Notes*:**—Among Brazilian *Bipinnula* spp., *B. montana* is easily recognized by its relatively robust habit (Fig. 7A), proportionally large flowers with twisted lateral sepals (Figs. 6A, 7B) and tomentose, black and white labellum usually with 4 (rarely 3 or 5) dark apical crests (Figs. 7B, D). Regarding vegetative features, this species is similar to *B. biplumata*, sharing with this species long, linear, well-developed leaves that are normally present during anthesis (Figs. 6A, 7A).

This species has frequently been cited in Brazilian literature (Rambo 1965, Pabst & Dungs 1975) as *B. canisii*. Schinini *et al*. (2008) placed this name under synonymy of *B. montana* without giving a rationale for this taxonomic decision. However, after a carefully examination of the protologue of *B. canisii* and its type specimen (*Orth 1180*, ICN) and further collections attributed to this species, we agree with these authors (Schinini *et al*. 2008) in considering *B. canisii* a synonym of *B. montana*.

1.4. ***Bipinnula penicillata*** (Rchb.f.) Cisternas & Salazar in Cisternas *et al*. (2012a: 10); basionym: *Chloraea pennicillata* Reichenbach 1878: 51. Type:—ARGENTINA. "*Tierra del Fuego*", 1838, *Wilkes s.n.* (holotype AMES 106737!).

Homotypic synonyms: *Geoblasta penicillata* (Rchb.f.) Hoehne (1940: 98).

Heterotypic synonyms: *Geoblasta teixeirana* Barbosa Rodrigues (1891: 133). Type:— BRAZIL. Paraná, Curitiba, *Barbosa Rodrigues s.n.* (destroyed, *fide* Sprunger *et al*., 1996; Buzatto *et al*., 2011, 2013). Lectotype, designated here, Barbosa Rodrigues's original illustration at Biblioteca Barbosa Rodrigues, JBRJ, *Iconographie des Orchidées du Brésil*, vol. 1, tab. 34, holotype cited as tab. 865 [unpub.], Barbosa Rodrigues, 1891, reproduced in Sprunger *et al*., 1996, vol. 1: 87); *Chloraea arechavaletae* Kraenzlin (1888: 316).Type:— URUGUAY. Montevideo, "*entre las piedras de Independencia*", November 1874, *Arechavaleta 2615* (holotype K); *Asarca arechavaletae* (Kraenzl.) Kuntze (1898: 298); *Geoblasta arechavaletae* (Kraenzl.) Szlachetko & Margonska (2001: 125); *Chloraea bergii* Hieronymus (1880: 54). Type:— ARGENTINA. Provincia de Buenos Aires, Carmen de Patagones, s.d., *Berg s.n.* (holotype CORD!); Figs. 1B, 8, 9.

Herbs 10–20 cm tall including inflorescence. Roots approx. 10, 3–10 cm long, asymmetric, cylindrical, and clavate to turbinate, apex conical. Leaves 3–4, dark green, prostrate, oblong, 2.0–3.0 × 1.5–2.0 cm, apex slightly cuspidate. Flower light green, with reticulate dark green veins and an insect-like labellum with a darker, blackish-brown, apical part. Dorsal sepal 3.0 × 1.2 cm. Lateral sepals 3.0 × 0.7–1.0 cm, flat, entire. Petals 2.5 × 0.8 cm, ovate-lanceolate, slightly asymmetric, entire. Labellum 2.2 × 1.3–1.5 cm, sessile, smooth, greenish, convex, shiny, 2–3 mm thick, entire; the basal part covered by short, slender, retrorse, pale green hairs; central disk with 6–7 naked ridges; apex covered with short, clavate, blackish-brown and yellow appendices. Column 1.3 × 0.3–0.5 cm; pollinia 0.4–0.5 × 0.4 cm. Ovary cone-shaped. Capsule 1.5–1.8 × 1.2 cm.

***Brazilian specimens examined*:**—Paraná: Curitiba, 6 September 1909, *Dusén 6685* (MBM, NY); Curitiba, 5 September 1914, *Dusén 15495* (P, K, NY); Curitiba, September 1945, *Hatschbach 166* (MBM); Curitiba, 19 September 1948, *Tessmann s.n.* (MBM 213786); São Mateus do Sul, 16 December 1969, *Hatschbach s.n.* (MBM 12508). Rio Grande do Sul: Cambará do Sul, 28 October 2010, *Buzatto 648 & Singer* (ICN); Cambará do Sul, 3 September 2011, *Buzatto 730 & Nervo* (ICN); Cambará do Sul, 7 October 2013, *Buzatto 914, Singer & Sanguinetti* (ICN, AMES); Porto Alegre, 5 October 1932, *Rambo 368* (PACA); Porto Alegre, 7 September 1944, *Rambo 27211* (PACA); Porto Alegre, 27 November 1945, *Rambo 30623* (PACA); São Francisco de Paula, 5 October 2011, *Buzatto 744 & Singer* (ICN); São Francisco de Paula, 11 October 2012, *Nervo s.n.* (ICN, AMES). Without locality data, 1816–1821, *Saint-Hilaire C2 2037* (P); s.d., *Nadeaud s.n.* (P 372181).

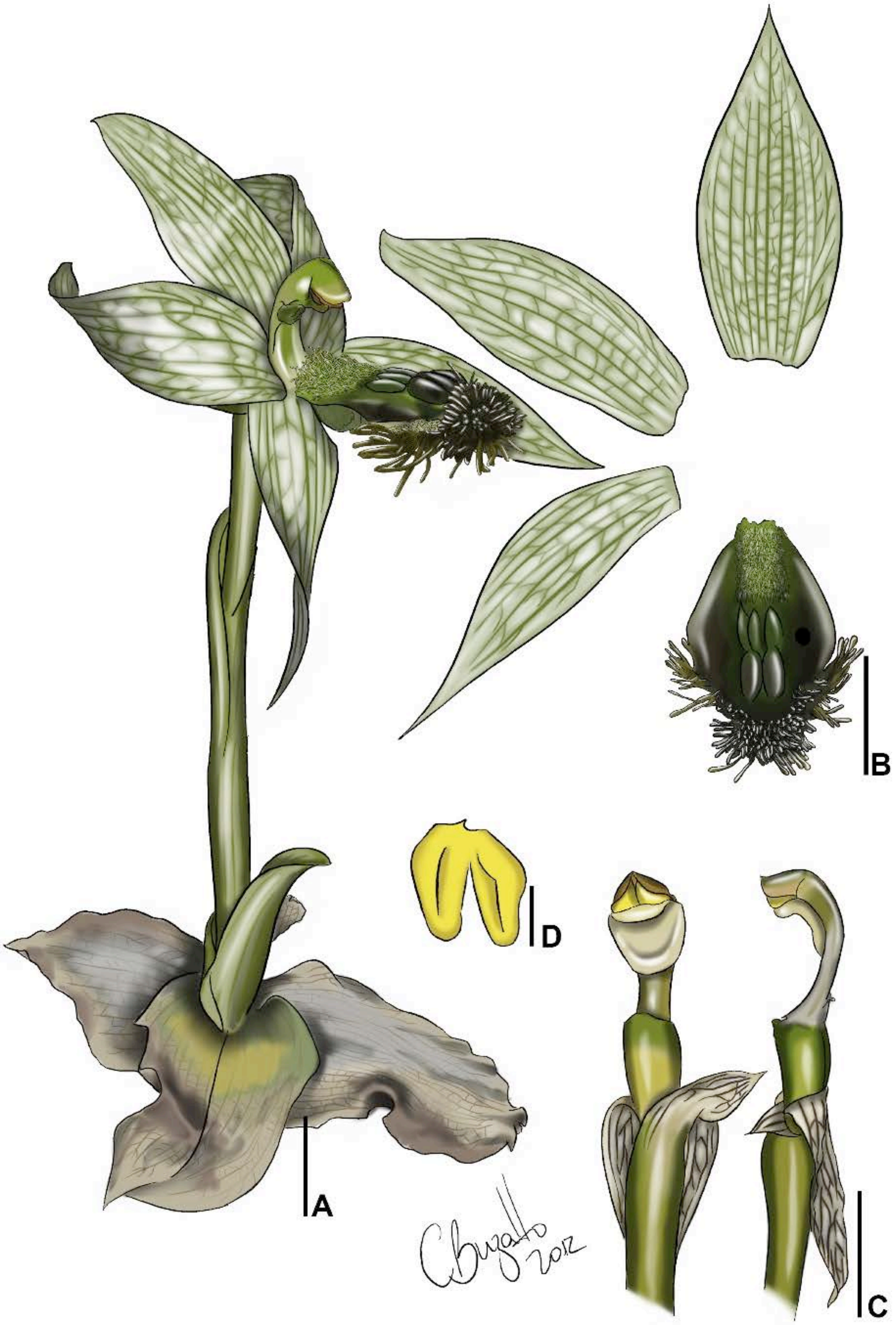

FIGURE 8. *Bipinnula penicillata* (Rchb.f.) Cisternas & Salazar. A. Habit; B. Dissected perianth; C. Column, dorsal and lateral views; D. Pollinarium. Scale bars, 1 cm (A–C); 2 mm (D). Drawn by C.R. Buzatto from *Buzatto 648 & Singer* (ICN).

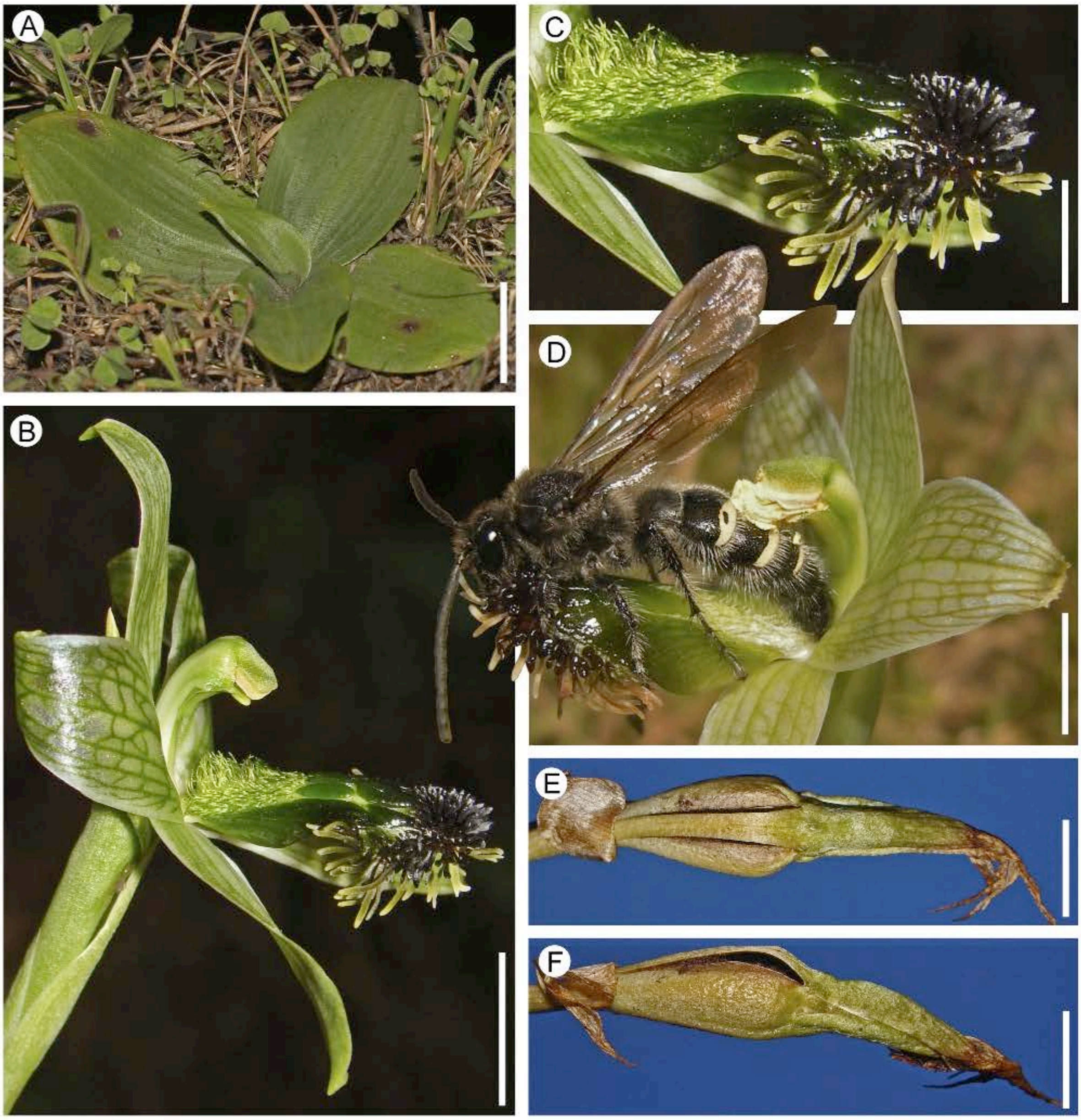

FIGURE 9. *Bipinnula penicillata* (Rchb.f.) Cisternas & Salazar. A. Leaves; B. Flower, side view; C. Detail of labellum; D. *Bipinnula penicillata* pollinated by *Campsomeris* (Scoliidae) male; E–F. Fruits. Scale bars, 1 cm (A–B); 5 mm (C–F).

Additional specimens examined:—ARGENTINA. Buenos Aires: Azul, 7 November 2012, *Sanguinetti 62* (BA); Balcarce, 31 October 1965, *Cabrera 17101* (LP); Coronel Suárez, 12 November 1928, *Scala s.n.* (LP 21791); General Alvarado, 3 December 1946, *Cabrera 10378* (SI); Olavarría, September 1909, *Spegazzini 2085* (LP); Pigüé, 8 November 1928, *Scala s.n.* (LP 21790); Saavedra, 4 November 1939, *Cabrera 5503* (LP); Tandil, 24 October 2012, *Sanguinetti 55* (BA); Tornquist, November 1895, *Spegazzini 2086* (LP); without locality data, 1893, *Gonnot s.n.* (P 372183, P 372184). URUGUAY. Banda Oriental: [Las Piedras], 1816–1821, *Saint-Hilaire C2 2140* (P); Canelones: Puerto Jackson, 31 October 1948, *s.col.* (MVFA B-5210); Colonia: Riachuelo, 11 October 1936, *Cabrera 3812* (SI); Lavalleja: Cerro Verdún, 20 October 1962, *Rosengurt & del Puerto 8665* (MVFA); Montevideo: Atahualpa, 27 October 1927, *Herter 631* (GOET); Carrasco, August 1871, *Fruchard s.n.* (P 372178); La Paz, 22 August 1876, *Fruchard s.n.* (P 372175); Montevideo, September 1828, *Gay s.n.* (P 372173); Montevideo, August 1871, *Fruchard s.n.* (P 372176); Montevideo, 31 August 1875, *Fruchard s.n.* (P 372174); Punta Gorda, 23 October 1910, *Osten 5290* (AMES); Rincon del Cerro, August 1871, *Fruchard s.n.* (P 372177); San José: Sierras de Mahoma, 21 October 1966. *s.col.* (MVFA 2602); Soriano: Vera, 25 October 1898, *s.col.*

(MVFA 435); Tacuarembó: Unidad Experimental La Magnolia, 11 October 1991, *sin col.* (MVFA 22146); *sin loc*., July 1868, *Gibert 488* (K).

Distribution:—*Bipinnula penicillata* occurs in northeastern Argentina, Uruguay and Brazil. In Brazil, this species is reported from Rio Grande do Sul to Paraná (Correa & Sánchez 2003). However, there are neither collections less than 50 years old from the state of Paraná, nor are there confirmed specimens from the neighbouring state of Santa Catarina. All recent collections come from the State of Rio Grande do Sul, where populations of this species have been found since 2006, after decades without records.

Phenology:—Flowering July to November; fruiting November and December.

Habitat and ecology:—*Bipinnula penicillata* grows in dry grasslands on rock outcrops in the pampas and Atlantic rain forest. *Bipinnula penicillata* is a sexually deceptive species, pollinated by males of *Campsomeris bistrimacula* (Lepeletier) (Scoliidae), as observed by Ciotek *et al*. (2006) in Argentina, that attempt copulation with the flowers. We have also observed the same wasps pollinating flowers of Brazilian populations (Fig. 9D).

Notes:—Among Brazilian species of *Bipinnula*, *B. penicillata* is easily distinguished by the insect-like labellum of the solitary flowers (rarely 2-flowered) devoid of fimbriate-pectinate lateral sepals (Figs. 8A,B, 9B). Regarding vegetative features, this species is similar to *B. gibertii* in its prostrate, ovate leaves that may be absent or deteriorating at anthesis (Figs. 8A, 9A). Indeed, *B. penicillata* normally has more leaves than *B. gibertii*, and its leaves have a slightly cuspidate apex.

When restablishing *Geoblasta*, Correa (1968) commented that this taxon shared a number of features with *Bipinnula* (especially with the single-flowered species group), suggesting a close relationship. This idea was later supported by phylogenetic analyses based on DNA sequence data (Chemisquy & Morrone 2010, 2012, Cisternas *et al*. 2012b). Consequently, Cisternas *et al*. (2012a) proposed the transfer of *G. penicillata* to *Bipinnula*. Remarkably, *B. penicillata* lacks the fimbriate-pectinate lateral sepals that are present in most species of the genus. However, the sepals of Chilean, multiflorous *B. apinnula* also are devoid of laciniae.

Most orchids described by Barbosa Rodrigues (1877, 1882, 1891, 1901, 1902, 1907, 1937 [1938]) have been lectotypified with the original illustrations (Buzatto *et al*. 2011, Koehler *et al*. 2012, Buzatto *et al*. 2013), currently housed at the Rio de Janeiro Botanical Garden, Brazil, and at the Orchid Herbarium of Oakes Ames, Harvard University (Cribb & Toscano 1996, Buzatto *et al*. 2011, Koehler *et al*. 2012, Buzatto *et al*. 2013). In agreement with these authors, we here designate this original watercolour (*Iconographie des Orchidées du Brésil*, vol. 1, tab. 34) as the lectotype of *Geoblasta teixeirana.* Szlachetko & Margonska (2001) considered two well-known synonyms (see Correa 1968) of *Bipinnula penicillata*, *Chloraea arechavaletae* and *C. bergii*, as species within *Geoblasta*. These combinations were later placed under synonymy of *B. penicillata* by Cisternas *et al*. (2012a). After having examined the respective type specimens, we conclude that these specimens in fact represent plants of *B. penicillata* and, therefore, we completely agree with the synonymy proposed by Cisternas *et al*. (2012a).

2. ***Chloraea*** Lindley (1827: 47)

Type species (designated by Correa 1969b):—*Chloraea virescens* (Willd.) Lindley (1827: 51); basionym: *Cymbidium virescens* Willdenow (1805: 106).

Asarca Lindley (1827: 52). Type species:—*Asarca speciosa* Lindley (1827: 52).

Ulantha Hooker (1830: 2956). Type species:—*Ulantha grandiflora* Hooker (1830: 2956).

Bieneria Reichenbach (1853: 3). Type species:—*Bieneria boliviana* Reichenbach (1853: 3).

Chileorchis Szlachetko (2008: 114). Type species:—*Chileorchis disoides* (Lindl.) Szlachetko (2008: 114); basionym: *Chloraea disoides* Lindley (1827: 47).

Correorchis Szlachetko in Szlachetko & Tukallo (2008: 115). Type species:—*Correorchis cylindrostachya* (Poepp.) Szlachetko (2008: 115); basionym: *Chloraea cylindrostachya* Poeppig (1833: 15).

Terrestrial herbs. Roots fascicled, cylindrical. Leaves more than one, erect in a basal rosette or cauline, lanceolate to elliptic, apex acute, margin entire; present or sometimes deteriorating at anthesis. Inflorescence terminal, more than 1-flowered, covered with several sheathing, reticulate bracts; floral bracts elliptic-lanceolate, acute. Flowers with dorsal slightly broader than lateral sepals, elliptic-lanceolate, slightly concave, margin entire, obtuse or acute. Lateral sepals free, narrower than dorsal sepal. Petals not longer than the dorsal sepal, obovate or lanceolate, equal or unequal, entire or with margin toothed, sometimes clawed, apex acute, membranous. Labellum with a claw, white, articulated to base of column, entire to trilobed, ecalcarate; lamina recurved, glabrous or with clavate projections with greyish-white stalks and black tips; margins entire or usually pinnately cleft, toothed or wavy.

Column curved, slightly swollen basally; column base generally bearing two cavities (foveae) that may extend to less than half the length of the ovary; column wings present and straight. Anther terminal, erect, persistent, with a reflexed apex, bilocular; pollinia two, oblong, imperfectly bipartite, yellow, granular and devoid of viscidium or any other pollinium stalk. Rostellum triangular. Stigma ventral, entire, concave, cuneate, ovate or oblong. Ovary plane-convex. Capsule erect, ellipsoid, opening by two dorsal lines of weakness (Sanguinetti et al. 2012). Seeds pale brown, club-shaped to kidney-shaped (Correa & Sánchez 2003).

Notes:—*Chloraea* has been revised by Correa (1969a). Some authors (Szlachetko & Tukallo 2008) proposed transfer of some *Chloraea* species to the newly proposed genera *Bieneria*, *Chileorchis*, *Correorchis* and *Ulantha*. However, these proposals were not based on a phylogenetic framework, but rather on biased interpretations of particular floral features (mostly labellum morphology). In spite of progress in phylogenetics of Chloraeinae (Chemisquy & Morrone 2010, 2012, Cisternas *et al*. 2012b), all recent analyses have important sampling gaps, such as species from northwestern Argentina, Bolivia and Peru. In this context, we concur with Cisternas *et al*. (2012b) on the need for refraining from making further taxonomic changes until relationships in the whole subtribe are better understood. In its current delimitation (Correa & Sánchez 2003), *Chloraea* comprises 45 species that, with two exceptions, are Andean.

Two basal cavities of the column (foveae, *sensu* Sanguinetti *et al*. 2012) have previously been considered a diagnostic feature of *Chloraea* (Correa & Sánchez 2003). However, some Patagonian *Chloraea* species lack these structures, whereas some Chilean and multiflorous species of *Bipinnula* have them (Cisternas *et al*. 2012b). This coupled with the fact that in some species, e.g. *C. membranacea*, *C. magellanica* Hooker (1847: 350–351), *C. philippii* Reichenbach (1849: 863) these foveae are shallow or poorly developed, suggests that this feature is more homoplasious than previously thought (Sanguinetti *et al*. 2012). Several authors have referred these cavities on several Andean *Chloraea* species (Correa 1969a, Clayton & Aizen 1996, Lehnebach & Riveros 2003, Humaña *et al*. 2008) as "nectariferous channels", even in clear absence of nectar. Since these structures are clearly non-secretory, the use of the term foveae has been suggested to replace "nectariferous channels" (Sanguinetti *et al*. 2012).

2.1. ***Chloraea membranacea*** Lindley (1840: 401). Type:—ARGENTINA. Buenos Aires: s.d., *Tweedie s.n*. (K!, illustration in W! and 68/6 in the microfiche edition, Burns-Balogh, 1987, available at AMES! and K!). Figs. 10, 11.

Heterotypic synonym: *Chloraea membranacea* var. *paranaensis* Schlechter (1927: 33). Type:—BRAZIL. Paraná: Curitiba, Nov. 1923, *Hatschbach 116* (B, not located, probably destroyed). Neotype, designated here: BRAZIL. Paraná: Curitiba, 2 Nov 1953, *Hatschbach 3291* (MBM 49065!).

Herbs 30–60 cm tall, including inflorescence. Roots 5–10, 4–12 cm long, oblanceolate, with basal annular thickenings. Leaves 4–7, greyish green, erect, elliptic-lanceolate, 15.0–21.0 × 3.0–4.5 cm. Inflorescence 8–15-flowered. Flowers greenish-white (rarely reddish-brown), with labellum covered by clavate, dark grey appendices. Dorsal sepal 2.1 × 0.9 cm, elliptic; acute. Lateral sepals 3.3 × 1.7 cm, elliptic, apex acute. Petals 1.5–2.0 × 0.8–1.0 cm, ovate, membranous, margin entire. Labellum 1.0 × 0.9 cm, shortly clawed, white, slightly trilobed, margin entire, wavy. Column 0.9–1.0 × 0.3–0.4 cm; pollinia 0.4 × 0.2 cm; stigma dumb-bell-shaped. Ovary 1.5–1.8 × 0.3–0.4 cm. Capsule erect, light brown, 1.8–2.3 × 1.5–2.0 cm.

***Brazilian specimens examined*:**—Paraná: Clevelândia, 22 November 1972, *Hatschbach 30832* (MBM); Curitiba, August 1909, *Dusén s.n.* (MBM); Curitiba, 25 October 1909, *Dusén 8900 & Lange* (AMES); Curitiba, 12 November 1977, *Kummrow 1203* (MBM); São Mateus do Sul, 16 December 1969, *Hatschbach 23261* (MBM). Rio Grande do Sul: Bom Jesus, 10 November 2009, *Buzatto 579 & Singer* (ICN); Canela, July 1952, *Richter s.n.* (AMES 2900); Farroupilha, 7 February 1950, *Rambo 45732* (PACA); Farroupilha, 5 November 1956, *Camargo 914* (PACA); Flores da Cunha, November 2011, *Pedron s.n.* (ICN); Ijuí, 5 November 1953, *Rambo 56055* (PACA); Jaquirana, 3 September 2011, *Buzatto 717 & Nervo* (ICN); Jaquirana, 1 October 2012, *Buzatto 849 & Sanguinetti* (ICN); Palmas, 31 October 2011, *Buzatto 745 & Singer* (ICN); Vacaria, 29 December 1951, *Rambo 52648* (PACA).

***Additional specimens examined*:**—ARGENTINA. Buenos Aires: Punta Lara, 29 October 1946, *Moreau s.n*. (AMES 628). URUGUAY. Canelones: Canelón Chico, 14 November 1908, *Berro 5431* (AMES); Montevideo: Durazno, 1888, *Alvarés s.n.* (P 340866); Malvin, November 1914, *Berro 7547* (AMES); Miguelete, October 1901, *Berro 1330* (AMES); Rochers de Malvino, November 1874, *Fruchard s.n.* (P 363403); Rochers de Malvino, 16 June 1877, *Fruchard s.n.* (P 363402). Without locality data, 1856, *Courbon 489* (P); November 1867, *Furchard s.n.* (P 363407); s.d., *Fruchard 489* (P).

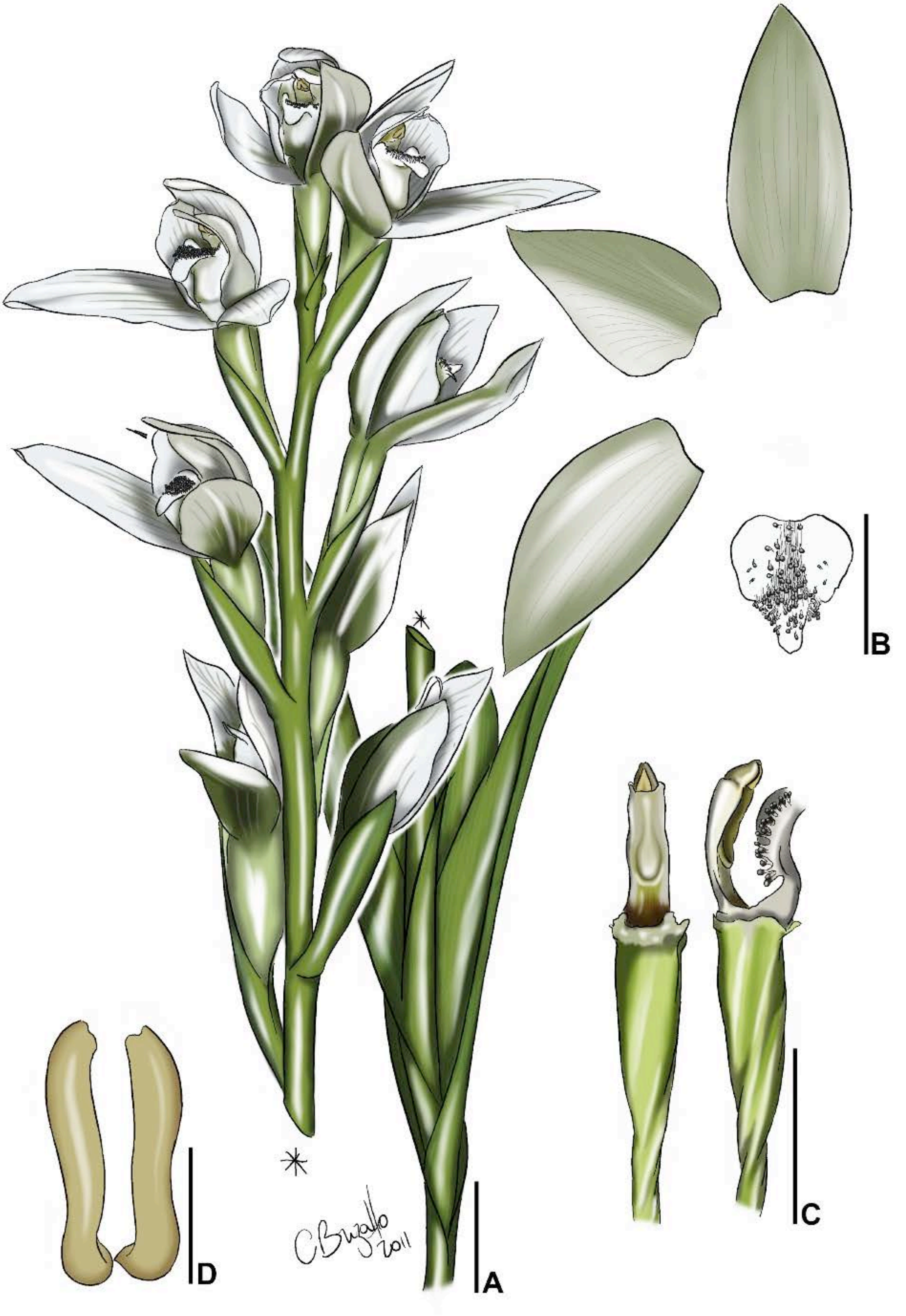

FIGURE 10. *Chloraea membranacea* Lindl. A. Habit; B. Dissected perianth; C. Column, dorsal and side views; D. Pollinarium. Scale bars, 1 cm (A–C); 2 mm (D). Drawn by C.R. Buzatto from *Buzatto 579 & Singer* (ICN).

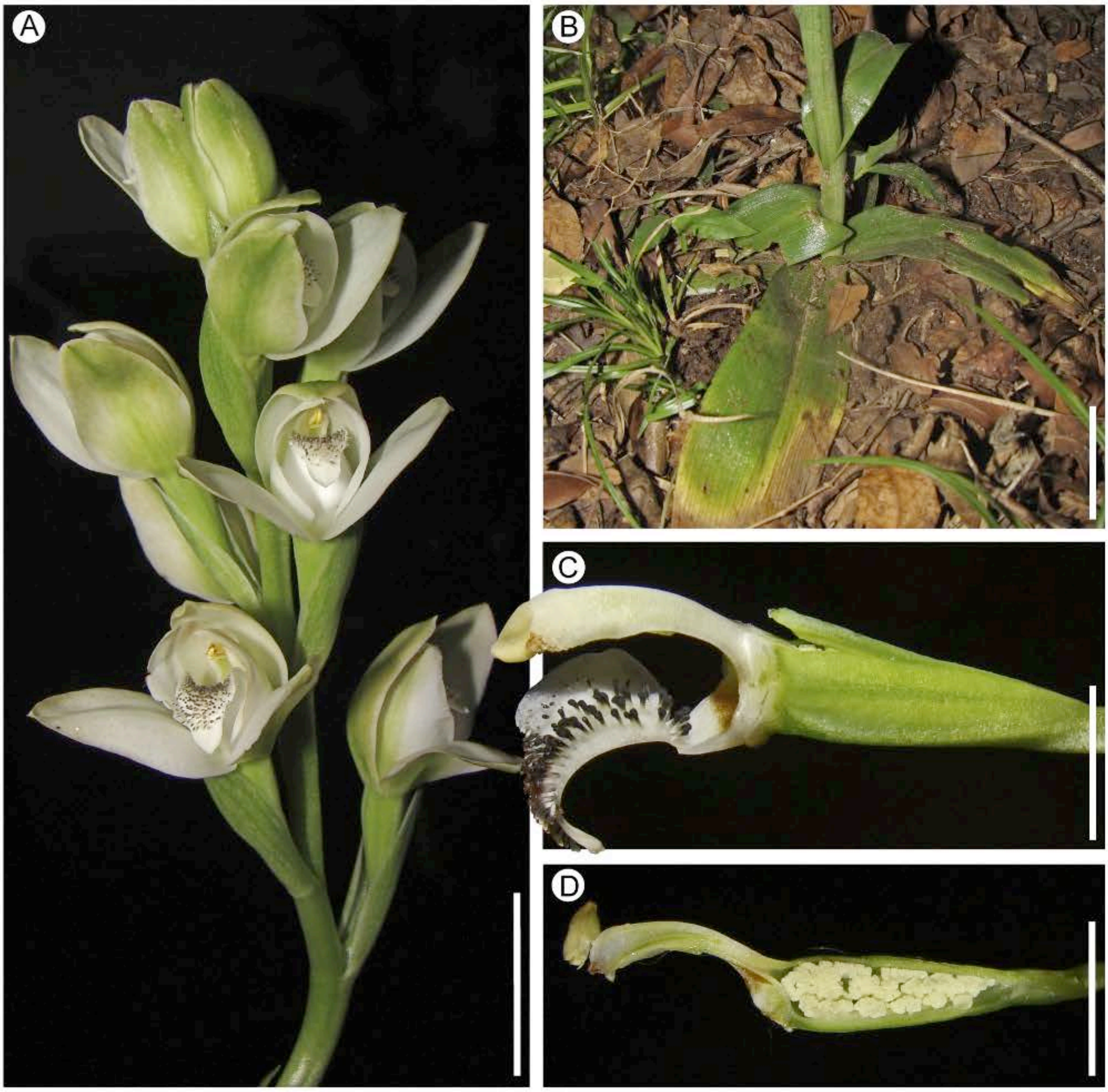

FIGURE 11. *Chloraea membranacea* Lindl. A. Inflorescence; B. Leaves; C. Column articulated with labellum; D. Section of ovary and column, lateral view. Scale bars, 1 cm (A–B); 5 mm (C–D).

Distribution*:—Chloraea membranacea* occurs in northeastern Argentina (from Buenos Aires Province northwards to Santa Fé, Entre Ríos and Corrientes), Uruguay and southern Brazil. In Brazil, this species has been recorded from Rio Grande do Sul and Paraná (Correa 1969a). *Chloraea membranacea* appears to be rare in Brazil, and all recent collections come from Rio Grande do Sul. The collections from Paraná date to more than three decades ago. To our knowledge, there are no known collections from Santa Catarina. We suggest the possibility of a continuous distribution of *C. membranacea* from Rio Grande do Sul to Paraná, similar to that of some *Bipinnula* species.

***Phenology*:—**Flowering July to December; fruiting November to February.

Habitat and ecology*:—Chloraea membranacea* grows in open grasslands or at the edge and interior of humid or xeric forests. Flowers of *C. membranacea* are nectarless and self-compatible, although pollinator-dependent (Sanguinetti *et al*. 2012). Pollinators are augochlorini bees (Halictidae; Sanguinetti *et al*. 2012). The basal foveae of the column are shallow and non-secretory, and dark-tipped appendices of the labellum are scent-emitting osmophores (Sanguinetti *et al*. 2012).

Notes*:—Chloraea membranacea* is the only multiple-flowered Chloraeinae species recorded for Brazil, and it is easily recognizable by its showy, greenish-white (rarely reddish-brown) membranous flowers with the labellum densely covered by grey-tipped appendices (Fig. 11C).

As for most orchid types deposited at B, the type collection of *C. membranacea* var. *paranaensis* was likely destroyed during World War II. After a detailed search there, we could not find this specimen, and this particular type is not mentioned by Butzin (1978, 1981) in his compilation of the extant orchid types at B. We therefore here propose *Hatschbach 3291* (MBM 49065) as neotype forof *C. membranacea* var. *paranaensis* because that specimen is in full agreement with the protologue and has the same provenance (Curitiba, Paraná) as the original type specimen.

Chloraea membranacea and *C. bella* (endemic to Entre Rios, Argentina and neighbouring areas of Uruguay) constitute the so-called "eastern-*Chloraea* group" (Correa 1969a, Hauman 1922). So far, these are the only non-Andean species of *Chloraea* and share some important floral features, such as the dumb-bell-shaped stigma that occupies 2/3 of the length of the column and column wings more or less adjacent to the stigma (as opposed to cuneate, ovate or oblong in other *Chloraea* species; Correa & Sánchez 2003).

Unrecognized taxon

Bipinnula ctenopetala Schlechter (1925: 24). Type:—BRAZIL. Rio Grande do Sul: Porto Alegre, January 1910, *Wendt s.n.* (B, probably destroyed).

***Notes*:—**The type collection of this species (*Wendt s.n.*) deposited at B, like most orchid types there, was probably destroyed during World War II (Merril 1943, Hiepko 1987, Butzin 1978, 1981). After an exhaustive search at B, we could not find this specimen. Our detailed search in Brazilian and foreign herbaria failed to bring to light additional specimens that could represent *B. ctenopetala.* Although Pabst & Dungs (1975, 1977) have referred this species to Brazil (Rio Grande do Sul), they did not provide voucher information and/or illustrations supporting its presence. Unfortunately, Schlechter (1925) only cited the specimen *Wendt s.n.* in the protologue and did not provide an illustration that could be considered as a candidate for a lectotype. Schlechter (1925) mentioned affinities to *B. montana* but considered that the main difference are clavate thickenings on the petals. Based on our observations in large populations of *B. montana*, this feature appears only abnormally (less than 1% of specimens examined) and can be attributed to a lateral heterotopy, that is, the expression of a labellar feature in the organs of the same whorl (Bateman & Rudall 2006; Fig. 7G). The description of the protologue together with the type locality strongly suggest that *B. ctenopetala* should probably be considered a synonym of *B. montana* based on the shared four glabrous carenae on a densely tomentose labellum (Figs. 6A,B, 7D; Schlechter 1925). However, due to the lack of definitive proof supporting *B. ctenopetala* as a synonym of *B. montana*, we prefer to consider this a dubious name.

