
**PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(BIOLOGIA VEGETAL)**

**FILOGENIA E BIOGEOGRAFIA DE *Barbosella* Schltr. (ORCHIDACEAE,
PLEUROTHALLIDINAE), GÊNERO COM DISTRIBUIÇÃO DISJUNTA NO
NEOTRÓPICO**

MÔNICA BOLSON

Tese apresentada ao Instituto de Biociências do
Campus de Rio Claro, Universidade Estadual Paulista,
como parte dos requisitos para obtenção do título de
Doutora em Ciências Biológicas (Biologia Vegetal).

Julho – 2018

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Orientador: Prof. Dr. Eric de Camargo Smidt

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Filogenia e Biogeografia
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gênero com distribuição disjunta
no Neotrópico



Mônica Bolson

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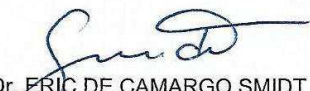
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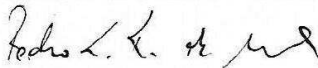
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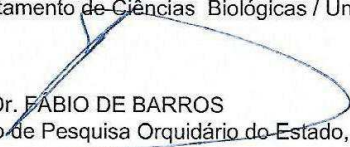


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RESUMO

Barbosella, um gênero pequeno dentro da subtribo Pleurothallidinae, compreende 20 espécies de orquídeas geralmente epífitas, de crescimento cespitoso ou reptante, inflorescência racemo unifloro, labelo com uma base côncava com um distinto sistema de articulação livre com o pé da coluna (*ball-and-socket*), e antera com quatro polínias. De ampla distribuição, desde a América Central através das florestas úmidas dos Andes, até o norte da Argentina e sul do Brasil na Floresta Atlântica, o gênero apresenta distribuição disjunta entre as florestas costeiras. As relações filogenéticas de *Barbosella* foram investigadas usando nrITS, cinco regiões plastidiais (*atpI-atpH*, *matK*, *psbD-trnT*^(GGU), *trnQ-5'-rps16* e *trnH-psbA*) e 43 caracteres morfológicos, analisadas por máxima verossimilhança, inferência bayesiana e máxima parcimônia. Também foram realizadas reconstruções de caracteres morfológicos, datação molecular e análises biogeográficas. O gênero é fortemente suportado como monofilético, com provável origem no Mioceno Médio-Superior (~ 11 Ma) onde a Floresta Atlântica e a Floresta Andina estão distribuídas e hoje são divididas por uma imensa área seca. A primeira diversificação de *Barbosella* ocorreu no Mioceno Superior (~ 8 Ma) envolvendo um evento de vicariância e, posteriormente, múltiplas dispersões em ambas as áreas, onde hoje as espécies são restritas. Com o intuito de investigar como a morfologia interna e externa dos órgãos vegetativos evoluiu entre as diferentes linhagens e habitats ocupados pelo gênero, a anatomia da raiz, rizoma, ramicaule e folha de representantes de *Barbosella* foram descritas. Durante a elaboração da monografia das espécies brasileiras de *Barbosella*, um nome negligenciado desde a sua publicação (*B. perinii*) é indicado como sinônimo de *B. macaheensis*. Visto que quase a metade do gênero ocorre no Brasil, um tratamento taxonômico é apresentado para as nove espécies brasileiras, incluindo descrições morfológicas, ilustrações, fotografias, chave de identificação, status de conservação e distribuição geográfica. Novos registros são descritos, dois lectótipos e um epítipo são designados. Por último, um guia de campo é apresentado com as espécies do Brasil.

Palavras-chave: análise filogenética, anatomia vegetativa, evolução de caracteres morfológicos, flora, monocotiledôneas, nomenclatura, padrões biogeográficos

ABSTRACT

Barbosella orchids, a small genus in the subtribe Pleurothallidinae with 20 species, are usually epiphytes, with long-repent or caespitose growth; inflorescence always a single-flowered raceme; and lip with basal cavity articulated to the column foot (*ball-and-socket*), and anther with four pollinias. It is distributed from Central America through the Andes rainforest to southeastern of Brazil in Atlantic Forest, the genus presents a disjunct distributed between coastal forests. Phylogenetic relationships of *Barbosella* species were investigated using nrITS, five plastid regions (*atpI-atpH*, *matK*, *psbD-trnT*^(GGU), *trnQ-5'-rps16* e *trnH-psbA*) and 43 morphological characters, analyzed using maximum likelihood, Bayesian inference and maximum parsimony to infer evolutionary relationships. Morphological characters reconstruction, molecular dating and biogeographical analyses were also performed. The genus is strongly supported as monophyletic, with probable origin through the Middle-Late Miocene (~ 11 Ma), where the Atlantic Forest and Andean Forest are nowadays distributed and separated by a dry area. The early diversification within the genus occurred in the Late Miocene (~ 8 Ma) involving a vicariance event and subsequently multiple dispersals in both areas, where the species are restricted nowadays. In order to investigate both internal and external morphology of the vegetative organs have evolved among different lineages and habitats occupied by the genus, the anatomy of the root, rhizome, ramicaul and leaf of *Barbosella* representatives were described, as well as morphological character evolution were analyzed. During a study to monograph the *Barbosella* species from Brazil, a name largely overlooked since its publication (*B. perinii*) is indicated here as a synonym of *B. macaheensis*. Since almost half of the genus occurs in Brazil, a taxonomic review of the nine Brazilian species including morphological descriptions, taxonomic discussions, illustrations, identification key, updated synonymy and distribution maps as well their conservation status are provided for each taxon. New records are described; two lectotypes and an epitype are designated. Lastly, a field guide with the Brazilian species is presented.

Key words: biogeography patterns, flora, monocots, morphological characters evolution, nomenclature, phylogenetic analysis, vegetative anatomy

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INTRODUÇÃO GERAL

Os mais recentes tratamentos taxonômicos de Orchidaceae são o de Pridgeon et al. (1999, 2001a, 2003, 2005, 2009, 2014), pela série *Genera Orchidacearum* (GO). Publicada em seis volumes, a série tem como intuito apresentar uma classificação mais robusta das orquídeas, em relação aos sistemas publicados anteriormente com a família (SCHLECHTER, 1926; GARAY, 1960; DRESSLER; DODSON, 1960; DRESSLER, 1979, 1981, 1986, 1993). Além do tratamento taxonômico, Pridgeon e colaboradores (1999, 2001a, 2003, 2005, 2009, 2014) propuseram uma verdadeira classificação filogenética para Orchidaceae, através da inclusão de dados moleculares.

Atualmente, a família é composta por 736 gêneros reconhecidos (CHASE et al., 2015), uma das maiores e mais diversas famílias vegetais, e está distribuída em quase todo o mundo, com exceção das regiões desérticas e polares (DRESSLER, 1993). Apresenta-se em maior abundância e diversidade nas regiões tropicais e subtropicais. No Neotrópico, a região dos Andes é a área mais rica em orquídeas, seguida da Floresta Atlântica brasileira. No Brasil, as orquídeas descritas até recentemente perfazem aproximadamente 2500 espécies distribuídas em 221 gêneros (FLORA DO BRASIL 2020, em construção).

Poucas famílias de plantas possuem tanta diversidade vegetativa quanto as orquídeas. A estrutura da flor é bastante uniforme em número e disposição de peças, mas há uma grande diversidade de tamanho e detalhes estruturais (DRESSLER, 1993). O monofiletismo da família é sustentado por numerosos estudos, baseado tanto em dados morfológicos quanto moleculares (DRESSLER, 1981, 1993; CAMERON et al., 1999; FREUDENSTEIN; RASMUSSEN, 1999; PRIDGEON et al., 1999; FREUDENSTEIN et al., 2000, 2004).

A família atualmente é dividida em cinco subfamílias: Apostasioideae Garay, Vanilloideae Szlach., Cypripedioideae Garay, Orchidoideae Lindl., e Epidendroideae Lindl., esta última constitui a maior subfamília do grupo com ampla distribuição geográfica (PRIDGEON et al., 2005). Epidendroideae está dividida em 16 tribos, dentre as quais a tribo Epidendreae está dividida em seis subtribos: Bletiinae Benth, Chysinae Schlechter, Coeliinae Dressler, Laeliinae Benth, Poneriinae Pfitzer e Pleurothallidinae Lindley (PRIDGEON et al., 2005; CHASE et al., 2015).

A subtribo Pleurothallidinae conta com aproximadamente 5100 espécies distribuídas em 44 gêneros (KARREMANS, 2016), sendo responsável por cerca de 20% das espécies de toda a família Orchidaceae (HIGGINS; WILLIAMS, 2009; KARREMANS, 2016). De distribuição exclusiva no Neotrópico, a maioria das espécies de Pleurothallidinae é composta por ervas de crescimento simpodial, unifolioladas com o caule secundário (ramicaule) não pseudobulboso, e a inflorescência apresenta uma articulação associada a uma zona de abscisão entre o ovário e o pedicelo (LUER, 1986; DRESSLER, 1993; PRIDGEON, 2005a).

Suas flores possuem características de miofilia, como por exemplo, o tamanho reduzido e a forma radial, e ainda o labelo que pode conter manchas, pequenas projeções, fendas e lóbulos fusionados (van der PIJL; DODSON, 1966; LUER, 1986; NEYLAND et al., 1995; PRIDGEON et al., 2005; KARREMANS et al., 2015). Muitas espécies de Pleurothallidinae apresentam estruturas com função de atração de polinizadores através de secreção de fragrâncias, que podem ser encontradas no labelo, pétalas e/ ou sépalas (PRIDGEON; STERN, 1983; DRESSLER, 1993; PRIDGEON; STERN, 1985; SCHIESTL et al., 2003; SCHIESTL; JOHNSON, 2013; CARDOSO-GUSTAVSON et al., 2017). Nectários também já foram identificados dentro da subtribo como atrativos para os polinizadores oferecendo recompensa (BARBOSA et al., 2009; MELO et al., 2010).

Barbosella Schltr., um dos gêneros reconhecidos em Pleurothallidinae, foi estabelecido em 1918 por Rudolf Schlechter em homenagem ao botânico pioneiro nos estudos de Arecaceae e Orchidaceae brasileiras, João Barbosa Rodrigues (SCHLECHTER, 1918). O gênero foi baseado em *Pleurothallis gardneri* Lindl. e *P. miersii* Lindl., duas espécies brasileiras, muito pequenas e similares entre si e que podem coexistir na natureza (LINDLEY, 1842; LUER, 2000). No entanto, Schlechter (1918) falhou em não designar o material tipo para o gênero, o que mais tarde levou Angely (1972) a estabelecer *Barbosella miersii* (Lindl.) Schltr. como lectótipo.

Atualmente, *Barbosella* compreende 20 espécies (TABELA 1) com ampla distribuição neotropical, ocorrendo nas florestas tropicais e subtropicais de altitudes variadas e umidade relativa do ar elevada. As espécies estão distribuídas desde a América Central através dos Andes e com uma espécie nas Antilhas, até o norte da Argentina e sul do Brasil, país onde cerca de 50% das espécies estão presentes, principalmente na Floresta Atlântica (LUER, 2000; PRIDGEON, 2005b; FLORA DO BRASIL 2020, em construção).

TABELA 1. Listagem e os países de ocorrência das espécies atualmente aceitas do gênero *Barbosella*.

Espécies	Ocorrência
<i>Barbosella australis</i> (Cogn.) Schltr.	Brasil
<i>B. circinata</i> Luer	Panamá
<i>B. cogniauxiana</i> (Speg. & Kraenzl.) Schltr.	Brasil e Argentina
<i>B. crassifolia</i> (Edwall) Schltr.	Brasil
<i>B. cucullata</i> (Lindley) Schltr.	Venezuela, Colômbia, Bolívia, Equador e Peru
<i>B. dolichorhiza</i> Schltr.	Costa Rica, Panamá, Colômbia, Equador e Peru
<i>B. dusenii</i> (A. Samp.) Schltr.	Brasil
<i>B. fuscata</i> Garay	Colômbia e Equador
<i>B. gardneri</i> (Lindl.) Schltr.	Brasil
<i>B. geminata</i> Luer	Costa Rica
<i>B. macaheensis</i> (Cogn.) Luer	Brasil
<i>B. miersii</i> (Lindl.) Schltr.	Brasil
<i>B. orbicularis</i> Luer	Panamá, Venezuela e Equador
<i>B. portillae</i> Luer	Equador
<i>B. prorepens</i> (Rchb.f.) Schltr.	República Dominicana, Guatemala, Costa Rica, Nicarágua, Panamá, Venezuela, Colômbia, Equador, Peru e Bolívia
<i>B. ricii</i> Luer & R.Vásquez	Bolívia
<i>B. schista</i> Luer & R.Escobar	Colômbia
<i>B. spiritu-sanctensis</i> (Pabst) F.Barros & Toscano	Brasil
<i>B. trilobata</i> Pabst	Brasil
<i>B. vasquezii</i> Luer	Bolívia

O gênero é composto por plantas geralmente epífitas, com crescimento cespitoso ou reptante, capazes de formar grandes e densos tapetes, característica esta presente em todas as espécies brasileiras. O ramicaule é reduzido em relação às folhas e não possui ânulo. As folhas são coriáceas, elípticas a suborbiculares ou semicilíndricas a cilíndricas, agudas a obtusas. É um dos únicos gêneros dentre a subtribo que apresenta caracteres vegetativos como o tamanho e forma das folhas, determinantes na identificação e reconhecimento de algumas espécies (LUER, 2000). A inflorescência é um racemo unifloro, com o pedúnculo mais longo que as folhas, delicado, que emerge lateralmente a partir do ramicaule. As flores são ressupinadas pela torção do ápice do pedúnculo (com exceção de *B. circinata*); as sépalas são similares, a dorsal livre, ereta ou patente, as laterais geralmente conadas formando sinsepálo (com exceção de *B. portillae*). Labelo inteiro a trilobado, disco com

sulco, lobos agudos a obtusos, glabros, base côncava com um distinto sistema de articulação livre com o pé da coluna, denominado *ball-and-socket*. Coluna com pé desenvolvido, geralmente bulboso, e ápice fimbriado. Antera incumbente, com quatro polínias de tamanhos iguais, clavadas, de ornamentação gemada, e caudículo presente (LUER, 2000; STENZEL, 2000; PRIDGEON, 2005b) (Figura 1).

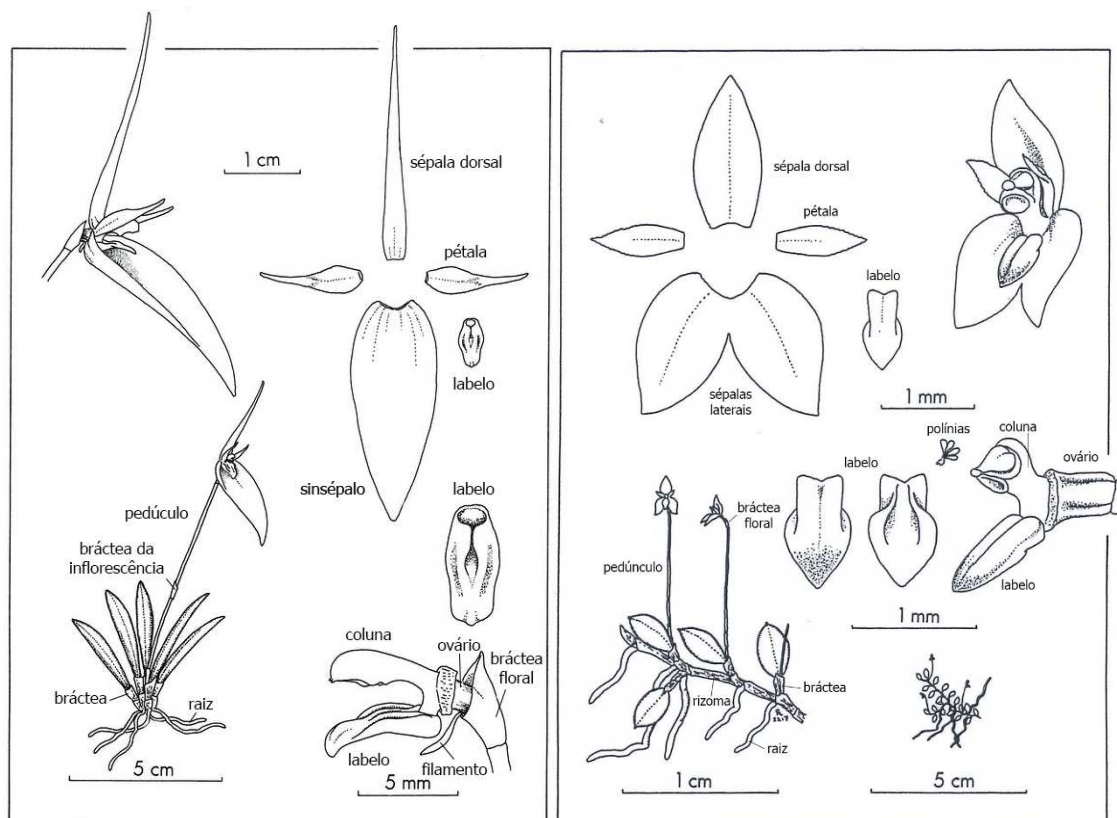


Figura 1 - Aspecto morfológico geral de *Barbosella cucullata* (esquerda) e *Barbosella miersii* (direita).

(Fonte: Adaptado de LUER, 2000).

Luer (1981) alocou *Barbosella miersii* em um gênero monoespecífico, denominado *Barbrodria* Luer devido a morfologia do labelo e da coluna ser distintas do que caracteriza o gênero *Barbosella*. A primeira revisão para o gênero foi realizada por Luer (2000), em um de seus volumes do *Icones Pleurothallidarum*, reconhecendo 18 espécies apenas. No entanto, a primeira filogenia molecular de Pleurothallidinae realizada por Pridgeon et al. (2001b) indicou *Barbrodria* altamente suportada dentro de *Barbosella*, e a partir daí o gênero *Barbrodria* passa a ser tratado como sinônimo. Dois anos após a revisão, Luer (2002) publica uma nova espécie, *B. portillae* Luer, encontrada apenas no Equador.

Com o desenvolvimento de técnicas moleculares, ferramentas estatísticas e a consolidação da sistemática filogenética como um método para estabelecer relações de parentesco entre organismos, os estudos em sistemática têm apresentado cada vez mais a abordagem filogenética e evolutiva na interpretação dos padrões morfológicos, de distribuição geográfica e delimitações taxonômicas. Filogenias moleculares permitem ainda inferir a ocorrência de processos biológicos como o surgimento de linhagens, a evolução de caracteres morfológicos e padrões ecológicos, incluindo processos de diversificação, tanto em ampla escala geográfica e níveis hierárquicos superiores, como em escala local e ao nível populacional (AVISE, 2000; FELSENSTEIN, 2004).

Pridgeon et al. (2001b) com o intuito de verificar o monofiletismo e a relação evolutiva dos gêneros da subtribo Pleurothallidinae, abrangeram 185 táxons na primeira filogenia realizada para o grupo. Os espaçadores internos transcritos ITS1 e ITS2 e o gene 5.8S do DNA ribossômico nuclear, adicionados a sequências do DNA plastial, como o gene *matK*, o intron *trnL* e o espaçador intergênico *trnL-F* foram utilizados nessa filogenia. Vários dos resultados corroboram as classificações baseadas na morfologia citadas por Luer (1986). Baseados ainda nessa primeira filogenia molecular, Pridgeon e Chase (2001) publicaram uma reclassificação para Pleurothallidinae, e através de cladogramas bem suportados, forneceram várias retribuições nomenclaturais, entre as quais a sinonimização do gênero *Barbrodria* em *Barbosella*.

Uma recente atualização sobre as relações filogenéticas de Pleurothallidinae foi a realizada por Karremans (2016), através de um compilado de estudos publicados baseados em filogenia molecular. A Figura 2 apresenta a atual visão geral das principais afinidades dentro da subtribo, com oito afinidades e seus respectivos gêneros. O posicionamento de alguns gêneros e espécies dentro da subtribo ainda é questionável, demonstrando a necessidade de maiores estudos, tanto moleculares quanto taxonômicos. O gênero *Barbosella* encontra-se posicionado na Afinidade *Restrepia* (Re), grupo que inclui ainda os gêneros *Chamelophyton* Garay, *Dresslerella* Luer, *Echinosepala* Pridgeon & M.W.Chase, *Myoxanthus* Poepp. & Endl., *Pleurothallopsis* Porto & Brade, *Restrepia* Kunth and *Restrepiella* Garay & Dunst.

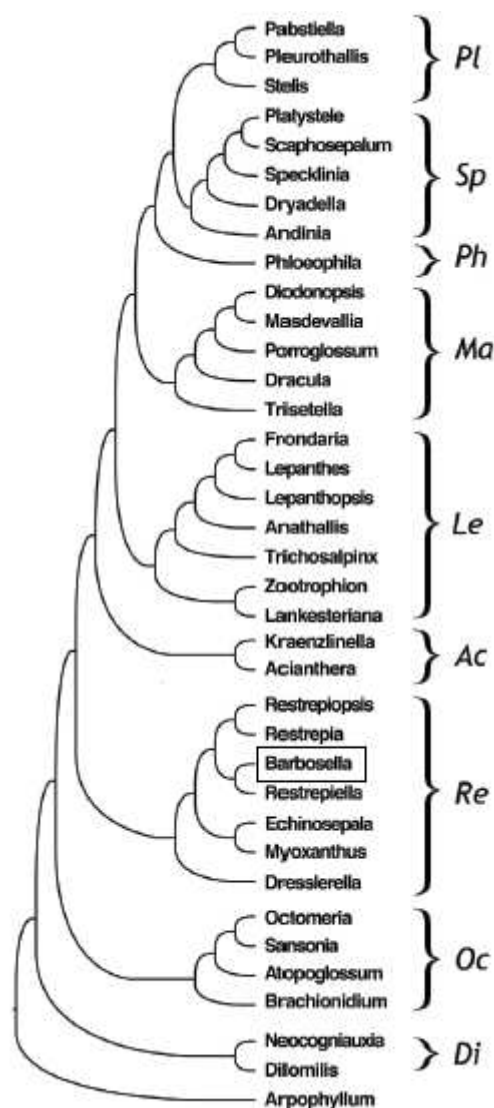


Figura 2 - Visão geral das principais afinidades em Pleurothallidinae.

(Fonte: Adaptado de KARREMANS, 2016).

Aliados às análises filogenéticas, nas últimas décadas, os métodos biogeográficos têm permitido inferir padrões de diversificação das linhagens de plantas atuais e como esses padrões se originaram (MORRONE, 2006; PENNINGTON et al., 2006). Esta diversificação provavelmente está associada a processos ecológicos (ANTONELLI; SAN MARTÍN, 2011) e eventos geológicos complexos que agiram em conjunto ao longo do tempo (HOORN et al., 2010; 2011).

A região neotropical é conhecida por sua notável biodiversidade, abrangendo os trópicos do Novo Mundo, que compreende a América do Sul, a América Central, o sul do México e o sul da Flórida (MORRONE, 2006). No entanto, a reconstrução da história

biogeográfica do Neotrópico não é uma tarefa fácil, por se tratar de uma região que não é única e nem homogênea, já que compartilha semelhanças com a flora e fauna da Nova Zelândia e sudeste da Austrália, e ainda com a porção tropical da África, Índia e Oceania (MORRONE, 2013).

Dessa forma, os estudos capazes de abordar informações filogenéticas e de datação molecular de diferentes grupos neotropicais podem auxiliar na compreensão de eventos que influenciaram a diversificação dos táxons no espaço e no tempo. Com a utilização de uma amostragem robusta, tanto em representatividade de táxons quanto em marcadores moleculares, e da utilização de registros fósseis (RAMÍREZ et al., 2007; CONRAN et al., 2009), Gustafsson et al. (2010) realizaram a datação molecular para Orchidaceae, indicando que a família pode ter se diversificado antes do que se pensava, há cerca de 80 Ma (milhões de anos) em um período de resfriamento global, no Cretáceo Superior. No entanto, as estimativas de idade de Chomicki et al. (2015) indicam que Orchidaceae compartilhou o ancestral comum mais recente (MRCA) por volta de 94 Ma. Já GIVNISH et al. (2015) apontam que a família divergiu de seu MRCA há cerca de 112 Ma e que as linhagens de orquídeas viventes divergiram uma da outra aproximadamente há 90 Ma.

Trabalhos recentes têm abordado os processos de diversificação dentro de alguns grupos da família (CHOMICKI et al., 2015; GIVNISH et al., 2015; PÉREZ-ESCOBAR et al., 2017). Em Pleurothallidinae esses estudos demonstraram que a subtribo tem uma das maiores taxas de diversificação de espécies dentro Orchidaceae, e que se originou no Mioceno Inferior (~ 20 Ma) (GIVNISH et al., 2015; PÉREZ-ESCOBAR et al., 2017).

Mesmo com poucos registros fósseis de orquídeas, sendo que nenhum deles está relacionado a Pleurothallidinae, existe a possibilidade de datar os eventos de cladogênese que originaram os gêneros da subtribo e também suas respectivas linhagens infragenéricas. Tendo em mãos as idades estimadas para tais cladogêneses é possível inferir sobre a dinâmica evolutiva de todo um grupo, como também definir possíveis rotas de migração e hipotetizar eventos que propiciaram a diversificação de linhagens (FOREST, 2009).

Apesar de Luer (2000) apresentar uma revisão para as espécies de *Barbosella*, até o momento, o gênero nunca foi estudado sob o ponto de vista da sistemática filogenética. Recentemente vários estudos filogenéticos abordaram Pleurothallidinae (PRIDGEON et al., 2001b; PRIDGEON; CHASE, 2001; PRIDGEON; CHASE, 2003; KARREMANS, 2016; PÉREZ-ESCOBAR et al., 2017) e, apesar do seu monofiletismo, ainda faltam estudos para entender a relação de todas as linhagens dentro dessa enorme subtribo.

A primeira filogenia molecular de Pleurothallidinae (PRIDGEON et al., 2001b) incluiu quatro espécies de *Barbosella*. Posteriormente, o gênero foi incluído com seis espécies, em uma análise filogenética de *Pleurothallis sensu lato* inferida a partir de sequências de ITS (nrDNA) (CHIRON et al., 2012), onde ficou evidenciado a colocação de *Barbosella* em um dos oito grupos reconhecidos em Pleurothallidinae.

Barbosella, por se tratar de um gênero com distribuição disjunta no neotrópico, a caracterização dos processos biogeográficos pode contribuir para o entendimento dos centros de riqueza e endemismo para Orchidaceae na região Neotropical. Sendo assim, este trabalho tem como objetivo geral investigar as relações filogenéticas e identificar os padrões biogeográficos do gênero baseado em caracteres morfológicos e moleculares e o estudo da distribuição dos táxons. Mais especificamente (i) testar o monofiletismo de *Barbosella*, utilizando o maior número de espécies possíveis, empregando análises baseadas em dados morfológicos e moleculares; (ii) dada a distribuição disjunta do gênero entre a região andina e a Floresta Atlântica, caracterizar os padrões de distribuição geográfica de *Barbosella*; (iii) investigar caracteres morfológicos e anatômicos vegetativos e sua importância para a sistemática do gênero; (iv) realizar um levantamento florístico e tratamento taxonômico das espécies do gênero para o Brasil; e (v) confeccionar um guia de campo (Field Museum) para as espécies brasileiras.

Dessa forma, esta tese tem os capítulos propostos da seguinte forma:

CAPÍTULO I: Relações filogenéticas e padrões biogeográficos de *Barbosella* (Orchidaceae, Pleurothallidinae), gênero com distribuição disjunta no Neotrópico;

CAPÍTULO II: Anatomia comparada dos órgãos vegetativos de *Barbosella* (Orchidaceae, Pleurothallidinae);

CAPÍTULO III: Um nome negligenciado e um novo sinônimo em *Barbosella* (Pleurothallidinae, Orchidaceae);

CAPÍTULO IV: O gênero *Barbosella* (Orchidaceae, Pleurothallidinae) no Brasil;

CAPÍTULO V: FieldGuide: Orchidaceae: Pleurothallidinae, *Barbosella* Schltr. do Brasil.

REFERÊNCIAS

- ANGELY, J. **Flora Analítica e Fitogeográfica do Estado de São Paulo**, vol. 6. Phytos, São Paulo, 336 pp., 1972.
- ANTONELLI, A.; SAN MARTÍN, I. Why are there so many plant species in the Neotropics? **Taxon**, v. 60, n. 2, p. 403–414, 2011.
- AVISE, J.C. **Phylogeography: the history and formation of species**. Cambridge: Harvard University Press, 447 pp., 2000.
- BARBOSA, A.R.; MELO, M.C.; BORBA, E.L. Self-incompatibility and myophily in *Octomeria* (Orchidaceae, Pleurothallidinae) species. **Plant Systematics and Evolution**, v. 283, n. 1, p. 1–8, 2009.
- CAMERON, K.M.; CHASE, M.W.; WHITTEN, W.M.; KORES, P.J.; JARELL, D.C.; ALBERT, V.A.; YUKAWA, T.; HILLS, H.G.; GOLDMAN, D.H. A phylogenetic analysis of the Orchidaceae: Evidence from *rbcL* nucleotide sequences. **American Journal of Botany**, v. 86, p. 208–224, 1999.
- CARDOSO-GUSTAVSON, P.; SOUZA, S.R.; BARROS, F. Floral volatile profile in Pleurothallidinae, an orchid subtribe pollinated by flies: ecological and phylogenetic considerations. **Phytochemistry Letters**, v. 22, p. 49–55, 2017.
- CHASE, M.W.; CAMERON, K.M.; FREUDENSTEIN, J.V.; PRIDGEON, A.M.; SALAZAR, G.; van den BERG, C.; SCHUITMAN, A. An updated classification of Orchidaceae. **Botanical Journal of the Linnean Society**, v. 177, p. 151–174, 2015.
- CHIRON, G.R.; GUIARD, J.; van den BERG, C. Phylogenetic relationships in Brazilian *Pleurothallis sensu lato* (Pleurothallidinae, Orchidaceae): evidence from nuclear ITS rDNA sequences. **Phytotaxa**, v. 46, p. 34–58, 2012.
- CHOMICKI, G.; BIDEL, L.P.R.; MING, F.; COIRO, M.; ZHANG, X.; WANG, Y.; BAISSAC, Y.; JAY-ALLEMAND, C.; RENNER, S.S. The velamen protects photosynthetic orchid roots against UV-B damage, and a large dated phylogeny implies multiple gains and losses of this function during the Cenozoic. **New Phytologist**, v. 205, p. 1330–1341, 2015.
- CONRAN, J.G.; BANNISTER, J.M.; LEE, D.E. Earliest orchid macrofossils: early Miocene *Dendrobium* and *Earina* (Orchidaceae: Epidendroideae) from New Zealand. **American Journal of Botany**, v. 96, p. 466–474, 2009.
- DRESSLER, R.L. The subfamilies of Orchidaceae. **Selbyana**, v. 5, n. 2, p. 197–206, 1979.
- DRESSLER, R.L. **The orchids: natural history and classification**. Cambridge: Harvard University Press., 332 pp., 1981.

- DRESSLER, R.L. **Phylogeny and classification of the orchid family**. Portland: Dioscorides Press., 312 pp., 1993.
- DRESSLER, R.L. Recent advances in orchid phylogeny. **Lindleyana**, v. 1, p. 520, 1986.
- DRESSLER, R.L.; DODSON, C.H. Classification and phylogeny in the Orchidaceae. **Annals of the Missouri Botanical Garden**, v. 47, p. 25–68, 1960.
- FELSENSTEIN, J. **Inferring phylogenies**. Sunderland: Sinauer, 664 pp., 2004.
- FLORA DO BRASIL 2020, em construção. Jardim Botânico do Rio de Janeiro. Disponível em: < <http://floradobrasil.jbrj.gov.br/> >. Acesso em: 19 Jun 2018.
- FOREST, F. Calibrating the Tree of Life: fossils, molecules, and evolutionary timescales. **Annals of Botany**, v. 104, p.789–794, 2009.
- FREUDENSTEIN, J.V.; RASMUSSEN, F.N. What does morphology tell us about orchid relationships? A cladistic analysis. **American Journal of Botany**, v. 86, p. 225–248, 1999.
- FREUDENSTEIN, J.V.; SENYO, D.M.; CHASE, M.W. Mitochondrial DNA and relationships in the Orchidaceae. *In*: WILSON, K.L.; MORRISON, D.A. **Monocots II: Systematics and Evolution**. Melbourne: CSIRO, p. 421–429, 2000.
- FREUDENSTEIN, J.V.; van den BERG, C.; GOLDMAN, D.H.; KORES, P.J.; MOLVRAY, M.; CHASE, M.W. An expanded plastid DNA phylogeny of Orchidaceae and analysis of jackknife branch support strategy. **American Journal of Botany**, v.91, p.149–157, 2004.
- GARAY, L. On the origin of the Orchidaceae. **Botanical Museum Leaflets**, v. 19, p. 57–88, 1960.
- GIVNISH, T.J.; SPALINK, D.; AMES, M.; LYON, S.P.; HUNTER, S.J.; ZULUAGA, A.; ILES, W.J.D, CLEMENTS, M.A.; ARROYO, M.T.K.; LEEBENS-MACK, J.; ENDARA, L.; KRIEBEL, R.; NEUBIG, K.M.; WHITTEN, W.M.; WILLIAMS, N.H.; CAMERON, K.M. Orchid phylogenomics and multiple drivers of their extraordinary diversification. **Proceedings of the Royal Society B: Biological Sciences**, v. 282, p. 1–10, 2015.
- GUSTAFSSON, A.L.S.; VEROLA, C.F.; ANTONELLI, A. Reassessing the temporal evolution of orchids with new fossils and a Bayesian relaxed clock, with implications for the diversification of the rare South American genus *Hoffmannseggella* (Orchidaceae: Epidendroideae). **BMC Evolutionary Biology**, v. 10, n. 177, p. 13, 2010.
- HIGGINS, W.E.; WILLIAMS, N.H. Pleurothallidinae: how many genera? *In*: SAULEDA, R.P.; SANDOW, L.A. (Eds.) **Proceedings of the 19th World Orchid Conference**. American Printing Arts, Miami, USA, p. 425–430, 2009.
- HOORN, C.; WESSELINGH, F.P.; TER STEEGE, H.; BERMUDEZ, M.A.; MORA A.; SEVINK, J.; SANMARTÍN, I.; SANCHEZ-MESEGUER, A.; ANDERSON, C.L.; FIGUEIREDO, J.P.; JARAMILLO, C.; RIFF, D.; NEGRI, F.R.; HOOGHMESTRA, H.;

LUNDBERG, J.; STADLER, T.; SÄRKINEN, T.; ANTONELLI, A. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. **Science**, v. 330, p. 927–931, 2010.

HOORN, C.; WESSELINGH, F.P.; TER STEEGE, H.; BERMUDEZ, M.A.; MORA, A.; SEVINK, J.; SANMARTÍN, I.; SANCHEZ-MESEGUER, A.; ANDERSON, C.L.; FIGUEIREDO, J.P.; JARAMILLO, C.; RIFF, D.; NEGRI, F.R.; HOOGHIEMSTRA, H.; LUNDBERG, J.; STADLER, T.; SÄRKINEN, T.; ANTONELLI, A. Origins of biodiversity. **Science**, v. 331, p. 398–400, 2011.

KARREMANS A.P. Genera Pleurothallidinarum: an updated phylogenetic overview of Pleurothallidinae. **Lankesteriana**, v. 16, n. 2, p. 219–242, 2016.

KARREMANS A.P.; PUPULIN, F.; GRIMALDI, D.; BEENTJES, K. K.; BUTO, R.; FAZZI, G. E.; KASPERS, K.; KRUIZINGA, J.; ROESSINGH, P.; SMETS, E. F.; GRAVENDEE, B. Pollination of *Specklinia* by nectar-feeding *Drosophila*: the first reported case of a deceptive syndrome employing aggregation pheromones in Orchidaceae. **Annals of Botany**, p. 1–19, 2015.

LINDLEY, J. *Pleurothallis gardneri*. *Pleurothallis miersii*. **Edwards's Botanical Register** 28: Misc. p. 83–84, 1842.

LUER, C.A. *Barbrodria*, a new genus in the Pleurothallidinae (Orchidaceae). **Selbyana**, v. 5, p. 386–387, 1981.

LUER, C.A. Icones Pleurothallidinarum I. Systematics of the Pleurothallidinae (Orchidaceae). **Monographs in Systematic Botany from the Missouri Botanical Garden**, v. 15, p. 29–34, 1986.

LUER, C.A. Icones Pleurothallidinarum XX. Systematics of *Jostia*, *Andinia*, *Barbosella*, *Barbrodria*, *Pleurothallis* subgen. *Antilla*, subgen. *Effusia*, subgen. *Restrepioidia*. Addenda to *Lepanthes*, *Masdevallia*, *Platystele*, *Pleurothallis*, *Restrepiopsis*, *Scaphosepalum* and *Teagueia*. **Monographs in Systematic Botany from the Missouri Botanical Garden**, v. 79, p. 7–38, 2000.

LUER, C.A. Icones Pleurothallidinarum XXIV: A First Century of New Species of *Stelis* of Ecuador, Part One. Addenda to *Barbosella*, *Dracula*, *Dresslerella*, *Lepanthopsis*, *Platystele*, *Pleurothallis*, *Restrepia*, *Scaphosepalum*, *Teagueia*, and *Trichosalpinx*. **Monographs in Systematic Botany from the Missouri Botanical Garden**, v. 88, p. 97–122, 2002.

MELO, M.C.; BORBA, E.L.; PAIVA, E.A.S. Morphological and histological characterization of the osmophores and nectaries of four species of *Acianthera* (Orchidaceae: Pleurothallidinae). **Plant Systematics and Evolution**, v. 286, n. 3–4, p.141–151, 2010.

MORRONE, J.J. Biogeographic areas and transition zones of Latin America and the Caribbean islands based on panbiogeographic and cladistic analysis of the entomofauna. **Annual Review of Entomology**, v. 51, p. 467–494, 2006.

MORRONE, J.J. Cladistic biogeography of the Neotropical region: identifying the main events in the diversification of the terrestrial biota. **Cladistics**, v. 30, p. 202–214, 2013.

NEYLAND, R.; URBATSCH, L.C.; PRIDGEON, A.M. A phylogenetic analysis of subtribe Pleurothallidinae (Orchidaceae). **Botanical Journal of the Linnean Society**, v. 117, p. 13–28, 1995.

PENNINGTON, R.T.; RICHARDSON, J.E.; LAVIN, M. Insights into the historical construction of species-rich biomes from dated plant phylogenies, neutral ecological theory and phylogenetic community structure. **New Phytologist**, v. 172, p. 605–616, 2006.

PÉREZ-ESCOBAR, O.A.; CHOMICKI, G.; CONDAMINE, F.L.; KARREMANS, A.P.; BOGARÍN, D.; MATZKE, N.J.; SILVESTRO, D.; ANTONELLI, A. Recent origin and rapid speciation of Neotropical orchids in the world's richest plant biodiversity hotspot. **New Phytologist**, v. 215, p. 891–905, 2017.

PRIDGEON, A.M. Subtribe Pleurothallidinae. In: PRIDGEON, A.M.; CRIBB, P.J.; CHASE, M.W.; RASMUSSEN, F.N. (Eds.), **Genera Orchidacearum**. Epidendroideae (Part One), Vol. 4. Oxford: Oxford University Press, p. 405–412, 2005a.

PRIDGEON, A.M. *Barbosella*. In: PRIDGEON, A.M.; CRIBB, P.J.; CHASE, M.W.; RASMUSSEN, F.N. (Eds.), **Genera Orchidacearum**. Epidendroideae (Part One), Vol. 4. Oxford: Oxford University Press, p. 336–338, 2005b.

PRIDGEON, A.M.; CHASE M.W. A phylogenetic reclassification of Pleurothallidinae (Orchidaceae). **Lindleyana**, v. 16, n. 4, p. 235–271, 2001.

PRIDGEON, A.M.; CHASE, M.W. Phylogenetics of the subtribe Pleurothallidinae (Epidendreae: Orchidaceae) based on combined evidence from DNA sequences. **Lankesteriana**, v. 7, p. 49–50, 2003.

PRIDGEON, A.M.; STERN, W.L. Ultrastructure of osmophores in *Restrepia* (Orchidaceae). **American Journal of Botany**, v. 70, n. 8, p. 1233–1243, 1983.

PRIDGEON, A.M.; STERN, W.L. Osmophores of *Scaphosepalum* (Orchidaceae). **Botany Gazette**, v. 146, p. 115–123, 1985.

PRIDGEON, A.M.; CRIBB, P.J.; CHASE, M.W.; RASMUSSEN, F.N. **Genera Orchidacearum**, Vol. 1. General introduction, Apostasioideae, Cyripedioideae, Oxford: Oxford University Press., 197 pp., 1999.

PRIDGEON, A.M.; CRIBB, P.J.; CHASE, M.W.; RASMUSSEN, F.N. **Genera Orchidacearum**, Vol. 2. Orchidoideae (*Part one*). Oxford: Oxford University Press., 464 pp., 2001a.

PRIDGEON, A.M.; SOLANO, R.; CHASE, M.W. Phylogenetic relationships in Pleurothallidinae (Orchidaceae): combined evidence from nuclear and plastid DNA sequences. **American Journal of Botany**, v. 88, n. 12, p. 2286–2308, 2001b.

PRIDGEON, A.M.; CRIBB, P.J.; CHASE, M.W.; RASMUSSEN, F.N. **Genera Orchidacearum**. Vol. 3. Orchidoideae (*Part two*). Vanilloideae, Oxford: Oxford University Press., 400 pp., 2003.

PRIDGEON, A.M.; CRIBB, P.J.; CHASE, M.W.; RASMUSSEN, F.N. **Genera Orchidacearum**. Vol. 4. Epidendroideae (*Part one*). Oxford: Oxford University Press., 696 pp., 2005.

PRIDGEON, A.M.; CRIBB, P.J.; CHASE, M.W.; RASMUSSEN, F.N. **Genera Orchidacearum**. Vol. 5. Epidendroideae (*Part two*). Oxford: Oxford University Press., 664 pp., 2009.

PRIDGEON, A.M.; CRIBB, P.J.; CHASE, M.W.; RASMUSSEN, F.N. **Genera Orchidacearum**. Vol. 6. Epidendroideae (*Part three*). Oxford: Oxford University Press., 576 pp., 2014.

RAMÍREZ, S.R.; GRAVENDEEL, B.; SINGER, R.B.; MARSHALL, C.R.; PIERCE, N.E. Dating the origin of the Orchidaceae from a fossil orchid with its pollinator. **Nature**, v. 448, p. 1042-5, 2007.

SCHIESTL, F.P.; JOHNSON, S.D. Pollinator-mediated evolution of floral signals. **Trends in Ecology & Evolution**, v. 28, n. 5, p. 307–315, 2013.

SCHIESTL, F.P.; PEAKALL, R.; MANT, J. G.; IBARRA, F.; SCHULZ, C.; FRANKE S.; FRANCKE W. The chemistry of sexual deception in an orchid-wasp pollination system. **Science**, v. 302, p. 437–438, 2003.

SCHLECHTER, F.R.R. Die Gattungen *Restrepia* H. B. u. Kth. **Repertorium Specierum Novarum Regni Vegetabilis**, v. 15, p. 255–270, 1918.

SCHLECHTER, F.R.R. Das System der Orchidaceen. **Notizblatt des Botanischen Gartens und Museums zu Berlin-Dahlem**, v. 9, p. 563–591, 1926.

STENZEL, H. Pollen morphology of the subtribe Pleurothallidinae Lindl. (Orchidaceae). **Grana**, v. 39, n. 2-3, p. 108–125, 2000.

van der PIJL, L.; DODSON, C.H. **Orchids flowers. Their pollination and evolution**. Coral Gables, FL: University of Miami Press., p. 101–122, 1966.

CAPÍTULO I / CHAPTER I

Relações filogenéticas e padrões biogeográficos de *Barbosella* (Orchidaceae, Pleurothallidinae), gênero com distribuição disjunta no Neotrópico

Phylogenetic relationships and biogeography patterns of *Barbosella* (Orchidaceae, Pleurothallidinae), genus with a disjunct distribution in the Neotropics

(Capítulo formatado segundo as normas do periódico *Cladistic*)

Phylogenetic relationships and biogeography patterns of *Barbosella* (Orchidaceae, Pleurothallidinae), genus with a disjunct distribution in the Neotropics¹

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¹*Part of the first author's Ph.D. dissertation.*

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Running title: Phylogenetics of the genus *Barbosella*

Abstract

Barbosella orchids, a small genus in the subtribe Pleurothallidinae with 20 species, are usually epiphytes, with long-repent or caespitose growth; inflorescence always a single-flowered raceme; and lip with a basal cavity articulated to the column foot. It is distributed from Central America through the Andes to southeastern of Brazil with a disjunct distribution between coastal forests. Thirteen species of *Barbosella* plus related genera and outgroup taxa were investigated using molecular nrITS, five plastid regions (5.5k bp) and 43 morphological characters, analyzed using maximum likelihood, Bayesian inference and maximum parsimony to infer evolutionary relationships. Morphological characters reconstruction, molecular dating and biogeographical analyses were also performed. Results obtained provide strong support for a monophyletic *Barbosella* and origin through the Middle-Late Miocene (~11 Ma), where the Atlantic Forest and Andean Forest are nowadays distributed and separated by a dry area. The early diversification within the genus occurred in the Late Miocene around 8 Ma involving a vicariance event and subsequently multiple dispersals in both areas, where the species groups are restricted today. All multiple specimens were recovered as monophyletic species with high support. The findings from this study contribute to a better understanding of the evolution of *Barbosella* and the evidence of historical connection of the coastal forests of South America.

Introduction

The isolation of South America from Central America and Africa during the Tertiary Period (Burnham and Graham, 1999) has done the Neotropical region stands out for its great biodiversity (Antonelli *et al.*, 2009; Antonelli and Sanmartín, 2011) and the spectacular richness of orchid species (Brieger, 1969; Ulloa Ulloa *et al.*, 2017). One of the fundamental questions about the biodiversity of a region is how this is distributed across major vegetation types. For this reason, identifying where and how orchid species are distributed could improve our understanding to elucidate the evolutionary history of the family (Hou, 2017).

The temporal and spatial distribution of the evolutionary events remains largely unknown (Hou, 2017). Characterize endemism patterns and their biogeographic units are thus extremely important for planning biological preserves (Knapp, 2002). The importance of studying taxa with wide or narrow distribution is broadly recognized from a conservation view and the determination of the vulnerability of species to extinction (Menini-Neto & Forzza, 2012). In the Neotropics, Serra do Mar mountain range in southeastern Brazil and small portions of the Andes are good examples at where should make extinction more likely (Givnish, 2017).

