UNIVERSIDADE FEDERAL DO PARANÁ

NICOLÁS GUTIÉRREZ MORALES

FILOGENIA MOLECULAR E BIOGEOGRAFIA DE *Pabstiella* BRIEGER & SENGHAS (ORCHIDACEAE)

CURITIBA

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Dissertação apresentada como requisito para a obtenção do título de mestre em Botânica no programa de Pós-Graduação em Botânica, Setor de Ciências Biológicas, Universidade Federal do Paraná.

Orientador: Dr. Eric de Camargo Smidt (UFPR)

Co-orientador: Dr. Antônio Luiz Vieira Toscano de Brito (MSBG-FL, EUA)

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Filogenia molecular e biogeografia de *Pabstiella* Brieger & Senghas (Orchidaceae)

por

Nicolás Gutiérrez Morales

Dissertação aprovada como requisito parcial
para obtenção do grau de Mestre no Programa
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DEDICATORIA

Dedico esse trabalho a Mamá Mariela, nossa mãe que nos deixou no mesmo ano que o Ben chegou, que me criou com a sabedoria das avós e o amor das mães, o melhor exemplo para essa grande e linda família. Tudo o que aprendi do teu lado vai ser transmitido para o Benjamín.

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Tierra es mi cuerpo, agua mi sangre,
aire mi aliento y fuego mi espíritu.

RESUMO

A subtribo neotropical Pleurothallidinae (Epidendroideae: Epidendrae) é a mais rica em espécies dentro da família Orchidaceae. Apesar dos avanços na sua filogenia, a circunscrição de alguns dos seus gêneros ainda é problemática. Com cerca de 135 espécies epífitas, *Pabstiella* é um dos gêneros mais ricos e variáveis morfologicamente das Pleurothallidineae do Brasil, e sua diversidade se concentra principalmente no Sudeste do país. Estudos filogenéticos prévios da subtribo Pleurothallidinae no Brasil sugeriram a monofilia do gênero, no entanto, não existem sistemas de classificação infragenéricos. Este trabalho apresenta uma análise filogenética baseada em sequências de nrITS, *matK* e *trnH-psbA*, de 102 espécies de orquídeas, das quais 59 pertencem ao gênero *Pabstiella*, usando máxima verossimilhança, inferência Bayesiana e máxima parcimônia. Também foram realizadas análises de biogeografia, datação molecular e de reconstrução de caracteres morfológicos. Os resultados confirmam a monofilia do gênero. Dez clados infragenéricos são reconhecidos e propostos como seções, definidas e sustentadas por evidência morfológica e molecular. O gênero provavelmente originou-se nos Andes e na Mata Atlântica no Mioceno tardio (7.93 Ma), em uma época em que os dois biomas estavam provavelmente conectados. Um evento principal de vicariância dividiu a linhagem basal do gênero que habitava os Andes, do clado Atlântico que se diversificou neste bioma no Plioceno e Mioceno. Esse trabalho sugere que a Mata Atlântica pode ter tido um papel importante na origem da subtribo Pleurothallidinae. A reconstrução de caracteres morfológicos indica altos níveis de homoplasia, com algumas sinapomorfias relacionadas às pétalas e ramicaules. Outros caracteres vegetativos foram identificados como importantes na história evolutiva do gênero.

Palavras-chave: Pleurothallidinae, Mata Atlântica, filogenia molecular, biogeografia, evolução de caracteres, datação molecular.

ABSTRACT

The neotropical subtribe Pleurothallidinae (Epidendroideae: Epidendrae) is the most species-rich of the Orchidaceae. Despite the advances in its phylogeny, the circumscription of some genera is still problematic. Consisting of ca. 135 epiphytic species, *Pabstiella* is one of the richest and most morphologically variable genera of the brazilian Pleurothallidinae, and its greatest diversity is concentrated in southeastern Brazil. Previous phylogenetic studies of the brazilian Pleurothallidinae suggest the monophily of the genus, but there are no infrageneric classification systems. In this study we present a phylogenetic analysis based on nrITS, matK and trnH-psbA sequences from 102 orchid species, of which 59 belong to the genus, using maximum likelihood, Bayesian inference and maximum parsimony. We also performed molecular dating, biogeographical analyses and historical character reconstruction. Our results confirm the monophyletic nature of *Pabstiella*. Ten infrageneric clades are recognised and are herein proposed as sections, supported by morphological and molecular evidence. The genus originated in the Andes and the Atlantic Rainforest in the Late Miocene (ca. 7.93 Mya), in an epoch were these biomes were probably connected. A main vicariant event divided an early diverging lineage that inhabited the Andes, from an Atlantic Rainforest lineage that diversified in this region during the Pliocene and Pleistocene. Our findings also suggest that the Atlantic forest may have played an important role in the origin of the subtribe Pleurothallidinae. The morphological character reconstruction showed high levels of homoplasy, with few recognised synapomorphies associated with petals and ramicauls. Other vegetative characters were identified as important in the evolutionary history of the genus.

Keywords: Pleurothallidinae, Atlantic forest, molecular phylogeny, biogeography, character evolution, molecular dating.