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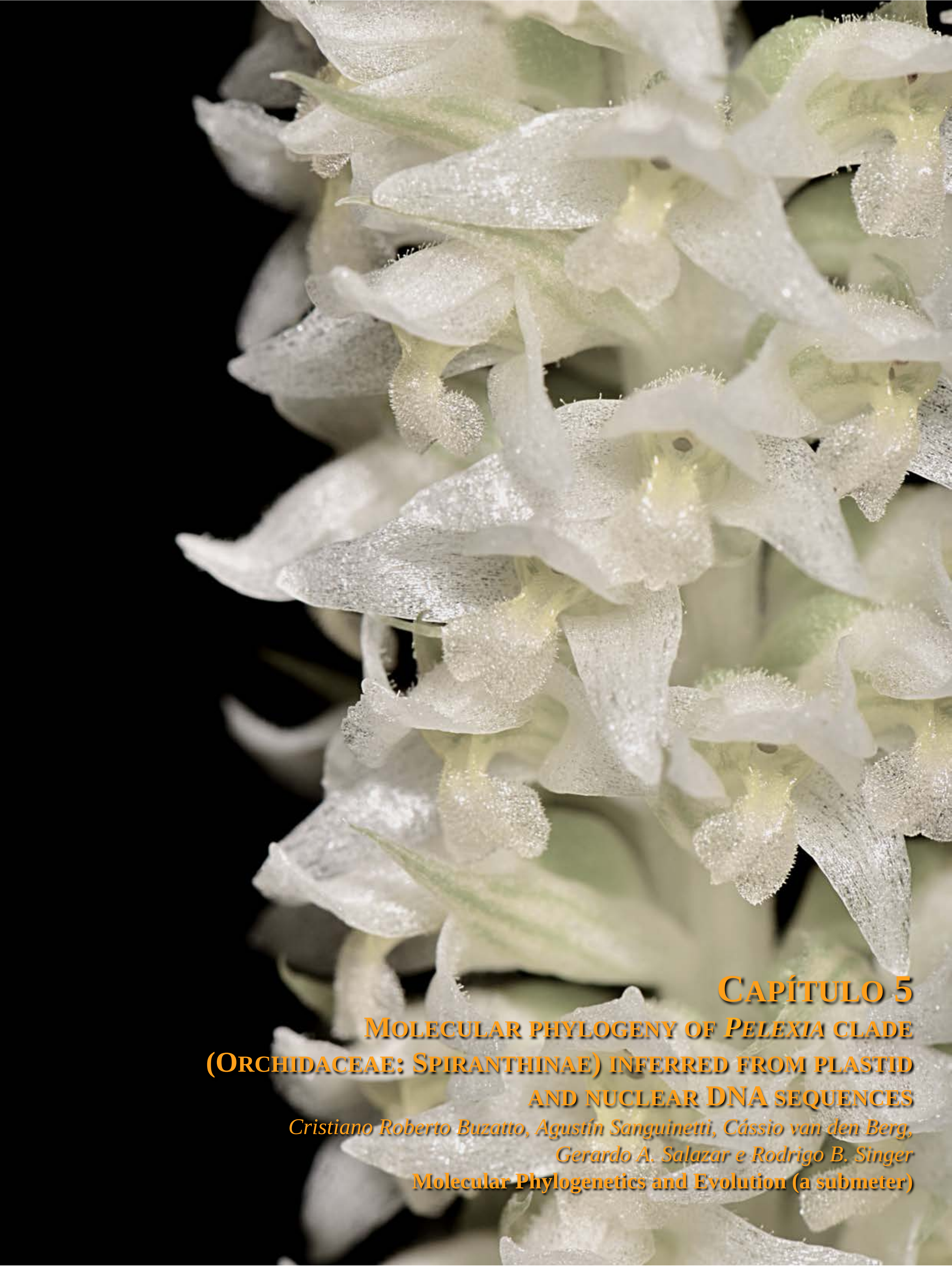
SEÇÃO 2

FILOGENIA DO CLADO *PELEXIA* E CHLORAEINAE



Capítulo 5

Molecular phylogeny of *Pelexia* clade (Orchidaceae: Spiranthinae) inferred from plastid and nuclear DNA sequences

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Molecular Phylogenetics and Evolution (a submeter)

Molecular phylogeny of *Pelexia* clade (Orchidaceae: Spiranthinae) inferred from plastid and nuclear DNA sequences

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Abstract: We sequenced nuclear ITS and the *matK-trnK* and *trnL-trnF* plastid regions for 154 samples representing *Brachystele*, *Coccineorchis*, *Cyclopogon*, *Odontorrhynchos*, *Pelexia*, *Sarcoglottis*, *Sauroglossum* and *Veyretia*, and representative species of generic segregates (e.g. *Beadlea*, *Cocleorchis* and *Warscaea*), in order to examine the phylogenetic relationships of *Pelexia* clade (Spiranthinae, Orchidaceae). Phylogenetic analyses indicate that both *Coccineorchis* and *Sarcoglottis* are monophyletic, whereas *Sauroglossum* is paraphyletic. A broad *Cyclopogon* circumscription including *Brachystele guayanensis* and all *Veyretia* species is strongly supported, whereas *Brachystele* and *Pelexia* are polyphyletic. *Odontorrhynchus* samples grouped to *Brachystele unilateralis*. Like in *Sauroglossum*, *Brachystele* flowers are protandrous and present a ventrally-adhesive viscidium. On the other hand, *Pelexia weberbaueri* is strongly supported in a group recognized here as *Synassa*. *Pelexia* sect. *Pachygenium* is monophyletic, and should be recognized as a separate genus *Pachygenium*. *Pelexia* sect. *Pelexia* and *P*. sect. *Centropelexia* constitute a single group, thus *Pelexia s.s*. Although the *Pelexia* clade is strongly supported as a monophyletic group in our analyses, most genera are clearly non-monophyletic in their current delimitation. Hopefully, the clades recovered in our analyses will be supported with sets of non-molecular characters that with help in a well-supported new generic delimitation.

Key words: *Brachystele*, *Coccineorchis*, *Cyclopogon*, nrITS, *matK-trnK*, *Sarcoglottis*, *Sauroglossum*, *Synassa*, *trnL-trnF*

1. Introduction

Spiranthinae is a predominantly terrestrial orchid subtribe of approximately 40 genera and 470 species, mainly restricted to the New World, with centres of diversity in Brazil and Mexico (Salazar, 2003; Salazar et al., 2003). Although most species are terrestrial, the species of *Lankesterella* Ames and *Eurystyles* Wawra are exclusively epiphytes (Salazar, 2003; Salazar and Dressler, 2011). In spite of its enormous morphological diversity (Fig. 1), most Spiranthinae taxonomic delimitations have largely relied on floral characters, especially those of the rostellum and viscidium (Salazar, 2003; Salazar et al., 2003).

The generic delimitation of Spiranthinae started with Lindley (1840), who recognised five genera, four of which (*Pelexia* Poit. ex Lindl., *Sauroglossum* Lindl., *Spiranthes* Rich. and *Stenorrhynchos* Rich. ex Spreng.) still remain placed in this subtribe. However, only in the early 20th century, when Schlechter (1920, 1926) proposed the first taxonomic revision of the subtribe, a sketch for the internal classification of Spiranthinae was proposed, recognizing 24 genera divided in four groups on the basis of rostellum and viscidium features. Similarly to Schlechter’s (1920, 1926) groups, Balogh (1982) accepted only 16 genera and in four alliances (*Brachystele*, *Eurystyles*, *Pelexia* and *Stenorrhynchos* alliances). Balogh (1982) slightly increased the number of characters to support her groups (rostellum and pollinarium shape, position of the stylar canal entrance, position of the lateral sepals, and type of viscidium). In the same year, Garay (1982) inflated Spiranthinae to 44 genera, many segregated by himself, but delimitated in a very superficial way. It is important to stress that Garay (1982) explicitly refrained from a phylogenetic classification and clearly stated that he intended for genera recognizable on morphological grounds only. Not surprisingly, several of his newly proposed taxa are ill-defined on morphological grounds. Although Garay (1982) mentioned the structure of rostellum as a significant character, he considered the position of stigma (“terminal” *vs.* “anterior”) and the fusion of dorsal sepal with the lateral sepals more important as a diagnostic character within Spiranthinae.

Recent molecular phylogenetic analyses have supported Spiranthinae as a monophyletic group made up by four major suprageneric groups: 1) *Stenorrhynchos* clade; 2) *Pelexia* clade; 3) *Eurystyles* and *Lankesterella* clade, and 4) *Spiranthes* clade (Salazar et al., 2003; Figueroa et al., 2008; Salazar et al., 2009; Batista et al., 2011; Salazar et al., 2011; Salazar and Dressler, 2011; Salazar and Jost, 2012; Borba et al., in press). Despite the fact that each clade is strongly supported, the circumscription of many genera has been controversial. However, in all precedent analyses, representatives of non-Andean South-American taxa are either lacking or have been poorly sampled.

This contribution presents a broad and representative sampling of the *Pelexia* clade. The aim of the present study is to use phylogenetic analysis to evaluate the generic circumscription within the *Pelexia* clade.

2. Material and methods

2.1. Taxon sampling

A total of 206 terminals were used representing 151 species from the six subtribes of Cranichideae (Lindl.) Endl. Of these species, 48 (20 genera) were considered outgroups and 101 (154 samples) belong to genera of the *Pelexia* clade (*sensu* Salazar, 2003; Salazar et al., 2003; Figueroa et al., 2008; Salazar et al., 2009; Borba et al., in press). They cover most of the morphological variability and geographical distribution of *Brachystele* Schltr., *Coccineorchis* Schltr., *Cyclopogon* C.Presl, *Odontorrhynchos* M.N. Correa, *Pelexia*, *Sarcoglottis* C.Presl, *Sauroglossum* and *Veyretia* Szlach., representing approximately 41 % of the species currently included in the aforementioned genera. Number of species, and species sampled in each genus are show in Table 1. It is important to stress that our sampling also includes representative species of generic segregates proposed by Garay (1982), Szlachetko (1994) and Rutkowski et al. (2008). Yet, for the purposes of this contribution within Spiranthinae we follow the generic circumscriptions of Salazar (2003). Leaf samples were collected from natural habitats, dried, and preserved in silica gel (Chase and Hills, 1991). Voucher information, geographic origin and GenBank accession numbers are listed in Table 2.

2.2. DNA extraction, amplification and sequencing

Total DNA was extracted from leaves dried in silica gel using standard method explained in Salazar et al. (2011). In this study, all analyses were performed with nucleotide sequences of nuclear (nrITS) and plastid (*matK-trnK* and *trnL-trnF*) DNA, which were amplified with the same primers and PCR programs described in Salazar et al. (2003) and Figueroa et al. (2008). PCR products were purified with PEG 11% (polyethylene glycol), and were bidirectionally sequenced with the same primers used for the PCR amplification. Sequence eletropherograms were produced in an automatic sequencer (ABI 3130XL Genetic Analyzer) using Big Dye Terminator 3.1 (Applied Biosystem). The resulting chromatograms were edited and assembled using Geneious 5.6.5 (Biomatters, Aukland, New Zealand available from www.geneious.com). The edited sequences were aligned with MAFFT (Katoh et al., 2002), and manually adjusted using Seaview v4 (Gouy et al., 2010). The aligned matrix consisted of 5,416 characters, of which 783 correspond to the ITS, 2,260 to the partial *matK-trnK* and 2,373 to the *trnL-trnF*.

2.3. Model test

All DNA regions were partitioned into four or three parts based on molecular evolution features (nrITS: ITS1, 5.8S, ITS2, and rRNA; *matK-trnK*: tRNA-Lys, matK, and tRNA-Lys; *trnL-trnF*: intron, tRNA-Leu, and intergenic spacer). The optimal evolutionary model for each partition was selected using jModelTest (Posada, 2008), under Bayesian Information Criterion (BIC). The substitution model SYM+G was selected for the ITS1, rRNA, matK, tRNA-Lys, intron and intergenic spacer, the model K80+G for both 5.8S and ITS2, and JC+G for tRNA-Leu.

2.4. Phylogenetic analysis

The data were analysed by means of maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI). We first inferred the trees for each of the three DNA regions separately to identify potential incongruence. As there were no instances of conflict at well-supported nodes, as well as showed in Salazar et al. (2003, 2011), the matrices of the three regions were concatenated and partitioned in their constituents as previously described (see Model test section).

Maximum parsimony analyses were performed using PAUP* version 4.0b 10 (Swofford, 2002) with Fitch parsimony (equal weights, unordered characters; Fitch, 1971) as the optimality criterion. The MP trees were obtained through heuristic searches, each with 2,000 random taxon-addition replicates, with branch swapping using the tree-bisection and reconnection (TBR) algorithm, saving up to 15 trees per replicate to prevent extensive swapping on suboptimal trees in islands with many trees. The resulting trees were then used in a second search with the same parameters of the first, however without limit for the number of trees per replicate. Parsimony bootstrap percentages (BP_P) were generated with 1,000 pseudoreplicates (Felsenstein, 1985), with simple taxon-addition and the TBR algorithm, saving up to 20 trees per replicate. The BP_P limits considered in this study were: 50–70% as weak, 71–85% as moderate, and >85% as strong (Kress et al., 2002).

The ML analysis was conducted with the program RAxML-HPC2 version 7.6.3 (Stamatakis, 2006) as implemented in the Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal 2.0 (Miller et al., 2010). Analysis of 1,000 rapid bootstrap replicates (BP_{ML}) (Stamatakis et al., 2008) was followed by a search for the tree that maximizes the likelihood function, with the default value of 30 rate categories and estimation of all free model parameters for three character partitions (ITS, *matK-trnK*, and *trnL-trnF*). Both the BP_{ML} searches and the search for the ML tree used the GTRGAMMA model for nucleotides.

The BI analysis of combined dataset was implemented in MrBayes v. 3.2.1 with mixed models (Huelsenbeck and Ronquist, 2001; Ronquist et al., 2012). Each analysis consisted of two independent runs with four chains for 5,000,000 generations, sampling one tree every 1,000 generations. In the combined analysis, in order to improve swapping of chains, the temperature parameter for heating the chains was lowered to 0.01. Convergence between the runs was evaluated by the average standard deviation of split frequencies (<0.01). After discarding the first 50% of the trees as burn-in, the remaining trees were used to assess topology and posterior probabilities (PPs) in a majority-rule consensus. PPs in Bayesian analysis are not equivalent to BPs, but are generally much higher (Erixon et al., 2003). Therefore, we used criteria similar to a standard statistical test, considering groups with PPs >0.95 as strongly supported, groups with PPs ranging from 0.90–0.95 as moderately supported, and groups with PPs <0.90 as weakly supported.

3. Results

Of the 5,416 characters of the aligned matrix, 1,560 were potentially informative to parsimony. The MP analysis found a single most-parsimonious tree (MPT) with a length of 3,925 steps, CI (excluding uninformative characters) of 0.38 and RI of 0.62. Both MP and ML trees found, are topologically identical to the BI tree, and are depicted in Figs. 2–5. Branch lengths for ML analysis and bootstrap support from the MP, ML and IB analyses are shown on that tree (Figs. 2–5).

Spiranthinae *sensu* Salazar (2003) obtained strong support (BP_P 100; BP_{ML} 100; PP 1.00), with *Cotylolabium lutzii* (Pabst) Garay as the sister of the rest of the subtribe. The same four major suprageneric groups recurrently found in previous molecular phylogenetic analyses of

Spiranthinae (e.g. Salazar et al., 2003; Salazar and Ballesteros-Barrera, 2010; Salazar and Dressler, 2011; Salazar et al., 2011; Salazar and Jost, 2012; Batista et al., 2011) were also recovered here; they are labelled in Figs. 2-5 as follows: a) *Eurystyles* and *Lankesterella* clade (BP_{ML} 62; PP 0.97; clade A; Fig. 2); b) *Spiranthes* clade (BP_{ML} 72; PP 0.72; clade B; Fig. 2); c) *Stenorrhynchos* clade (BP_{P} 91; BP_{ML} 94; PP 1.00; clade C; Fig. 2); and d) *Pelexia* clade (BP_{P} 68; BP_{ML} 95; PP 1.00; clade D; Figs. 2-5). The clades A and B that grouped with *Degranvillea dermaptera* Determann formed a moderate supported group (BP_{P} 71; BP_{ML} 71; PP 0.74).

The *Pelexia* clade (*sensu* Salazar, 2003) was recovered as a highly-supported monophyletic group in both ML and BI analyses (BP_{ML} 95; PP 1.00; clade D; Figs. 2-5). Yet, all analyses pointed out that the genera *Brachystele*, *Cyclopogon*, *Pelexia*, *Sarcoglottis* and *Sauroglossum* are not monophyletic in their current delimitation (Salazar, 2003) (see Discussion for each respective genus).

4. Discussion

4.1. Relationships of Spiranthinae including Cotylolabium *and* Degranvillea

With the inclusion of both monospecific genera *Cotylolabium* Garay and *Degranvillea* Determann in our analyses, the internal relationships of Spiranthinae are changed, when compared to previously published phylogenies (Salazar et al., 2003; Figueroa et al., 2008; Salazar et al., 2009; Batista et al., 2011; Salazar et al., 2011; Salazar and Dressler, 2011; Salazar and Jost, 2012; Borba et al., in press). The phylogenetic position of *Cotylolabium* as sister of the rest of Spiranthinae has been corroborated, as recently shown by Borba et al. (in press). *Cotylolabium lutzii* has a lip divided in two chambers by a trabecula, a unique feature in Cranichideae (Borba et al., in press).

The position of *Degranvillea* is still unclear. Our MP analysis (not shown) has placed *Degranvillea dermaptera* embedded within the *Eurystyles* and *Lankesterella* clade, as sister of

Eurystyles species. Nevertheless, a detailed morphological study of this taxon is lacking. Conversely, the inclusion of other DNA plastid regions should provide a more reliable overview of its affinities within Spiranthinae.

4.2. Phylogenetic relationships, taxonomic implications and pollination systems within the Pelexia *clade*

The *Pelexia* clade comprises eight subclades herein treated as follows: *Coccineorchis* (clade D1; Fig. 2), *Sauroglossum* (clade D2; Fig. 2), *Cyclopogon s.l.* (clade D3; Fig. 3), *Sarcoglottis* (clade D4; Fig. 4), *Synassa* Lindl. (formerly, part of *Sauroglossum*) (clade D5; Fig. 5), *Pelexia* sect. *Pachygenium* Schltr. (clade D6; Fig. 5), *Pelexia s.s.* (clade D7; Fig. 5) and *Brachystele* (clade D8; Fig. 5).

Our phylogenetic analyses confirm the overall Spiranthinae generic relationships proposed by Salazar et al. (2003, 2009, 2011) and Borba et al. (in press). However, the sampling of representatives of the *Pelexia* clade was very small in all preceding phylogenetic studies (Salazar et al., 2003; Figueroa et al., 2008; Salazar et al., 2009; Batista et al., 2011; Salazar et al., 2011; Salazar and Dressler, 2011; Salazar and Jost, 2012; Borba et al., in press). Therefore, it has not been possible, so far, to address intra and intergeneric relationships within the *Pelexia* clade (Salazar, 2003; Salazar et al., 2003). Our sampling represents approximately 41% of the species referable to this group. Indeed, taxa such as *Veyretia* (clade D3; Fig. 3), many of the genera segregated from *Cyclopogon* (*sensu* Garay, 1982; Szlachetko, 1994; Rutkowski et al., 2008; clade D3; Fig. 3) and representative species of *Pelexia* sect. *Pachygenium* (Schlechter, 1920; clade D6; Fig. 5) were sampled for the first time. All our analyses recovered *Synassa* (formerly, part of *Sauroglossum*) (clade D5; Fig. 5) as the sister-group of *Pelexia* sect. *Pachygenium*, *Pelexia s.s.* and *Brachystele* (clades D6, D7 and D8, respectively; Fig. 5). Discussions regarding each genus are presented below.

4.2.1. Coccineorchis*: clade D1*

Despite the low support for the MP analysis (BP_P 68), *Coccineorchis* is strongly supported by ML and BI analyses (BP_{ML} 95; PP 1.00, respectively) in which it is sister to the rest of the *Pelexia* clade genera (Fig. 2), as has been previously demonstrated (Salazar et al., 2003, 2009, 2011; Batista et al., 2011; Borba et al., in press).

Coccineorchis comprises about four species, occurring in mountainous regions from Nicaragua to Bolivia and, it is characterized for their tubular, bright yellow to orange-red coloured flowers (Salazar, 2003). *Coccineorchis* was initially inserted into "Raphiorhyncha" group (Schlechter, 1920), with *Lyroglossa* Schltr., *Pteroglossa* Schltr. and *Stenorrhynchos*. More recently, was considered as a section of *Stenorrhynchos s.l.* (Balogh, 1982). Nonetheless, characters such as presence of a rhizome, long-petiolate leaves, and others have been used to justify its separation from *Stenorrhynchos* (Salazar, 2003). Its bright yellow flowers are similar to these found in *Cotylolabium lutzii*, however, this latter species lacks a sepaline tube (Borba et al. in press). Conversely, *C. lutzii* is the sister-group of the whole Spiranthinae (Borba et al., in press).

Regarding pollination system, Salazar (2003) has suggested that the shared floral features between *Coccineorchis* and *Dichromanthus* Garay (inserted in *Spiranthes* clade; Fig. 2) could be a result from convergent adaptation to hummingbird pollination.

4.2.2. Sauroglossum*: clade D2*

Our analyses have demonstrated that *Sauroglossum* is paraphyletic, with *Sauroglossum corymbosum* (Lindl.) Garay not grouping with *Sauroglossum elatum* Lindl., the type species of this genus, as discussed below (see *Synassa*: clade D5). Another important finding is the strongly supported relationship of *Sarcoglottis sancta* (Rchb.f & Warm.) Schltr. grouping with *Sauroglossum elatum* (BP_P 96; BP_{ML} 99; PP 1.00). *Sarcoglottis sancta* has flowers arranged horizontally, in which the most apical flowers are surrounded by green and glabrous bracts, as long as the slightly tubular flowers. Remarkably, the lateral sepals are curved upwards, ascendent and light green coloured, similarly to *S. elatum* (Singer, 2002).

In its current delimitation, *Sauroglossum* comprises approximately 12 species restricted to South America (Salazar, 2003). This genus shares with *Brachystele* the presence of a ventrally-adhesive viscidium and both genera have been considered related by Schlechter (1920) and Balogh (1982). However, our analyses have indicated that *Sauroglossum* (clade D2; Fig. 2) is more related to *Cyclopogon* (clade D3; Fig. 3) than to *Brachystele* (clade D8; Fig. 5).

Characteristically, *Sauroglossum* is one of the few protandrous genera of Spiranthinae (Singer, 2002, Catling, 1983; Catling & Catling, 1991). Protandry likely favours cross-pollination (Singer & Cocucci, 1999). In contrast to *Spiranthes*, which are pollinated by bumble-bees (Catling, 1983; Catling & Catling, 1991), *Sauroglossum elatum* is mainly pollinated by moths (Singer, 2002).

4.2.3. Cyclopogon s.l.*: clade D3*

The species sampled in clade D3 formed a strongly supported group (BP_P 100; BP_{ML} 100; PP 1.00; Fig. 3). However, our analyses did not recover the infrageneric classifications or generic segregates previously proposed (Balogh, 1982; Garay, 1982; Szlachetko, 1993, 1994, 1995). Furthermore, our analyses have shown *Brachystele guayanensis* (Lindl.) Schltr. To be sister of all samples *Veyretia*, a relationship is strongly supported (BP_P 100; BP_{ML} 100; PP 1.00; clade D3; Fig. 3).

Cyclopogon is one of the largest genera of the *Pelexia* clade, with approximately 75 species, distributed from southeastern United States to South America, excluding Chile (Salazar, 2003). Despite displaying extensive morphological diversity, *Cyclopogon* is easily distinguished from other genera of Spiranthinae for its habit and overall floral morphology. However, its infrageneric classification has been constantly under discussion. Balogh (1982) considered that the species *Cyclopogon* were divided in two sections [*C*. sect. *Cyclopogon* and *C*. sect. *Beadlea*

(Small) Burns-Bal.], based on the presence or not of a sepaline tube. In contrast, Garay (1982) segregated *Cyclopogon* into three genera. Garay (1982) re-established *Beadlea* Small, that comprised the largest number of species (54 names), whereas two species were placed into *Stigmatosema* Garay, rendering *Cyclopogon* monospecific (*C. ovalifolium*, the type species). Later, Szlachetko (1993) modified Balogh's (1982) proposal, raising her sections to subgenera. Nevertheless, not satisfied with its subgenera, Szlachetko (1994) proposed two new genera: *Cocleorchis* Szlach. and *Warscaea* Szlach. Further on, Szlachetko (Szlachetko, 1995) grouped all these genera in his subtribe Cyclopogoninae Szlach., which also included *Stigmatosema*.

The group recovered as clade D3, here called as *Cyclopogon s.l.* (Fig. 3) is formed by three major subclades: a species group formed by *Cyclopogon variegatus* Barb.Rodr. and *Cyclopogon cranichoides* (Griseb.) Schltr. [as *Bradlea cranichoides* (Griseb.) Small] (BP_P 100; BP_{ML} 100; PP 1.00); a second large group comprising *Cyclopogon apricus* (Lindl.) Schltr., *C. congestus* (Vell.) Hoehne and other (BP_P 96; BP_{ML} 98; PP 1.00); and a third formed by *Cyclopogon ovalifolium* C. Presl (the type species of *Cyclopogon*), as well as *Brachystele guayanensis* and *Veyretia* spp. (BP_P 94; BP_{ML} 98; PP 1.00). Although such big subclades are strongly supported, we do not favour a generic fragmentation. Many inner subclades are composed of one or few taxa and consequently many genera with one or a few species would have to be created. Furthermore, there are no unequivocal morphological characters (or sets of morphological characters) to support a robust generic fragmentation, and therefore we prefer a broadly defined *Cyclopogon*.

In its broad delimitation, all *Cyclopogon* species have a dorsally-adhesive, wedge-like viscidium and is more diverse in Brazil and adjacent countries. All studied taxa so far are pollinated by Augochlorine Halictidae bees (Galetto et al., 1977; Singer & Cocucci, 1999; Singer & Sazima, 1999; Buzatto et al., in prep.)

4.2.4. Sarcoglottis*: clade D4*

Veyretia species (clade D3; Fig. 3) were formerly included in *Sarcoglottis* sect. *Aphylla* Burns-Bal. However, our analyses have indicated that *Veyretia* spp. are nested within *Cyclopogon s.l.* (BP_P 63; BP_{ML} 63; PP 1.00; clade D3; Fig. 3). Our phylogenetic analyses have also indicated that *Sarcoglottis* (*sensu* Salazar 2003) is non-monophyletic, since *Sarcoglottis sancta* grouped with *Sauroglossum elatum* (clade D2; Fig. 2) and *Sarcoglottis juergensis* Schltr. is nested within *Pelexia s.s.* (see below; clade D7; Fig. 5). Nevertheless, the rest of *Sarcoglottis* spp. are placed in a strongly supported clade (BP_P 100; BP_{ML} 99; PP 1.00; clade D4; Fig. 4), which includes the type species.

Sarcoglottis contains about 45 species, distributed from Mexico to southern South America, except Chile (Salazar, 2003). This genus is unequivocally diagnosed for its overall floral morphology. The flowers are fleshy, inclined upward, with a lip curved down, arranged in a sub-corymbose short raceme (Salazar, 2003). Regarding the pollination biology, the few studies of *Sarcoglottis* species have been showing the pollination by Euglossini bees (Dressler, 1993; Singer & Sazima, 1999).

4.2.5. Synassa*: clade D5*

Our analyses grouped *Sauroglossum corymbosum* to *Pelexia weberbaueriana* (Kraenzl. ex Schltr.) Schltr. and *Brachystele chlorops* (Rchb.f.) Schltr. in a strongly supported clade (BP_P 100; BP_{ML} 100; PP 1.00; clade D5; Fig. 5). *Synassa* was established by Lindley (1933), based in *Synassa corymbosa* Lindl. The same author (Lindley, 1940) after examining more material of this taxon considered that there was no characters to separate *S. corymbosa* from *Pelexia*. Garay (1978) analysed the type collections of *Synassa corymbosa* and *Sauroglossum elatum* and did not find any reason to keep these taxa separated generically, and then proposed the combination *Sauroglossum corymbosum*, which has been accepted up to the moment. Based on our phylogenetic analyses, *Synassa* should be re-established as a genus. This group is essentially Andean.

4.2.6. Pelexia*: clades D6 and D7*

Our analysis has indicated the polyphyletic nature of *Pelexia*, with samples grouped in three clades (D5, D6 and D7; Fig. 5), of which *Pelexia weberbaueri* has already been discussed above (clade D5; see above). In its current delimitation (Salazar, 2003) *Pelexia* comprises about 75 species, distributed from Mexico to Uruguay (Salazar, 2003). Schlechter (1920) proposed an infrageneric classification mostly based on floral features. This author proposed five sections, three of which are currently included in *Pelexia* (*P*. sect. *Pelexia, P*. sect. *Pachygenium* and *P*. sect. *Centropelexia* Schltr.). The other sections were subsequently combined into *Sarcoglottis* (*P*. sect. *Potosia* Schltr.) and *Pteroglossa* (*P*. sect. *Cogniauxiocharis* Schltr.), as circumscribed by Salazar (2003).

Pelexia sect. *Pachygenium* has emerged in our trees as a monophyletic group (clade D6). Our MP and ML analyses are weakly and moderately supported (BP_P 65; BP_{ML} 82, respectively), while BI have a strong support (PP 1.00). Thus, our results support the recognition of *Pachygenium* as a segregate genus, as proposed by Szlachetko et al. (2001). The genus has about 38 species and is the sister-group of *Pelexia s.s.* (clade D7) plus *Brachystele* (clade D8).

Pelexia sect. *Pelexia* and *P*. sect. *Centropelexia* are comprised in *Pelexia s.s*. (clade D7), which is strongly supported in all analyses (BP_P 96; BP_{ML} 100; PP 1.00; clade D7; Fig. 5). As aforementioned, *Sarcoglottis juergensis* grouped with *Pelexia s.s*., as sister to a basically south American group (BP_P 99; BP_{ML} 98; PP 1.00), comprising no more than 30 species.

Since *Pelexia* can be divided in two well-defined clades (clades D6 and D7), no information is available on the pollination of *Pelexia s.s.* However, the presence of dorsally-adhesive viscidia could be considered indicative of bee-pollination (Buzatto et al., in prep). On the other hand, the species of *Pachygenium* are pollinated by bumble-bees and other big-sized Apidae bees (Dressler, 1993; Singer & Sazima, 1999; Buzatto et al., in prep.).

4.2.7. Brachystele*: clade D8*

Our phylogenetic analyses have demonstrated that *Brachystele* (*sensu* Salazar 2003) is polyphyletic (clades B, D3 and D8). The Mexican species *Brachystele affinis* (C.Schweinf.) Burns-Bal. & R.González (clade B; Fig. 2) is sister to *Schiedeella* Schltr. (BP_P 100; BP_{ML} 100; PP 1.00). The floral morphology of *Brachystele affinis* differs substantially from other members of the *Spiranthes* clade (clade B). The difference, however, may be a result of the strong reduction in flower size. *Brachystele guayanensis* grouped to *Cyclopogon s.l.*, as sister to *Veyretia* (BP_P 100; BP_{ML} 100; PP 1.00; clade D3; Fig. 5). In all analyses, *Odontorrhynchus* samples grouped to *Brachystele unilateralis* (Poir.) Schltr. (BP_P 100; BP_{ML} 100; PP 1.00). *Odontorrhynchus castillonii* (Hauman) M.N.Correa (type species of the genus, not available for molecular studies) has an overall rostellum structure similar to these of the genera of the *Stenorrhynchos* clade (*sensu* Balogh, 1982). Like in *Sauroglossum*, *Brachystele* flowers are protandrous and present a ventrally-adhesive viscidium (Singer & Cocucci, 1999). As a whole, *Brachystele* presents the smaller flowers within the *Pelexia* clade.

In its current delimitation, *Brachystele* comprises about 15 species (Salazar, 2003). In this delimitation, it is the only genus with disjunct distribution, with a small species-group occurring in North of South America, extending to Mexico, while other group occurs in southern South America, mainly in Chilean Andes, Argentina, Uruguay and southern Brazil (Salazar, 2003). Regarding its pollination system, Sanguinetti and Singer (in prep.) and Buzatto et al. (in prep.) have recorded introduced bees acting as pollinators for *Brachystele unilateralis* and *B. camporum* (Lindl.) Schltr., respectively.

4.5. Conclusions and perspectives

This is the first comprehensive sampling of the *Pelexia* clade, including all genera (and proposed segregates) currently placed in this group. The *Pelexia* clade is strongly supported as a monophyletic group in our analyses, but most genera are clearly non-monophyletic in their current delimitation and require taxonomic adjustments. Future studies should plot and explore character evolution of morphological and ecological (pollination) features in the phylogenies. We hope that the clades recovered in our analyses will be supported with sets of non-molecular characters that with help in a well-supported new generic delimitation.

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LEGENDS

Figure 1. Representative species of *Pelexia* Clade. A, *Brachystele camporum*. B, *Brachystele dilatata*. C, *Brachystele ulaei*. D, *Cyclopogon apricus*. E, *Cyclopogon chloroleuchus*. F, *Cyclopogon congestus*. G, *Cyclopogon eugenii*. H, *Cyclopogon variegatus*. I, *Cyclopogon warmingii*. J-K, *Pachygenium ekmanii*. J, Habit. K, Inflorescence. L, *Pachygenium orthosepalum*. M, *Pelexia lindmanii*. N, *Pelexia cf. macropoda*. O-P, *Pelexia novofriburgensis*. O, Inflorescence. P, Habit. Q, *Sarcoglottis sancta*. R, *Sarcoglottis uliginosa*. S, *Sauroglossum elatum*. T, *Veyretia hassleri*.

Figure 2. Bayesian tree from the combined ITS, *matK-trnK* and *trnL-trnF* datasets. Numbers above branches are bootstrap values of >50 % (in both instances: MP/BI); number below branches are bootstrap values of > 50 % of ML. Asterisk represent values of 100 % (to both MP and ML analyses) and PP 1.00 in BI. Dash represent nodes with bootstrap and PP values >50 % and >0.50, respectively or collapsed. The phylogram on the upper left hand of the main tree represents the single MPT with branches drawn proportional to their lengths. The three letter abbreviation to the right of the species name indicates its geographic origin of the sample. The type species of *Pelexia* clade genera are highlighted in bold.

Figure 3. Continuation of the tree of Fig. 2. The type species of *Pelexia* clade genera are highlighted in bold. For abbreviations, see Fig. 1.

Figure 4. Continuation of the tree of Fig. 3. The type species of *Pelexia* clade genera are highlighted in bold. For abbreviations, see Fig. 1.

Figure 5. Continuation of the tree of Fig. 4. The type species of *Pelexia* clade genera are highlighted in bold. For abbreviations, see Fig. 1.