Neotropical rainforests are currently not continuous, in fact are separated by open vegetation with a diagonal distribution and a drier climate zone (Werneck, 2011). The Seasonally Dry Tropical Forests or Caatinga (northeastern Brazil) and the Cerrado savanna (central Brazil) separate the Atlantic Forest from Amazon; whereas the Chaco (northeastern Argentina, western Paraguay and south-eastern Bolivia) divides the Atlantic Forest from the Andean Forest (Werneck, 2011, Turchetto-Zolet *et al.*, 2012). This disjunction may to contribute as a barrier to gene flow between these rainforests region.

Orchidaceae is one of the largest angiosperm families, arranged in five subfamilies and placed in 736 genera with over 27,000 species described (Chase *et al.*, 2015). Most species which are found in tropical forests and over 80% are epiphytes (Givnish *et al.*, 2015). For the Americas, Orchidaceae is the most diverse family, including 12,983 species, which is also the most diverse representative between the largely epiphytic plants in the tropical Andean countries (Ulloa Ulloa *et al.*, 2017).

The subtribe Pleurothallidinae, placed in Epidendroideae subfamily, is the most diverse Neotropical group in all orchid family, with over 5,000 currently accepted species

in 44 genera (Karremans, 2016). It includes about 20% of all known orchids (Higgins and Williams, 2009, Karremans, 2016). Several phylogenetic studies have recently focused on the Pleurothallidinae (*e.g.* Pridgeon *et al.*, 2001, Pridgeon & Chase, 2001, Pridgeon & Chase, 2003, Karremans, 2016, Pérez-Escobar *et al.*, 2017) and despite its monophyly, it have shown the lack on the studies to understand the systematic placement of all lineages within this huge subtribe. Moreover, Chomick *et al.* (2015), Givnish *et al.* (2015) and Pérez-Escobar *et al.* (2017) showed that Pleurothallidinae has one of the highest species diversification rates in the Orchidaceae, with about 20 My (millions of years) of age.

Barbosella Schltr. is a small genus in Pleurothallidinae with 20 species distributed from Central America through the Andes to northern Argentina, with a few species in the Antilles, and approximately half of the species occurs in a centre of diversity in the Atlantic Forest in southeastern Brazil (Pridgeon, 2005). *Barbosella* orchids are usually epiphytes, with long-repent or caespitose growth, and sometimes form large, dense masses on the tree trunks. The main features for the genus are: inflorescence always a single-flowered raceme, with a long and delicate peduncle; lip with basal cavity articulated to the column foot, which one the base is prolonged into a prominent and bulbous foot, with “ball-and-socket” configuration; and anther with four clavate pollinia of equal size (Luer, 2000, Stenzel, 2000, Pridgeon, 2005) (Fig. 1).

The only taxonomic revision of *Barbosella* was published by Luer (2000), in a volume of *Icones Pleurothallidarum*. He recognized 18 species published at that moment, since he had previously moved *B. miersii* (Lindl.) Schltr. to the monospecific genus *Barbrodria* Luer (Luer, 1981). The first molecular phylogeny of Pleurothallidinae (Pridgeon *et al.*, 2001), with combined nuclear and plastid DNA sequences included four species of *Barbosella*, it have indicated with strong support that *Barbrodria miersii* Luer was embedded within *Barbosella*. The genus was later included with six species, into a phylogenetic relationships analyses inferred with only ITS sequences (nrDNA) of *Pleurothallis sensu lato* by Chiron *et al.* (2012) evidencing its monophyletic placement in one of eight groups recognized in Pleurothallidinae.

In a recent update of the phylogenetic relationships within Pleurothallidinae, Karremans (2016) recognized nine major clades, which *Barbosella* is embedded in *Restrepia* (Re) affinity, including seven more accepted genera: *Chamelophyton* Garay, *Dresslerella* Luer, *Echinosepala* Pridgeon & M.W.Chase, *Myoxanthus* Poepp. & Endl., *Pleurothallopsis* Porto & Brade, *Restrepia* Kunth and *Restrepiella* Garay & Dunst. This

affinity has undergone minor changes since the first molecular phylogeny of Pleurothallidinae was published (Pridgeon *et al.*, 2001, Karremans, 2016).

This study firstly aimed to test the monophyly of the *Barbosella* genus and to inspect relationships inside the group among Neotropical region. Reconstructing the phylogeny, we aimed to apply biogeographic principles and analyses to summarize the patterns of geographic distribution, its diversification times, and arrivals in this region. To do this, we collected DNA sequence data from nrITS region (ITS1, 5.8S, ITS2), and five plastid regions (*atpI-atpH*, *matK*, *psbD-trnT*^(GGU), *trnQ-5'-rps16*, *trnH-psbA*).

Materials and methods

Taxon sampling

Based on previous phylogenetic studies in the subtribe Pleurothallidinae (Pridgeon *et al.*, 2001; Karremans, 2016), five (including *Barbosella*) of eight accepted genera in *Restrepia* affinity (Karremans, 2016) were therefore included as ingroup to investigate the monophyly of genus. At least three individuals of *Barbosella* from distantly populations were sampled when were possible, to analyze the monophyletic nature of the species. In addition, an enlarged outgroup sampling, including different Pleurothallidinae genera and representatives of the subtribes Bletiinae and Laeliinae, were used to better accommodate molecular dating calibrations. A total of 46 specimens (32 species) were sampled with 248 new sequences. Vouchers were deposited in the Herbarium of the Botany Department at the Federal University of Paraná (UPCB), Herbarium Rioclarense (HRCB) and Marie Selby Botanical Gardens Herbarium (SEL) (acronyms following Thiers, 2018). All information about the sequences is listed in Appendix S1 with herbarium vouchers, geographic origin and GenBank accession numbers.

Molecular Methods

Total genomic DNA was extracted from fresh-frozen leaves or leaves stored in CTAB solution (Rogstad, 1992, adapted) using the 2× CTAB protocols (Doyle and Doyle, 1987), without the addition of RNase A and scaled to 2 ml tubes.

PCR amplification of nrITS region (internal transcribed spacers ITS1 and ITS2, and 5.8S gene) was performed in 20 µl reactions mixtures containing 1× buffer, 1.5 mM

MgCl₂, 0.2 mM dNTPs, 0.2 µM of each primer, 0.08 mg/mL BSA, 1 M betaine, 0.08% DMSO, 0.025 unit of *Taq* DNA Polymerase (Invitrogen, Life Technologies Corporation), and 20–50 ng genomic DNA. The plastid regions (*matK* gene, and intergenic spacers *atpI-atpH*, *psbD-trnT*^(GGU), *trnQ-5'-rps16*, *trnH-psbA*) were amplified in 20 µl reactions mixtures using the TopTaq Master Mix kit (QIAGEN Biotechnology) (0.5 units of TopTaq DNA polymerase, PCR buffer with 0.6 mM MgCl₂ and 0.08 mM dNTPs), 0.2 µM of each primer and 20–50 ng genomic DNA. Information about each region and primers are listed in Appendix S2. The choice of these regions was based in previous *Pleurothallidinae* studies (e.g., Pridgeon *et al.*, 2001, Shaw *et al.*, 2007).

PCR amplifications for all regions were performed under initial 94°C pre-melt for 1 min followed by 40 cycles of (i) 94°C denaturation for 30s, (ii) 51°C (nrITS) or 53°C (plastids) annealing for 40s, and (iii) 72°C extension for 40s, followed by 72°C a final extension for 5 min. Amplified products were purified using polyethylene glycol (PEG 20%) and ethanol 80% (Paithankar and Prasad, 1991) and sequenced with Big Dye Terminator version 3.1 (Applied Biosystems, California, USA), using the same primers used for PCR, by the company Macrogen Inc. (Seoul, South Korea - <http://dna.macrogen.com>). Forward and reverse sequences were assembled in Geneious v7.1.3 (<http://www.geneious.com>, Kearse *et al.*, 2012).

All individual region alignments were conducted using MAFFT v7.017 (Katoh and Standley, 2013) as implemented in Geneious, and then concatenated in SequenceMatrix (Vaidya *et al.*, 2011), exporting sequences with external gaps coded as question marks for phylogenetic analysis. The G-INS-i algorithm, the most accurate MAFFT algorithm for aligning loci was used for all regions, including rDNA. The 1PAM nucleotide scoring matrix was used. Gaps were treated as missing data. Final alignments were visually checked in Geneious to minimize the number and size of gaps.

Morphological Character Evolution

Morphological characteristics (Appendix S3, Appendix S4) were selected based on the most common diagnostic features used in generic and species descriptions from the literature and herbarium and live specimens. We use the proposal of de Pinna (1999) to establish the homologies between the states of character and coded according to Sereno (2007). The matrix of morphological character states was prepared with Mesquite v3.04

(Maddison and Maddison, 2015). The terminology of characters follows Luer (1986, 2000), Stearn (2004) and Gonçalves and Lorenzi (2007).

Phylogenetic analyses

The analyses were performed for all individual regions and the results are summarized in Appendix S5, however the results and discussion were emphasized in four analyses as follows: ITS, plastid dataset, molecular combined (plastid + nuclear) dataset and total evidence (molecular + morphological).

Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian inference (BI) were used to estimate tree topology on the individual and concatenated matrix. All analyses were performed on CIPRES Science Gateway server (Miller *et al.*, 2010).

ML analyses were performed using RAxML v8.0 (Stamatakis, 2014) using the graphical user interface raxmlGUI v1.3 (Silvestro and Michalak, 2012). The model of nucleotide substitution applied was the GTR+G and for estimating support, we used the rapid bootstrapping algorithm with 1,000 nonparametric bootstrap replicates (BPML). We considered and emphasized bootstrap percentages (BS) ≥ 70 . The polymorphisms of the morphological characters matrix were treated as missing data.

For the Bayesian inference each region was considered as one partition and the best-fitting model was conducted under the AIC (Akaike, 1974) using jModelTest 2 (Darriba *et al.*, 2012). The GTR+G model was found to be the most appropriate one for the *atpI-atpH*, *psbD-trnT*^(GGU) and *trnQ-5'-rps16* data matrices, and the GTR+I+G model for the ITS, *matK* and *trnH-psbA* data matrices. MrBayes v3.2.6 (Ronquist *et al.*, 2012) was used to estimate tree topology and posterior probability distribution. Bayesian analyses started from random trees and employed with the Markov Chain Monte Carlo (MCMC) runs over 10,000,000 generations, sampling trees every 1000 generations. From the initial generations, 25% were discarded as burn-in period, after visual inspection of the stabilization of the log-likelihood of the trees, as measured by the Stdev(s) and PSRF values (Gelman and Rubin, 1992) and by TreeAnnotator (Drummond *et al.*, 2012). The remaining trees were used to produce a 50% majority-rule consensus tree with posterior probabilities of the clades. The posterior probability (PP) ≥ 0.99 was considered here.

MP analyses were performed with Fitch (1971) parsimony using the software PAUP 4.0b10a (Swofford, 2002). Analyses included 1,000 random taxon-addition replicates (holding 10 trees per replication) and TBR algorithm, followed by a second search to explore all topologies from the previous search (limited to 10,000 trees). The

support was estimated from 1,000 bootstrap replications (BP) (Felsenstein, 1985), simple addition, and TBR algorithm, holding 20 trees per replicate in PAUP, and bootstrap percentages ≥ 70 (BS) were considered. Trees were visualized and edited with FigTree v1.4.3 (Rambaut, 2009).

Apomorphies and homoplasies of morphological characters were mapped on the total evidence tree using WinClada v1.00.08 (Nixon, 2002). The discussion of trees under accelerated transformation (ACCTRAN) was emphasized on the results, which supports reversals to parallelism when the choice is parsimonious equally.

Geographical data

To estimate the probable geographic origin of *Barbosella*, species ranges were coded from the literature and from herbarium specimens. We examined more than 946 specimens, including duplicates, deposited in 54 herbaria, added to observations in the field, with special effort in Brazil. Specimens from the following herbaria were examined: AAU, AMES, BHCB, BHZB, BM, BR, C, CESJ, COL, CRI, CUVC, ECT, ESA, F, FLOR, FURB, GB, GUA, HB, HBG, HCF, HPBR, HRCB, HUCP, HUEFS, HUEM, HVAT, ICN, JOI, K, LD, M, MBM, MBML, MO, NY, P, PACA, QCA, QCNE, R, RB, RFA, S, SEL, SP, SPF, SPSF, U, UEC, UPGB, US, VIES and W (acronyms following Thiers, 2018). The geographical areas delimitation for all species represents the range occurrence of that species and not for each individual analyzed in this study.

Molecular dating

We estimated the lineage divergence times of *Barbosella* using a secondary calibration method since the few known unambiguous Orchidaceae macrofossils are not closely related to Pleurothallidinae (Ramírez *et al.*, 2007, Conran *et al.*, 2009). The same complete matrix of one nuclear and five plastid regions of 46 taxa including 34 representatives of *Restrepia* affinity and 12 as outgroup was time-calibrated. Secondary calibration points were obtained from Perez-Escobar *et al.* (2017), due its most representatives for Orchidaceae with a wide taxon-sampling. The most recent common ancestor (MRCA) of the crown node of Pleurothallidinae + Laeliinae clade is dated at 24.85 million years (Ma) (95% Highest Posterior Density Interval (95% HPD): 18.68 – 31.38) and Pleurothallidinae clade is dated at 19.61 Ma (95% HPD: 10.52 – 27.69). These

ages are similar with Chomick *et al.* (2015) and Givnish *et al.* (2015). The calibration points and constraints are depicted in Fig. 2.

A normal distribution prior was applied to secondary calibrations nodes, with values as follows: of the crown node of Pleurothallidinae + Laeliinae group (Mean= 24.85, Stdev=3.8 (95% HPD 18.6 – 31.1) and Pleurothallidinae group (Mean= 19.61, Stdev=4.9 (95% HPD 11.55 – 27.67). Divergence times were inferred under relaxed uncorrelated lognormal clock model (Drummond *et al.*, 2006) in BEAST 1.8.3 (Drummond *et al.*, 2012) implemented in the CIPRES server (Miller *et al.*, 2010), using a Yule tree model of speciation (the appropriate model for species level studies relationships) (Yule, 1925, Gernhard, 2008), and the appropriate model of nucleotide substitution with empirical base frequencies. The Markov Chain Monte Carlo (MCMC) chains were run for 100 million generations, sampling every 10,000 steps. Convergence was assessed using effective sample sizes (ESS) values ≥ 200 in Tracer 1.6 (Rambaut *et al.*, 2014). Maximum clade credibility tree was created in TreeAnnotator (BEAST package) with a burn-in of 25%, and visualized and edited in FigTree v1.4.3 (Rambaut, 2009).

Ancestral area reconstruction

Ancestral area estimation on the multimodel approach performed by package BioGeoBEARS (Matzke, 2013) implemented in RASP 4.0 beta (Yu *et al.*, 2015) across the BEAST chronogram of the genus *Barbosella*. Six models for the evolution of geographic range were conducted in independent runs using Dispersal-Extinction-Cladogenesis (DEC) model (Ree and Smith, 2008); a modified version of Dispersal-Vicariance Analysis (DIVA) (Ronquist, 1997), named DIVA-like; and a modified version of BayArea (Landis *et al.*, 2013) or BayArea-like. The founder-event-speciation parameter j was also added separately. Fit of the models was compared using the Akaike information criterion corrected for sample size (AICc).

Nine biogeographical areas were defined based on literature and distribution patterns observed in other plant studies grounded on the Neotropical regionalization proposed by Morrone (2014) and Antonelli *et al.* (2009): A- Mexican Transition Zone; B- Mesoamerica; C- Pacific; D- Boreal Brazilian; E- South-Brazilian; F- Paraná (Atlantic Forest); G- South- American Transition Zone; H- Antillean; I- Chacoan. Definitions of areas were based on the current distribution of *Barbosella* species (Appendix S1).

Results

Phylogenetic analyses

The Q-INS-i algorithm, implemented in MAFFT (Katoh and Standley, 2013), which considers inferred secondary structure of rDNA (Katoh and Toh, 2008), was not efficient for the alignment and the phylogenetic reconstruction of ITS. Differently than evidenced by Pridgeon *et al.* (2001) and Karremans (2016), the alignment with this algorithm has shown a distinct tree arrangement for the following Pleurothallidinae affinities: *Pl*, *Ma* and *Oc*; and for this reason, we have used the G-INS-i algorithm for aligning ITS. Outgroup in the *trnQ-rps16* alignment needed to be removed due to the numbers of divergent sequences. The final matrix of morphological characters is composed of 43 characters, including 18 binary and 25 multistate characters. The aligned data matrices will be available at TreeBASE (<https://www.treebase.org/>).

The ML, BI and MP analyses for ITS, plastid, molecular combined and total evidence data did not differ significantly on tree topologies and all figures show the ML topology with the summary of ML bootstrap support, followed by Bayesian posterior probability and MP bootstrap percentages on the branches. The individual analyses results are summarized in the Appendix S5 and the tree topologies in the Figure 2 and Appendix S6, S7 and S8.

The results of concatenated molecular dataset and total evidence analyses were very similar (Appendix S8, Fig. 2, Appendix S5), and for that we will discuss the results based on total evidence. The total matrix comprised 5543 characters (5500 molecular plus 43 morphological) derived 46 taxa (32 spp.), of these, 3907 (70%) were constant and 986 (18%) were potentially parsimony informative (Appendix S5). The MP analysis generated nine most parsimonious trees of 3321 steps, CI = 0.63 and RI = 0.77 (Appendix S5). Inside *Restrepia* affinity, *Myoxanthus* appears as sister genus to all other genera of the affinity with high support in molecular combined dataset (BS-ML 100%, PP 1, BS-MP 94%) and total evidence (BS-ML 100%, PP 1, BS-MP 95%) (Fig. 2), followed by *Restrepia* and *Pleurothallopsis*, a group also highly supported in molecular combined dataset (BS-ML 98%, PP 1, BS-MP 88%) and total evidence (BS-ML 100%, PP 1, BS-MP 88%) (Fig. 2).

Barbosella was always recovered as a monophyletic with high support (Fig. 2) and *Restrepiella* appears as sister genus, also highly supported, except in ITS analyses (BS-ML 69%, PP 0.97, BS-MP 54%) (Appendix S6). *Barbosella* species form a strongly supported

clade (BS-ML 100%, PP 1, BS-MP 100%) (Fig. 2), with three internal main groups, and here we called as Clades A, B and C.

All species sampled with two or three individuals (*B. australis*, *B. cogniauxiana*, *B. crassifolia*, *B. dusenii*, *B. gardneri*, *B. macaheensis*, *B. miersii*, *B. prorepens* and *B. trilobata*) were recovered in monophyletic groups highly supported (BS-ML 100%, PP 1, BS-MP 100%).

The Clade A is composed for three species with trilobed lip (*B. crassifolia*, *B. spiritu-sanctensis* and *B. trilobata*), all endemic from Brazilian Atlantic Forest. This group is highly supported (BS-ML 100%, PP 1, BS-MP 100%) and appears as sister group of all other *Barbosella* species. Except for *B. orbicularis*, the Andean species (*B. cucullata*, *B. dolichorhiza* and *B. prorepens*) (Clade B) were recovered in a group with strong support (BS-ML 100%, PP 1, BS-MP 100%). *Barbosella orbicularis* appears associated highly supported as sister of all species from Atlantic Forest (excepting species of Clade A) (BS-ML 100%, PP 1, BS-MP 100%).

The main clade of Atlantic Forest species (Clade C) showed *B. macaheensis* associated of *B. australis* and forming a small internal group with moderate support values (BS-ML 87%, PP 1, BS-MP 76%). In MP analysis of plastid dataset, *B. dusenii* is positioned closely related with *B. macaheensis* following for *B. australis*, however both branches present weak supports (Appendix S7). Inside Clade C, the subclade of *B. macaheensis* and *B. australis* is the sister group of a well supported subclade (BS-ML 87% and PP 1) composed for *B. cogniauxiana*, followed by *B. gardneri*, which one is related of *B. dusenii* and *B. miersii*, all relationship well supported here (Fig. 2). In ITS analyses *B. cogniauxiana* was recovered with weak supports (BS ML and MP < 50%) as sister species of *B. australis* and *B. macaheensis*. The two individuals of *Barbosella miersii* not recovered in a monophyletic group in BI and MP (BS-ML < 50%) and it is positioned as sister species of *B. gardneri* with weak supports (Appendix S6).

Morphological character evolution

Figure 3 represents the total evidence ML tree where all morphological characters were mapped. *Barbosella* appears with six homoplastic character states: repent habit (character 1), ascending secondary stem (character 3), single-flowered raceme inflorescence (character 12), oblique inflorescence floral bract (character 17), the connation of lateral sepal in the middle (character 27), denticulate column apex (character

40); and four synapomorphic characters: suberect leaves disposal (character 8), leaf with minutely emarginate and mucronate apex (character 10), lip base articulation with the column foot (character 37), and bulbous column foot (character 38). Among Clades A, and Clades B-C they are supported by only homoplastic character states: trilobed and ovate lip (character 33) (character 34); and ovate petal (character 30) and presence of wings on the column (character 39), respectively. Inside Clade A, *B. crassifolia* is the only one supported by a autapomorphic character state: uncinatate lip lateral lobes (character 36). Clade B is supported only by homoplastic character states. *Barbosella orbicularis* is supported by the autapomorphic character states of orbicular leaves (character 9). Inside Clade C the autapomorphies appears only in *B. gardneri* supported by the character states of subpandurate lip (character 34); and in *B. miersii* supported by three character states: dorsal and lateral sepal with one venation (character 23 and 29), and synsepal with flat shape (character 28).

Divergence times and ancestral area reconstruction

Two clades were forced to be monophyletic since they were well supported in the phylogenetic analyses but were not recovered in the BEAST trees without applying the constraints: 1) Pleurothallidinae + Laeliinae, and 2) a group with Pleurothallidinae representatives, excluding species of *Brachionidium* and *Octomeria*. All ages described refer to the crown node ages.

Of the six biogeographical models evaluated using BioGeoBEARS, the BayArea-like model resulted the best supported (AICc_{wt} 0.76, Appendix S9). The inclusion of the parameter *j* was not significantly to improve the models (Appendix S9), suggesting for *Barbosella* the models with founder-event speciation (or jump dispersal) are not adequate to account for all movements to new areas.

Ancestral range estimation under the BayArea-like model (Fig. 4) indicates the origin of *Barbosella* approximately at 11 Ma in the Late Miocene (crown age: 11.28; HPD: 9.3–18.06) suggests the Pacific and Paraná region (Atlantic Forest) as the most probable ancestral area of the MRCA of the genus. All three clades inside *Barbosella* are estimated to have originated in Miocene/ Pliocene border (~ 5 Ma). The Pacific and Atlantic Forest also appears as ancestral area on the first main diversification within *Barbosella* (crown age: 9.61 Ma; HPD: 7.61–15.18), between the Clades B and C.

Diversification of *B. orbicularis* (species restricted to the Andean Forest) and Clade C (all species from Atlantic Forest) occurred in Later Miocene at approximately 8 Ma (crown age: 7.8; HPD: 5.12–10.8). Inside Clade C the MRCA is restricted to the Atlantic Forest and the two subclades diversified successively in Middle Pliocene at 4.9 Ma (crown age: 4.9; HPD: 3.02–7.02) (*B. cogniauxiana*, *B. gardneri*, *B. dusei* and *B. miersii*) and 4.8 Ma (crown age: 4.84; HPD: 2.87–7.09) (*B. australis* and *B. macaheensis*). *Barbosella cogniauxiana* was found to be the younger diversification in the genus, originated at 0.3 Ma (crown age: 0.29; HPD: 0.05–0.76).

On the other hand, the MRCA of Clade B is restricted to the Andean Forest, i.e. Pacific, Boreal Brazilian, South-Brazilian and South-American Transition Zone (crown age: 5.51; HPD: 2.93–8.64). Diversification of the Clade A was also in Middle Pliocene approximately at 4.7 Ma (crown age: 4.75; HPD: 2.44–7.61), and the ancestral area is the Atlantic Forest, most probably diversified in Espírito Santo State, by *B. spiritu-sanctensis* and *B. crassifolia* (crown age: 2.43 Ma; HPD: 1.1–4.21), which the first one is restricted to this state.

Discussion

Phylogenetic analyses

Morphological and molecular matrices combined improve the branches support. State characters such as the lip base articulation with the bulbous column foot have been frequently mentioned as diagnostic for the genus (Luer, 2000, Pridgeon *et al.*, 2001). However, the state characters as suberect leaves disposal and the minutely emarginate and mucronate apex leaf are also synapomorphic for *Barbosella* in this study, although it occurs independently in other unrelated genera of the subtribe (Luer, 2000).

In all phylogenetic analyses, the *Barbosella* species here investigated were recovered with moderate (few nodes) to high support. In this study, the highly supported monophyly of *Barbosella* was recovered including 13 species (nine species from Atlantic Forest and four from Andean region), and this result refute the probable hypothesis about the segregation of the genus due its disjunction distribution in the Neotropics. As seen by Chiron *et al.* (2012), in the ITS analysis with six species (four from Atlantic Forest and two from Andean), the tree topologies of the genus *Barbosella* are maintained here. *Barbosella*

miersii and *B. trilobata*, species which do not share the ball-and-socket articulation of the lip, are embedded in *Barbosella*, placed in different internal groups, showing a plesiomorphic nature of this character state. Pridgeon *et al.* (2001) have already elucidated the position of *B. miersii* in the genus *Barbosella*, disagreeing with Luer's segregation (Luer, 1981, Luer, 2000) when he proposed the monospecific genus, *Barbrodria* Luer, due to its simply hinged lip and apical anther.

Clade A appears as sister of all other *Barbosella* species, which are recognized for its feature of the lip shape and it composed for the Brazilian species with trilobed lip, which all of them present the lip with a striking lateral lobes (Luer, 2000, Bolson *et al.*, in press). *Barbosella crassifolia* is morphologically related to *B. spiritu-sanctensis* sharing the same repent habitat, and the prostate and suborbicular leaves. While *B. crassifolia* is the most widely distributed species in Brazil, *B. spiritu-sanctensis* presents a restrict distribution in the states of Espírito Santo and Minas Gerais, southeast region (Bolson *et al.*, in press). Inside this clade, *Barbosella trilobata* shares the same habitat, however the leaves are suberect and elliptical, and this species presents a restrict distribution in southern Brazil, in Paraná and Santa Catarina States (Bolson *et al.*, in press).

Barbosella orbicularis, a species distributed in Panama, Ecuador and Venezuela, is morphological related to *B. crassifolia* and *B. spiritu-sanctensis* for sharing the same repent habitat, the prostate and suborbicular leaves, as well as the trilobed lip (Luer 2000). However, the phylogenetic analyses show this species positioned as sister of clade A, as evidenced by Chiron *et al.* (2012).

Internally clade C is organized in only two small clades, the first composed of closely related species *B. australis* and *B. macaheensis*. Both species are morphologically similar in shape of leaves and floral morphology, although the flowers are considerably distinct in size. Also, *B. australis* presents the lip apex bifid into two obtuse angles, while in *B. macaheensis* is truncated (Bolson *et al.*, in press). The second clade is composed of *B. cogniauxiana*, *B. gardneri*, *B. dusenii* and *B. miersii* with a distinct morphological relationship.

Barbosella cogniauxiana is endemic to Atlantic Forest of southern Brazil and northern Argentina, of all nine species occurring in Brazil, this is the only one not exclusively Brazilian in distribution. *Barbosella cogniauxiana* is morphologically related with *B. australis* and *B. dusenii* in habit and leaf shape and size, but with thicker leaves than in the last two (Bolson *et al.*, in press). Although the analyses have not shown *B.*

cogniauxiana closely related with *B. dusenii*, these species are also similar in flower shape, mainly to share a very similar entire lip where both species share a pair of longitudinal calli in the middle of the lip, however it is more prominent in *B. dusenii* (Luer, 2000).

Barbosella gardneri and *B. miersii* appearing vegetatively related, both species present minute leaves very variable in size, and sterile plants of the two species are sometimes indistinguishable. These two species present the same and the broader occupation area in Brazil. Nevertheless, *B. miersii* produces the smallest flowers of the Brazilian species and are easily distinguished from those of *B. gardneri* (Bolson *et al.*, in press). However, in the present study both species are not positioning closely related as sister species, also evidenced in Chiron *et al.* (2012).

Origin and diversification of Barbosella

Results obtained here suggest that early diversification of *Barbosella* occurred through the Middle-Late Miocene (~ 11 Ma), where the Atlantic Forest and Andean Forest are nowadays distributed and separated by a dry area, and principally close to the warm period of the Middle Miocene climatic optimum, which coincides with radiation of several plants in the Neotropics (Antonelli *et al.*, 2009, Gustafsson *et al.*, 2010, Pérez-Escobar *et al.*, 2017). At the same time intensified mountain building started in the Andes (Hoorn *et al.*, 2010) and the forested biomes of South America were partially separated by the open vegetation biomes like Cerrado (Simon *et al.*, 2009).

At present, the Andean Forest/Amazonian and Atlantic Forest are separated by the 'dry diagonal' biomes (Chaco, Cerrado and Caatinga) (Werneck, 2011, Werneck *et al.*, 2012), causing a disjunct pattern of distribution between many groups of Neotropical forested biomes (Costa, 2003, Nihei & Carvalho, 2007, Fiaschi & Pirani, 2009, Pires & Marinoni, 2010, Pellegrino *et al.*, 2011, Turchetto-Zolet *et al.*, 2012).

In *Barbosella*, dispersals were recovered as the most frequent biogeographic event for range change. The migration by dispersal in Pleurothallidinae and other orchids was reported in the extensive biogeographical analyses between the Andes and Amazonia presented by Pérez-Escobar *et al.* (2017). These authors concluded that the uplift of the Andes did not act as a major dispersal barrier for Cymbidieae and Pleurothallidinae orchids.

However, for the genus *Barbosella*, vicariance events seem to have played an important role in the biogeographic diversification. The first main group formed within the genus, *B. orbicularis* + Clade C, for which a Late Miocene origin was estimated (~ 8 Ma), were characterized by a vicariance event from Atlantic Forest and Pacific + South American. The ancestral area estimation of the *Barbosella* provides evidence of past connections as well (Batalha-Filho *et al.*, 2013, Costa, 2003).

The second group of *Barbosella*, Clade B, seems to have dispersed from the Pacific, Boreal Brazilian, South Brazilian and South American regions by the end of the Late Miocene (~ 5.5 Ma), exhibiting subsequent dispersions principally to Northern Andes in the Pliocene. The colonisation of Mesoamerican and Mexican regions occurred by *B. prorepens*, probably dispersed using the recently formed Isthmus of Panama, which was completely established around 3 Ma (Hoorn *et al.* 2010, O'Dea *et al.*, 2016). Antillean region was also colonized by dispersal by the same species.

Clade C exhibited younger divergences, with their crown node ages recovered mainly through the Middle Pliocene.

Evidences of a recent connection do not exist between species of *Barbosella* of Atlantic Forest and Andean Forest given the currently total isolation of Atlantic species. Even though the gallery forests of Chacoan region it seems to be an important route for some groups (Oliveira-Filho and Ratter, 1995, Costa, 2003) making a connection to Andes and Atlantic Forest, for *Barbosella* is not a relevant corridor since the genus does not occur in these galleries at present.

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References

- Akaike, H. 1974. A new look at the statistical model identification. *IEEE Trans. Automat. Contr.* 19, 716–723.
- Antonelli, A., Sanmartín, I. 2011. Why are there so many plant species in the Neotropics? *Taxon* 60, 403–414.
- Antonelli, A., Nylander, J.A.A., Persson, C., Sanmartín, I. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proc. Natl. Acad. Sci. USA* 106, 9749–9754.
- Batalha-Filho, H., Fjeldså, J., Fabre, P., Miyaki, C.Y. 2013. Connections between the Atlantic and the Amazonian forest avifaunas represent distinct historical events. *J. Ornithol.* 154, 41–50.
<http://doi.org/10.1007/s10336-012-0866-7>
- Bolson, M.; Toscano de Brito, A.L.V.; Machnicki-Reis, M.; Smidt, E.C. (in press). The genus *Barbosella* (Orchidaceae, Pleurothallidinae) in Brazil. *Phytotaxa*.
- Burnham, R.J., Graham, A. 1999. The history of Neotropical vegetation: new developments and status. *Ann. Mo. Bot. Gard.* 86, p. 546–589.
- Brieger, F.G. 1969. Patterns of evolutionary and geographical distribution in neotropical orchids. *Biol. J. Linnean Soc.* 1, 197–217.
- Chase, M.W., Cameron, K.M., Freudenstein, J.V., Pridgeon, A.M., Salazar, G., van den Berg, C., Schuitman, A. 2015. An updated classification of Orchidaceae. *Bot. J. Linnean Soc.* 177, 151–174.

- Chiron, G.R., Guiard, J., van den Berg, C. 2012. Phylogenetic relationships in Brazilian *Pleurothallis sensu lato* (Pleurothallidinae, Orchidaceae): evidence from nuclear ITS rDNA sequences. *Phytotaxa* 46, 34–58.
- Chomicki, G., Bidel, L.P.R., Ming, F., Coiro, M., Zhang, X., Wang, Y., Baissac, Y., Jay-Allemand, C., Renner, S.S. 2015. The velamen protects photosynthetic orchid roots against UV-B damage, and a large dated phylogeny implies multiple gains and losses of this function during the Cenozoic. *New Phytol.* 205, 1330–1341.
<http://dx.doi.org/10.1111/nph.13106>
- Conran, J.G., Bannister, J.M., Lee, D.E. 2009. Earliest orchid macrofossils: early Miocene *Dendrobium* and *Earina* (Orchidaceae: Epidendroideae) from New Zealand. *Am. J. Bot.* 96, 466–474.
<http://dx.doi.org/10.3732/ajb.0800269>
- Costa, L.P. 2003. The historical bridge between the Amazon and the Atlantic Forest of Brazil: a study of molecular phylogeography with small mammals. *J. Biogeogr.* 30, 71–86.
- Darriba, D., Taboada, G.L., Doallo, R., Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9, 772.
- Doyle, J., Doyle, J., 1987. A rapid DNA isolation procedure for small amounts of leaf tissue. *Phytochem. Bull.* 19, 810–815.
- Drummond, A.J., Ho, S.Y.W, Phillips, M.J., Rambaut, A. 2006. Relaxed phylogenetics and dating with confidence. *PLOS Biology* 4, e88.
<https://doi.org/10.1371/journal.pbio.0040088>.
- Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29, 1969–1973.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach to using bootstrap. *Evolution* 39, 783–791.
- Fiaschi, P., Pirani, J.R. 2009. Review of plant biogeographic studies in Brazil. *J. Syst. Evol.* 47, 477–496.
<http://dx.doi: 10.1111/j.1759-6831.2009.00046.x>
- Fitch, W.M. 1971. Towards defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* 20, 406–416.
- Gelman, A., Rubin, D.B. 1992. Inference from iterative simulation using multiple sequences. *Stat. Sci.* 7, 457–472.

- Gernhard, T. 2008. The conditioned reconstructed process. *J. Theor. Biol.* 253, 769–778.
- Givnish, T.J. 2017. A New World of plants. *Science* 358, 1535–1536.
<http://dx.doi.org/10.1126/science.aar4191>
- Givnish, T.J., Spalink, D., Ames, M., Lyon, S.P., Hunter, S.J., Zuluaga, A., Iles, W.J.D., Clements, M.A., Arroyo, M.T.K., Leebens-Mack, J., Endara, L., Kriebel R., Neubig, K.M., Whitten, W.M., Williams, N.H., Cameron, K.M. 2015. Orchid phylogenomics and multiple drivers of their extraordinary diversification. *Proc. R. Soc. Lond. B Biol. Sci.* 282, 20151553.
<http://dx.doi.org/10.1098/rspb.2015.1553>
- Gonçalves, E.G., Lorenzi, H. 2007. *Morfologia vegetal: organografia e dicionário ilustrado de morfologia das plantas vasculares*. 1st ed. Instituto Plantarum de Estudos da Flora, Nova Odessa, 416 pp.
- Gustafsson, A.L.S., Verola, C.F., Antonelli, A. 2010. Reassessing the temporal evolution of orchids with new fossils and a Bayesian relaxed clock, with implications for the diversification of the rare South American genus *Hoffmannseggella* (Orchidaceae: Epidendroideae). *BMC Evol. Biol.* 10, 177.
<https://doi.org/10.1186/1471-2148-10-177>
- Higgins, W.E., Williams, N.H. 2009. Pleurothallidinae: how many genera? In: Saulea, R.P., Sandow, L.A. (Eds.) *Proceedings of the 19th World Orchid Conference*. American Printing Arts, Miami, USA, pp. 425–430.
- Hoorn, C., Wesselingh, F.P., ter Steege, H., Bermudez, M.A., Mora, A., Sevink, J., Sanmartin, I., Sanchez-Meseguer, A., Anderson, C.L., Figueiredo, J.P., Jaramillo, C., Riff, D., Negri, F.R., Hooghiemstra, H., Lundberg, J., Stadler, T., Sarkinen, T., Antonelli, A. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* 330, 927–931.
<https://doi.org/10.1126/science.1194585>
- Hou, B., Luo, J., Zhang, Y., Niu, Z., Xue, Q., Ding, X. 2017. Iteration expansion and regional evolution: phylogeography of *Dendrobium officinale* and four related taxa in southern China. *Sci. Rep.* 7, 43525.
<http://dx.doi.org/10.1038/srep43525>
- Karremans, A.P. 2016. Genera Pleurothallidinarum: An updated phylogenetic overview of Pleurothallidinae. *Lankesteriana* 16, 219–241.

- Katoh, K., Toh, H., 2008. Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT based framework. *BMC Bioinformatics* 9, 212.
- Katoh, K., Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28, 1647–1649.
- Knapp, S. 2002. Assessing patterns of plant endemism in Neotropical uplands. *Bot. Rev.* 68, 22–37.
- Landis, M.J., Matzke, N.J., Moore, B.R., Huelsenbeck, J.P. 2013. Bayesian analysis of biogeography when the number of areas is large. *Syst. Biol.* 62, 789–804. <http://doi:10.1093/sysbio/syt040>
- Luer, C.A. 1981. *Barbrodria*, a new genus in the Pleurothallidinae (Orchidaceae). *Selbyana* 5, 386–387.
- Luer, C.A. 1986. Icones Pleurothallidarum I. Systematics of the Pleurothallidinae (Orchidaceae). *Monogr. Syst. Bot. Mo. Bot. Gard.* 15, 29–34.
- Luer, C.A. 2000. Icones Pleurothallidarum XX. Systematics of *Jostia*, *Andinia*, *Barbosella*, *Barbrodria*, *Pleurothallis* subgen. *Antilla*, subgen. *Effusia*, subgen. *Restrepioidia*. Addenda to *Lepanthes*, *Masdevallia*, *Platystele*, *Pleurothallis*, *Restrepiopsis*, *Scaphosepalum* and *Teagueia*. *Monogr. Syst. Bot. Mo. Bot. Gard.* 79, 7–38.
- Maddison, W.P., Maddison, D.R. 2015. Mesquite: a modular system for evolutionary analysis, version 3.04. Available at: <http://mesquiteproject.org>
- Matzke, N.J. 2013. Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Front. Biogeogr.* 5, 242–248.
- Menini-Neto, L., Forzza, R.C. 2012. Biogeography and conservation status assessment of *Pseudolaelia* (Orchidaceae). *Bot. J. Linnean Soc.* 171, 191–200.

- Miller, M.A., Pfeiffer, W., Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, USA, pp. 1–8.
- Morrone, J.J. 2014. Cladistic biogeography of the Neotropical region: identifying the main events in the diversification of the terrestrial biota. *Cladistics* 30, 202–214.
- Nihei, S.S., Carvalho, C.J.B. 2007. Systematics and biogeography of the Neotropical genus *Polietina* Schnabl & Dziedzicki (Diptera, Muscidae): Neotropical areas relationships and Amazonia as a composite area. *Syst. Entomol.* 32, 477–501. <http://doi:10.1111/j.1365-3113.2006.00376.x>
- Nixon, K.C. 2002. WinClada, version 1.00.08. Published by the author. Ithaca.
- O’Dea, A., Lessios, H.A., Coates, A.G., Eytan, R.I., Restrepo-Moreno, S.A., Cione, A.L., Collins, L.S., de Queiroz, A., Farris, D.W., Norris, R.D., Stallard, R.F., Woodburne, M.O., Aguilera, O., Aubry, M.P., Berggren, W.A., Budd, A.F., Cozzuol, M.A., Coppard, S.E., Duque-Caro, H., Finnegan, S., Gasparini, G.M., Grossman, E.L., Johnson, K.G., Keigwin, L.D., Knowlton, N., Leigh, E.G., Leonard-Pingel, J.S., Marko, P.B., Pyenson, N.D., Rachello-Dolmen, P.G., Soibelzon, E., Soibelzon, L., Todd, J.A., Vermeij, G.J., Jackson, J.B.C. 2016. Formation of the Isthmus of Panama. *Sci. Adv.* 2, e1600883. <http://dx.doi.org/10.1126/sciadv.1600883>
- Oliveira-Filho, A.T., Ratter, J.A. 1995. A study of the origin of central Brazilian forests by the analysis of plant species distribution patterns. *Edinburgh J. Bot.* 52, 141–194. <https://doi.org/10.1017/S0960428600000949>
- Paithankar, K.R., Prasad, K.S. 1991. Precipitation of DNA by polyethylene glycol and ethanol. *Nucleic Acids Res.* 19, 1346.
- Pellegrino, K.C.M., Rodrigues, M.T., Harris, D.J., Yonenaga-Yassuda, Y., Sites, J.W. 2011. Molecular phylogeny, biogeography and insights into the origin of parthenogenesis in the Neotropical genus *Leposoma* (Squamata: Gymnophthalmidae): ancient links between the Atlantic forest and Amazonia. *Mol. Phylogenet. Evol.* 61, 446–459.
- Pérez-Escobar, O.A., Chomicki, G., Condamine, F.L., Karremans, A.P., Bogarín, D., Matzke, N.J., Silvestro, D., Antonelli, A., 2017. Recent origin and rapid speciation of Neotropical orchids in the world’s richest plant biodiversity hotspot. *New Phytol.* 215, 891–905.

- <http://dx.doi.org/10.1111/nph.14629>
- Pinna, M.G.G. de 1991. Concepts and tests of homology in the cladistic paradigm. *Cladistics* 7, 367–394.
- Pires, A.C., Marinoni, L. 2010. Historical relationships among Neotropical endemic areas based on *Sepedonea* (Diptera: Sciomyzidae) phylogenetic and distribution data. *Zoologia* 27, 681–690.
<http://dx.doi.org/10.1590/S1984-46702010000500003>
- Pridgeon, A.M. 2005. *Barbosella*. In: Pridgeon, A.M., Cribb, P.J., Chase, M.W., Rasmussen, F.N. (Eds.), *Genera Orchidacearum*, 4. Epidendroideae (*Part one*), Oxford University, Oxford, pp. 336–338.
- Pridgeon, A.M., Chase, M.W. 2001. A phylogenetic reclassification of Pleurothallidinae (Orchidaceae). *Lindleyana* 16, 235–271.
- Pridgeon, A.M., Chase, M.W. 2003. Phylogenetics of the subtribe Pleurothallidinae (Epidendreae: Orchidaceae) based on combined evidence from DNA sequences. *Lankesteriana* 7, 49–50.
- Pridgeon, A.M., Solano, R., Chase, M.W. 2001. Phylogenetic relationships in Pleurothallidinae (Orchidaceae): combined evidence from nuclear and plastid DNA sequences. *Am. J. Bot.* 88, 2286–2308.
- Rambaut, A. 2009. FigTree version v1.4.3 [computer program]. Available at: <http://tree.bio.ed.ac.uk/software/figtre>
- Rambaut, A., Suchard, M.A., Drummond, A.J. 2014. Tracer version 1.6. Available at: <http://beast.bio.ed.ac.uk/Tracer>
- Ramírez, S.R., Gravendeel, B., Singer, R.B., Marshall, C.R., Pierce, N.E. 2007. Dating the origin of the Orchidaceae from a fossil orchid with its pollinator. *Nature* 448, 1042–1045.
- Ree, R.H., Smith, S.A. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction and cladogenesis. *Syst. Biol.* 57, 4–14.
<http://dx.doi.org/10.1080/10635150701883881>
- Rogstad, S.H. 1992. Saturated NaCl-CTAB solution as a means of field preservation of leaves for DNA analyses. *Taxon* 41, 701–708.
- Ronquist, F. 1997. Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. *Syst. Biol.* 46, 195–203.

- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* 61, 539–542.
- Sang, T., Crawford, D.J., Stuessy, T.F. 1997. Chloroplast DNA phylogeny, reticulate evolution and biogeography of *Paeonia* (Paeoniaceae). *Am. J. Bot.* 84, 1120–1136.
- Sereno, P.C. 2007. Logical basis for morphological characters in phylogenetics. *Cladistics* 23, 565–587.
- Shaw, J., Lickey, E.B., Schilling, E.E., Small, R.L. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The Tortoise and the hare III. *Am. J. Bot.* 94, 275–288.
- Silvestro, D., Michalak, I. 2012. raxmlGUI: a graphical front-end for RAxML. *Org. Divers. Evol.* 12, 335–337.
- Simon, M.F., Grether, R., de Queiroz, L.P., Skema, C., Pennington, R.T., Hughes, C.E. 2009. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proc. Natl. Acad. Sci.* 106, 20359–20364. <https://doi.org/10.1073/pnas.0903410106>
- Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30, 1312–1313. <http://dx.doi.org/10.1093/bioinformatics/btu033>.
- Stearn, W.T. 2004. *Botanical Latin*. Timber Press, Portland, 546 pp.
- Stenzel, H. 2000. Pollen morphology of the subtribe Pleurothallidinae Lindl. (Orchidaceae). *Grana* 39, 108–125. <http://dx.doi.org/10.1080/001731300300045247>
- Sun, Y., Skinner, D.Z., Liang, G.H., Hulbert, S.H. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theor. Appl. Genet.* 89, 26–32.
- Swofford, D.L. 2002. PAUP*: Phylogenetic analysis using parcimony and others methods, version 4.0b10. Sinauer Associates, Inc, Sunderland, MA.
- Tate, J.A. 2002. Systematics and evolution of *Tarasa* (Malvaceae): an enigmatic Andean polyploid genus. Ph.D. dissertation. The University of Texas at Austin, pp. 183.