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Figure 2. Maximum-likelihood tree of *Pabstiella* plus outgroups.

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Supplementary Material

Figure S1. Maximum-likelihood tree resulting from the analysis of cpDNA (*matK+trnH-psbA*) (A) and nrITS (B) for *Pabstiella* plus outgroups.

Figure S2. Biogeographic reconstruction under DEC model for *Pabstiella* plus outgroups based on Bayesian maximum clade credibility tree.

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INTRODUÇÃO GERAL

Orchidaceae é uma das maiores famílias das angiospermas com aproximadamente 28.000 espécies distribuídas em todos os continentes exceto a Antártica e em todos os biomas terrestres exceto os desertos verdadeiros (PRIDGEON, 2014; GIVNISH *et al*., 2016). É considerado que a evolução do habito epífito mediante adaptações morfológicas e fisiológicas, junto com a evolução das polinias e o desenvolvimento dos diferentes mecanismos de polinização foi o que promoveu a rápida diversificação da família, particularmente nas regiões montanhosas tropicais (TREMBLAY *et al*., 2004; GIVNISH *et al*., 2015).

No neotrópico essa rápida especiação está relacionada ao soerguimento dos Andes há cerca de 15 milhões de anos, particularmente da região norte, onde se originaram várias linhagens adaptadas a altitude e que depois migraram para outras regiões de Sul e Centro América (GIVNISH *et al*., 2016; PEREZ-ESCOBAR *et al*., 2017). Em algumas subtribos exclusivamente neotropicais como Pleurothallidinae e Laeliinae a complexidade topográfica e climática resultante desta região megadiversa gerou muitas espécies de distribuição restrita devido a processos de especiação *in situ* (GIVNISH *et al*., 2015; GIVNISH *et al*., 2016; PEREZ-ESCOBAR *et al*., 2017).

Evidências de endemismo em Pleurothallidinae do Brasil em seções de *Anathallis* Barb.Rodr. (1877), *Pabstiella* Brieger e Senghas (1976) e *Acianthera* Scheidw. (1842), assim como análises filogenéticas para outros grupos como a subtribo Spiranthinae Lindl. ex Meisn. (1842) e o clado Ornithocephalinae da subtribo Oncidiinae Benth. & Hook.f. (1883), sugerem que a história evolutiva da família na América do Sul não está só relacionada ao soerguimento dos Andes, mas também a eventos posteriores de diversificação na Mata Atlântica (CHIRON *et al*., 2012; RODRIGUES *et al*., 2017; SALAZAR *et al*., 2018; SMIDT *et al*., 2018).

Pleurothallidinae Lindl. contém mais de 44 gêneros e 5100 espécies concentradas nas montanhas do norte dos Andes, da América Central e da Mata Atlântica, constituindo o maior dos grupos de Orchidaceae que apresenta especialização à polinização por Diptera (PRIDGEON *et al*., 2001; BORBA *et al*., 2011; KARREMANS *et al*., 2016, KARREMANS E DIAZ-MORALES, 2019). As plantas geralmente possuem crescimento simpodial, raízes com velame, caule unifoliado e sem pseudobulbo (chamado de ramicaule), folhas conduplicadas e uma articulação entre o pedicelo e ovário. Essa articulação permite diferenciá-las do grupo irmão composto por *Dilomilis* Raf. (1836), *Neocogniauxia* Schltr. (1913) e *Tomzanonia* Nir. (1997), gêneros endêmicos das Antilhas, e recentemente incorporados à subtribo (PRIDGEON *et al.*, 2001; PRIDGEON *et al.*, 2005; KARREMANS, 2016).

A identificação de sinapomorfias morfológicas e anatômicas para alguns grupos da subtribo é difícil devido às várias homoplasias, tanto de caracteres florais em resposta a pressões seletivas impostas por polinizadores de comportamento semelhante, quanto de caracteres vegetativos devido às adaptações ao hábito epifítico. Tais convergências levaram a interpretações taxonômicas erradas em vários gêneros como *Andinia* (Luer) Luer (2000) ou *Pabstiella* (PRIDGEON E CHASE, 2001; LUER, 2006; SCHIESTL E JOHNSON, 2013; KARREMANS, 2016; WILSON *et al.*, 2017).

Pabstiella foi inicialmente proposto como gênero monotípico por Brieger e Senghas (1975) no livro “Die Orchideen” e validado um ano depois pelos mesmos autores na revista “De Orchidee” (1976) com a finalidade de diferenciar a *Pleurothallis mirabilis* Schltr. (Figura 1), uma espécie atípica no gênero *Pleurothallis* R. Br (1813) devido ao mento conspícuo formado pela sinsepala côncava em conjunto com o pé da coluna. No entanto, Luer (1986) considerou inválido o nome *Pabstiella* devido à ausência de uma descrição latina na primeira publicação, sem perceber da validação posterior, e criou o subgênero *Mirabilia* Luer (1986) para acomodar essa espécie novamente dentro do gênero *Pleurothallis*. Assim o nome *Pabstiella* foi esquecido por vários anos e *Pleurothallis* foi mantido como grupo artificial até a aparição das primeiras análises filogenéticas (BARROS, 2012; CHIRON *et al.*, 2013, CHIRON *et al.*, 2016, KARREMANS, 2016).