Figure 1

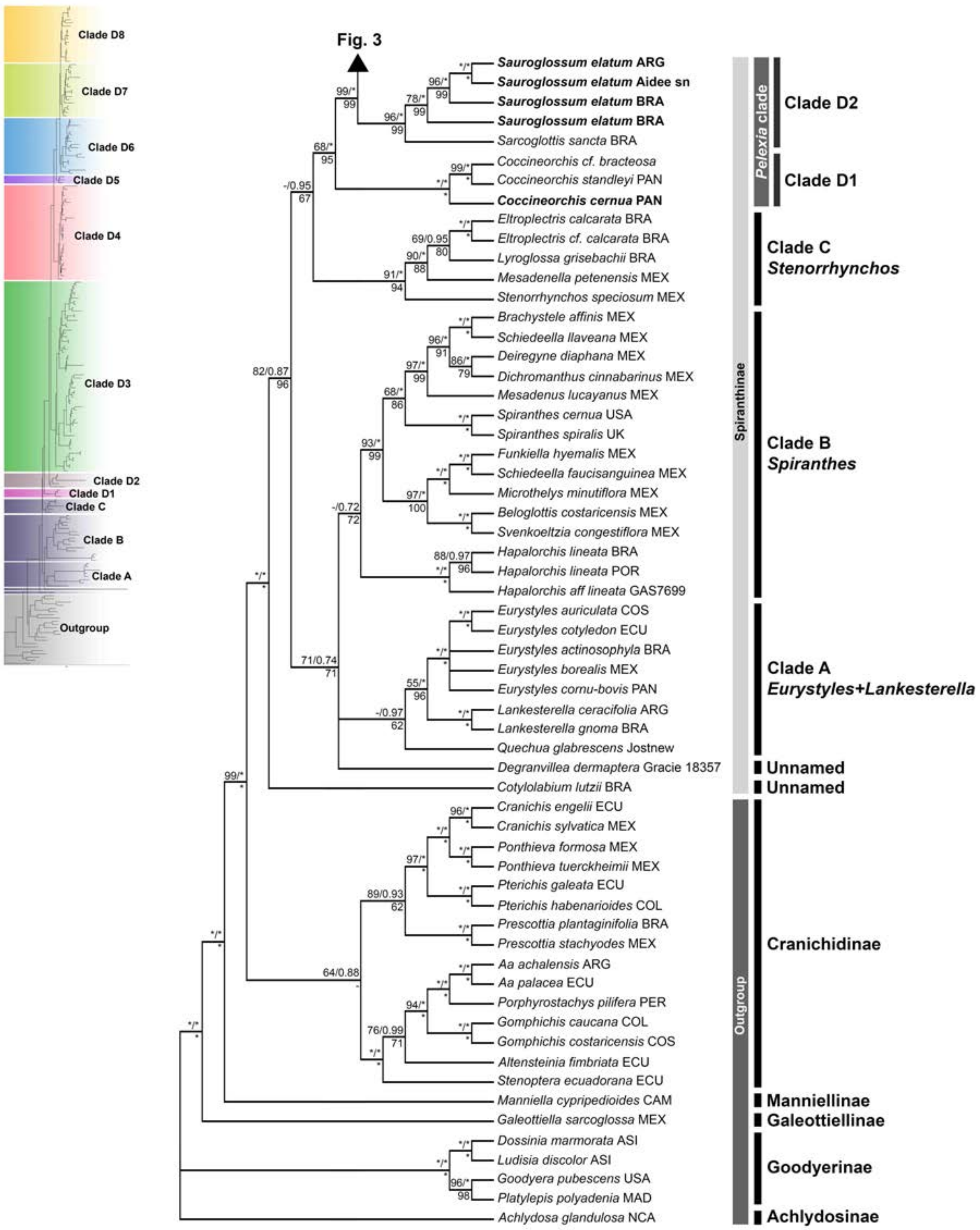
Clade D8
Clade D7
Clade D6
Clade D5
Clade D4
Clade D3
Clade D2
Clade D1
Clade C
Clade B
Clade A
Outgroup
Fig. 3
Sauroglossum elatum ARG
Sauroglossum elatum Aidee sn
Sauroglossum elatum BRA
Sauroglossum elatum BRA
Sarcoglottis sancta BRA
Coccineorchis cf. bracteosa
Coccineorchis standleyi PAN
Coccineorchis cernua PAN
Eltroplectris calcarata BRA
Eltroplectris cf. calcarata BRA
Lyroglossa grisebachii BRA
Mesadenella petenensis MEX
Stenorrhynchos speciosum MEX
Brachystele affinis MEX
Schiedeella llaveana MEX
Deiregyne diaphana MEX
Dichromanthus cinnabarinus MEX
Mesadenus lucayanus MEX
Spiranthes cernua USA
Spiranthes spiralis UK
Funkiella hyemalis MEX
Schiedeella faucisanguinea MEX
Microthelys minutiflora MEX
Beloglottis costaricensis MEX
Svenkoeltzia congestiflora MEX
Hapalorchis lineata BRA
Hapalorchis lineata POR
Hapalorchis aff lineata GAS7699
Eurystyles auriculata COS
Eurystyles cotyledon ECU
Eurystyles actinosophyla BRA
Eurystyles borealis MEX
Eurystyles cornu-bovis PAN
Lankesterella ceracifolia ARG
Lankesterella gnoma BRA
Quechua glabrescens Jostnew
Degranvillea dermaptera Gracie 18357
Cotylolabium lutzii BRA
Cranichis engelii ECU
Cranichis sylvatica MEX
Ponthieva formosa MEX
Ponthieva tuerckheimii MEX
Pterichis galeata ECU
Pterichis habenarioides COL
Prescottia plantaginifolia BRA
Prescottia stachyodes MEX
Aa achalensis ARG
Aa palacea ECU
Porphyrostachys pilifera PER
Gomphichis caucana COL
Gomphichis costaricensis COS
Altensteinia fimbriata ECU
Stenoptera ecuadorana ECU
Manniella cypripedioides CAM
Galeottiella sarcoglossa MEX
Dossinia marmorata ASI
Ludisia discolor ASI
Goodyera pubescens USA
Platylepis polyadenia MAD
Achlydosa glandulosa NCA
Pelexia clade
Spiranthinae
Outgroup
Clade D2
Clade D1
Clade C
Stenorrhynchos
Clade B
Spiranthes
Clade A
Eurystyles+Lankesterella
Unnamed
Unnamed
Cranichidinae
Manniellinae
Galeottiellinae
Goodyerinae
Achlydosinae

Figure 2

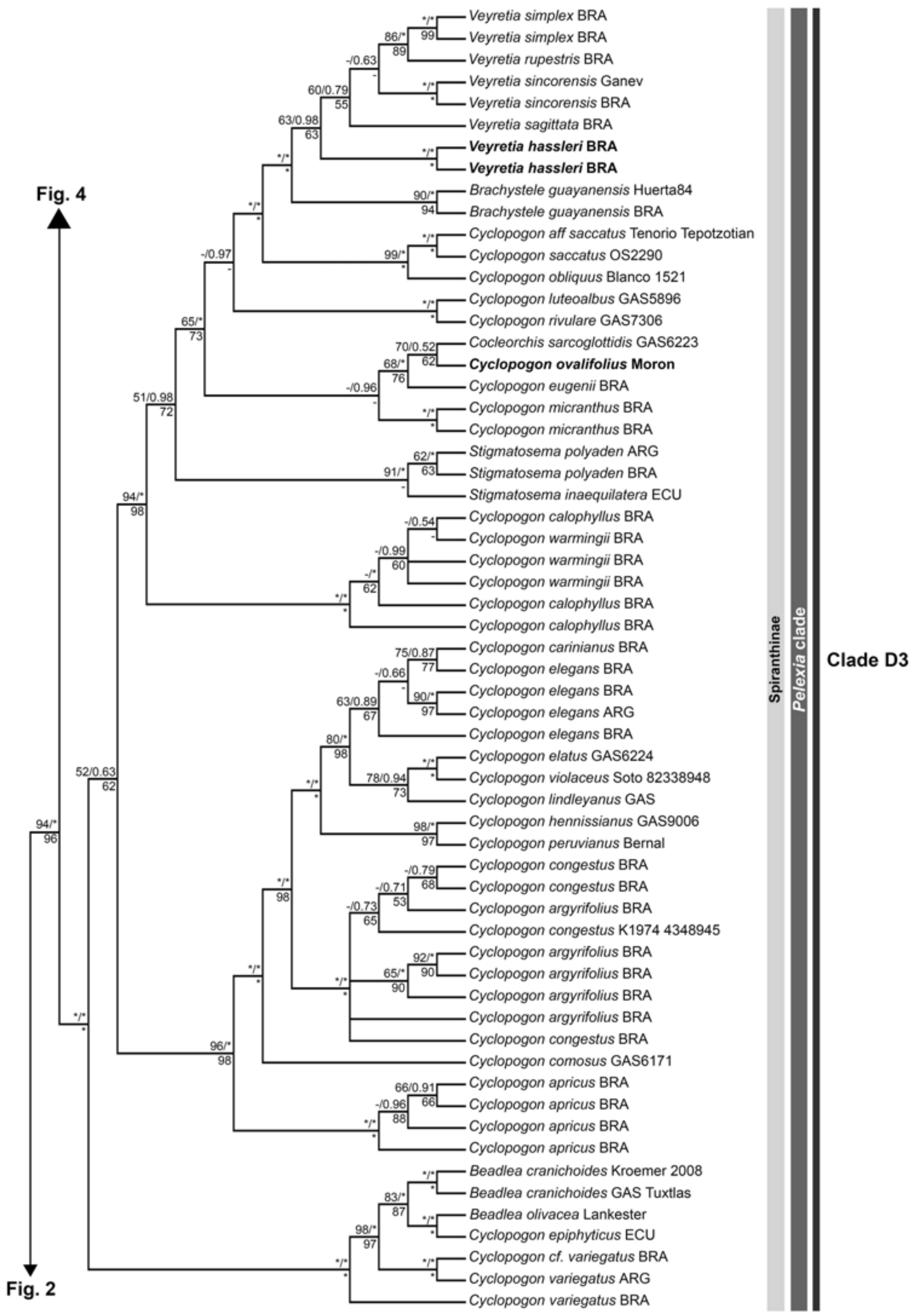
Veyretia simplex BRA
Veyretia simplex BRA
Veyretia rupestris BRA
Veyretia sincorensis Ganev
Veyretia sincorensis BRA
Veyretia sagittata BRA
Veyretia hassleri BRA
Veyretia hassleri BRA
Brachystele guayanensis Huerta84
Brachystele guayanensis BRA
Cyclopogon aff saccatus Tenorio Tepotzotian
Cyclopogon saccatus OS2290
Cyclopogon obliquus Blanco 1521
Cyclopogon luteoalbus GAS5896
Cyclopogon rivulare GAS7306
Cocleorchis sarcoglottidis GAS6223
Cyclopogon ovalifolius Moron
Cyclopogon eugenii BRA
Cyclopogon micranthus BRA
Cyclopogon micranthus BRA
Stigmatosema polyaden ARG
Stigmatosema polyaden BRA
Stigmatosema inaequilatera ECU
Cyclopogon calophyllus BRA
Cyclopogon warmingii BRA
Cyclopogon warmingii BRA
Cyclopogon warmingii BRA
Cyclopogon calophyllus BRA
Cyclopogon calophyllus BRA
Cyclopogon carinianus BRA
Cyclopogon elegans BRA
Cyclopogon elegans BRA
Cyclopogon elegans ARG
Cyclopogon elegans BRA
Cyclopogon elatus GAS6224
Cyclopogon violaceus Soto 82338948
Cyclopogon lindleyanus GAS
Cyclopogon hennissianus GAS9006
Cyclopogon peruvianus Bernal
Cyclopogon congestus BRA
Cyclopogon congestus BRA
Cyclopogon argyrifolius BRA
Cyclopogon congestus K1974 4348945
Cyclopogon argyrifolius BRA
Cyclopogon argyrifolius BRA
Cyclopogon argyrifolius BRA
Cyclopogon argyrifolius BRA
Cyclopogon congestus BRA
Cyclopogon comosus GAS6171
Cyclopogon apricus BRA
Cyclopogon apricus BRA
Cyclopogon apricus BRA
Cyclopogon apricus BRA
Beadlea cranichoides Kroemer 2008
Beadlea cranichoides GAS Tuxtlas
Beadlea olivacea Lankester
Cyclopogon epiphyticus ECU
Cyclopogon cf. variegatus BRA
Cyclopogon variegatus ARG
Cyclopogon variegatus BRA
Fig. 4
Fig. 2
Spiranthinae
Pelexia clade
Clade D3

Figure 3

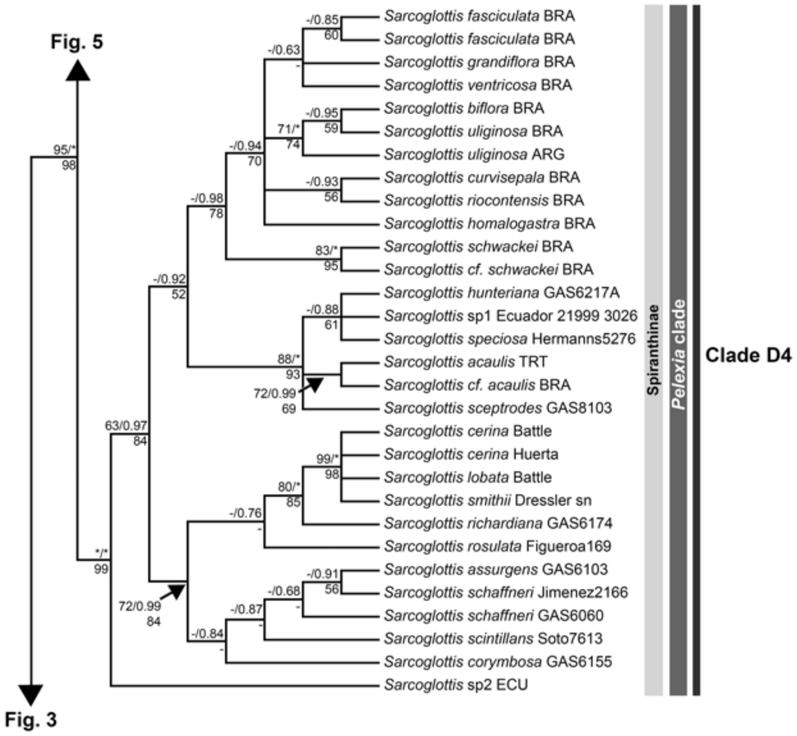
Fig. 5
95/*
98
-/0.85
60
Sarcoglottis fasciculata BRA
Sarcoglottis fasciculata BRA
-/0.63
-
Sarcoglottis grandiflora BRA
Sarcoglottis ventricosa BRA
-/0.95
59
Sarcoglottis biflora BRA
71/*
74
Sarcoglottis uliginosa BRA
Sarcoglottis uliginosa ARG
-/0.94
70
-/0.93
56
Sarcoglottis curvisepala BRA
Sarcoglottis riocontensis BRA
-/0.98
78
Sarcoglottis homalogastra BRA
83/*
95
Sarcoglottis schwackei BRA
Sarcoglottis cf. schwackei BRA
-/0.92
52
Sarcoglottis hunteriana GAS6217A
-/0.88
61
Sarcoglottis sp1 Ecuador 21999 3026
Sarcoglottis speciosa Hermanns5276
88/*
93
Sarcoglottis acaulis TRT
72/0.99
69
Sarcoglottis cf. acaulis BRA
Sarcoglottis sceptrodes GAS8103
63/0.97
84
Sarcoglottis cerina Battle
99/*
98
Sarcoglottis cerina Huerta
Sarcoglottis lobata Battle
80/*
85
Sarcoglottis smithii Dressler sn
-/0.76
-
Sarcoglottis richardiana GAS6174
Sarcoglottis rosulata Figueroa169
/
99
-/0.91
56
Sarcoglottis assurgens GAS6103
-/0.68
-
Sarcoglottis schaffneri Jimenez2166
72/0.99
84
-/0.87
-
Sarcoglottis schaffneri GAS6060
-/0.84
-
Sarcoglottis scintillans Soto7613
Sarcoglottis corymbosa GAS6155
Sarcoglottis sp2 ECU
Fig. 3
Spiranthinae
Pelexia clade
Clade D4

Figure 4

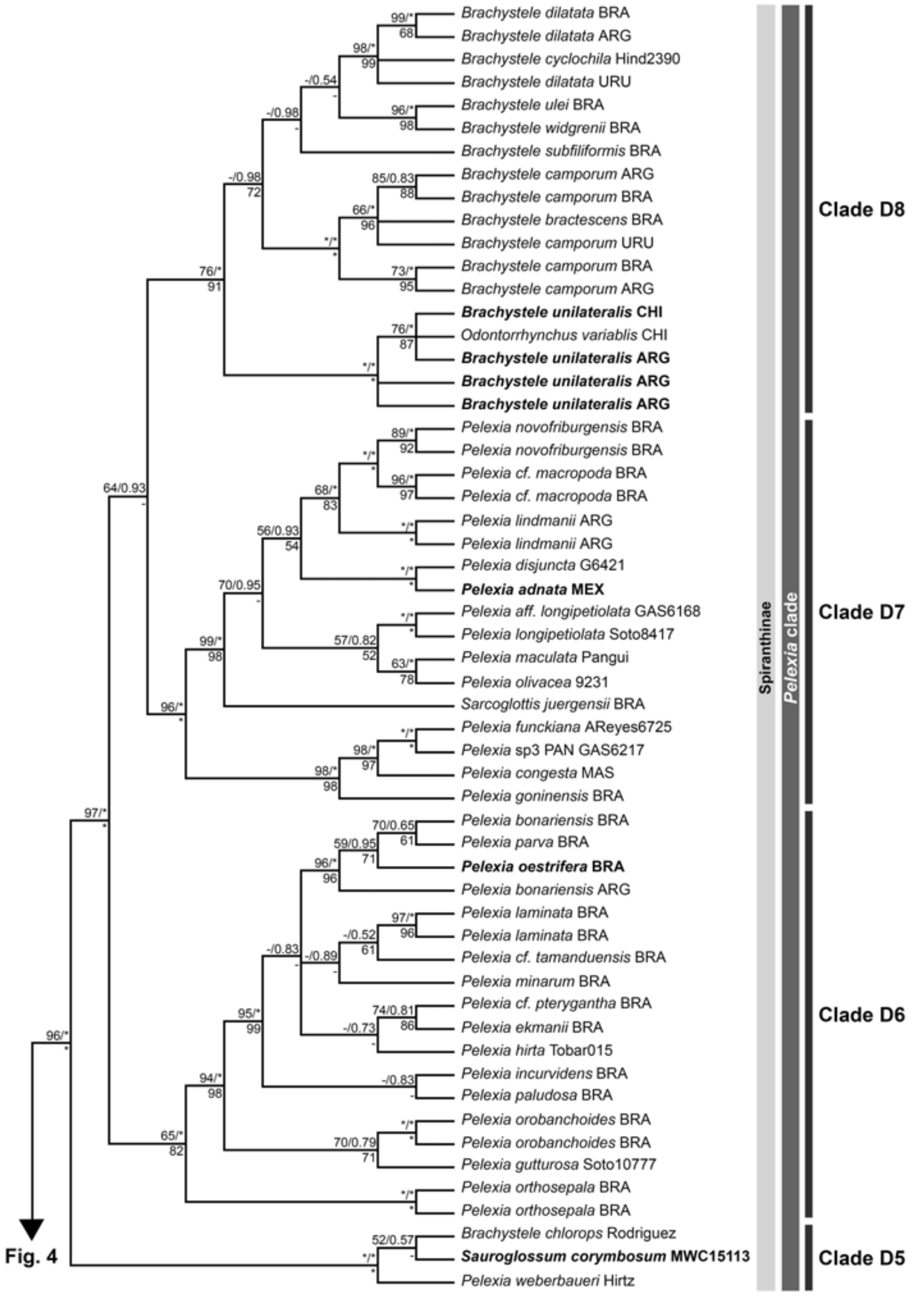
Brachystele dilatata BRA
Brachystele dilatata ARG
Brachystele cyclochila Hind2390
Brachystele dilatata URU
Brachystele ulei BRA
Brachystele widgrenii BRA
Brachystele subfiliformis BRA
Brachystele camporum ARG
Brachystele camporum BRA
Brachystele bractescens BRA
Brachystele camporum URU
Brachystele camporum BRA
Brachystele camporum ARG
Brachystele unilateralis CHI
Odontorrhynchus variablis CHI
Brachystele unilateralis ARG
Brachystele unilateralis ARG
Brachystele unilateralis ARG
Pelexia novofriburgensis BRA
Pelexia novofriburgensis BRA
Pelexia cf. macropoda BRA
Pelexia cf. macropoda BRA
Pelexia lindmanii ARG
Pelexia lindmanii ARG
Pelexia disjuncta G6421
Pelexia adnata MEX
Pelexia aff. longipetiolata GAS6168
Pelexia longipetiolata Soto8417
Pelexia maculata Pangui
Pelexia olivacea 9231
Sarcoglottis juergensii BRA
Pelexia funckiana AReyes6725
Pelexia sp3 PAN GAS6217
Pelexia congesta MAS
Pelexia goninensis BRA
Pelexia bonariensis BRA
Pelexia parva BRA
Pelexia oestrifera BRA
Pelexia bonariensis ARG
Pelexia laminata BRA
Pelexia laminata BRA
Pelexia cf. tamanduensis BRA
Pelexia minarum BRA
Pelexia cf. pterygantha BRA
Pelexia ekmanii BRA
Pelexia hirta Tobar015
Pelexia incurvidens BRA
Pelexia paludosa BRA
Pelexia orobanchoides BRA
Pelexia orobanchoides BRA
Pelexia gutturosa Soto10777
Pelexia orthosepala BRA
Pelexia orthosepala BRA
Brachystele chlorops Rodriguez
Sauroglossum corymbosum MWC15113
Pelexia weberbaueri Hirtz
Fig. 4
Spiranthinae
Pelexia clade
Clade D8
Clade D7
Clade D6
Clade D5
99/*
68
98/*
99
-/0.54
-
96/*
98
-/0.98
-
-/0.98
72
85/0.83
88
66/*
96
/
*
76/*
91
73/*
95
76/*
87
/
*
89/*
92
/
*
96/*
97
64/0.93
-
68/*
83
/
*
56/0.93
54
/
*
70/0.95
-
/
*
99/*
98
57/0.82
52
63/*
78
96/*
*
/
*
98/*
97
98/*
98
97/*
*
70/0.65
61
59/0.95
71
96/*
96
97/*
96
-/0.52
61
-/0.83
-
-/0.89
-
74/0.81
86
-/0.73
-
95/*
99
96/*
*
94/*
98
-/0.83
-
65/*
82
/
*
70/0.79
71
/
*
52/0.57
-
/
*

Figure 5

TABLES

Table 1. Sampled genera of the *Pelexia* clade (*sensu* Salazar, 2003; Salazar et al., 2003) with the respective number of sampled species per genus

Genus	**No. of taxa/No. of taxa sampled**	**% of taxa sampled**
Brachystele Schltr.	15/11	73
Coccineorchis Schltr.	4/3	75
Cyclopogon C.Presl	75/27	36
Odontorrhynchus M.N.Correa	10/1	10
Pelexia Poit. ex Lindl.	75/27	36
Sarcoglottis C.Presl	45/26	58
Sauroglossum Lindl.	12/2	17
Veyretia Szlach.	10/5	50

Table 2. Species and gene regions for this study, voucher information, geographic origin and GenBank accession numbers. Asterisks indicate the type of the genera

Taxon	**nrITS**	***matK-trnK***	***trnL-trnF***	**Voucher**
Tribe Cranichideae (Lindl.) Endl.				
Subtribe Achlydosinae M.A.Clem. & D.L.Jones				
Achlydosa glandulosa (Schltr.) M.A.Clem. & D.L.Jones*	AJ539525	AJ543950	AJ544506	New Caledonia, *Clements D285*, CANB
Subtribe Cranichidinae Lindl.				
Aa achalensis Schltr.	Pending	-	Pending	Argentina, *Singer s.n.*, ICN
Aa paleacea (Kunth) Rchb.f.*	AJ539515	AJ309989	AJ544497	Ecuador, *Chase 535*, K
Altensteinia fimbriata Kunth*	AM419765	AM900801	AM412737	Ecuador, *Salazar 6789*, MEXU (spirit)
Cranichis engelii Rchb.f.	AM419779	AM900814	AM412721	Ecuador, *Schott s.n.*, K (spirit)
Cranichis sylvatica A.Rich. & Galeotti	AM419781	AM900816	AM412734	Mexico, *Suarez 2443*, MEXU (photograph)
Gomphichis caucana Schltr.	AM419770	AM900805	AM412736	Colombia, *Dıaz 159*, ANDES
Gomphichis costaricensis (Schltr.) Ames, F.T.Hubb. & C.Schweinf.	AM419769	AM900804	AM412729	Costa Rica, *Soto s.n.*, AMO
Ponthieva formosa Schltr.	AM419793	AM900828	AM412707	Mexico, *Salazar et al. 6250*, MEXU
Ponthieva tuerckheimii Schltr.	AM419794	AM900829	AM412716	Mexico, *Salazar et al. 6512*, MEXU
Porphyrostachys pilifera (Kunth) Rchb.f.*	AJ539514	AJ543942	AJ544496	Peru, *Whalley s.n.*, K (photograph)
Prescottia plantaginifolia Lindl. ex Hook.*	AJ539511	AJ543939	AJ544493	Brazil, *Salazar 6350*, K (spirit)
Prescottia stachyodes (Sw.) Lindl.	AM419774	AM900809	AM412726	Mexico, *Salazar et al. 7312*, MEXU
Pterichis galeata Lindl.*	AM419796	AM900831	AM412732	Ecuador, *Schott s.n*, K (spirit)
Pterichis habenarioides (F.Lehm. & Kraenzl.) Schltr.	AJ539509	AJ543937	AJ544491	Colombia, *Aldana 12*, COL
Stenoptera ecuadorana Dodson & C.Vargas	AJ539512	AJ543940	AJ544494	Ecuador, *Salazar 6357*, K (spirit)

Subtribe Galeottielinae Salazar & M.W.Chase				
Galeottiella sarcoglossa (A.Rich. & Galeotti) Schltr.*	AJ539518	AJ543945	AJ544500	Mexico, *Jimenez 2334*, AMO
Subtribe Goodyerinae Klotzsch				
Dossinia marmorata Lindl.*	AJ539521	AJ543947	AJ544502	Tropical Asia (cultivated specimen), *Munich Bot. Gard. 94/1190*, M
Goodyera pubescens (Willd.) R.Br.	AJ539519	AJ543954	AM419815	USA, *Chase 212*, NCU
Ludisia discolor (Ker Gawl.) Blume*	AJ539483	AJ543911	AJ544466	Tropical Asia (cultivated specimen), *Salazar 6354*, K (spirit)
Platylepis polyadenia Rchb.f.	AJ539520	AJ543946	AJ544501	Madagascar, *Salazar 6352*, K (spirit)
Subtribe Manniellinae Schltr.				
Manniella cypripedioides Salazar, T.Franke, Zapfack & Beenken	AJ539516	AJ543943	AJ544498	Cameroon, *Salazar et al. 6323*, YA
Subtribe Spiranthinae Lindl.				
Beadlea cranichoides (Griseb.) Small [=*Cyclopogon cranichoides* (Griseb.) Schltr.]	Pending	Pending	-	Pending
Beadlea cranichoides (Griseb.) Small [=*Cyclopogon cranichoides* (Griseb.) Schltr.]	Pending	-	-	Pending
Beadlea olivacea (Rolfe) Garay [=*Cyclopogon olivaceus* (Rolfe) Schltr.]	Pending	Pending	Pending	Pending
Beloglottis costaricensis (Rchb.f.) Schltr.*	AJ539492	AJ543920	AJ544475	Mexico, *Soto 8129*, MEXU
Brachystele affinis (C.Schweinf.) Burns-Bal. & R.González	Pending	Pending	Pending	Pending
Brachystele bracteosa Schltr.	Pending	-	Pending	Brazil, *Buzatto 625*, ICN
Brachystele camporum (Lindl.) Schltr.	-	HG425366	HG425369	Argentina, Insaurralde s.n., MEXU (spirit)
Brachystele camporum (Lindl.) Schltr.	Pending	Pending	-	Uruguay, *Sanguinetti 37*, BA
Brachystele camporum (Lindl.) Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 641 & Singer*, ICN
Brachystele camporum (Lindl.) Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 642 & Singer*, ICN
Brachystele camporum (Lindl.) Schltr.	Pending	Pending	Pending	Pending
Brachystele chlorops (Rchb.f.) Schltr.	-	Pending	Pending	Pending
Brachystele cyclochila (Kraenzl.) Schltr.	Pending	Pending	-	Pending
Brachystele dilatata (Lindl.) Schltr.	Pending	Pending	Pending	Uruguay, *Sanguinetti 36*, BA
Brachystele dilatata (Lindl.) Schltr.	Pending	Pending	Pending	Argentina, *Sanguinetti 49*, BA
Brachystele dilatata (Lindl.) Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 735*, ICN

Brachystele guayanensis (Lindl.) Schltr.	Pending	-	-	Pending
Brachystele guayanensis (Lindl.) Schltr.	Pending	Pending	Pending	Brazil, *Batista 2928*, BHCB
Brachystele subfiliformis (Cogn.) Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 649 & Singer*, ICN
Brachystele ulaei (Cogn.) Schltr.	Pending	Pending	-	Brazil, *Buzatto 763 & Nervo*, ICN
Brachystele unilateralis (Poir.) Schltr.*	Pending	Pending	Pending	Argentina, *Sanguinetti 21*, BA
Brachystele unilateralis (Poir.) Schltr.*	Pending	Pending	Pending	Argentina, *Sanguinetti 42*, BA
Brachystele unilateralis (Poir.) Schltr.*	Pending	Pending	Pending	Argentina, *Sanguinetti s.n.*, BA
Brachystele unilateralis (Poir.) Schltr.*	Pending	Pending	Pending	Pending
Brachystele widgrenii (Rchb.f.) Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 700*, ICN
Coccineorchis cf *bracteosa* (Ames & C.Schweinf.) Garay	Pending	-	-	Pending
Coccineorchis cernua (Lindl.) Garay*	AJ539502	AJ543930	AJ544485	Panama, *Salazar 6249*, MEXU
Coccineorchis standleyi (Ames) Garay	FN996949	-	FN996961	Panama, *Salazar 6248*, MEXU
Cocleorchis sarcoglottidis Szlach.	Pending	Pending	Pending	Pending
Cotylolabium lutzii (Pabst) Garay*	HG425360	HG425363	HG425367	Brazil, Martins da Costa 326, BHCB
Cyclopogon apricus (Lindl.) Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 762 & Nervo*, ICN
Cyclopogon apricus (Lindl.) Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 765 & Nervo*, ICN
Cyclopogon apricus (Lindl.) Schltr.	Pending	-	-	Brazil, *Buzatto 582 & Singer*, ICN
Cyclopogon apricus (Lindl.) Schltr.	Pending	Pending	-	Brazil, *Batista 3222*, BHCB
Cyclopogon argyrifolius Barb.Rodr.	Pending	Pending	Pending	Brazil, *Buzatto 873*, ICN
Cyclopogon argyrifolius Barb.Rodr.	Pending	Pending	-	Brazil, *Buzatto 874*, ICN
Cyclopogon argyrifolius Barb.Rodr.	Pending	Pending	-	Brazil, *Buzatto 875*, ICN
Cyclopogon argyrifolius Barb.Rodr.	Pending	-	Pending	Brazil, *Buzatto 876*, ICN
Cyclopogon argyrifolius Barb.Rodr.	Pending	Pending	-	Brazil, *Buzatto 895*, ICN
Cyclopogon calophyllus Barb.Rodr.	Pending	Pending	-	Brazil, *Buzatto 830*, ICN
Cyclopogon calophyllus Barb.Rodr.	Pending	-	Pending	Brazil, *Singer s.n.*, ICN
Cyclopogon calophyllus Barb.Rodr.	Pending	Pending	Pending	Brazil, *van den Berg s.n.*, HUEFS
Cyclopogon carinianus Pansarin	Pending	-	-	Brazil, *Pansarin 1291*, SPFR
Cyclopogon comosus (Rchb.f.) Burns-Bal. & E.W.Greenw.	Pending	Pending	Pending	Pending
Cyclopogon congestus (Vell.) Schltr.	Pending	-	Pending	Brazil, *Buzatto 833*, ICN
Cyclopogon congestus (Vell.) Schltr.	Pending	Pending	Pending	Brazil, *Stancato s.n.*, RB

Cyclopogon congestus (Vell.) Schltr.	Pending	Pending	Pending	Pending
Cyclopogon congestus (Vell.) Schltr.	Pending	Pending	Pending	Brazil, *Batista 2902*, BHCB
Cyclopogon elatus (Sw.) Schltr.	Pending	-	Pending	Pending
Cyclopogon elegans Hoehne	Pending	-	-	Brazil, *Singer s.n.*, ICN
Cyclopogon elegans Hoehne	Pending	-	Pending	Brazil, *Grings 1066*, ICN
Cyclopogon elegans Hoehne	Pending	Pending	Pending	Argentina, *Salazar 8946*, MEXU
Cyclopogon cf *elegans* Hoehne	Pending	Pending	Pending	Brazil, *Batista 2904*, BHCB
Cyclopogon epiphyticus (Dodson) Dodson	AJ539499	AJ543927	AJ544482	Ecuador, *Salazar 6355*, K
Cyclopogon eugenii (Rchb.f. & Warm.) Schltr.	Pending	-	Pending	Brazil, *Buzatto 828*, ICN
Cyclopogon hennisianus (Sandt) Dodson	Pending	Pending	Pending	Pending
Cyclopogon lindleyanus (Link, Klotzsch & Otto) Schltr.	Pending	Pending	Pending	Pending
Cyclopogon luteoalbus (A.Rich. & Galeotti) Schltr.	Pending	Pending	Pending	Pending
Cyclopogon micranthus Barb.Rodr.	Pending	-	-	Brazil, *Batista 2891*, BHCB
Cyclopogon micranthus Barb.Rodr.	Pending	Pending	Pending	Brazil, *Batista 2903*, BHCB
Cyclopogon obliquus (J.J.Sm.) Szlach.	Pending	Pending	Pending	Pending
Cyclopogon ovalifolius C.Presl*	Pending	Pending	-	Pending
Cyclopogon peruvianus (C.Presl) Schltr.	Pending	Pending	Pending	Pending
Cyclopogon rivulare Salazar & Soto Arenas	Pending	-	-	Pending
Cyclopogon saccatus (A.Rich. & Galeotti) Schltr.	Pending	Pending	Pending	Pending
Cyclopogon aff *saccatus* (A.Rich. & Galeotti) Schltr.	-	Pending	Pending	Pending
Cyclopogon variegatus Barb.Rodr.	Pending	-	Pending	Brazil, *Buzatto 883*, ICN
Cyclopogon variegatus Barb.Rodr.	Pending	Pending	Pending	Argentina, *Radins s.n.*, MEXU (spirit)
Cyclopogon aff. *variegatus* Barb.Rodr.	Pending	Pending	Pending	Brazil, *Smidt s.n.*, UPCB
Cyclopogon violaceus (A.Rich. & Galeotti) Schltr.	Pending	Pending	Pending	Pending
Cyclopogon warmingii (Rchb.f.) Schltr.	Pending	-	Pending	Brazil, *Buzatto 665 & Singer*, ICN
Cyclopogon warmingii (Rchb.f.) Schltr.	Pending	-	Pending	Brazil, *Buzatto 838*, ICN
Cyclopogon warmingii (Rchb.f.) Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 845*, ICN
Degranvillea dermaptera Determann	Pending	-	-	Pending
Deiregyne diaphana (Lindl.) Garay*	AJ539484	AJ543912	AJ544467	Mexico, *Salazar et al. 6172*, MEXU
Dichromanthus cinnabarinus (La Llave & Lex.) Garay*	AJ539486	AJ543914	AJ544469	Mexico, *Linares 4469*, MEXU
Eltroplectris calcarata (Sw.) Garay & H.R.Sweet*	AJ519448	AJ519450	AJ519452	Brazil, *Soares s.n.*, K (photograph)

Eltroplectris cf *calcarata* (Sw.) Garay & H.R.Sweet*	Pending	Pending	-	Brazil, *Buzatto 870*, ICN
Eurystyles actinosophila (Barb.Rodr.) Schltr.	Pending	Pending	Pending	Brazil, *Nervo s.n.*, ICN
Eurystyles auriculata Schltr.	FN556161	FN556171	FN556166	Costa Rica, *Dressler s.n.*, MEXU (spirit)
Eurystyles borealis A.H.Heller	AJ539497	AJ543925	AJ544480	Mexico, *Soto 9149*, AMO
Eurystyles cornu-bovis Szlach.	FN556162	FN556172	FN556167	Panama, *Maduro 230*, FLAS
Eurystyles cotyledon Wawra*	HE575514	HE575501	HE575524	Ecuador, *Salazar 7642*, MEXU (spirit)
Funkiella hyemalis (A.Rich. & Galeotti) Schltr.*	AJ539495	AJ543923	AJ544478	Mexico, *Salazar et al. 6128*, MEXU
Hapalorchis lineata (Lindl.) Schltr.	Pending	Pending	-	Brazil, *Buzatto 643 & Singer*, ICN
Hapalorchis lineata (Lindl.) Schltr.	FN556165	FN556175	FN556170	Porto Rico, *Ackerman 3648*, UPRRP
Hapalorchis aff *lineata* (Lindl.) Schltr.	HE575515	HE575502	HE575525	Guatemala, *Salazar et al. 7699*, MEXU, (spirit)
Lankesterella ceracifolia (Barb.Rodr.) Ames	FN556164	FN556174	FN556169	Argentina, *Salazar 7535*, MEXU (spirit)
Lankesterella gnomus (Kraenzl.) Hoehne	FN556163	FN556173	FN556168	Brazil, *Warren s.n.*, K (spirit)
Lyroglossa grisebachii (Cogn.) Schltr.*	Pending	Pending	Pending	Brazil, *Buzatto 606 & Singer*, ICN
Mesadenella petenensis (L.O.Williams) Garay	AJ539503	AJ543931	AJ544486	Mexico, *Salazar 6069*, MEXU
Mesadenus lucayanus (Britton) Schltr.	AJ539488	-	AJ544471	Mexico, *Salazar 6043*, MEXU
Microthelys minutiflora (A.Rich. & Galeotti) Garay*	AJ539494	AJ543922	AJ544477	Mexico, *Salazar et al. 6129*, MEXU
Odontorrhynchus variabilis Garay	AJ539498	AJ543926	AJ544481	Chile, *Wallace 130/85*, CANB
Pelexia adnata (Sw.) Spreng.*	AJ539501	AJ543929	AJ544484	Mexico, *Salazar 6012*, MEXU
Pelexia bonariensis (Lindl.) Schltr.	-	Pending	Pending	Brazil, *Buzatto 622*, ICN
Pelexia bonariensis (Lindl.) Schltr.	Pending	Pending	Pending	Argentina, *Radins s.n.* MEXU (spirit)
Pelexia congesta Ames & C.Schweinf.	Pending	Pending	Pending	Pending
Pelexia disjuncta???	Pending	Pending	Pending	Pending
Pelexia ekmanii (Kraenzl.) Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 904*, ICN
Pelexia funckiana (A.Rich. & Galeotti) Schltr.	Pending	Pending	Pending	Pending
Pelexia goninensis (Pulle) Schltr.	Pending	Pending	Pending	Brazil, *Smidt s.n.*, UPCB
Pelexia gutturosa (Rchb.f.) Garay	Pending	Pending	Pending	Pending
Pelexia hirta (Lindl.) Schltr.	Pending	Pending	-	Pending
Pelexia incurvidens Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 711 & Singer*, ICN
Pelexia laminata Schtr.	Pending	Pending	Pending	Brazil, *Buzatto 822*, ICN
Pelexia laminata Schtr.	Pending	Pending	Pending	Brazil, *Buzatto 823*, ICN
Pelexia lindmanii Kraenzl.	-	Pending	Pending	Brazil, *Nervo s.n.*, ICN
Pelexia lindmanii Kraenzl.	Pending	Pending	Pending	Argentina, *Radins s.n.* MEXU (spirit)
Pelexia longipetiolata (Rchb.f.) Schltr.	Pending	Pending	Pending	Pending
Pelexia aff *longipetiolata*	Pending	Pending	Pending	Pending

(Rchb.f.) Schltr.				
Pelexia cf *macropoda* (Barb.Rodr.) Schltr.	Pending	Pending	Pending	Brazil, *Nervo s.n.*, ICN
Pelexia cf *macropoda* (Barb.Rodr.) Schltr.	Pending	Pending	-	Brazil, *Buzatto 755 & Nervo*, ICN
Pelexia maculata Rolfe	Pending	Pending	Pending	Pending
Pelexia minarum (Kraenzl.) Schltr.	Pending	Pending	Pending	Brazil, *Batista 3178*, BHCB
Pelexia novofriburgensis (Rchb.f.) Garay	Pending	Pending	Pending	Brazil, *Buzatto 658*, ICN
Pelexia novofriburgensis (Rchb.f.) Garay	Pending	Pending	Pending	Brazil, *Batista 3164*, BHCB
Pelexia oestrifera (Rchb.f. & Warm.) Schltr.	Pending	Pending	Pending	Brazil, *Singer s.n.*, ICN
Pelexia olivacea	Pending	Pending	Pending	Pending
Pelexia orobanchoides (Kraenzl.) Schltr.	Pending	Pending	Pending	Brazil, *Batista 2922*, BHCB
Pelexia orobanchoides (Kraenzl.) Schltr.	Pending	Pending	Pending	Brazil, *Batista 3039*, BHCB
Pelexia orthosepala (Rchb.f. & Warm.) Schltr.	Pending	Pending	-	Brazil, *Buzatto 589*, ICN
Pelexia orthosepala (Rchb.f. & Warm.) Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 694*, ICN
Pelexia paludosa M.N.Correa	-	-	Pending	Brazil, *Singer s.n.*, ICN
Pelexia parva (Cogn.) Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 770 & Nervo*, ICN
Pelexia cf *pterygantha* (Rchb.f. & Warm.) Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 891*, ICN
Pelexia cf *tamanduensis* (Kraenzl.) Schltr.	Pending	-	Pending	Brazil, *Buzatto 889*, ICN
Pelexia weberbaueriana (Kraenzl. ex Schltr.) Schltr.	Pending	Pending	Pending	Pending
Pelexia sp1	Pending	Pending	Pending	Panama, *Salazar 6217*, MEXU
Quechua glabrescens (T.Hashim.) Salazar & L.Jost*	HE575516	HE575503	HE575526	Ecuador, Jost 7916, QCNE
Sarcoglottis acaulis (Sm.) Schltr.	AJ539500	AJ543928	AJ544483	Trinidad, *Salazar 6356*, K (spirit)
Sarcoglottis assurgens (Rchb.f.) Schltr.	Pending	Pending	Pending	Pending
Sarcoglottis biflora (Vell.) Schltr.	Pending	-	Pending	Brazil, *Batista 342*, CEN
Sarcoglottis cerina (Lindl.) P.N.Don	Pending	Pending	Pending	Pending
Sarcoglottis cerina (Lindl.) P.N.Don	Pending	Pending	Pending	Pending
Sarcoglottis corymbosa Garay	Pending	Pending	Pending	Pending
Sarcoglottis curvisepala Szlach. & Rutk.	Pending	Pending	Pending	Brazil, *Smidt s.n.*, UPCB
Sarcoglottis fasciculata (Vell.) Schltr.	Pending	-	Pending	Brazil, *Buzatto 835*, ICN
Sarcoglottis fasciculata (Vell.) Schltr.	Pending	Pending	Pending	Brazil, *Guimarães 184*, ICN
Sarcoglottis grandiflora (Lindl.) Klotzsch	Pending	Pending	Pending	Brazil, *Smidt s.n.*, UPCB
Sarcoglottis homalogastra (Rchb.f. & Warm.) Schltr.	Pending	Pending	Pending	Brazil, *Batista 419*, CEN
Sarcoglottis hunteriana Schltr.	Pending	-	Pending	Pending

Sarcoglottis juergensii Schltr.	Pending	Pending	Pending	Brazil, *Buzatto 719 & Nervo*, ICN
Sarcoglottis lobata (Lindl.) P.N.Don	Pending	Pending	Pending	Pending
Sarcoglottis richardiana (Schltr.) Salazar & Soto Arenas	Pending	Pending	Pending	Pending
Sarcoglottis riocontensis E.C.Smidt & Toscano	-	Pending	Pending	Pending
Sarcoglottis rosulata (Lindl.) P.N.Don	Pending	Pending	Pending	Pending
Sarcoglottis sancta (Rchb.f. & Warm.) Schltr.	Pending	Pending	Pending	Brazil, *Nervo s.n.*, ICN
Sarcoglottis sceptrodes (Rchb.f.) Schltr.	Pending	Pending	Pending	Pending
Sarcoglottis schaffneri (Rchb.f.) Ames	Pending	Pending	-	Pending
Sarcoglottis schaffneri (Rchb.f.) Ames	-	-	Pending	Pending
Sarcoglottis schwackei (Cogn.) Schltr.	-	Pending	-	Brazil, *Batista 2921*, BHCB
Sarcoglottis cf *schwackei* (Cogn.) Schltr.	Pending	Pending	Pending	Brazil, *van den Berg s.n.*, HUEFS
Sarcoglottis scintillans (E.W.Greenw.) Salazar & Soto Arenas	Pending	Pending	Pending	Pending
Sarcoglottis smithii (Rchb.f.) Schltr.	Pending	Pending	Pending	Pending
Sarcoglottis speciosa C.Presl	Pending	Pending	Pending	Pending
Sarcoglottis uliginosa (Barb.Rodr.) Barb.Rodr.	Pending	Pending	Pending	Brazil, *Buzatto 693*, ICN
Sarcoglottis uliginosa (Barb.Rodr.) Barb.Rodr.	Pending	Pending	Pending	Argentina, *Krapovickas s.n.*, MEXU (spirit)
Sarcoglottis ventricosa (Vell.) Hoehne	Pending	-	-	Brazil, *Smidt 956*, UPCB
Sarcoglottis sp1	Pending	-	Pending	Pending
Sarcoglottis sp2	Pending	-	Pending	Brazil, *da Silva s.n.*, MEXU (spirit)
Sarcoglottis sp3	Pending	-	Pending	Pending
Sauroglossum corymbosum (Lindl.) Garay	Pending	Pending	Pending	Pending
Sauroglossum elatum Lindl.*	Pending	Pending	-	Brazil, *Guimarães 167*, SP
Sauroglossum elatum Lindl.*	HG425361	HG425365	HG425368	Argentina, *Cabassi s.n.*, MEXU (spirit)
Sauroglossum elatum Lindl.*	-	Pending	Pending	Pending
Sauroglossum elatum Lindl.*	Pending	Pending	Pending	Brazil, *van den Berg s.n.*, HUEFS
Schiedeella faucisanguinea (Dod) Burns-Bal.	AJ539496	AJ543924	AJ544479	Mexico, *Jimenez s.n.*, AMO
Schiedeella llaveana (Lindl. ex Benth.) Schltr.	AJ539487	AJ543915	-	Mexico, *Salazar 6105*, MEXU
Spiranthes cernua (L.) Rich.	AJ539489	AJ543916	AJ544472	USA, *Nickrent 4188*, MEXU
Spiranthes spiralis (L.) Chevall.*	AJ539490	AJ543918	AJ544473	UK, *Bateman s.n.*, K (spirit)
Stenorrhynchos speciosum (Jacq.) Rich. ex Spreng.*	AJ539505	-	AJ544487	Mexico, *Salazar 6090*, MEXU
Stigmatosema inaequilatera (Poepp. & Endl.) Szlach.	-	Pending	-	Ecuador, *Ecuagenera*, MEXU (spirit)

[=*Cyclopogon inaequilaterus* (Poepp. & Endl.) Schltr.]				
Stigmatosema polyaden (Vell.) Garay (=*Cyclopogon chloroleuchus* Barb.Rodr.)	-	Pending	Pending	Argentina, *Radins s.n*., MEXU (spirit)
Stigmatosema polyaden (Vell.) Garay (=*Cyclopogon chloroleuchus* Barb.Rodr.)	Pending	Pending	Pending	Brazil, *Batista 2901*, BHCB
Svenkoeltzia congestiflora (L.O.Williams) Burns-Bal.*	AJ539493	AJ543921	AJ544476	Mexico, *Salazar 6143*, MEXU
Veyretia hassleri (Cogn.) Szlach.*	Pending	Pending	-	Brazil, *Buzatto 841 & Nervo*, ICN
Veyretia hassleri (Cogn.) Szlach.*	Pending	Pending	Pending	Brazil, *Buzatto 677 & Nervo*, ICN
Veyretia rupicola (Garay) F.Barros	Pending	-	Pending	Brazil, *Smidt s.n.*, UPCB
Veyretia sagittata (Rchb.f. & Warm.) Szlach.	Pending	Pending	-	Brazil, *Batista 1651*, BHCB
Veyretia simplex (Griseb.) Szlach.	Pending	Pending	Pending	Brazil, *Batista 3068,* BHCB
Veyretia cf *simplex* (Griseb.) Szlach.	Pending	Pending	Pending	Brazil, *van den Berg 1477*, HERB
Veyretia sincorensis (Schltr.) Szlach.	Pending	Pending	Pending	Pending
Veyretia sincorensis (Schltr.) Szlach.	Pending	Pending	Pending	Brazil, *van den Berg s.n*., HUEFS

CAPÍTULO 6

AN UPDATED PHYLOGENY OF CHLORAEINAE (ORCHIDACEAE)

Cristiano Roberto Buzatto, Agustín Sanguinetti,
Cássio van den Berg e Rodrigo B. Singer

An updated phylogeny of Chloraeinae (Orchidaceae)

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Abstract - Recent phylogenetic studies based on molecular features have confirmed the position Chloraeinae in Cranichideae. However, the uniflorous or Pampean *Bipinnula* species, including the type species of the genus, were not sampled. Here, all the extra-Andean species are sampled and their relationships are accessed. Our analyses confirm *Chloraea* as non-monophyletic. However, the sampling of the species from northern Argentina, Bolivia and Peru is necessary until a taxonomic decision is made. On the other hand, both *Gavilea* and *Bipinnula* are confirmed as monophyletic. *Bipinnula* sect. *Bipinnula*, *B.* sect. *Trilobatae* and *B*. sect. *Multiflorae* are recovered as monophyletic in our analyses.