- Thiers, B. [continuously updated] *Index Herbariorum: A global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. Available at: <http://sweetgum.nybg.org/ih/> (Accessed 17 Jan 2018).
- Turchetto-Zolet, A.C., Cruz, F., Vendramin, G.G., Simon, M.F., Salgueiro, F., Margis-Pinheiro, M., Margis, R. 2012. Large-scale phylogeography of the disjunct Neotropical tree species *Schizolobium parahyba* (Fabaceae-Caesalpinioideae). *Mol. Phylogenet. Evol.* 65, 74–182.
- Ulloa Ulloa, C., Acevedo-Rodríguez, P., Beck, S., Belgrano, M.J., Bernal, R., Berry, P.E., Brako, L., Celis, M., Davidse, G., Forzza, R.C., Gradstein, S.R., Hokche, O., León, B., León-Yáñez, S., Magill, R.E., Neill, D.A., Nee, M., Raven, P.H., Stimmel, H., Strong, M.T., Villaseñor, J.L., Zarucchi, J.L., Zuloaga, F.O., Jørgensen, P.M. 2017. An integrated assessment of the vascular plant species of the Americas. *Science* 358, 1614–1617.
- Vaidya, G., Lohman, D.J., Meier, R. 2011. SequenceMatrix: concatenation software for the fast assembly of multigene datasets with character set and codon information. *Cladistics* 27, 171–180.
- Werneck, F.P. 2011. The diversification of eastern South American open vegetation biomes: historical biogeography and perspectives. *Quat. Sci. Rev.* 30, 1630–1648. <http://doi.org/10.1016/j.quascirev.2011.03.009>
- Werneck, F.P., Gamble, T., Colli, G.R., Rodrigues, M.T., Sites, J.W. 2012. Deep diversification and long-term persistence in the south american ‘Dry Diagonal’: integrating continent-wide phylogeography and distribution modeling of geckos. *Evolution* 66-10: 3014–3034. doi:10.1111/j.1558-5646.2012.01682.x
- Yu, Y., Harris, A.J., Blair, C., He, X.J. 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. Version 4.0 beta. *Mol. Phylogenet. Evol.* 87, 46–49.
- Yule, G.U. 1925. A mathematical theory of evolution, based on the conclusions of Dr. J.C. Willis, F.R.S. *Philos. Trans. R. Soc. Lond. B. Biol. Sc.* 213, 21–87.

Figures

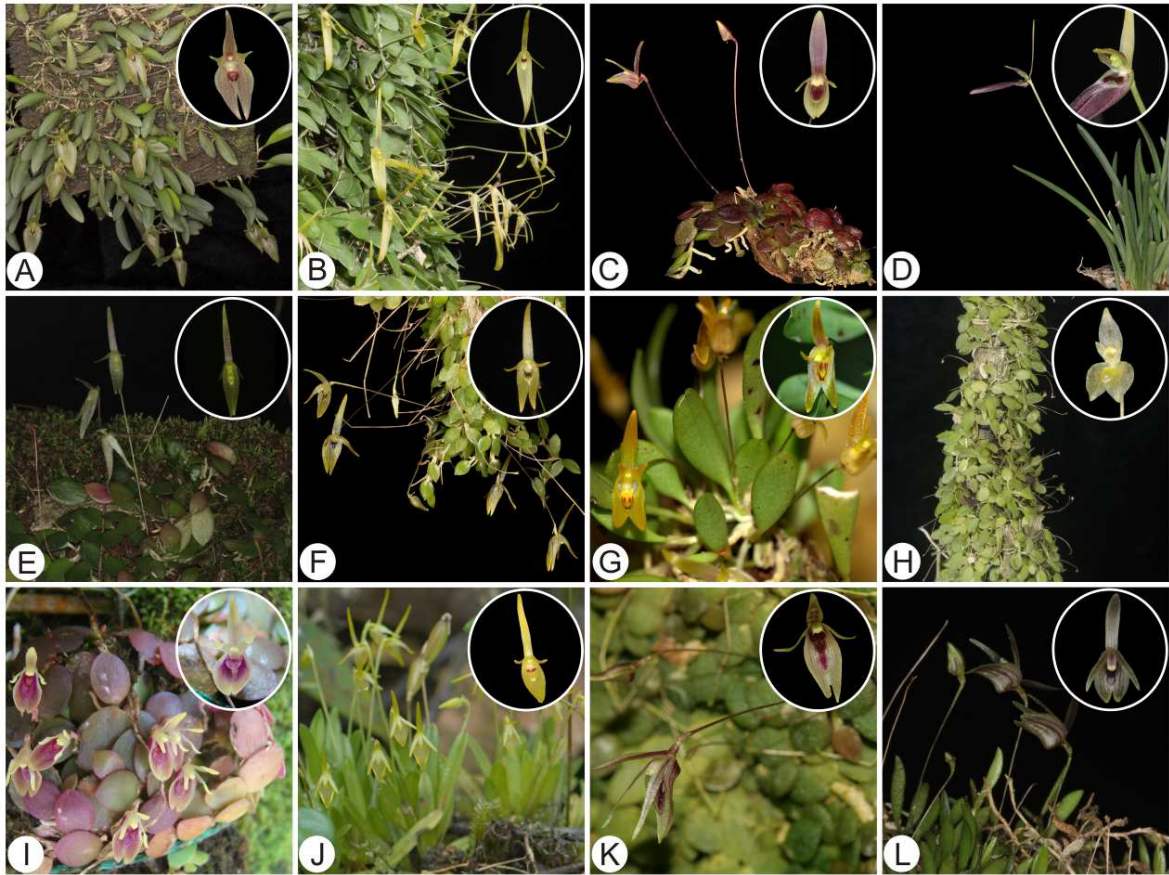


Fig. 1. Species of *Barbosella* showing habit and the flower in detail. A, *Barbosella australis*. B, *Barbosella cogniauxiana*. C, *Barbosella crassifolia*. D, *Barbosella cucullata*. E, *Barbosella dusenii*. F, *Barbosella gardneri*. G, *Barbosella macaheensis*. H, *Barbosella miersii*. I, *Barbosella orbicularis*. J, *Barbosella prorepens*. K, *Barbosella spiritus-sanctensis*. L, *Barbosella trilobata*.

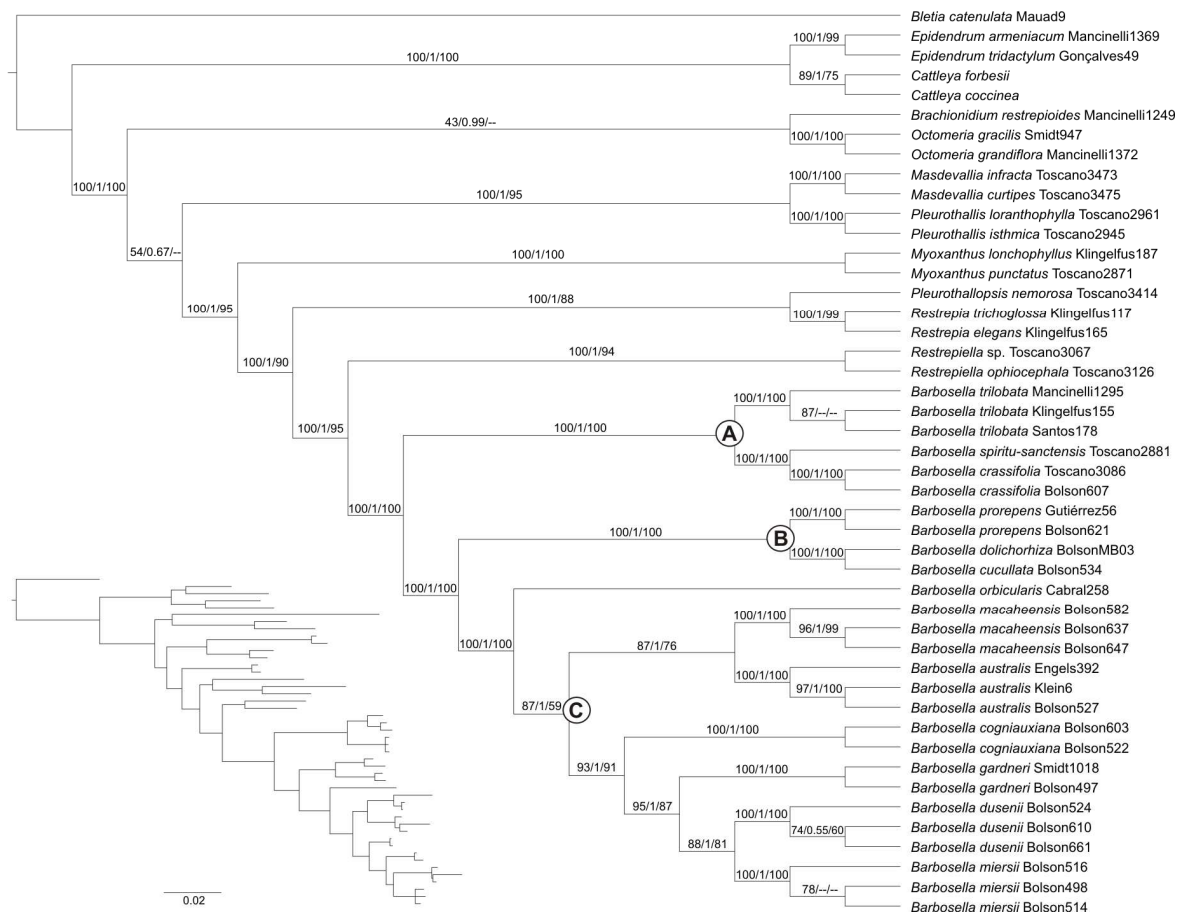


Fig. 2. Total evidence maximum likelihood tree of *Barbosella* and affinities plus outgroups. Numbers above branches represent maximum-likelihood bootstrap percentages, posterior probability from Bayesian inference, and maximum parsimony bootstrap percentages (values >50 are shown). *Barbosella* clades are represented by letters A, B and C on nodes. In detail, the BI tree has proportional branch lengths.

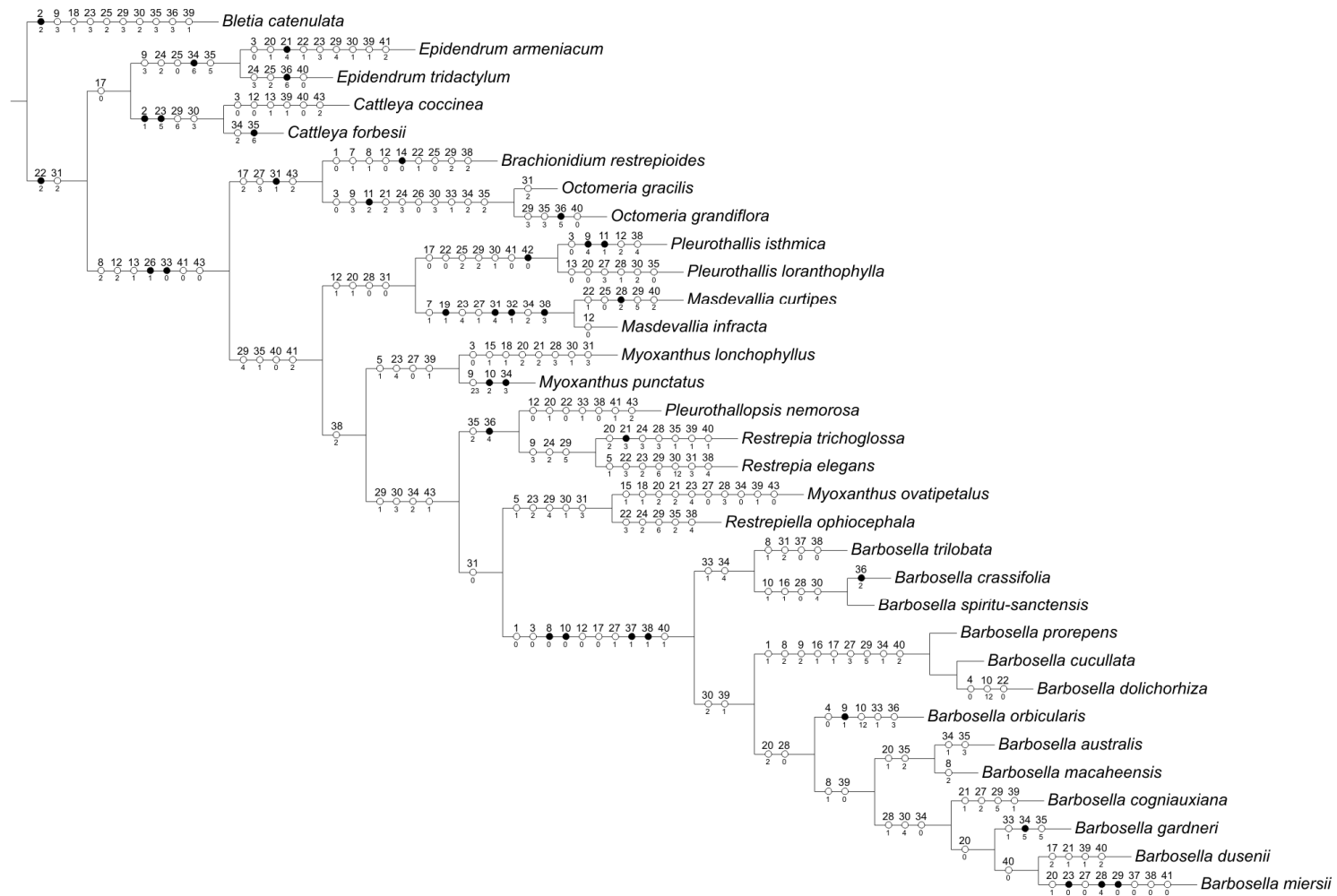


Fig. 3. Total evidence MP tree of *Barbosella* and affinities plus outgroups, resulting from the morphological matrix with characters (numbers above the circles) and character states (numbers below the circles) mapped. Character numbers are listed in Appendix S3. Black circles are apomorphies, white circles are homoplasies.

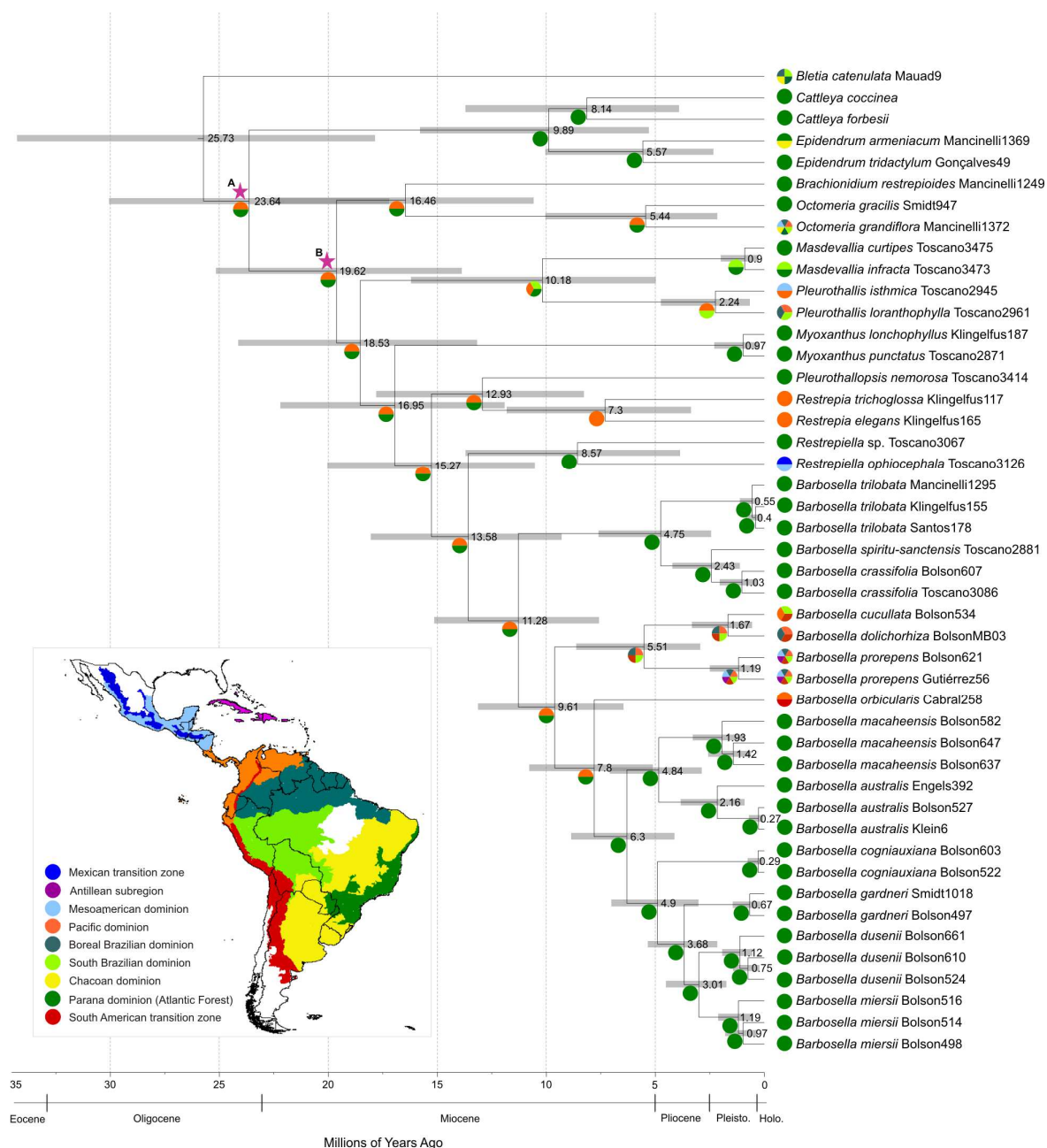


Fig. 4. Time calibrated tree for *Barbosella* (13 spp.) and related genera (7 spp.) plus outgroup inferred under uncorrelated lognormal clock model in BEAST with biogeographic inference. Stars show the points of calibration and constraints: A. Pleurothallidinae + Laeliinae clade and B. Pleurothallidinae clade. Numbers in front of the nodes indicate the estimated age and grey bars indicate the 95% highest posterior density interval. Coloured circles on tips represent the occurrence of that species in the delimited geographical areas. Coloured circles below the nodes show the most probable ancestral region using the best model BayArea-like according to the BioGeoBEARS analysis, and colour code follows the legend on the map. Pleisto., Pleistocene; Holo., Holocene.

Supporting Information

Appendix S1. Species for which sequences were newly produced for this study, with voucher information, locality of origin, geographic distribution and biogeographic coded areas, and GenBank Accession Numbers. Herbarium acronyms follows the *Index Herbariorum* (Thiers *et al.*, 2018 - continuously updated). Geographic regions following Morrone's (2014) proposal for Neotropic regions: A- Mexican Transition Zone; B- Mesoamerica; C- Pacific; D- Boreal Brazilian; E- South-Brazilian; F- Paraná (Atlantic Forest); G- South- American Transition Zone; H- Antillean; I- Chacoan.

Species	Subtribe	Voucher (Herbarium)	Locality of origin	Area	ITS	<i>atpI</i> - <i>atpH</i>	<i>matK</i>	<i>psbD</i> - <i>trnT</i> ^(GGU)	<i>trnH</i> - <i>psbA</i>	<i>trnQ</i> -5'- <i>rps16</i>
Ingroup										
<i>B. australis</i> (Cogn.) Schltr.	Pleurothallidinae	M. Bolson 527 (UPCB)	Brazil, Santa Catarina, Luzerna	F	OK	OK	OK	OK	OK	OK
<i>B. australis</i>	Pleurothallidinae	M.E. Engels 392 (UPCB)	Brazil, Paraná, Turvos do Paraná		OK	OK	OK	OK	OK	OK
<i>B. australis</i>	Pleurothallidinae	J. Klein 6 (UPCB)	Brazil, Rio Grande do Sul, São Francisco de Paula		OK	OK	OK	OK	OK	OK
<i>B. cogniauxiana</i> (Speg. & Kraenzl.) Schltr.	Pleurothallidinae	M. Bolson 522 (UPCB)	Brazil, Santa Catarina, Ibicaré	F	OK	OK	OK	OK	OK	OK
<i>B. cogniauxiana</i>	Pleurothallidinae	M. Bolson 603 (UPCB)	Brazil, Santa Catarina, Benedito Novo		OK	OK	OK	OK	OK	OK

<i>B. crassifolia</i> (Edwall) Schltr.	Pleurothallidinae	M. Bolson 607 (UPCB)	Brazil, Minas Gerais, Uberlândia	F	-	OK	OK	OK	OK	OK
<i>B. crassifolia</i>	Pleurothallidinae	A.L.V. Toscano de Brito 3086 (UPCB)	Brazil, Espírito Santo		OK	OK	OK	OK	OK	OK
<i>B. cucullata</i> (Lindl.) Schltr.	Pleurothallidinae	M. Bolson 534 (UPCB)	Ecuagenera Cia. Ltda., without locality	C,E,G	OK	OK	OK	OK	OK	OK
<i>B. dolichorhiza</i> Schltr.	Pleurothallidinae	M. Bolson MB 03 (UPCB)	Ecuagenera Cia. Ltda., without locality	C,D,G	OK	OK	OK	OK	OK	OK
<i>B. dusenii</i> (A. Samp.) Schltr.	Pleurothallidinae	M. Bolson 661 (UPCB)	Brazil, Minas Gerais, Santana do Riacho	F	OK	OK	-	OK	-	OK
<i>B. dusenii</i>	Pleurothallidinae	M. Bolson 524 (UPCB)	Brazil, Santa Catarina, Canoinhas		OK	OK	OK	OK	OK	OK
<i>B. dusenii</i>	Pleurothallidinae	M. Bolson 610 (UPCB)	Brazil, Paraná, Piraquara		OK	OK	OK	OK	OK	OK
<i>B. gardneri</i> (Lindl.) Schltr.	Pleurothallidinae	M. Bolson 497 (UPCB)	Brazil, Rio de Janeiro, Teresópolis, Parque Nacional da Serra dos Órgãos	F	OK	OK	OK	OK	OK	OK
<i>B. gardneri</i>	Pleurothallidinae	E.C. Smidt 1018 (UPCB)	Brazil, Paraná, Curitiba		OK	OK	OK	OK	OK	OK
<i>B. macaheensis</i> (Cogn.) Luer	Pleurothallidinae	M. Bolson <i>et al.</i> 582 (UPCB)	Brazil, Espírito Santo, Santa Teresa, Reserva Biológica Augusto Ruschi	F	OK	OK	OK	OK	OK	-

<i>B. macaheensis</i>	Pleurothallidinae	M. Bolson 637 (UPCB)	Brazil, Rio de Janeiro, Nova Friburgo		OK	-	-	OK	OK	OK
<i>B. macaheensis</i>	Pleurothallidinae	M. Bolson <i>et al.</i> 647 (UPCB)	Brazil, Rio de Janeiro, Nova Friburgo		OK	OK	OK	OK	OK	OK
<i>B. miersii</i> (Lindl.) Schltr.	Pleurothallidinae	M. Bolson 498 (UPCB)	Brazil, Rio de Janeiro, Teresópolis, Parque Nacional da Serra dos Órgãos	F	OK	OK	OK	OK	OK	OK
<i>B. miersii</i>	Pleurothallidinae	M. Bolson 514 (UPCB)	Brazil, Paraná, São José dos Pinhais		-	OK	OK	OK	OK	OK
<i>B. miersii</i>	Pleurothallidinae	M. Bolson 516 (UPCB)	Brazil, Santa Catarina, Benedito Novo		OK	OK	OK	OK	OK	OK
<i>B. orbicularis</i> Luer	Pleurothallidinae	M.R.P.F. Cabral 258 (UPCB)	Ecuagenera Cia. Ltda., without locality	C,G	OK	OK	OK	OK	OK	OK
<i>B. prorepens</i> (Rchb.f.) Schltr.	Pleurothallidinae	M. Bolson 621 (UPCB)	Ecuagenera Cia. Ltda., without locality	B,C,D,E,G ,H	OK	OK	OK	OK	OK	OK
<i>B. prorepens</i>	Pleurothallidinae	N. Gutiérrez M. 56 (JBB)	Colômbia, Santander, La Belleza		OK	OK	OK	-	OK	OK
<i>B. spiritu-sanctensis</i> (Pabst) F.Barros & Toscano	Pleurothallidinae	A.L.V. Toscano de Brito 2881 (UPCB)	Brazil, Espírito Santo, Santa Teresa	F	OK	OK	OK	OK	OK	OK
<i>B. trilobata</i> Pabst	Pleurothallidinae	M.L. Klingelfus, 155 (UPCB)	Brazil, Paraná, Ponta Grossa	F	OK	OK	OK	OK	OK	OK
<i>B. trilobata</i>	Pleurothallidinae	W.S. Mancinelli 1295 (JOI)	Brazil, Santa Catarina, Joinville		OK	OK	OK	OK	OK	OK

<i>B. trilobata</i>	Pleurothallidinae	T.F. Santos 178 (UPCB)	Brazil, Paraná, Tijucas do Sul		OK	OK	OK	OK	OK	OK
<i>Myoxanthus lonchophyllus</i> (Barb.Rodr.) Luer	Pleurothallidinae	M.L. Klingelfus 187 (UPCB)	Orquidário Marcos Klingelfus, without locality	F	OK	OK	OK	OK	OK	OK
<i>M. punctatus</i> (Barb.Rodr.) Luer	Pleurothallidinae	A.L.V. Toscano de Brito 2871 (UPCB)	Brazil, Espírito Santo, Santa Teresa, Estação Biológica de Santa Lúcia	F	OK	OK	OK	OK	OK	OK
<i>Pleurothallopsis nemorosa</i> (Barb.Rodr.) Porto & Brade	Pleurothallidinae	A.L.V. Toscano de Brito 3414 (UPCB)	Orquidário Sítio Paraíso das Microorquídeas, without locality	F	OK	OK	OK	OK	OK	OK
<i>Restrepia trichoglossa</i> F. Lehm. ex Sander	Pleurothallidinae	M.L. Klingelfus 117 (UPCB)	Orquidário Marcos Klingelfus, without locality	C	OK	-	OK	OK	OK	OK
<i>R. elegans</i> H.Karst.	Pleurothallidinae	M.L. Klingelfus 165 (UPCB)	Orquidário Marcos Klingelfus, without locality	C	OK	OK	OK	OK	OK	OK
<i>Restrepiella ophiocephala</i> (Lindl.) Garay & Dunst.	Pleurothallidinae	A.L.V. Toscano de Brito 3126 (SEL-spirit)	Panama, without locality	A,B	OK	OK	OK	OK	OK	OK
<i>Restrepiella</i> sp.	Pleurothallidinae	A.L.V. Toscano de Brito 3067 (UPCB)	Brazil, Espírito Santo, Santa Teresa	F	OK	OK	OK	OK	OK	OK
Outgroup										
<i>Bletia catenulata</i> Ruiz	Bletiinae	A.V.M.S. Mauad	Alvim Seidel Orquidário	D,E,F,I	OK	-	OK	OK	OK	-

& Pav.		09 (UPCB)	Catarinense, without locality							
<i>Brachionidium restrepioides</i> (Hoehne)	Pleurothallidinae	W.S. Mancinelli 1249 (UPCB)	Brazil, Santa Catarina, Garuva, Pico Garuva	F	OK	OK	OK	OK	OK	OK
Pabst										
<i>Cattleya coccinea</i> Lindl.	Laeliinae	T.F. Santos <i>et al.</i> 146 (UPCB)	Brazil, Paraná, Quatro Barras	F	-	-	OK	OK	OK	-
<i>C. coccinea</i>	Laeliinae	MWC6089	GenBank database		AY008646	-	-	-	-	-
<i>C. forbesii</i> Lindl.	Laeliinae	E.C. Smidt 967 (UPCB)	Brazil, Paraná, Pontal do Paraná	F	OK	-	OK	OK	-	-
<i>C. forbesii</i>	Laeliinae	-	GenBank database		-	-	-	-	EU140032	-
<i>Epidendrum armeniacum</i> Lindl.	Laeliinae	W.S. Mancinelli 1369 (UPCB)	Brazil, Santa Catarina, Joinville, Morro da Tromba	F,I	OK	-	OK	OK	-	-
<i>E. tridactylum</i> Lindl.	Laeliinae	G.F. Gonçalves 49 (SP)	Orquidário Frederico Carlos Hoehne, Instituto de Botânica de São Paulo, without locality	F	OK	-	OK	OK	-	-
<i>Masdevallia curtipes</i> Barb.Rodr.	Pleurothallidinae	A.L.V. Toscano 3475 (UPCB)	Brazil, Rio de Janeiro, Paty do Alferes	F	OK	-	OK	OK	OK	OK
<i>M. infracta</i> Lindl.	Pleurothallidinae	A.L.V. Toscano 3473 (UPCB)	Brazil, Rio de Janeiro, Paty do Alferes	E,F	OK	-	OK	OK	OK	OK
<i>Octomeria gracilis</i>	Pleurothallidinae	E.C. Smidt 947	Brazil, Paraná, Pontal do	F	OK	OK	OK	OK	OK	OK

Lodd.		(UPCB)	Paraná							
<i>O. grandiflora</i> Lindl.	Pleurothallidinae	W.S. Mancinelli 1372 (UPCB)	Brazil, Santa Catarina, Joinville, Morro da Tromba	B,C,D,E,F, I	OK	OK	OK	OK	OK	OK
<i>Pleurothallis isthmica</i> Luer	Pleurothallidinae	A.L.V. Toscano de Brito 2945 (SEL-spirit)	Panama, Chiriquí, La Fortuna	B,C	OK	OK	OK	OK	-	OK
<i>P. loranthophylla</i> Rchb. f	Pleurothallidinae	A.L.V. Toscano de Brito 2961 (SEL-spirit)	Venezuela, Guatopo	C,D,E	OK	-	OK	OK	-	-

* OK sequences to submit to GenBank; - missing data.

Appendix S2. List of primers used for PCR amplification and sequencing.

Region	Primer	Sequence (5' – 3')	Reference
ITS	17SE	ACG AAT TCA TGG TCC GGT GAA GTG TTC G	Sun <i>et al.</i> (1994)
	26SE	TAG AAT TCC CCG GTT CGC TCG CCG TTA C	Sun <i>et al.</i> (1994)
<i>atpI-atpH</i>	atpI	TAT TTA CAA GYG GTA TTC AAG CT	Shaw <i>et al.</i> (2007)
	atpH	CCA AYC CAG CAG CAA TAA C	Shaw <i>et al.</i> (2007)
<i>matK</i>	19F	CGT TCT CAT ATT GCA CTA TG	Pridgeon <i>et al.</i> (2001)
	881R	TMT TCA TCA GAA TAA GAG T	Pridgeon <i>et al.</i> (2001)
<i>psbD-trnT^(GGU)</i>	psbD	CTC CGT ARC CAG TCA TCC ATA	Shaw <i>et al.</i> (2007)
	trnT ^(GGU) -R	CCC TTT TAA CTC AGT GGT AG	Shaw <i>et al.</i> (2007)
<i>trnH-psbA</i>	trnHf_05	CGC GCA TGG TGG ATT CAC AAT CC	Tate (2002)
	psbA3F	GTT ATG CAT GAA CGT AAT GCT C	Sang <i>et al.</i> (1997)
<i>trnQ-5'-rps16</i>	trnQ ^(UUG)	GCG TGG CCA AGY GGT AAG GC	Shaw <i>et al.</i> (2007)
	rpS16x1	GTT GCT TTY TAC CAC ATC GTT T	Shaw <i>et al.</i> (2007)

Appendix S3. List of morphological characters and their corresponding states of the species used in this study.

Character and states
1. Plant, habit: (0) repent, (1) caespitose
2. Rhizome, secondary stem, type: (0) simple, (1) pseudobulbous, (2) corm
3. Secondary stem, form: (0) ascending, (1) erect
4. Secondary stem, sheath: (0) absent, (1) present
5. Secondary stem, sheaths indument: (0) absent, (1) present
6. Secondary stem, in pairs: (0) absent, (1) present
7. Annulus, association: (0) absent, (1) present
8. Leaf, disposal: (0) prostrate, (1) suberect, (2) erect
9. Leaf, form: (0) elliptical, (1) orbicular, (2) obovate, (3) lanceolate, (4) ovate
10. Leaf, apex: (0) minutely emarginate and mucronate, (1) acute, (2) obtuse
11. Leaf, base: (0) cuneate, (1) rounded, (2) attenuate
12. Inflorescence, type: (0) single-flowered raceme, (1) multi-flowered raceme, (2) single-flowered fascicle, (3) multi-flowered fascicle
13. Inflorescence, flower, anthesis: (0) simultaneous, (1) successive
14. Inflorescence, flowers resupinate: (0) absent, (1) present
15. Inflorescence, peduncle indument: (0) glabrous, (1) pubescent
16. Inflorescence, bract position: (0) basal, (1) below the middle
17. Inflorescence, floral bract form: (0) oblique, (1) tubular, (2) infundibular and mucronate, (3) amplexicaul
18. Inflorescence, floral bractea indument: (0) glabrous, (1) pubescent
19. Flower, dorsal sepal conation with lateral sepals: (0) free, (1) united
20. Flower, dorsal sepal disposal: (0) erect, (1) suberect, (2) slightly recurved
21. Flower, dorsal sepal form: (0) ovate, (1) linear, (2) oblong, (3) elliptical, (4) lanceolate
22. Flower, dorsal sepal apex: (0) acute, (1) acuminate, (2) obtuse, (3) thickened
23. Flower, dorsal sepal venation (n) : (0) 1, (1) 3, (2) 5, (3) 6, (4) 7, (5) multiple
24. Flower, lateral sepal form: (0) ovate, (1) triangular, (2) lanceolate, (3) oblong
25. Flower, lateral sepal apex: (0) acuminate, (1) obtuse, (2) acute
26. Flower, lateral sepal connation: (0) absent, (1) present
27. Flower, lateral sepal connation form: (0) at the base, (1) in the middle, (2) near the apex, (3) completely
28. Flower, lateral sepal synsepal shape: (0) forming a mentum, (1) slightly concave at the base, (2) deeply concave, (3) more and less revolute, (4) flat
29. Flower, lateral sepal venation (n) : (0) 1, (1) 3, (2) 4, (3) 5, (4) 6-each, (5) 6-together, (6) multiple
30. Flower, petal form: (0) oblong, (1) linear, (2) ovate, (3) elliptical, (4) lanceolate, (5) subfalcate
31. Flower, petal apex: (0) acute, (1) acuminate, (2) obtuse, (3) thickened, (4) truncate

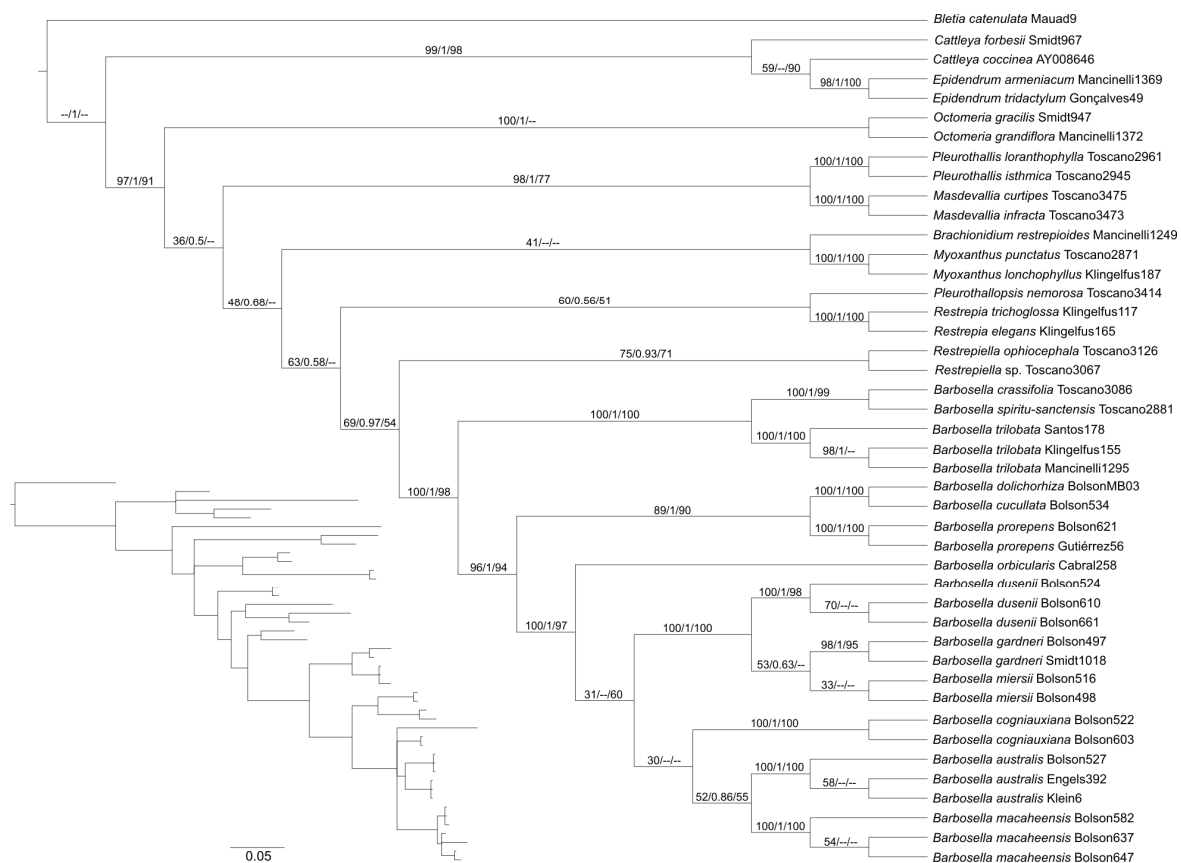
- 32. Flower, petals calli: (0) absent, (1) present
 - 33. Flower, lip: (0) entire, (1) trilobed
 - 34. Flower, lip form: (0) elliptical, (1) suboblong, (2) oblong, (3) ligulate, (4) ovate, (5) subpandurate, (6) obovate
 - 35. Flower, lip apex: (0) subacute, (1) obtuse, (2) truncate, (3) two obtuse angles, (4) apiculate, (5) acuminate, (6) denticulated
 - 36. Flower, lip lateral lobes form: (0) rounded, (1) uncinat, (2) triangular, (3) oblong, (4) subfalcate, (5) falciform, (6) obovate
 - 37. Flower, lip base articulation with the column foot: (0) absent, (1) present
 - 38. Column, foot shape: (0) not bulbous, (1) bulbous, (2) stout, (3) curved, (4) pedestal-like
 - 39. Column, wings: (0) absent, (1) present
 - 40. Column, apex: (0) rounded, (1) denticulate, (2) apiculate
 - 41. Column, anther position: (0) apical, (1) subapical, (2) ventral
 - 42. Column, stigma position: (0) apical, (1) ventral
 - 43. Column, pollinia (n) : (0) 2, (1) 4, (2) 8
-

Appendix S4. Matrix of morphological characters used in this study.

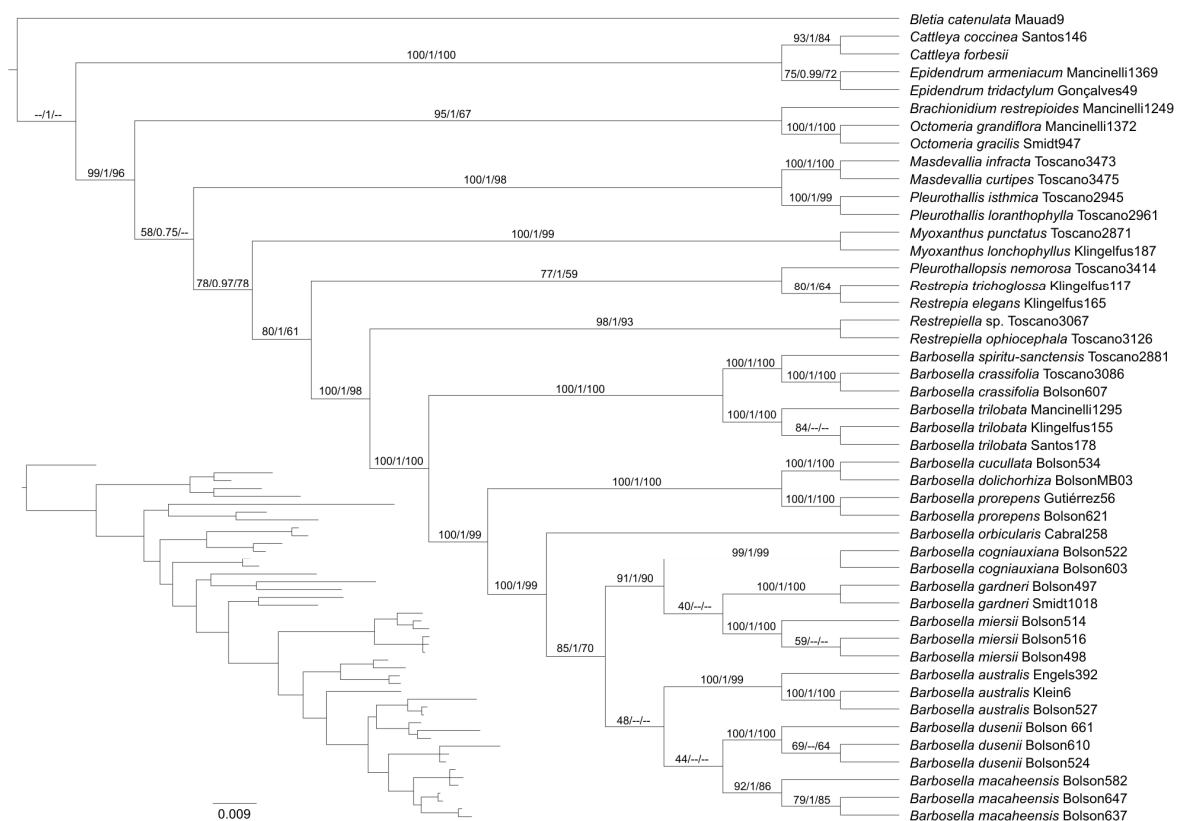
<i>Barbosella australis</i>	00010001000011000001021011101{2 4}00013?1101211
<i>Barbosella cogniauxiana</i>	00010001000011000002121{0 3}11215{1 4}0000{0 1}?111{1 2}211
<i>Barbosella crassifolia</i>	00010000{0 1}{1 2}{0 1}01101000002101110140014121101211
<i>Barbosella cucullata</i>	{0 1}0010002{2 3}00011011000{0 1}21011315{2 4}00011?1112211
<i>Barbosella dolichorhiza</i>	10000002{2 3}{1 2}0011011000{0 1}01{0 1}11315{2 4}00011?1112211
<i>Barbosella dusenii</i>	00010001{0 1}00011002000121{0 2}11{1 2}114000{0 2}{0 1}?1112211
<i>Barbosella gardneri</i>	00010001{0 3}00011000000021011111{0 4}0015501101211
<i>Barbosella macaheensis</i>	00010002{0 3}00011000001021{0 1}11{1 2}01{2 5}00022?1101211
<i>Barbosella miersii</i>	00010001{0 3}000110000010{0 2}00{1 2}1040{2 4}000{0 2}1?0000011
<i>Barbosella orbicularis</i>	000000001{1 2}{0 1}01100{0 1}002021011{1 2}01{2 4}0012{0 1}31111211
<i>Barbosella prorepens</i>	{0 1}0010{0 1}02{2 3}0001101100{0 2}{0 1}21011315{1 2}00011?1112211
<i>Barbosella spiritu-sanctensis</i>	00010000{0 1}{1 2}{0 1}011010000021011{1 2}0140014101101211
<i>Barbosella trilobata</i>	00010001000011000000021011{1 2}1132014{0 1}00001211
<i>Bletia catenulate</i>	1211???13101010{0 1}1100003020??320014330011111
<i>Brachionidium restrepioides</i>	001100110100100{0 1}2000011001312010040?0201012
<i>Cattleya coccinea</i>	1101???10{1 2}001100{0 1}000025010??632014{0 1}00010112
<i>Cattleya forbesii</i>	1111???10{1 2}010100{0 1}000025010??632012600001111
<i>Epidendrum armeniacum</i>	1001???1310101000001413200??412016500011211
<i>Epidendrum tridactylum</i>	1011???131010100{0 1}000021320??10201??60000111
<i>Masdevallia infracta</i>	101100120{1 2}011100{0 1}011{0 2}140011250410{2 5}{1 2}?0302210
<i>Masdevallia curtipes</i>	10110012010011001011{0 2}240111040{1 4}10{2 5}{1 2}?0300210
<i>Myoxanthus lonchophyllus</i>	1001100201021110{0 1}102224011034130041?0210210
<i>Myoxanthus punctatus</i>	1011100201021110{0 1}1022240{0 1}1034130001?0210210
<i>Octomeria gracilis</i>	100100023122110???00221310??132012200001012
<i>Octomeria grandiflora</i>	100100023122{0 1}10???00221310??3{3 4}{0 1}012350000012
<i>Pleurothallis isthmica</i>	10010002411211000001001021202{1 3}00041?0400000
<i>Pleurothallis loranthophylla</i>	10110002010101000000001021312200040?0000000
<i>Pleurothallopsis nemorosa</i>	101100020{1 2}001100{0 1}001001011211{0 3}2012240000112
<i>Restrepiella ophioccephala</i>	101100023{1 2}021100{0 1}0023213112353200{2 3}1?0211211
<i>Restrepiella</i> sp.	10111002{2 3}2021100{0 1}00002?01101?020031?0210210
<i>Restrepia trichoglossa</i>	10111002{0 3}{1 2}0211001000032211216{1 2}30022?0400211
<i>Restrepia elegans</i>	10111002{0 3}{1 2}{0 1}211001000032211216{1 2}30022?0400211

Appendix S5. Molecular characteristics of the all regions and combined matrices.