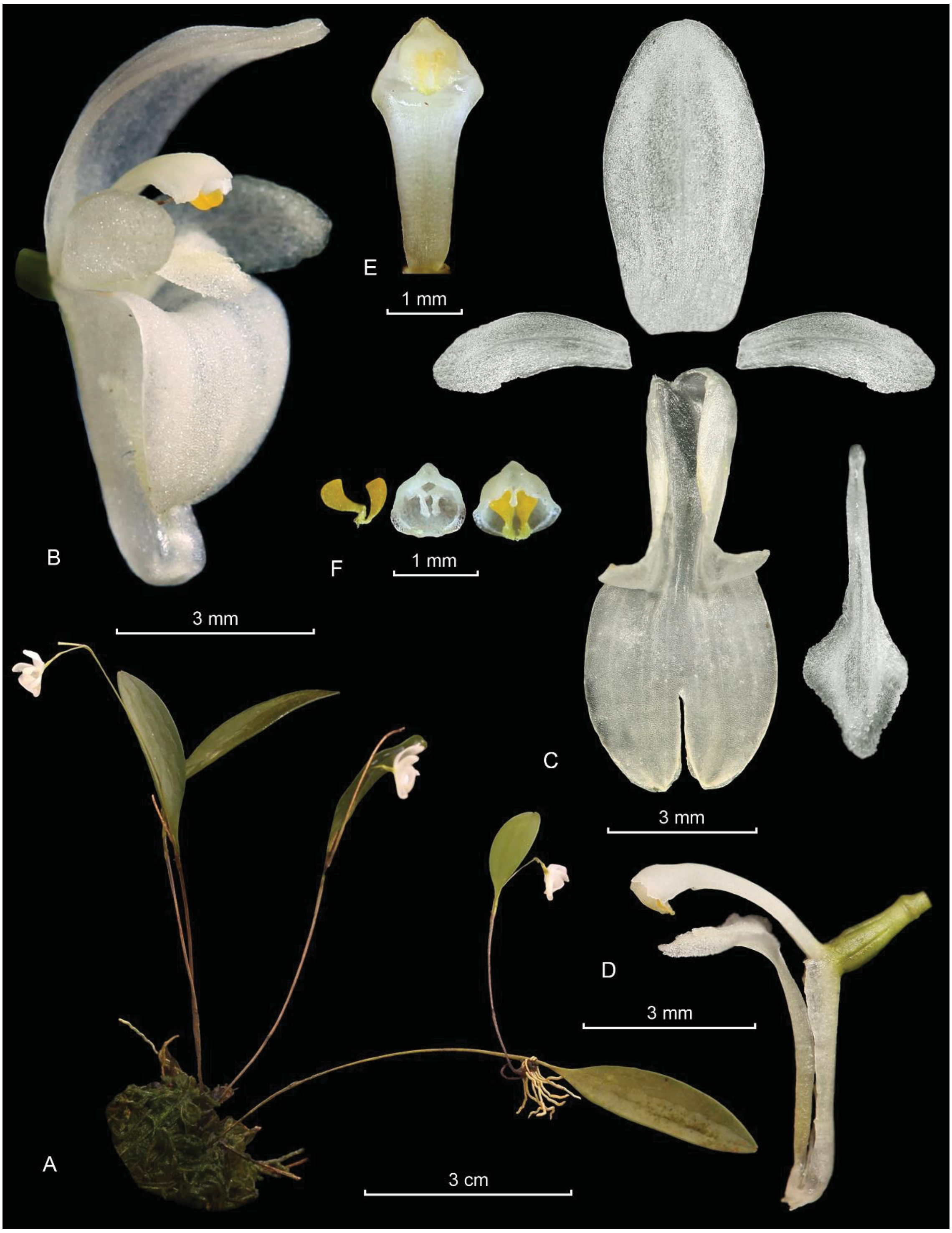

Figura 1. *Pabstiella mirabilis*. **A.** Hábito. **B.** Flor. **C.** Perianto. **D.** Coluna e labelo (mento). **E.** Coluna, vista ventral **F.** Antera e polinário.

A filogenia molecular da subtribo Pleurothallidinae feita por Pridgeon *et al.* (2001), seguido da proposta de Pridgeon e Chase (2001) para redefinir a subtribo resultou na divisão do gênero *Pleurothallis* e na criação do gênero *Anthereon* Pridgeon e Chase (2001). *Anthereon* (sinônimo de *Pabstiella*) permitiu agrupar seis espécies que, até então, correspondiam a subgêneros diferentes de *Pleurothallis* (Figura 2), como *Pleurothallis mirabilis* Schltr., do subgênero *Mirabilia*, quatro espécies das secções *Mentosae* Luer. (1986) e *Tripteranthae* Luer. (1986) do subgênero *Specklinia* (Lindl.) Garay (1974) e *Pleurothallis syringodes* Luer. (1999), caracterizadas, em sua maioria, pelo mento formado pelo sinsepalo e o pé da coluna (PRIDGEON *et al*, 2001; PRIDGEON *et al*, 2005; KARREMANS 2016).