Keywords – *Bipinnula*, *Chloraea*, *Gavilea*, *matK-trnR*, nrITS, Pampa, *trnL-trnF*

Introduction

The species of Chloraeinae Pfitzer are particularly peculiar for its complex floral morphology. In its current circumscription, the subtribe comprises approximately 70 species in three genera, (Correa & Sánchez 2003, Chemisquy 2012, Chemisquy & Morrone 2010, 2012 Cisternas *et al*. 2012a, Cisternas *et al*. 2012b): *Chloraea* Lindl. (45 species), *Bipinnula* Comm. ex Juss. (11–12 species) and *Gavilea* Poepp. (17 species). These genera are essentially Andean (Correa & Sánchez, 2003), with species occurring in southern Brazil, northeastern Argentina and Uruguay (Buzatto *et al*., 2014).

In the last decades, Chloraeinae has been defined and interpreted in different ways. The three genera above mentioned plus *Geoblasta* Barb. Rodr. and *Codonorchis* Lindl. were referred by Dressler (1981, 1993), Szlachetko (1995), Kores *et al*. (2001) and Clements *et al*. (2002) to the subtribe Chloraeinae, based on the presence of multiple leaves, embryos similar to these of Spiranthinae and roots not tuberous. However, although Dressler (1981, 1993) inserted Chloraeinae in tribe Diurideae Endl., Szlachetko (1995) inserted it in Geoblasteae Barb. Rodr. More recently, Clements *et al*. (2002) re-circumscribed Chloraeinae, including it in subtribe Cranichideae Lindl. ex Meisn. and inserted *Codonorchis* in a separate subfamily, Codonorchidoideae Clement & Jones. Later, Correa & Sanchez (2003) re-established the status of the tribe Codonorchideae Cribb based on the molecular results of Kores *et al*. (2001). Recent phylogenetic analyses based on nuclear and plastid sequences (Chemisquy & Morrone 2010, 2012, Cisternas *et al*. 2012b) confirmed the proposal of Clements *et al*. (2002), with Chloraeinae being a sister group to subtribes Spiranthinae Lindl. ex Meisn., Manniellinae Schltr., Cranichidinae Lindl. ex Meisn. and Goodyerinae Klotz, collectively. Recently, Cisternas *et al*. (2012b) demonstrated that *Geoblasta penicillata* (Rchb.f) Hoehne is nested within *Bipinnula*. Therefore, part of these authors (Cisternas *et al*. 2012a) proposed the combination *B. penicillata* (Rchb.f.) Cisternas & Salazar. However, it is important to stress that the sampling of the uniflorous *Bipinnula* spp. so far has been incomplete, with most species missing in phylogenetic analyses (Cisternas *et al*., 2012b; Buzatto *et al*., 2014).

The main goal of this paper is to build a more complete and representative molecular phylogeny of Chloraeinae, with the inclusion of the extra-Andean taxa, especially the type species of *Bipinnula* (*B. biplumata*).

Material and methods

Taxon sampling

Seventy-two ingroup taxa representing 46 species were sampled including representatives of the three genera (*Bipinnula*, *Chloraea* and *Gavilea*) accepted by Cisternas *et al*. (2012b). The sample included the types of three currently accepted genera, i.e., *Bipinnula biplumata* (L.f.) Rchb.f., *Chloraea virescens* (Willd.) Lindl. and *Gavilea leucantha* Poepp. Leaf samples were collected from natural habitats, dried, and preserved in silica gel (Chase and Hills 1991).

DNA extraction, amplification and sequencing

Total DNA was extracted from leaves dried in silica gel using standard method explained in Salazar *et al*. (2011). In this study, all analysis were performed with nucleotide sequences of nuclear (nrITS) and plastid (*matK*-*trnK*, *rbcL* and *trnL*-*trnF*) DNA, which were amplified as explained in Cisternas *et al*. (2012b). PCR products were purified by precipitation with PEG 11% (polyethylene glycol), and were bidirectionally sequenced with the same primers used for the PCR amplification. Sequence eletropherograms were produced in an automatic sequencer (ABI 3130XL Genetic Analyzer) using Big Dye Terminator 3.1 (Applied Biosystem). Sequence reads were obtained for all of the DNA regions, and the resulting chromatograms were edited and assembled using the Geneious 5.6.5 (Biomatters, Aukland, New Zealand available from www.geneious.com). The edited sequences were aligned with MAFFT (Katoh *et al*., 2002), and manually adjusted using Seaview v4 (Gouy *et al*., 2010). The aligned matrix consisted of 5,773 characters, of which 687 correspond to the nrITS, 1,954 to the partial *matK-trnK*, 1,400 to the *rbcL* and 1,732 to the *trnL-trnF*. Taxa, voucher information, geographic origin, and GenBank numbers are listed in Table 1.

Model test

All DNA regions were partitioned according to molecular evolution patterns (nrITS: ITS1, rRNA and ITS2; *matK-trnR*: tRNA-LysA, *matK* and tRNA-LysB; *rbcL*; *trnL-trnF*: tRNA-Leu intron, tRNA exon, intergenic spacer and tRNA-Phe). The optimal evolutionary model for each partition was selected using jModelTest (Posada, 2008), using the Bayesian Information Criterion (BIC). The substitution models are shown in Table 2.

Phylogenetic analysis

The data were analysed using maximum parsimony (MP) and Bayesian inference (BI). We first inferred the trees for each of the three DNA regions separately to identify potential incongruence. As there were no instances of conflict at well-supported nodes, the matrices of the three regions were concatenated and analysed taking into account partitions for each of the DNA regions (see Model test).

Maximum parsimony (MP) analyses were performed using PAUP* version 4.0b 10 (Swofford, 2002) with Fitch parsimony (equal weights, unordered characters; Fitch, 1971) as the optimality criterion. The MP trees were obtained through heuristic searches, each with 2,000 random taxon-addition replicates, with branch swapping using the tree-bisection and reconnection (TBR) algorithm, saving up to 15 trees per replicate to prevent extensive swapping on suboptimal trees in islands with many trees. The resulting trees were then used in a second search with the same parameters of the first, however without limit for the number of trees per replicate. Parsimony bootstrap percentages (BP_P) were used on 1,000 replicates of character bootstrapping (Felsenstein, 1985), with simple taxon-addition and the TBR algorithm, saving up to 20 trees per replicate. The BP_P considered in this study were: 50–70% as weak, 71–85% as moderate, and >85% as strong (Kress *et al.*, 2002).

The BI analysis of the combined dataset was implemented in MrBayes v. 3.2.1 (Huelsenbeck & Ronquist, 2001; Ronquist *et al.*, 2012). Each analysis consisted of two independent runs with four chains for 5,000,000 generations, sampling one tree every 1,000 generations. In the combined analysis, in order to improve swapping of chains, the temperature parameter for heating the chains was lowered to 0.01. Convergence between the runs was evaluated by the average standard deviation of split frequencies (<0.01). After discarding the first 50% of the trees as burn-in, the remaining trees were used to assess topology and posterior probabilities (PPs) in a majority-rule consensus. PPs in Bayesian analysis are not equivalent to BPs, but are generally much higher (Erixon *et al.*, 2003). Therefore, we used criteria similar to a standard statistical test, considering groups with PPs >0.95 as strongly supported, groups with PPs ranging from 0.90–0.95 as moderately supported, and groups with PPs <0.90 as weakly supported.

Results

Parsimony analysis, Bayesian Inference and tree topology

Table 3 gives details of the sequence and alignment lengths and information content of the data included in this study. The combined matrix includes 5,773 characters analysed. In total, 933 were variable of which 981 were potentially parsimony informative. The parsimony search produced 3,882 trees with a length of 161 steps, a consistence index (CI) of 0.6517 and a retention index (RI) of 0.7686 (Table 3). The MP trees found are topologically identical with the BI tree, and are depicted in Figs. 2. The main difference was the placement of the clade formed by *Chloraea grandiflora* Poepp., *Chloraea speciosa* Poepp., *Chloraea magellanica* Hook. f. and *Chloraea gaudichaudii* Brongn. as sister to *Gavilea* + *Bipinnula* (BP_P 71).

The relationships generated by the combined matrix are less supported than those generated by nuclear and plastid regions isolated. All bootstrap values are shown in Fig. 2. In the combined dataset, Chloraeinae is strongly supported in BI analyses (PP 1.00; Fig. 2). With the exception of the *trnL-trnF* plastid gene, in both individual or combined data sets, Chloraeinae is recovered as a monophyletic group. The group is weakly supported (BP_P 68) by plastid *rbcL* data, while with nrITS it is moderately supported (BP_P 81) and with *matK-trnK* and all concatenated regions were strongly supported (BP_P 100).

Chloraea is non-monophyletic, forming a grade with five distinct subclades in both MP and BI analyses (Fig. 2): Clade 1) *Chloraea cylindrostachya* Poepp. and *Chloraea leptopetala* Rchb.f. are placed together in both individual and combined analyses, and this group is sister to remaining *Chloraea* species + *Gavilea* + *Bipinnula*. This cluster is weakly supported (BP_P 69) in nrITS trees, whereas in *matK-trnK*, *trnL-trnF* and combined datasets it is strongly supported (BP_P >95). This relationship collapsed in the *rbcL* trees; Clade 2) *Chloraea reticulata* Schltr. was not included in any of the three clades in both BI and MP and is sister of the all *Chloraea* species (except *Chloraea cylindrostachya* + *Chloraea leptopetala*), more *Gavilea* and *Bipinnula*, and is weakly supported in nrITS and *rbcL* sets (BP_P 69 and BP_P 66, respectively) and strongly supported for the other datasets (BP_P >95); Clade 3) the group formed by *Chloraea gaudichaudii*, *Chloraea magellanica*, *Chloraea speciosa* and *Chloraea grandiflora* is strongly supported in all analyses (BP_P >94), with the exception of *rbcL* in which it is not recovered. The position of this clade is contrasting between the MP and BI analysis, being sister to *Gavilea* + *Bipinnula* in MP and unresolved in relation to *Gavilea* + *Bipinnula* and Clade 3 in the BI analysis; Clade 4) *Chloraea membranacea* Lindl. is closely related to the core-*Chloraea*, but always in separated datasets analyses; Clade 5) the main subclade is named herein as the *Chloraea*-core. This group includes *Chloraea virescens* (Willd.) Lindl. (the type species of the genus), *Chloraea nudilabia* Poepp., *Chloraea alpina* Poepp., *Chloraea volkmanii* Phil. ex Kraenzl., *Chloraea cristata* Lindl., and others. Indeed, the *Chloraea*-core is a polytomy strongly supported by nrITS, *matK-trnR* and combined analyses (BP$_{P}$ 100, BP$_{P}$ 86 and BP$_{P}$ 99, respectively) but moderately and weakly supported in separate analyses of *rbcL* and *trnL-trnF* (BP$_{P}$ 79 and BP$_{P}$ 51, respectively).

Gavilea is monophyletic, moderately supported by the nrITS and *matK-trnR* datasets (BP$_{P}$ 75 and BP$_{P}$ 70 respectively). In analyses with *rbcL* and *trnL-trnF* sets it is not recovered. However, *Gavilea* is strongly supported in the combined analysis of nuclear and plastid regions (BP$_{P}$ 97, PP 1.00; Fig. 2), of which *Gavilea chica* and *Gavilea australis* (Skottsb.) M.D. Correa (PP 1.00) form a group which in turn is sister of the other *Gavilea* species.

Bipinnula is monophyletic in combined analyses (BP$_{P}$ 75, PP 1.00; Fig. 2). For individual datasets, the internal relationships are unclear. In general, two groups are established: a clade formed by *Bipinnula biplumata* (the type species of the genus) along with other uniflorous or Pampean species (BP$_{P}$ 100, PP 1.00; Fig. 2), and the other clade composed for multi-flowered species (*Bipinnula apinnula* Gosewijn and *Bipinnula volkmannii* Kraenzl.). The uniflorous or Pampean group is strongly supported in individual analyses of nrITS and *matK-trnR* (BP$_{P}$ 99 and BP$_{P}$ 97, respectively), as well as the group formed by multi-flowered species (BP$_{P}$ 99 and BP$_{P}$ 100, respectively).

Discussion

Non-monophyly of Chloraea

Our analyses (both MP and BI) show *Chloraea* is non-monophyletic, a pattern also obtained in phylogenies previously published (Chemisquy & Morrone, 2010, 2012; Cisternas *et al.*, 2012b). In phylogenetic studies aforementioned, *Chloraea cyclindrostachya* occupied the most basal position in the Chloraeinae. In our analyses, *Chloraea cylindrostachya* grouped with *Chloraea leptopetala*, sampled for the first time in the present study. Both taxa are the only species of the western group (*sensu* Hauman, 1922). Thus, our results recover basically the same five subclades species of Chemisquy & Morrone (2010, 2012), which differs from the relationships shown by Cisternas *et al*. (2012b). In the analysis of the latter authors, *Bipinnula* + *Gavilea* are a sister-group to the core *Chloraea*. Although we have a sampling that is very similar to Cisternas *et al*. (2012b), it is possible that these differences are because not sampled plastid region *rpoB-trnC*. Nevertheless, the internal relationships of the core *Chloraea* are not clearly resolved. We understand that the inclusion of further plastid and nuclear regions can help in resolving this group.

Our analyses did not recover the circumscription of *Chloraea* proposed by Correa (1969). For a strict concept of *Chloraea* (so called as *Chloraea* core), it would be necessary to fragment this genus in four or five new genera, based on subgroups that our analysis has been showing, as well as those already published phylogenies (Chemisquy & Morrone, 2010, 2012; Cisternas *et al.*, 2012b). However, we agree with Cisternas *et al*. (2012b) that a more representative sample available is needed to reach a taxonomic decision, once all recent analyses have important sampling gaps, such as species from northwestern Argentina, Bolivia and Peru (Buzatto *et al.*, 2014).

Phylogenetic position of uniflorous or Pampean Bipinnula *species*

All non-Andean *Bipinnula* spp. were sampled here, including the type species of the genus, *Bipinnula biplumata*. Our results grouped *Bipinnula biplumata* on a broad generic concept, in which this species is grouped with the rest of uniflorous or Pampean species. Our phylogenetic analyses support the transfer of *Geoblasta penicillata* to *Bipinnula*, proposed by Cisternas *et al*. (2012b). The multiflorous species (*Bipinnula fimbriata* (Phil.) I.M. Johnst., *Bipinnula volkmanii* and *Bipinnula apinnula*) are more divergent than those corresponding to Pampean group. However, the multiflorous species form a grade and our analyses thus did not support the recognition of *Jouyella* Szlach. (Szlachetko & Margonska, 2001). On the other hand, the sections proposed by Nieuwenhuizen (1993) are recovered, in which *Bipinnula* Sect. *Bipinnula* accommodates all uniflorous or Pampean species including *Bipinnula penicillata*

(BPP 100, PP 1:00); *Bipinnula* sect. *Trilobatae* Nieuwenhuizen includes the Chilean species *Bipinnula apinnula* and *Bipinnula volkmanii* (BPP 100; 1:00 PP), and *Bipinnula* sect. *Multiflorae* Nieuwenhuizen encompassing *Bipinnula fimbriata*.

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Table 1. Species and gene regions for this study, voucher information, geographic origin and GenBank accession numbers. Asterisks indicate the type of the genera

Taxon	**Country**	**nrITS**	***matK-trnR***	***rbcL***	***trnL-trnF***
Tribe Codonorchideae P.J.Cribb					
Codonorchis lessonii (Brongn.) Lindl.*	Ryan 002, K (spirit)	AF348005	-	AJ542399	AJ544508
Codonorchis lessonii (Brongn.) Lindl.*	Argentina, Sanguinetti 72, BA	Pending	Pending	Pending	Pending
Subtribe Achlydosinae M.A.Clem. & D.L.Jones					
Achlydosa glandulosa (Schltr.) M.A.Clem. & D.L. Jones*	New Caledonia, Clements D-285, CANB	AJ539525	AJ543950	AJ542401	AJ544506
Subtribe Prasophyllinae Schltr.					
Microtis parviflora R. Br.	Australia, MA21, CANB	DQ104550	-	-	-
Subtribe Orchidinae Dressler & Dodson					
Ophrys apifera Huds.	UK, Chase 536, K	AJ539529	AJ543953	AJ542396	AJ544511
Subtribe Pterostylidinae Pfitz.					
Pterostylis curta R. Br.*	Australia, Chase 572, K	AJ539526	AJ543951	AJ542400	AJ544507
Subtribe Diuridinae Lindl.					
Diuris sulphurea R. Br.	Chase 554, K	AJ539527	AJ543952	AJ542398	AJ544509
Tribe Cranichideae Endl.					
Subtribe Cranichidinae Lindl.					
Aa achalensis Schltr.	Argentina, Singer s.n., ICN	Pending	Pending	Pending	Pending
Aa paleacea (Kunth) Rchb. f.*	Chase 535, K	AJ539515	AJ309989	AJ542410	AJ544497
Cranichis revoluta Hamer & Garay	Mexico, Soto 10097, AMO	AM419783	AM900818	AM778147	AM412718
Gomphichis caucana Schltr.	Colombia, Dıaz 159, ANDES	AM419770	AM900805	AM778136	AM412736
Ponthieva elata Schltr.	Colombia, Salazar s.n., MEXU (photograph)	AM419792	AM900827	AM778158	AM412708
Porphyrostachys pilifera (Kunth) Rchb. f.*	Peru, Whalley s.n., K (photograph)	AJ539514	AJ543942	AJ542411	AJ544496
Prescottia plantaginifolia Lindl. ex Hook.*	Brazil, Salazar 6350, K (spirit)	AJ539511	AJ543939	AJ542414	AJ544493
Pterichis habenarioides (F. Lehm. & Kraenzl.) Schltr.	Colombia, Aldana 12, COL	AJ539509	AJ543937	AJ542416	AJ544491
Stenoptera ecuadorana Dodson & C. Vargas	Ecuador, Salazar 6357, K (spirit)	AJ539512	AJ543940	AJ542413	AJ544494
Subtribe Galeottiellinae Salazar & M.W.Chase					
Galeottiella sarcoglossa (A. Rich. & Galeotti) Schltr.*	Mexico, Jimenez 2334, AMO	AJ539518	AJ543945	AJ542407	AJ544500
Subtribe Goodyerinae Klotzsch					
Goodyera pubescens (Willd.) R. Br.	USA, Chase 212, NCU	AJ539519	AJ543954	AF074174	AM419815
Ludisia discolor (Ker Gawl.) Blume*	Tropical Asia, Salazar 6354, K (spirit)	AJ539483	AJ543911	AJ542395	AJ544466
Subtribe Manniellinae Schltr.					
Manniella gustavi Rchb. f.*	Cameroon, Etuge 4515R, YA	AJ539517	AJ543944	AJ542408	AJ544499
Subtribe Chloraeinae Rchb. f.					
Bipinnula apinnula Gosewijn	Chile, Ryan 002, K (spirit)	FR832100	FR832063	FR831957	-
Bipinnula biplumata (L. f.) Rchb. f.*	Argentina, Sanguinetti 47, BA	Pending	Pending	Pending	Pending
Bipinnula biplumata (L. f.) Rchb. f.*	Argentina, Sanguinetti 46, BA	Pending	Pending	Pending	Pending
Bipinnula biplumata (L. f.) Rchb. f.*	Brazil, Buzatto 561, ICN	Pending	Pending	Pending	Pending
Bipinnula biplumata (L. f.) Rchb. f.*	Argentina, Sanguinetti s.n., BA	Pending	Pending	Pending	Pending

Bipinnula fimbriata (Phil.) I.M. Johnst.	Chile, Cisternas 111, CONC	FR832101	FR832064	FR831958	FR832135
Bipinnula fimbriata (Phil.) I.M. Johnst.	Chile, V Región, Valparaíso, Concon, Chemisquy 01 (SI)	JQ045521	-	-	-
Bipinnula giberti Rchb. f.	Brazil, Buzatto 652 & Singer, ICN	Pending	Pending	Pending	Pending
Bipinnula giberti Rchb. f.	Brazil, Buzatto 725 & Nervo, ICN	Pending	Pending	Pending	Pending
Bipinnula montana Arechav.	Uruguay, Cisternas 112, CONC	FR832102	FR832065	FR831959	FR832137
Bipinnula montana Arechav.	Brazil, Buzatto 645 & Singer, ICN	Pending	Pending	Pending	Pending
Bipinnula penicillata (Rchb. f.) Cisternas & Salazar	Argentina, Benitez s.n., CORD	FR832134	FR832098	FR831993	FR832167
Bipinnula penicillata (Rchb. f.) Cisternas & Salazar	Argentina, Sanguinetti 44, BA	-	Pending	Pending	Pending
Bipinnula penicillata (Rchb. f.) Cisternas & Salazar	Brazil, Buzatto 648 & Singer, ICN	Pending	Pending	Pending	Pending
Bipinnula polysyka Kraenzl.	Argentina, Sanguinetti 65, BA	Pending	Pending	Pending	Pending
Bipinnula polysyka Kraenzl.	Argentina, Sanguinetti 56, BA	Pending	Pending	Pending	Pending
Bipinnula volkmanni Kraenzl.	Chile, Rodríguez & Marticorena 2259, CONC	FR832099	FR832062	FR831956	-
Chloraea alpina Poepp.	Argentina, Sanguinetti 73, BA	Pending	Pending	Pending	Pending
Chloraea alpina Poepp.	Chile, Saavedra & Pauchard 296, CONC	FR832114	FR832079	FR831974	FR831974
Chloraea barbata Lindl.	Chile, Cisternas 106, CONC	-	FR832070	FR831965	FR832142
Chloraea bicallosa Phil. ex Kraenzl.	Chile, Cisternas 113, CONC	FR832108	FR832071	FR831966	FR832143
Chloraea bidentata (Poepp. & Endl.) M.D. Correa	Chile, Cisternas 109, CONC	-	FR832072	FR831967	FR832144
Chloraea bletioides Lindl.	Chile, Cisternas 104, CONC	FR832111	FR832075	FR831970	FR832147
Chloraea chrysantha Poepp.	Chile, Novoa s.n., CONC	FR832113	FR832078	FR831973	FR832150
Chloraea crispa Lindl.	Chile (cultivated specimen), Cisternas 103, CONC	FR832109	FR832073	FR831968	FR832145
Chloraea cristata Lindl.	Chile, Novoa 177, CONC	FR832110	FR832074	FR831969	FR832146
Chloraea cuneata Lindl.	Chile, Espejo s.n., CONC	-	FR832076	FR831971	FR832148
Chloraea cylindrostachya Poepp.	Chile, Cisternas 123, CONC	FR832118	FR832083	FR831978	FR832154
Chloraea cylindrostachya Poepp.	Argentina, Sanguinetti 2, BA	Pending	Pending	Pending	Pending
Chloraea cylindrostachya Poepp.	Argentina, Sanguinetti 79, BA	Pending	Pending	Pending	Pending
Chloraea disoides Lindl.	Chile, Cisternas 122, CONC	FR832115	FR832080	FR831975	FR832152
Chloraea gaudichaudii Brongn.	Argentina, Sanguinetti 82, BA	Pending	Pending	Pending	Pending
Chloraea gaudichaudii Brongn.	Chile, Cisternas 120, CONC	FR832116	FR832081	FR831976	-
Chloraea gavilu Lindl.	Chile (cultivated specimen), Cisternas 102, CONC	FR832121	FR832086	FR831981	FR832157
Chloraea grandiflora Poepp.	Chile, Espejo 21, CONC	FR832103	FR832061	FR831960	-

Chloraea heteroglossa Rchb. f.	Chile (cultivated specimen), Cisternas 105, CONC	FR832107	FR832069	FR831964	FR832141
Chloraea incisa Poepp.	Chile (cultivated specimen), Cisternas s.n., CONC	FR832125	FR832089	FR831984	FR832159
Chloraea lamellata Lindl.	Chile, Cisternas 116, CONC	FR832120	FR832085	FR831980	FR832156
Chloraea lechleri Lindl. ex Kraenzl.	Chile, Cisternas 107, CONC	FR832124	FR832088	FR831983	FR832158
Chloraea leptopetala Reiche	Argentina, Sanguinetti 80, BA	Pending	Pending	Pending	Pending
Chloraea longipetala Lindl.	Chile, Cisternas 115, CONC	FR832112	FR832077	FR831972	FR832149
Chloraea magellanica Hook. f.	Chile, Ryan 1, K (spirit)	AJ539523	AJ543948	AJ542403	AJ544504
Chloraea magellanica Hook. f.	Argentina, Sanguinetti 3, BA	Pending	Pending	Pending	Pending
Chloraea magellanica Hook. f.	Argentina, Sanguinetti 74, BA	Pending	Pending	Pending	Pending
Chloraea membranacea Lindl.	Chile, Cisternas 108, CONC (photograph and dissected flower)	FR832126	FR832091	FR831986	FR832161
Chloraea membranacea Lindl.	Brazil, Buzatto 745 & Singer, ICN	Pending	Pending	Pending	Pending
Chloraea membranacea Lindl.	Brazil, Buzatto 579 & Singer, ICN	Pending	Pending	Pending	Pending
Chloraea membranacea Lindl.	Argentina, Sanguinetti 48, BA	Pending	Pending	Pending	Pending
Chloraea multiflora Lindl.	Chile, Novoa 126, CONC	FR832119	FR832084	FR831979	FR832155
Chloraea nudilabia Poepp.	Chile, Cisternas 114, CONC	-	FR832090	FR831985	FR832160
Chloraea philippii Rchb. f.	Chile, Cisternas 119, CONC	FR832106	FR832068	FR831963	FR832140
Chloraea philippii Rchb. f.	Argentina, Sanguinetti 76, BA	Pending	Pending	Pending	Pending
Chloraea reticulata Schltr.	Peru, Weigend 2000-361, NY	FJ473314	-	FJ571318	FJ571268
Chloraea speciosa Poepp.	Chile, Cisternas 121, CONC	FR832105	FR832067	FR831962	FR832139
Chloraea virescens (Willd.) Lindl.*	Argentina, Sanguinetti 81, BA	Pending	Pending	Pending	Pending
Chloraea virescens (Willd.) Lindl.*	Chile, Cisternas 117, CONC	FR832122	FR832087	-	-
Chloraea virescens (Willd.) Lindl.*	Argentina, Sanguinetti 70, BA	Pending	Pending	Pending	Pending
Chloraea volkmanni Phil. ex Kraenzl.	Chile, Cisternas 118, CONC	FR832104	FR832066	FR831961	FR832138
Gavilea araucana (Phil.) M.D. Correa	Argentina, Sanguinetti 83, BA	Pending	Pending	Pending	Pending
Gavilea araucana (Phil.) M.D. Correa	Chile, Cisternas 124, CONC	FR832129	FR832093	FR831988	FR831988
Gavilea australis (Skottsb.) M.D. Correa	Chile, Cisternas 125, CONC	FR832128	FR832092	FR831987	-
Gavilea chica (Speg. & Kraenzl.) Chemisquy	Chile, Tellier & Márquez 5328, CONC	FR832117	FR832082	FR831977	FR832153
Gavilea glandulifera (Poepp.) M.D. Correa	Argentina, Sanguinetti 6, BA	Pending	Pending	Pending	Pending
Gavilea glandulifera (Poepp.) M.D. Correa	Argentina, Sanguinetti 77, BA	Pending	Pending	Pending	Pending
Gavilea glandulifera (Poepp.)	Chile, Cisternas 126,	FR832131	FR832095	FR831990	FR832164

M.D. Correa	CONC				
Gavilea leucantha Poepp.*	Chile, Novoa 259, CONC	FR832130	FR832094	FR831989	FR832163
Gavilea lutea (Comm. ex Pers.) M.D. Correa	Chile, Ryan 3, K (spirit)	AJ539524	AJ543949	AJ542402	AJ544505
Gavilea lutea (Comm. ex Pers.) M.D. Correa	Argentina, Sanguinetti 75, BA	Pending	Pending	Pending	Pending
Gavilea odoratissima Poepp.	Chile, Cisternas 127, CONC	FR832133	FR832097	FR831992	FR832166
Gavilea trullata (Garay) Ormerod	Argentina, Sanguinetti 12, BA	Pending	Pending	Pending	Pending
Gavilea trullata (Garay) Ormerod	Argentina, Sanguinetti 10, BA	Pending	Pending	Pending	Pending
Gavilea venosa (Lam.) Garay & Ormerod	Chile, Novoa 81, CONC	FR832132	FR832096	FR831991	FR832165

Table 2. Model test selected

Region/categories	Model	PB
nrITS		
its1	SYM+I+G	290pb
rRNA	K80+G	168pb
its2	K80+G	229pb
matK-trnR		
tRNA-LysA	JC	39pb
matK	SYM+G	1653pb
tRNA-LysB	JC+G	262pb
rbcL	K80+I+G	1400pb
trnL-trnF		
tRNA-Leu intron	SYM+G	850pb
tRNA exon	K80+G	61pb
intergenic spacer	SYM+G	656pb
tRNA-Phe	K80	165pb

Table 3. Description of nuclear and plastid data sets

Data set	**Aligned length**	**Constant characters**	**Variable but parcimony uninformative/%**	**Parcimony informative/ %**	**CI**	**RI**	**Tree length**	**Steps**	**Number of trees retained**
nrITS	687	258	117/17.03	312/45.41	0.5647	0.7857	1236	157	13380
matK-trnR	1954	1289	380/19.45	285/14.59	0.6983	0.7721	1147	168	9855
rbcL	1400	1188	131/9.36	81/5.79	0.7926	0.8520	299	134	13650
trnL-trnF	1732	1196	307/17.73	229/13.22	0.7564	0.7751	903	160	13590
combined	5773	3859	933/16.16	981/16.99	0.6517	0.7686	3882	161	12855

LEGENDS

Figure 1. Representative species of Chloraeinae. A, *Bipinnula biplumata*. B, *Bipinnula giberti*. C, *Bipinnula montana*. D, *Bipinnula polysyka*. E, *Chloraea alpina*. F, *Chloraea cylindrostachya*. G, *Chloraea virescens*. H, *Codonorchis lessonii*. I, *Gavilea araucana*. J, *Gavilea lutea*. K, *Gavilea glandulifera*.

Figure 2. Phylogenetic relationships of Chloraeinae inferred from combined analyses of plastid and nuclear sequences. A, strict consensus of the 12,855 most parcimonious trees (MPTs) found by the parsimony analysis (numbers above branches are bootstrap proportions, not shown when < 50%). B, Bayesian summary tree (numbers above branches are posterior probabilities).