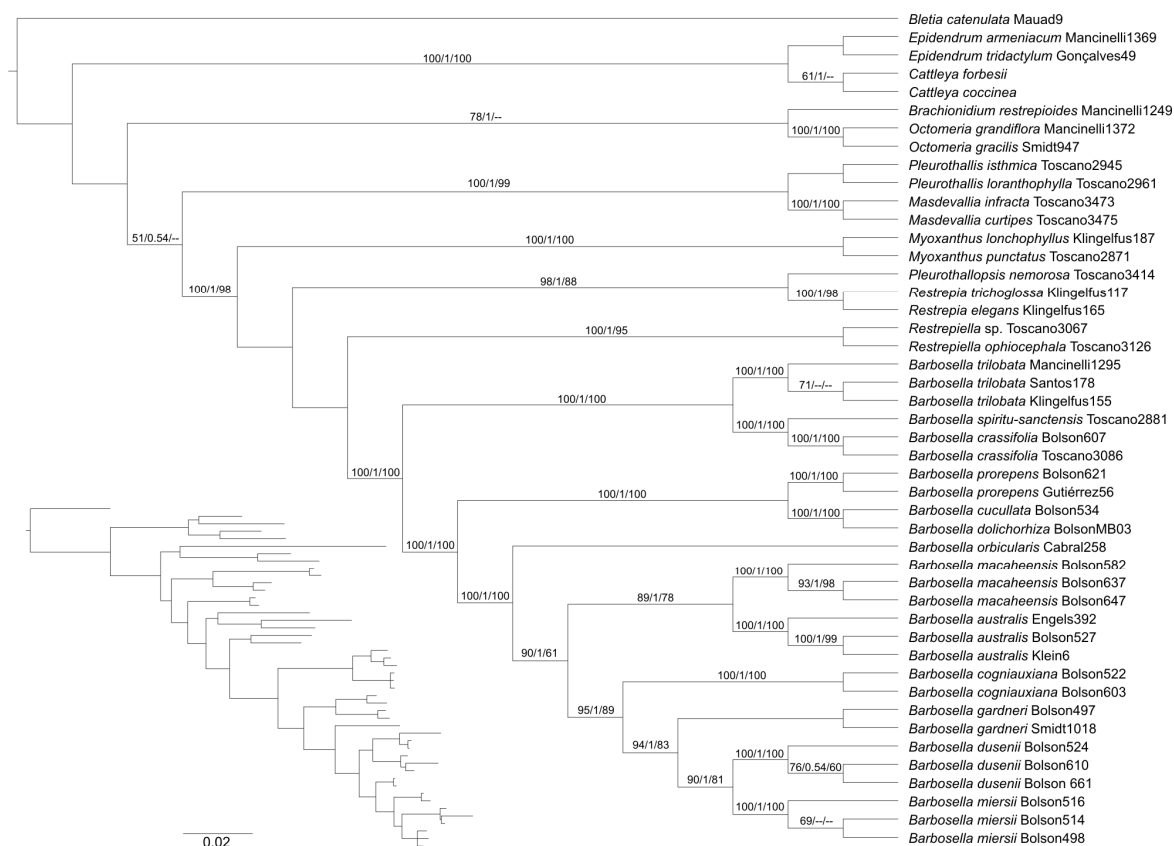
Region	<i>N</i> individuals in the matrix (<i>N</i> species)	Alignment length (bp)	<i>N</i> conserved characters (CC)	<i>N</i> variable characters parsimony- uninformative (VCPU)	<i>N</i> parsimony- informative characters (PIC)	<i>N</i> trees retained	Tree length	Consistency index (CI)	Retention index (RI)
ITS	44 (32)	797	454	95	248	4	935	0.56	0.76
<i>atpI-atpH</i>	36 (23)	999	726	135	138	6	376	0.80	0.88
<i>matK</i>	43 (31)	963	758	70	135	72	341	0.68	0.84
<i>psbD-trnT^(GGU)</i>	45 (32)	1230	902	152	176	2908	519	0.73	0.83
<i>trnH-psbA</i>	41 (28)	932	698	125	109	10000	382	0.78	0.81
<i>trnQ-5'-rps16</i>	39 (26)	579	392	50	137	191	313	0.69	0.85
Plastid	46 (32)	4703	3476	532	695	9	2079	0.68	0.80
Molecular combined	46 (32)	5500	3930	627	943	27	3032	0.64	0.78
Total evidence	46 (32)	5543	3907	650	986	9	3321	0.63	0.77



Appendix S6. Nuclear (ITS) ML tree. Numbers on branches represent ML bootstrap percentages, posterior probability from BI, and MP bootstrap percentages (values >50 are shown). In detail, the BI tree has proportional branch lengths.



Appendix S7. Plastid ML tree. Numbers on branches represent ML bootstrap percentages, posterior probability from BI, and MP bootstrap percentages (values >50 are shown). In detail, the BI tree has proportional branch lengths.



Appendix S8. Molecular combined ML tree. Numbers on branches represent ML bootstrap percentages, posterior probability from BI, and MP bootstrap percentages (values >50 are shown). In detail, the BI tree has proportional branch lengths.

Appendix S9. Results of the fit of the six biogeographic models tested in BioGeoBEARS, including Log-likelihood $\ln(L)$, Dispersal rate (d), Extinction rate (e), Founder-event speciation rate (j), Akaike information criterion corrected for sample size ($AICc$), and Akaike weights ($AICc_{wt}$) showing the relative likelihood of each model. In bold the preferred model.

Model	$\ln L$	N parameters	d	e	j	$AICc$	$AICc_{wt}$
DEC	-117.4	2	0.010	2.0e-08	0	239	3.0e-05
DEC+j	-117.4	3	0.010	1.0e-12	1.0e-05	241.3	9.7e-06
DIVA-like	-121.5	2	0.012	1.0e-12	0	247.2	5.2e-07
DIVA-like+j	-121.5	3	0.012	1.0e-12	1.0e-05	249.5	1.7e-07
BayArea-like	-107.3	2	0.0069	0.051	0	218.8	0.76
BayArea-like+j	-107.3	3	0.0069	0.051	1.0e-05	221.1	0.24

CAPÍTULO II / CHAPTER II

**Anatomia comparada dos órgãos vegetativos de *Barbosella* (Orchidaceae,
Pleurothallidinae)**

Comparative vegetative anatomy of *Barbosella* (Orchidaceae, Pleurothallidinae)

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Anatomia comparada dos órgãos vegetativos de *Barbosella* (Orchidaceae, Pleurothallidinae)¹

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Abstract

Barbosella comprises 20 species of epiphytic orchids occurring in the rainforest of the Andes and Atlantic Forest. We examined the anatomy of root, rhizome, ramicaul, and leaf of 13 species of *Barbosella* plus five species of Pleurothallidinae as outgroup taxa, with the aim to describe the anatomy of the vegetative organs. Ten anatomical characters are exclusively shared among *Barbosella* species: five layers of root cortex; conspicuous rhizome; ramicaul with absence of the sclerified outer layer of the cortex, absence of vascular bundles connected by perivascular fibers and absence of pith; and flat leaf in cross-section, absence of longitudinal groove on upper surface, thin cuticle thickness, absence of bundles in margins. Although the vegetative anatomy is considered homoplastic for the subtribe, the results show us the phylogenetic value when analyzed among species of the same genus.

Key words: *Anatomical characters, leaf, Monocots, roots, ramicaul, rhizome*

Resumo

Barbosella compreende 20 espécies de orquídeas epífitas, com ocorrência na região dos Andes e na Floresta Atlântica. Neste trabalho examinamos a anatomia da raiz, rizoma, ramicaule e folha de 13 espécies de *Barbosella*, mais cinco espécies de Pleurothallidinae como grupo externo, com o objetivo de descrever anatomicamente os órgãos vegetativos. Dez caracteres anatômicos são compartilhados exclusivamente entre os representantes de *Barbosella*, como: raiz com cinco camadas de córtex; rizoma conspícuo; ramicaule com camada externa esclerificada do córtex ausente, ramicaule com os feixes vasculares não conectados por fibras e medula ausente; folha com formato plano em secção transversal, ausência de sulco na porção mediana longitudinal da face adaxial, cutícula fina, feixes no bordo foliar não predominantes, mas quando presentes são do tipo fibroso. Os resultados mostraram que anatomia vegetativa, apesar de ser considerada homoplástica para a subtribo, possui valor filogenético quando analisada entre espécies de um mesmo gênero.

Palavras-chave: *Caracteres anatômicos, folha, Monocotiledôneas, raiz, ramicaule, rizoma*

Introdução

A subtribo Pleurothallidinae Lindl. ex G.Don, considerada a mais diversa dentro da família Orchidaceae, atualmente compreende 44 gêneros e cerca de 5100 espécies (Karremans 2016), representando aproximadamente 20% de toda a família (Pridgeon et al. 2001, Higgins & Williams 2009, Karremans 2016). Embora distribuída exclusivamente no Neotrópico, as espécies de Pleurothallidinae ocorrem em diferentes habitats e apresentam diferentes formas de crescimento (Luer 1986, Pridgeon et al. 2001).

Barbosella Schltr. um dos gêneros reconhecidos em Pleurothallidinae, compreende 20 espécies com ampla distribuição, desde a América Central através das florestas úmidas dos Andes, até o norte da Argentina e sul do Brasil, distribuídas pela Floresta Atlântica (Pridgeon 2005, Brazilian Flora 2020, in construction). São ervas geralmente epífitas, com crescimento cespitoso ou reptante, capazes de formar grandes e densos tapetes. O ramicaule é abreviado, sem ânulos, e sempre menor do que a folha. As folhas são geralmente coriáceas, elípticas a suborbiculares. A inflorescência é sempre um racemo unifloro, com um pedúnculo longo e delicado, que emerge lateralmente a partir do ramicaule; o labelo possui uma base côncava com um distinto sistema de articulação livre com o pé da coluna, denominado *ball-and-socket*; e a antera possui quatro polínias de tamanhos iguais (Luer 2000, Pridgeon 2005) (Figura 1).

Recentemente vários estudos filogenéticos evidenciam o monofiletismo de Pleurothallidinae (Pridgeon et al. 2001, Pridgeon & Chase 2001, Pridgeon & Chase 2003, Karremans 2016, Pérez-Escobar et al. 2017), no entanto, mostram também que ainda há necessidade de mais esforços para entendermos a posição de todas as linhagens dentro dessa enorme subtribo. A mais recente atualização das relações filogenéticas em Pleurothallidinae reconhece nove clados principais, sendo *Barbosella* posicionado dentro da afinidade *Restrepia* (Re) juntamente com mais sete gêneros: *Chamelophyton* Garay, *Dresslerella* Luer, *Echinosepala* Pridgeon & M.W.Chase, *Myoxanthus* Poepp. & Endl., *Pleurothallopsis* Porto & Brade, *Restrepia* Kunth and *Restrepiella* Garay & Dunst. (Karremans 2016).

Reconstruções filogenéticas em Orchidaceae têm demonstrado que caracteres morfológicos internos e/ou externos podem ser ferramentas importante na taxonomia da família (Pridgeon 1982, Neyland et al. 1995, Figueroa et al. 2008, Carlsward & Stern 2009, Smidt et al. 2013). Os principais estudos anatômicos realizados com Pleurothallidinae (Pridgeon & Williams 1979, Pridgeon 1981, 1982, Benzing & Pridgeon 1983, Neyland et

al. 1995) enfatizam que as raízes e folhas apresentam características anatômicas importantes que auxiliam na taxonomia e podem ser determinantes para a identificação de alguns gêneros.

Descrições anatômicas dos órgãos vegetativos de representantes de *Barbosella* foram realizadas apenas por Pridgeon (1982) e Stern (2014). A morfologia vegetativa, tanto quanto a morfologia floral, é muito variável dentro do gênero; no entanto, Luer (2000) enfatiza que *Barbosella* é um dos únicos gêneros dentro da subtribo que apresenta caracteres vegetativos, como o tamanho e forma das folhas, determinantes na identificação e reconhecimento de algumas espécies. Tendo em vista esta grande variação morfológica entre as espécies de *Barbosella*, estudos anatômicos com uma maior amostragem podem colaborar na compreensão das relações evolutivas do gênero, bem como para a subtribo.

O objetivo deste trabalho foi descrever anatomicamente os órgãos vegetativos (raiz, rizoma, ramicaule e folha) de representantes de *Barbosella*, bem como de gêneros relacionados e o grupo externo.

Material e métodos

Amostragem

As amostras foram coletadas em campo ou em coleções privadas de diferentes localidades. Um total de 13 espécies de *Barbosella* foram amostradas devido a disponibilidade de material, somado a mais três espécies de gêneros da afinidade *Restrepia* (Karremans 2016) como grupo interno e, *Octomeria grandiflora* Lindl. amostrada como grupo externo (Tabela I). Para cada espécie, sempre que possível pelo menos mais de um espécime de localidade diferente foi analisado (ver Tabela I).

Microscopia de luz (ML)

Amostras de raiz, rizoma, ramicaule e lâmina foliar foram fixadas em FAA 50 (Johansen 1940) e estocadas em etanol 70%. A região mediana de cada material foi seccionado à mão livre com o auxílio de lâmina de barbear, corado com azul de alcian 8GX e fucsina básica (Luque et al. 1996) e montado em lâminas semi-permanentes usando gelatina glicerina (Kaiser 1880). Algumas amostras de raiz, rizoma e ramicaule foram desidratadas em série etílica, processadas em Leica Historesin® de acordo com as instruções do fabricante e

seccionadas de 5 a 7 μm de espessura em micrótomo rotativo. Os cortes foram corados com azul de toluidina (O' Brien et al. 1965). Testes histoquímicos foram realizados com lugol (Johansen 1940) para a detecção de amido e floroglucinol acidificado (Foster 1949) para a detecção de lignina. A dissociação do tecido foliar realizada de acordo com Franklin (1945) e corada com Safranina 1% em etanol a 50% e montada em lâminas semi-permanentes. As fotografias foram realizadas usando o microscópio Olympus EX41 acoplado a uma câmera digital usando o software analySISgetIT.

O rizoma e o ramicaule são inconspícuos em algumas das espécies estudadas, e por isso não foram descritos anatomicamente para as espécies que apresentam este órgão abreviado (ver Tabela I).

Microscopia Eletrônica de Varredura (MEV)

Para as observações em MEV, a região mediana de amostras foliares já fixadas foram desidratadas em série etílica, submetidas ao ponto crítico (CPD 030, Leica®) e metalizadas com ouro (Balzers Union FL 9496 SCD030). O registro eletromicrográfico foi realizado com o microscópio eletrônico de varredura TESCAN VEGA3 LMU.

Resultados

Raiz

As raízes da maioria das espécies estudadas, em secção transversal, apresentam duas a quatro camadas de velame (Figura 2 (A-E)), com exceção de *B. trilobata* que apresenta apenas uma camada de células quadradas (Figura 2 (F)). As camadas do velame podem ser diferenciadas apresentando células quadradas na camada externa e células radialmente alongadas na camada interna (*B. australis*, *B. cogniauxiana*, *B. macaheensis* e *R. elegans*) (Figura 2 (A, C)). Camadas lignificadas do velame foram observadas em *M. lonchophyllus* (Figura 2 (D)).

Em secção transversal, a exoderme também sofre variação quanto ao formato de suas células, sendo o formato quadrado o mais comum entre as espécies (Figura 2 (B, C, D, G)), exceto *B. australis*, *B. cogniauxiana*, *B. crassifolia*, *B. prorepens*, *B. spiritu-sanctensis* e *R. elegans* que apresentam células radialmente alongadas (Figura 2 (A)). O espessamento da exoderme apresenta-se em forma de U em quase todas as espécies (Figura 2 (A)),

exceto em *B. trilobata* que possui espessamento em forma de U invertido (Figura 2 (F)), e em *B. crassifolia*, *R. elegans* e *O. grandiflora* que possuem espessamento em forma de O (Figura 2 (E)). O número de camadas do córtex pode variar de quatro (*B. miersii* e *O. grandiflora*) (Figura 2 (E)) a oito camadas (*M. lonchophyllus*). A endoderme apresenta células em formato irregular e espessamento em forma de O (Figura 2 (E)), apenas em *R. elegans* o formato das células da endoderme é retangular. As células do apresentam paredes não espessadas em todas as espécies (Figura 2 (H)).

O número de pólos do protoxilema também varia entre as espécies (Figura 2 (F, G, H)), em menor número em *B. dusenii*, *B. gardneri*, *B. miersii* (três) e em maior em *B. cucullata* (12). A medula das raízes possui células de paredes espessadas (Figura 2 (A, E, F, G)), exceto *B. cucullata* e *M. lonchophyllus* que apresentam células somente com parede primária (Figura 2 (H)).

Rizoma

Todas as espécies apresentam rizoma em formato circular em secção transversal (Figura 3 (A, B)), com cutícula espessa (Figura 3 (B)), epiderme uniestratificada de células com parede periclinal convexa (Figura 3 (C, D)), com exceção de *B. gardneri* que apresenta células com parede periclinal reta (Figura 3 (A)). As camadas de células do córtex variam de quatro (*B. orbicularis*, *B. spiritu-sanctensis* e *B. trilobata*) (Figura 3 (C)) a sete camadas (*B. macaheensis*).

O número de feixes vasculares pode variar de seis (*B. miersii*) (Figura 3 (B)) até 17 feixes (*B. australis*) (Figura 3 (D)), todos organizados em círculo e envoltos por fibras perivasculares. Os feixes medulares ocorrem apenas em *B. australis* (Figura 3 (D)), *B. cogniauxiana*, *B. macaheensis*, *B. spiritu-sanctensis* e *B. trilobata*.

Ramicaule

Em secção transversal, a maioria das espécies apresenta ramicaule em formato circular (Figura 3 (E)), exceto *B. australis*, *B. cucullata*, *B. dolichorhiza* e *B. gardneri* das quais apresentam formato semicircular (Figura 3 (G)). A epiderme é uniestratificada em todas as espécies e esclerificada apenas em *M. lonchophyllus* (Figura 3 (F)), apresentando células com parede periclinal convexa, no entanto, células com parede periclinal reta são encontradas em *B. cucullata*, *B. dolichorhiza* e *B. gardneri* (Figura 3 (G)), e papilosas

apenas em *B. prorepens*. Nas espécies de *Barbosella* a cutícula é mais fina do que nas espécies dos demais gêneros (Figura 3 (F, H)).

Em todas as espécies, as células do córtex apresentam formato arredondado e apresentam variação de três (*B. cogniauxiana*) até 14 camadas (*B. prorepens*). Camadas subepidérmicas esclerificadas são encontradas em *R. elegans* e *O. grandiflora* (uma camada) e, *M. lonchophyllus* e *P. nemorosa* (duas camadas) (Figura 3 (E, H)). O parênquima aerífero está presente somente no córtex interno de *B. australis*, *B. dolichorhiza*, *B. cucullata*, *P. Nemorosa* (Figura 3 (H)).

Os feixes vasculares apresentam-se dispostos na região central, variando de três (Figura 3 (G)) a cinco feixes entre espécies de *Barbosella*, e em maior número nos demais gêneros, 13 feixes em *P. nemorosa*, 32 feixes em *M. lonchophyllus*, 35 feixes em *R. elegans* e 44 feixes em *O. grandiflora* (Figura 3 (E, I)). Os feixes vasculares apresentam-se organizados em anel (Figura 3 (E, I)), mas em *B. australis*, *B. cucullata*, *B. dolichorhiza* e *B. gardneri* organizam-se em semicírculo (Figura 3 (G)). Fibras perivasculares foram observadas em todas as espécies e podem ser interligadas em *B. cogniauxiana*, *B. macaheensis*, *M. lonchophyllus*, *P. nemorosa*, *R. elegans* e *O. grandiflora* (Figura 3 (E, H, I)). A medula está presente apenas em *B. cogniauxiana*, *M. lonchophyllus*, *P. nemorosa*, *R. elegans* e *O. grandiflora* (Figura 3 (E, I)); com exceção dessa última que apresenta células com parede espessada (lignificada), as demais apresentam células somente com parede delgada.

Folha

Em secção transversal, a folha na maioria das espécies é de formato plano (Figura 4 (A)), enquanto *B. cucullata*, *B. dolichorhiza*, *B. prorepens*, *M. lonchophyllus*, *P. nemorosa*, *R. elegans* e *O. grandiflora* possuem formato semiplano (Figura 4 (B)). As espécies com folha de formato semiplano apresentam um sulco na região mediana da face adaxial (Figura 4 (B)), e aparece de forma menos aparente em *B. australis* e *B. macaheensis* (Figura 4 (C)).

Todas as espécies possuem cera em ambas as superfícies foliares, com diferentes variações dentro da mesma espécie, sendo o tipo em grânulos o mais comum (Figura 4 (D)). O tipo de cera camada suave ocorre em *B. crassifolia*, *B. cucullata*, *B. dolichorhiza*, *B. dusenii*, *B. gardneri*, *B. orbicularis*, *M. lonchophyllus* e *R. elegans*. (Figura 4 (E)), já o

tipo de cera em plaqueta é o menos comum, presente somente em *B. cucullata*, *B. gardneri* e *B. prorepens* (Figura 4 (E)).

As folhas geralmente são cobertas por uma cutícula fina (Figura 4 (F)), no entanto em *B. cucullata*, *B. dolichorhiza*, *M. lonchophyllus*, *P. nemorosa*, *R. elegans* e *O. grandiflora* a cutícula é espessa (Figura 4 (G)). A ornamentação da cutícula ocorre em ambas as faces em *B. australis*, *B. cogniauxiana*, *B. gardneri*, *B. macaheensis*, *B. orbicularis*, *B. prorepens* e *M. lonchophyllus* (Figura 4 (H)), e apenas na face abaxial em *B. miersii*, *B. spiritu-sanctensis*, *B. trilobata*, *P. nemorosa* e *O. grandiflora*. Nas demais espécies a cutícula não é lisa.

Em vista frontal, o formato das células é irregular em todas as espécies (Figura 4 (I)), ou alongado como em *B. cucullata*, *B. dolichorhiza* e *B. prorepens* (Figura 4 (J)). As paredes anticlinais das células da epiderme apresentam-se curvas na maioria das espécies de *Barbosella* (Figura 4 (I)), exceto em *B. cogniauxiana*, *B. dolichorhiza*, *B. prorepens* e *B. trilobata* e os demais gêneros que as apresentam retas (Figura 4 (J)).

A epiderme foliar é uniestratificada em ambas as faces (Figura 4 (B, C)) e em corte transversal, a epiderme da face adaxial apresenta células com parede periclinal convexa na maioria das espécies, mas células com parede periclinal reta ocorre em *B. cogniauxiana*, *B. crassifolia*, *B. dolichorhiza*, *B. dusenii*, *B. spiritu-sanctensis* e *P. nemorosa* (Figura 4 (G)). A epiderme da face abaxial também apresenta células com parede periclinal convexa na maioria das espécies (Figura 4 (F)), porém células com parede periclinal reta foram observadas em *B. dolichorhiza*, *B. dusenii*, *B. orbicularis* e *O. grandiflora*, e células com parede periclinal em formato elíptico foram encontradas em *P. nemorosa*.

As folhas de todas as espécies analisadas são hipoestomáticas e na maioria os estômatos encontram-se no mesmo nível das demais células epidérmicas; abaixo do nível das células epidérmicas em *B. cucullata* e *B. dolichorhiza* (Figura 4 (K)); ou acima da epiderme como em *B. trilobata* (Figura 4 (L)). A partir da dissociação da epiderme foi possível observar que, em geral, os estômatos possuem quatro células subsidiárias em todas as espécies (Figura 4 (I, J)).

O número de camadas do mesofilo pode variar de oito (*B. macaheensis* e *O. grandiflora*) a 20 camadas (*B. dolichorhiza*), e todas as espécies apresentam uma única camada de hipoderme na face abaxial, e o espessamento das células ocorre em *B. crassifolia*, *B. cucullata*, *B. dusenii*, *B. macaheensis*, *B. orbicularis*, *B. prorepens*, *B. spiritu-sanctensis* e *B. trilobata*.

O parênquima clorofiliano é homogêneo em todas as espécies (Figura 4 (B, C); Figura 5 (A, B, C)), exceto *P. nemorosa*. O parênquima aquífero se encontra disperso por todo o mesofilo na maioria das espécies (Figura 5 (A, C)) ou encontrado apenas na face adaxial como em *B. cucullata*, *B. dolichorhiza*, *B. gardneri*, *B. miersii*, *B. orbicularis*, *M. lonchophyllus* e *O. grandiflora* (Figura 5 (B)).

O formato dos idioblastos traqueoidais com espessamento helicoidal apresenta formato cilíndrico e globoso na maioria das espécies (Figura 5 (C)), no entanto, em *B. cucullata*, *B. dolichorhiza*, *B. macaheensis*, *B. miersii*, *B. orbicularis*, *B. prorepens* e *B. trilobata* possuem idioblastos somente em formato globoso (Figura 5 (D, G)).

O número de feixes vasculares pode variar de três (*B. miersii*, *B. spiritu-sanctensis* e *B. trilobata*) a 12 feixes (*B. cucullata*), e estão dispostos em fileira na região mediana da folha, em secção transversal (Figura 4 (B, C); Figura 5 (A, C)). Em *B. cogniauxiana*, *B. gardneri* e *B. miersii* os feixes estão organizados próximos à face abaxial (Figura 5 (B)). A maioria das espécies apresenta células em arranjo radial em torno do feixe vascular (Figura 5 (D)), com exceção de *B. cucullata*, *B. dolichorhiza*, *B. macaheensis*, *B. miersii*, *B. prorepens*, *B. trilobata* e *R. elegans*. O calibre dos feixes vasculares pode ser variável na maioria das espécies, alternando feixes de calibre maiores com feixes de menor calibre (Figura 5 (A, C)), ou apenas apresentar feixes de mesmo calibre como em *B. cogniauxiana*, *B. gardneri*, *B. miersii*, *B. spiritu-sanctensis* e *B. trilobata* (Figura 5 (B)).

Os bordos da folha, em secção transversal podem apresentar um feixe vascular em *M. lonchophyllus*, *P. nemorosa*, *R. elegans* e *O. grandiflora* (Figura 5 (E)); e um feixe de fibras na maioria das espécies de *Barbosella* (Figura 4 (H); Figura 5 (F)). As demais espécies possuem os bordos preenchidos por tecido parenquimático (Figura 5 (G)).

Discussão

O velame da raiz em *Barbosella* apresentou variações no número de camadas de células. Camadas uni- e biestratificadas já haviam sido relatadas para o gênero (Pridgeon 1982), no entanto, em *B. cogniauxiana*, *B. cucullata* e *B. macaheensis* três camadas de células foram observadas. O espessamento da exoderme em formato “U” é o mais comum entre as espécies de Pleurothallidinae (Pridgeon 1982, Stern 2014), como também já observado em *Barbosella* (Pridgeon 1982). No entanto, variações dos tipos de espessamento podem ocorrer dentro do mesmo gênero, como ocorre nas espécies de *Barbosella* aqui analisadas,

onde algumas espécies apresentaram espessamento em formato “U” invertido e outras em formato “O”.

Algumas espécies de *Barbosella* possuem o ramicaule abreviado, porém quando conspícuos são sempre muito menores do que a folha. O córtex externo do ramicaule das espécies de *Barbosella* não apresentou espessamento de parede lignificada, diferentemente do que foi observado nos demais gêneros. A presença de córtex externo esclerificado confere resistência aos órgãos vegetativos que geralmente são mais delgados (Stern & Pridgeon 1984, Oliveira & Sajo 2001). Para as espécies de *Barbosella* aqui estudadas, bem como observado em outros trabalhos (Pridgeon 1982, Stern & Pridgeon 1984), fica evidente que em Pleurothallidinae apenas os representantes que possuem ramicaule longo é que apresentam córtex com essas células de sustentação. O diâmetro do ramicaule também está diretamente relacionado a quantidade de feixes vasculares presentes (Stern 2014), visto que em *Barbosella* a quantidade de feixes é bem menor do que em *M. lonchophyllus*, *P. nemorosa*, *R. elegans* e *O. grandiflora*, as quais possuem ramicaules longos e com maior diâmetro.

Stern (2014) menciona que espécies de Pleurothallidinae podem ou não apresentar tricomas na superfície foliar, variação essa aqui verificada. Os tricomas registrados na superfície foliar de algumas das espécies assemelham-se aos tricomas glandulares registrados por Pridgeon (1981). Esse tipo de tricoma pode ocorrer em ambas as faces da folha, e indica papel absorvente ao longo do desenvolvimento foliar Pridgeon (1981).

Os idioblastos traqueoidais com espessamento helicoidal até então não haviam sido registrados no mesófilo foliar das espécies de *Barbosella* (Pridgeon 1982). Os idioblastos ou elementos traqueoidais são células que se diferenciam das demais em forma, tamanho, conteúdo ou estrutura de parede, sendo registrados com frequência em Orchidaceae (Olantui & Neguin 1980, Scatena & Nunes 1996, Stern 2014). Acredita-se que a função destas estruturas esteja relacionada ao suporte mecânico associado ao parênquima aquífero que a circundam, uma vez que a parede com espessamento secundário previne o colapso durante o período de dessecação (Olantui & Neguin 1980).

A presença de feixe vascular no bordo foliar, envoltos por fibras, foi observada em *M. lonchophyllus*, *P. nemorosa*, *R. elegans* e *O. grandiflora*. Em *Barbosella* o bordo foliar de apenas algumas espécies apresentou feixes de fibras, caráter sinapomórfico para as espécies do clado formado por *B. orbicularis* e as demais espécies brasileiras. *Barbosella*

australis foi a única espécie de todos os gêneros de Pleurothallidinae analisados por Stern (2014) em que feixes fibrosos foram registrados.

Levando em consideração que as espécies de *Barbosella* são muito semelhantes morfológicamente e que a identificação das espécies nem sempre é uma tarefa fácil, por se tratarem de plantas diminutas, as características anatômicas vegetativas aqui observadas, são um acréscimo de caracteres diagnósticos que podem auxiliar na taxonomia, bem como na interpretação da história evolutiva dos caracteres anatômicos não só do gênero, mas como para a subtribo em geral. Em conclusão, descrevemos em detalhes a morfologia interna dos órgãos vegetativos até então pouco conhecida para o gênero *Barbosella*.

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Referências

- Benzing DH, Pridgeon AM. 1983. Foliar trichomes of Pleurothallidinae (Orchidaceae): functional significance. *Am. J. Bot.* 70: 173–180.
- Brazilian Flora 2020 (in construction) Jardim Botânico do Rio de Janeiro. Available: <http://floradobrasil.jbrj.gov.br/>. Accessed Jun 2018.
- Carlsward BS, Stern WL. 2009. Vegetative anatomy and systematics of Triphorinae (Orchidaceae). *Bot. J. Linnean Soc.* 159: 203-210.
- Figueroa C, Salazar GA, Zavaleta HÁ, Engleman EM. 2008. Root character evolution and systematic in Cranichidinae, Prescottiinae and Spiranthinae (Orchidaceae, Cranichideae). *Ann. Bot.* 101: 509-520.

- Foster AS. 1949. Practical plant anatomy. Princeton: D. Van Nostrand Company Inc., 228 pp.
- Franklin GL. 1945. Preparation of thin sections of synthetic resins and wood-resin composites, and a new macerating method for wood. *Nature* 155: 51.
- Higgins WE, Williams NH. 2009. Pleurothallidinae: how many genera? In: Saulea RP, Sandow LA (Eds.) Proceedings of the 19th World Orchid Conference. American Printing Arts, Miami, USA, pp. 425–430.
- Johansen DA. 1940. Plant microtechnique. New York: McGraw-Hill Book Co. Inc., 523 pp.
- Kaiser E. 1880. Verfahren zur Herstellung einer tadellosen glycerin-gelatine. *Botanisch Zentralb* 180: 25–26.
- Karremans AP. 2016. Genera Pleurothallidinarum: An updated phylogenetic overview of Pleurothallidinae. *Lankesteriana* 16: 219–241.
- Luer CA. 1986. Icones Pleurothallidinarum I. Systematics of the Pleurothallidinae (Orchidaceae). *Monogr. Syst. Bot. Mo. Bot. Gard.* 15: 29–34.
- Luer CA. 2000. Icones Pleurothallidinarum XX. Systematics of *Jostia*, *Andinia*, *Barbosella*, *Barbrodria*, *Pleurothallis* subgen. *Antilla*, subgen. *Effusia*, subgen. *Restrepioidia*. Addenda to *Lepanthes*, *Masdevallia*, *Platystele*, *Pleurothallis*, *Restrepiopsis*, *Scaphosepalum* and *Teagueia*. *Monogr. Syst. Bot. Mo. Bot. Gard.* 79: 7–38.
- Luque RL, Sousa HC, Kraus JE. 1996. Métodos de coloração de Roeser (1972)-modificado- e Kropp (1972) visando a substituição do azul de astra por azul de alcião 8GS ou 8GX. *Acta Bot. Bras.* 10: 199–212.
- Neyland R, Urbatsch LC, Pridgeon AM. 1995. A phylogenetic analysis of subtribe Pleurothallidinae (Orchidaceae). *Bot. J. Linnean Soc.* 117: 13–28.
- O'Brien TP, Feder N, McCully ME. 1965. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma* 59: 368–373.
- Olantui AO, Nengui RO. 1980. Occurrence and distribution of tracheoidal elements in the Orchidaceae. *Bot. J. Linnean Soc.* 80: 357–370.
- Oliveira VC, Sajo MG. 2001. Morfoanatomia caular de nove espécies de Orchidaceae. *Acta Bot. Bras.* 15: 177–188.

- Pérez-Escobar OA, Chomicki G, Condamine FL, Karremans AP, Bogarín D, Matzke NJ, Silvestro D, Antonelli A. 2017. Recent origin and rapid speciation of Neotropical orchids in the world's richest plant biodiversity hotspot. *New Phytol.* 215: 891–905.
<http://dx.doi.org/10.1111/nph.14629>
- Pridgeon AM. 1981. Absorbing Trichomes in the Pleurothallidinae (Orchidaceae). *Am. J. Bot.* 68: 64–71.
- Pridgeon AM. 1982. Diagnostic Anatomical Characters in the Pleurothallidinae (Orchidaceae). *Am. J. Bot.* 69: 921–938.
- Pridgeon AM. 2005. *Barbosella*. In: Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (Eds.), *Genera Orchidacearum*, 4. Epidendroideae (*Part one*), Oxford University, Oxford, pp. 336–338.
- Pridgeon AM, Chase MW. 2001. A phylogenetic reclassification of Pleurothallidinae (Orchidaceae). *Lindleyana* 16: 235–271.
- Pridgeon AM, Chase MW. 2003. Phylogenetics of the subtribe Pleurothallidinae (Epidendreae: Orchidaceae) based on combined evidence from DNA sequences. *Lankesteriana* 7: 49–50.
- Pridgeon AM, Williams NH. 1979. Anatomical aspects of *Dresslerella* (Orchidaceae). *Selbyana* 5: 120–134.
- Pridgeon AM, Solano R, Chase MW. 2001. Phylogenetic relationships in Pleurothallidinae (Orchidaceae): combined evidence from nuclear and plastid DNA sequences. *Am. J. Bot.* 88: 2286–2308.
- Scatena VL, Nunes AC. 1996. Anatomia foliar de *Pleurothallis rupestris* Lindl. (Orchidaceae) dos campos rupestres do Brasil. *Boletim de Botânica da Universidade de São Paulo* 15: 35–43.
- Smidt EC, Gallo LW, Scatena VL. 2013. Leaf anatomical and molecular studies in *Bulbophyllum* section *Micranthae* (Orchidaceae) and their implications for systematic. *Braz. J. Bot* 36: 75–82.
- Stern WL. 2014. *Anatomy of the Monocotyledons. Volume X: Orchidaceae*. Oxford University Press, Oxford, pp. 130–135.
- Stern WL, Pridgeon AM. 1984. Ramicaul, a better term for the pleurothallid “secondary stem”. *Am. Orchid Soc. Bull.* 53: 397–401.

Figuras

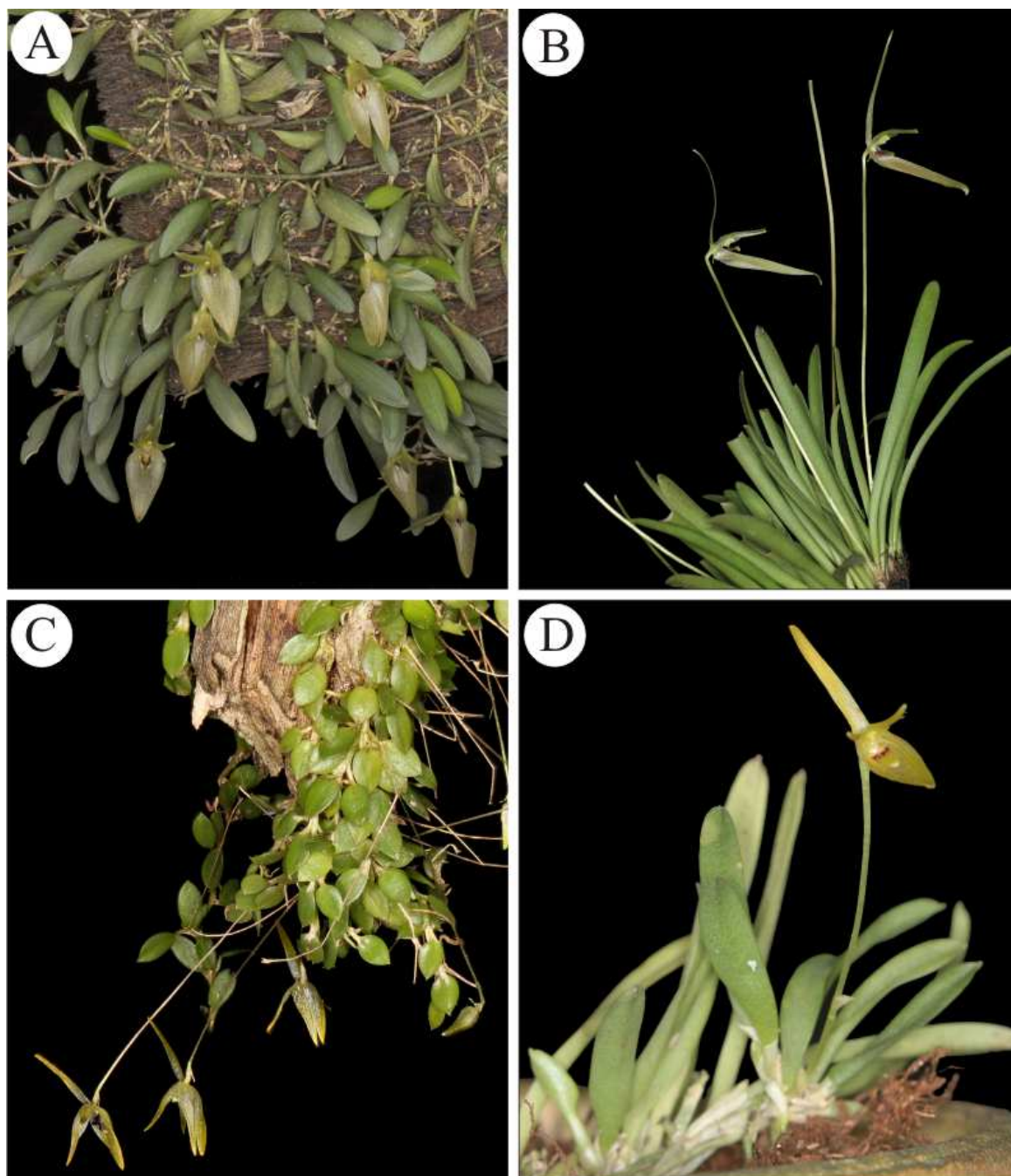


Figura 1. Espécies de *Barbosella* e suas diferentes formas de crescimento. (A) *Barbosella australis*. (B) *Barbosella cucullata*. (C) *Barbosella gardneri*. (D) *Barbosella prorepens*. Fotos de E.C. Smidt (B, D) e W. Collier & A. Toscano de Brito (A, C).

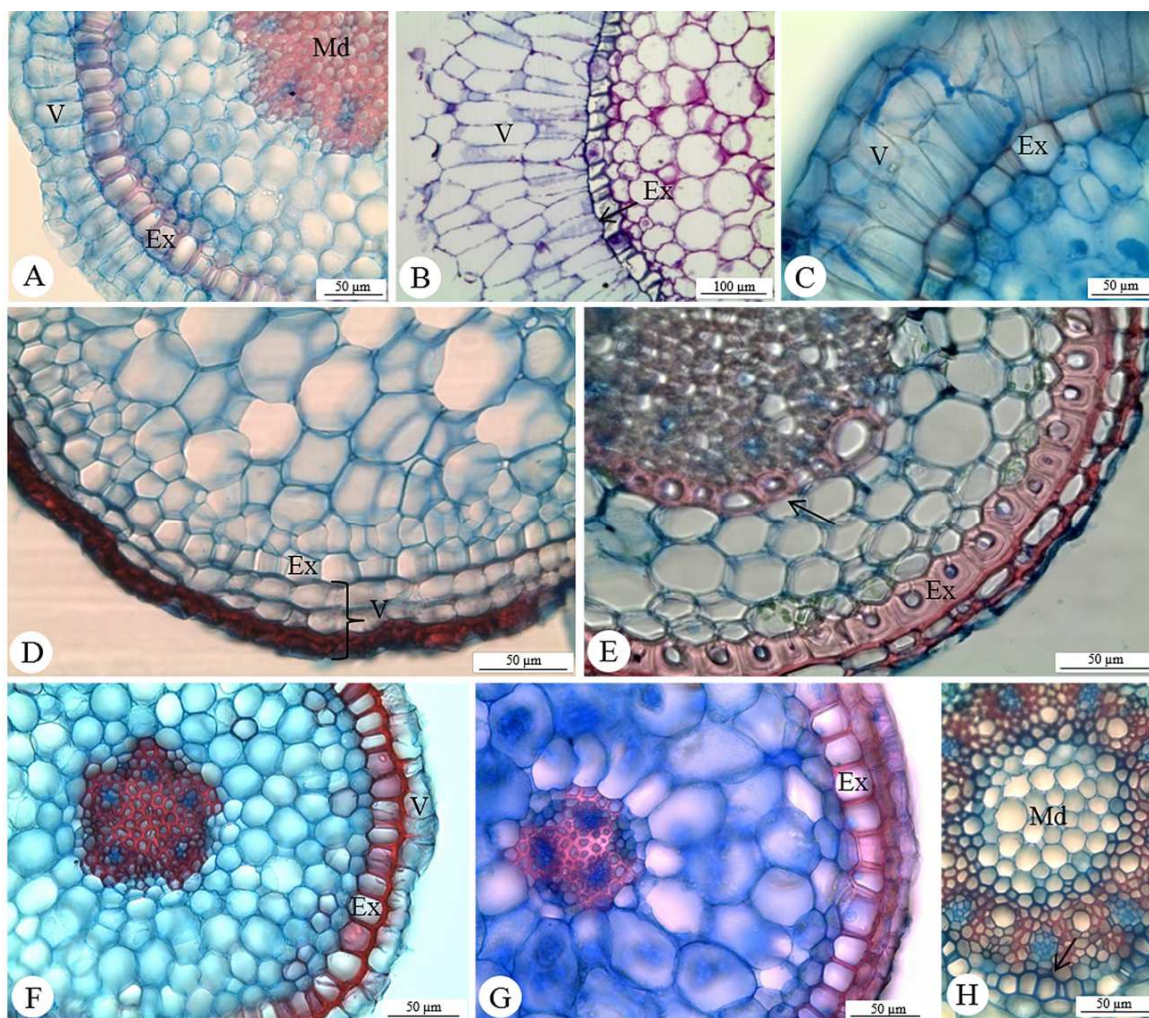


Figura 2. Aspectos anatômicos da raiz em secção transversal. (A) Detalhes do número de camadas do velame e espessamento da exoderme em *B. australis*. (B) Número de camadas do velame em *B. cucullata*. (C) Formato das células do velame em *B. macaheensis*. (D) Detalhe da camada externa do velame lignificada em *M. lonchophyllus*. (E) Detalhes do velame e parede da exoderme com espessamento em O em *O. grandiflora*. (F) Detalhe do velame e exoderme com espessamento em U invertido em *B. trilobata*. (G) Número de polos do protoxilema em *B. dusenii*. (H) Detalhe da endoderme com espessamento em O (seta), células do periciclo sem espessamento, e medula com parede primária em *M. lonchophyllus*. V=Velame, Ex=Exoderme, Md= Medula.

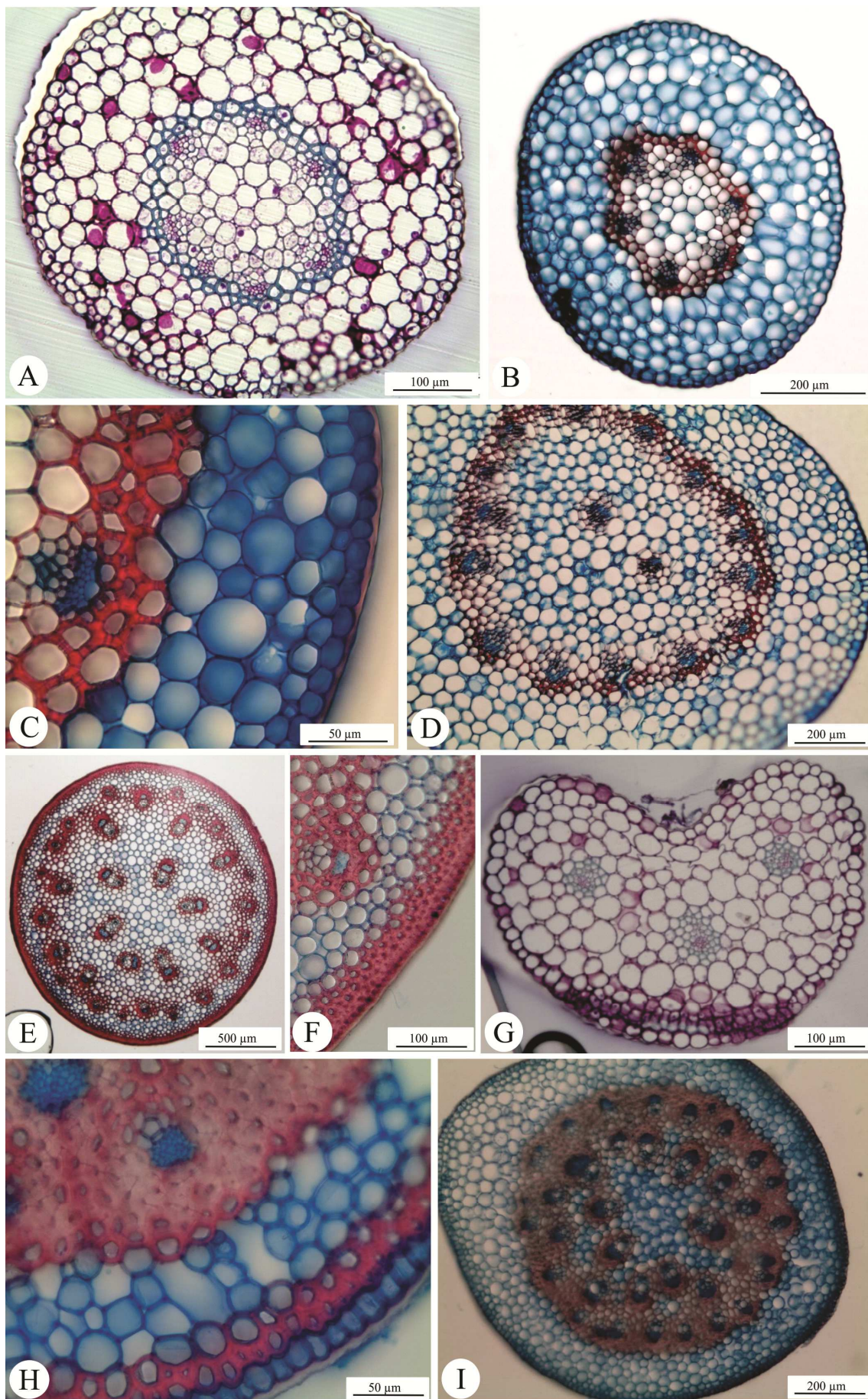


Figura 3. Aspectos anatômicos do rizoma (A, B, C, D) e ramicaule (E, F, G, H) em secção transversal. (A) Formato do rizoma e detalhes da epiderme e medula em *B. gardneri*. (B) Formato do rizoma e detalhe dos feixes vasculares em *B. miersii*. (C) Detalhes da cutícula e feixes na medula em *B. spiritu-sanctensis*. (D) Detalhes dos feixes colaterais na região central e feixes vasculares medulares em *B. australis*. (E, F) Formato do ramicaule e detalhes da epiderme e feixes vasculares medulares em *M. lonchophyllus*. (G) Formato do ramicaule e detalhe da epiderme em *B. gardneri*. (H) Parênquima aerífero e detalhe do número de feixes e das camadas de fibras espessa ao redor dos feixes em *P. nemorosa*. (I) Detalhe dos anéis de feixes vasculares em *R. elegans*.