Fig. 2. Continuation of the most parsimonious tree in Fig. 1. Taxa indicated by numbered bars were formerly known under epithets as follows: **3.** *Pleurothallis*; **4.** *Condylago*; **5.** *Salpistele*; **6.** *Acostaea* (*costaricensis*).

Figura 2. Modificado de Pridgeon e Chase (2001). *Anthereon* constituído por *Anthereon mentosus* (Cogn.) Pridgeon e M.W.Chase (*Pabstiella yauaperyensis* (Barb.Rodr.) F.Barros), *Anthereon tripteranthus* (Rchb.f.) Pridgeon e M.W.Chase (*Pabstiella tripterantha*) e *Anthereon mirabilis* (Schltr) Pridgeon e M.W.Chase (*Pabstiella mirabilis*).

No ano seguinte Luer (2002) discute o problema nomenclatural dessa transferência e Barros (2002) reconhece a validade e revive o nome *Pabstiella,* transferindo as seis espécies de *Anthereon* para *Pabstiella* (BARROS, 2002, LUER, 2002). Essa circunscrição do gênero foi reconhecida por Pridgeon e colaboradores na obra Genera Orchidacearum, Volume 4. Epidendroideae (2005) e por Luer (2006). Luer (2007) reconhece a variabilidade floral e vegetativa presente no gênero e transfere para *Pabstiella* 68 táxons pertencentes em sua maioria a *Pleurothallis* subgênero *Effusia* Luer. (2000), que tinham sido transferidas erroneamente a *Stelis* Sw. (1800) por Pridgeon e Chase (2001), assim como outras espécies inicialmente descritas como *Pleurothallis*, *Lepanthes* Sw. (1799) e *Restrepia* Kunth. (1816) (LUER, 2007). A maioria dessas espécies são aceitas atualmente no gênero *Pabstiella* exceto *Acianthera crinita* (Barb.Rodr.) Pridgeon e M.W.Chase (2001), *Acianthera aveniformis* (Hoehne) C.N.Gonç. e Waechter (2004), *Acianthera variegata* (Barb.Rodr.) Campacci (2008), *Acianthera leptotifolia* (Barb.Rodr.) Pridgeon e M.W.Chase (2001), *Acianthera macuconensis* (Barb.Rodr.) F.Barros (2003), *Acianthera dutrae* (Pabst) C.N.Conç. e Waechter (2004) e *Stelis gomesii-ferreirae* (Pabst) Pridgeon e M.W.Chase (2001).

Delimitar o número exato de espécies de *Pabstiella* é complicado, pois as várias fontes recentes apontam para divergências no número de espécies. Ex: Karremans (2016) 138 espécies, Trópicos (2019) 140 espécies, Flora do Brasil (2019) 110 espécies, International Plant Names Index (2019) 120 espécies, World Checklist of Selected Plant Families (2019) 126 espécies. Hoje o gênero está composto por cerca de 135 espécies (GOVAERTS, 2019), distribuídas principalmente no sul e sudeste da Mata Atlântica, sendo o gênero das Pleurothallidineas brasileiras com maior porcentagem de endemismo (90%) (CHIRON *et al.*, 2012; KARREMANS, 2016; TOSCANO E LUER, 2016). Poucas espécies estão presentes na Amazônia, nos Andes e na América Central. Vários problemas taxonômicos permanecem sem resolução, e várias espécies reconhecidas ainda não foram descritas formalmente, fazendo com que o estudo do gênero seja muito interessante na medida em que ainda há muito a fazer.

Torna-se difícil definir caracteres morfológicos que definam a totalidade das espécies de *Pabstiella*, visto que a espécie tipo, (*Pabstiella mirabilis*) possui os caráteres atípicos já mencionados (Figura 1). No entanto reconhece-se que muitas espécies produzem uma inflorescência em racemo que normalmente ultrapassa a folha, com raque fractiflexa e flores sucessivas dispostas em zig-zag (PABST E DUNGS, 1975; CHIRON *et al.*, 2012), sépala dorsal livre e sépalas laterais fusionadas formando um sinsépalo (Figura 3), às vezes formando um mento em conjunção com o pé da coluna (Figura 1) (LUER, 2006), labelo oblongo, côncavo e trilobado, antera incumbente e polínias com caudículo (KARREMANS obs. pers).