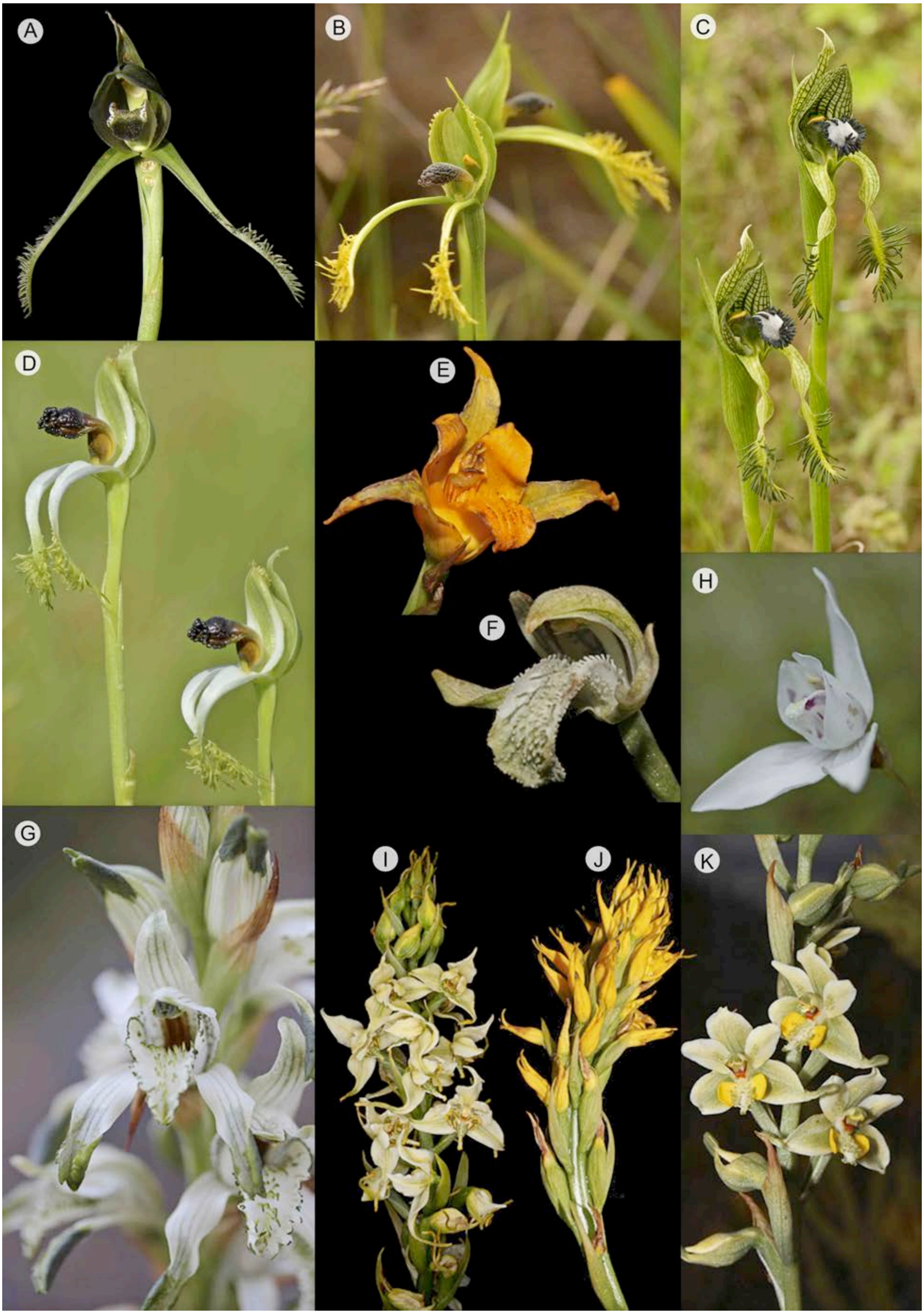


Figure 1

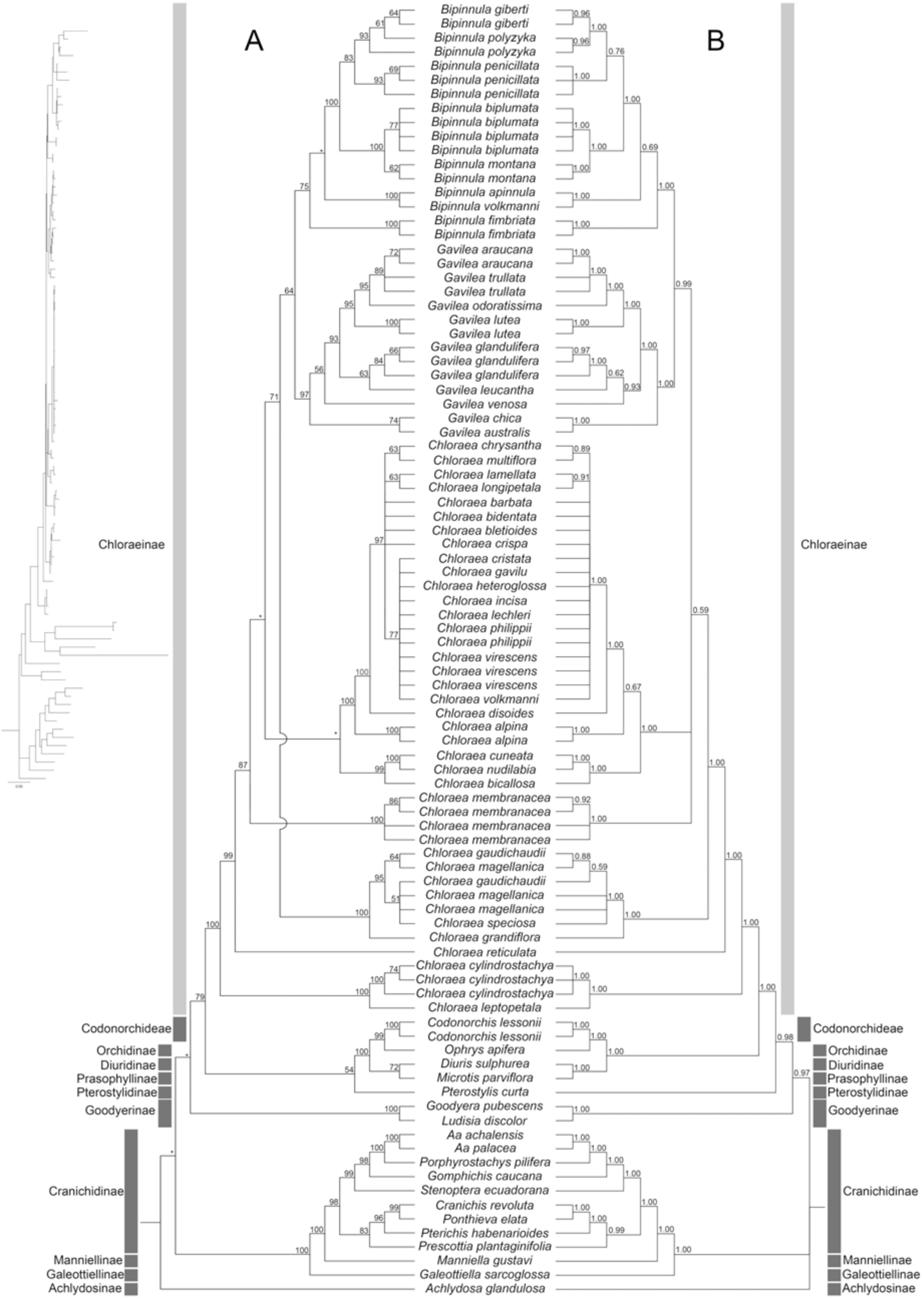
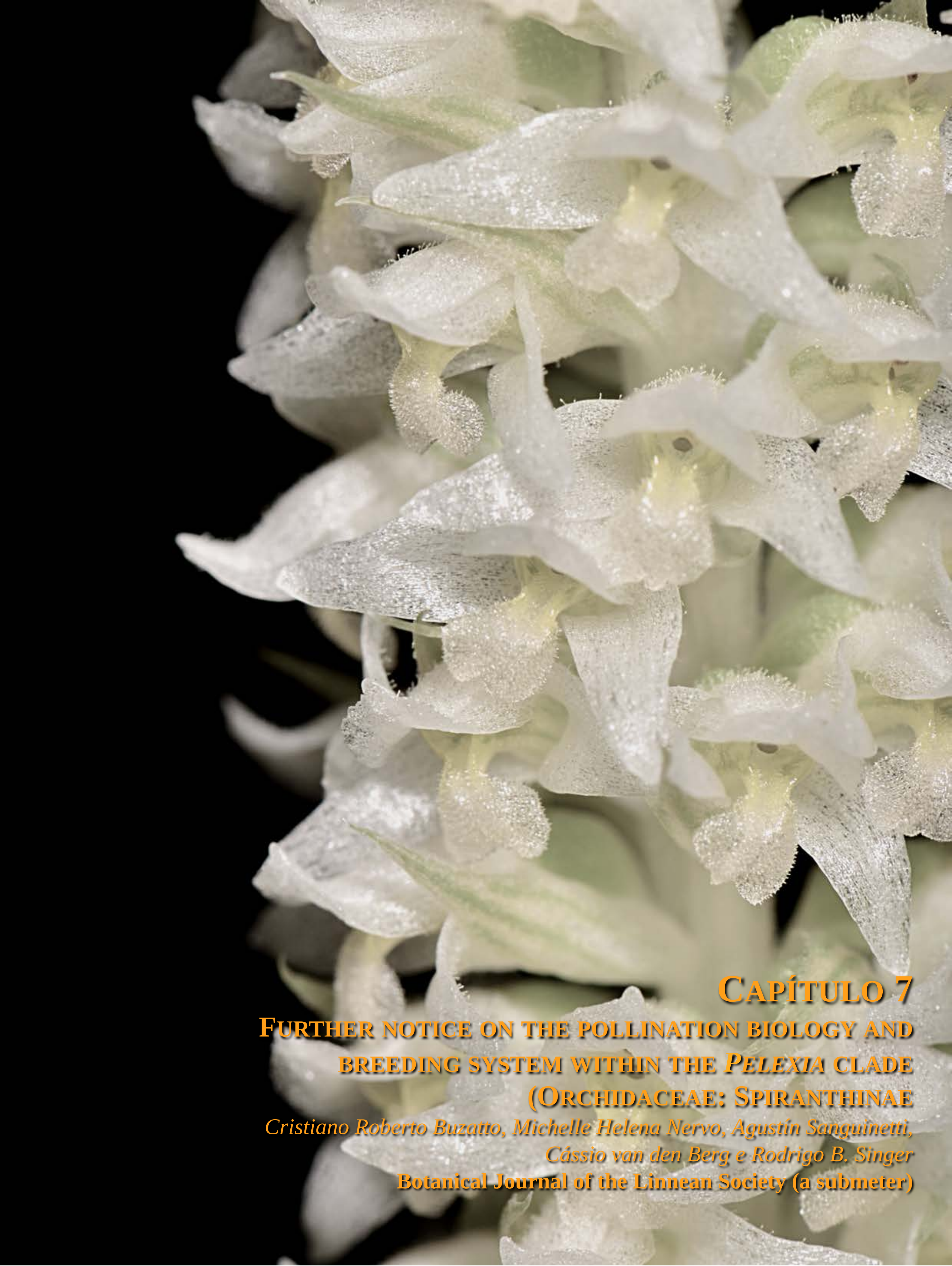

A
B
Bipinnula giberti
Bipinnula giberti
Bipinnula polyzyka
Bipinnula polyzyka
Bipinnula penicillata
Bipinnula penicillata
Bipinnula penicillata
Bipinnula biplumata
Bipinnula biplumata
Bipinnula biplumata
Bipinnula biplumata
Bipinnula montana
Bipinnula montana
Bipinnula apinnula
Bipinnula volkmanni
Bipinnula fimbriata
Bipinnula fimbriata
Gavilea araucana
Gavilea araucana
Gavilea trullata
Gavilea trullata
Gavilea odoratissima
Gavilea lutea
Gavilea lutea
Gavilea glandulifera
Gavilea glandulifera
Gavilea glandulifera
Gavilea leucantha
Gavilea venosa
Gavilea chica
Gavilea australis
Chloraea chrysantha
Chloraea multiflora
Chloraea lamellata
Chloraea longipetala
Chloraea barbata
Chloraea bidentata
Chloraea bletioides
Chloraea crispa
Chloraea cristata
Chloraea gavilu
Chloraea heteroglossa
Chloraea incisa
Chloraea lechleri
Chloraea philippii
Chloraea philippii
Chloraea virescens
Chloraea virescens
Chloraea virescens
Chloraea volkmanni
Chloraea disoides
Chloraea alpina
Chloraea alpina
Chloraea cuneata
Chloraea nudilabia
Chloraea bicallosa
Chloraea membranacea
Chloraea membranacea
Chloraea membranacea
Chloraea membranacea
Chloraea gaudichaudii
Chloraea magellanica
Chloraea gaudichaudii
Chloraea magellanica
Chloraea magellanica
Chloraea speciosa
Chloraea grandiflora
Chloraea reticulata
Chloraea cylindrostachya
Chloraea cylindrostachya
Chloraea cylindrostachya
Chloraea leptopetala
Codonorchis lessonii
Codonorchis lessonii
Ophrys apifera
Diuris sulphurea
Microtis parviflora
Pterostylis curta
Goodyera pubescens
Ludisia discolor
Aa achalensis
Aa palacea
Porphyrostachys pilifera
Gomphichis caucana
Stenoptera ecuadorana
Cranichis revoluta
Ponthieva elata
Pterichis habenarioides
Prescottia plantaginifolia
Manniella gustavi
Galeottiella sarcoglossa
Achlydosa glandulosa
Chloraeinae
Codonorchideae
Orchidinae
Diuridinae
Prasophyllinae
Pterostylidinae
Goodyerinae
Cranichidinae
Manniellinae
Galeottiellinae
Achlydosinae

Figure 2

SEÇÃO 3

BIOLOGIA DA POLINIZAÇÃO DE ESPÉCIES DE SPIRANTHINAE E CHLORAEINAE



Capítulo 7

Further notice on the pollination biology and breeding system within the *Pelexia* clade (Orchidaceae: Spiranthinae

Cristiano Roberto Buzatto, Michelle Helena Nervo, Agustín Sanguinetti,
Cássio van den Berg e Rodrigo B. Singer

Botanical Journal of the Linnean Society (a submeter)

POLLINATION IN *PELEXIA* CLADE (ORCHIDACEAE)

Further notice on the pollination biology and breeding system within the *Pelexia* clade (Orchidaceae: Spiranthinae)

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ABSTRACT

The pollination process and breeding system of *Pachygenium bonariense* and *P. pteryganthum* was documented for native populations from Rio Grande do Sul, Brazil. The flowers offer a nectar reward (mean values of total sugars ranging from 65 to 67 %) concealed into an incurved spur. Both species are pollinated by big-sized Apidae bees that adhere the pollinaria under the ventral surface of the labrum while trying to reach the nectar. *Pachygenium bonariense* is pollinated by native bumble-bees [*Bombus* (*Fervidobombus*) *morio*] and *P. pteryganthum* by females of oil-gathering bees [*Centris* (*Melacentris*) sp.]. Both orchid species are self-compatible, but pollinator dependent; this is, unable to set fruit and seed in absence of pollinators. They also displayed high natural reproductive success (ranging from 22 to 90 %) and male efficiency factors higher than 1 (ranging from 2.2 and 3.6), suggesting that, during our observations, more than one flower was pollinated for each flower acting as pollen donor. In order to enrich the discussion, unpublished data on the pollination and breeding system of other orchid species of the *Pelexia* clade are also presented and briefly illustrated.

ADDITIONAL KEYWORDS: *Brachystele*, *Cyclopogon*, Orchidaceae, orchids, *Pachygenium*, *Pelexia*, pollination, *Veyretia*

INTRODUCTION

Within Neotropical Spiranthinae orchids, the column structure of genera such as *Cyclopogon* C.Presl, *Pelexia* Poit. ex Lindl. and *Sarcoglottis* C.Presl is characteristic and highly diagnostic (Dressler, 1993; Singer & Sazima, 1999; Singer & Cocucci, 1999). In these orchids, the viscidium (the detachable, adhesive part of the rostellum that glues the pollinarium to the pollinator) is wedge-like shaped and adhesive through its dorsal surface (*vs*. adhesive through the ventral surface, as in all remaining Spiranthinae orchids, see Singer & Sazima, 1999; Catling, 1982, 1987). The biomechanics of this particular kind of viscidium configuration was elucidated by means of pollination studies in the aforementioned genera (Singer & Cocucci, 1999; Singer & Sazima, 1999). Irrespective of their size differences, these orchids are pollinated exclusively by nectar-seeking bees that press their labrum (plate-like structure that partially hides the insect's mouthparts) against the dorsal (and adhesive) surface of the viscidium, thus adhering the pollinarium at the underside of this structure (Singer & Cocucci, 1999; Singer & Sazima, 1999). The viscidium structure in the aforementioned orchid genera is so characteristic that has been considered of phylogenetic value, supporting the affinity of these taxa (Dressler, 1993; Balogh, 1982; Burns-Balogh & Robinsin, 1983). The term "*Pelexia* Alliance" was then coined to embrace the genera *Cyclopogon*, *Pelexia* and *Sarcoglottis* (Dressler, 1993; Balogh, 1982; Burns-Balogh & Robinson, 1983). However, subsequent phylogenetic studies have clearly demonstrated that, in order to achieve monophyly, the "*Pelexia* Alliance" (hereafter, the *Pelexia* clade) should also include genera with differently constructed flowers (namely, with ventrally-adhesive viscidia) such as *Brachystele* Lindl. and *Sauroglossum* Lindl. (Salazar *et al*., 2003). So delimitated, the *Pelexia* clade has received strong support in recent phylogenetic studies (Salazar *et al*., 2003; Figueroa *et al*., 2008; Salazar *et al*., 2009; Batista *et al*., 2011; Salazar *et al*., 2011; Salazar & Dressler, 2011; Salazar & Jost, 2012; Borba *et al*., in press; Buzatto *et al*., in prep.).

Few reports exist on the breeding system and pollination biology of representatives of the *Pelexia* clade. *Cyclopogon* species are largely pollinated by Augochlorine Halictidae bees

(Galetto *et al*., 1997; Singer & Cocucci, 1999; Singer & Sazima, 1999). Dressler (1993) mentions bumble-bees pollinating *Pachygenium ekmanii* (Kraenzl.) Szlach., Tamayo & Rutk. [as *Pelexia ekmanii* (Kraenzl.) Schltr.] and Euglossini bees captured bearing *Sarcoglottis* sp. pollinaria "behind their mouth-parts" (Dressler, 1993). *Pachygenium oestriferum* (Rchb.f. & Warm.) Szlach., Tamayo & Rutk. [as *Pelexia oestrifera* (Rchb.f. & Warm.) Cogn.] is regularly pollinated by bumble-bees in Southeastern Brazil and *Sarcoglottis fasciculata* (Vell.) Schltr. is regularly pollinated by Euglossine bees of the genus *Euglossa* (Singer & Sazima, 1999). In all these cases the orchids secrete a nectar reward and the pollinarium adheres at the underside of the labrum while bees try to reach the nectar with their proboscises (Singer & Cocucci, 1999; Singer & Sazima, 1999). Conversely, the flowers of *Sauroglossum elatum* Lindl. display scent production during the night hours and short, ventrally-adhesive viscidia (Singer, 2002). In agreement with these features, these flowers are pollinated by moths that carry the pollinaria on the surface of the proboscis (Singer, 2002). All orchid species of the *Pelexia* clade whose breeding system has been studied so far have proven to be self-compatible yet pollinator-dependent (this is, unable to set fruit in absence of pollinators) (Galetto *et al*., 1997; Singer & Sazima, 1999; Singer, 2002). Remarkably, no other orchid species from this clade has been studied since then regarding its pollination biology and breeding system (Singer & Cocucci, 1999; Singer & Sazima, 1999; Singer, 2002).

As part of a series of biosystematic studies of the *Pelexia* clade, during the last 3 years we have focused our attention on the breeding system and pollination biology of two *Pachygenium* species [*Pachygenium bonariense* (Lindl.) Szlach., Tamayo & Rutk. and *P. pteryganthum* (Rchb.f. & Warm.) Szlach., Tamayo & Rutk.] native to Rio Grande do Sul (Southern Brazil). In addition, we managed to collect complementary information on the pollination biology and/or breeding system in other species of other genera of the clade and we believe that it may be opportune to briefly present these data in order to enrich and update the discussion. The aims of the present contribution are thus twofold: 1) to elucidate the pollination biology and breeding system of two as yet unstudied *Pachygenium* species and, 2) to present accumulative, complementary data on other species of the same clade.

MATERIAL AND METHODS

***Study sites* -** A large natural population of *Pachygenium bonariensis* was studied at the Parque Estadual das Guaritas (Torres Municipality, Rio Grande do Sul, S29º21'08" W49º43'51") and a natural population of *P. pteryganthum* was studied at the municipality of Tramandaí (Rio Grande do Sul, S30^{o}00'42" W50^{o}08'12"). Both populations are very close (less to 100 m) to the seashore and are placed within the Atlantic Rain Forest Domain (Moreno, 1961). The surrounding vegetation is grassy and the soil is sandy. Annual rainfall is ca. 1,600 mm and the annual average temperature is approximately 25 ^{o}C (Moreno, 1961). The climate is characterized by a hot summer and the lack of a well-defined dry season (Nimer, 1989; Almeida, 2009).

***Studied species* -** Both studied species were formerly placed in *Pelexia* (see Hoehne, 1945; Pabst & Dungs, 1975). Szlachetko (2001) transferred these species to *Pachygenium*, based on floral features. Whereas this transfer was only weakly justified (Szlachetko, 2001), ongoing molecular studies of the whole *Pelexia* clade (Buzatto *et al.*, in prep.) clearly support the separation of *Pachygenium* from *Pelexia*. Thus, we herein accept the placement of the studied species within *Pachygenium*. *Pachygenium bonariense* dwells from Southern Brazil to Paraguay, Uruguay and Central Argentina (Córdoba) (Hoehne, 1945; Pabst & Dungs, 1975). Conversely, *P. pterygantum* seems to be restricted to Southern, Southeastern and Central Brazil (Hoehne, 1945; Pabst & Dungs, 1975). Throughout this paper we follow the orchid systematics concepts of Chase *et al.* (2003) and (unless otherwise indicated) the Spiranthinae generic concepts of Salazar (2003).

***Floral features and nectar properties* -** As a whole, we used the same methods our team already applied in precedent studies on terrestrial orchids (Singer & Sazima, 1999; Pedron *et al.*, 2012;

Sanguinetti *et al*., 2012). Ten fresh, intact flowers from each species were used to record flower features as well as nectar volume and concentration. All flowers came from five bagged specimens of each species, and nectar parameters were always measured for each species during the period when their pollinators had been recorded in activity. Nectar volume was measured with the help of a capillary tube and measuring the length of the column (7µl; 75 mm), and nectar concentration (total sugar) was measured using a manual pocket refractometer.

Flower morphology was studied using fresh and alcohol-preserved flowers [70 % (v/v) ethanol]. Flower features were recorded with the help of a digital camera. Plant vouchers were deposited at the ICN Herbarium of the Universidade Federal do Rio Grande do Sul (UFRGS) under the following accession numbers: *Pachygenium bonariense* (*CR Buzatto 839 & MH Nervo*); *P. pteryganthum* (*CR Buzatto 676 MH Nervo*). Throughout this paper, we follow the orchid morphology concepts of Dressler (1993).

Pollination observations **-** The pollination of both studied species was studied in the field during the flowering seasons of 2011 to 2013. The observation period began in February (*Pachygenium pteryganthum*) and extended until May (*P. bonariense*). Preceding literature (Singer & Sazima, 1999; Singer & Cocucci, 1999) as well as preliminary observations with cultivated specimens (see Flower features) clearly pointed out that both studied species should be pollinated during the day-hours. In agreement with this no perfume secretion was noticed in the cultivated specimens after the twilight. Therefore, only diurnal observations were performed, between 06:00 and 18:00 h (Table 1). The specific observation period for each species is detailed in Table 1.

Pollinator behaviour was documented for both species studied using field notes, photography and video. As in recent studies by our team (Pedron *et al*., 2012; Sanguinetti *et al*., 2012) in other terrestrial orchids, video record was used to obtain a more accurate record of pollinator behaviour and a better understanding of the pollination process as a whole (see Supplementary material, Videos 1–3). Some pollinating insects were collected and sacrificed for taxonomic identification and were later deposited at the entomological didactic collection of the Insects Ecology Laboratory, Zoology Department, UFRGS and at the Zoology Department of the Universidade Federal de Minas Gerais.

***Breeding system, fruiting success and pollination efficiency* -** Breeding system experiments were performed by means of specimens cultivated at the UFRGS. Four treatments were applied to these inflorescences, following the same procedures already applied in preceding studies with terrestrial orchids (Singer & Sazima, 1999; Pedron *et al*., 2012; Sanguinetti *et al*., 2012): intact flowers (control); emasculation; manual self-pollination; and cross pollination (Table 2). Treatments that set fruit were compared using a Chi-square-test ($\alpha = 0.05$). The number of plants per species and flowers used per treatment are shown in Table 2. 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 the observation periods. Fruiting success (mean fruit set per inflorescence) in both years was statistically compared using an independent, two-sample *t*-test (unequal sample sizes, equal variance) by means of PAST software (Hammer, Harper & Ryan, 2001). Nilsson’s male efficiency factor (percentage of pollinated flowers divided by the percentage of flowers acting as pollen donors; Nilsson *et al*., 1992) was also used to calculate pollination effectiveness for both studied species studied. The total number of flowers used in these calculations and the percentages of flowers that were pollinated and that acted as pollen donors are shown in Table 3.

***Ecological and morphological ancestral state reconstruction* -** A total of 18 terminals were used, representing 15 genera of Spiranthinae, and three genera to outgroup. Of these sampled, seven genera are placed into the current generic circumscription of *Pelexia* clade (*sensu* Salazar, 2003; Salazar *et al*., 2003; Figueroa *et al*., 2008; Salazar *et al*., 2009; Borba *et al*., in press;

Buzatto *et al*., in prep.). All sequences were obtained from GenBank (Supplementary material, Table S1) of already published phylogenies (Salazar *et al*., 2003; Salazar *et al*., 2011; Borba *et al*., in press). The analysis was carried with nucleotide sequences of nuclear (nrITS) and plastid (*matK-trnK* and *trnL-trnF*) DNA, of which the combined matrix consisted of 4,270 characters. Voucher information, geographic origin and GenBank accession numbers are listed in Supplementary material, Table S1. The data were analysed by means of maximum likelihood (ML), conducted with the program RAxML-HPC2 version 7.6.3 (Stamatakis, 2006) as implemented in the Cyberinfrastructure for Phylogenetic Research (CIPRES) Portal 2.0 (Miller *et al*., 2010). We chose one ecological and two morphological characters (Appendix 1). Both ecological and morphological data were obtained from specimen observations and the literature (Salazar, 2003), and all characters were discrete and coded as binary or multistate (Appendix 1). We mapped the occurrence of these characters using Mesquite v.2.75 (Maddison & Maddison, 2011). The trace character history function was used with the 50 % majority-rule consensus tree from the maximum likelihood analysis. The ancestral state was inferred using maximum likelihood under the Markov k-state one-parameter (Mk1; Lewis, 2001) model, in which all changes are equally probable.

RESULTS

***Plant and flower features* -** In this contribution we will focus on details pertinent to the pollination process. Readers interested in more details regarding either vegetative or floral details are referred to Hoehne (1945), where both species were treated under the genus *Pelexia*. Both studied species are terrestrial and bear a rosette of erect, lanceolate leaves that may wither during the anthesis. The flowers are clustered in dense spikes (Fig. 1A, E), a single individual producing 1–3 inflorescences. Inflorescences of *Pachygenium bonariense* and *P. pteryganthum* may bear up to 51 and 77 flowers, respectively. The flowers are resupinate, externally pilose and bear a noticeable incurved spur (Fig. 1B, F). In both studied species the lateral petals are adherent to the dorsal sepal, forming a helmet-like structure that hides most of the column. The lateral sepals are spreading and incurved. The labellum is cochleate and basally prolonged in two nectaries (auricles) (Fig. 1C). The base of the lateral sepals and the sides of the base of the labellum are connivent into the incurved spur (Fig. 1B, F). The column is erect. The anther holds a pollinarium made up by two bipartite, powdery, whitish pollinia, short elastic caudicles and a terminal viscidium (Fig. 1D, G). As a whole, the pollinarium is wishbone-shaped (Fig. 1D). In both studied species the viscidium is wedge-like shaped (Fig. 1D) and adhesive through its dorsal surface. Indeed, the dorsal surface of the viscidium has to be pressed in order to liberate the glue that adheres the pollinarium to the pollinator (see Supplementary material, Video 1). The stigmatic surface is ventral, bilobed, flat to slightly convex. The flowers of both studied species emit a faint, unpleasant fragrance during the day-hors that is best perceived by enclosing flowers in a vial.

Flower lifespan reaches 13–32 (average: 19.8 ± 11.56) days in *P. bonariense* and 8–15 (average: 12.14 ± 2.25) in *P. pteryganthum* (Table 4). Both studied species are nectariferous and dense drops of nectar are noticeable around the auricles during the day-hours (Fig. 1B, F). The flowers of *P. bonariense* secrete 0.75–3.32 (average: 1.51 ± 0.67) microliters with a concentration 44.38–93.86 (average: 67.46 ± 10.16) % (Table 4). The flowers of *P. pteryganthum* secrete 1.59–5.88 (average: 4.57 ± 1.53) microliters with a concentration of 23.1–94.2 (65.06 ± 26.82) % (Table 4). The fruits are brown, dehiscent capsules (Fig. 1F) and the minute seeds are brownish and dust-like.

Pollination mechanism - The pollination mechanism is the same in both studies species, in spite of the found different pollinators (see Supplementary material, Videos 1–3). The withdrawal of the pollinarium takes place when the labrum of an appropriately-sized bee is pressed against the dorsally-adhesive viscidium (Figs. 2–3; see also Supplementary material, Video 1). Bees can only introduce the mouthparts into the flowers and use the external part of the flower to get a hold (Figs. 2A–C and 3A–C). A landing bee opens its mandibles and extends its proboscis in order to reach the nectar into the floral spur. In doing so, the underside of the bee labrum is pressed against the dorsal surface of the viscidium. The viscidium thus glues to the underside of the labrum and the whole pollinarium is removed (Figs. 2–3; see also Supplementary material, Video 1). When leaving the flower the bee retracts its mouthparts, folding the labrum. Just removed pollinaria remain thus hidden below bee’s head. Both, pollinarium size and its place of adherence (the underside of the labrum) preclude the removal of a high number of pollinaria by the same pollinating bee. During the observation period, bees were recorded carrying 1–4 pollinaria. Pollination takes place when a pollinarium-laden bee visits a flower and inserts its mouthparts. The distal part of the pollinia then contact the sticky stigmatic surface, leaving pads of pollen. Since the pollinia are friable, only a fraction of the pollen-content of the pollinarium is left during a pollination event.

***Pollinators and pollinator behaviour* -** Queens and workers of the native bumble-bee *Bombus* (*Fervidobombus*) *morio* (Swederus, 1787) were recorded as the sole pollinators of *P. bonariense* during the whole observation period (Fig. 2; see also Supplementary material, Video 2). Remarkably, drones of the same bees were recorded visiting the flowers without removing pollinaria, even when clearly manipulating the flowers in the same way of the workers and queens. In all cases, bees hovered for a few moments around the inflorescences prior to landing. No clear visitation pattern (e.g. from the base of the inflorescence toward the top) was perceived. Bumble-bees were recorded visiting 2–5 inflorescences and 2–17 flowers per inflorescence, spending 1.3–66 per visited flower and 2–122.6 seconds during the whole visitation event. Bees were recorded bearing up to four pollinaria adhered at the underside of the labrum (Fig. 2; see also Supplementary material, Video 2). In some instances, pollinarium-laden bumble-bees were recorded hanging in the nearby vegetation trying to remove the pollinaria, a fact that suggests that they may disturb the bees in some way (see Supplementary material, Video 2).

Females of the oil-gathering bees of *Centris* (*Melacentris*) sp. were the only pollinators recorded for *P. pteryganthum* during the whole observation period (Fig. 3). Overall, bees hovered in front of the inflorescences prior to landing. Bees visited 2–6 inflorescences and 2–16 flowers per inflorescence, spending 1.1–12.3 per visited flower and 1.5–16.2 seconds per visitation event. Unlike the bumble-bee pollinators of *P. bonariense*, *Centris* bees tended to start visiting the inflorescence from the basal-most flowers, proceeding towards the apex. Other than this, *Centris* bees worked the flowers much in the same way of the bumble-bee pollinators of *P. bonariense*: the insects inserted their mouth-parts into the floral tube and used the external part of the flower to get a hold (Fig. 2, Supplementary Material, Video 3). *Centris* bees were recorded bearing up to four pollinaria and, unlike the bumble-bee pollinators of *P. bonariense*, they were not recorded trying to remove the pollinaria adhered to their labri. Another remarkable finding is that in several instances *Centris* bees were also recorded carrying 1–2 pollinaria of an unidentified *Gomesa* sp. (Orchidaceae: Oncidiinae) (see also Supplementary material, Video 3). In one instance a female *Xylocopa* sp. (Apidae) bee was found piercing the spur of some flowers robbing nectar. Since this bee did not disturb the column, it was not carrying pollinaria of *P. pteryganthum*. Yet, this animal was carrying a pollinarium morphologically matching these of *Eulophia alta* (L.) Fawc. & Rendle (Epidendroideae: Eulophiinae), a relatively common orchid in the study area.

Breeding systems - The results of the breeding system experiments are summarized at the Table 2. Both studied species presented very similar results. No fruits were formed from either intact or emasculated flowers, thus indicating that both species are pollinator-dependent, this is, unable to set fruit in absence of pollinators. High fructifications were obtained either through manual self-pollinations or cross-pollinations. Whereas both studied species are clearly self-compatible, no statistically significant differences were observed between self and cross-pollinations (Table 2).

***Fruiting success and pollination efficiency* -** Results are summarized in Table 3. Fruiting success in *P. bonariense* reached 90.49 % in 2012. Mean fruit set per inflorescence was also high for this species, reaching 85.99 in 2012 (Table 3). Fruiting success in *P. pteryganthum* reached 50.5 (53.9 ± 26.01) % in 2011 and 22.5 (23.63 ± 24.66) % in 2012. Mean fruit set per inflorescence reached 53.9 in 2011 and 23.63 in 2012 (Table 3). It is to be stressed, however, that natural fruiting success in 2012 should likely be higher in *P. pteryganthum*, but several fruits in development were attacked (eaten) by caterpillars and pierced by Hemiptera. During the observation period both studied species displayed male efficiency factors higher than 1 (Table 3), suggesting that more than one flower was pollinated per pollinarium removed (or per flower acting as pollen-donor).

DISCUSSION

Regarding their gross floral morphology, floral features in *P. bonariense* and *P. pteryganthum* are quite consistent with these already reported for *P. oestriferum* (as *Pelexia oestrifera*) and other related species of the *Pelexia* clade (Singer & Cocucci, 1999; Singer & Sazima, 1999). In particular, the dorsally-adhesive viscidium observed in both studied species is, from a functional point of view, identical to this already observed in *P. oestriferum* (Singer & Sazima, 1999). It is important to keep in mind, however, that the current delimitation of the *Pelexia* clade is significantly different to that used in the 90’s (Singer & Sazima, 1999). Following this clade’s current delimitation (Salazar, 2003; Salazar *et al*., 2003), dorsally-adhesive viscidia within the *Pelexia* clade are present in the genera *Cyclopogon*, *Pachygenium*, *Pelexia*, *Sarcoglottis* (Fig. 5) and *Veyretia*. Yet, the clade also holds genera with ventrally-adhesive viscidia, such as *Brachystele* and *Sauroglossum* (Fig. 5)(Singer, 2002; Salazar, 2003; Salazar *et al*., 2003).

The flowers of *Pachygenium bonariense* and *P. pteryganthum* are nectariferous, as already documented for other species in the clade (Singer & Cocucci, 1999; Singer & Sazima,

1999) and for the whole subtribe Spiranthinae (Catling, 1982, 1987; Dressler, 1993). It is remarkable that the found nectar concentration (total sugars) is relatively high (≥ 50 %), in both studied species. Yet, concentrated nectars have already been documented for Argentinean plants of *P. bonariense* and the distantly related (and hummingbird-pollinated) *Sacoila lanceolata* (Aubl.) Garay [as *Stenorrhynchos orchioides* (Sw.) Rich.] (Galetto *et al*., 1997). To our knowledge, no other Spiranthinae orchids have been studied regarding their nectar properties. Yet, the values obtained are consistent with these that could be expected in bee-pollinated flowers (Proctor *et al*., 1996).

The pollination mechanism observed in *Pachygenium bonariense* and *P. pteryganthum* is essentially the same already reported for *P. oestriferum* (as *Pelexia oestrifera*) and other species of the *Pelexia* clade with dorsally-adhesive viscidia (Singer & Cocucci, 1999; Singer & Sazima, 1999). These findings, plus some additional unpublished observations of us (see below) clearly support that all Spiranthinae orchids of the *Pelexia* clade with dorsally-adhesive viscidia may be pollinated by bees (Fig. 5). Among all pollinator categories only bees have an appropriate plate-like structure (the labrum) suited to exert a pressure against dorsally-adhesive viscidia (Singer & Sazima, 1999). In other words, the presence of dorsally-adhesive viscidia should be considered diagnostic of bee-pollination (Fig. 5). Remarkably, dorsally adhesive viscidia have recently been reported for some Brazilian species of *Aspidogyne* Garay and *Ligeophila* Garay and at least one of these species [*A. longicornu* (Cogn.) Garay] was shown to be pollinated by Euglossini bees in a similar way to the Spiranthinae orchids of the *Pelexia* clade; this is, with the bees adhering the pollinaria at the underside of the labrum (Singer & Sazima, 2001). Singer & Sazima (1999) suggested two ecological advantages of ventrally-adhesive viscidia: 1) the ventral surface of the labrum is a difficult place for the bees to clean and get rid of the pollinaria and 2) while a pollinarium-laden bee leaves a flower, it retracts its mouth-parts and the pollinarium remains hidden (and protected) under the head of the insect (Singer & Sazima, 1999). These features should prevent or diminish pollinarium wastage (Singer & Sazima, 1999). The high fruiting success and relatively high male efficiency factors (see Results and Table 2) found in both studied species apparently give support to these ideas.

The pollinators of *P. bonariense* are native bumble-bees, like in the already studied *P. oestriferum* (Singer & Sazima, 1999) and, overall, the bee behaviour herein reported is very similar to this already described for *P. oestriferum* (Singer & Sazima, 1999). It is remarkable; however, that unlike the case of *P. oestriferum* (Singer & Sazima, 1999), males of *Bombus* (*Fervidobombus*) *morio* were also recorded as flower visitors in *P. bonariense*. Our filmic record indicates that male bees work the flowers in the same way of the females, inserting their mouth-parts and covering the viscidium with their labrum during the process, without, however, removing the pollinarium. After a comparative examination of male and female bees under stereomicroscope, we noticed that male *labri* are smaller. Although a bigger sample would be desirable to confidently ascertain this, we tentatively suggest that male bumble-bees are not pollinators of *P. bonariense* because the smaller size of their *labri* precludes pollinarium removal. Bumble-bee pollination was recently confirmed for *P. oestriferum* at the Itatiaia Mountains (Rio de Janeiro State, ca. 1500 m. a.s.l) (P. Sierra, pers. comm.). In addition, Dressler (1993) mentions bumble-bees pollinating *Pachygenium ekmanii* flowers, but without giving more specific details on the pollination process. Hoehne (1945) at his introduction of the genus *Pelexia* (where, until recently, most *Pachygenium* species were placed) for his Flora Brasilica mentions that pollination is promoted by “mamangavas”. Unhappily, it is difficult to ascertain to which specific bees Hoehne (1945) refers, since the Tupi-Guarani indigenous term “mamangava” designates big-sized bees, either solitary (as carpenter bees) or social (as bumble-bees).

Remarkably, *Pachygenium pteryganthum* is pollinated by solitary, oil-collecting bees. These bees are frequently associated with the pollination of oil-secreting orchids and Malpighiaceae (Renner & Schaeffer 2010 and literature cited therein) but, to our knowledge, this is the first record of these bees pollinating nectar-secreting terrestrial orchids. In agreement with the oil-collecting habits of these bees, some insects were recorded bearing the pollinaria of an as yet unidentified *Gomesa* sp. (Orchidaceae: Oncidiinae, see Supplementary material, Video 3), an orchid genus where oil-secretion has been already documented (Torretta *et al*., 2011). Overall, *Centris* bees behaved much in the same way of the bumble-bees, showing, however, a tendency (not observed in the bumble-bees pollinators of *P. bonariense*) to visit the inflorescences from the base towards the apex. The latter behaviour has been well-documented in *Spiranthes* species mostly pollinated by bumble-bees (Catling, 1983). Since these *Spiranthes* flowers are protandrous (Fig. 5)(a condition which is absent in the species studied herein), basal most, older flowers tend to act as pollen-receivers and younger, more apical flowers tend to act as pollen-donors (Catling, 1983). Thus, bumble-bee behaviour in these *Spiranthes* species tends to promote cross-pollination (Catling, 1982).