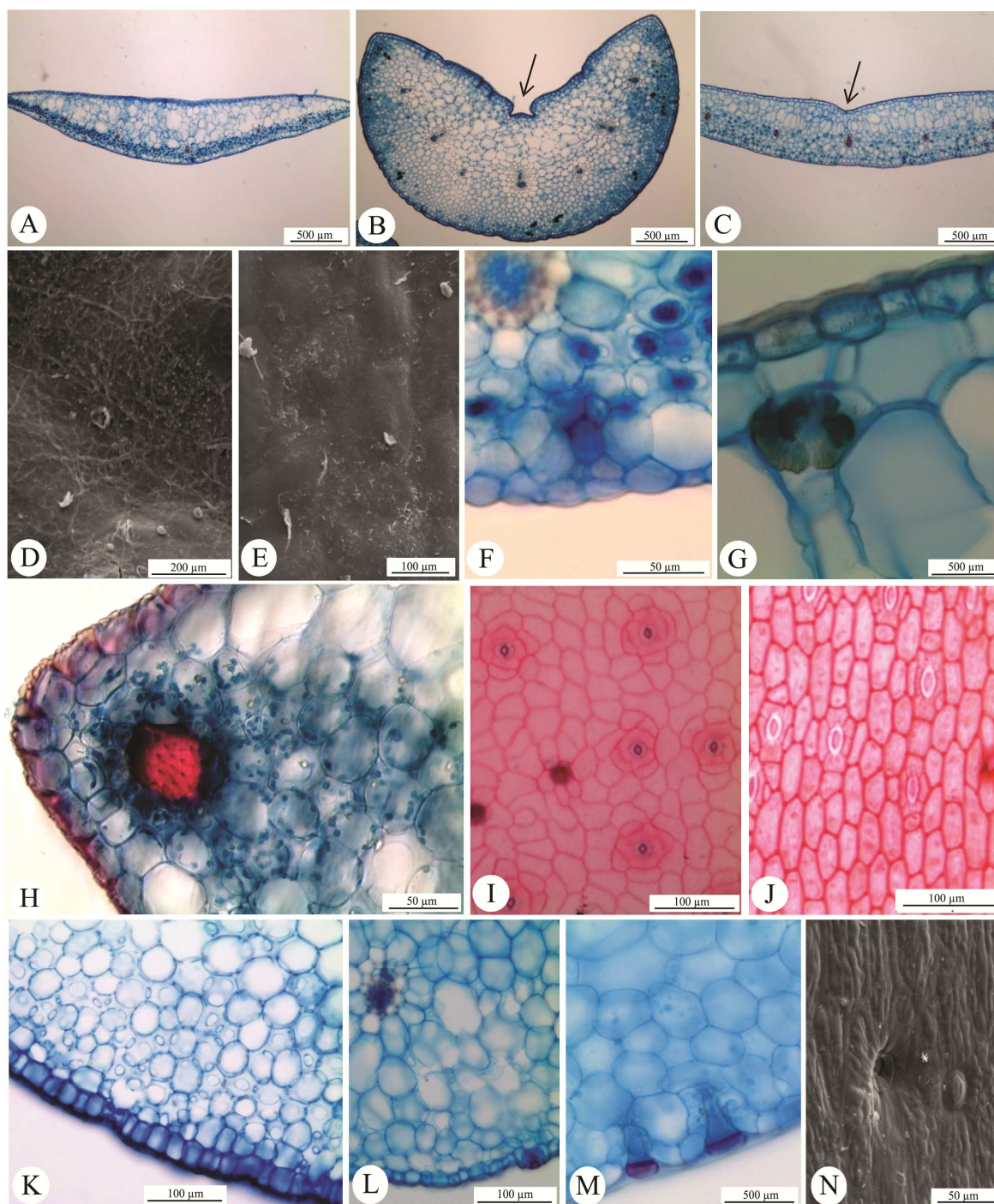


Figura 4. Micromorfologia e anatomia da folha. (A) Forma da folha em secção transversal de *B. gardneri*. (B) Forma da folha em secção transversal, detalhe do sulco (seta) de *B. dolichorhiza*. (C) Detalhe do sulco (seta), em secção transversal, em *B. macaheensis*. (D) Detalhe da cera da epiderme na face adaxial em *B. spiritu-sanctensis*, foto em MEV. (E) Detalhe da cera da epiderme na face adaxial em *B. gardneri*, foto em MEV. (F) Detalhe da cutícula e células epidérmicas na face abaxial, em secção transversal, em *B. gardneri*. (G) Detalhe da cutícula e hipoderme em *M. lonchopyllus*. (H) Detalhe da cutícula em ambas as

faces em *B. cogniauxiana*. (I) Dissociação da epiderme foliar em vista frontal de *B. gardneri*. (J) Dissociação da epiderme foliar em vista frontal de *B. dolichorhiza*. (K) Detalhe do estômato abaixo do nível das demais células epidérmicas em *B. cogniauxiana*. (L) Detalhe do estômato acima do nível das demais células epidérmicas em *B. trilobata*. (M) Detalhe do tricoma inteiro em folha jovem de *B. prorepens*. (N) Detalhe da base do tricoma em *B. australis*, foto em MEV.

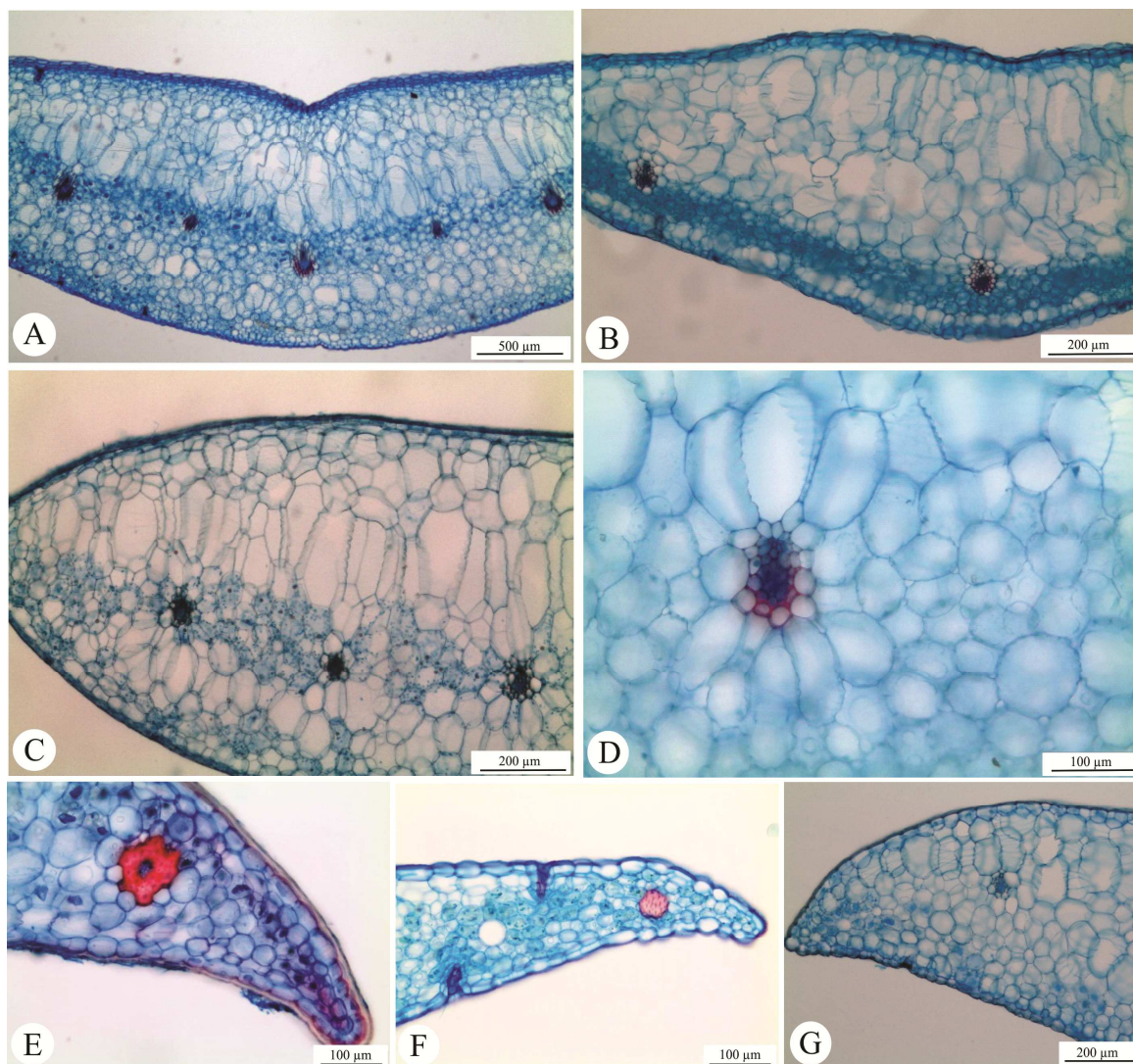


Figura 5. Anatomia da folha em secção transversal. (A) Detalhe do parênquima clorofiliano e parênquima aquífero em *B. australis*. (B) Detalhe do parênquima clorofiliano e parênquima aquífero na face adaxial em *B. gardneri*. (C) Detalhe do parênquima clorofiliano e parênquima aquífero, e idioblastos em *B. dusenii*. (D) Detalhe das células radiadas ao redor do feixe vascular em *B. orbicularis*. (E) Detalhe do feixe vascular no bordo foliar em *P. nemorosa*. (F) Detalhe do feixe de fibras no bordo foliar em *B. macaheensis*. (G) Detalhe do bordo foliar parenquimático em *B. prorepens*.

Tabela

Tabela I. Lista das espécies estudadas e dados de coleta.

Espécies	Voucher	Localidade	Hábito
Grupo interno			
<i>Barbosella australis</i> (Cogn.) Schltr.	<i>M. Bolson</i> 525 (UPCB)	Alvim Seidel Orquidário Catarinense, sem localidade	Reptante
<i>B. australis</i>	<i>M. Bolson</i> 527 (UPCB)	Brasil, Santa Catarina, Luzerna	
<i>B. cogniauxiana</i> (Speg. & Kraenzl.) Schltr.	<i>M. Bolson</i> 522 (UPCB)	Brasil, Santa Catarina, Ibicaré	Reptante
<i>B. cogniauxiana</i>	<i>M.L. Klingelfus</i> 113 (UPCB)	Brasil, Paraná, Ponta Grossa	
<i>B. cogniauxiana</i>	<i>M.L. Klingelfus</i> 114 (UPCB)	Brasil, Paraná, Ponta Grossa	
<i>B. crassifolia</i> (Edwall) Schltr.	<i>M. Bolson et al.</i> 545 (UPCB)	Brasil, Paraná, Pontal do Paraná	Reptante
<i>B. crassifolia</i>	<i>A.L.V. Toscano de Brito</i> 3479 (UPCB)	Brasil, Espírito Santo, sem localidade	
<i>B. cucullata</i> (Lindley) Schltr.	<i>M. Bolson</i> 534 (UPCB)	Ecuagenera Cia. Ltda., sem localidade	Cespitoso
<i>B. dolichorhiza</i> Schltr.	<i>M. Bolson</i> MB03 (UPCB)	Ecuagenera Cia. Ltda., sem localidade	Cespitoso
<i>B. dusenii</i> (Samp.) Schltr.	<i>M. Bolson</i> 524 (UPCB)	Brasil, Santa Catarina, Canoinhas	Reptante
<i>B. gardneri</i> (Lindl.) Schltr.	<i>M. Bolson & D.F Lima</i> 493 (UPCB)	Brasil, Rio de Janeiro, Teresópolis, Parque Nacional da Serra dos Órgãos	Reptante
<i>B. macaheensis</i> (Cogn.) Schltr.	<i>M. Bolson et al.</i> 582 (UPCB)	Brasil, Espírito Santo, Santa Teresa, Reserva Biológica Augusto Ruschi	Reptante
<i>B. macaheensis</i>	<i>M. Bolson et al.</i> 647 (UPCB)	Brasil, Rio de Janeiro, Nova Friburgo	

<i>B. miersii</i> (Lindl.) Schltr.	<i>M. Bolson et al. 519</i> (UPCB)	Brasil, Santa Catarina, Joinville	Reptante
<i>B. miersii</i>	<i>M. Bolson et al. 516</i> (UPCB)	Brasil, Santa Catarina, Benedito Novo	
<i>B. miersii</i>	<i>M. Bolson et al. 577</i> (UPCB)	Brasil, Espírito Santo, Santa Teresa, Reserva Biológica Augusto Ruschi	
<i>B. orbicularis</i> Luer	<i>M. Bolson MB04</i> (UPCB)	Ecuagenera Cia. Ltda., sem localidade	Reptante
<i>B. prorepens</i> (Rchb.f.) Schltr	<i>M. Bolson MB05</i> (UPCB)	Ecuagenera Cia. Ltda., sem localidade	Cespitoso
<i>B. spiritus-sanctensis</i> (Pabst) F. Barros & Toscano	<i>A.L.V. Toscano de Brito 2881</i> (UPCB)	Brasil, Espírito Santo, Santa Teresa	Reptante
<i>B. trilobata</i> Pabst	<i>M.L. Klingelfus 155</i> (UPCB)	Brasil, Paraná, Ponta Grossa	
<i>Myoxanthus lonchophyllus</i> (Barb.Rodr.) Luer	<i>M.L. Klingelfus 199</i> (UPCB)	Orquidário Marcos Klingelfus, sem localidade	Cespitoso
<i>Pleurothallopsis nemorosa</i> (Barb.Rodr.) Porto & Brade	<i>A.L.V. Toscano de Brito 3414</i> (UPCB)	Orquidário Sítio Paraíso das Microorquídeas, sem localidade	Cespitoso
<i>Restrepia elegans</i> H.Karst.	<i>M.L. Klingelfus 165</i> (UPCB)	Orquidário Marcos Klingelfus, sem localidade	Cespitoso
Grupo externo			
<i>Octomeria grandiflora</i> Lindl.	<i>M. Bolson 609</i> (UPCB)	Brasil, Paraná, Bocaiúva do Sul	Cespitoso

CAPÍTULO III / CHAPTER III

**Um nome negligenciado e um novo sinônimo em *Barbosella* (Orchidaceae,
Pleurothallidinae)**

A neglected name and new synonym in *Barbosella* (Orchidaceae, Pleurothallidinae)

Mônica Bolson, A. L. V. Toscano de Brito & Eric C. Smidt

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A neglected name and new synonym in *Barbosella* (Pleurothallidinae, Orchidaceae)

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Abstract

During a study to monograph the *Barbosella* species from Brazil, historical material and protologues of various species were analyzed. Among these, the type material of *B. perinii*, a name largely overlooked since its publication, was examined. This name is indicated here as a synonym of *B. macaheensis*. Taxonomical discussions, updated distribution, illustrations and conservation status are provided for *B. macaheensis*.

Resumo

Durante a elaboração da monografia das espécies brasileiras de *Barbosella*, os materiais históricos e protólogos de várias espécies foram analisados. Entre eles, examinamos o material tipo de *B. perinii*, um nome negligenciado desde a sua publicação. Neste trabalho, *B. perinii* é indicada como sinônimo de *B. macaheensis*. Além disso, atualizamos a distribuição de *B. macaheensis*, apresentamos uma detalhada ilustração e discutimos o estado de conservação deste táxon.

Key words: *Barbosella macaheensis*, *Barbosella perinii*, IUCN, nomenclature, taxonomy.

Introduction

Barbosella Schlechter (1918: 259) comprises 20 species distributed from Central America to Southern Brazil, a country where approximately 50% of the species occur (Pridgeon 2005, Brazilian Flora 2020 in construction).

They are usually epiphytes, with long, repent or caespitose growth, and sometimes form large, dense masses. The ramicaul is abbreviated, without an annulus. The inflorescence is always a single-flowered raceme, with a long and delicate peduncle, emerging laterally from the ramicaul. The flower is resupinate due to a 180° bending of the flower near the peduncle apex (except in *B. circinata* Luer 1977: 204). The sepals are similar to each other, the dorsal sepal is free and erect and the lateral sepals are partially or completely connate (except in *B. portillae* Luer 2002: 97). The petals are narrow, usually linear-ovate to linear-lanceolate. The lip is entire to trilobed, with obtuse to acute lobes, a sulcate disc, and a basal cavity that is articulate to the column-foot. The column is usually broadly and longitudinally winged with an entire or usually dentate apex, and the base is prolonged into a prominent and bulbous foot (rarely footless); the anther is incumbent, with four clavate pollinia of equal size, distinct caudicles and gemmate sculpture (Luer 2000, Stenzel 2000, Pridgeon 2005).

The only taxonomic revision of *Barbosella* is the one of Luer (2000), in which he recognized 18 species, besides reviewing history and morphology of the genus. Because of differences in column and lip morphology, especially the way the lip articulates to the column-foot, *B. miersii* (Lindley 1842: 84) Schlechter (1918: 262) was not included in Luer's monograph but rather treated as a monospecific genus, *Barbrodria* Luer (1981: 386) (Luer 2000). However, molecular studies (Pridgeon *et al.* 2001) indicated with strong support that *Barbrodria* is embedded within *Barbosella*, and for this reason placed in synonymy. Subsequently, Luer (2002) published another species in the genus, *B. portillae*, which is known only from Ecuador.

While treating *Barbosella* for Flora of Brazil 2020, specimens from the main national and international herbaria were analyzed and the type material of *B. perinii* Ruschi (1977: 1) was examined. This name was neither included in the revision of the genus nor cited in any publication dealing with Brazilian orchids and international plant databases. We also studied the material of *B. macaheensis* (Cogniaux 1907: 310) Luer (2000: 23), which until recently was only known from the type material and one other collection made approximately 120 years ago. The present work discusses the history of the two names and

proposes synonymizing *B. perinii* under *B. macaheensis*. The conservation status of *B. macaheensis* is also provided.

Methods

The interpretation of the species were based on literature review, examination of living collections and comparisons with type and other specimens deposited at BR, GUA, MBML, P, RB and UPCB (acronyms according to Thiers 2017). For scanning electron microscope (SEM) analysis, the lip sample was dehydrated in an ethanol series and submitted to critical point drying with CO₂. It was then affixed to metal supports with adhesive copper tape, sputter coated with gold (BALZERS SCD030), and analyzed with a TESCAN VEGA3 LMU Scanning Electron Microscope. The terminology for morphological criteria followed Stearn (2004) and Gonçalves & Lorenzi (2007). The conservation status assessment of the species was based on categories and criteria according to IUCN (IUCN 2001, 2017) based on all records numbers, in extent of occurrence (EOO), in area of occupancy (AOO) and quality of habitat occupied of each specimen. These measures were obtained using GeoCAT (Geospatial Conservation Assessment Tool – Bachman *et al.* 2011). The default is 2 km² cell size.

Synonymy and Taxonomy Note

Barbosella macaheensis (Cogniaux 1907: 310) Luer (2000: 23). Type:—BRAZIL. Rio de Janeiro: Alto Macahé, 6 July 1891, A.F.M. Glaziou *s.n.* (Holotype: BR 997274 [digital image!]; Fig. 1).

= *Barbosella perinii* Ruschi (1977: 1). Type:—BRAZIL. Espírito Santo: Santa Teresa, Reserva Biológica de Nova Lombardia, do IBDF, 16 July 1975, O. Perini *et al. s.n.* (Holotype: MBML 10044!, spirit).

Cogniaux (1907) first described *B. macaheensis* in the genus *Pleurothallis* Brown, in Aiton (1813: 211) based on a collection (Fig. 1) by Auguste F. M. Glaziou, in 1891, in Macaé de Cima (“Alto Macahé”), a locality in the municipality of Nova Friburgo, state of Rio de Janeiro. Until recently, this species was known from only two Glaziou collections: *Glaziou s.n.*, the type specimen at BR (Fig. 1), and *Glaziou 18535a*, which is also deposited at BR (Fig. 2) with a duplicate at P (Fig. 3). Luer (2000) provided an illustration

of *B. macaheensis* based on the specimen *Glaziou 18535a* at BR and noted that the lip was distorted and the description needed to be updated if new collections were made.

Barbosella perinii was described and illustrated (Fig. 4) by Augusto Ruschi (1977) based on a collection by Orlando Perini and his sons, Eber and Evandro, in 1975. It was collected in the Augusto Ruschi Biological Reserve (formerly Nova Lombardia Biological Reserve), which is located in the municipality of Santa Teresa, state of Espírito Santo. This name was overlooked by Pabst & Dungs (1975, 1977), who published a comprehensive, illustrated checklist of the orchids of Brazil, and also by Luer (2000). With exception of TROPICOS, this name does not appear in any other online plant database such as ePIC, IPNI, WCSP, The Plant List, and Flora of Brazil 2020. It is not cited by Chiron & Bolsanello (2013), who recently published an account of the orchids of Espírito Santo State.

During a recent visit to MBML, the holotype specimen of *B. perinii*, a spirit preserved collection numbered 10044, was located and examined. This number is correctly cited in the Latin description in the protologue, but is erroneously cited as 10040 in the Portuguese description that is part of the protologue.

Based on additional material collected in the Augusto Ruschi Biological Reserve, the type locality of *B. perinii*, as well specimens from the state of Rio de Janeiro, we decided that this species and *B. macaheensis* are inseparable and, therefore, conspecific.

Barbosella macaheensis is a small, epiphytic herb, with repent growth, to 30 mm long, with a thick, conspicuous rhizome, ramicaul 2–7 mm long, and leaf 8–24 × 2–7 mm (Figs. 5A–6A). The inflorescence is a single-flowered raceme with a peduncle up to 34 mm long. The sepals and petals are light yellow-green to light orange, occasionally with purple primary veins (Fig. 5B, C). The sepals are ovate with an obtuse to acute apex, and 3-veined. The dorsal sepal is suberect and the lateral sepals are connate into an ovate-triangular, bifid synsepal (Fig. 6B–E). The petals are porrect or occasionally patent, narrowly ovate to lanceolate, usually subfalcate, with an acute apex (Fig. 5C–6F). The lip is yellow suffused with purple (Fig. 5B), 3.3–4.3 × 1.9–2.5 mm, entire, oblong, with erect sides at the middle, the disc with a pair of subtrapeziform callosities at base connecting the concave base with the rest of the lip, it is then concave and minutely papillose between the erect sides, with a pair of low, pyramidal calli more or less above the middle, the base is rounded and deeply concave (Figs. 5D–6G). The column is 2.7–3.5 mm long and not winged (Fig. 6H).

Additional material examined:—BRAZIL. Espírito Santo: Santa Teresa, Reserva Biológica Augusto Ruschi, Nova Lombardia, Trilha da divisa, lado direito, Goipabo, 10 January 2016, *Bolson et al.* 582 (UPCB). Rio de Janeiro: Nova Friburgo, Alto Macahé, 6 July 1891, *Glaziou* 18535a (BR [digital image; Fig. 2], P [digital image; Fig. 3]). *Idem*, 5 June 1893, *Glaziou* 20497a (P [digital image]). *Idem*, Macaé de Cima, 7 July 1978, *Araujo et al.* 2075 (GUA). *Idem*, 18 June 1986, *Martinelli et al.* 11756 (RB). *Idem*, 1 August 2016, *Bolson* 637 (UPCB). *Idem*, Sítio Bacchus, April 1998, *Miller s.n.* (RB [543, spirit]). *Idem*, Sítio 1, 6 June 2006, *Bocayuva et al.* 185 (RB). *Idem*, Trilha atrás do S. Bacchus, 1 May 2001, *Cattan* 81 (RB). *Idem*, Trilha do Pico do Faraó, 2 June 2017, *Bolson et al.* 647 (UPCB).

This species inhabits the humid forests in the Atlantic Forest domain of the states of Rio de Janeiro and Espírito Santo. Based on an analysis of GeoCAT, *B. macaheensis* has an EOO of approximately 2,393 km² and an AOO of 12 km². According to IUCN criteria, its restricted distribution, even though concentrated in an environmental protected area in Rio de Janeiro and a biological reserve in Espírito Santo, indicates that the conservation status of this species is Endangered [EN B2ab(i,ii,iii,iv)] (IUCN 2017).

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References

- Bachman, S., Moat, J., Hill, A.W., De La Torre, J. & Scott, B. (2011) Supporting Red List threat assessment with GeoCAT: geospatial conservation assessment tool. *ZooKeys* 150: 117–126.
<https://doi.org/10.3897/zookeys.150.2109>
- Brazilian Flora 2020 (in construction) Jardim Botânico do Rio de Janeiro. Available from: <http://floradobrasil.jbrj.gov.br/> (Accessed 12 Dec 2016).
- Brown, R. (1813) *Pleurothallis*. In: Aiton, W.T. (Ed.) *Hortus Kewensis; a Catalogue of the Plants Cultivated in the Royal Botanic Garden at Kew*, 5. 2nd ed. London, 568 pp.
- Chiron, G.R. & Bolsanello, R.X. (2013) *Orchidées du Brésil - As orquídeas da Serra do Castelo (Espírito Santo, Brasil)*, 2. Tropicalia, Voreppe, 416 pp.
- Cogniaux, C.A. (1907) Notes sur les Orchidées du Brésil et des régions voisines. *Bulletin de La Société Royale de Botanique de Belgique* 43: 310–311.
- Gonçalves, E.G. & Lorenzi, H. (2007) *Morfologia vegetal: organografia e dicionário ilustrado de morfologia das plantas vasculares*. 1st ed. Instituto Plantarum de Estudos da Flora, São Paulo, 416 pp.
- IUCN (2001) *IUCN Red List categories and criteria*. Version 3.1. 2nd ed. IUCN Species Survival Commission, Gland, Switzerland and Cambridge, UK. 33 pp.
- IUCN (2017) The IUCN Red List of Threatened Species. Version 2017-1. Available from: <http://www.iucnredlist.org>. (Accessed 07 Jun 2017).
- Lindley, J. (1835) *Pleurothallis hymenantha*. *Edwards's Botanical Register* 21: sub t. 1797.
- Lindley, J. (1842) *Pleurothallis articulata*. *Pleurothallis miersii*. *Edwards's Botanical Register* 28: Misc. 83–84.
- Luer, C.A. (1977) Icones Pleurothallidarum (Orchidaceae). Miscellaneous species in the Pleurothallidinae. Addenda et corrigenda. *Selbyana* 3: 203–412.
- Luer, C.A. (1981) *Barbrodria*, a new genus in the Pleurothallidinae (Orchidaceae). *Selbyana* 5: 386–387.

- Luer, C.A. (2000) Icones Pleurothallidarum XX. Systematics of *Jostia*, *Andinia*, *Barbosella*, *Barbrodria*, *Pleurothallis* subgen. *Antilla*, subgen. *Effusia*, subgen. *Restrepioidia*. Addenda to *Lepanthes*, *Masdevallia*, *Platystele*, *Pleurothallis*, *Restrepiopsis*, *Scaphosepalum* and *Teagueia*. *Monographs in Systematic Botany from the Missouri Botanical Garden* 79: 7–38.
- Luer, C.A. (2002) Icones Pleurothallidarum XXIV: A First Century of New Species of *Stelis* of Ecuador, Part One. Addenda to *Barbosella*, *Dracula*, *Dresslerella*, *Lepanthopsis*, *Platystele*, *Pleurothallis*, *Restrepia*, *Scaphosepalum*, *Teagueia*, and *Trichosalpinx*. *Monographs in Systematic Botany from the Missouri Botanical Garden* 88: 97–122.
- Pabst, G.F.J. & Dungs, F. (1975) *Orchidaceae Brasilienses*, 1. Kurt Schmersow, Hildesheim, 408 pp.
- Pabst, G.F.J. & Dungs, F. (1977) *Orchidaceae Brasilienses*, 2. Kurt Schmersow, Hildesheim, 418 pp.
- Pridgeon, A.M. (2005) *Barbosella*. In: Pridgeon, A.M., Cribb, P.J., Chase, M.W. & Rasmussen, F.N. (Eds.), *Genera Orchidacearum*. 4, *Epidendroideae (Part 1)*, Oxford University, Oxford, pp. 336–338.
- Pridgeon, A.M., Solano, R. & Chase, M.W. (2001) Phylogenetic relationships in Pleurothallidinae (Orchidaceae): combined evidence from nuclear and plastid DNA sequences. *American Journal of Botany* 88: 2286–2308.
- Ruschi, A. (1977) Orquidáceas novas do Estado do Espírito Santo. *Boletim do Museu de Biologia Mello Leitão* 87: 1–3.
- Schlechter, F.R.R. (1918) Die Gattungen *Restrepia* H. B. u. Kth. *Repertorium Specierum Novarum Regni Vegetabilis* 15: 255–270.
- Stenzel, H. (2000) Pollen morphology of the subtribe Pleurothallidinae Lindl. (Orchidaceae), *Grana* 39: 108–125. <http://dx.doi.org/10.1080/001731300300045247>
- Stearn, W.T. (2004) *Botanical Latin*. Timber Press, Portland, 546 pp.
- Thiers, B. [continuously updated] *Index Herbariorum: A global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. Available from: <http://sweetgum.nybg.org/ih/> (Accessed 17 Mar 2017).

Figures

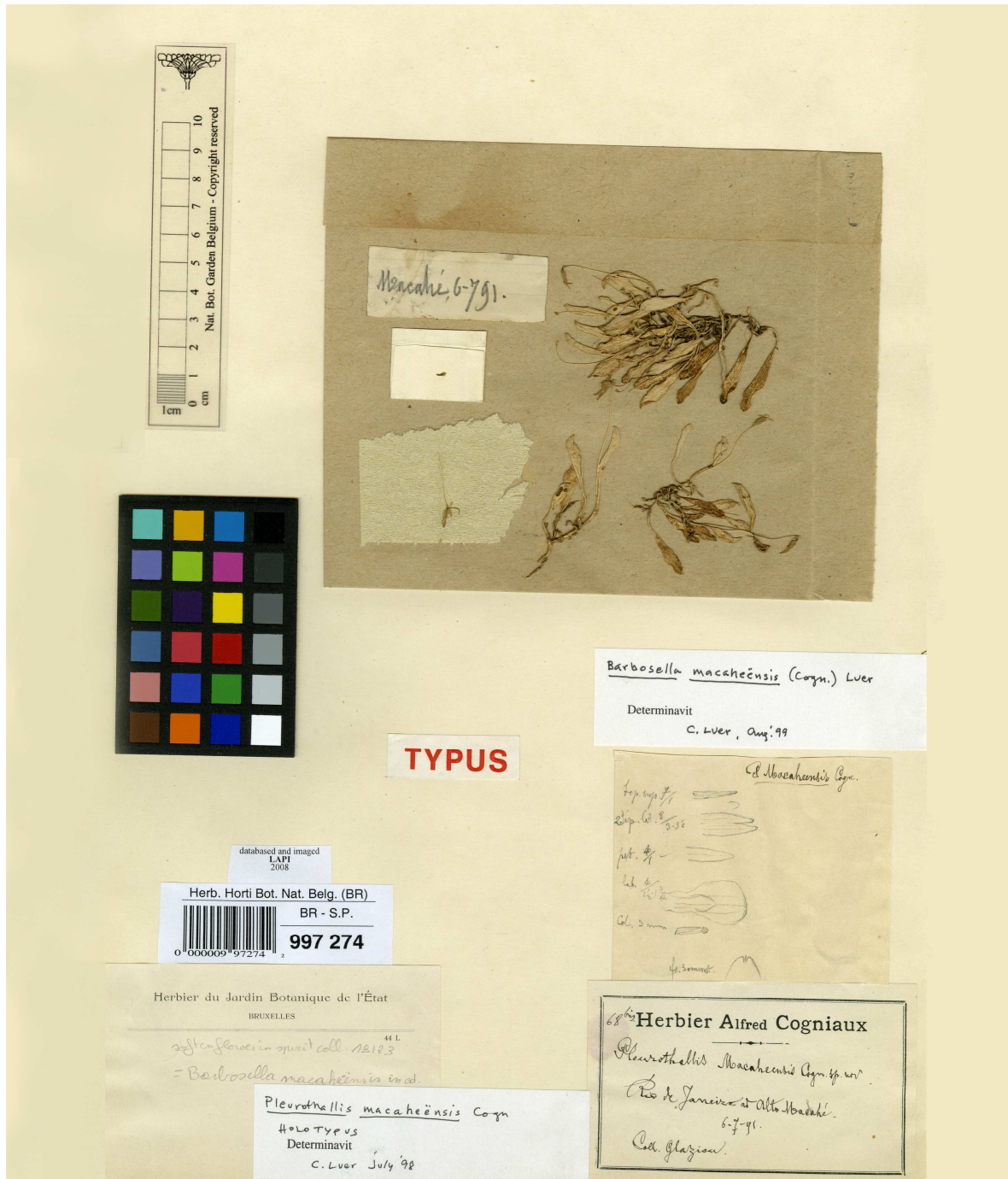


FIGURE 1. Holotype of *Barbosella macaheensis* deposited at BR (*Glaziou s.n.*).
Reproduced with permission of the Botanic Garden Meise.



FIGURE 2. Specimen of *Barbosella macaheensis* collected by Glaziou (18535a) and deposited at BR. It has been misidentified as *Pleurothallis articulata* Lindley (1842: 83). An illustration based on this collection was reproduced in Luer (2000). Reproduced with permission of the Botanic Garden Meise.



FIGURE 3. Duplicate specimen of *Barbosella macaheensis* collected by Glaziou (18535a) and deposited at P. It has been misidentified as *Pleurothallis hymenantha* Lindley (1835: sub t. 1797). Reproduced with permission of the Muséum National d'Histoire Naturelle (Paris). National infrastructure: e-ReColNat (ANR-11-INBS-0004).

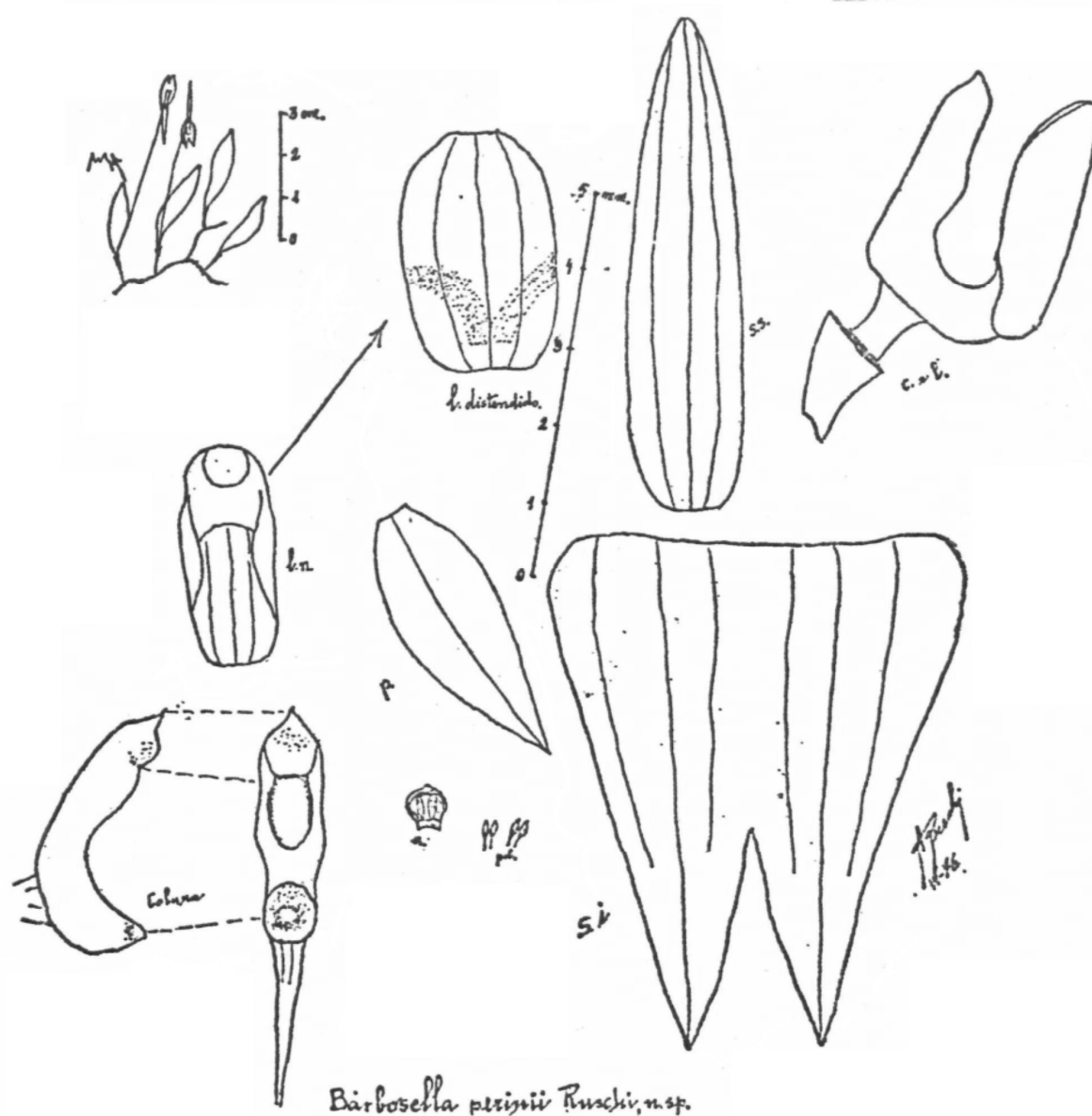


FIGURE 4. Augusto Ruschi's illustration which appeared in the protologue of *Barbosella perinii*. Reproduced with permission of the Boletim do Museu de Biologia Mello Leitão.

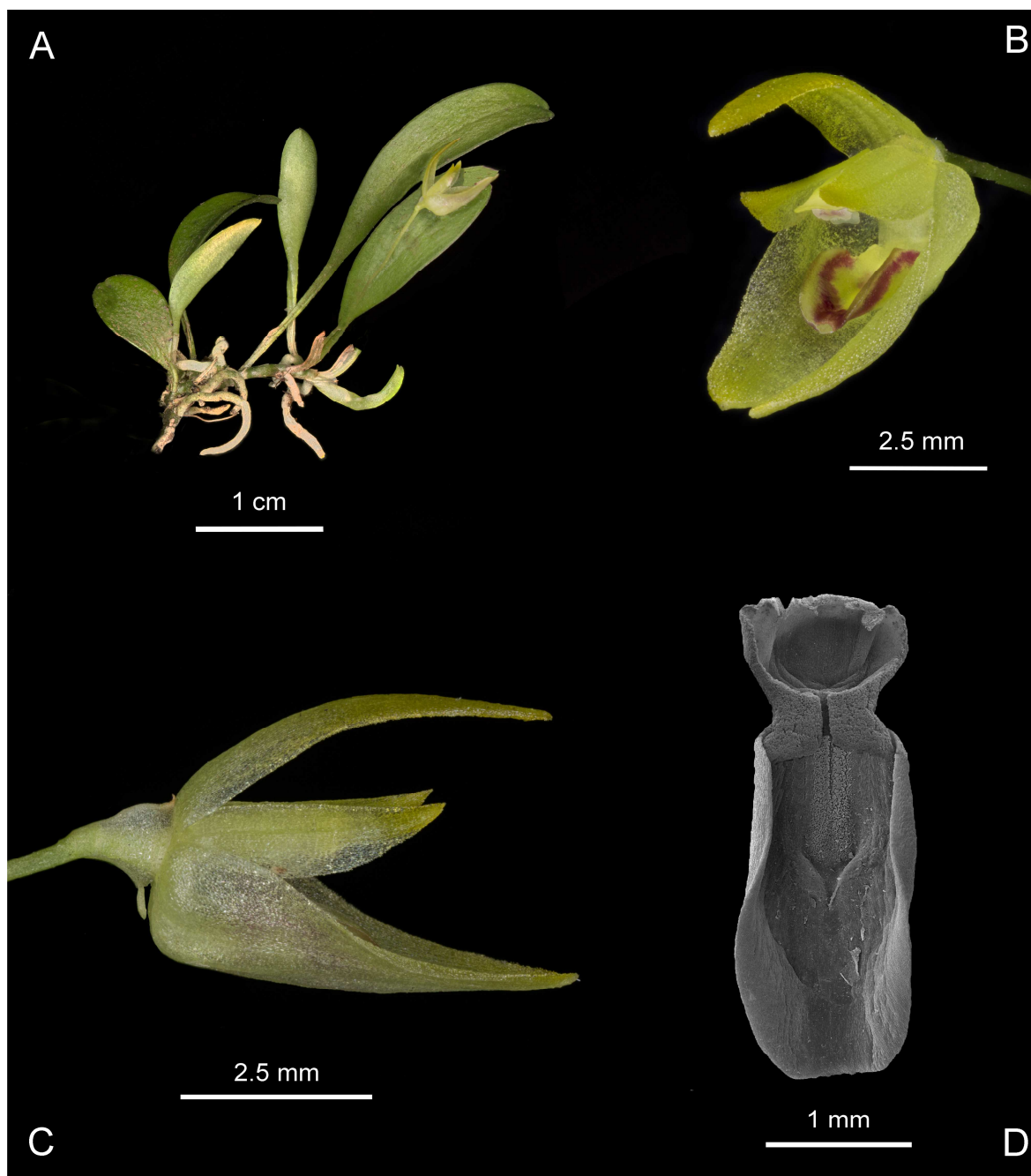


FIGURE 5. *Barbosella macaheensis*. A. Habit. B. Flower, 3/4 view. C. Flower, lateral view. D. Lip, frontal view (Scanning Electron Micrograph). Photos A–C by W. Collier & A. Toscano de Brito, based on *Bolson et al.* 582.

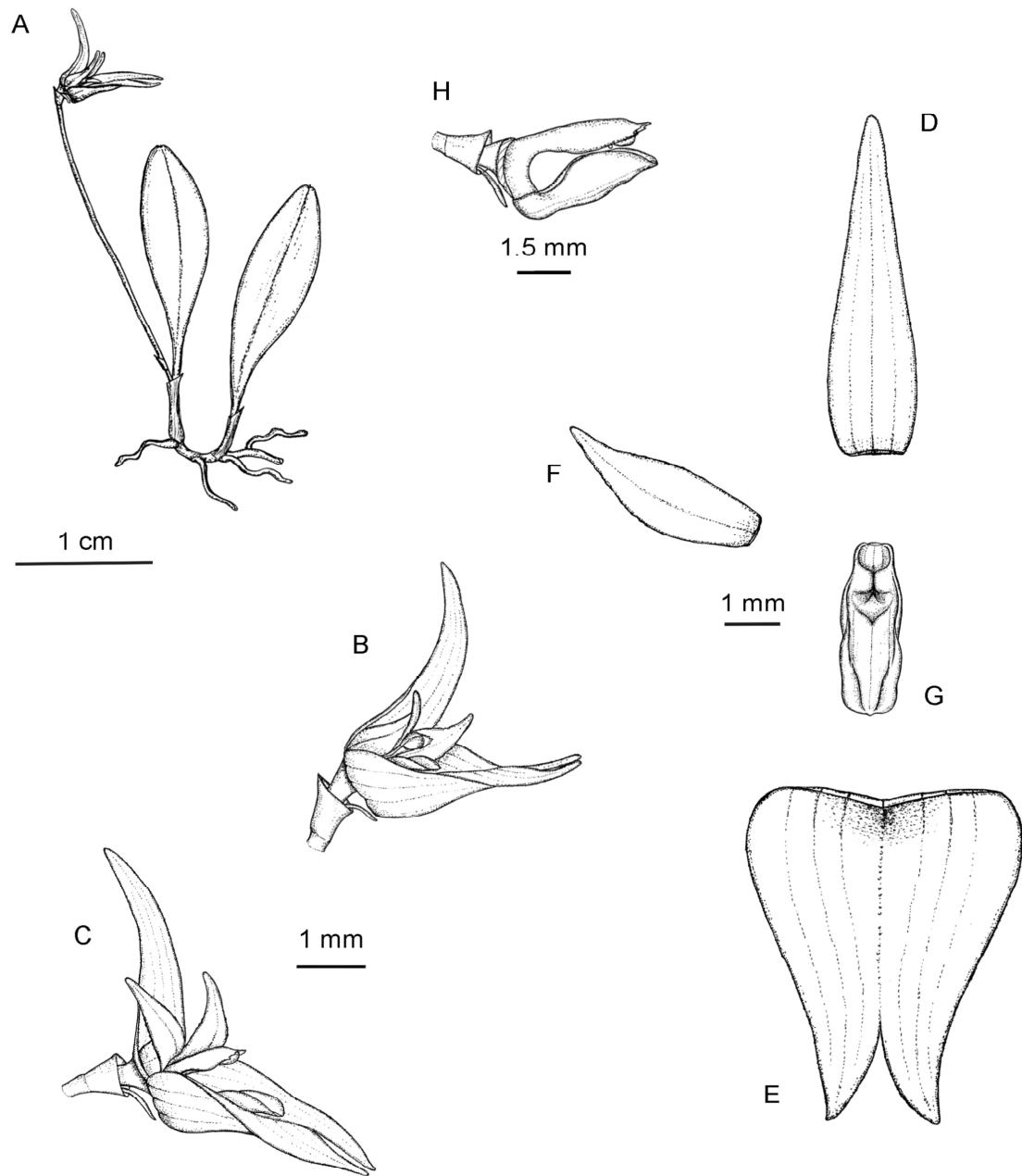


FIGURE 6. *Barbosella macaheensis*. A. Habit. B–C. Flower variation, lateral view. D. Dorsal sepal. E. Synsepal. F. Petal. G. Lip. H. Floral bract, ovary, lip and column in lateral view, based on *Bolson et al.* 582, except C based on *Bolson* 637.