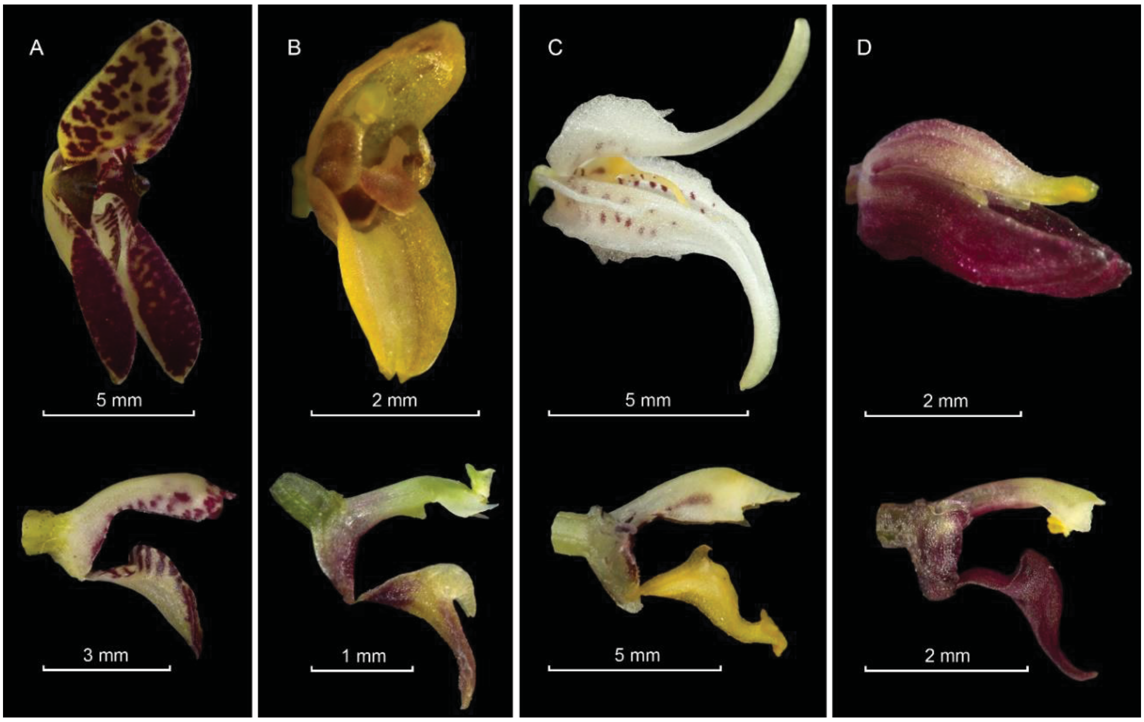

Figura 3. Flor e vista lateral do labelo e a coluna em *Pabstiella*. **A.** *Pabstiella hians*. **B.** *P. bradei*. **C.** *P. carinifera* **D.** *P. recurviloba*.

Com respeito à circunscrição de *Pabstiella* e das suas relações na subtribo Pleurothallidinae, vários estudos filogenéticos que avaliaram o gênero concluíram, apesar da baixa amostragem, que ele está fortemente suportado como grupo monofilético (Figura 4) (KARREMANS, 2016). As mesmas análises filogenéticas mostraram que o gênero conforma junto a *Pleurothallis* e *Stelis* a "Afinidade" *Pleurothallis*, e que algumas espécies de ampla distribuição como *Pabstiella yauaperyensis* e *P. tripterantha* formam um clado bem suportado, basal dentro do gênero (Figura 5) (CHIRON *et al.*, 2012; PEREZ-ESCOBAR *et al.*, 2017).

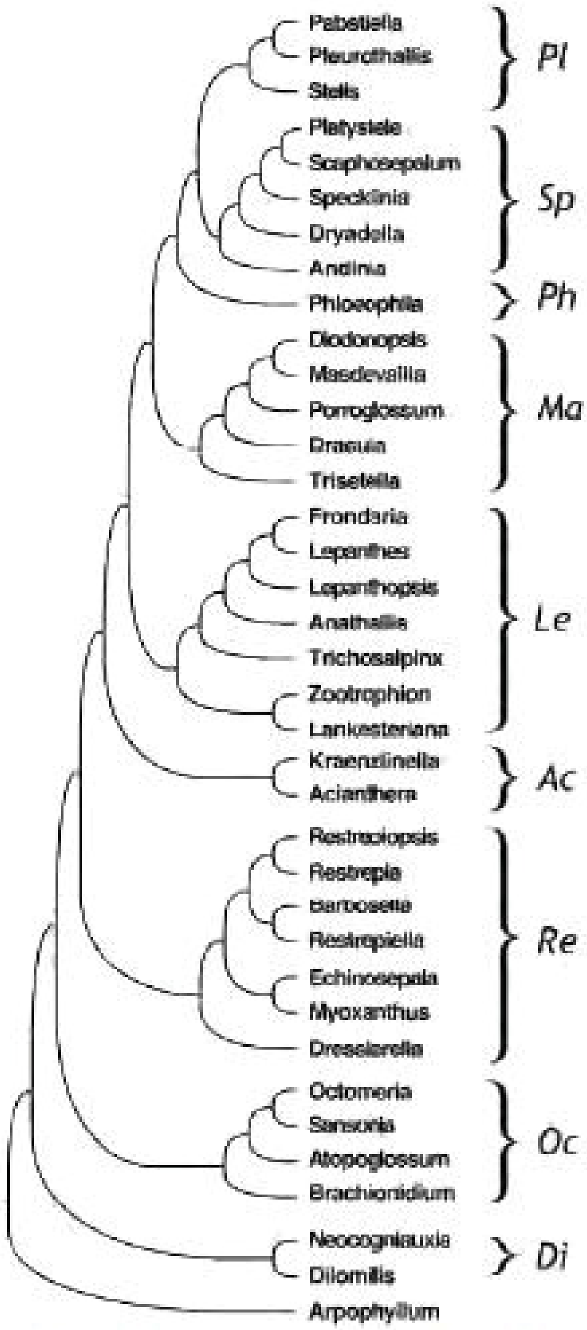

FIGURE 2. Phylogenetic overview of the main affinities within the Pleurothallidinae based on the available DNA in literature.