Both studied species are self-compatible and pollinator-dependent. Yet, no statistically significant differences were found between fruiting through self and cross-pollination. This is in fully agreement with preceding reports on other species of the *Pelexia* clade (Singer & Sazima, 1999; Singer, 2002). Indeed, self-compatibility seems to be widespread among Spiranthinae orchids as a whole (see Catling, 1982, 1987). In fact, additional data gathered by our team suggest that other species in the *Pelexia* clade are pollinator-dependent, yet self-compatible. Specimens cultivated of *Cyclopogon chloroleucus* Barb. Rodr. and *C.* aff. *trilineatus* Barb. Rodr. have produced essentially the same results regarding their breeding systems (R.B. Singer, pers. obs., Table 5). In addition, cultivated specimens of *Brachystele camporum* (Lindl.) Schltr. and *B. dilatata* (Lindl.) Schltr. are also pollinator-dependent and self-compatible (C.R. Buzatto & A. Sanguinetti, pers. obs., Table 5). Remarkably, the two latter species are protandrous (Fig. 5); a condition already documented in *Spiranthes* species (Catling, 1982) and, within the *Pelexia* clade, in *Sauroglossum elatum* (Singer, 2002), but absent in all the genera of the *Pelexia* clade with dorsally-adhesive viscidium, such as *Cyclopogon* (Singer & Cocucci, 1999; Singer & Sazima, 1999), *Pachygenium* and *Sarcoglottis* (Singer & Sazima, 1999). Within the orchid genera of the *Pelexia* clade with dorsally adherent viscidia (Fig. 5), all studied species of *Cyclopogon* are pollinated by Augochlorine Halictidae bees (Galetto *et al*., 1977; Singer & Cocucci, 1999; Singer & Sazima, 1999) and the few studies species of *Sarcoglottis* are pollinated by Euglossini bees (Dressler, 1993; Singer & Sazima, 1999). Unpublished observations on the pollination biology of *Veyretia hassleri* (Cogn.) Szlach. (C.R. Buzatto & M.H. Nervo, unpublished, Fig. 4B–C) have shown that this terrestrial orchid is pollinated by Megachilidae bees (Fig. 4B–C). In all these cases, the bee pollinators bear the pollinaria adhered on the ventral surface of the labrum, in agreement with the presence of dorsally-adhesive viscidia (Dressler, 1999; Singer & Sazima, 1999; Singer & Cocucci, 1999, and Fig. 4B–C). Within the orchid genera of the *Pelexia* clade with ventrally-adhesive viscidia, *Sauroglossum,* is remarkable by presenting moth-pollinated flowers (Fig. 5) which secrete an attractive fragrance during the crepuscule and night hours (Singer, 2002). Schlindwein (1995) mentions Halictidae bees foraging on nectar at the flowers of *Brachystele camporum*. In addition, unpublished observations on the same orchid species (C.R. Buzatto & A. Sanguinetti) have shown that workers of the introduced *Apis mellifera* L. are efficient pollinators of this orchid species In Rio Grande do Sul (Southern Brazil) (Fig. 4A, Table 5). In this latter case, bees carry the orchid pollinaria onto the galeae.

Both studied species have shown high fruiting success, exceeding the normal fruiting success in rewarding orchids (less than 40 %; Tremblay *et al*., 2005). Indeed, very few Spiranthinae orchids have been studied in precedence regarding their natural fruiting success. Calvo (1990) found 26–61.6 % of fruiting success/inflorescence in a long-term field study on *Cyclopogon cranichoides* (Griseb.) Schltr. Singer & Sazima (2000) found that 74 % of the available flowers of the hummingbird-pollinated *Sacoila lanceolata* [as *Stenorrhynchos lanceolatus* (Aubl.) Rich. ex Spreng.] were pollinated during the observation period. High fruiting success has also been documented for non-Spiranthinae, nectariferous terrestrial orchids (namely, some Brazilian *Habenaria* species; Pedron *et al*., 2012). These orchids showed fruiting successes ranging from 64 to 97 % and factors such as the presence of friable pollinaria, self-compatibility and pollinator behaviour were suggested to promote such high scores (Pedron *et al*., 2012). In our opinion, similar factors may be operating in the high natural fruit sets observed in both studied *Pachygenium* species Both species herein studied showed male efficiency factors higher than 1, thus indicating that more than one flower was pollinated per pollinarium removed. Similarly, a male efficiency factor of 1.8 was found in the very well-pollinated *Sacoila lanceolata* (as *Stenorrhynchos lanceolatus*) (Singer & Sazima, 2000). This latter species is pollinated by hummingbirds, but shares with the two studied species features like self-compatibility (Catling, 1987) and friable pollinia whose pollen-content id deposited along several flower visits (Singer & Sazima, 2000). Thus, we herein propose that, as previously suggested for other recently studied terrestrial orchids (Pedron *et al*., 2012; Sanguinetti *et al*., 2012), a combination of factors such as self-compatibility, presence of a nectar reward, friable pollinaria and pollinator behaviour and abundance may explain the high natural fruit set observed in *P. bonariense* and *P. pteryganthum*.

CONCLUDING REMARKS

When the first studies on the pollination biology of representatives of the *Pelexia* clade came to light (Singer & Cocucci, 1999; Singer & Sazima, 1999), the group was considered in a very different way of its current delimitation (Balogh, 1982; Burns-Balogh & Robinson, 1983; Dressler, 1993; Salazar *et al.*, 2011 and literature cited therein). Then, the presence of a dorsally adhesive viscidium was considered a synapomorphy for the group (Balogh, 1982; Burns-Balogh & Robinson, 1983; Dressler, 1993). Yet, this notion was challenged by more recent phylogenetic studies (Salazar *et al*., 2011 and literature cited therein) that clearly pointed out that the *Pelexia* clade also includes orchid genera with differently flower morphology (this is, without dorsally-adherent viscidium). In its current delimitation, the *Pelexia* clade embraces genera with dorsally-adhesive viscidia (*Cyclopogon*, *Pachygenium*, *Pelexia*, *Sarcoglottis* and *Veyretia*) and devoid of protandry, plus genera with protandrous flowers and ventrally-adhesive viscidium (*Brachystele* and *Sauroglossum*). Regarding the pollinators, all available information so far indicates that all the genera with dorsally-adhesive viscidium are pollinated by bees (see Results and Discussion). However, the flowers of *Brachystele* are also pollinated by bees and these of *Sauroglossum* are pollinated by moths (see Discussion). However, we would like to stress that, among Spiranthinae orchids, dorsally-adherent viscidia are only present in genera of the *Pelexia* clade in its current delimitation (Salazar, 2003). This is, even if the delimitation of the clade underwent significant modifications, this feature still has an important diagnostic value in this orchid group. Phylogenetic analyses of the *Pelexia* clade with denser and more representative sampling are on the way (C.R. Buzatto *et al*., in prep). When these studies become available, it will be possible to use them as a framework to propose well-supported scenarios for the evolution of pollination-related (e.g. dorsally-adhesive *vs*. ventrally adhesive viscidium; protandry *vs*. absence of protandry, etc.). This will be the next step our team intends to make.

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Table 1. Observation localities and observation period for both studied species

Species/locality	Period	Observation period	Observation hours (total)
Pachygenium bonariense (Lindl.) Szlach., Tamayo & Rutk./Brazil, Rio Grande do Sul, Torres	25–27 May 2012	6–18 h	32 h
Pachygenium pteryganthum (Rchb.f. &Warm.) Szlach., Tamayo & Rutk./Brazil, Rio Grande do Sul, Tramandai	26-27 February and 7–9 March 2012	6–18 h	56 h

Table 2. Breeding system experiments. Fruiting success (%) in *Pachygenium* species for intact flowers (Control), emasculated flowers (Emasculation), hand self-pollinated (Self-pollination) and cross-pollinated (Cross-pollination) treatments

Species	*N*	Control	Emasculation	Self-pollination	Cross-pollination	Chi-square (self × cross pollination) values, $P < 0.05$)
Pachygenium bonariense (Lindl.) Szlach., Tamayo & Rutk.	8	0 (0/138)	0 (0/40)	100 (45/45)	100 (44/44)	NS
Pachygenium pteryganthum (Rchb.f. & Warm.) Szlach., Tamayo & Rutk.	11	0 (0/161)	0 (0/47)	100 (59/59)	100 (49/49)	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 3. Pollinators and fruiting success in *Pachygenium* species

Species	Pollinators	Fruiting success (%)		Male efficiency factor		
		2011	**2012**	**2011**	**2012**	**2013**
Pachygenium bonariense (Lindl.) Szlach., Tamayo & Rutk.	*Bombus morio* (Apidae)	*	90.49	*	1.318 (*N*: 41, 39.5/29.97)	*
Pachygenium pteryganthum (Rchb.f. & Warm.) Szlach., Tamayo & Rutk.	*Centris* (Melacentris) sp. (Apidae)	50.5 (53.9±26.01)^{a}	22.5 (23.63±24.66)^{b}	3.619 (*N*: 21, 66.09/18.26)	2.482 (*N*: 41, 69.58/28.04)	2.245 (*N*: 32, 59.5/26.51

Species not sharing the same letter (^{a} or ^{b}) within traits differ significantly (Kruskall–Wallis, $P < 0.05$).
N represents the number of individuals used in the experiments.
* Not observed

Table 4. Flower features in *Pachygenium* species

Species	Flower lifespan (days)	Nectar volume (µl)	Nectar concentration (total sugars)
Pachygenium bonariense (Lindl.) Szlach., Tamayo & Rutk.	13–32 (19.8±11.56)	0.75–3.32 (1.51±0.67)	44.38–93.86 (67.46±10.16)
Pachygenium pteryganthum (Rchb.f. & Warm.) Szlach., Tamayo & Rutk	8–15 (12.14±2.25)	1.59–5.88 (4.57±1.53)	23.1–94.2 (65.06±26.82)

Table 5. Resume of inedit data on the pollination and breeding system of orchids of the *Pelexia* clade

Species	**N**	**Control**	**Emasculation**	**Self-pollination**	**Cross-pollination**	**Chi-square (self × cross pollination) values, *P* < 0.05)**
Brachystele camporum (Lindl.) Schltr.	5	0 (0/40)	0 (0/32)	93.94 (31/33)	100 (30/30)	NS
Brachystele dilatata (Lindl.) Schltr.	6	0 (0/104)	0 (0/50)	86 (43/50)	98 (49/50)	NS
Cyclopogon elegans Hoehne	5	0 (0/47)	0 (0/47)	55.81 (24/43)	89.47 (34/38)	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 S1. Taxa studied, voucher information and GenBank accession

		GenBank accession		
Taxon	**Voucher**	**nrITS**	***matK-trnK***	***trnL-trnF***
Goodyera pubescens (Willd.) R. Br.	USA, *Chase 212,* NCU	AJ539519	AJ543954	AM419815
Cranichis engelii Rchb.f.	Ecuador, *Schott s.n.*, K (spirit)	AM419779	AM900814	AM412721
Prescottia stachyodes (Sw.) Lindl.	Mexico, *Salazar et al. 7312*, MEXU	AM419774	AM900809	AM412726
Dichromanthus cinnabarinus (La Llave & Lex.) Garay	Mexico, *Linares 4469,* MEXU	AJ539486	AJ543914	AJ544469
Spiranthes spiralis (L.) Chevall.	UK, *Bateman s.n.*, K (spirit)	AJ539490	AJ543918	AJ544473
Eurystyles borealis A.H. Heller	Mexico, *Soto 9149*, AMO	AJ539497	AJ543925	AJ544480
Hapalorchis aff. lineata (Lindl.) Schltr.	Guatemala, *Salazar et al. 7699*, MEXU, (spirit)	HE575515	HE575502	HE575525
Lankesterella ceracifolia (Barb. Rodr.) Ames	Argentina, *Salazar 7535,* MEXU (spirit)	FN556164	FN556174	FN556169
Eltroplectris calcarata (Sw.) Garay & H.R. Sweet	Brazil, *Soares s.n.*, K (photograph)	AJ519448	AJ519450	AJ519452
Mesadenella petenensis (L.O. Williams) Garay	Mexico, *Salazar 6069,* MEXU	AJ539503	AJ543931	AJ544486
Stenorrhynchos speciosum (Jacq.) Rich. ex Spreng.	Mexico, *Salazar 6090,* MEXU	AJ539505	-	AJ544487
Coccineorchis cernua (Lindl.) Garay	Panama, *Salazar 6249,* MEXU	AJ539502	AJ543930	AJ544485
Sauroglossum elatum Lindl.	Argentina, *Cabassi s.n.*, MEXU (spirit)	Pending	Pending	Pending
Sarcoglottis acaulis (Sm.) Schltr.	Trinidad, *Salazar 6356*, K (spirit)	AJ539500	AJ543928	AJ544483
Cyclopogon comosus (Rchb. f.) Burns-Bal. & E.W. Greenw.	Mexico, *Salazar et al. 6171*, MEXU	HE575509	HE575510	HE575519
Pachygenium bonariense (Lindl.) Szlach., R. González & Rutk.	Argentina, *Morrone & Giussani 5785*, SI	-	GQ917035	-
Pelexia adnata (Sw.) Spreng.	Mexico, *Salazar 6012,* MEXU	AJ539501	AJ543929	AJ544484
Brachystele unilateralis (Poir.) Schltr.	Chile, *Wallace 130/85*, CANB	AJ539498	AJ543926	AJ544481

LEGENDS

Figure 1. A–D) Floral features in *Pachygenium bonariense*. A) Inflorescence. B) Flower in longitudinal section exposing the spur and the column. C) Section of the spur showing the auricles surrounded by dense drops of nectar. D) Dorsal view of the column, showing the pollinarium and the wedge-like, dorsally adhesive viscidium. E–H) Flower features in *Pachygenium pteryganthum*. E) Inflorescence. F) Longitudinal section of the flower, showing the spur and the column. G) Detail of the wedge-like, dorsally adhesive viscidium. H) Dehiscent fruits.

Figure 2. Pollination of *Pachygenium bonariense* by bees of *Bombus morio* (Apidae). A–B) Bees removing pollinaria. C) Polinarium-laden bumble-bee arriving to an inflorescence. D) Detail of the head of a pollinarium-laden bumble-bee showing a pollinarium adhered at the ventral surface of its labrum.

Figure 3. Pollination of *Pachygenium pteryganthum* by *Centris* sp. (Apidae) bees. A) Bee visiting an inflorescence. B) Bee removing a pollinarium. C) Pollinarium-laden bee arriving to an inflorescence. D) Detail of the head of a pollinarium-laden bee showing a pollinarium adhered at the ventral surface of its labrum.

Figure 4. Other pollinators within the *Pelexia* clade. A) *Apis mellifera* visiting an inflorescence of *Brachystele camporum*. B) Augochlorini Halictidae bee visiting *Cyclopogon elegans*. C–D) *Anthidium* sp. (Megachilidae) pollinating *Veyretia hassleri*. C) Bee visiting an inflorescence. D) Captured bee showing two pollinaria attached at the underside of the labrum.

Figure 5. Ancestral state reconstruction mapped on the Maximum Likelihood tree. A) Protandry; B) Viscidum; C) Pollinators.

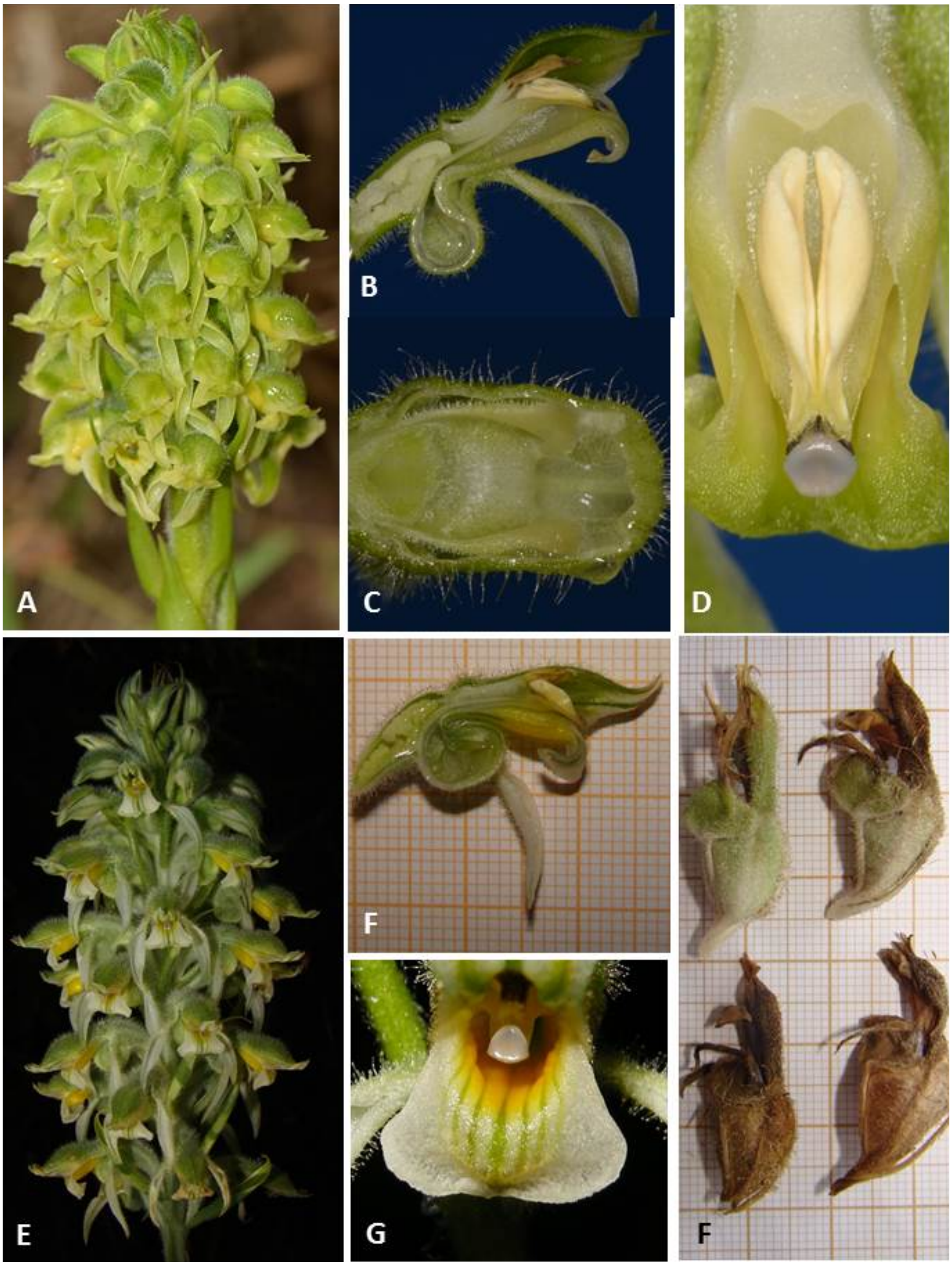


Figure 1

Figure 2

Figure 3

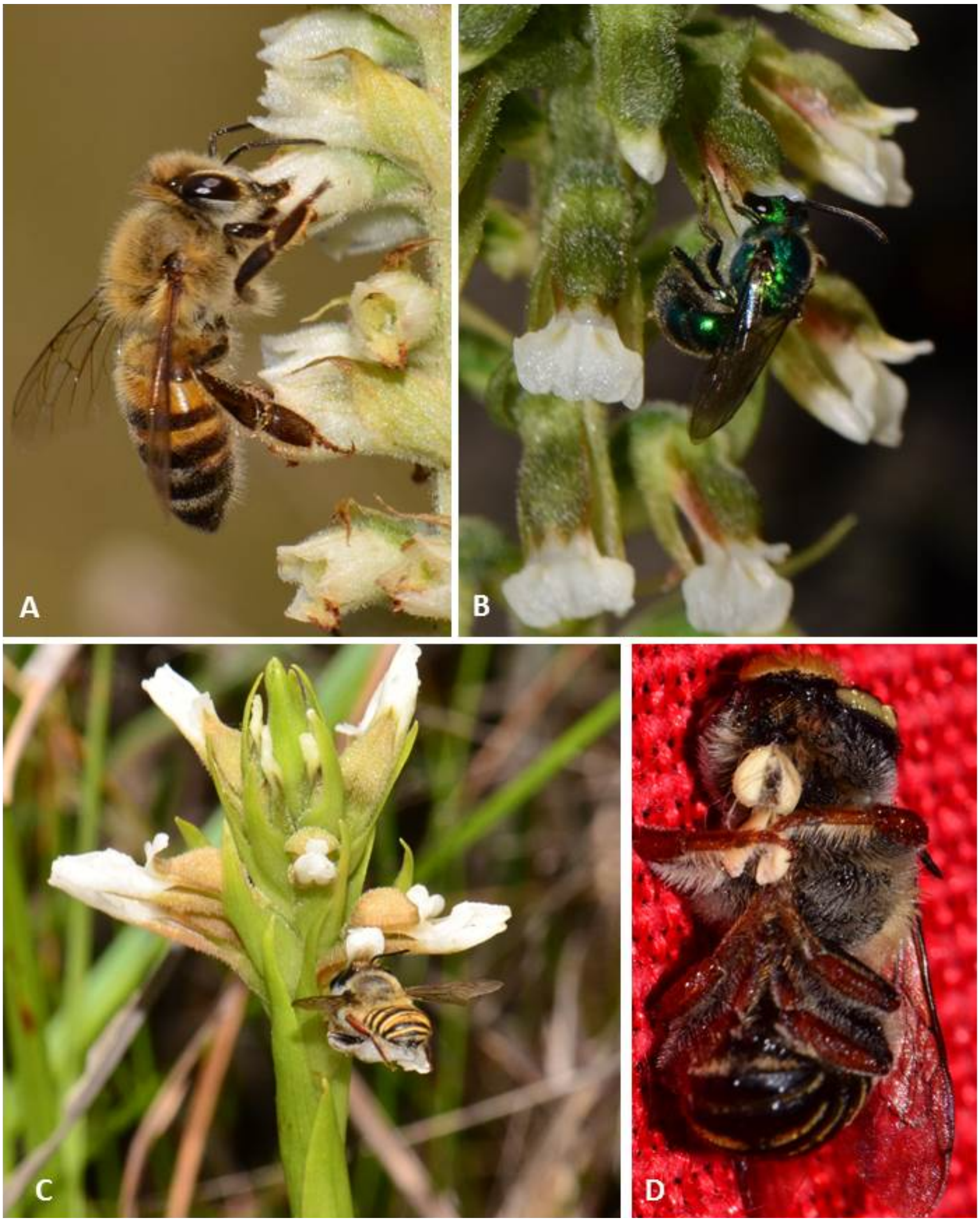


Figure 4

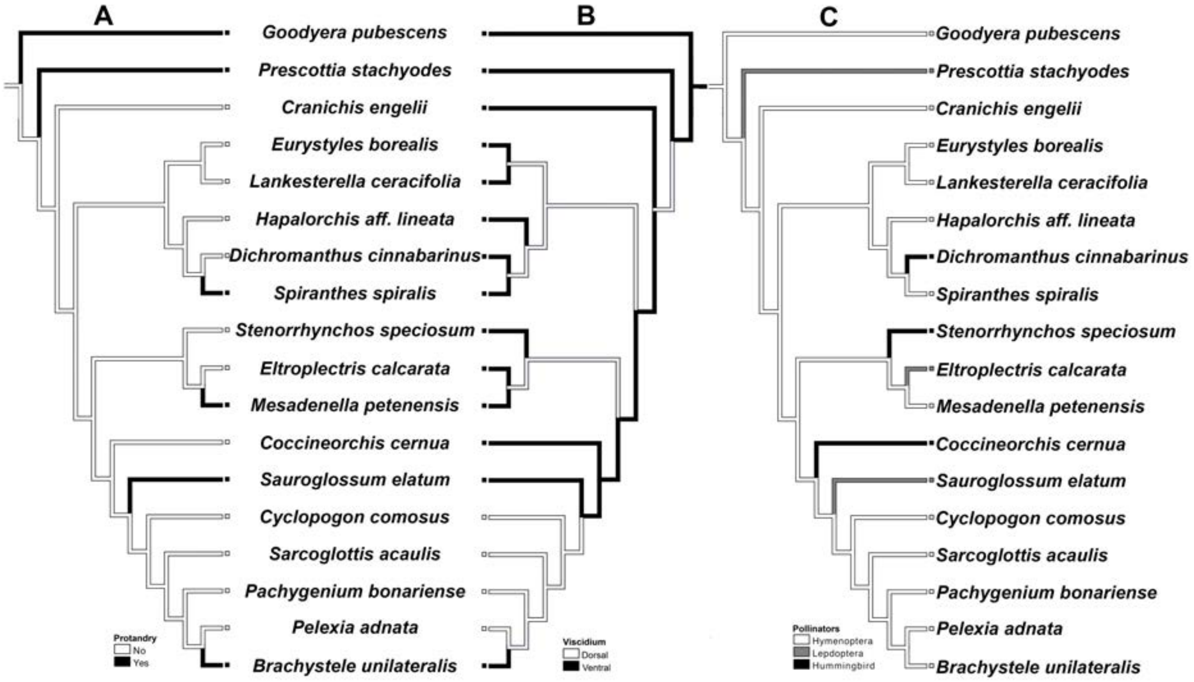
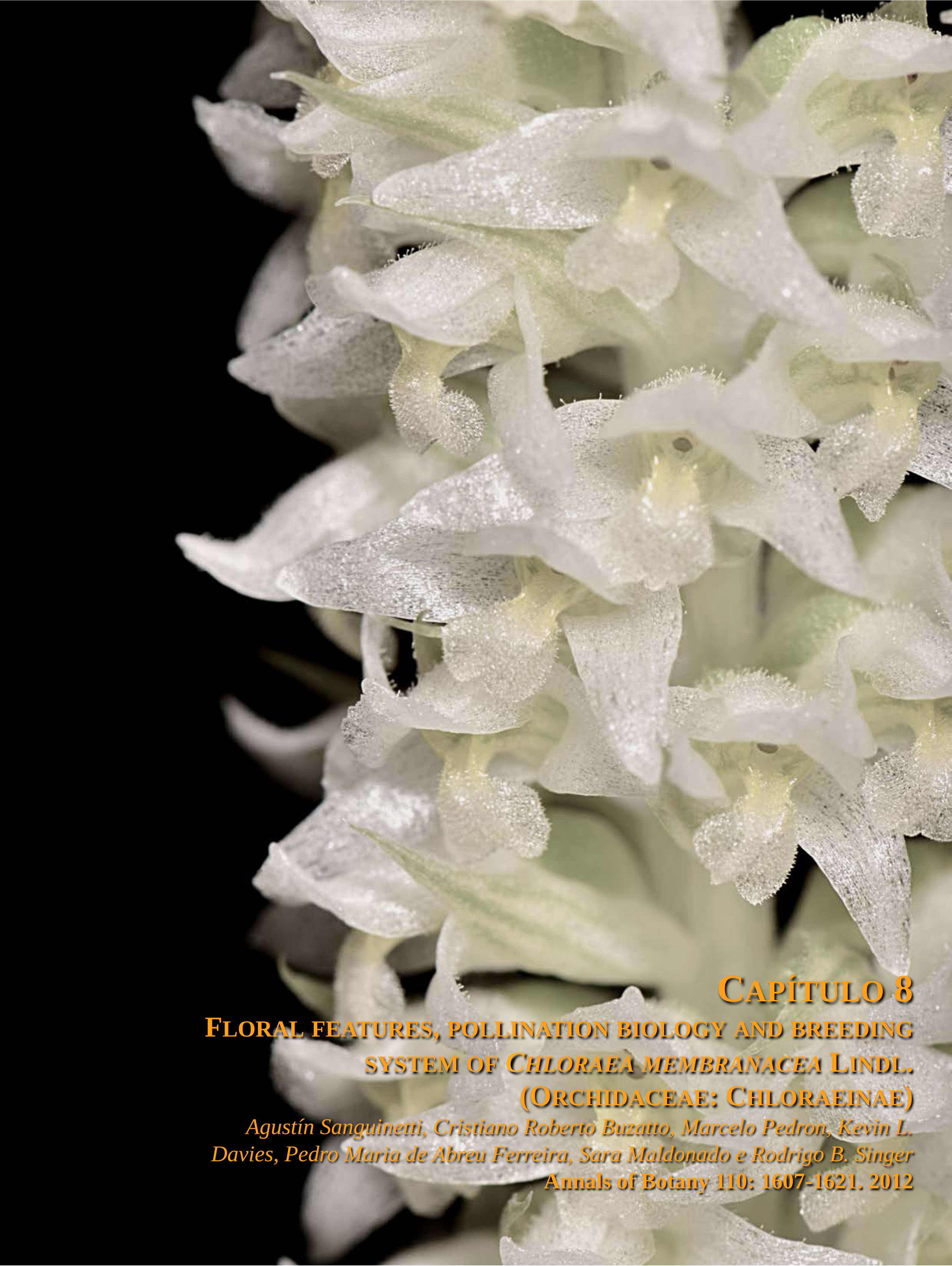

A
B
C
Goodyera pubescens
Prescottia stachyodes
Cranichis engelii
Eurystyles borealis
Lankesterella ceracifolia
Hapalorchis aff. lineata
Dichromanthus cinnabarinus
Spiranthes spiralis
Stenorrhynchos speciosum
Eltroplectris calcarata
Mesadenella petenensis
Coccineorchis cernua
Sauroglossum elatum
Cyclopogon comosus
Sarcoglottis acaulis
Pachygenium bonariense
Pelexia adnata
Brachystele unilateralis
Protandry
No
Yes
Viscidium
Dorsal
Ventral
Goodyera pubescens
Prescottia stachyodes
Cranichis engelii
Eurystyles borealis
Lankesterella ceracifolia
Hapalorchis aff. lineata
Dichromanthus cinnabarinus
Spiranthes spiralis
Stenorrhynchos speciosum
Eltroplectris calcarata
Mesadenella petenensis
Coccineorchis cernua
Sauroglossum elatum
Cyclopogon comosus
Sarcoglottis acaulis
Pachygenium bonariense
Pelexia adnata
Brachystele unilateralis
Pollinators
Hymenoptera
Lepdoptera
Hummingbird

Figure 5

Appendix 1. Characters and corresponding states used in this study for the ecological and morphological ancestral state reconstruction. **1. Viscidium**: Dorsal (0); Ventral (1). **2. Protandry**: No (0); Yes (1). **3. Pollinator**: Hymenoptera (0); Lepdoptera (1); Hummingbird (2).



CAPÍTULO 8

FLORAL FEATURES, POLLINATION BIOLOGY AND BREEDING SYSTEM OF *CHLORAEA MEMBRANACEA* LINDL. (ORCHIDACEAE: CHLORAEINAE)

Agustín Sanguinetti, Cristiano Roberto Buzatto, Marcelo Pedron, Kevin L. Davies, Pedro Maria de Abreu Ferreira, Sara Maldonado e Rodrigo B. Singer

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Floral features, pollination biology and breeding system of *Chloraea membranacea* Lindl. (Orchidaceae: Chloraeinae)

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- *Background and Aims* The pollination biology of very few Chloraeinae orchids has been studied to date, and most of these studies have focused on breeding systems and fruiting success. *Chloraea membranacea* Lindl. is one of the few non-Andean species in this group, and the aim of the present contribution is to elucidate the pollination biology, functional floral morphology and breeding system in native populations of this species from Argentina (Buenos Aires) and Brazil (Rio Grande do Sul State).
- *Methods* Floral features were examined using light microscopy, and scanning and transmission electron microscopy. The breeding system was studied by means of controlled pollinations applied to plants, either bagged in the field or cultivated in a glasshouse. Pollination observations were made on natural populations, and pollinator behaviour was recorded by means of photography and video.
- *Key Results* Both Argentinean and Brazilian plants were very consistent regarding all studied features. Flowers are nectarless but scented and anatomical analysis indicates that the dark, clavate projections on the adaxial labellar surface are osmophores (scent-producing glands). The plants are self-compatible but pollinator-dependent. The fruit-set obtained through cross-pollination and manual self-pollination was almost identical. The main pollinators are male and female Halictidae bees that withdraw the pollinarium when leaving the flower. Remarkably, the bees tend to visit more than one flower per inflorescence, thus promoting self-pollination (geitonogamy). Fruiting success in Brazilian plants reached 60·78 % in 2010 and 46 % in 2011. Some pollinarium-laden female bees were observed transferring pollen from the carried pollinarium to their hind legs. The use of pollen by pollinators is a rare record for Orchidaceae in general.
- *Conclusions Chloraea membrancea* is pollinated by deceit. Together, self-compatibility, pollinarium texture, pollinator abundance and behaviour may account for the observed high fruiting success. It is suggested that a reappraisal and re-analysis of important flower features in Chloraeinae orchids is necessary.

Key words: Breeding system, *Chloraea membrancea*, Chloraeinae, Halictidae, Orchidaceae, orchids, pollination.

INTRODUCTION

In its current delimitation, the orchid subtribe Chloraeinae Rchb. f. is, in essence, an Andean group that embraces approx. 70 species distributed in three genera: *Bipinnula* Comm. ex Juss., *Chloraea* Lindl. and *Gavilea* Poepp. (Correa and Sánchez, 2003; Chemisquy and Morrone, 2012; Cisternas *et al.*, 2012*a*, *b*). The most recent monograph of *Chloraea* was prepared by Correa (1969) and recognizes 46 valid species. Correa (1969) stressed that only two species, *C. bella* Hauman – restricted to Uruguay and the Argentinean province of Entre Rios – and *C. membranacea* Lindl., occur outside the Andean range. Most of the remaining species inhabit Chilean–Argentinean Patagonia, and some species grow in north-western Argentina, Bolivia and Peru (Correa, 1969; Correa and Sánchez, 2003). Pollination biology has previously been investigated for only a handful of Patagonian *Chloraea* species, and these studies focused mainly on breeding systems and fruiting success, but unfortunately yielded little pollinator behaviour data (Clayton and Aizen, 1996; Lehnebach and Riveros, 2003; Humaña *et al.*, 2008). They nevertheless agreed that all *Chloraea* spp. studied to date lack nectar and are self-compatible, yet are pollinator-dependent; i.e. a pollinator is required to set fruit and produce viable seed (Gumprecht, 1975; Clayton and Aizen, 1996; Lehnebach and Riveros, 2003; Humaña *et al.*, 2008). Bees (Apidae and Colletidae), flies and Coleoptera have been observed pollinating these orchids, but such insect visits were rare and, consequently, few details of pollinator behaviour at the flowers are available (Clayton and Aizen, 1996; Lehnebach and Riveros, 2003; Humaña *et al.*, 2008).

As all *Chloraea* spp. studied to date lack nectar (Lehnebach & Riveros, 2003; Humaña *et al.*, 2008), it is likely that these flowers are pollinated by deceit, but due to the scarcity of pollination data this remains to be proved. Therefore, detailed analyses of floral features coupled with complete pollinator observations are necessary to fully ascertain the pollination biology of these orchids. The aim of the present contribution is to present a detailed study of the floral functional morphology, pollination biology and breeding system of *C. membranacea*, one of the few Chloraeinae orchids having a non-Andean distribution.

MATERIALS AND METHODS

Study sites

The pollination biology and breeding system of *Chloraea membranacea* was studied for natural populations occurring in Brazil (Flores da Cunha, Rio Grande do Sul: 29 °1′50″S, 51 °11′30″W, alt. approx. 756 m) and Argentina (Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, city of Buenos Aires: 34 °32′40″S, 58 °26′25″W, alt. approx. 6 m). Supplementary observations were also made on a cultivated plant grown at the city of Porto Alegre (State of Rio Grande do Sul: 30 °01′59″S, 51 °13′48″W, alt. approx. 10 m). For conservation purposes, we cite here only the coordinates of Brazilian municipalities and omit the exact location details of populations. These data, however, are available on request.

The Flores da Cunha locality occurs within the Atlantic Rain Forest biome/domain, and the vegetation consists of forests dominated by *Araucaria angustifolia* (Bertol.) Kuntze (Araucariaceae). Average annual rainfall is about 2400 mm and annual average temperature is approximately 14·5 °C (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, 2009). Both the Brazilian locality of Porto Alegre and Buenos Aires (Argentina) occur within the Pampa Biome. The Argentinean population occurs in the understorey of an elm (*Ulmus* sp.) forested park at the Campus of the Facultad de Ciencias Exactas y Naturales, near the Rio de la Plata river. Average annual rainfall is about 1306 mm and annual average temperature is approx. 18·05 °C (DCAO-FCEN-UBA, 2012). In Porto Alegre, the vegetation consists of a combination of grassy areas (‘*campos*’) surrounded by granitic outcrops and forests. Average annual rainfall is about 1321 mm and annual average temperature is approx. 19·3 °C (PMPA, 2012).

Species studied

Chloraea membranacea grows in north-eastern Argentina (from Buenos Aires Province, northwards to Santa Fé, Entre Rios and Corrientes), Uruguay and southern Brazil, reaching its northernmost limit at the State of Paraná (Ponta Grossa and Curitiba; Correa, 1969). Although common in Argentina and Uruguay, this plant is rare in Brazil, and all recent herbarium records (10 years or less) come from the State of Rio Grande do Sul. In the Brazilian State of Paraná, this species has not been collected for decades (the most recent voucher for Paraná State, as far as we can ascertain, was pressed in 1977; C. R. Buzatto, pers. observ.), and there are no known pressed vouchers from Santa Catarina State. Generally, flowering plants may reach a height of 40–70 cm. The lanceolate leaves are 10–15 cm long and 2–3 cm wide (see Supplementary Data Fig. S1A) and may be dry or absent during anthesis (Correa, 1969). The inflorescence may bear up to 20 greenish-white flowers. Morphologically, Hauman (1921) and Correa (1969) considered this species to be very similar to *C. bella*, the only other non-Andean species of *Chloraea*. Together, *C. membranacea* and *C. bella* constitute the so-called ‘eastern’ *Chloraea* species-group (Hauman, 1922; Correa, 1969), the only non-Andean species-group in the genus. Apart from its distribution, this ‘eastern’ group is characterized by distinctive column features: the stigmatic surface is long (two-thirds of the total length of the column) and dumb-bell-shaped, and the column wings are narrower and thicker towards the apex (Correa, 1969).

Recent phylogenetic studies (Chemisquy and Morrone, 2010, 2012; Cisternas *et al.*, 2012*a*, *b*) cast doubt on the monophyly of the genera assigned to Chloraeinae as currently circumscribed. Indeed, the same analyses (see Chemisquy and Morrone, 2012; Cisternas *et al.*, 2012*a*, *b*) place *C. membranacea* either as sister to, or in the same clade as, *Chloraea virescens* (Willd.) Lindl., the type species of the genus. In other words, even if *Chloraea* is redefined and some groups are segregated (as already proposed by Szlachetko & Margonska, 2001, and Szlachetko and Tukałło, 2008), it is likely that *C. membranacea* will remain within *Chloraea*. Plant vouchers were deposited at the ICN Herbarium of the Universidade Federal do Rio Grande do Sul (UFRGS) and also at the Museo Argentino de Ciencias Naturales – Bernardino Rivadavia Herbarium (BA).

Floral morphology and anatomy

Fresh flowers and their parts were photographed using a digital camera. They were then examined for secretory structures using a stereomicroscope and subsequently prepared for light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM; see below).

Light microscopy and histochemistry

Following macroscopic observations, pieces of labellar tissue were tested for lipids and starch by treating each with a saturated solution of Sudan III in 70 % (v/v) ethanol and IKI, respectively, and examined microscopically (Jensen, 1962). Other labellar samples (approx. 1 mm^3) were fixed in 2·5 % glutaraldehyde/4 % freshly de-polymerized paraformaldehyde in phosphate buffer (pH 7·2; 0·05 M) for 5 h at room temperature (initially under reduced pressure to facilitate penetration), carefully washed three times in phosphate buffer (30 min each) and post-fixed in 1 % (w/v) osmium tetroxide solution for 2 h at
0 °C. They were then dehydrated using a graded ethanol series, and infiltrated and embedded in Historesin (Leica). Sections were cut at 1 µm thickness on a Leica RM2155 microtome using a Jung carbide knife, stained with 0·25 % (w/v) toluidine blue O (TBO) in 0·25 % (w/v) aqueous sodium tetraborate solution, air-dried and mounted in synthetic Canada balsam.

Transmission electron microscopy

Pieces of labellar projections (approx. 1 mm^3) were fixed as above and, following dehydration using a graded ethanol series, infiltrated and embedded in Spurr’s resin. Following polymerization at 60 °C, sections were cut at 70 nm for TEM using a Sorvall Porter-Blum MT2-B ultramicrotome and Diatome diamond knife. Sections were then stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined using a Zeiss EM 109T transmission electron microscope at an accelerating voltage of 80 kV.