CAPÍTULO IV / CHAPTER IV

O gênero *Barbosella* (Orchidaceae, Pleurothallidinae) no Brasil

The genus *Barbosella* (Orchidaceae, Pleurothallidinae) in Brazil

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(Capítulo formatado segundo as normas do periódico *Phytotaxa*)

The genus *Barbosella* (Orchidaceae, Pleurothallidinae) in Brazil¹

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Abstract

Barbosella comprises 20 orchid species widely distributed in the Neotropics, of which almost half are endemic to the Atlantic Forest of southeastern Brazil. A taxonomic review of the Brazilian species of *Barbosella* is presented herein. Morphological descriptions, taxonomic discussions, illustrations, identification key, updated synonymy and distribution maps as well their conservation status are provided for each taxon. Nine species are recognized as occurring in Brazil. With exception of *Barbosella cogniauxiana*, which also reaches northern Argentina, all others are Brazilian endemics. Lectotypes are designated for two names: *Restrepia dusenii* (= *Barbosella dusenii*) and *Restrepia microphylla* (= *Barbosella gardneri*). An epitype is selected for *Restrepia dusenii* (= *Barbosella dusenii*). All nine species here reviewed are considered Endangered according to IUCN categories.

Resumo

Barbosella compreende 20 espécies com ampla distribuição no Neotrópico, das quais a metade é endêmica da Floresta Atlântica brasileira. Neste trabalho apresentamos um tratamento taxonômico para as espécies brasileiras de *Barbosella* constituído de descrições morfológicas, ilustrações, fotografias, chave de identificação, status de conservação e distribuição geográfica. Novos registros são descritos para alguns estados brasileiros. São confirmadas nove espécies para o Brasil, todas endêmicas, exceto *Barbosella cogniauxiana*. Lectótipos são designados para duas espécies: *Restrepia dusenii* (= *Barbosella dusenii*) e *Restrepia microphylla* (= *Barbosella gardneri*). Um epítipo é selecionado para *Restrepia dusenii* (= *Barbosella dusenii*). De acordo com os critérios da IUCN, todas as espécies são consideradas em perigo.

Key words: Atlantic Forest, IUCN, Monocots, Neotropics, Taxonomy.

Introduction

Barbosella consists of about 20 species distributed in the Neotropical region, from Central America through the Andes to northern Argentina and southern Brazil. Approximately half of the species occur in the Brazilian Atlantic forest (Pridgeon 2005, Brazilian Flora 2020 in construction). It belongs to subtribe Pleurothallidinae, the most diverse Neotropical group in Orchidaceae, with 44 genera and over 5,000 currently accepted species (Karremans 2016).

The genus comprises epiphytic or rarely lithophytic herbs, with long-repent or caespitose growth, which sometimes form large, dense masses on the tree trunks. The ramicaul is abbreviated, without an annulus. The inflorescence is always a single-flowered raceme, with a long and delicate peduncle, emerging laterally from the ramicaul. The flower is resupinate due to a 180° bending of the flower near the apex of the peduncle (except in Panamanian *B. circinata* Luer 1977: 204). The sepals are similar, the dorsal is free, erect or patent, and the laterals are partially or completely connate into a synsepal (except in Ecuadorian *B. portillae* Luer 2002: 97). The petals are narrow, usually linear-ovate to linear-lanceolate. The lip is entire to trilobed, with obtuse to acute lobes, a sulcate disc, and basal cavity articulate to the column foot. The column is usually broadly and longitudinally winged with an entire or usually dentate apex, and in almost all species the base is prolonged into a prominent and bulbous foot, which is jointed to the basal cavity of the lip through a “ball-and-socket” articulation. The only exceptions are the Brazilian *B. miersii* (Lindl. 1842: 84) Schltr. (1918: 262) and *B. trilobata* Pabst (1956: 21), whose lips lack a basal cavity and are simply hinged to the column foot. The anther is incumbent and carries four clavate pollinia of equal size, with distinct caudicles and gemmate sculpture (Luer 2000, Stenzel 2000, Pridgeon 2005).

The genus was established by Rudolf Schlechter (1918: 259) based on two small and vegetatively similar plants, which had been previously described in *Pleurothallis* R.Br. (1813: 211): *P. gardneri* Lindl. (1842: 83) and *P. miersii* Lindl. As Schlechter did not indicate a nomenclatural type when describing the genus, Angely (1972) designated the latter species as lectotype. The generic name honors João Barbosa Rodrigues, a Brazilian botanist who specialized in Arecaceae and Orchidaceae in the 19th century.

The Brazilian species of *Barbosella* were described and reviewed by a number of studies: Barbosa Rodrigues (1882), Cogniaux (1896, 1906a), who treated the then known

species under the genus *Restrepia* Kunth (1816: 366), Hoehne (1947), and Pabst & Dungs (1975, 1977).

The only taxonomic revision of *Barbosella* is that of Luer (2000) in which 18 species are recognized. He followed his previous works (Luer 1981) and treated *Barbosella miersii* as the monospecific genus *Barbrodria* Luer (1981: 386) because of differences in the position of the anther, the absence of a lip basal cavity and the way it articulates with a not bulbous column foot. However, *Barbosella trilobata*, another unusual species whose flowers also lack a bulbous column foot and lip basal cavity, transferred to *Restrepiopsis* Luer (1978: 199) in Luer (1981), was then placed in *Barbosella* subgen. *Cardinella* Luer (2000: 10), a monospecific subgenus proposed to accommodate this single species. Nevertheless, molecular evidence suggests that *B. miersii* (Pridgeon *et al.* 2001) and *B. trilobata* (Bolson *et al.* in prep.) are nested within *Barbosella*.

The present work is a taxonomic review of the *Barbosella* species of Brazil. We provide morphological descriptions, taxonomic discussions, illustrations, an identification key, updated synonyms and distribution maps for all taxa as well their conservation status.

Methods

We examined specimens deposited in 46 herbaria, in addition to specimens collected in the field from 2014 to 2017. Specimens from the following herbaria were examined: AMES, BHCB, BHZB, BM, BR, C, CESJ, CRI, ECT, ESA, F, FLOR, FURB, GUA, HB, HBG, HCF, HPBR, HRCB, HUCP, HUEFS, HUEM, HVAT, ICN, JOI, K, M, MBM, MBML, MO, NY, P, PACA, R, RB, RFA, S, SEL, SP, SPF, SPSF, U, UEC, UPGB, US and VIES (acronyms following Thiers 2018). All examined specimens are listed under the taxonomic treatment for each species. The terminology for morphological characters follows Stearn (2004) and Gonçalves & Lorenzi (2007). The descriptions were generated using DELTA (Dallwitz *et al.* 2000).

When the specimen label did not present locality information, the locality record was geo-referenced using coordinates obtained from Google Earth (<http://earth.google.com/>). In some cases the position had to be approximated to municipality coordinates. When geographic information was too vague on the labels for coordinate determination, the specimens were not included in maps. The distribution maps and richness analyze (total number of species per grid square) were created by plotting the collection localities using DIVA-GIS 7.5 (Hijmans *et al.* 2012).

The conservation status was based on categories and criteria defined by the IUCN (2017), which are based on number of records, extent of occurrence (EOO), area of occupancy (AOO) and quality of habitat occupied by each species. The precautionary principle was applied; therefore, when categories were met resulting in different status assessments, the most threatened category was assigned (IUCN 2001). These estimates were obtained using GeoCAT (Bachman *et al.* 2011) applying the default 2 km² cell size.

Results

A total of 520 specimens from eight states were analyzed. Nine species of *Barbosella* occur in Brazil (Fig. 1). All but one species, *B. cogniauxiana* (Speg. & Kraenzl. 1908: 127) Schltr. (1918: 260), whose distribution extends to northern Argentina, are Brazilian endemics and mostly restricted to the Atlantic forest of the southeast (Fig.2). The only exception is *B. crassifolia* (Edwall 1903: 193) Schltr. (1918: 261), which is also found outside this range limit (Fig. 3–C). This species is the more widely distributed species in Brazil, extending from Rio de Contas, state of Bahia, in the northeast, to Capão do Leão, state of Rio Grande do Sul, in the south, whereas *B. macaheensis* (Cogniaux 1906b: 310) Luer (2000: 23), *B. spiritu-sanctensis* (Pabst 1975: 54) F.Barros & Toscano (1990: 23) and *B. trilobata* Pabst (1956: 21) seem to be the more restricted ones, with populations represented by few individuals distributed in few locations. The most richness states are Espírito Santo, Paraná and Santa Catarina, with six species each, followed by São Paulo, with five species (Fig. 2–I).

Silva & Silva (2004) reported the presence of *Barbosella orbicularis* Luer (1976: 10) in Brazil. This record was based on a misidentification, as the photograph presented by the authors refers to a species of *Anathallis* Barbosa Rodrigues (1877: 23). Apparently, no voucher of this collection has ever been made.

Taxonomy

Barbosella Schlechter (1918: 259–260)

Type species: *Pleurothallis miersii* Lindley (1842: 84) = *Barbosella miersii* (Lindley)

Schlechter (1918: 262). Lectotype selected by Angely (1972: 1282).

= *Restrepia* Kunth sect. *Ramulosae* Lindley (1859: 3). Type species: *Restrepia cucullata* Lindley (1845: 108).

= *Restrepia* Kunth sect. *Prorepentes* Reichenbach.f. (1877: 810). Type species: *Restrepia prorepens* Reichenbach. f. (1877: 810).

= *Barbrodria* Luer (1981: 386), *nom. illeg.* Type species: *Pleurothallis miersii* Lindley.

Plants very small to medium sized, perennial, epiphytic, long-repent; roots slender or stout, produced at the base of the ramicaul or along the rhizome; rhizome conspicuous or inconspicuous, slender, enclosed by tubular sheaths. Ramicaul unifoliate, ascending or erect, considerably shorter than the leaf, enclosed by small, tubular sheaths, without an annulus. Leaf suberect or prostrate, coriaceous, elliptical, oblanceolate or occasionally orbicular, apex obtuse or mucronate, base cuneate or rounded. Inflorescence a single-flowered raceme that emerges laterally below the apex of the ramicaul; peduncle erect, long and slender, with a bract at the base or occasionally below the middle; floral bract thin, tubular, oblique, enclosing the pedicel and often much of the ovary; pedicel usually shorter than the floral bract, with a small filament (remnant of a vestigial flower bud) at the apex; ovary trivalvate. Flowers resupinate due to a 180° bending of the flower near the peduncle apex; sepals translucent, green, yellow, orange, brown or purple, membranaceous, the dorsal sepal free, suberect or erect, linear, lanceolate or ovate, 1–3-veined, obtuse at the apex, the lateral sepals variously connate, lanceolate or ovate, concave at the base and sometimes forming a mentum, 1–3-veined, obtuse at the apex; petals similar in color and consistence to the sepals, lanceolate or ovate, the apex acute or obtuse, 1-veined; lip entire or trilobed, suboblong, oblong or elliptical, the base rounded or subcordate, often with a rounded cavity that articulates with the column foot (rarely simply hinged), the disc bicallos near the middle, the apex obtuse; column stout, broadly and longitudinally winged above the middle or not winged, the apex usually hooded and variously dentate, the base prolonged into a prominent and bulbous foot (rarely not bulbous), the anther ventral (rarely apical), the stigma ventral, the pollinia 4, arranged in two equal pairs, yellow, free, clavate. Capsule not seen.

Identification Key

1. Lip simply hinged to the apex of a not bulbous column foot 2
- Lip with a concave base articulated with a bulbous column foot 3
2. Lip entire *B. miersii*
- Lip trilobed *B. trilobata*

- 3. Lip trilobed 4
- Lip entire 6
- 4. Mid-lobe of the lip with acuminate apex..... *B. gardneri*
- Mid-lobe of the lip with the apex rounded or obtuse 5
- 5. Lateral sepals 2–4.5 mm long; lip with mid-lobe rounded at the apex, lateral lobes triangular, near the middle of the lip *B. crassifolia*
- Lateral sepals usually 6.8–7.35 mm long; lip with mid-lobe obtuse at the apex, lateral lobes rounded, below the middle of the lip *B. spiritu-sanctensis*
- 6. Dorsal sepal suberect and ovate.....7
- Dorsal sepal erect, linear to lanceolate.....8
- 7. Lip more than 6.2 mm long, with apex bifid divided into two obtuse angles
..... *B. australis*
- Lip less than 4.3 mm long, with truncated apex *B. macaheensis*
- 8. Lateral sepals 11.1–16 mm long; lip with a pair of low, indistinct, longitudinal calli near the middle, the disc shallowly concave and minutely papillose..... *B. cogniauxiana*
- Lateral sepals 6.95–9.1 mm long; lip with a pair of protruding longitudinal calli more or less below the middle, the disc concave and minutely papillose at base..... *B. dusenii*

1. *Barbosella australis* (Cogniaux) Schlechter (1918: 260). $\equiv$ *Restrepia australis* Cogniaux (1906a: 564). Type:—BRAZIL. Rio Grande do Sul: Ferromecco, 1889, A. Kunert 40 (BR [digital image!]).
- = *Restrepia loefgrenii* Cogniaux (1906a: 565). Type:—BRAZIL. São Paulo: Invernada Pinhal, Campos da Bocaina, 26 March 1994, A. Loeftgren & G. Edwall ex Comm. Geogr. & Geolog. São Paulo nr. 2303 (Holotype: BR [digital image!]; Isotypes: SP [digital image!], BR [digital image!]). = *Barbosella loefgrenii* (Cogniaux) Schlechter (1918: 262).
- = *Barbosella australis* var. *latipetala* Hoehne (1947: 74). Type:—BRAZIL. São Paulo, Jardim Botânico, 10 May 1938, O. Handro s.n. (Holotype: SP 46127 [digital image!]).
- = *Barbosella australis* var. *genuina* Hoehne (1947: 74), not validly publ.
- = *Barbosella australis* var. *loefgrenii* (Cogniaux) Hoehne (1947: 74).

Figure 3 A–D.

Plant small, 13–52 mm long. Roots stout, produced singly at the base of the ramicaul. Rhizome 4–21 mm long between ramicauls, stout. Ramicaul 3–15 mm long, ascending, 1–2 sheaths. Leaf 11–43.5 $\times$ 3–11 mm, suberect, coriaceous, elliptical to oblanceolate, the base cuneate, the apex minutely emarginate and mucronate. Inflorescence 22–71 mm long; peduncle 8–40 mm long, with a basal bract 2.4–8.4 mm long; floral bract 1.2–4.2 mm long; pedicel 0.48–1.66 mm long, with a filament 1–3.8 mm long; ovary 1.5–3.1 mm long; sepals yellowish-green, with purple primary veins, minutely papillose and ciliate, the dorsal sepal 14.7–21.6 $\times$ 2.1–3.7 mm, suberect, ovate, 3-veined, the apex obtuse, the lateral sepals 15.5–22.2 $\times$ 4–5.95 mm, ovate, connate for about half of their length, forming a deep mentum around the column foot, 3-veined, the apex obtuse; petals same color as sepals, 8.4–15.4 $\times$ 2.15–8 mm, ovate to lanceolate, minutely papillose and ciliate, the apex acute; lip yellow suffused with purple, 6.2–8 $\times$ 2.2–4.4 mm, entire, suboblong, the base rounded, deeply concave, articulated with the apex of the column foot, the disc with a pair of conspicuous, elongate and subpyramidal, obtuse callosities, the middle third concave, the apical third with a pair of inconspicuous, elongate, low callosities, the apex bifid into two obtuse angles; column 4.2–6.8 mm long, not winged, the base with a prominent and bulbous foot, the apex hooded, denticulate to slightly emarginate, the anther ventral.

Additional specimens examined:—BRAZIL. *s.loc.*, obtained from Orquidário Colibri, São Lourenço da Serra, São Paulo, fl. cult. in Curitiba, Paraná, 17 April 2015, *Bolson 523* (UPCB). *s.loc.*, obtained from Alvim Seidel Orquidário Catarinense, Corupá, Santa Catarina, fl. cult. in Curitiba, Paraná, 21 May 2015, *Bolson 525* (UPCB). *s.loc.*, obtained from Orquidário Três Irmãos, Garuva, Santa Catarina, fl. cult. in Curitiba, Paraná, 7 July 2015, *Bolson 532* (UPCB). Paraná: Araucária, 24 April 2004, *Kersten 850* (UPCB). Campina Grande do Sul, 21 May 1967, *Hatschbach 16468* (HB, MBM). *Idem*, 6 May 1960, *Hatschbach 7092* (HB, MBM). Curitiba, 20 May 2010, *Kersten 1433* (HUCP). *Idem*, Barigui de Santa Felicidade, 15 May 1958, *Leinig 28* (HB). Guaratuba, 16 June 1957, *Hatschbach 4049* (HB, MBM). *Idem*, 22 June 2001, *Santos et al. 1006* (UPCB). Jaguariaíva, 13 July 2005, *Barbosa et al. 1076* (MBM). Morretes, 1 July 2016, *Santos & Machnicki-Reis 148* (UPCB). *Idem*, 7 May 1961, *Hatschbach 8015* (MBM, U). Piraquara, 18 May 1909, *Dusén 8138* (HBG, S). *Idem*, Mananciais da Serra, 21 June 2006, *Imig 451* (UPCB). Quatro Barras, 20 June 1965, *Hatschbach 12634* (F, MBM, MO, UPCB, US). São José dos Pinhais, 19 July 1967, *Hatschbach 16716* (MBM). *Idem*, 3 June 1970, *Hatschbach 24383* (MBM). *Idem*, 26 May 2014, *Ariati 983* (MBM). São Mateus do Sul, 29 June 1987, *Souza & Britez 1627* (MBM). Telêmaco Borba, 2012, *Bochorny s.n.* (MBM394155). Tijucas do Sul, 10 June 1963, *Klein 3620* (HB). *Idem*, 7 July 2016, *Santos 147* (UPCB). *Idem*, 11 July 2016, *Toscano de Brito 3548* (UPCB). Rio de Janeiro: Petrópolis, cultivated by J & L Orchids, obtained from Verboonen, 21 October 1988, *Luer 13756* (MO). Resende, Serra do Itatiaia, 1953, *Segadas-Vianna et al. 264* (R). Rio Grande do Sul: Camaquã, 21 June 2001, *Jurunitz 217* (ICN). Dois Irmãos, 15 April 1979, *Saalfeld s.n.* (ICN 44532). Torres, 8 July 1977, *Waetcher 560* (ICN). Guaíba, 16 May 1994, *Nunes 1406* (ICN). Pelotas, 10 May 2008, *Perleberg 248* (ECT, RB). *Idem*, 31 May 2008, *Perleberg 250* (ECT). *Idem*, 31 May 2008, *Perleberg 264* (ECT). *Idem*, 31 May 2008, *Perleberg 260* (ECT, RB). *Idem*, 4 June 1959, *Sacco 1317* (HB). São Francisco de Paula, s.d., *Klein 6* (UPCB). São José do Herval, 18 April 2001, *Klein & Freitas s.n.* (HVAT 707). São Leopoldo, s.d., *Dutra 1112* (ICN). *Idem*, 12 May 1947, *Orth S.J. s.n.* (PACA 2850). *Idem*, 15 May 1937, *Orth S.J. s.n.* (PACA 2853 — mixed collection with *B. cogniauxiana*). Km 58 da estrada Porto Alegre-Taquara, 2 June 1964, *Birk 19* (HB). Cultivado no Jardim Botânico de São Paulo, March 1951, *s.coll. s.n.* (SP 56257). Santa Catarina: Doutor Pedrinho, 25 May 2010, *Cadorin et al. 2335* (FURB). Lages, June 1961, *Seidel s.n.* (HB 19647). Luzerna, 21 May 2015, *Bolson 527* (UPCB). *s.loc.*, l.a.h.i., June

1962, *Seidel* 872 (HB). São Paulo: Santo André, Estação Biológica do Alto da Serra de Paranapiacaba, 4 May 1921, *Gehrt* 5612 (AMES, NY, SP). Serra da Bocaina, 23 April 1951, *Brade* 20688 (ICN, RB).

Barbosella australis is recognized by its relatively large habit and flowers for the genus. The ramicauls may reach ca. 20 mm in length and the leaves are elliptical or oblanceolate. The flowers possess an entire, suboblong lip with a bifid apex and a pair of prominent subpyramidal calli on the disc next to the basal cavity. It was collected in flower from March to July and in October.

Alfred Cogniaux described *Restrepia loefgrenii* (= *Barbosella australis*) in his orchid monograph for Martius's *Flora Brasiliensis* in 1906. He based his description on a collection by Albert Löfgren (1854-1918) and Gustaf Edwall (1862-1946), who worked as scientists for Comissão Geográfica e Geológica de São Paulo. Orchid specimens collected by the Comissão are deposited mainly at SP with duplicates in several herbaria including AMES, BR, C, S, and others. The original materials cited in the protologue of *Restrepia loefgrenii* comprises the holotype, i.e., the actual specimen collected by Löfgren and Edwall (nr. 2303) and an illustration by Löfgren (nr. 36). These two materials are found mounted on the same herbarium sheet (BR barcode BR0000009972773) deposited at BR, where Cogniaux worked as curator. Isotypes exist at BR (barcode BR0000009894815) and SP (barcode SP002408), the latter specimen erroneously cited as holotype by Luer (2000). The type sheet at BR also contains an additional illustration, a pencil drawing of the habit and floral segments, by the German artist Joseph Pohl (1864-1939), who prepared many orchid illustrations that appeared in *Flora Brasiliensis*. This illustration was apparently meant for publication in Cogniaux (1906a), but it remains unpublished until today. Study of these materials and the additional specimens cited herein supports the view of Hoehne (1947) and Luer (2000), who considered this taxon conspecific with *Barbosella australis*. It is worth mentioning here that the names *Barbosella loefgrenii* and *Restrepia loefgrenii* are not cited anywhere in Pabst & Dungs (1975, 1977), who provided an illustrated checklist of the Brazilian Orchidaceae.

Barbosella australis is a common and widely distributed species in the southeast and south of Brazil, and it mainly inhabits humid, high altitude areas of Atlantic forest. It occurs in the states of Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, and São Paulo (Fig. 2A). Following the IUCN categories, this taxon is here classified as

Endangered [EN B2b(ii,iii)] despite its estimated EOO of 353,333 km² and estimated AOO of 156 km². Almost all collections studied came from outside protected areas, which are impacted by anthropic actions such as dams, proximity of railways and farms.

2. *Barbosella cogniauxiana* (Spegazzini & Kraenzlin) Schlechter (1918: 260). $\equiv$ *Restrepia cogniauxiana* Spegazzini & Kraenzlin, in Kraenzlin (1908: 127). Type:—ARGENTINA. Misiones: San Pedro, June 1908, *C.L. Spegazzini s.n.* (Holotype: HBG 501979!).

= *Restrepia porschii* Kraenzlin (1911: 51). Type:—BRAZIL. Paraná: Ponta Grossa, Vila Velha, 4 February 1909, *P.K.H. Dusén 8032* (Holotype: S!; Isotypes: AMES [digital image!], MBM!, NY!). = *Barbosella porschii* (Kraenzlin) Schlechter (1918: 263).

= *Barbosella handroi* Hoehne (1933[1934]: 613). Type:—BRAZIL. São Paulo: São Paulo, “Parque do Estado e Jardim Botânico”, 15 December 1930, *O. Handro s.n.* (Holotype: SP 25476!; Isotypes: AMES 54830!, 61620!, F 895973!, HB 55001!, MO 5463776!, NY barcode NY0008586 [digital image!]).

= *Pleurothallis spegazziniana* L.O.Williams (1941: 243), replacement name for *Restrepia cogniauxiana* Spegazzini & Kraenzlin.

= *Barbosella riograndensis* Dutra ex Pabst (1959: 133), *nom. nud.*

Figure 3 E–H.

Plant small, 5–33 mm long. Roots stout, produced from nodes along the rhizome. Rhizome 2–14 mm long, stout. Ramicaul 1–8 mm long, ascending, 1–2 sheaths. Leaf 5–24.5 $\times$ 2–7 mm, suberect, thickly coriaceous, elliptical, the base cuneate, the apex minutely emarginate. Inflorescence 27–90 mm long; peduncle 22–74 mm long, with a basal bract 2–8 mm long; floral bract 1.2–3.8 mm long; pedicel 0.59–1 mm long, with a filament 0.7–2.5 mm long; ovary 0.72–1.40 mm long; sepals yellow to yellowish-green, the dorsal sepal suffused with pale purple near the base, 7.7–15.7 $\times$ 0.7–1.6 mm, erect, linear to lanceolate, slightly recurved toward the apex, 3-veined, the apex obtuse, the lateral sepals 11.1–16 $\times$ 1.95–3.6 mm, oblong-ovate to oblong-lanceolate, slightly concave at base, connate to near the apex (rarely connate at base only) into a narrow, shortly bifid synsepal, 6-veined all together, the apex obtuse; petals same color as lateral sepals, 5.5–10 $\times$ 0.55–1.3 mm, linear to lanceolate, the apex acute; lip yellow, suffused with red-brown on the basal third, 2.55–

3.8 × 1–1.7 mm, entire, elliptical, slightly dilated near the middle, the base rounded, deeply concave, articulated with the apex of the column foot, the disc with a pair of short, obtuse callosities next to the concave lip base, concave and microscopically papillose above the callosities, with a pair of low, indistinct, longitudinal calli near the middle, apex obtuse to subacute; column 1.95–2.7 mm, winged on the apical third, the base with a prominent and bulbous foot, the apex hooded, apiculate slightly denticulate, the anther ventral.

Additional specimens examined:—ARGENTINA. Misiones: San Pedro, 9 March 1991, *Johnson 168* (MO). BRAZIL. *s.loc.*, cultivated by Florália, 26 January 1997, *Luer & Toscano de Brito 18270* (MO). *s.loc.*, flowered in cultivation by J & L Orchids, Easton, C.T., 28 September 1991, *Luer 15426* (MO). *s.loc.*, flowered in cultivation by J & L Orchids, Easton, CT, 28 September 1991, *Luer 15424* (MO). *s.loc.*, purchased from Orquidário Binot, flowered in cultivation by J & L Orchids, Easton, C.T., 5 May 1990, *Luer 14750* (MO). *s.loc.*, obtained from Orquidário Colibri, São Lourenço da Serra, São Paulo, fl. cult. in Curitiba, Paraná, 11 February 2016, *Bolson 604* (UPCB). *s.loc.*, obtained from Orquidário Três Irmãos, Garuva, Santa Catarina, fl. cult. in Curitiba, Paraná, 11 February 2016, *Bolson 605* (UPCB). Espírito Santo: Domingos Martins, 15 June 1976, *Kautsky 547* (HB). *s.loc.*, cultivated at Orquidário Caliman, Venda Nova do Imigrante, Espírito Santo, 7 June 2012, *Toscano de Brito 3087* (UPCB). Minas Gerais: Oliveira, originally collected by Márcio Silveira, cultivated at Orquidário Caliman, Venda Nova do Imigrante, Espírito Santo, 26 March 2015, *Toscano de Brito 3383* (UPCB). Paraná: Campo Mourão, 7 March 2016, *Geraldino 328* (HCF, MBM). *Idem*, Parque Municipal do Lago, 19 May 2005, *Paulino 58* (MBM). Curitiba, 30 March 1965, *Grupo Piracicaba 7761* (HB). Jaguariáiva, 5 March 1916, *Dusén 17962* (AMES, HB, MBM, NY, S). *Idem*, 20 March 1912, *Dusén 14007* (S). Luiziana, Estação Ecológica Luiziana, 21 March 2012, *Caxambu 3929* (HCF). Mauá da Serra, December 2009, *Milaneze-Guitierre 68* (HUEM). Piraquara, 5 January 1953, *Hatschbach 2921* (HB, MBM). *Idem*, 16 March 1911, *Dusén 11552* (AMES, S). Ponta Grossa, 13 January 1949, *Brade 19674* (RB). *Idem*, 13 February 1949, *Brade 19676* (HB). *Idem*, 2014, *Klingelfus 113* (UPCB). *Idem*, 2014, *Klingelfus 114* (UPCB). *Idem*, Vila Velha, 4 February 2010, *Mancinelli 1137* (JOI). *Idem*, 30 March 1965, *Grupo Piracicaba 6343* (HB). *Idem*, January 1964, *Frank s.n.* (HB 20560). *Idem*, 6 March 1963, *Leinig 301* (HB). *Idem*, 30 March 1965, *Grupo Piracicaba 11740* (HB). *Idem*, 20 November 1967, *Grupo Piracicaba 11742* (M). *Idem*, 27 April 1909, *Dusén 8473* (HBG). *Idem*, 9 June 2003, *Breier 1366* (UEC, digital image). Quedas do Iguaçu, 12 May 1999,

Carneiro 675 (MBM). Telêmaco Borba, February 2012, *Milaneze-Guitierre s.n.* (HUEM 29102). *Idem*, 26 February 1910, *Dusén* 9826 (S). *Idem*, 10 February 2009, *Bonnet* 630102 (UPCB). *Idem*, 24 April 2012, *Bochorny* 82 (MBM). *Idem*, 30 January 2012, *Ariati* 576 (MBM). Tibagi, 19 February 1953, *Hatschbach* 3228 (HB, MBM, U). Toledo, Bom Princípio, 6 January 1959, *Leinig* 84 (HB). *s.loc.*, 01 March 2011, *Smidt* 1029 (UPCB). *s.loc.*, fl. cult., 20 April 1944, *Guimarães s.n.* (RB 53488). *s.loc.*, 3 March 1916, *Dusén s.n.* (S 16-23418). Rio Grande do Sul: Estrela, 3 February 2002, *Freitas s.n.* (HVAT 903). Forquentina, 19 May 2001, *Freitas s.n.* (HVAT 683). Lajeado, 01 April 2000, *Freitas s.n.* (HVAT 227). Marcelino Ramos, Estação Ecológica Teixeira Soares, 8 March 1999, *Scariot s.n.* (HPBR 6141). Rolante, s.d., *Klein* 203 (UPCB). São José do Herval, 14 April 2001, *Freitas s.n.* (HVAT 684). *Idem*, 18 May 2001, *Freitas s.n.* (HVAT 705). São Leopoldo, 16 April 1925, *Dutra* 860 (ICN, RB). *Idem*, 15 May 1937, *Orth S.J. s.n.* (PACA 2853 — mixed collection with *B. australis*). *Idem*, 1940, *Eugenio* 117 (R). *Idem*, 1940, *Leite* 378 (SP). *Idem*, s.d., *Dutra s.n.* (ICN 192827). Sapiranga, 2 April 1991, *Nunes et al.* 1283 (PACA). Santa Catarina: Benedito Novo, 11 February 2016, *Bolson et al.* 603 (UPCB). Florianópolis, 18 March 1951, *Rohr* 2107 (HB). Ibicaré, 17 April 2015, *Bolson* 522 (UPCB). Ibirama, Horto Forestal INP, 6 March 1956, *Klein* 1904 (HB, NY, S, US). Nova Teutônia, 8 April 1944, *Plaumann* 510 (RB). Orleans, 02 March 1993, *Citadini-Zanette & Martinello s.n.* (CRI 1653). Peritiba, 12 February 2016, *Imig* 414 (UPCB). Santo Amaro da Imperatriz, 1 March 2013, *Lenzi et al.* 104 (FLOR). *s.loc.*, 1951, *Reitz* 4805 (AMES, HB). São Paulo: Jundiaí, 3 March 2000, *Pansarin* 708 (UEC, digital image).

Barbosella cogniauxiana resembles *B. australis* and *B. dusenii* in habit and leaf shape and size, but its leaves are smaller and thicker than in the last two. *Barbosella cogniauxiana* can also be distinguished from them by the shape of the lateral sepals, which are usually fused to almost the apex to form a shortly bifid synsepal. The lateral sepals were found to be only fused at base in one collection studied (*Luer & Toscano de Brito* 18270, MO), but this condition seems to be very rare or even an abnormality. The lip is variable in shape and very similar to that of *B. dusenii*, with which *B. cogniauxiana* shares a pair of longitudinal calli more or less in the middle of the lip, but these callosities are more distinct in *B. dusenii*. With *B. australis*, it shares a pair of obtuse, subpyramidal callosities next to the concave base, but they are not as evident as in *B. cogniauxiana*. This species was collected in flower throughout the year.

Barbosella cogniauxiana was first described in the genus *Restrepia* by Carlo Luigi Spegazzini (1858-1926), an Italian-born Argentine botanist who collected extensively in Argentina, and the German botanist Friedrich W. L. Kraenzlin (1847-1934). The description appeared in Kraenzlin's work entitled "Neue und Kristische Arten" published in 1908 and was based on a collection by Spegazzini from the district of San Pedro, Argentinian province of Misiones. Spegazzini's personal herbarium of vascular plants was transferred from LPS to LP around 1966 (Gutiérrez *et al.* 2002; Katinas *et al.* 2001, 2004). Despite the citation of Luer (2000) that the holotype is deposited at LP, we were not successful in locating this specimen. According to the staff at LP, no collection of *R. cogniauxiana* collected by Spegazzini exists at this herbarium (L. Iharlegui 2017, pers. comm.). The only specimen of *R. cogniauxiana* that agrees with the protologue, and dully annotated by Kraenzlin as "*typus*", is the one cited by Christenson (1994), which is part of Kraenzlin's personal herbarium deposited at HBG (barcode 50199). This is most probably the only existing specimen of *R. cogniauxiana* collected by Spegazzini, and there is no evidence that duplicates are deposited in other herbaria.

Barbosella cogniauxiana is endemic to Atlantic forest of southern Brazil and also reaches the Argentinian province of Misiones, where it was first discovered. This name was not included in Hoehne (1947) and Pabst & Dungs (1975) most probably because it was believed to be exclusively Argentinian in distribution. Luer (2000) reported it to the states of Paraná, Rio Grande do Sul, Santa Catarina, and São Paulo, and was the first to recognize it as conspecific with *B. handroi* and *B. porschii*, two later synonyms. It is herein for the first time recorded for the state of Espírito Santo (Fig. 2B) based on a collection (Kautsky 547, HB) previously identified by Guido Pabst in the herbarium as *B. porchii*. It is also herein recorded for the state of Minas Gerais, based on a single collection cultivated at Orquidário Caliman, located in Espírito Santo, southeast Brazil. Following the IUCN categories, based on its estimated EOO of 560,700 km² and estimated AOO of 160 km², *B. cogniauxiana* is here classified as Endangered [EN B2b(ii,iii)].

3. *Barbosella crassifolia* (Edwall) Schlechter (1918: 261). $\equiv$ *Restrepia crassifolia* Edwall (1903: 193). Type:—BRAZIL. São Paulo: Itirapina, near Morro Pelado, January 1907, G. Edwall ex Comissão Geográfica e Geológica de São Paulo nr. 6025 (Holotype: SP 23536 [digital image!]).

- = *Pleurothallis hamburgensis* Kraenzlin (1911: 49). Type:—BRAZIL. Rio Grande do Sul: Novo Hamburgo, 27 October 1892, *C.A.M. Lindman A.459* (Holotype: S [digital image!]; Isotypes: AMES!, HB, HBG!, MO!, S [digital image!]). = *Barbosella hamburgensis* (Kraenzlin) Hoehne (1947: 75).
- = *Barbosella crassifolia* var. *genuina* Hoehne (1947: 75), not validly publ.
- = *Barbosella crassifolia* var. *minor* Hoehne (1947: 75). Type:—BRAZIL. São Paulo: São Paulo, Butantã, 05 September 1917, *A. Gehrt s.n.* (Holotype: SP 495 [digital image!]; Isotypes: AMES 54827!, MO 5463777!, NY barcode NY00414711!, R 3369!).
- = *Barbosella crassifolia* var. *aristata* Hoehne (1947: 75). Type:—BRAZIL. São Paulo: São Paulo, Bosque da Saúde, 08 June 1921, *F.C. Hoehne s.n.* (Holotype: SP 5614 [digital image!]; Isotypes: AMES 54829! — mixed collection with *B. miersii*, NY barcode NY00414717! — mixed collection with *B. miersii*).
- = *Barbosella crassifolia* var. *hamburgensis* (Kraenzlin) Garay (1952: 178).

Figure 3 I–L.

Plant very small, 3–7 mm long. Roots slender, produced singly at nodes along the rhizome. Rhizome 1–5 mm long between ramicauls, slender. Ramicaul 0.5–2 mm long, ascending, 1–3 sheaths. Leaf 3–5.5 × 1–4 mm, prostrate, thickly coriaceous, suborbicular to elliptical, the base rounded, the apex obtuse to acute, minutely apiculate. Inflorescence 13–32 mm long; peduncle 11–27 mm long, with a bract below the middle, 0.3–1.7 mm long; floral bract 0.6–1.2 mm long; pedicel 0.4–0.66 mm long, with a filament 0.5–1.5 mm long; ovary 0.43–0.77 mm long; sepals yellowish-green, occasionally light purple, with purple primary veins, the dorsal sepal 3.3–5.7 × 0.6–1.2 mm, erect, ovate, 3-veined, apex obtuse, the lateral sepals 2.5–4.5 × 0.8–2.9 mm, ovate, connate at base for about two-thirds of their length, forming a deep mentum around the column foot, 6-veined all together, the apex obtuse; petals same color as sepals, 2.5–4.8 × 0.4–0.8 mm, lanceolate, apex acute; lip light purple with yellowish-green margin, 1.35–3.2 × 0.5–1.9 mm expanded, trilobed, the base truncate and slightly concave, articulated with the apex of the column foot, the disc with a pair of low longitudinal calli near the base, the lateral lobes near the middle, erect, triangular, the apical lobe ovate to oblong, rounded at apex; column 1–2.4 mm long, winged, the base with a prominent and bulbous foot, the apex hooded, denticulate, the anther ventral.

Additional specimens examined:—BRAZIL. *s.loc.*, 1 March 2014, *Varella s.n.* (UPCB 91641). *s.loc.*, cultivated by Rafael Bianchi Galati - Embu das Artes/ São Paulo, 11 March 2007, *Luer et al. 21081* (SEL). Bahia: Rio de Contas, Pico das Almas, 12 December 1988, *Harley & Hind 27219* (K, SPF). Espírito Santo: Santa Teresa, 18 March 1940, *Ruschi 44* (SP — mixed collection with *B. gardneri*). Venda Nova do Imigrante, collected locally and cultivated by Orquidário Caliman, 7 June 2012, *Toscano de Brito 3086* (UPCB). Minas Gerais: *s.loc.*, cultivated by Orquidário Caliman - Venda Nova do Imigrante, Espírito Santo, 22 November 2015, *Toscano de Brito 3479* (UPCB). Belo Horizonte, Serra da Canastra, 14 December 2009, *Ferreira Junior et al. s.n.* (BHZB 7894). Betim, 7 October 1945, *Williams & Assis 7608* (AMES, HB, K, MO, R, RB, S, SP, US). Carrancas, 20 January 2001, *Borba et al. s.n.* (UEC 118850, digital image). *Idem*, 16 January 2006, *Batista et al. 2421* (BHCB). Conceição das Alagoas, cultivated by Ademar Dantas, University of São Paulo, Piracicaba, São Paulo, 14 March 2007, *Luer et al. 21114* (SEL). Córrego Fundo, próximo Formiga, 27 April 1956, *Welter 170* (HB). Ibiraci, December 1999, *Silva s.n.* (SP 342965). Uberlândia, 23 October 2015, *Bolson 607* (UPCB). Serra do Guapé, September 1961, *Andra & Emmerich 1119* (R). Paraná: Pontal do Paraná, 23 October 2015, *Bolson et al. 545* (UPCB). Rio Grande do Sul: Cambará do Sul, Parque Nacional dos Aparados da Serra, 20 September 1980, *Fleig s.n.* (ICN 48476). Capão do Leão, Horto Bot. Irmão. Teodoro Luís, 29 September 1986, *Waetcher 2224* (ICN). São Leopoldo, 13 October 1926, *Dutra 880* (ICN). *Idem*, 23 April 1905, *Pivetta S.J. s.n.* (RB 3695). Torres, 29 July 1977, *Waetcher 572* (ICN). Amaral Ribeiro, 23 November 1927, *Dutra s.n.* (SP 20941). São Paulo: São Paulo, s.d., *Brade 8127* (AMES, US). Santo Amaro, 20 September 1942, *Krieger 138* (CESJ).

Barbosella crassifolia is distinguished by the small leaves and relatively large flowers. The leaves are usually appressed on the substrate, quite variable in shape, ranging from orbicular to elliptical, and are usually fleshy and rugous or smooth. When sterile, *B. crassifolia* can be confused with *B. spiritu-sanctensis*, from which it can be distinguished by the position of the sterile bract of the peduncle, which is placed more or less in the middle, and the obliquely triangular, lateral lobes of the lip. In *B. spiritu-sanctensis*, the sterile bract is located near the base of the peduncle and the lateral lobes are more rounded and obtuse. Petals in *B. crassifolia* are about the same length or longer than sepals. Collected with flowers year round.

Barbosella crassifolia is a Brazilian endemic species distributed from northeast to south of the country, previously known to the states of Bahia (Toscano de Brito 1995; Toscano de Brito & Cribb 2005), Minas Gerais, São Paulo, Santa Catarina and Rio Grande do Sul (Pabst & Dungs 1975). It is here recorded for the first time to the states of Espírito Santo and Paraná (Fig. 2C). This species was first recorded for the state of Santa Catarina by Pabst & Dungs (1975) and then by Reis *et al.* (2011) and Siqueira *et al.* (2014), all based on *Rohr 2030* (HB), a specimen collected in Campo de Massiambú, in Serra do Tabuleiro State Park, in municipality of Palhoça. Unfortunately, we were unable to locate this collection during a recent visit to HB, and this precluded us to confirm its identity. *Barbosella crassifolia* is the most widely distributed species in Brazil, with an estimated EOO of 886,484 km² and estimated AOO of 88 km². Following the IUCN categories, this species is here classified as Endangered [EN B2b(ii,iii)].

4. *Barbosella dusenii* (A. Sampaio) Schlechter (1918: 261). $\equiv$ *Restrepia dusenii* A. Sampaio (1909: 189). Type:—BRAZIL. Paraná, s.d., *P.K.H. Dusén s.n.* (Holotype: Lost; Lectotype here designated: original illustration by Santos Lahera, which appeared in *Arq. Mus. Nac. Rio de Janeiro* 15: 193. 1909; Epitype here designated: BRAZIL. Paraná: Ponta Grossa, Vila Velha, 27 April 1909, *P.K.H. Dusén 8070* (S [digital image!]; AMES!, HBG!, K, MBM!, MO!, P [digital image!], U!).
= *Barbosella gardneri* var. *dusenii* (A. Sampaio) Hoehne (1947: 75).

Figure 3 M–O.

Plant small, 4.5–16 mm long. Roots slender, produced singly at the base of ramicaul. Rhizome 1.5–9 mm long, slender. Ramicaul 2–4 mm long, ascending, 1 sheath. Leaf 3–11 $\times$ 2–5 mm, suberect to prostrate, thickly coriaceous, elliptical to suborbicular, base cuneate, apex mucronate. Inflorescence 22–60 mm long; peduncle 17–52 mm long, with a basal bract 1.1–4.4 mm long; floral bract 0.9–2.4 mm long; pedicel 0.6–1.09 mm long, with a filament, 0.7–1.9 mm long; ovary 0.78–1.42 mm long; sepals yellowish-green, minutely papillose, the dorsal sepal occasionally light purple at the base, 5.9–8.45 $\times$ 0.65–1.2 mm, erect, linear to lanceolate, 3-veined, apex obtuse, the lateral sepals 6.95–9.1 $\times$ 1.2–2.7 mm, narrowly ovate-lanceolate to lanceolate, slightly concave at the base, connate to just below the middle or to near the apex, sometimes forming a deeply bifid synsepal, 6-veined all together, the apex obtuse; petals same color as lateral sepals, 3.95–7.7 $\times$ 0.6–1.3

mm long, minutely papillose, lanceolate, apex acute; lip yellowish-green, $1.8\text{--}3.2 \times 0.7\text{--}1.1$ mm, entire, oblong to elliptical, the base rounded to truncate, with a shallow cavity, articulated with the apex of the column foot, the disc concave and minutely papillose at base, with a pair of longitudinal calli more or less below the middle, apex obtuse to subacute; column $1.5\text{--}2.8$ mm long, winged, the base with a prominent and bulbous foot, the apex hooded, apiculate to slightly emarginated, the anther ventral.

Additional specimens examined:—BRAZIL. *s.loc.*, obtained from Florália Orquídeas, flowered in cultivation at Selby Gardens 2017–0217–A, 12 January 2018, *Toscano de Brito 3707* (SEL). *s.loc.*, cultivated by Marcos Klingelfus in Pinhais, Paraná, flowered in 2014, *Klingelfus 116* (UPCB). Bahia: Sul do Estado, 6 July 1980, *Silva s.n.* (SP 247569). Espírito Santo: Domingos Martins, 15 June 1976, *Kautsky 548* (HB). *Idem*, Pedra Azul, 10 August 1988, *Milaneze et al. s.n.* (VIES 3009). Mimoso do Sul, 9 June 2004, *Couto 137* (MBML). *Idem*, 16 June 2006, *Couto 314* (MBML). *Idem*, 29 June 2008, *Couto 618* (MBML). Santa Teresa, 28 July 2007, *Kollmann & Brahim 9502* (MBML). Minas Gerais: Santana do Riacho, Serra do Cipó, originally collected by Francisco Bicalho *s.n.*, flowered in cultivation at Instituto de Botânica de São Paulo nr. 5089, 7 June 2014, *Bolson 661* (UPCB). Paraná: Bituruna, 29 April 1969, *Hatschbach 21449* (C, F, HB, MBM, RFA, SP, UPCB, US). Morretes, 18 November 1909, *Dusén 9535*, mixed collection with *B. miersii* (S). *Idem*, 6 June 2016, *Santos 144* (UPCB). Piraquara, Mananciais da Serra, May 2005, *Reginato & Matos 451* (UPCB). *Idem*, 22 May 2009, *Bianchi 56* (HUCP). *Idem*, 23 March 2016, *Bolson et al. 610* (UPCB). Ponta Grossa, Vila Velha, 27 April 1914, *Dusén 14767* (AMES, F, K, MO, NY, P, S, U). Prudentópolis, 10 April 2003, *Goldenberg & Labiak 590* (MBM, UPCB). São Mateus do Sul, 20 April 1983, *Pirani & Yano 581* (SP). Próximo a Ventania, 20 May 1972, *Seidel 1035* (HB). Banhado, 1 April 1911, *Dusén s.n.* (S 16-3443, AMES 1855). Rio Grande do Sul: Maquiné, 2014, *Klein 66* (UPCB). Torres, 14 May 1977, *Waetcher et al. 541* (ICN). *Idem*, 1 April 1978, *Waetcher & Baptista 800* (ICN). Santa Catarina: Blumenau, Parque Nacional da Serra do Itajaí, 23 November 2009, *Cadorin et al. 2043* (FURB). *Idem*, 23 November 2009, *Cadorin et al. 763* (FURB). Canoinhas, 6 May 2015, *Bolson 524* (UPCB). Jacinto Machado, 14 April 1979, *Waetcher 1213* (ICN). Presidente Nereu, 7 April 1958, *Reitz & Klein 6655* (FLOR, HB, MBM, NY, PACA, RB, U, US). São Paulo: *s.loc.*, cultivada no Parque do Estado e Jardim Botânico de São Paulo, 30 August 1932, *Hoehne s.n.* (SP 29823). Itararé, Bairro

das Almas, collected by E.L.M. Catharino in August 1982, flowered in cultivation at the São Paulo Botanical Garden, 23 May 1986, *Luer 11620* (MO).