Figura 4. Posicionamento de *Pabstiella* na subtribo Pleurothallidinae como parte da afinidade Pleurothallis (*Pl*). Tomado de Karremans (2016).

Chiron e colaboladores (2012) avaliaram a classificação proposta por Pridgeon e Chase (2001) para as Pleurothallidinae brasileiras mediante uma análise de nrITS incluindo 260 espécies no total, das quais 32 espécies pertencem a *Pabstiella* (Figura 5). Os resultados permitiram aos autores validar a transferência para *Pabstiella* feita por Luer

(2007), e também, pela primeira vez, reconhecer algumas das relações entre as espécies do gênero, embora os autores reconheçam que a identificação de grupos infragenéricos só será possível com a inclusão de mais espécies e mais regiões de DNA, principalmente de plastídio.

Outras tentativas para entender as relações infragenéricas foram baseadas em semelhanças morfológicas. Pabst e Dungs (1975), usando exclusivamente espécies nativas do Brasil, estabeleceram seções dentro do gênero *Pleurothallis* e agruparam a maioria das espécies de *Pabstiella* dentro de *Pleurothallis* seção *Fractiflexae*, caracterizada por uma "inflorescência crescendo constantemente em zig-zag durante meses, sempre com uma flor perto do ápice e um botão novo em formação". A seção está constituída por quinze "Alianças" definidas por combinações de poucos caracteres como hábito, tamanho, tipo das bainhas no ramicaule, presença de mento, comprimento da inflorescência em relação ao tamanho das folhas e tipo das folhas (PABST E DUNGS, 1975). Também Chiron e Bolsanello (2010) discutem e ilustram um grupo de dez espécies relacionadas a *Pabstiella hians* (Lindl.) Luer (2007), caracterizadas pelo tamanho médio a grande, de inflorescência fractiflexa e flores sucessivas, sépalas pubescentes internamente e pétalas mais ou menos romboides entre outros caracteres, tentando propor uma classificação sistemática para esse grupo (CHIRON E BOLSANELLO, 2010).