Scanning electron microscopy

Labella and columns ($n = 3$, each) were dehydrated using a graded ethanol series, transferred to pure acetone, subjected to critical-point drying using liquid CO_2 and sputter-coated with 15 nm gold (Davies and Stpiczyńska, 2009). The samples were examined using a Jeol JSM-6060 scanning electron microscope, at an accelerating voltage of 20 kV, located at the Centro de Microscopia Eletrônica of the Universidade Federal do Rio Grande do Sul (UFRGS).

Floral life span, pollination, fruiting success, pollination efficiency and breeding system

Floral life span was established in 2011 by monitoring 15 tagged, untouched flowers occurring on five bagged individuals (three flowers per inflorescence) from the Flores da Cunha population (see Table 1).

Pollination biology of native Argentinean and Brazilian populations was studied in the field. The number of hours spent observing each population is indicated in Table 1. Preliminary observations carried out in 2009 clearly showed that the flowers were attractive to pollinators during daylight hours (1000 to 1800 h), a fact that was subsequently confirmed. Consequently, further observations were planned accordingly, and no crepuscular or nocturnal observations were made. Generally, the observation period extended from 0800 to 1830 h.

In Brazil (Flores da Cunha), the pollination biology of *C. membranacea* was studied for a period of two consecutive years (2010, 2011; Table 1). In 2010, observations were made on five plants that produced a total of 51 flowers. In 2011, 11 individuals that produced a total of 213 flowers were monitored. Fruiting success (fruits/flowers produced) was recorded at the Flores da Cunha locality for both years, as a measure of pollinator efficiency. A t-test was applied to analyse possible differences in fruiting success for each of the years observed. Between 17 and 19 November 2010, Nilsson's male efficiency factor (percentage of pollinated flowers divided by the percentage of flowers acting as pollen donors) was also calculated as another measure of pollinator efficiency (Nilsson *et al.*, 1992). Complementary observations were made in 2011 at the Brazilian locality of Porto Alegre by exposing to the outdoors a cultivated specimen bearing a single inflorescence of seven flowers (Table 1). In Buenos Aires (Argentina), the pollination of *C. membranacea* was studied in 2011 (see Table 1) by monitoring two individuals naturally growing at the Campus of the Facultad de Ciencias Exactas y Naturales. During the observations, these plants produced a total of 27 flowers.

TABLE 1. *Summary of pollination observations in populations of* Chloraea membranacea. *Data of voucher specimens are detailed below each locality*

Locality/country	Year	Observation days	Observation period	Hours spent per locality
Flores da Cunha (RS, Brazil) *M. Pedron s.n.* (ICN)	2010	2, 7, 8, 11, 13, 15, 19 and 20 November	0800 to 1930 h	57
Flores da Cunha (RS, Brazil) *M. Pedron s.n.* (ICN)	2011	31 October and 1–5 November and 7–11 November	1030 to 1700 h	33
Porto Alegre (RS, Brazil) *C.R. Buzatto 745* (ICN)	2011	5 and 6 November	1030 to 1700 h	13
UBA (CABA, Argentina) *A. Sanguinetti s.n.* (BA)	2011	17, 22–25 October	1100 to 1800 h	15
Total (h)				118

Pollinator behaviour was documented by means of field notes, photography and video. In general, this video record made it possible to gain a better understanding of both the pollination process and pollinator behaviour. Individuals of pollinating insects were collected and sacrificed for further taxonomic identification. These insect vouchers were deposited at the entomological didactic collections of the Zoology Department, UFRGS (Brazilian specimens) and at the Museo Argentino de Ciencias Naturales, entomological section (Argentinean specimens).

Breeding system experiments on Brazilian plants were performed *in situ* at Flores da Cunha, by bagging seven plants (totalling 97 flowers) in order to exclude natural pollinators (see Supplementary Data Fig. S1B). Plants cultivated in a greenhouse based in the experimental field of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina (13 plants totalling 112 flowers), were bagged and used for the same purpose. Four treatments were applied to these inflorescences: intact flowers (control), emasculation, hand self-pollination and cross-pollination (Table 2). Only hand self-pollinations and cross-pollinations yielded fruit in the plants of both countries (see Results and Table 2) and the percentages of fruiting success under both treatments are almost identical, rendering statistical comparisons unnecessary (Table 2). The number of plants and flowers used per treatment are detailed in Table 2.

RESULTS

Floral features

Only floral features involved in the pollination process will be discussed. Readers interested in a more detailed description of vegetative and floral features of *C. membranacea* are referred to the monograph by Correa (1969: 456–459; see also Supplementary Data Fig. S1A). The flowers at the Flores da Cunha locality had a mean life span of 22·4 d. However, flowers cultivated at Porto Alegre kept their fresh appearance for 6–7 d only (R. B. Singer, pers. observ.), while the flowers of specimens cultivated at Buenos Aires lasted for

TABLE 2. *Breeding system experiments; fruiting success (%) in* Chloraea membranacea *for control (intact flowers) and following emasculation, self- and cross-pollination treatments*

Locality/country	*n*	Control	Emasculation	Self-pollination	Cross-pollination
UBA (Buenos Aires, Argentina)	13	0 (0/28)	0 (0/28)	96·43 (27/28)	100 (28/28)
Flores da Cunha (RS, Brazil)	7	0 (0/25)	0 (0/24)	100 (24/24)	100 (24/24)

Numbers in parentheses represent the number of fruit obtained over the number of flowers used in each treatment. *n* represents the number of plants used in the experiments.

10–17 d (A. Sanguinetti, pers. observ.). This suggests that the feature is not constant and is probably affected by environmental conditions. The flowers are greenish-white (Fig. 1A) and membranous in texture. The sepals are 16–20 mm long and 7–8 mm wide (Correa, 1969). The lateral petals are asymmetric, 15–18 mm long and 9–11 mm wide. The labellum is entire to slightly three-lobed, 13–15 mm long and 7–9 mm wide, and is articulated (hinged) at the base of the column (Fig. 1B).

Examination of fresh material revealed that the adaxial surface of the labellum is white with numerous, clavate projections that have greyish-white stalks and black tips (Fig. 1B), the latter coated with translucent or whitish, glistening, secreted material. The projections are concentrated distally (Fig. 2A) and the epidermal cells of both labellar surface and projections have a striated cuticle (Fig. 2B, D). The projections, which may be flattened along one side, are usually unbranched and multicellular, with clavate or expanded heads (Fig. 2C). The stalk cells are narrow and elongate (Fig. 2C), whereas the head cells are more or less isodiametric and angular in surface view, often with scant amorphous residues or secretory droplets on their surface (Figs 2D and 4A). This surface material stained with Sudan III, and often accumulated between the isodiametric head cells (Fig. 4A, B). Occasionally, the projections have bifid heads (Fig. 2E-F), or are branched and deeply bifurcate (Fig. 2G), or are fused, with laterally compressed, flabelliform heads (Fig. 2H). Often, the heads consist of several confluent lobes (Fig. 2I). SEM of the adaxial labellar surface confirmed the absence of nectar-secreting cells and/or other nectariferous structures (Fig. 2).

The column is arcuate, greenish, about 10 mm long, and has a well-developed column-foot (Fig. 1B, C) and two small, lateral column wings (Fig. 3A). The cuticle overlying the epidermal cells of the column is striated (Fig. 3C). The anther is erect and terminal (Figs 1C and 3A). The pollinarium comprises two, bipartite, yellow, powdery pollinia that lack pollinium stalks (Fig. 1D), and the removal of pollinia is facilitated by a rostellar secretion. No detachable viscidium (*sensu* Dressler, 1993) is present. The stigmatic surface is extensive, dumb-bell- or hourglass-shaped and slightly convex (Figs 1C and 3A, B). It is borne ventrally on the column, is glandular (Fig. 3A, B) and bears numerous, densely packed, unbranched papillae or trichomes. These are usually smooth-walled, unicellular, clavate and secretory (Fig. 3D). SEM revealed that the latter are occluded by a homogeneous film of stigmatic fluid (Fig. 3A, B, D). At the base of the column occur two, so-called 'nectariferous channels' (*sensu* Cisternas *et al.*, 2012*a*, *b*, and references therein; Fig. 3E), which here are merely two shallow depressions or cavities (Fig. 3E, F). Despite the name given to these structures, the absence of nectar was confirmed. The flowers of *C. membranacea* emit a faint, sweet fragrance which is strongest during the warmer daylight hours (1000–1600 h), and this coincides with the greatest number of visits by pollinators. The fruit of *C. membranacea* specimens observed in this study dehisced along two dorsal lines of weakness (see Supplementary Data Fig. S1E), and the seeds were minute and dust-like.

Labellum anatomy

Transverse sections revealed that the labellum consists largely of mesophyll in which are embedded collateral vascular bundles (Fig. 4C), and is bound by a single-layered epidermis consisting of somewhat rounded and often-nucleated cells (Fig. 4C). Longitudinal and transverse sections through the labellar projections revealed that the head consists largely of a layer, some 1–4 cells deep, of isodiametric, epidermal secretory cells. At the base of the head, there is a marked distinction between these cells and the elongate, narrow cells of the stalk (Fig. 4D). The epidermis of the stalk is continuous with the adaxial epidermis of the labellum (Fig. 4C). Basally, the stalk is usually 6–10 cells wide, with a core of parenchymatous cells that is continuous with the labellar mesophyll (Fig. 4C). Like the mesophyll, the parenchymatous core of the stalk also contains idioblasts with raphides (Fig. 4D). This indicates that the projections are not epidermal, multiseriate trichomes, but rather outgrowths of the labellum. Moreover, they do not receive a direct vascular supply (Fig. 4C). Transverse sections of the projections reveal that acropetally, the secretory tissue is confined to a single layer of epidermal cells surrounding one or two core cells, the latter often containing raphides (Fig. 4E). Basipetally, however, several layers of subepidermal tissue are present, and these cells often contain amorphous, intravacuolar precipitates (Fig. 4D). LM revealed that the secretory cells are highly vesiculate, contain relatively large nuclei, numerous plastids (usually displaying perinuclear distribution) and lipid bodies (Fig. 4E, F). Starch was not detected.

TEM confirmed LM observations. A cuticle is present on the outer tangential walls of the secretory head cells (Fig. 5A–C, E–G). This is highly folded or striated, giving the cuticle a ridged appearance in section (Fig. 5B, C, E–G). Heterogeneous, secreted material is present on the surface of the cuticle, and this often collects between the cuticular ridges (Fig. 5B, C, E–G). Both secreted material and cuticle stained with Sudan III, demonstrating that they contain lipid (Fig. 4A, B). The outer tangential cellulose cell walls of the secretory cells lack ectodesmata (Fig. 5C).

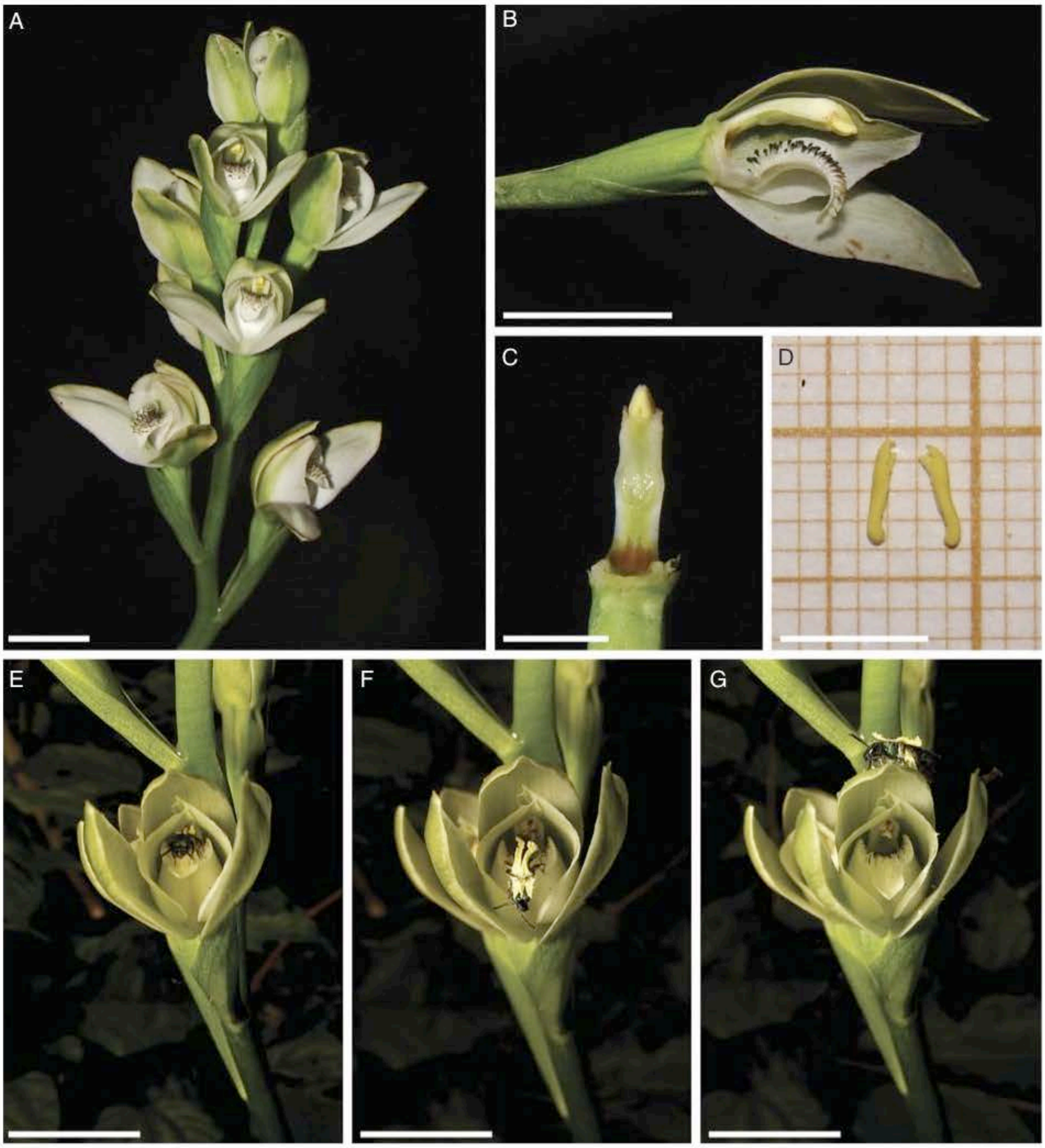

FIG. 1. Floral features and pollination of *Chloraea membranacea*. (A) Racemose, multi-flowered inflorescence. (B) Single flower with lateral sepal and lateral petal removed to show the column with hinged labellum attached at its base. Note the black-tipped, clavate, labellar projections (osmophores). (C) Column. Note also the ventral, dumb-bell-shaped, sticky stigmatic surface. (D) Two elongate pollinia. (E–G) Pollination by Halictid bee. (E) Bee prior to pollinarium removal. (F) Bee leaving the flower. The insect is attempting to remove the pollinarium cemented to its dorsum. (G) Pollinarium-laden bee. Note that the hind legs are loaded with pollen. Scale bars: (A, B, E–G) = 1 cm; (C, D) = 5 mm.

However, radial walls and inner tangential walls are often pitted, the primary pit-fields containing plasmodesmata that allow communication and transfer of material between adjacent cells. The outermost region of the outer tangential wall is clearly lamellate, and the cuticle is reticulate (Fig. 5E–G). The cells of the head contain relatively large, prominent nuclei, often with nucleoli, and highly vesiculate cytoplasm with a complement of organelles consistent with that of secretory cells (Fig. 5A, B). There is a well-developed vacuome comprising small vesicles and larger vacuoles (Fig. 5A, D). Indeed, these cells often display a very characteristic and atypical type of vacuolation during which the cytoplasm appears to become divided into somewhat angular segments (Fig. 4E). Components of the vacuome may contain membranous inclusions, spherical, osmiophilic bodies and amorphous precipitates (Fig. 5A, D). The

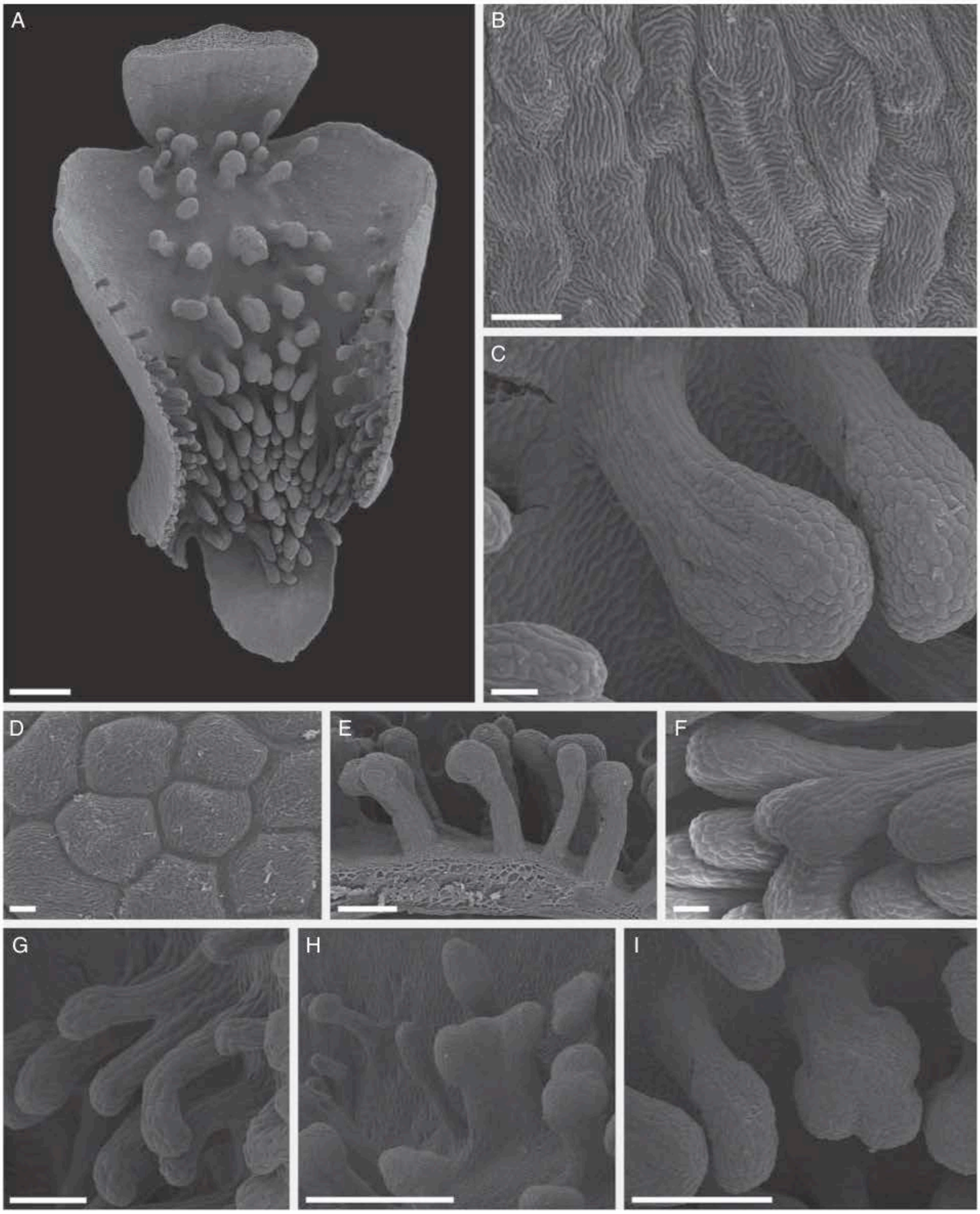

FIG. 2. Labellar features of *C. membranacea* (SEM). (A–I) Labellum bearing clavate projections (osmophores) that are concentrated distally and centrally. (B) Epidermal cells of distal, adaxial surface of labellum showing striated cuticle. (C) Detail of typical, unbranched, multicellular, clavate osmophore. Note the narrow, elongate stalk cells and the isodiametric head cells. (D) Detail of head showing angular cells with striated cuticle and scant residues of secreted material. (E,F) Osmophores with bifid heads. (G) Branched, deeply bifurcate osmophore. (H) Flabelliform, laterally compressed osmophore. (I) Osmophore with head composed of three confluent lobes. Scale bars: (A, H) = 1 mm; (B) = 20 μm; (C, F) = 100 μm; (D) = 10 μm; (E, I) = 500 μm, (G) = 200 μm.

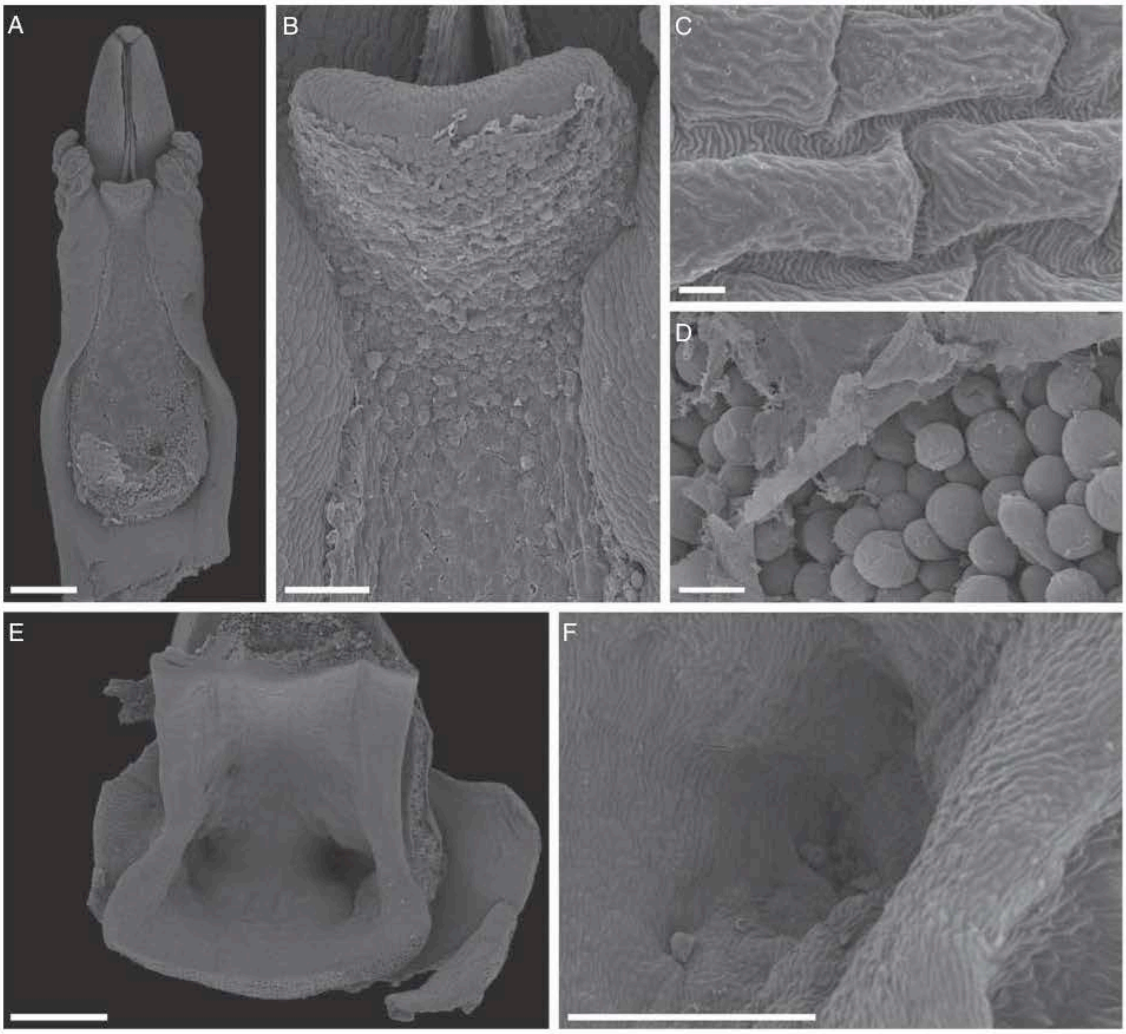

FIG. 3. Column features of *C. membranacea* (SEM). (A) Ventral surface of column, showing anther, lateral column wings and hourglass- or dumb-bell-shaped stigmatic surface. (B) Detail of part of stigmatic surface showing homogeneous residue film of stigmatic fluid. (C) Epidermal cells of column with striated cuticle. (D) Detail of stigmatic surface showing smooth-walled, unicellular, secretory, clavate papillae and overlying, residual, homogeneous film of stigmatic fluid. (E) 'Nectariferous channels' (in fact, two shallow depressions or cavities) at the base of the column. (F) Detail of one of these depressions, showing the absence of secretory cells. Scale bars: (A, E) = 1 mm; (B) = 200 μm; (C) = 10 μm; (D) = 50 μm; (F) = 500 μm.

numerous, oval, dumb-bell-shaped or irregularly shaped elaioplasts generally display a perinuclear distribution (Fig. 5A, B, D). They have an osmiophilic stroma and contain lipid droplets and/or numerous plastoglobuli and membranes (Fig. 5B, D), Much greater numbers of more or less spherical, but sometimes crescent-shaped, lipid bodies are also present (Fig. 5A, D, E–G). Often, these are less intensely osmiophilic than the elaioplasts. Under LM, they stain red with Sudan III, and are interpreted as droplets of fragrance precursors (terpenoids). They often aggregate adjacent to the plasmalemma (Fig. 5A, F). The abundant mitochondria contain large numbers of well-developed cristae (Fig. 5B–E, G), reflecting the high metabolic activity of these cells during secretion. Both smooth (SER) and rough (RER) endoplasmic reticulum are present, but the former predominates (Fig. 5C, F). The SER cisternae become dilated with secretory material (Fig. 5C). Dictyosomes (Golgi apparatus), however, were rarely observed (Fig. 5F), but ribosomes were abundant. Aggregates of small vesicles often became associated with the plasmalemma and cell wall (Fig. 5G), and a darkly staining, osmiophilic layer of lipid was often visible directly beneath the plasmalemma, or in the periplasmic space (Fig. 5A, E, G), and another to the outside of the cell wall, directly beneath the cuticle (Fig. 5A, E–G). Localized sub-cuticular accumulations of osmiophilic material coincided with cuticular ridges and partial detachment of the cuticle from the outer tangential wall (Fig. 5A–C, E–G). These accumulations are triangular in section and, under LM, stain intensely with TBO (Fig. 4F). Osmiophilic extensions pass from these into the cuticle (Fig. 5E-G), strongly suggesting the presence of

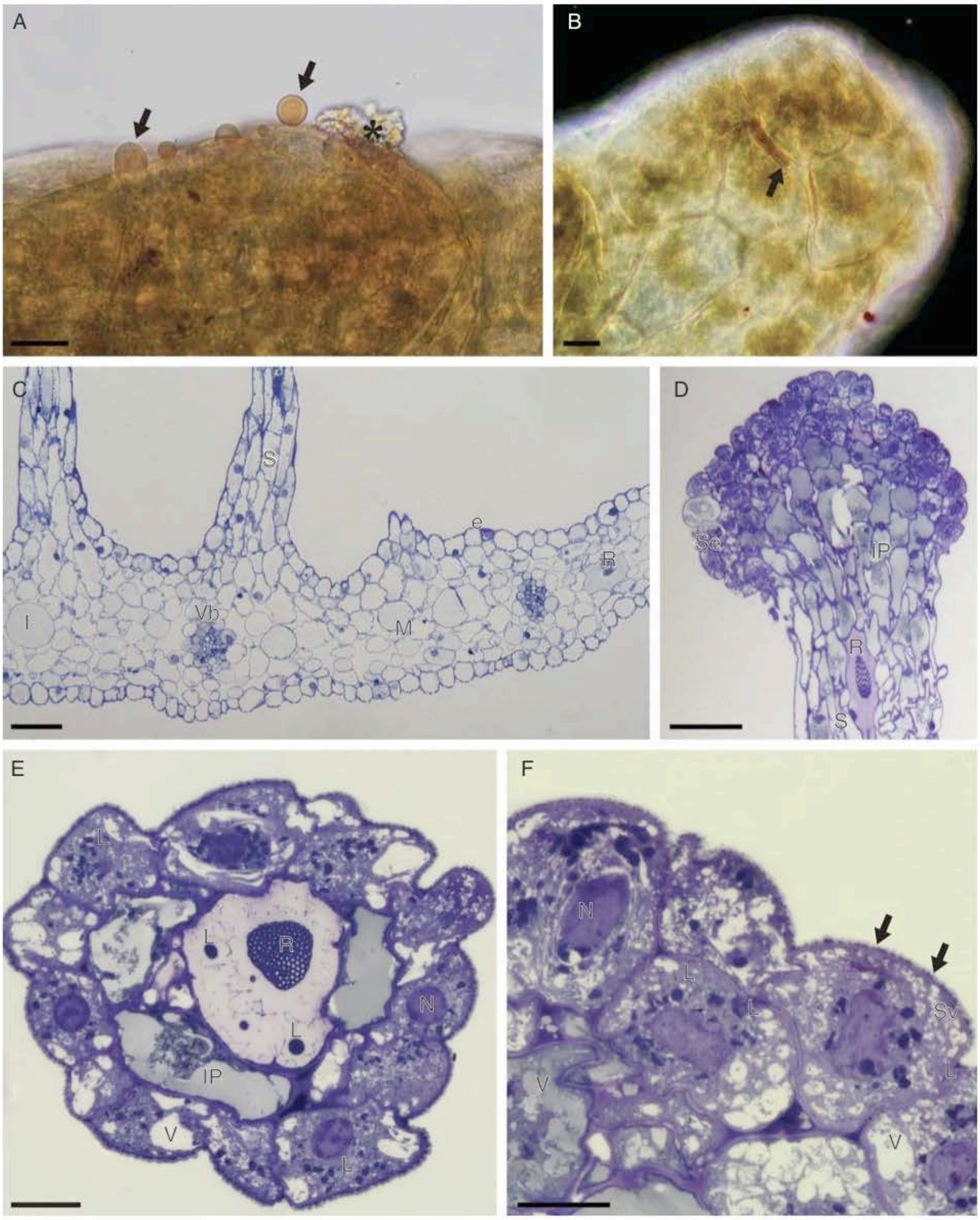

FIG. 4. (A, B) Detail of head of labellar osmophore of *C. membranacea*, treated with Sudan III (LM). (A) Osmophore tissue with secreted lipid droplets (arrows) that stain orange-red with Sudan III; also note the secretory residues (asterisk) present on the osmophore surface. (B) Surface view of osmophore head showing the accumulation of secreted lipid material between isodiametric cells (arrow). Note that the cuticle also stains red with Sudan III. (C–F) Labellar anatomy of *C. membranacea* (LM). (C) Transverse section of labellum with bases of stalked osmophores and collateral vascular bundles. (D) Vertical section through apex of osmophore showing head comprising intensely stained secretory cells and subsecretory layer containing amorphous, intravacuolar precipitates. The stalk contains idioblasts with raphides. (E) Transverse section of osmophore showing outer layer of secretory cells, subsecretory layer with intravacuolar precipitates, and a central cell with intravacuolar raphides and spherical lipid bodies. (F) Detail of secetory cells with perinuclear plastids. Note the vesicles that aggregate close to the cell wall and the lipid material (arrows) that accumulates beneath the cuticle. Scale bars: (A, B, E, F) = 20 µm; (C, D) = 100 µm. Abbreviations: e = epidermis; I = idioblast; IP = intravacuolar precipitate; L = lipid body; M = mesophyll; N = nucleus; R = raphide; S = stalk of osmophore; Se = secretory layer; Sv = secretory vesicles; V = vacuole; Vb = vascular bundle.

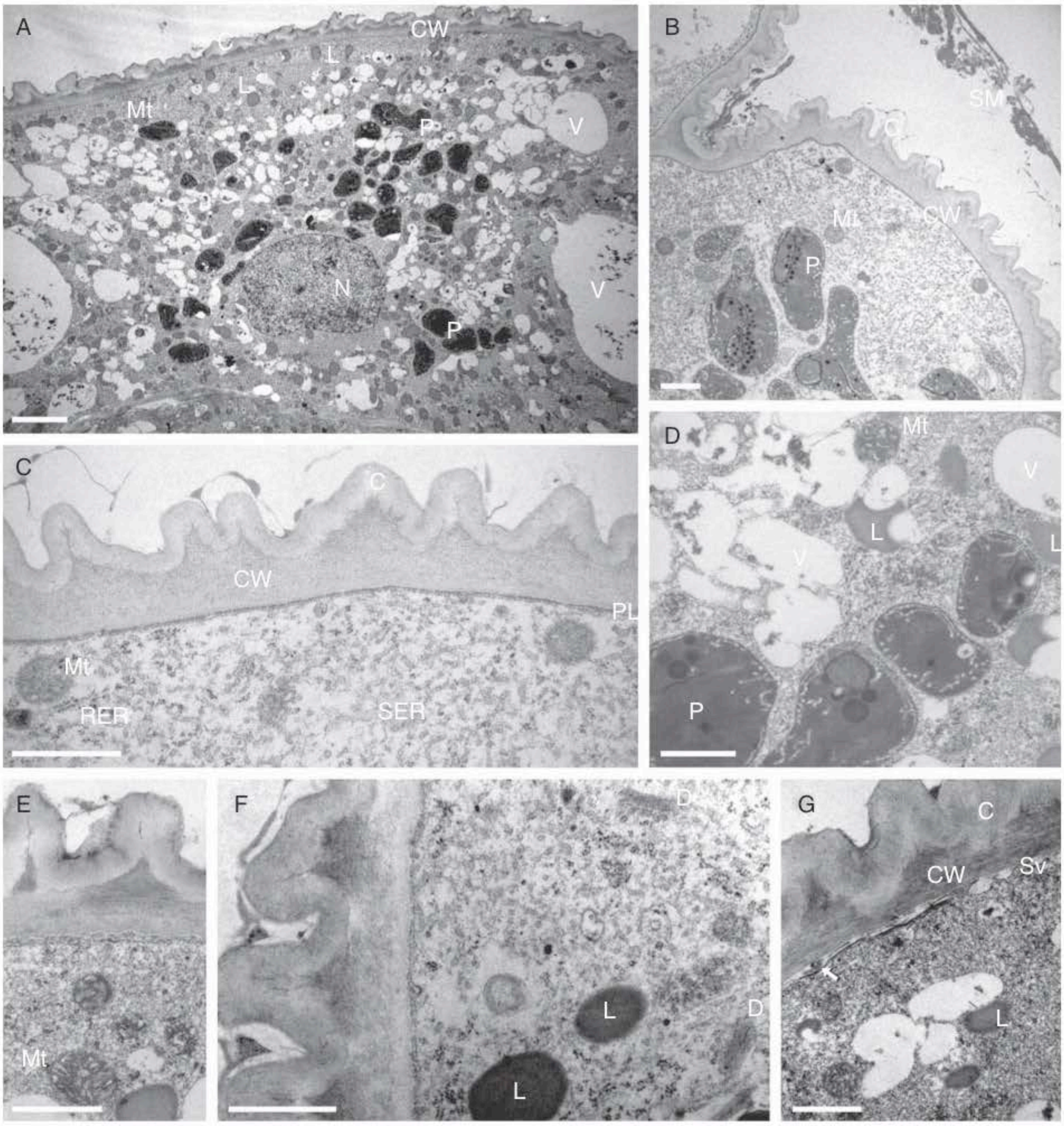

Fig. 5. (A–G) Ultrastructure of secretory osmophore cells of *C. membranacea* (TEM). (A) General section through secretory cell showing ridged cuticle and distribution of organelles. Abundant, spherical, osmiophilic lipid bodies, thought to be precursors of fragrance production, are present. (B) Similar cell showing heterogeneous surface secretion and elaioplasts. (C) Detail of cell wall, cuticle and plasmalemma with associated organelles. (D) Detail of vesiculate cytoplasm showing elaioplasts containing lipid bodies and membranes. (E) Osmiophilic material accumulates between the cell wall and cuticle. (F) Secretory cytoplasm with lipid bodies and dictyosomes (Golgi apparatus). (G) Small vesicles become associated with the plasmalemma. Osmiophilic material accumulates here and in the periplasmic space (arrow). The cell wall is more obviously lamellate and osmiophilic material accumulates beneath the cuticular ridges. Micro-channels penetrate the latter. Scale bars: (A) = 2 μm; (B–G) = 1 μm. Abbreviations: C = cuticle; CW = cell wall; D = dictyosome (Golgi apparatus); L = lipid body; Mt = mitochondrion; N = nucleus; P = plastid; PL = plasmalemma; RER = rough endoplasmic reticulum; SER = smooth endoplasmic reticulum; SM = secreted material; Sv = secretory vesicles; V = vacuole.

cuticular micro-channels, and possibly pores through which the secretion can pass. The localized presence of secreted lipoidal material on the surface of the cuticle supports this hypothesis. Although no cuticular pores were detected, this does not necessarily mean that they are absent, as the highly folded cuticle, in conjunction with the very small size of such structures and the presence of secreted material, would have frustrated our efforts to find them using SEM. Lipids, as well as occurring within plastids, ER and vesicles, or becoming associated with the plasmalemma, cell wall and cuticle, also accumulate within the larger vacuoles of the inner cells of the head, where they either occur as spherical bodies

(Fig. 4E) or become associated with the tonoplast. Likewise, lipids are also present in the vacuole and along the plasmalemma and tonoplast of raphide-containing idioblasts. Some lipids found in these cells are not associated with membranes, but form numerous, free, spherical lipid bodies or droplets scattered throughout the cytosol. The innermost cells of the head are lined by a narrow layer of parietal cytoplasm enclosing well-developed and much larger vacuoles than those of the outer cells. Intravacuolar, amorphous precipitates are frequently present here (Fig. 4D–E). This combination of cellular features is typical of osmophores (scent-producing glands).

Breeding system

The results are summarized in Table 2. Both Brazilian and Argentinean plants had a similar breeding system. They were self-compatible but pollinator-dependent. No fruit was produced either by intact or emasculated flowers, thus supporting the view that *C. membranacea* is not able to set fruit in the absence of pollinators. The percentage fruiting success resulting from manual self-pollination and cross-pollination was almost identical (Table 2), making statistical comparisons unnecessary.

Pollination biology

Pollinator observations are summarized in Table 3. For both Argentinean and Brazilian plants, the pollinators were male and female Augochlorini (Halictidae) bees (Fig. 1E–G, and Supplementary Data Video S1). Bees visited the flowers during the warmer daylight hours, especially when the inflorescences were receiving direct sunlight. Pollinator visits took place between 1048 and 1630 h, i.e. during the period when the flowers emit their sweet fragrance (see Floral features). In Argentina, the main pollinators were *Augochloropsis multiplex* (Vachal 1903) bees. In Brazil, the pollinators were also bees of the genus *Augochloropsis* (three morphospecies). In both countries, the pollination mechanism was similar: the bees briefly hovered in front of the flowers, alighted and entered the floral cavity. The hinged labellum moved noticeably relative to the movements of the bee inside the flower. Bees were repeatedly seen inspecting the clavate projections on the labellar surface. Remarkably, the vast majority of Halictid bees that alighted positioned themselves so that the abdomen pointed towards the column (Fig. 1E). Bees positioned in this way pressed their bodies against the rostellum when leaving the flower. The rostellum then released a sticky fluid that glued the pollinarium onto the dorsum of the bee (Fig. 1F, G). We would like to stress here that the orientation of the bee on entering the flower supports the view that the so-called ‘nectariferous channels’ are actually devoid of nectar. Also, that for all visits resulting in the removal of the pollinarium, the head of the bee projected outside the floral cavity, i.e. in the opposite direction to the ‘nectariferous channels’. In other words, Halictidae bees were not observed gathering or attempting to gather nectar from the presumed nectaries. Bees clearly spent considerable time inspecting the clavate labellar osmophores, but no attempts at nectar-collecting (or any other kind of collecting activity) at these structures were observed. The extra weight of the pollinarium upset the balance of the pollinator, and bees were often seen falling to the ground or actively attempting to remove the pollinaria with their forelegs (Fig. 1F). This latter behaviour was recorded at all three localities. Pollination occurs when a pollinarium-laden bee enters a flower and repeats the behaviour described above. As the stigmatic surface is long and broad, pollinia easily make contact with the stigmatic surface, leaving large pads of pollen.