Alberto José de Sampaio (1881–1946) first described *Barbosella dusenii* in the genus *Restrepia* based on a collection by the Swedish botanist and explorer Per Karl Hjalmar Dusén (1855–1926). Sampaio did not designate a type specimen in the protologue. Following standard rules of his time, he only cited provenance and flowering time of Dusén's collection, which was said to have come from the state of Paraná where it was growing on forest trees and found in flower in June. No precise location, collector's number or year of collection were provided by Sampaio. However, the protologue contains a detailed description and also an illustration by Santos Lahera y Castillo, who worked as photographer and artist at Museu Nacional (Santos 2011). Lahera's illustration (Fig. 5) provides drawings not only of the habit but also of the floral segments, including outlines of sepals and petals, and also of the lip and column in side view. The column is shown to be short, somewhat stout and winged, with a hooded apex, and the lip is clearly shown to be entire, oblong and obtuse.

Among the dried specimens of *Barbosella* preserved in the herbarium of Museu Nacional (R), there is a Dusén's collection (R35880) labelled "*Restrepia dusenii* A.Samp. n.sp." (Fig. 6). This collection, initially thought to be the holotype of this taxon, comprises several portions of a *Barbosella* specimen and are kept in a pack glued on the herbarium sheet. An additional specimen, which bears a too flattened, sole flower, is mounted on top of the same pack. During a recent visit to R, we examined all available flowers that remain on this material. They proved to be all badly flattened, but a few proved to be serviceable for study. We concluded that the specimen deposited at R does not conform to the description and illustration in the protologue. It actually refers to *B. gardneri* (Lindley 1842: 83) Schlechter (1918: 261). This corroborates with Hoehne (1947 *in sched.*), who also studied this collection and came to the same conclusion. A quite detailed drawing of the lip of one of the flowers of this collection was prepared by Irene Swentorzecky, a Russian born Brazilian artist who worked extensively with Hoehne. This drawing is now mounted on the lower, left side of the herbarium sheet and depicts all features commonly found on the lip of *B. gardneri*: it is trilobed, subpandurate, the disc presents a minutely papillose, longitudinal cavity that extends from the base to near the middle, and the apical lobe is ovate and acuminate. Nonetheless, Hoehne failed to recognize that the above-

mentioned specimen did not represent the type of *R. dusenii* and, consequently, proposed this name as a new variety of the former species: *B. gardneri* var. *dusenii* (A. Sampaio) Hoehne (1947: 75).

The herbarium collection deposited at R (35880) is said to have been made from a cultivated specimen at the Museu's *Horto Botânico*. It is possible that a mixture occurred when samples were collected for the preparation of the herbarium specimen. A mixture in the field cannot be discarded either, as some *Barbosella* species look very much alike and may grow side by side or even intermixed. In any case, this specimen does not represent the type specimen of *R. dusenii*, which is lost or destroyed.

Luer (2000) erroneously cited the collection *Dusén 8070* as the nomenclatural type of *R. dusenii*. In 1993 (Luer *in sched.*), he annotated the herbarium sheet at S (056358) as the lectotype of *R. dusenii*, but never effectively published that lectotypification. This collection comes from Vila Velha, which is located in the municipality of Ponta Grossa, state of Paraná. It dates from April 1909, at least two years after the collection of the type. Although the protologue of *R. dusenii* dates from 1909, the article is signed and dated 20 July 1907 (Sampaio 1909: 192), meaning that the specimen upon which the description and illustration were based was available either on that date or before it. Also, the collector's number does not match the information in the protologue.

In the absence of an actual specimen, the only original, extant materials of *R. dusenii* are the published description and illustration in the protologue. The illustration prepared by Lahera is here designated as lectotype (Fig. 5). However, as the drawings of a number of floral segments in this illustration can be misleading, especially of the lip, we believe that the illustration and description alone cannot be used to recognize this taxon. Therefore, we select as epitype the specimen *Dusén 8070* deposited at S (Fig. 7). It also comes from Paraná, duplicates are distributed in various herbaria, and a detailed black and white illustration based on this specimen has been recently published by Luer (2000).

Barbosella dusenii is very similar to *B. cogniauxiana*, with which it can be easily confused. In habit and flower size, the former is usually relatively smaller than the latter. Leaves of *B. dusenii* are suborbicular to elliptical, with a mucronate apex; lateral sepals are variously connate, usually to the apical third to form a deeply bifid synsepal, which resembles that of *B. cogniauxiana*. The lip is also similar in these two species, but in *B. dusenii*, the shallow, basal cavity is pubescent and the pair of longitudinal callosities on the

disc is more protruding and noticeable. It was collected in flower from March to August and in November.

Barbosella dusenii is a Brazilian endemic so far known to occur in the states of Espírito Santo, Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina, and São Paulo (Fig. 2D), with estimated EOO of 460,746 km² and estimated AOO of 80 km², and therefore classified as Endangered [EN B2b(ii,iii)].

5. *Barbosella gardneri* (Lindley) Schlechter (1918: 261). $\equiv$ *Pleurothallis gardneri* Lindley (1842: 83). Type:—BRAZIL. Rio de Janeiro: Organ Mountains, collected by Miers, March 1841, *G. Gardner* 5865 (Holotype: K-Lindl. [digital image!], Isotypes: BM [digital image!], BR [digital image!], K-Benth., 1854 [digital image!], K-Hook., 1867 [digital image!]). $\equiv$ *Restrepia gardneri* (Lindley) Benth., in Benth. & Hooker (1883: 491). $\equiv$ *Humboldtia gardneri* (Lindley) Kuntze (1891: 667).
 $\equiv$ *Restrepia microphylla* Barbosa Rodrigues (1881: 110). Type:—BRAZIL. Rio de Janeiro: Paulo de Frontin (formerly Rodeio), s.d., *J. Barbosa Rodrigues s.n.* (Holotype: Lost; Lectotype here designated: Barbosa Rodrigues's original illustration in Iconogr. Orchid. Brésil, vol. 3, tab. 183, at the Library of Rio de Janeiro Botanical Garden, cited as tab. 695 (unpublished) in Barb. Rodr. loc.cit; copied and reproduced in black and white in Cogn., Fl. Bras. (Mart.) 3(4), tab. 122, fig. 3. 1896; digitally restored image of the original reproduced in color in Sprunger *et al.* (1996), vol. 1: 241). $\equiv$ *Barbosella microphylla* (Barbosa Rodrigues) Schlechter (1918: 262).

$\equiv$ *Barbosella gardneri* var. *genuina* Hoehne (1947: 75), not validly publ.

$\equiv$ *Pleurothallis capillipes* Kraenzlin, *nom. in sched.*

Figure 3 P–R.

Plant very small, 3.5–24.5 mm long. Roots slender, produced singly at the base of the ramicauls. Rhizome 0.8–9.3 mm long, slender. Ramicaul 1.35–4 mm long, ascending, 1–2 sheaths. Leaf 2.1–15 $\times$ 1.2–3.3 mm, suberect, membranaceous, elliptical to oblanceolate, the base cuneate, the apex minutely emarginate and mucronate. Inflorescence 15–46 mm long; peduncle 9–41 mm long, with a basal bract 0.8–3.5 mm long; floral bract 0.6–2 mm long; pedicel 0.45–0.8 mm long, filament 0.7–2.5 mm long; ovary 0.5–0.8 mm long; sepals yellow to yellowish-green, occasionally light purple, the dorsal sepal 4.3–8 $\times$ 0.5–1.55

mm, erect, ovate, 3-veined, apex obtuse, the lateral sepals $3.9\text{--}8.4 \times 0.7\text{--}3.2$ mm, obliquely ovate, concave at base and forming a mentum with the prominent, bulbous column foot, variously connate, from near the base to above the middle to form an ovate, deeply bifid synsepal, 6-veined all together; apex obtuse; petals same color as sepals, $2.8\text{--}6.2 \times 0.2\text{--}1.7$ mm, oblong-lanceolate, apex acute; lip yellow with purple lateral lobes, $1.5\text{--}3.25 \times 0.7\text{--}1.6$ mm expanded, trilobed, subpandurate, the base rounded and concave, articulated with the apex of the column foot, the lateral lobes below the middle, erect, rounded, the disc with a minutely papillose, longitudinal cavity that extends from the base to near the middle, the apical lobe ovate, provided at base with a shallow, longitudinal depression, the apex acuminate; column $1.7\text{--}2.35$ mm long, not winged, the base with a bulbous foot the apex not hooded, slightly emarginated, the anther ventral.

Additional specimens examined:—BRAZIL. *s.loc.*, cultivated in the living collection of the Free University of Amsterdam, 4 September 1984, *Luer 10454* (MO). *s.loc.*, cultivated by P. Jesup in Bristol, C.T., 28 June 1998, *Luer 18839* (MO). *s.loc.*, cultivated by P. Jesup in Britol, C.T., 28 June 1998, *Luer 18840* (MO). *s.loc.*, submitted by Great Lakes Orchids, Mich., to the OIC, in cult. at SEL, 25 May 1981, *Luer 6320* (SEL). *s.loc.*, flowered in cultivation at the Hamburg Botanical Gardens, 12 July 1921, *Purpus 1912* (HBG). *s.loc.*, flowered in cultivation at the Hamburg Botanical Gardens, 7 July 1921, *s.coll.* (HBG barcode HBG501880). *s.loc.*, s.d., *Löfgren 2216* (BR [digital image]). *s.loc.*, s.d., *Glaziou s.n.* (BR 13702748 [digital image]). *s.loc.*, s.d., *Glaziou s.n.* (BR 13702755 [digital image]). *s.loc.*, s.d, *s.coll.* (K barcode K000847307). *s.loc.*, obtained from Alvim Seidel Orquidário Catarinense, Corupá, Santa Catarina, fl. cult. in Curitiba, Paraná, 3 November 2015, *Bolson 549* (UPCB). *s.loc.*, obtained from Orquidário Três Irmãos, Garuva, Santa Catarina, fl. cult. in Curitiba, Paraná, 12 November 2015, *Bolson 551* (UPCB). Espírito Santo: Alfredo Chaves, 23 August 2011, *Chiron & Tesch 11270* (MBML). *Idem*, 24 June 1995, *Hatschbach 62864* (MBM). Mimoso do Sul, 29 June 2008, *Couto 618* (MBML). Santa Teresa, 18 March 1940, *Ruschi 44* (SP — mixed collection with *B. crassifolia*). *Idem*, Reserva Biológica Augusto Ruschi, 5 April 2000, *Kollmann et al. 2801* (MBML). *Idem*, 7 January 2003, *Vervloet & Bausen 1603* (MBML). *Idem*, 6 February 2003, *Vervloet et al. 1789* (MBML). *Idem*, 16 April 2003, *Vervloet et al. 2234* (MBML). *Idem*, 29 January 2002, *Kollmann & Bausen 5380* (MBML). *Idem*, 30 January 2002, *Kollmann & Bausen 5393* (MBML). *Idem*, 14 January 2016, *Bolson et al. 576* (UPCB). *Idem*, 14 January 2016, *Bolson et al. 578* (UPCB). *Idem*, 14 January 2016, *Bolson*

et al. 579 (UPCB). *Idem*, 21 August 2012, *Flores & Romão* 995 (ESA). *Idem*, 22 August 2012, *Flores & Romão* 1015 (ESA). *Idem*, 14 April 2016, *Bolson et al.* 614 (UPCB). *Idem*, Reserva Biológica Santa Lúcia, 11 April 2003, *Oliveira et al.* 844 (HUEFS, MBML). *Idem*, 16 July 2011, *Toscano de Brito & Kollmann* 2879 (UPCB). *Idem*, 6 April 2000, *Kollmann & Vervloet* 2808 (MBML). Pedra Azul, 1 April 1970, *Kautsky* 255 (HB). Minas Gerais: Caldas, November 1854, *Lindberg* 514 (BR, digital image). Vale do Paraíba, Est. Benj. Constant., November 1933, *Campos Porto & Horta s.n.* (RB 3655). Paraná: *s.loc.*, cultivada no Horto Botânico antigo do Museu Nacional, *s.d.*, *Dusén s.n.* (R35880). Antonina, 11 September 1970, *Hatschbach* 24701 (MBM). Campina Grande do Sul, September 1961, *Leinig* 262 (HB). Curitiba, October 2010, *Smidt* 1018 (UPCB). Guaratuba, 21 October 1953, *Hatschbach* 3278 (HB, MBM). Guaratuba, 8 November 1983, *Kummrow* 2375 (MBM). Morretes, 11 December 2015, *Bolson et al.* 556 (UPCB). *Idem*, 18 November 1909, *Dusén* 9850 A (MBM, S). *Idem*, 30 August 1961, *Hatschbach* 8202 (HB, MBM, U, UPCB). *Idem*, 25 May 1966, *Hatschbach* 14457 (MBM). *Idem*, Parque Estadual Pico do Marumbi, 28 August 2016, *Bolson* 623 (UPCB). Serra do Mar, 3 November 1909, *Dusén* 8618 (HBG). Piraquara, Mananciais da Serra, 22 May 2009, *Bianchi* 56 (HUCP). São José dos Pinhais, RPPN Nhandara Guaricana, 10 August 2015, *Bolson et al.* 535 (UPCB). *Idem*, 10 August 2015, *Bolson et al.* 536 (UPCB). Serra de Paranaguá, 12 August 1968, *Seidel* 977 (HB). Rio de Janeiro: Itatiaia, 8 December 1942, *Brade* 17156 (HB, RB). *Idem*, 11 January 1970, *Emmerich s.n.* (HB 43038). Nova Friburgo, November 1954, *Lima s.n.* (HB 2562). *Idem*, 6 January 1998, *Toscano de Brito s.n.* (RB [32, spirit]). *Idem*, Macaé de Cima, *s.d.*, *A. Glaziou* 6917 (BR [digital image], C, K [digital image], P [digital image], S). *Idem*, *s.d.*, *Glaziou* 18532 (C, K, P [digital image]). *Idem*, 24 January 2016, *Bolson et al.* 589 (UPCB). *Idem*, 24 February 2016, *Bolson et al.* 591 (UPCB). *Idem*, cultivated by David Miller, 24 January 1997, *Luer* 18235 (MO). *Idem*, 25 January 1997, *Luer et al.* 18240 (MO). Paty do Alferes, 18 November 2015, *Toscano de Brito* 3469 (UPCB). Petrópolis, cultivated by Verboonen, 21 October 1988, *Luer* 13758 (MO). Teresópolis, July 1888, *Glaziou* 17256 (BR [digital image], C, HBG, K [digital image], P [digital image], RB). *Idem*, 17 June 1887, *Moura* 6 (BR [digital image]). *Idem*, August 1940, *Brade* 16737 (RB). *Idem*, 1918, *Frazão s.n.* (RB barcode RB00246984). *Idem*, Parque Nacional da Serra dos Órgãos, 19 March 2014, *Bolson & Lima* 493 (UPCB). *Idem*, 19 March 2014, *Bolson & Lima* 497 (UPCB). *Idem*, 19 March 2014, *Bolson et al.* 509 (UPCB). *Idem*, January 1958, *Abendroth P-5* (HB). *Idem*, 24 February 1958,

Abendroth P-15 (HB). *Idem*, 8 January 1983, *Miranda et al.* 108 (GUA). *Idem*, 28 November 2016, *Royer 160* (UPCB). *Idem*, 24 November 2017, *Royer 170* (UPCB). *Idem*, 1 February 2003, *Nilce s.n.* (RB 386207). *Idem*, 23 February 1887, *Schenk 2706* (BR [digital image]). *Idem*, 24 February 1887, *Schenk 2717* (BR [digital image]). *Idem*, January 1888, *Moura 76* (AMES, BR [digital image]). Serra dos Órgãos, October 1949, *Pabst 440* (HB). *Idem*, s.d., *Burchell 2283* (AMES, HB, K, P [digital image], U). *Idem*, s.d., *Schenk s.n.* (SP 29276). *Idem*, July 1878, *Miers s.n.* (K barcode K000847312). *Idem*, July 1878, *Miers 3476* (K). Between Teresópolis and Salvador, cultivated by the Jesups in Bristol, C.T., 2 December 1989, *Luer 14546* (MO). Above Petrópolis, collected in May 1986, flowered in cultivation by P. Jesup in Bristol, C.T. 1 June 1997, *Luer 18518* (MO). *s.loc.*, s.d., *Glaziou 20499* (C, K, P [digital image]). Santa Catarina: Benedito Novo, 12 October 2011, *Caetano s.n.* (FURB 37329). Jaraguá do Sul, 13 January 1958, *Reitz & Klein 6201* (HB). Joinville, 10 April 2015, *Toscano de Brito et al.* 3394 (UPCB). *Idem*, 10 April 2015, *Bolson et al.* 519 (UPCB). São Paulo: Apiaí, 21 April 1965, *Grupo de Piracicaba 10840* (M). *Idem*, PETAR, s.d., *Engels & Mazziero 668* (UPCB). Barra do Turvo, 7 September 2008, *Barbosa & Costa 2357* (MBM). Bertioga, 5 September 2006, *Barros s.n.* (SP 401812). Iguape, February 1920, *Brade s.n.* (HB 8280). São Luís do Paraitinga, Parque Estadual da Serra do Mar, 13 October 1999, *Martinelli et al.* 15913 (RB). São Miguel Arcanjo, Parque Estadual Carlos Botelho, 31 August 2011, *Moraes et al.* 3270 (HRCB, UEC [digital image]). Sete Barras, Parque Estadual Intervales, May 2002, *Guilherme 311* (HRCB). *Idem*, 15 December 2002, *Breier & Breier 796* (UEC [digital image]). Salesópolis, Estação Biológica da Boraceia, 26 September 1966, *Handro 1153* (NY). Piassaguera, 2 October 1922, *Kuhlmann s.n.* (RB 825). *s.loc.*, March 1899, *Edwall s.n.*, mixed collection with *B. miersii* (SP 29274).

In habit *B. gardneri* is very similar to *B. miersii*, with which it is easily confused. Its leaves are small and variable in size, sometimes as tiny as those of *B. miersii*, which can make sterile plants of these two species practically indistinguishable. They are sympatric and frequently found growing intermixed. They were first collected by John Miers in March 1841, on the huge Organ Mountains of the state of Rio de Janeiro, and most probably in the same locality. However, *B. gardneri* is easily distinguished from *B. miersii* by its trilobed, pandurate lip with round, evident lateral lobes and acuminate apical lobe.

The lip is entire and obtuse in *B. miersii*. *Barbosella gardneri* was collected in flower year round.

The name *Restrepia microphylla* (= *Barbosella gardneri*) was first published by Barbosa Rodrigues (1881) based on a specimen collected in Rodeio, today's Paulo de Frontin, in the state of Rio de Janeiro. Schlechter later transferred it to *Barbosella* in 1918. The type specimen of *Restrepia microphylla* was lost (Cribb & Toscano de Brito 1996), and the only extant original material is the illustration (Fig. 8) which appeared in Barbosa Rodrigues's *Iconographie des orchidées du Brésil*, which is now deposited in the library of Rio de Janeiro Botanical Garden. This illustration was copied and reproduced in black and white in Cogniaux (1896), and later digitally restored and reproduced in color in Sprunger *et al.* (1996). It is here selected as the lectotype.

Barbosella gardneri is also a Brazilian endemic found in the states of Espírito Santo, Minas Gerais, Paraná, Rio de Janeiro, Santa Catarina and São Paulo (Fig. 2E). This species and *B. miersii* present the highest occupation area, with an estimated EOO of 153,912 km² and estimated AOO of 184 km². Following the IUCN categories, *B. gardneri* is here classified as Endangered [EN B2b(ii,iii)].

6. *Barbosella macaheensis* (Cogniaux) Luer (2000: 23). ≡ *Pleurothallis macaheensis* Cogniaux (1906b: 310). Type:—BRAZIL. Rio de Janeiro: Alto Macahé, 6 July 1891, A.F.M. Glaziov s.n. (BR 9972742, digital image!).
- = *Barbosella perinii* Ruschi (1977: 1). Type:—BRAZIL. Espírito Santo: Santa Teresa, Reserva Biológica de Nova Lombardia, do IBDF, 16 July 1975, O. Perini *et al.* s.n. (MBML 10044!, spirit).

Figure 4 A–D.

Plant small, 11–31 mm long. Roots stout, produced at the base of ramicaul and from nodes along the rhizome. Rhizome 2–5 mm long, stout. Ramicaul 2.5–6 mm long, ascending, 1–2 sheaths. Leaf 8–23 × 3–6 mm, suberect, coriaceous, elliptical to oblanceolate, the base cuneate, the apex minutely emarginate and mucronate. Inflorescence 26–34 mm long; peduncle 19–25 mm long, with a basal bract 1.9–3.8 mm long; floral bract 1.65–2.1 mm long; pedicel 0.17–0.44 mm long, with a filament 1.6–1.9 mm long; and ovary 0.41–0.64 mm long; sepals yellowish-green to light orange, occasionally with purple primary veins, the dorsal sepal 6.4–7 × 1.3–1.7 mm, suberect, ovate, 3-veined, apex obtuse, the lateral

sepals $7.2\text{--}9.3 \times 3.6\text{--}5.4$ mm, concave at base and forming a mentum with the prominent, bulbous column foot, connate to just below the middle or to near the apex into an ovate-triangular, deeply bifid synsepal, 6-veined all together, the apex obtuse to subacute; petals same color as sepals, $4.3\text{--}6 \times 1\text{--}1.7$ mm, porrect or occasionally patent, narrowly ovate to lanceolate, usually subfalcate, apex acute; lip yellow suffused with purple, $3.3\text{--}4.3 \times 1.9\text{--}2.4$ mm, entire, oblong, with erect sides at the middle, the base rounded, deeply concave, articulated with the apex of the column foot, disc with a pair of subtrapeziform callosities at base, then concave and minutely papillose between the erect sides, and with a pair of low, converging calli more or less above the middle, the apex truncated; column $2.7\text{--}3.5$ mm long, not winged, the base with a bulbous foot, the apex hooded, denticulate, the anther ventral.

Additional specimens examined:—BRAZIL. Espírito Santo: Santa Teresa, Reserva Biológica Augusto Ruschi, 10 January 2016, *Bolson et al.* 582 (UPCB). Rio de Janeiro: Nova Friburgo, Alto Macaé, 6 July 1891, *Glaziou 18535a* (BR [digital image], P [digital image]). *Idem*, 5 June 1893, *Glaziou 20497a* (P [digital image]). Macaé de Cima, 1 August 2016, *Bolson 637*. *Idem*, 1 May 2001, *Cattan 81* (RB [spirit]). *Idem*, 6 June 2006, *Bocayuva et al.* 185 (RB). *Idem*, 18 June 1986, *Martinelli et al.* 11756 (RB). *Idem*, 7 July 1978, *Araujo et al.* 2075 (GUA). *Idem*, April 1998, *Miller s.n.* (RB [543, spirit]). *Idem*, Trilha do Pico do Faraó, 2 June 2017, *Bolson et al.* 647 (UPCB).

Barbosella machaeensis is similar to *B. australis* in habit and floral morphology, but it can be readily distinguished by its considerably smaller flowers and truncate lip. In contrast, the flowers of *B. australis* are twice the size and the lip is markedly bifid at the apex. It was collected in flower in January and from April to August.

This species is endemic to southeast Brazil and so far has been only found in the states of Espírito Santo and Rio de Janeiro (Fig. 2F). Until recently, it was believed to be known only from two collections made over a century ago in Macaé de Cima (“Alto Machaé”), a district in the municipality of Nova Friburgo, state of Rio de Janeiro. New collections of *B. machaeensis* have been made in the field and additional herbarium specimens, misidentified either as *B. australis* or *B. gardneri*, have been also located (Bolson *et al.* 2018). *Barbosella perinii*, a name published by Ruschi (1977) based on a collection from Santa Teresa, Espírito Santo, has been shown to be conspecific with *B. machaeensis* (Bolson *et al.* 2018).

Barbosella macaheensis is known from populations represented by few individuals. Its estimated EOO is 2,393 km² and estimated AOO, 12 km². Based on this apparent restricted distribution, and following the IUCN categories, this species was recently classified as Endangered [EN B2ab(i,ii,iii,iv)] (Bolson *et al.* 2018).

7. *Barbosella miersii* (Lindley) Schlechter (1918: 262). $\equiv$ *Pleurothallis miersii* Lindley (1842: 84) Type:—BRAZIL. Rio de Janeiro: Organ Mountains, *J. Miers s.n.* (Holotype: K barcode K000079981!). $\equiv$ *Restrepia miersii* (Lindley) Reichenbach.f. (1866: 150). $\equiv$ *Humboltia miersii* (Lindley) Kuntze (1891: 667). $\equiv$ *Barbrodria miersii* (Lindley) Luer (1981: 386), *nom. illeg.*

Figure 4 E–H.

Plant very small, 3–15 mm long. Roots slender, produced along the rhizome. Rhizome 1–6 mm long, slender. Ramicaul 1–4 mm long, ascending, 1 sheath. Leaf 2–10 $\times$ 1–3.5 mm, suberect to prostrate, coriaceous, elliptical to oblanceolate, the base cuneate, the apex minutely emarginate and mucronate. Inflorescence 8–23 mm long; peduncle 6–20 mm long, with a basal bract 0.5–1.6 mm long; floral bract 0.2–1 mm long; pedicel 0.17–0.44 mm long, with a filament 0.2–1.2 mm long; ovary 0.41–0.64 mm long; sepals translucent yellowish-green to translucent yellowish-pink, minutely papillose, the dorsal sepal 0.8–2.6 $\times$ 0.5–1.15 mm, suberect, ovate, the apex subacute to obtuse, 1-veined, the lateral sepals 1.65–2.35 $\times$ 0.5–1.9 mm, ovate, connate at base, 1-veined, apex subacute to obtuse; petals same color as sepals, 0.6–1.2 $\times$ 0.2–0.4 mm, narrowly ovate, ovate-lanceolate to lanceolate, minutely papillose, the apex acute; lip same color as sepals and petals, 0.6–1.7 $\times$ 0.3–0.6 mm, entire, oblong to elliptical, minutely papillose, the base subcordate, simply hinged to the apex of the column foot, the disc slightly sulcate, the margins revolute below the middle, the apex obtuse; column 0.55–1 mm long, not winged, the base without a bulbous foot, not hooded; anther apical.

Additional specimens examined:—BRAZIL. *s.loc.*, flowered in cultivation by P. Jesup, Bristol, Conn., 18 November 1977, *Luer 2217* (SEL). Espírito Santo: Alfredo Chaves, 2009, *Guiard 9670* (MBML). *Idem*, 15 February 2012, *Bolsanello s.n.* (MBML 49887). *Idem*, 15 February 2012, *Chiron 12023* (MBML). Santa Teresa, Estação Biológica de Santa Lúcia, 6 April 2000, *Kollmann & Vervloet 2813* (MBML). *Idem*, 17 June 2005, *Fontana et al. 1482* (MBML). *Idem*, Reserva Biológica Augusto Ruschi, 14 January 2016,

Bolson et al. 577 (UPCB). *Idem*, 8 June 1987, *Boudet Fernandes* 2150 (MBML). Venda Nova, collected by M. Frey, R. Kaustky *et al.*, s.n., s.d., *Luer* 20649 (SEL). Paraná: Adrianópolis, Parque Estadual das Lauráceas, 19 May 2010, *Mancinelli & Engels* 1233 (JOI [digital image], UPCB). *Idem*, 27 January 2011, *Mancinelli* 1377 (JOI, digital image). *Idem*, 9 December 2015, *Brotto & Vieira* 2163 (UPCB). Bocaiúva do Sul, 14 January 1969, *Hatschbach & Koczicki* 20718 (MBM). Campina Grande do Sul, 27 December 1967, *Hatschbach* 18183 (MBM). *Idem*, 30 January 1969, *Hatschbach* 20969 (MBM). *Idem*, February 1963, *Leinig* 297 (HB). Guaratuba, 31 December 1952, *Hatschbach* 2910 (HB, MBM). Morretes, Itupava, 18 November 1909, *Dusén* 9535 (S — mixed collection with *B. dusenii*). *Idem*, Parque Estadual Pico do Marumbi, 16 May 2009, *Keller* 67 (HUCP). *Idem*, 29 December 2015, *Bolson et al.* 560 (UPCB). *Idem*, 4 February 2016, *Bolson et al.* 601 (UPCB). *Idem*, Serra do Mar, 12 February 1910, *Dusén* 9850 (HBG, S). Quatro Barras, 29 November 2015, *Bolson et al.* 552 (UPCB). *Idem*, 1 April 1969, *Hatschbach* 21307 (MBM). *Idem*, 30 December 1969, *Hatschbach* 23282 (NY). São José dos Pinhais, 3 February 1967, *Hatschbach* 15937 (MBM). *Idem*, RPPN Nhandara Guaricana, 14 March 2015, *Bolson et al.* 514 (UPCB). *Idem*, 15 December 2015, *Bolson et al.* 557 (UPCB). *Idem*, 14 January 2016, *Bolson et al.* 598 (UPCB). *Idem*, 14 January 2016, *Bolson et al.* 599 (UPCB). Tijucas do Sul, 04 February 2017, *Santos & Kujaski* 256 (UPCB). Rio de Janeiro: Organ Mountains, *Miers s.n.* (K barcode K0000847314 — mixed collection with *Pabstiella* sp., digital image). *Idem*, *Miers? s.n.* (BM barcode BM000082608, digital image). *Idem*, *Miers? s.n.* (BM barcode 000923752, digital image). Itatiaia, 29 March 1974, *Windisch s.n.* (HB 60577). *Idem*, Parque Nacional do Itatiaia, 8 December 2015, *Bolson et al.* 555 (UPCB). Miguel Pereira, Rebio do Tinguá, 11 July 2005, *Bocayuva & Saddi* 136 (RB). Nova Friburgo, October 1974, *Dungs s.n.* (HB 63019). *Idem*, 15 April 1973, *Dungs s.n.* (HB 59406). *Idem*, Macaé de Cima, 15 April 2016, *Bolson et al.* 615 (UPCB). *Idem*, 15 April 2016, *Bolson et al.* 617 (UPCB). *Idem*, s.d., *Bocayuva* 231 (RB). *Idem*, 25 January 1997, *Luer et al.* 18251 (MO). Paty do Alferes, 16 July 2014, *Toscano de Brito* 3262 (UPCB). Petrópolis, 10 April 1928, *Spannagel* 139 (SP). Teresópolis, 8 January 1982, *Araujo* 4764 (GUA). *Idem*, Parque Nacional da Serra dos Órgãos, 19 March 2014, *Bolson & Lima* 494 (UPCB). *Idem*, 19 March 2014, *Bolson & Lima* 496 (UPCB). *Idem*, 19 March 2014, *Bolson & D.F. Lima* 498 (UPCB). *Idem*, 19 March 2014, *Bolson et al.* 507 (UPCB). *Idem*, 24 January 2016, *Royer & Matos* 117 (UPCB). *Idem*, 22 March 2016, *Royer & Matos* 133 (UPCB). *Idem*, 20 October 1977, *Martinelli & Mass* 3362 (RB). *Idem*,

17 August 1983, *Martinelli et al.* 9308 (RB). *Idem*, July 1888, *Moura* 287 (BR). Between Teresópolis and Salvador, 2 December 1989, *Luer & Luer* 14545 (MO). Santa Catarina: Benedito Novo, 10 April 2015, *Bolson et al.* 516 (UPCB). *Idem*, 10 April 2015, *Bolson et al.* 517 (UPCB). *Idem*, 10 April 2015, *Bolson et al.* 518 (UPCB). *Idem*, 3 November 2015, *Bolson et al.* 547 (UPCB). *Idem*, 3 November 2015, *Bolson et al.* 548 (UPCB). *Idem*, 3 November 2015, *Bolson et al.* 550 (UPCB). *Idem*, 7 December 2015, *Bolson et al.* 554 (UPCB). *Idem*, 29 March 2010, *Schmitt et al.* 1749 (FURB). *Idem*, 12 October 2011, *Caetano s.n.* (FURB 37334). Joinville, 15 June 2009, *Mancinelli* 906 (JOI, digital image). *Idem*, 25 January 2005, *Vieira* 784 (JOI, digital image). *Idem*, 4 October 2015, *Bolson et al.* 520 (UPCB). Rio do Campo, 22 February 2010, *Korte & Kniess* 1966 (FURB). Próximo a Mafra, 23 August 1975, *Seidel* 1148 (HB). São Paulo: Apiaí, PETAR - Parque Estadual Turístico do Alto Ribeira, Núcleo Caboclos, 10 March 2017, *Bolson et al.* 638 (UPCB). Caraguatatuba, 14 March 2015, *Bolson* 515 (UPCB). Eldorado, Parque Estadual Jacupiranga, 23 March 2005, *Oriani* 557 (ESA, SPSF). Santo André, Estação Biológica do Alto da Serra de Paranapiacaba, s.d., *Usteri s.n.* (SP 26662). São Miguel Arcanjo, Parque Estadual Carlos Botelho, 24 April 2002, *Savassi et al.* 370 (ESA). *Idem*, 23 April 2002, *Udulutsch et al.* 672 (ESA). São Paulo, Bosque da Saúde, 8 June 1921, *Hoehne s.n.*, mixed collection with *B. crassifolia* (AMES 54829, NY barcode NY00414717). Serra da Mantiqueira, s.d., *Rodrigues* 65 (UPCB). Alto da Serra, 6 January 1907, *Usteri s.n.* (K barcode K000847313). *s.loc.*, March 1899, *Edwall s.n.*, mixed collection with *B. gardneri* (SP 29274).

Barbosella miersii was first described in the genus *Pleurothallis* by John Lindley (1842) based on a specimen collected by John Miers in the Organ Mountains at the state of Rio de Janeiro. This specimen is preserved in Lindley's herbarium at K. Three other unnumbered collections from the Organ Mountains, are found at K (barcode K0000847314) and BM (barcode BM000082608, BM000923752). The specimen at Kew was collected by Miers and might well represent a duplicate of the original gathering and therefore an isotype. However, this cannot be ascertained with the present information. This collection at Kew is actually a mixture of two taxa as specimens of *Barbosella miersii* are mounted together with an unidentified *Pabstiella* Brieger & Senghas (1976: 195) specimen. The two collections at BM are mounted together and have no collector's name or number. They were bequeathed by Miers in 1879 and it is possible that they were

collected by him. One of them, BM000082608, may even represent a duplicate of the type, but this is difficult to determine at this point.

This species is vegetatively very similar to *B. gardneri*. Their leaves are very variable in size, and sterile plants of the two species are sometimes inseparable. Nevertheless, *B. miersii* produces the smallest flowers of the Brazilian species and are easily distinguished from those of *B. gardneri*. The apical anther of *B. miersii* is unique in the genus and the lip is simply hinged to the footless column, lacking the typical ball-and-socket articulation found in *B. gardneri* and in almost all other members of the genus, the only exception being *B. trilobata* of which *B. miersii* can be also readily separated not only by its much smaller flowers but especially by its entire lip (versus trilobed in *B. trilobata*) with revolute margins. It was collected in flower year round.

Barbosella miersii is a Brazilian endemic found in states of Espírito Santo, Paraná, Rio de Janeiro, Santa Catarina and São Paulo (Fig. 2G), with estimated EOO of 153,614 km² and estimated AOO of 188 km². Following the IUCN categories, *B. miersii* is here classified as Endangered [EN B2b(ii,iii)].

8. *Barbosella spiritu-sanctensis* (Pabst) F.Barros & Toscano (1990: 23). $\equiv$ *Pleurothallis spiritu-sanctensis* Pabst (1975: 54). Type:—BRAZIL. Espírito Santo: Domingos Martins, Pedra Azul, 12 February 1970, *R. Kautsky* 236 (Holotype: HB!; Photocopy of the holotype and drawing by C. A. Luer based on the holotype: MO!).

Figure 4 I–L.

Plant very small, 4–6 mm long. Roots slender, produced singly along the rhizome. Rhizome 0.5–4 mm long, slender. Ramicaul 0.1–0.9 mm long, ascending, 1 sheath. Leaf 4–5 $\times$ 3–4 mm, prostrate, thickly coriaceous, suborbicular to elliptical, the base rounded, the apex obtuse to acute. Inflorescence 13–34 mm long; peduncle 10.5–30 mm long, bract below the middle, 0.6–1.8 mm long; floral bract 0.8–1.5 mm long; pedicel 0.3–0.4 mm long, with a filament 0.85–1.6 mm long; ovary 0.3–0.5 mm long; sepals yellowish-green, with purple primary veins, the dorsal sepal 7.5–7.7 $\times$ 1.4–1.8 mm, erect, ovate, 3-veined, apex obtuse, the lateral sepals 6.8–7.35 $\times$ 1.7–4 mm, ovate, concave at base and forming a conspicuous mentum with the column foot, connate to approximately the middle or to about two-thirds of their length to form an ovate, deeply bifid synsepal, 6-veined all together, apex subacute; petals same color as sepals, 7.1–8.8 $\times$ 0.7–1.3 mm, lanceolate,

apex obtuse; lip purple, $2.4\text{--}4.1 \times 1.5\text{--}2.6$ mm expanded, trilobed, base slightly concave, articulated with the apex of the column foot, the lateral lobes below the middle, erect, rounded, the disc with a pair of low, longitudinal calli that extend from near the base to the middle of the lip, the apical lobe ovate, obtuse at apex; column $2.2\text{--}2.8$ mm long, not winged, the base with a prominent and bulbous foot, the apex hooded, denticulate; anther ventral.

Additional specimens examined:—BRAZIL. Espírito Santo: Santa Maria de Jetibá, 5 May 2011, *Freitas 186* (MBML). Santa Teresa, Estação Biológica de Santa Lúcia, 17 May 2005, *Fontana 1490* (MBML). *s.loc.*, collected by M. Frey nr. NC 497, fl. cult. 27 May 2003, *Toscano de Brito 2678* (UPCB). Minas Gerais: Bonsucesso, 3 November 1991, *Tameirão Neto & Werneck 718* (BHCB, MBM).

The prostrate, thickly coriaceous, suborbicular to elliptical, tiny leaves of *Barbosella spiritu-sanctensis* are very similar to those of *B. crassifolia*, with which it can be confused when sterile. The lip is trilobed in both species, but in *B. spiritu-sanctensis* the lateral lobes are smaller and more rounded, and the longitudinal calli on the disc are less noticeable. Collected with flowers in February, May and August.

Barbosella spiritu-sanctensis is a Brazilian endemic until recently known to occur only in the state of Espírito Santo in the southeast. It is newly recorded here for the state of Minas Gerais, also in the southeast (Fig. 2H). Apparently this species presents a restrict distribution with populations consisting of scattered individuals with estimated EOO of $8,854\text{ km}^2$ and estimated AOO of 16 km^2 . Following the IUCN categories, it is here classified as Endangered [ENB2ab(i,ii,iii,iv)].

9. *Barbosella trilobata* Pabst (1956: 21). Type:—BRAZIL. Paraná: São José dos Pinhais, Saltinho, 21 November 1952, *G. Hatschbach 2732* (Holotype: HB!; Isotype: MBM!; Drawing by C. A. Luer based on the holotype: MO!). $\equiv$ *Restrepiopsis trilobata* (Pabst) Luer (1981: 387).

Figure 4 M–O.

Plant very small, 6–8 mm long. Roots slender, produced along the rhizome. Rhizome 3–6 mm long, slender. Ramicaul 1–2 mm long, ascending, 2–3 sheaths. Leaf $4\text{--}6 \times 1\text{--}1.5$ mm,

suberect, membranaceous, elliptical, base cuneate, apex obtuse. Inflorescence 13–15 mm long; peduncle 8.5–14 mm long, basal bract, 0.7–0.9 mm long; floral bract 0.9–1.4 mm long; pedicel 0.45–0.8 mm long, filament 0.3–0.9 mm long; and ovary 0.7–1.1 mm long; sepals yellowish-green, with purple primary veins, the dorsal sepal $5.8\text{--}7.1 \times 0.9\text{--}1.7$ mm, ovate, 3-veined, apex obtuse, the lateral sepals $5.3\text{--}5.4 \times 2.1\text{--}5.1$ mm, concave at base and forming a mentum with the prominent but not bulbous column foot, connate to above the middle to form a broadly ovate, bifid synsepal, 6-veined all together, apex obtuse; petals same color as sepals, $5.6\text{--}5.7 \times 0.6\text{--}1$ mm, lanceolate, apex obtuse; lip purple, $2.5\text{--}3 \times 2.3\text{--}3$ mm expanded, trilobed, the base simply hinged to the apex of the column foot, the lateral lobes below the middle, erect, rounded, falcate, the disc with a tall, thick pair of parallel calli extending from the base to the middle of the lip, the apical lobe ovate, minutely papillose, erose, obtuse to subacute; column 2.9–3.2 mm long, not winged, the base without a bulbous foot, apex hooded, denticulate to slightly emarginate; anther ventral.

Additional specimens examined:—BRAZIL. *s.loc.*, flowered in cultivation at J & L Orchids, Easton, CT, 5 May 1990, *Luer 14740* (MO). Paraná: Ponta Grossa, s.d., *Klingelfus 155* (UPCB). Tijucas do Sul, 25 October 1971, *Hatschbach 27578* (C, K, MBM, S, SP). *Idem*, 10 September 2016, *Santos 178* (UPCB). Rio de Janeiro: Near Petrópolis, cultivated at Orquidário Binot by Jorge and Maurício [“Marcel”] Verboonen, 30 November 1989, *Luer 14498* (MO). Santa Catarina: Joinville, 30 September 2010, *Mancinelli & Mancinelli 1295* (JOI, digital image).

Vegetatively, this species resembles *B. gardneri* and *B. miersii* with which it shares similar leaves. It is readily distinguished from them, however, by its floral morphology: From *B. miersii* is easily separated by the much larger flowers, the trilobed lip, and the long column foot; and from *B. gardneri* by the quite distinct lip that possesses rounded, falcate lateral lobes and a never acuminate apical lobe. The lip in *B. trilobata* is simply articulated to the tip of a long but not bulbous column foot, lacking the typical ball-and-socket articulation found in all other but one species (*B. miersii*) in the genus. It was collected in flower in May, September, October and November.

Barbosella trilobata was until recently known only to the states of Paraná and Rio de Janeiro (Luer 2000). It is here newly recorded for the state of Santa Catarina (Fig. 2H). Populations seem to be represented by scattered individuals with estimated EOO is 135 km² and estimated AOO is 12 km². All populations were found outside protected areas

exposed to impacts of anthropic activity. Following the IUCN categories, it is here classified as Endangered [EN B2ab(i,ii,iii,iv)].

Excluded Name

Barbosella gracilis Schlechter, *nom.*

This name appears in TROPICOS and in The PLANT LIST databases as unresolved and without publication details. We were unable to find it in any other online plant database. Apparently it has never been validly published and possibly has only appeared in a checklist or on a herbarium specimen. It is therefore excluded from this treatment.

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References

- Angely, J. (1972) *Flora analítica e fitogeográfica do Estado de São Paulo*, 6. Edições Phytton, São Paulo, 336 pp.
- Bachman, S., Moat, J., Hill, A.W., De La Torre, J. & Scott, B. (2011) Supporting Red List threat assessment with GeoCAT: geospatial conservation assessment tool. *ZooKeys* 150: 117–126.
<https://doi.org/10.3897/zookeys.150.2109>
- Barbosa Rodrigues, J. (1877) *Genera et Species Orchidearum Novarum*, 1. Fleiuss, Rio de Janeiro, 208 pp.
- Barbosa Rodrigues, J. (1881) Orchideae rodeienses et alterae ineditae. *Revista de Engenharia* 3: 109–110.
- Barbosa Rodrigues, J. (1882) *Genera et Species Orchidearum Novarum*, 2. Typographia Nacional, Rio de Janeiro, 295 pp.
- Barros, F. & Toscano de Brito, A.L.V. (1990) Notas taxonômicas no gênero *Pleurothallis* R.Br. (Orchidaceae). *Anais do Congresso Nacional da Sociedade de Botânica do Brasil* 35: 23–33.
- Bentham, G. (1883) *Restrepia gardneri*. In: Bentham, G. & Hooker, J.D. *Genera plantarum: ad exemplaria imprimis in Herberiis Kewensibus servata definite*, 3, part 2. A. Black, London, pp. 448–1258.
- Bolson, M., Toscano de Brito, A.L.V. & Smidt, E.C. (2018). A neglected name and new synonym in *Barbosella* (Pleurothallidinae, Orchidaceae). *Phytotaxa* 340: 246–254.
<https://doi.org/10.11646/phytotaxa.340.3.4>
- Brazilian Flora 2020 (in construction) Jardim Botânico do Rio de Janeiro. Available from: <http://floradobrasil.jbrj.gov.br/> (Accessed 17 Mar 2018).
- Brieger, F.G. & Senghas, K. (1976) *Pabstiella*, eine neue Orchideengattung aus Brasilien. *Orchidee (Hamburg)* 27: 193–196.
- Brown, R. (1813) *Pleurothallis*. In: Aiton, W.T. (Ed.) *Hortus Kewensis; a Catalogue of the Plants Cultivated in the Royal Botanic Garden at Kew*, 5. 2nd ed. London, 568 pp.
- Christenson, E.A. (1994) Significant collections of Orchidaceae conserved in Herbarium Hamburgense (HBG). *Brittonia* 46: 344–354.