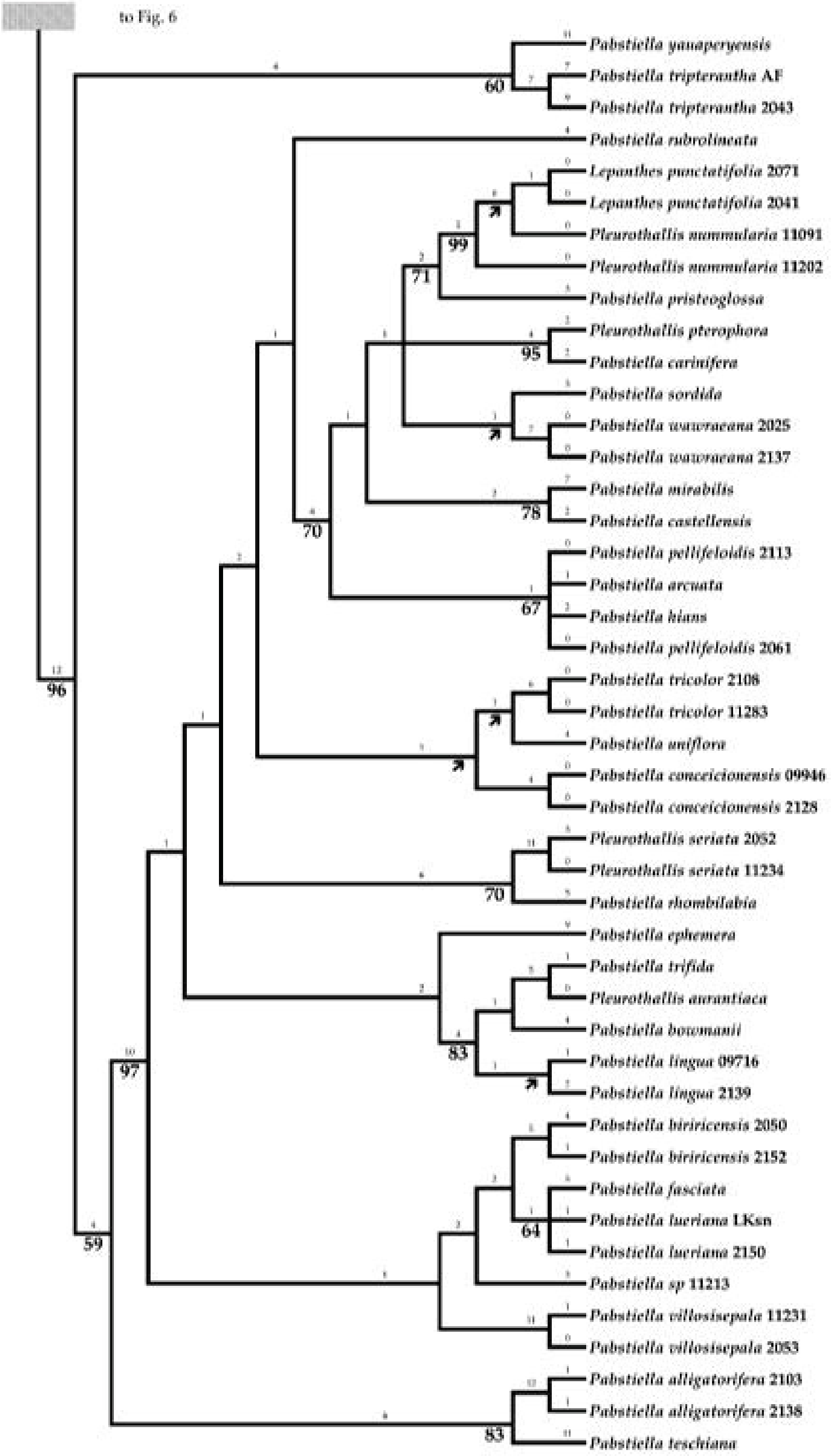

FIGURE 7. Portion B3, second part (*Pabstiella*) of the most parsimonious tree presented in Fig. 1.

Figura 5. Primeira aproximação filogenética de *Pabstiella* baseada em sequências de nrITS de 32 espécies do gênero. Retirado de Chiron et al. (2012).

Por outro lado, análises de tempo de divergência por datação molecular para a família foram realizadas recentemente (RAMIREZ *et al*., 2007; GUSTAFSSON *et al*., 2010; CHOMICKI *et al*., 2014; GIVNISH *et al*., 2015), com o fim de contextualizar e compreender a heterogeneidade dos grupos taxonômicos numa escala evolutiva. De acordo com essas análises foi estimado que Orchidaceae surgiu no final do período Cretáceo (cerca de 80 Ma), e a subtribo Pleurothallidinae no início do Mioceno (aproximadamente 19 Ma), tendo a subtribo Ponerinae como grupo-irmão (GIVNISH *et al*., 2015). As idades obtidas para a subtribo podem ser utilizadas para a calibração dos ramos de filogenias de menor escala, desde que conhecida a margem de erro das estimativas, segundo o método de calibração secundária (FOREST, 2009), permitindo se aproximar à dinâmica evolutiva de um grupo, hipotetizando eventos que propiciaram a diversificação das linhagens e suas possíveis rotas de migração (FOREST, 2009).

Portanto, e tendo em vista a grande variabilidade do gênero, este projeto teve como objetivo geral propor uma hipótese filogenética para *Pabstiella,* permitindo o reconhecimento de linhagens infragenéricas a partir do sequenciamento de regiões de DNA nuclear e do plastídio e suportadas por evidência morfológica. E ainda especificamente, com o fim de complementar a proposta filogenetica, e procurando compreender a história biogeográfica do gênero, foi realizada uma análise de tempo de divergência por datação molecular, junto com uma optimização dos principais biomas Neotropicais, para sugerir a origem espacial e temporal de *Pabstiella* e dos diferentes agrupamentos infragenéricos.

CAPÍTULO 1

Molecular phylogeny and biogeography of *Pabstiella* (Pleurothallidinae: Orchidaceae) highlight the importance of Atlantic Rainforest in the speciation of the genus

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Abstract

Pabstiella consists of ca. 130 epiphytic orchid species from the neotropics. We present a phylogenetic analysis based on nrITS, *matK* and *trnH-psbA* sequences from 59 species of the genus and 40 species from Pleurothallidinae, two from Laeliinae and one Bletiinae as an outgroup, using maximum likelihood, Bayesian inference and maximum parsimony. We also performed molecular dating, biogeographical analyses and ancestral morphological character reconstruction. Our results confirm the monophyly of *Pabstiella* with strong support. Ten clades are inferred and are herein proposed as sections. *Pabstiella* originated in the Andes and the Atlantic Rainforest in the Late Miocene (ca. 7.93 Mya), in an epoch were these biomes were probably connected. A main vicariant event divided an early diverging lineage that inhabited the Andes, from an Atlantic Rainforest lineage that diversified in this region during the Pliocene and Pleistocene, mainly in the Serra do Mar in southeastern Brazil. Our findings also suggest that the Atlantic Rainforest may have played an important role in the origin of the subtribe Pleurothallidinae. The morphological character reconstruction showed high levels of homoplasy, with few recognised synapomorphies associated with stems and petals. Other characters related to the habit and stems were identified as important in the evolutionary history of the genus.

ADDITIONAL KEYWORDS: Atlantic Rainforest – biogeography – character evolution – molecular phylogenetics – Neotropics

INTRODUCTION

Orchidaceae are one of the largest families of Angiosperms and occur in all continents except Antarctica, and in all biomes except true deserts (Givnish *et al*., 2016). Adaptation to the epiphytic niche along with the evolution of the pollinarium and the development of the different mechanisms of pollination are the main factors that promoted the rapid diversification of the family, particularly in the tropical mountainous regions (Tremblay *et al*., 2004; Givnish *et al*., 2015). In the American continent, this diversification is linked to the uplift of the Andes 15 million years ago and subsequent events of speciation in Central America and the Atlantic Rainforest (Givnish *et al*., 2016; Perez-Escobar *et al*., 2017; Rodrigues *et al*., 2017; Salazar *et al*., 2018; Smidt *et al*., 2018).