The bees may spend 6–79 s inside each flower (see Table 3 for observation details) and visit 1–3 flowers per inflorescence, and 1–3 inflorescences per visit to the population. The bees spend considerable time grooming outside the flowers and, generally, may stay at a single inflorescence for several (more than 8) minutes. It is worth noting that most bees visited more than one flower per visit to each inflorescence, promoting a variable degree of geitonogamy. The film record shows that, depending on locality, the bees self-pollinated 0·47–14·8 % of available flowers (Table 3). Remarkably, some pollinarium-laden female Halictid bees were often seen performing the same behaviour, both in Brazil and in Argentina. These bees alighted on the inflorescence and used their forelegs to transfer part of the pollen content of the pollinarium to their hind legs (see Supplementary Data Video S1). This behaviour strongly suggests that female Halictidae bees may eventually use in some way the pollen of *C. membranacea* during their life

TABLE 3. *Summary of pollinator observations for plants of* C. membranacea *in Brazil and Argentina*

Locality/country	Year	Pollinators	Period of observed insect activity	Observed pollinarium withdrawals†	Observed insect-mediated self-pollinations†	Time (s) spent by pollinators inside the flowers‡
Flores da Cunha (RS, Brazil)	2010	Halictidae bees	1048–1634 h	6	5 (9·80 %)	6–84 (25·33 ± 23·01)
Flores da Cunha (RS, Brazil)	2011	Halictidae bees, Vespidae wasp*	1130–1523 h	3	1 (0·47 %)	61–79 (70 ± 12·72)
Porto Alegre (RS, Brazil)	2011	Halictidae bees	1200–1500 h	5	1 (11·11 %)	6–65 (33 ± 19·08)
UBA Campus (CABA, Argentina)	2011	Halictidae bees	1200–1500 h	4	4 (14·81 %)	16–49 (29 ± 11·71)

* Observed only once.

† During video record.

‡ Values in parentheses represent mean ± s.d.

cycles. Pollinated flowers characteristically display a closed perianth with lateral petals hiding the column (see Supplementary Data Fig. S1C). In Brazil, unidentified flies were recorded as non-pollinating visitors to these flowers. The flies explored the outside of the flower, or licked the labellum, but made no contact with the column. An unidentified Vespidae wasp was seen once in Flores da Cunha dislodging a pollinarium (see Supplementary Data Fig. S1D, Table 3). On one occasion, in Argentina, a queen of *Bombus atratus* Franklin was observed to visit an inflorescence and probe three flowers, but without removing pollinia (see also Supplementary Data Video S1).

Pollination success

In 2010, of the 51 available flowers, 31 (60·78 %) developed into fruit. Individual fruiting success ranged from 22·22 to 100 %. Mean fruiting success per inflorescence was also very high (64·25 ± 31·64 %). Between 17 and 19 November 2010, 45 flowers (88·23 %) acted as pollen-donors, and 31 (60·78 %) as pollen receivers. Therefore, Nilsson's efficiency factor was 0·688, indicating that during this period, approx. 0·7 flowers were pollinated for each pollinarium removed, suggesting some pollen-loss.

In 2011, of the 213 available flowers, 98 (46 %) developed into fruit. Individual fruiting success ranged from 0 to 88·88 %. Mean fruiting success per inflorescence was also lower (42·068 ± 22·97 %). Despite the striking numerical disparity in mean fruiting success between consecutive years, the *t*-test revealed that this was not statistically significant ($P = 0{\cdot}096$, $t = 1{\cdot}747$, 20 d.f.). However, the test also revealed that a type II error was highly probable ($\alpha = 0{\cdot}05$; 0·262), and it is likely that this was related to sample size.

DISCUSSION

The flowers of *C. membranacea* display a set of morphological features that are widespread and mostly consistent with those already reported for the genus (Correa, 1969; Correa and Sánchez, 2003). However, some of our findings suggest that certain morphological features of *Chloraea* spp. have been incorrectly interpreted in the past. Contrary to the claim of Szlachetko & Rutkowski (2000: 62), there is no detachable viscidium, and the pollinia become glued to the pollinator by means of a rostellar secretion (see Results). Moreover, Correa (1969) states that the fruit of *Chloraea* is a capsule, each having six lines of dehiscence. However, our study of *C. membranacea* showed that the fruit of this species splits along two dorsal lines of weakness.

Absence of nectar on the labellar surface generally agrees with earlier reports of pollination in Chloraeinae (Lehnebach and Riveros, 2003; Ciotek *et al.*, 2006; Humaña *et al.*, 2008), and also fully agrees with previous reports relating to the pollination biology of Patagonian *Chloraea* spp. All *Chloraea* species for which pollination biology has already been studied (Clayton and Aizen, 1996; Lehnebach and Riveros, 2003; Humaña *et al.*, 2008) presented the so-called 'nectariferous channels', but these lacked nectar (see also Ciotek *et al.*, 2006). In describing the genus, Correa (1969: 380–381) remarked that these 'nectariferous channels' extend to 'less than the middle of the length of the ovary' and illustrated this (p. 381), but did not state upon which species she had based her diagram. Later, in the same text, Correa (1969: 470) claims that *Chloraea praecincta* Speg. & Kranzl. is the only species in the genus to lack 'nectariferous channels'. Fortunately, one of us (A. Sanguinetti, pers. observ.) had the opportunity to examine fresh flowers of the Patagonian species *C. cylindrostachya* Poepp., and found no 'nectariferous channels'. This observation, together with the fact that these structures are clearly non-secretory and almost vestigial in *C. membranacea*, suggests that this character is, perhaps, much more variable than expected. Recently, Cisternas *et al.* (2012*a*) have commented that similar 'nectariferous channels' may be present in some Chilean species of the genus *Bipinnula*. All this suggests that the presence of the so-called 'nectariferous channels' may be more homoplasious than hitherto thought.

Labellar projections such as those reported here for *C. membranacea* are widespread amongst *Chloraea* spp., and within Chloraeinae in general (Correa, 1969; Correa and Sánchez, 2003). Even so, as far as we know, this is the first time for the floral anatomy of Chloraeinae species, and the biological importance of the labellar projections in pollination, to be described. Superficially similar labellar projections are widespread in distantly related terrestrial orchids from Oceania, namely members of the genera *Caladenia* R. Br. (Diuridae: Caladeniinae) and *Chiloglottis* R. Br. (Diuridae: Thelymitrinae – see photographs and drawings in Jones, 1991, 2006), although, to our knowledge, anatomical studies of these genera have not yet been undertaken. The flowers of *Chiloglottis* spp. are exclusively pollinated by sexual mimicry (see Mant *et al.*, 2005), whereas *Caladenia* spp. are pollinated either by food-deceit strategies or by sexual mimicry (literature reviewed by Dixon and Tremblay, 2009; Phillips *et al.*, 2009). Similar labellar projections have also been reported for Epidendroid orchids, such as *Camaridium ochroleucum* Lindl. and *Camaridium pulchrum* Schltr. (as *Maxillaria camaridii* Rchb. f. and *M. pulchra* (Schltr.) L.O. Williams ex Correll, respectively; Singer and Koehler, 2004), where they are thought to mimic stamens (Davies and Turner, 2004; Davies, 2009).

Based on anatomical studies, we herein suggest that the labellar projections of *C. membranacea* are osmophores (scent-producing glands), and consequently are involved in pollinator attraction. This is supported by the presence of large numbers of intracellular, spherical, osmiophilic bodies (interpreted as droplets of a fragrance precursor) identical to those found in the osmophores of *Stanhopea* J. Frost ex Hook. (Epidendroideae: Stanhopeinae; Stern *et al.*, 1987) and *Brasiliorchis picta* (Hook.) R. Singer, S. Koehler & Carnevali (Epidendroideae: Maxillariinae; Davies and Stpiczyńska, 2012). Moreover, the secretory cells have relatively large nuclei, often with nucleoli, together with elaioplasts containing lipid bodies and plastoglobuli. In section, the cuticle is ridged, and secretory vesicles aggregate next to the plasmalemma or within the periplasmic space. Osmiophilic material may also collect both here and between the cuticle and the outer tangential wall. Walls between secretory cells and cells of the subsecretory layer display primary pit-fields with plasmodesmata. Extensive arrays of ER are present (mainly SER, but frequently RER also), with dilated cisternae, together with mitochondria possessing well-developed cristae. The cuticle becomes distended as secreted material accumulates between it and the outer tangential wall. The presence of dictyosomes (Golgi apparatus) may change as secretion progresses (Pridgeon and Stern, 1983, 1985; Stern *et al.*, 1987; Stpiczyńska, 1993, 2001). In many respects, the anatomical organization of the labellar secretory tissue of *Chloraea membranacea* thus resembles that of the fragrance-secreting tissues of several other orchid species. The osmophore tissue of orchids generally displays a granulocrine mode of fragrance secretion (i.e. with the ER producing secretory vesicles), as in *Restrepia* Kunth and *Gymnadenia conopsea* (L.) R.Br. (Pridgeon and Stern, 1983; Stpiczyńska, 2001). However, to date, eccrine fragrance secretion (involving active transport through the plasma membrane) is known to occur only in a few orchid taxa, such as *Stanhopea* (Stern *et al.*, 1987). The numerous secretory vesicles associated with the plasmalemma of *C. membranacea*, the abundant mitochondria with well-developed cristae and the absence of visible secretory particles within the cell wall are all indicative of granulocrine secretion.

In general, our results fully agree with previous reports on the breeding system both of Chilean species of *Chloraea* (Clayton and Aizen, 1996; Lehnebach and Riveros, 2003; Humaña *et al.*, 2008) and other Chloraeinae orchids (Valdivia *et al.*, 2010). All these earlier studies indicate that *Chloraea* orchids are self-compatible, but pollinator-dependent (Clayton and Aizen, 1996; Lehnebach and Riveros, 2003; Humaña *et al.*, 2008; Valdivia *et al.*, 2010). Reiche (1910) suggested that flowers of *Chloraea fonckii* Phil. may be either cleistogamous or autogamous, whereas those of *C. philippii* Rchb. f. may also be autogamous. However, Reiche (1910) did not provide evidence in support of this claim. Within Chloraeinae, automatic self-pollination was reported for *Gavilea araucana* (Phil.) M.N. Correa (Valdivia *et al.*, 2010). However, the authors did not state how this was achieved. One of us (A. Sanguinetti, pers. observ.) has documented automatic self-pollination in *G. glandulifera* (Poepp.) M.N. Correa. In this species, the pollinarium partially disaggregates, and pieces of the pollinia fall onto the swollen and prominent stigmatic surface. None of the floral features that are associated with automatic self-pollination were recorded for *C. membranacea*.

This is the first report of Halictid bees unequivocally pollinating Chloraeinae orchids. Previous reports have listed Colletidae (Lehnebach and Riveros, 2003) and Apidae (*Bombus* Latreille) bees, as well as Coleoptera and Diptera (Tabanidae and Sarcophagidae) as the natural pollinators of some Chilean species of *Chloraea* (Lehnebach and Riveros, 2003; Humaña *et al.*, 2008). Males of *Campsomeris bistrimacula* (Lepeletier) (Scoliidae) are the pollinators of *Bipinnula penicillata* (Hoehne ex M.N. Correa) Cisternas (as *Geoblasta penicillata* Hoehne ex M.N. Correa) (Ciotek *et al.*, 2006). The breeding system of some *Gavilea* species has been studied in detail, but the pollinators are not known (Valdivia *et al.*, 2010). Overall, the flowers of *C. membranacea* present a set of floral features (relatively small, greenish-white, partially closed, and sweetly scented flowers) that are widespread amongst orchids pollinated by sweat-bees (Singer and Cocucci, 1999; Singer and Sazima, 2000). Based on floral features, it is likely that other Chloraeinae orchids, such as *C. bella*, are also pollinated by Halictidae bees. During our observations, some pollinarium-laden Halictidae females were recorded actively transferring pollen from the pollinarium to their hindlegs. The pollen of monandrous orchids normally occurs in pollinia and therefore is rarely used by bee pollinators. However, the pollen of *Psilochilus modestus* Barb. Rodr. (Epidendroideae: Triphoreae) is often gathered by its Meliponine bee pollinators (Pansarin and Amaral, 2008). On occasion, Meliponine bees were also observed collecting pollen from the pollinaria of *Sauroglossum elatum* Lindl. (Spiranthinae), an orchid usually pollinated by noctuid moths (Singer, 2002). Remarkably, flowers of the orchid subfamily Apostasioideae (sister-group to the remaining Orchidaceae) present their pollen loose and free (i.e. not packed in pollinia), and all available evidence to date suggests that these orchids reward their pollinators with pollen (Kocyan and Endress, 2001). The present observations indicate that *C. membranacea* is a rewardless plant that is pollinated by food-seeking Halictidae bees. The fact that some pollinarium-laden female bees were seen actively collecting pollen from the pollinaria should be interpreted with caution. All our evidence suggests that pollen collection is a by-product of grooming activities. As already explained, the extra weight of the pollinarium has an adverse effect on the bee which, in turn, attempts to remove it with its forelegs. It could be argued that the bees were actively seeking pollen, but our photographic and film records indicate otherwise, in that all recorded bees interacted with the clavate projections on the labellar surface and ignored the anther or the 'nectariferous channels'. Furthermore, male bees also pollinated *C. membranacea*, and male bees do not collect pollen at all.

A variable degree of insect-mediated self-pollination was observed at the three locations where pollinator activities were recorded (see Table 3). In view of this, and considering that the plants are self-compatible and that there were no mechanical barriers to hinder self-pollination, we can safely assume that the observed natural fruit-set was partly a consequence of insect-mediated self-pollination.

It is important to stress that the observed fruiting percentages (60·78 and 46 % for 2010 and 2011, respectively) are remarkably high for a rewardless orchid. As a rule (Neiland and Wilcock, 1998; Tremblay *et al.*, 2005), rewardless orchids have low fruiting success (<21 %, compared with a mean of 37 % for rewarding species). However, Humaña *et al.* (2008) have already reported high fruiting success for four Chilean *Chloraea* taxa [approx. 35 % in *C. bletiodes* Lindl. (white form), approx. 80 % in *C. bletiodes* (yellow form), approx. 90 % in *C. chrysantha* Poepp. and approx. 90 % in *C. galeata* Lindl.], despite the fact that these orchids are rewardless and pollinator-dependent. Humaña *et al.* (2008) have also suggested that these high percentages may be explained in terms of good availability/abundance of natural pollinators. This may also partly explain the high fruiting values observed for *C. membranacea.* Augochlorini Halictid bees are common and widespread, even in anthropized or urban areas, where they are common residents of gardens and orchards. However, it is our opinion that some flower features, such as pollinarium texture, may also account for the high pollination success observed. As in many terrestrial orchids (e.g. Spiranthinae; Singer and Cocucci, 1999; Singer and Sazima, 1999, 2000), the pollinarium is friable, and thus the pollen content of a single pollinarium can be transferred to the stigmatic surfaces of several flowers. Even if there is some pollen loss (as shown by the calculated Nilsson's index = 0·68), the pollen content of a single pollinarium is still able to pollinate several flowers. Lehnebach and Riveros (2003) reported a very low visitation frequency for the Chilean species *C. lamellata* Lindl. These authors did not provide the final fruiting success value for this species, but reported that 71·3 % of the observed flowers acted as pollen donors, whereas 28·6 % acted as pollen receivers (Lehnebach and Riveros, 2003). From these data, a Nilsson's male efficiency factor of 0·4 can be calculated for *C. lamellata*, a value that is considerably lower than that obtained here for *C. membranacea*. These data indicate that pollinator availability/abundance is a key factor in the fruiting success of Chloraeinae. A low (approx. 12 %) fruiting success was reported for the Chilean species *C. crispa* Lindl. (Humaña *et al.*, 2008), a taxon for which pollination events were only rarely observed. With the exception of *Chloraea*, the natural pollination success of few other Chloraeinae orchids has been studied. A fruiting success of approx. 98 % has been reported for self-pollinating *Gavilea araucana* (Phil.) M.N. Correa, and one of approx. 28 % for allogamous *G. venosa* (Lam.) Garay & Ormerod (Valdivia *et al.*, 2010). This latter value is slightly higher than the average obtained worldwide for other non-rewarding orchids (Tremblay *et al.*, 2005). The considerable (albeit non-significant in statistical terms) numerical difference in fruiting success between 2010 and 2011 observed at the Flores da Cunha population could have been caused by climatic differences. Climatic data gathered at a local station support this idea (full data available on request). The results of *t*-tests indicate that the temperature was significantly higher during the 2011 sampling period ($P = 0{\cdot}006$, 0·033 and 0·005 for minimum, maximum and averages, respectively) and that wind speed was also higher in 2011 ($P = 0{\cdot}004$). These factors may have influenced pollinator availability and behaviour.

Pollination strategies within the Chloraeinae orchids

All the evidence indicates that the pollination of *C. membranacea* flowers involves a generalized food-fraud strategy, as already proposed for other species of the genus (Lehnebach and Riveros, 2003; Humaña *et al.*, 2008). In fact, all Chloraeinae orchids studied to date lack nectar (Lehnebach and Riveros, 2003; Ciotek *et al.*, 2006; Humaña *et al.*, 2008; Valdivia *et al.*, 2010). It should be stressed, however, that the beetle pollinators of *C. bletioides* actually use the flowers as a shelter and may even mate inside them. Therefore, it is likely that flowers of this particular species reward pollinators by these means, and this may account for its high fruiting success (Humaña *et al.*, 2008). Hitherto, the flowers of only two species of *Gavilea* have been studied (Valdivia *et al.*, 2010), both of which are said to be nectarless. One of the most remarkable distinguishing features of this genus is the presence of two, swollen, so-called 'nectaries' located at the base of the column (Correa and Sánchez, 2003; Cisternas *et al.*, 2012*a*,*b*). Among the Chloraeinae, the pollination of *Bipinnula penicillata* (Ciotek *et al.*, 2006) is noteworthy. Male wasps of *Campsomeris bistrimacula* (Scoliidae) attempt to copulate with the insect-like labellum, thereby precipitating pollination (Ciotek *et al.*, 2006). This is the first report of pollination by sexual mimicry in terrestrial Neotropical orchids (Ciotek *et al.*, 2006). Even though the pollination and breeding system of fewer than 15 % of Chloraeinae orchids have been studied, all those species investigated lack nectar and most employ deception of some kind (Lehnebach and Riveros, 2003; Ciotek *et al.*, 2006; Humaña *et al.*, 2008; Valdivia *et al.*, 2010). Surprisingly, recent phylogenetic analyses suggest that Chloraeinae orchids are best placed within the tribe Cranichideae, as sister-group to a clade containing the subtribes Spiranthinae, Manniellinae, Cranichidinae and Goodyerinae (Chemisquy and Morrone, 2012; Cisternas *et al.*, 2012*a*). By contrast, the flowers of all these latter subtribes are nectar-secreting (Singer and Cocucci, 1999; Singer and Sazima, 1999, 2000, 2001*a*, *b*; Singer, 2002).

A greater number of studies involving more species are now necessary if we are to understand fully the evolution of pollination strategies within Chloraeeae. Fortunately, the resolution of the phylogeny of this orchid group is imminent (Chemisquy and Morrone, 2012; Cisternas *et al.*, 2012*a*). Hopefully, once a complete, representative and robust phylogeny becomes available, it can be used as a framework to understand both the evolution of pollination strategies and that of floral characters (features) associated with them. There remains, however, one further important task, namely the complete reappraisal and re-evaluation of the floral characters of these orchids. Contrary to many authors (e.g. Correa, 1969; Correa and Sánchez, 2003; Chemisquy and Morrone, 2010, 2012; Cisternas *et al.*, 2012*a*), we have placed certain morphological terms used in this text between quotation marks, e.g. 'nectariferous channels' and 'nectary'. The reason for this is that all available bibliography relating to pollination and breeding systems, as well as this present contribution, clearly indicates that the use of these terms is misleading. Indeed, when viewed from a functional standpoint, they are blatantly incorrect, as none of the species of *Chloraea* and *Gavilea* studied so far presents nectar either at so-called 'nectariferous channels' (Lehnebach and Riveros, 2003; Ciotek *et al.*, 2006; Humaña *et al.*, 2008) or at 'nectaries' located at the base of the column (Valdivia *et al.*, 2010). Only by the careful reappraisal and scrutiny of these features in representative species can their true nature and their importance in the pollination process be properly assessed. In the meantime, the incorrect use of these terms in Chloraeinae orchids is to be discouraged. More specifically, we suggest here that terms such as *foveae* (plural of *fovea*, Latin for pit) or *fossae* (plural of *fossa*, Latin for trench or ditch) be used to replace the incorrect term 'nectariferous channels' in *Chloraea* species.

Future prospects and concluding remarks

This contribution represents the first detailed report of the breeding system and pollination of a non-Patagonian species of *Chloraea*, as well as the first detailed report of Halictidae bees pollinating members of the subtribe Chloraeinae. To our knowledge, this is also the first detailed account of floral anatomy for that subtribe. Shortly, we intend to extend our studies to other species, so as to investigate the entire floral diversity of Chloraeinae. As stated previously, a complete resolution of the phylogeny of Chloraeinae is imminent (Chemisquy and Morrone, 2010, 2012; Cisternas *et al*., 2012*a*). When a robust, fully representative and reliable phylogeny of Chloraeae becomes available, it will be possible to plot documented pollination strategies onto phylogenetic trees and propose possible pathways for their evolution. To achieve this, a complete re-evaluation of those floral characters involved in pollination is essential. These are tasks that we intend to address in the near future.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Figure S1: (A) vegetative features of *C. membranacea*, (B) cased specimen used in breeding system experiments in 2010, (C) pollinated flowers, (D) unidentified Vespidae wasp leaving a flower laden with a pollinarium, (E) fruit showing two lines of dehiscence. Video S1: pollination of *Chloraea membranacea* in Brazil (Porto Alegre) and Argentina (Buenos Aires) by Halictidae bees. Bees attempt to remove the adhered pollinarium and eventually transfer some pollen to their hind legs. A non-pollinating queen of *Bombus atratus* is also shown. (Note: the video is 29 Mb in size.)

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CONSIDERAÇÕES FINAIS

CONSIDERAÇÕES FINAIS

Este trabalho apresentou a primeira compilação taxonômica, nomenclatural, filogenética e de biologia reprodutiva sobre orquídeas terrestres das subtribos Chloraeinae e Spiranthinae, para esta última, especialmente sobre o clado *Pelexia*. Foram analisadas aproximadamente 2.000 exsicatas, coletadas cerca de 150 representantes de Cranichideae e processadas 732 sequências de DNA.

A partir de um detalhado estudo das espécies descritas pelos botânicos João Barbosa Rodrigues e do Frei José Mariano da Conceição Vellozo, foi possível entender o controverso histórico dos trabalhos deste autores. Além disso, uma parte importante dessa história foi a descoberta de mais uma exsicata de Barbosa Rodrigues, depositada no herbário de Estocolmo (S). Assim, além das propostas de novas tipificações, novas sinonímias, nomes excluídos e *nomem nudum* para as espécies de Goodyerinae e Spiranthinae descritas por estes dois autores, é reconhecido também o holótipo de *Pelexia hypnophila*. Infelizmente, até o momento, a localização da coleção de José Vellozo é desconhecida, o que torna difícil a interpretação das suas espécies.

Todos os trabalhos de campo e consultas realizadas em herbários e bibliografias permitiram trazer ao conhecimento a presença de táxons ainda não registrados para o Brasil (*Bipinnula giberti*) e para o Rio Grande do Sul (*Pteroglossa* e *Lyroglossa*). No entanto, são necessários mais esforços para a busca de táxons ainda não registrados e estudos morfológicos mais detalhados. Além disso, estes estudos detalhados permitiram desvendar questões nomenclaturais, como proposto no Capítulo 3. Por outro lado, muitos problemas taxonômicos/nomenclaturais que tem sido detectados, também estão em conflito com o Código Internacional de Nomenclatura Botânica. Desta forma, novas propostas deverão ser feitas com o objetivo de seguir as normas que regem a nomenclatura botânica.

Este conjunto de trabalhos, é a primeira amostragem significativa de espécies para estudos moleculares que incluiu todos os gêneros reconhecidos para o clado *Pelexia*, bem como espécies extra-Andinas de *Bipinnula*. Enquanto o clado *Pelexia* é fortemente suportado, a delimitação genérica ainda está incerta, no qual, alguns gêneros não são recuperados como monofiléticos e outros deverão ser sinonimizados e reestabelecidos. Além disso, a morfologia floral tem demonstrado diferentes estratégias de polinização para os gêneros do clado *Pelexia*, que pode ocorrer por abelhas, lepidópteros ou beija-flores. Apesar de compartilharem características como a presença de protandria e viscídio ventralmente adesivo, as espécies de *Sauroglossum* e *Brachystele* são polinizadas de diferentes formas (lepidópteros e abelhas, respectivamente). Isso demonstra que alguns gêneros podem ser suportados não apenas por caracteres da coluna, e sim por um conjunto de caracteres associados com estruturas vegetativas e florais. Por outro lado, as análises filogenéticas de Chloraeinae tem suportado *Bipinnula* e *Gavilea* como monofiléticos, enquanto as amostras de *Chloraea* formam um grado que, no qual, não pode ser resolvido até que uma nova amostragem de táxons e sequências plastidiais seja realizada.

Assim, todos os capítulos que formam esta tese, além dos trabalhos apresentados em congressos e capítulos de livro (ver Anexos) trouxeram uma nova perspectiva para o entendimento das relações filogenéticas, estratégias de polinização, bem como a delimitação genérica das orquídeas que compreendem as subtribos Chloraeinae e Spiranthinae.

ANEXOS

ANEXO I

composed by trees bearing bipinnate leaves and monothecate anthers. This section includes two clades, one with exclusive South American distribution, and the other distributed from northern Amazonia to Central America. Most species within section Dilobos are shrubs bearing pinnate leaves and anthers with two thecae. This section is distributed in central and eastern South America. Morphological features of *Digomphia* species (simple or pinnate leaves, dithecate anthers) are herein interpreted as probable reversals within section *Jacaranda*. This study was conducted at the laboratory of Molecular Evolution, Genetic Department, Universidade Federal do Rio Grande do Sul. Funding was provided by CNPq (processo 0151978/208 5).

P0975 – ePoster

The Brazilian Chloraeeae orchids revisited

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As a whole, orchid tribe Chloraeeae embraces four genera (*Bipinnula*, *Chloraea*, *Gavilea* and *Geoblasta*) which mainly dwell along the Andes, from Tierra del Fuego (Argentina) to Peru. Remarkably, eight species in this tribe are found well beyond the Andes, at the grassy Pampa Biome (which comprises Southern Brazil, Uruguay and part of Eastern Argentina). Six of these species, in turn, are found in Southern Brazil (states of Rio Grande do Sul, Santa Catarina and Paraná). As a rule, these taxa are seldom collected and, therefore, poorly represented at local herbaria. Literature (either local or international) regarding these species is scant and often misleading. Having all this in mind, we started a research line aimed to elucidate nomenclatural and taxonomical issues within Brazilian Chloraeeae orchids. In addition, we are documenting biological aspects of these plants (vegetative features, floral morphology, pollination, etc.) which were unknown or poorly known to date. Starting in late 2005, populations have been located during several field trips. Brazilian Chloraeeae orchids are protected by local laws and, therefore, no complete individuals were pressed as vouchers. Only some inflorescences and leaves were pressed. In addition, some leaves were silica-dried for future phylogenetic studies. Floral and vegetative features were recorded in the field, with the help of a digital camera Sony H7. Pollination studies are being made in the natural populations, with the help of a digital camera and a dcr-sr45 Sony video camera as well. In Brazil, there are six Chloraeeae orchid species: *Bipinnula biplumata*, *B. montana*, *B. gibertii*, *B. ctenopetala*, *Chloraea membranacea* and *Geoblasta pennicillata*. All these species are quite consistent regarding floral features. With the exception of *G. pennicillata*, all other species present the labellum articulated at the base of the column. The anther is incumbent and holds a single pollinarium made up by four yellow, granular pollinia. The pollinarium is devoid of any pollinium stalks and the pollinia adhere onto the pollinator due to a rostellar secretion which is released when the rostellum gets involuntarily pressed by the pollinators. Floral features (color, indument, etc) in all species except *C. membranacea* are suggestive of pollination through sexual mimicry. Natural pollination is very good in *G. pennicillata* and *B. gibertii*. Pollination in *B. montana* seems moderated. Ongoing research on the pollination of *C. membranacea* points that this orchid is pollinated through a food-fraud system promoted by deceived halictid bees (M. Pedron, pers. comm.). Preliminary phylogenetic studies suggest that *Chloraea* is not monophyletic, with *Gavilea* merged within. However, the taxonomic sampling is yet quite incomplete. We hope that the inclusion of the non-Andean Chloraeeae spp. helps to improve the resolution of this orchid group in future phylogenetic analyses.

P0976 – ePoster

Phylogeny of the *Bouteloua curtipendula* complex diploids (Gramineae: Chloridoideae) based on nuclear ribosomal and chloroplast DNA sequences

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The *Bouteloua curtipendula* complex (BCC) is a group of 12 closely related species within the New World genus *Bouteloua*, a member of Poaceae subfamily Chloridoideae. The BCC is distributed in various ecological settings from southern Canada to Argentina, and its center of diversity is Mexico. The complex is not only highly variable morphologically, but also exhibits a wide range of chromosome numbers ($2n = 20$ to 103). Most species, however, are diploids with $2n = 20$ and only *Bouteloua curtipendula* sensu stricto ($2n = 20$ to 103), *B. warnockii* ($2n = 20$ to 40), and *B. purpurea* ($2n = 40$) are polyploids. Due to considerable morphological variation, putative hybridization, polyploidy and apomixis, circumscription of and relationships among taxa has been uncertain. In order to elucidate the evolution of the BCC, the main goals of this study were to to explore phylogenetic relationships among the BCC diploids, based on nuclear ribosomal and chloroplast sequences. The resulting phylogenetic pattern has been then used to explore the evolution of morphological traits and their usefulness to circumscribe species. Two non-coding DNA regions, ITS and *trn*T-L-F representing the nuclear and chloroplast genomes respectively, were sequenced and subjected to maximum parsimony analyses. Character state reconstruction analysis was carried out to examine the main morphological characters used in species circumscription. According to these molecular data, the BCC constitutes a monophyletic group. However, the monophyly of the majority of its taxa is not supported, except B. triaena which monophyly is strongly assessed. Although resolution within the BCC was limited with the *trn*T-L-F data set, the ITS sequences and the combination of the two nuclear and plastid data sets provided a fairly well-resolved phylogenetic pattern of relationships, with the main clades strongly supported. Present phylogenetic results suggest that the BCC has originally evolved into two main evolutionary lines, which arose from a common diploid ancestor with $2\ n = 20$ chromosomes, and most likely having yellow anthers and multiple spikelets per branch. Both lineages appear to have mainly diversified in Mexico, with subsequent

Singer RB, Buzatto CR. 2011. The Brazilian Chloraeeae orchids revisited. *In*: XVIII International Botanical Congress, Melbourne. *Abstract book* 18: 643-643.

ANEXO II

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CARACTERES FLORAIS, BIOLOGIA DA POLINIZAÇÃO E SISTEMA REPRODUTIVO DE *Chloraea membranacea* (ORCHIDACEAE: CHLORAEINAE)

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Introdução

A biologia da polinização de poucas espécies de Chloraeinae tem sido estudada, e muitos desses estudos tem focado no sistema reprodutivo e sucesso de frutificação [1, 2]. *Chloraeinae membranacea* Lindl. é uma das poucas espécies de distribuição não-Andina deste grupo.

O objetivo deste trabalho é elucidar a biologia da polinização, morfologia floral funcional e o sistema reprodutivo em populações nativas desta espécie na Argentina (Buenos Aires) e no Brasil (Rio Grande do Sul).

Metodologia

Os caracteres florais foram examinados utilizando microscopia óptica, eletrônica de varredura e de transmissão. O sistema reprodutivo foi estudado através de polinizações controladas, em campo e cultivadas e em casa de vegetação. As observações de polinização foram feitas em populações naturais e o comportamento dos polinizadores foi registrado através de fotografia e vídeo.

Resultados e Discussão

Ambos espécimens, da Argentina e do Brasil, foram muito consistentes sobre todos os caracteres estudados (Fig. 1). As flores não são nectaríferas e as análises anatômicas e de fragrância indicam que as projeções escuras e clavadas sobre a superfície adaxial do labelo são osmóforos (Fig. 1A-B and E-G).

As plantas são auto-compatíveis e dependentes de polinizadores. Os frutos obtidos pelos tratamentos de polinização manual e auto-polinização foram quase idênticos. O principal polinizador são machos e fêmeas de abelhas Halictidae que retiram o polinário quando saem das flores (Fig. 1E-G). Estas abelhas tendem a visitar mais que uma flor por inflorescência, promovendo assim auto-polinização (geitonogamia). O sucesso de frutificação nas plantas Brasileiras atingiram 60,78% em 2010 e 46% em 2011.

Algumas abelhas fêmeas que carregavam os polinários foram observadas transferindo o pólen para suas pernas traseiras (Fig 1G). O uso do pólen pelo polinizador é um registro raro para Orchidaceae.

Chloraea membranacea é polinizada por engodo. A auto-polinização, a textura do polinário, a abundância de polinizadores e o seu comportamento podem refletir o alto sucesso reprodutivo observado.

Conclusões

A partir do momento que uma filogenia mais robusta e representativa de *Chloraea* estiver disponível, será possível traçar estratégias de polinização já documentadas nas árvores filogenéticas e propor possíveis cenários para sua evolução. Para alcançar este objetivo, é necessário uma reavaliação completa dos caracteres florais envolvidos na polinização, no qual serão abordados em trabalhos futuros.

Figura 1. Caracteres florais e polinização de *Chloraea membranacea*. (A) Inflorescência racemose e multi-florada. (B) Flor com sépala e pétala lateral removidas para mostrar o labelo articulado com a coluna e projeções labelares (osmóforos). (C) Coluna. (D) Polinário. (E-G) Polinização por abelhas Halictidae. (E) Abelha antes da remoção do polinário. (F) Abelha retirando o polinário. O inseto tenta remover o polinário grudado em seu dorso. (G) Abelha carregando o polinário. Note que as pernas traseiras estão carregadas com pólen. Escalas: (A, B, E–G). 1 cm; (C, D). 5 mm.

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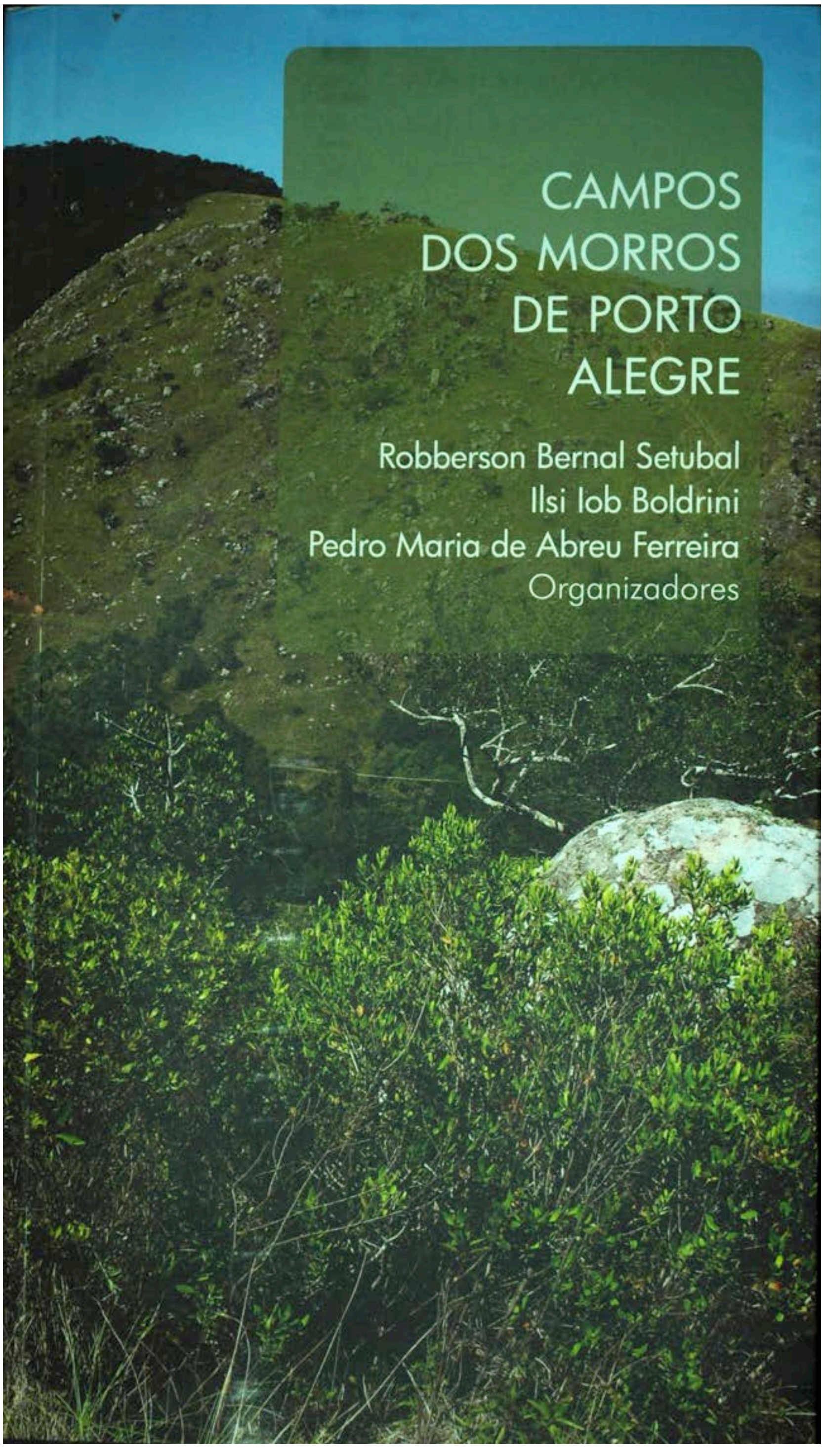
ANEXO III



CAMPOS
DOS MORROS
DE PORTO
ALEGRE

Robberson Bernal Setubal
Ilsi Iob Boldrini
Pedro Maria de Abreu Ferreira
Organizadores

Setubal RB, Boldrini II, Ferreira PMA, Trevisan R, Schneider AA, Grings M, Mello AS, Buzatto CR. 2011. Guia ilustrado. *In*: RB Setubal, II Boldrini, PMA Ferreira (Org.) *Campos dos morros de Porto Alegre*. 1ed. Porto Alegre, RS: Pallotti, p. 103-213.

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