- Cogniaux, C.A. (1896) Orchidaceae. *In*: Martius, C.F.P., Eichler, A.G. & Urban, I. (Eds.). *Flora brasiliensis*. Typographia Regia, Monachii et Lipsiae, 3: 1–672.
- Cogniaux, C.A. (1906a) *Restrepia*. *In*: Martius, C.F.P., Eichler, A.W. & Urban, I. (Eds.) *Flora Brasiliensis*. Typographia Regia, Monachii et Lipsiae, 3: 563–566.
- Cogniaux, C.A. (1906b) Notes sur les Orchidées du Brésil et des régions voisines. *Bulletin de La Société Royale de Botanique de Belgique* 43: 266–356.
- Cribb, P.J. & A.L.V. Toscano de Brito (1996) Introduction and history. *In*: Sprunger, S., Cribb, P.J. & Toscano de Brito, A.L.V. (Eds.), *João Barbosa Rodrigues – Iconographie des Orchidées du Brésil. The illustrations. 1*. Friedrich Reinhardt Verlag, Basle, pp. 23–30.
- Dallwitz, M.J., Paine, T.A. & Zurcher, E.J. (2000) Principles of interactive keys. Available from: <http://delta.intkey.com> (Accessed 10 Mar 2018).
- Edwall, G. (1903) Plantas paulistas novas ou menos conhecidas. *Revista do Centro de Ciencias, Letras e Artes de Campinas* 2: 191–195.
- Garay, L.A. (1952) Notatio Orchidologica II. *Archivos do Jardim Botânico do Rio de Janeiro* 12: 169–186.
- Gonçalves, E.G. & Lorenzi, H. (2007) *Morfologia vegetal: organografia e dicionário ilustrado de morfologia das plantas vasculares*. 1st ed. Instituto Plantarum de Estudos da Flora, São Paulo, 416 pp.
- Gutiérrez, D. G., Katinas, L. & Robles S.S.T. (2002) Type material of Carlos L. Spegazzini in the Museo de La Plata Herbarium (LP), Argentina. II: Fabaceae. *Darwiniana* 40: 77–101.
- Hijmans, R.J., Guarino, L., Bussink, C., Mathur, P., Cruz, M., Barrentes, I. & Rojas, E. (2012) DIVA-GIS: A geographic information system for the analysis of species distribution data. Version 7.5. Available from: <http://www.diva-gis.org/> (Accessed 28 Mar 2018).
- Hoehne, F.C. (1933[1934]) Contribuições para o conhecimento da flora orchidologica brasílica III. *Boletim de Agricultura* 34: 600–638.
- Hoehne, F.C. (1947) Barbosellae brasiliae asutralus novae varietates commutationesque in ipsius specierum nomenclatione. *Arquivos de Botânica do Estado de São Paulo* 2: 74–76.
- IUCN (2001) *IUCN Red List categories and criteria*. Version 3.1. 2nd ed. IUCN Species Survival Commission, Gland, Switzerland and Cambridge, UK. 33 pp.

- IUCN (2017) The IUCN Red List of Threatened Species. Version 2017-1. Available from: <http://www.iucnredlist.org/> (Accessed 07 Jun 2018).
- Karremans, A.P. (2016) Genera Pleurothallidinarum: An updated phylogenetic overview of Pleurothallidinae. *Lankesteriana* 16: 219–241.
- Katinas, L., Gutierrez, D.G. & Robles, S.S.T. (2001) Asteraceae type material of Carlos L. Spegazzini in the Museo de la Plata Herbarium (LP), Argentina. *Compositae Newsletter* 36: 31–68.
- Katinas, L., Gutierrez, D.G. & Robles, S.S.T. (2004) Type material of Carlos L. Spegazzini in the Museo de La Plata Herbarium (LP), Argentina. III. Cactaceae. *Darwiniana* 42: 177–200.
- Kraenzlin, F.W.L. (1911) Beiträge zur Orchideenflora Südamerikas. *Kongliga Svenska Vetenskaps Akademiens Handlingar* 46: 1–105.
- Kunth, K.S. (1816) *Restrepia*. In: Humboldt, F.W.H.A. von, Bonpland, A.J.A., Kunth, K.S. *Nova Genera et Species Plantarum (quarto ed.)*, 1. D'Hautel, Paris, 543 pp.
- Kuntze, C.E.O. (1891) Monocotyledones. In: *Revisio Generum Plantarum*, 2. A. Felix, Leipzig, 1011 pp.
- Lindley, J. (1842) Miscellaneous matter of the Botanical Register. *Edwards's Botanical Register* 28: 1–86.
- Lindley, J. (1845) A century of new genera and species of orchidaceous plants. *The Annals and Magazine of Natural History* 15: 106–108.
- Lindley, J. (1859) *Restrepia*. In: Lindley, J. (Ed.) *Folia Orchidacea*. J. Matthews, London, 1–46.
- Luer, C.A. (1976) Icones pleurothallidinarum: miscellaneous new species in *Barbosella*, *Lepanthes*, *Masdevallia*, *Platystele* and *Scaphosepalum*. *Selbyana* 3: 10–37.
- Luer, C.A. (1977) Icones Pleurothallidinarum (Orchidaceae). Miscellaneous species in the Pleurothallidinae. Addenda et corrigenda. *Selbyana* 3: 203–412.
- Luer, C.A. (1978) *Restrepiopsis*, a new genus in the Pleurothallidinae (Orchidaceae). *Selbyana* 2: 199–204.
- Luer, C.A. (1981) *Barbrodria*, a new genus in the Pleurothallidinae (Orchidaceae). *Selbyana* 5: 386–387.
- Luer, C.A. (2000) Icones Pleurothallidinarum XX. Systematics of *Jostia*, *Andinia*, *Barbosella*, *Barbrodria*, *Pleurothallis* subgen. *Antilla*, subgen. *Effusia*, subgen. *Restrepioidia*. Addenda to *Lepanthes*, *Masdevallia*, *Platystele*, *Pleurothallis*,

- Restrepiopsis*, *Scaphosepalum* and *Teagueia*. *Monographs in Systematic Botany from the Missouri Botanical Garden* 79: 7–38.
- Luer, C.A. (2002) Icones Pleurothallidarum XXIV: A First Century of New Species of *Stelis* of Ecuador, Part One. Addenda to *Barbosella*, *Dracula*, *Dresslerella*, *Lepanthopsis*, *Platystele*, *Pleurothallis*, *Restrepia*, *Scaphosepalum*, *Teagueia*, and *Trichosalpinx*. *Monographs in Systematic Botany from the Missouri Botanical Garden* 88: 97–122.
- Pabst, G.F.J. (1956) Additamenta ad Orchidologiam Brasiliensem – II. *Archivos do Jardim Botânico do Rio de Janeiro* 14: 21–22.
- Pabst, G.F.J. (1959) Orchidaceae novae riograndenses a cl. João Dutra descriptae vel nomiatae se nunquam luci editae – II. *Sellowia* 10: 133.
- Pabst, G.F.J. (1975) Additamenta ad Orchideologiam Brasiliensem – XX. *Bradea* 2: 49–56.
- Pabst, G.F.J. & Dungs, F. (1975) *Orchidaceae Brasilienses*. 1. Kurt Schmersow, Hildesheim, 408 pp.
- Pabst, G.F.J. & Dungs, F. (1977) *Orchidaceae Brasilienses*. 2. Kurt Schmersow, Hildesheim, 418 pp.
- Pridgeon, A.M. (2005) *Barbosella*. In: Pridgeon, A.M., Cribb, P.J., Chase, M.W. & Rasmussen, F.N. (Eds.), *Genera Orchidacearum*. 4, *Epidendroideae (Part 1)*, Oxford University, Oxford, 696 pp.
- Pridgeon, A.M., Solano, R. & Chase, M.W. (2001) Phylogenetic relationships in Pleurothallidinae (Orchidaceae): combined evidence from nuclear and plastid DNA sequences. *American Journal of Botany* 88: 2286–2308.
- Reichenbach, H.G. (1866) *Restrepia miersii*. In: Wawra, H. *Botanische Ergebnisse der Reise Seiner Majestat des Kaisers von Mexico Maximilian I. nach Brasilien (1859–60)*. 234 pp.
- Reichenbach, H.G. (1877) New garden plants: *Restrepia prorepens*. *The Gardeners' Chronicle*, new series 7: 810.
- Reis, A., Boeira, A.F., Dametto, A., Sanches, A.C., Coutinho Souza, A.C., Neto, A.A., Neves, B.T., Longo, B.L., Barcellos, C.S., Perart, D., Freitas, D.M., Zech, D.F., Matos, E., Pansera, E.G., Marchioreto, L., Bileski, M.K.S., Machado, M.S.C., Heinz, M.K., Cury, R.K., Spuldaro, S.C., Quevedo, T.C., Zanotto, T.L. &

- Soboleski, V.F. (2011) Lista das espécies vegetais catarinenses da divisão Angiospermas. *Sellowia* 56-63: 09–256.
- Ruschi, A. (1977) Orquidáceas novas do Estado do Espírito Santo. *Boletim do Museu de Biologia Mello Leitão* 87: 1–3.
- Sampaio, G.A.S.F. (1909) Uma Orchidacea nova. *Arquivos do Museu Nacional do Rio de Janeiro* 15: 187–192.
- Santos, R.C.M. (2011) *No coração do Brasil: Roquette-Pinto e a expedição à Serra do Norte (1912)*. Dissertation. Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 149 pp.
- Schlechter, F.R.R. (1918) Die Gattungen *Restrepia* H. B. u. Kth. *Repertorium Specierum Novarum Regni Vegetabilis* 15: 255–270.
- Silva, M.F.F. & Silva, J.B.F. (2004) *Orquídeas nativas da Amazônia Brasileira II*. Universidade Federal Rural do Pará, Museu Paraense Emílio Goeldi, Belém, 540 pp.
- Siqueira, C. E., A. Zanin, L. Menini Neto. (2014) Orchidaceae in Santa Catarina: Update, geographic distribution and conservation. *Check List* 10: 1452–1478.
<http://dx.doi.org/10.15560/10.6.1452>.
- Spegazzini, C.L. & Kraenzlin, F.W.L. (1908). In: Kraenzlin, F.W.L. Neue und kritische Arten. *Orchis: Monatsschrift der Deutschen Gesellschaft für Orchideenkunde* 2: 127–129.
- Sprunger, S., Cribb, P.J. & Toscano de Brito, A.L.V. (1996) *João Barbosa Rodrigues – Iconographie des Orchidées du Brésil. The illustrations. 1*. Friedrich Reinhardt Verlag, Basle, 540 pp.
- Stenzel, H. (2000) Pollen morphology of the subtribe Pleurothallidinae Lindl. (Orchidaceae), *Grana* 39: 108–125.
<http://dx.doi.org/10.1080/001731300300045247>
- Stearn, W.T. (2004) *Botanical Latin*. Timber Press, Portland, 546 pp.
- The Plant List. Version 1.1. Available from: <http://www.theplantlist.org/> (Accessed 12 Apr 2018).
- Thiers, B. [continuously updated] *Index Herbariorum: A global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. Available from: <http://sweetgum.nybg.org/ih/> (Accessed 17 Jan 2018).

- Toscano de Brito, A.L.V. (1995) Orchidaceae. *In*: Stannard, B.L., Harvey, Y.B & Harley R.M. (Eds.) *Flora of the Pico das Almas – Chapada Diamantina, Bahia, Brazil*. Kew Royal Botanic Gardens, London, pp. 725–767.
- Toscano de Brito, A.L.V. & Cribb, P. (2005) *Orquídeas da Chapada Diamantina*. Nova Fronteira, Rio de Janeiro, 399 pp.
- Tropicos.org. Missouri Botanical Garden. Available from: <http://www.tropicos.org/> (Accessed 12 Apr 2018).
- Williams, L.O. (1941) Orchidaceae austro-americanae - III. *Lilloa* 6: 241–246.

Figures

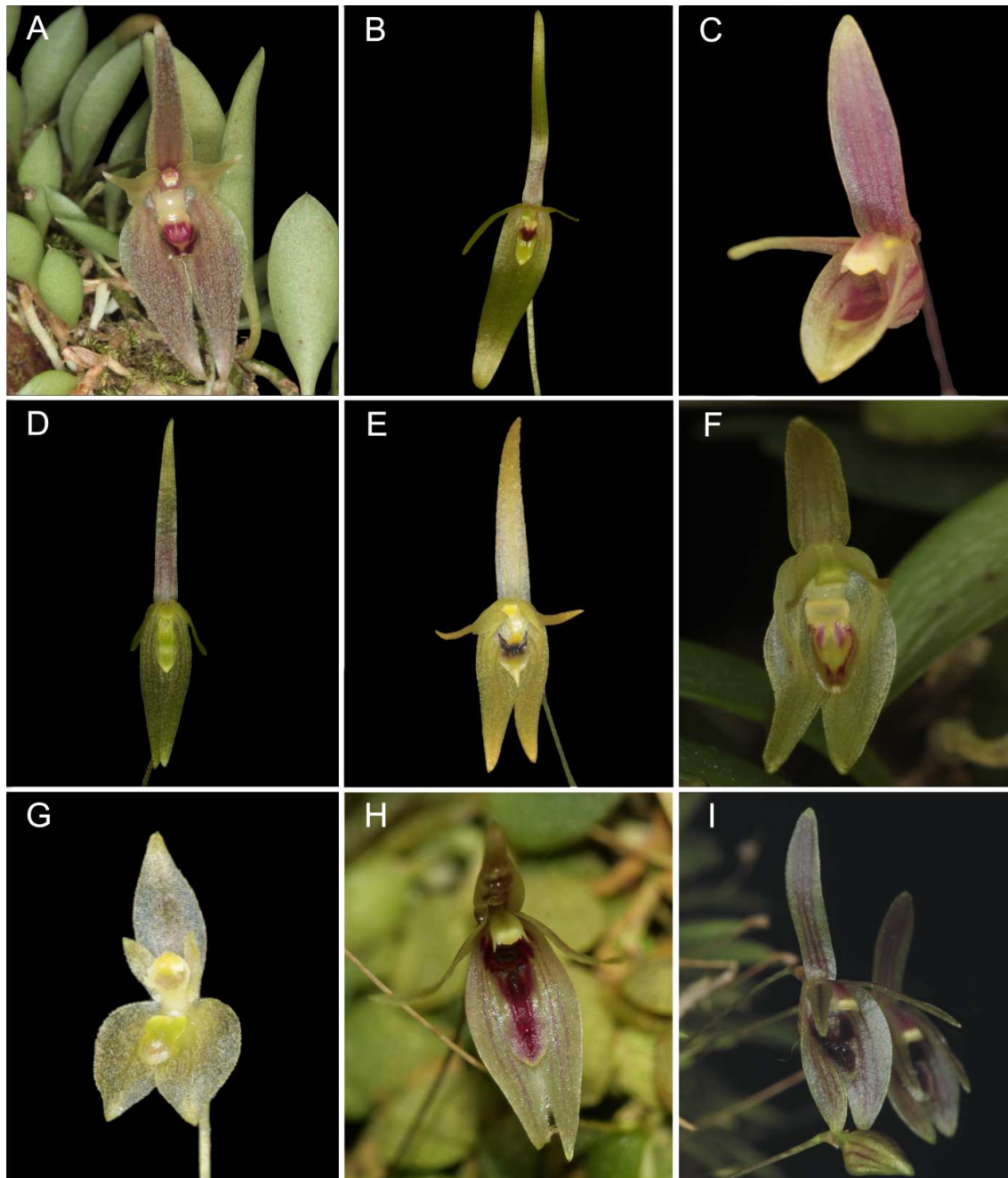


FIGURE 1. Flowers of *Barbosella* species from Brazil. A. *B. australis* (Bolson 525). B. *B. cogniauxiana* (Klingelfus 114). C. *B. crassifolia* (Toscano de Brito 3479). D. *B. dusenii* (Klingelfus 116). E. *B. gardneri* (Bolson et al. 535). F. *B. macaheensis* (Bolson et al. 582). G. *B. miersii* (Bolson et al. 518). H. *B. spiritu-sanctensis* (Chiron s.n.). I. *B. trilobata* (Santos 178). Photos by E.C. Smidt, except C by W. Collier & A. Toscano de Brito, and H by G.R. Chiron.

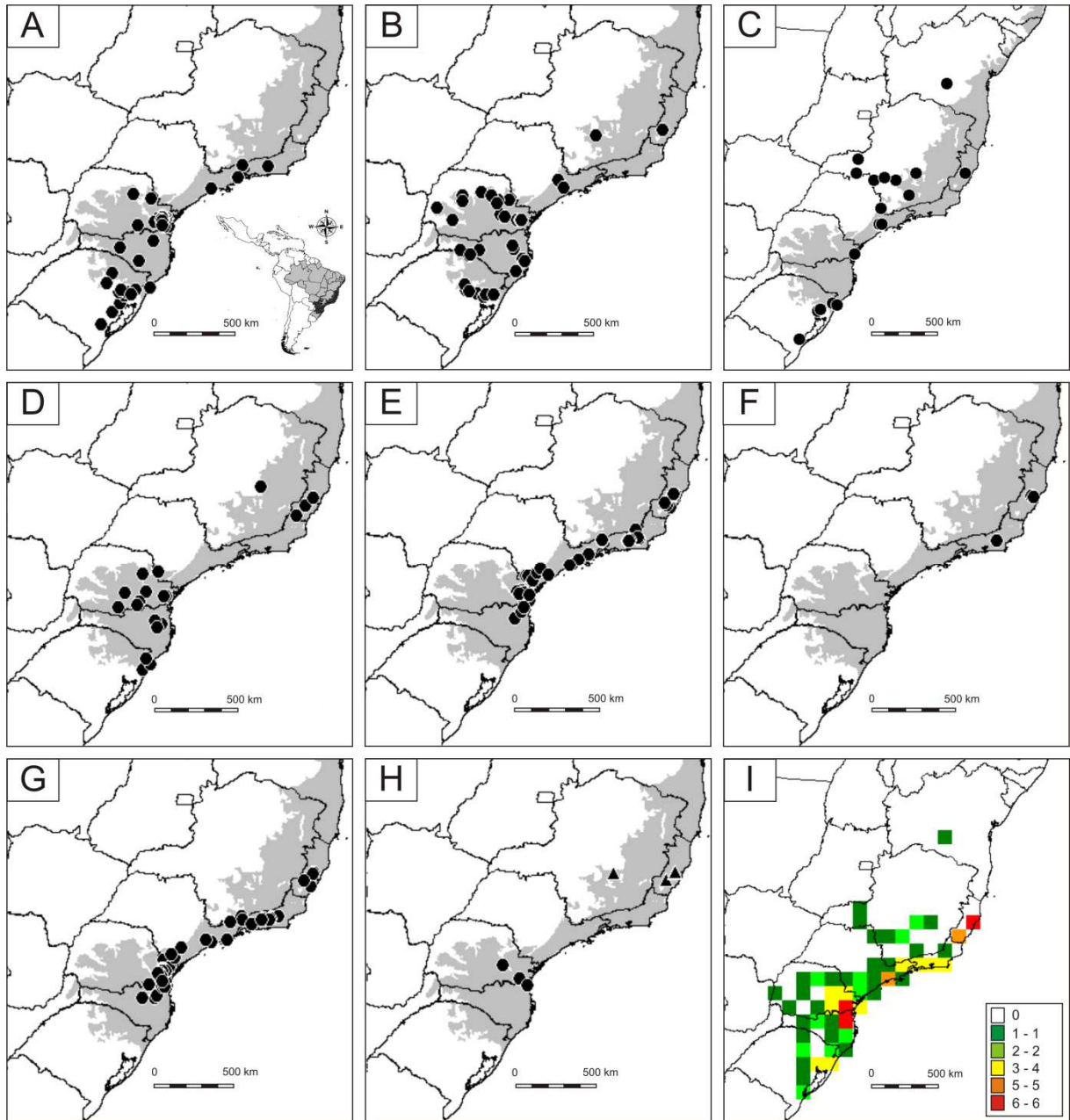


FIGURE 2. Distribution maps of *Barbosella* in Brazil. Atlantic Forest domain represented in gray. A. *B. australis*. B. *B. cogniauxiana*. C. *B. crassifolia*. D. *B. dusenii*. E. *B. gardneri*. F. *B. macaheensis*. G. *B. miersii*. H. *B. spiritu-sanctensis* (triangle) and *B. trilobata* (circle). I. Richness of *Barbosella* species.

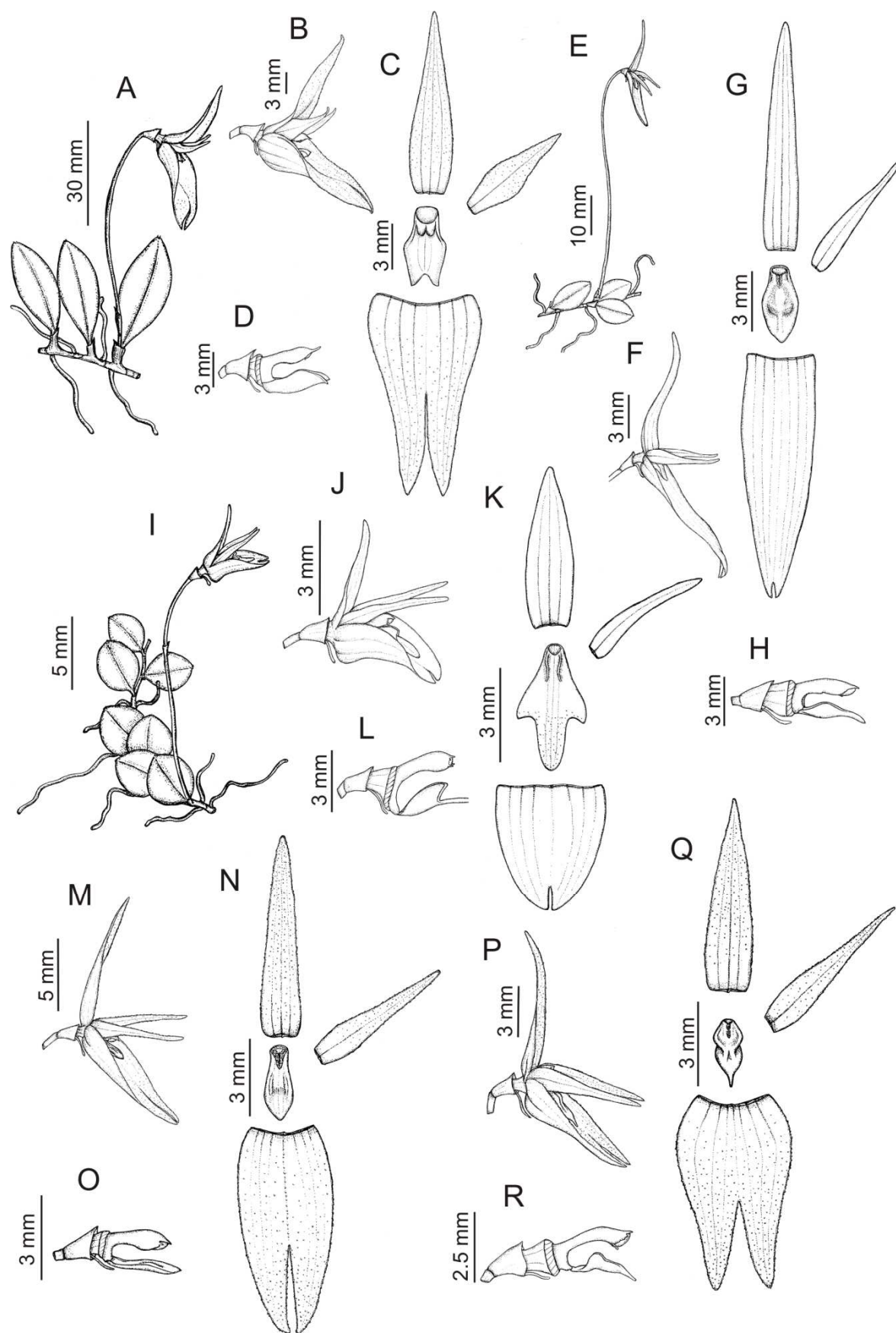


FIGURE 3. *Barbosella*. A–D. *B. australis* (Santos 147). A. Habit. B. Flower, lateral view. C. Floral parts. D. Floral bract, ovary, column and lip, lateral view. E–H. *B. cogniauxiana* (Klingelfus 113). E. Habit. F. Flower, lateral view. G. Floral parts. H. Floral bract, ovary, column and lip, lateral view. I–L. *B. crassifolia* (Toscano de Brito 3479). I. Habit. J. Flower, lateral view. K. Floral parts. L. Floral bract, ovary, column and lip, lateral view. M–O. *B. dusenii* (Bolson et al. 610). M. Flower, lateral view. N. Floral parts. O. Floral bract, ovary, column and lip, lateral view. P–R. *B. gardneri* (Bolson et al. 556). P. Flower, lateral view. Q. Floral parts. R. Floral, bract, ovary, column and lip, lateral view.

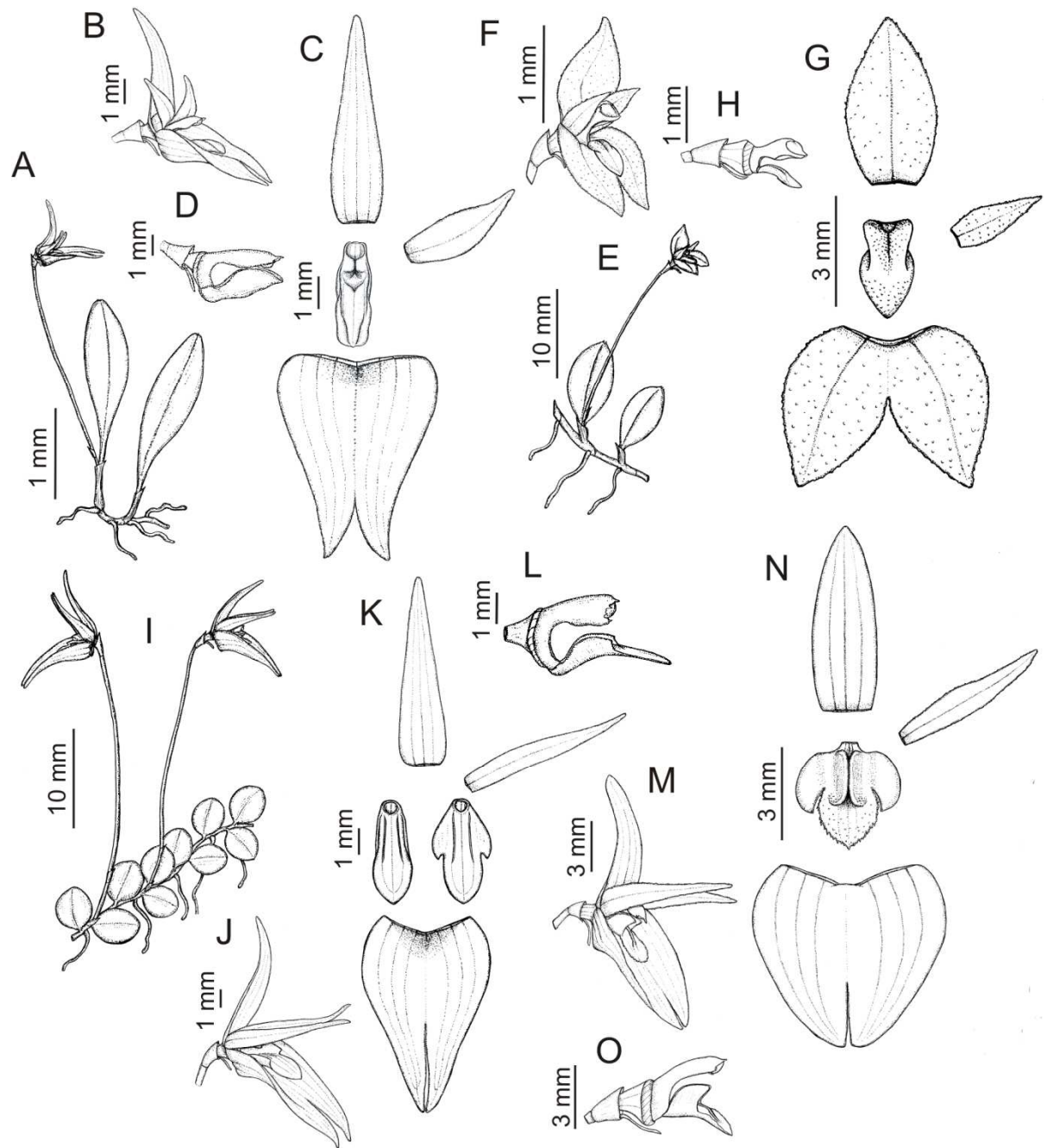


FIGURE 4. *Barbosella*. A–D. *B. macaheensis* (A, C and D from Bolson *et al.* 582; B from Bolson 637). A. Habit. B. Flower, lateral view. C. Floral parts. D. Floral bract, ovary, column and lip, lateral view. E–H. *B. miersii* (Bolson *et al.* 560). E. Habit. F. Flower, lateral view. G. Floral parts. H. Floral bract, ovary, column and lip, lateral view. I–L. *B. spiritu-sanctensis* (Toscano de Brito 2678). I. Habit. J. Flower, lateral view. K. Floral parts. L. Ovary, column and lip, lateral view. M–O. *B. trilobata* (Santos 178). M. Flower, lateral view. N. Floral parts. O. Floral bract, ovary, column and lip, lateral view.

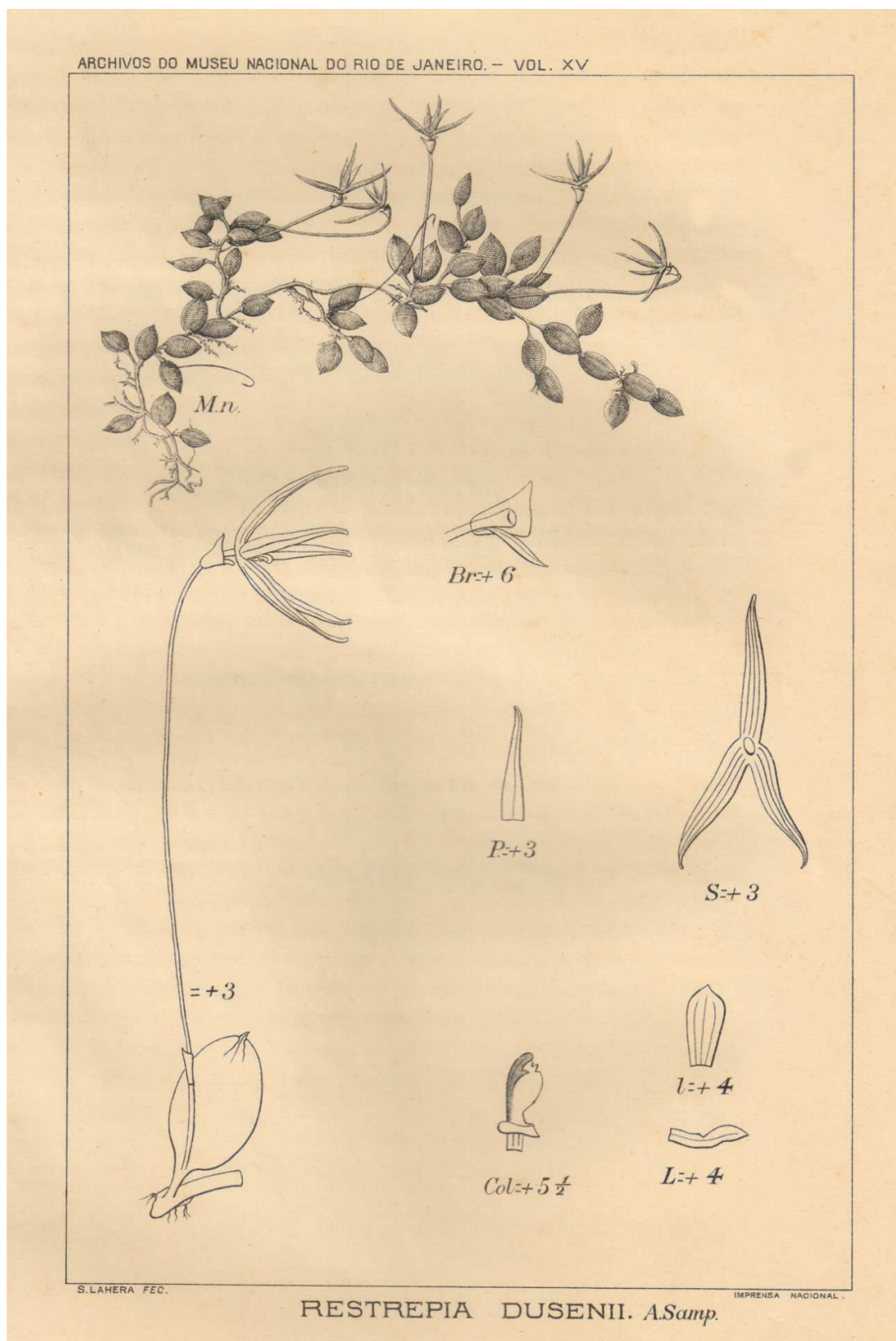


FIGURE 5. Lectotype of *Restrepia dusenii*, selected here. Original plate prepared by Santos Lahera y Castillo which appeared in the protologue. Reproduced with permission of Biblioteca do Museu Nacional (BMN)/UFRJ.

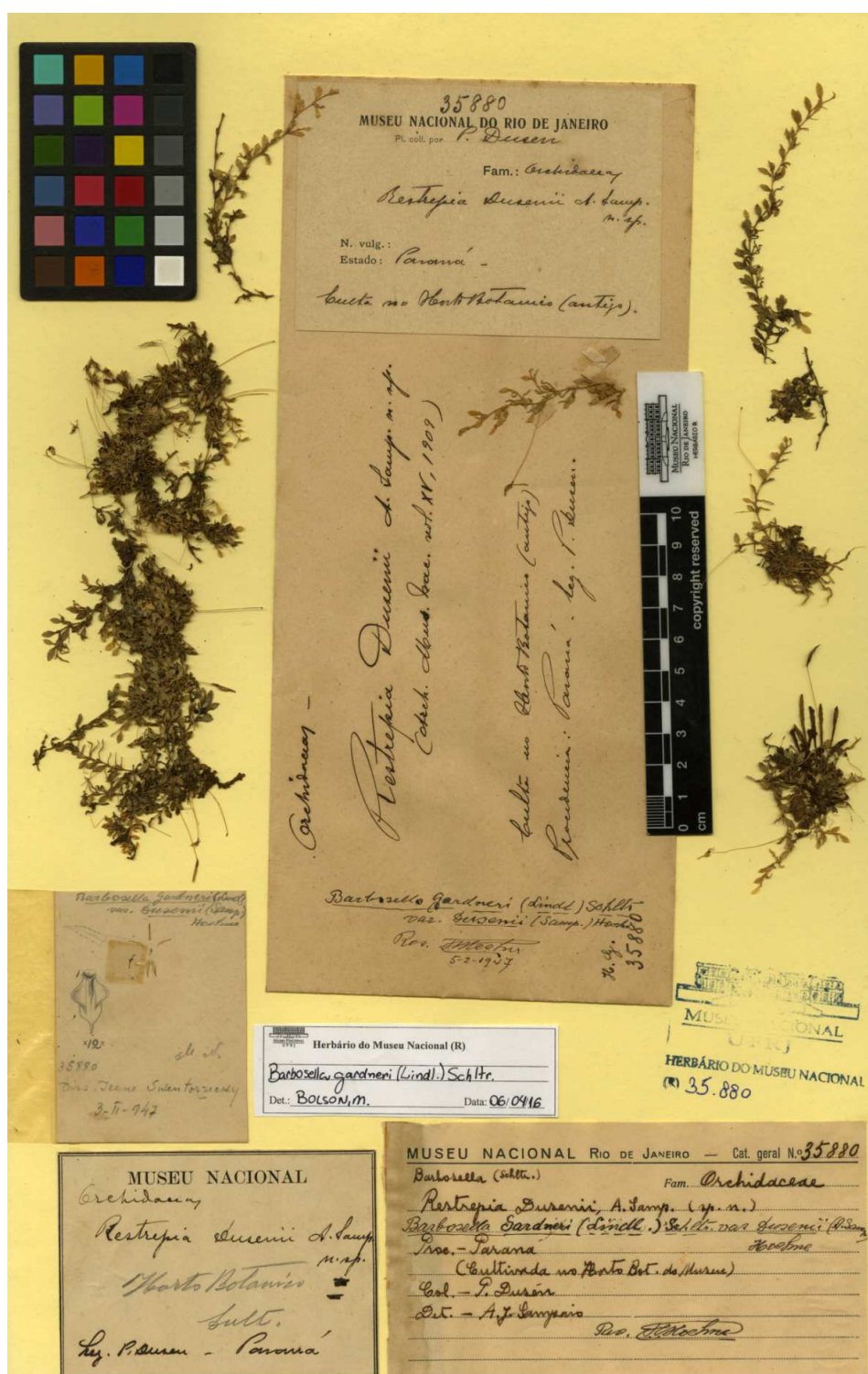


FIGURE 6. Herbarium sheet at R (35880) containing a collection of *Barbosella gardneri* by Dusén (s.n.), labelled “*Restrepia dusenii* A.Samp. n.sp.”, and drawing of lip of one of the flowers by Irene Swentorzecky (lower, left side). Reproduced with permission of Museu Nacional (Rio de Janeiro).



FIGURE 7. Dusén's collection nr. 8070 deposited at S and here designated as epitype of *Restrepia dusenii*. Scanned by Åsa Dalsätt and reproduced with permission of the Keeper, Herbarium of the Swedish Museum of Natural History.



Restrepia microphylla, Barb. Rod.

FIGURE 8. Lectotype of *Restrepia microphylla*, here designated: Barbosa Rodrigues's original illustration in *Iconographie des orchidées du Brésil*. Reproduced with the permission of the Reinhardt Verlag, Basel.

CAPÍTULO V / CHAPTER V

Field Guide:

ORCHIDACEAE: Pleurothallidinae

***Barbosella* Schltr. do Brasil**

Field Guide:

ORCHIDACEAE: Pleurothallidinae

***Barbosella* Schltr. from Brazil**

(FieldGuide formatado segundo as normas de *fieldguides.fieldmuseum.org*)

ORCHIDACEAE: Pleurothallidinae

Barbosella Schltr. from Brazil

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Photos by Eric de Camargo Smidt, except where indicated. Production by: M. Bolson, with support from E.C Smidt.

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1 *Barbosella australis*



2 *Barbosella australis*



3 *Barbosella australis*



4 *Barbosella australis*



5 *Barbosella australis*



6 *Barbosella cogniauxiana*



7 *Barbosella cogniauxiana*



8 *Barbosella cogniauxiana*



9 *Barbosella cogniauxiana*



10 *Barbosella cogniauxiana*



11 *Barbosella crassifolia*

photo: A. Toscano de Brito & Wade Collier



12 *Barbosella crassifolia*



13 *Barbosella crassifolia*



14 *Barbosella crassifolia*



15 *Barbosella crassifolia*



16 *Barbosella dusenii*



17 *Barbosella dusenii*



18 *Barbosella dusenii*



19 *Barbosella dusenii*



20 *Barbosella dusenii*

ORCHIDACEAE: Pleurothallidinae

Barbosella Schltr. from Brazil

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21 *Barbosella gardneri*



22 *Barbosella gardneri*



23 *Barbosella gardneri*



24 *Barbosella gardneri*



25 *Barbosella gardneri*



26 *Barbosella macaheensis*



27 *Barbosella macaheensis*



28 *Barbosella macaheensis*



29 *Barbosella macaheensis*
photo: Jorge Gastin



30 *Barbosella macaheensis*



31 *Barbosella miersii*



32 *Barbosella miersii*



33 *Barbosella miersii*



34 *Barbosella miersii*



35 *Barbosella miersii*



36 *Barbosella spiritus-sanctensis*
photo: Guy. R. Chiron



37 *Barbosella spiritus-sanctensis*
photo: Mônica Bolson



38 *Barbosella spiritus-sanctensis*
photo: Guy. R. Chiron



39 *Barbosella spiritus-sanctensis*
photo: Guy. R. Chiron



40 *Barbosella spiritus-sanctensis*
photo: Guy. R. Chiron

ORCHIDACEAE: Pleurothallidinae

Barbosella Schltr. from Brazil

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41 *Barbosella trilobata*



42 *Barbosella trilobata*



43 *Barbosella trilobata*



45 *Barbosella trilobata*



45 *Barbosella trilobata*

CONSIDERAÇÕES FINAIS

A presente tese foi organizada em cinco capítulos e, de modo geral, aborda as relações filogenéticas e os padrões biogeográficos do gênero *Barbosella*, o qual está distribuído em dois principais centros de biodiversidade na região neotropical, a Floresta Atlântica e as florestas úmidas dos Andes. Além, de também apresentar a reconstrução evolutiva de caracteres vegetativos anatômicos e uma monografia das espécies brasileiras.

O Capítulo I aborda a filogenia e a biogeografia do gênero onde, a partir de 13 espécies de *Barbosella* em conjunto com outros gêneros relacionados, análises filogenéticas foram realizadas usando dados moleculares e morfológicos, a fim de verificar o monofiletismo do gênero, dada a sua distribuição disjunta. Os resultados obtidos demonstram que *Barbosella* é um grupo monofilético, altamente suportado, e que as relações dos clados internos não ocorrem totalmente de acordo com a distribuição geográfica das espécies. A partir da datação molecular e as análises de biogeografia, os resultados demonstraram que a provável origem de *Barbosella* ocorreu no Mioceno Médio-Superior, por volta de 11 Ma, onde a Floresta Atlântica e a Floresta Andina estão distribuídas e hoje são divididas por uma imensa área seca. A diversificação inicial do gênero ocorreu por um evento de vicariância e, posteriormente, por múltiplas dispersões em ambas as áreas. Os resultados deste capítulo contribuem para o nosso melhor entendimento da evolução de *Barbosella*, bem como evidências sobre a conexão histórica das florestas costeiras da América do Sul.

O Capítulo II descreve e compara a anatomia de raiz, rizoma, ramicaule e folha de 13 espécies de *Barbosella*, bem como gêneros relacionados, a fim de investigar como a morfologia interna e externa dos órgãos vegetativos evoluiu entre as diferentes linhagens e habitats ocupados pelo gênero. Os resultados demonstraram dez caracteres anatômicos sinapomórficos para *Barbosella* e, internamente, o clado das espécies de que ocorre nos Andes diferenciam-se do clado das espécies da Floresta Atlântica por apresentarem caracteres sinapomórficos como ramicaule com células da epiderme em formato papiloso e presença de 14 camadas no córtex; e folha com células da epiderme de formato alongado. As sinapomorfias encontradas nas espécies da Floresta Atlântica são rizoma com seis camadas no córtex e presença de sete feixes vasculares. Dada a quantidade de caracteres anatômicos vegetativos sinapomórficos dentro gênero, a partir

deste estudo foi possível compreender mais a fundo as relações evolutivas em *Barbosella*.

O Capítulo III trata da sinonimização de *B. perinii*, um nome negligenciado desde a sua publicação, son *B. macaheensis*, uma espécie brasileira até então conhecida apenas por duas coletas realizadas há mais de 120 anos. *Barbosella perinii* foi descrita por Augusto Ruschi em 1977, baseada em uma coleta do estado do Espírito Santo, e não foi mencionada na revisão do gênero realizada por Luer no ano de 2000. Durante a elaboração da monografia das espécies brasileiras de *Barbosella*, examinamos os materiais históricos e protólogos de várias espécies, entre eles o material tipo de *B. perinii*; bem como coletas realizadas durante este estudo. Visto as semelhanças encontradas em espécimes analisados, a partir deste estudo foi possível indicar a sinonimização de *B. perinii* em *B. macaheensis*.

O Capítulo IV trata da monografia das espécies brasileiras de *Barbosella*, trabalho este resultante de esforços de coleta em campo, do estudo dos materiais históricos e protólogos, bem como da análise de exsicatas provenientes de vários herbários do mundo. A identificação das espécies de *Barbosella* não é uma tarefa trivial, já que são espécies diminutas e muito semelhantes morfológicamente. Anteriormente eram citadas 10 espécies para o Brasil, mas no presente trabalho atualizamos a listagem para nove espécies reconhecidas no país: *B. australis*, *B. cogniauxiana*, *B. crassifolia*, *B. dusenii*, *B. gardneri*, *B. macaheensis*, *B. miersii*, *B. spiritu-sanctensis* e *B. trilobata*. Através deste tratamento taxonômico atualizamos as descrições morfológicas, realizadas por Luer em 2000; apresentamos ilustrações mais detalhadas, juntamente com fotografias; elaboramos uma chave de identificação; atualizamos o status de conservação das espécies através das categorias e critérios da IUCN, por meio de um levantamento robusto da distribuição geográfica das *Barbosella* brasileiras. A partir disso, apresentamos novos registros para alguns estados brasileiros, dois lectótipos foram designados e um epítipo selecionado, visto que erroneamente outros materiais eram designados como holótipos para as espécies na revisão de Luer.

O Capítulo V apresenta um Guia de Campo com o intuito de auxiliar, não só pesquisadores e cultivadores de orquídeas, como também a comunidade em geral, na identificação das espécies de *Barbosella* do Brasil. Através desse guia ilustrado, de fácil acesso e manuseio, proporcionamos às pessoas conhecimento científico de uma maneira fácil e rápida.

Vale ressaltar que, apesar dos esforços recentes em Pleurothallidinae, muito ainda tem a ser feito para compreender a relação entre os gêneros da subtribo. Os resultados aqui apresentados com o gênero *Barbosella*, embora de grande importância e filogeneticamente bem suportados, representam apenas uma pequena parcela no conhecimento geral deste enorme grupo, levando em consideração que dezenas de espécies de Pleurothallidinae são descritas a cada ano. Como um todo, esta tese também reforça que estudos filogenéticos e biogeográficos têm auxiliado na compreensão de eventos que influenciaram a diversificação de Orchidaceae no espaço e no tempo. Entretanto, outros estudos sobre biosistemática e polinização são fortemente encorajados e podem contribuir ainda mais para as hipóteses filogenéticas. Os trabalhos taxonômicos e nomenclaturais também são essenciais para a compreensão da diversidade em geral, pois, sem esses trabalhos, é impossível estimar a diversidade real. Tendo como a base a monografia realizada nesta tese, percebemos que 65% das nove espécies do Brasil (30% de *Barbosella*) apresentaram algum problema nomenclatural e/ou taxonômico. Extrapolando para Pleurothallidinae, se 65% das espécies da subtribo ainda apresentarem problemas, isso significa que aproximadamente 3300 espécies ainda precisam ser revisadas taxonomicamente. Desta forma fica evidente que esse tipo de abordagem ainda é crucial para o entedimento do grupo.