The neotropical Pleurothallidinae is the most diverse subtribe within the family (44 genera and 5114 species, divided in nine genera "affinities" proposed by Karremans (2016)) and is pollinated by Diptera (Borba *et al*., 2011; Karremans *et al*., 2016; Karremans & Diaz-Morales, 2019). Despite the advances in its phylogeny, the circumscription and the identification of morphological synapomorphies for some genera of the subtribe is problematic. Homoplasies, both of floral characters in response to selective pressures imposed by pollinators, and of vegetative characters due to the adaptations to the epiphytic niche, have led to artificial systematic arrangements (Pridgeon & Chase, 2001; Schiestl & Johnson, 2013).

This is the case of *Pabstiella* Brieger & Senghas. (Fig. 1), of which many species have already been treated in *Pleurothallis* R.Br., *Lepanthes* Sw., *Specklinia* Lindl., *Acianthera* Sw., *Stelis* Sw., and *Restrepia* Kunth (Chiron, Guiard & van der Berg, 2012, Chiron & van der Berg, 2013, Chiron & Ximenes Bolsanello, 2010, Luer

2002, Toscano de Brito & Luer, 2016). The genus was only widely accepted after the first phylogenetic analyses of the subtribe Pleurothallidinae (Pridgeon, Solano & Chase, 2001; Chiron *et al*., 2012; Karremans *et al*., 2016). From then on, dozens of new species have been described, and today there are approximately 135 valid species (Govaerts, 2019), distributed mainly in the Brazilian Atlantic Rainforest, with few species in the Amazon, the Andes and Central America (Chiron *et al*., 2012; Karremans, 2016; Toscano de Brito & Luer, 2016).

It is challenging to select morphological characters present in all *Pabstiella* species that allow distinguish the genus from the other genera of the Pleurothallidinae. Nevertheless, all species produce racemose inflorescences, several with a flexuous or fractiflex rachis that usually surpasses the leaves, and successive flowers (Chiron *et al*., 2012) (Fig. 1). The dorsal sepal is free, and the laterals are fused into a synsepal, sometimes forming a mentum or spur with the usually elongate column foot (Luer, 2006) (Fig. 1D, N). The lip is oblong, usually concave and three-lobed. The column is hooded or winged, the anther apical, incumbent, and the pollinarium consists of two pollinia with caudicles (Pridgeon *et al*., 2005).

Despite the modest sampling (32 species and using only nr ITS) used in previous phylogenetic studies (Chiron *et al*., 2012), the genus is well-supported as monophyletic, and constitutes, along with *Pleurothallis* (465 species) and *Stelis* (1030 species), the *Pleurothallis* "affinity" proposed by Karremans (2016). Some widely distributed species, such as *Pabstiella yauaperyensis* (Barb.Rodr.) F.Barros, *P. tripterantha* (Rchb.f.) F.Barros and *P. determannii* (Luer) F.Barros, form a well-supported and basal clade within the genus (Pridgeon *et al*., 2001; Chiron *et al*., 2012; Chiron, Karremans & van den Berg, 2016; Karremans, 2016; Perez-Escobar *et al*., 2017), but otherwise, no infrageneric classification is currently available for *Pabstiella*. It is worth mentioning here that in their illustrated checklist of the orchids of Brazil, Pabst & Dungs (1975) recognised under *Pleurothallis* some alliances or morpho-groups consisting mostly of *Pabstiella* species, such as section *Pleurothallis* sect. *Fractiflexae*, unfortunately invalidly published.

Several molecular divergence time analyses have been used to estimate the ages of the Orchidaceae (Chomicki *et al.*, 2014; Givnish *et al.*, 2015; Gustafsson *et al.,* 2010; Ramirez *et al.*, 2007) and also of the Pleurothallidinae, whose stem age is estimated around 18-19 Mya in the early Miocene (Givnish *et al.*, 2015; Perez-Escobar *et al.*, 2017). The ages obtained for the subtribe can be used for node calibration in smaller scale molecular dating analysis, allowing the study of evolutionary dynamics of a group or genus, hypothesising events that allowed its diversification (Forest, 2009).

In this study, we propose a phylogenetic hypothesis for *Pabstiella*, based on combined molecular analysis of nuclear and plastid sequence data of an increased sampling of taxa, and supported by morphological evidence. Through molecular dating analysis, together with the optimisation of the main Neotropical Biomes, we also hypothesise the origin in time and space of the genus and its lineages.

MATERIAL AND METHODS

TAXON SAMPLING

Sampling was composed of 99 species of subtribe Pleurothallidinae, with representatives of 29 genera of all the subtribal "affinities" proposed by Karremans (2016). Of these, 59 species belong to *Pabstiella,* and five species belong to *Stelis* and five to *Pleurothallis*. Species from closely related subtribes according to the phylogenetic proposal of Chase *et al.* (2015), were chosen for the outgroup, including *Cattleya forbesii* Lindl., and

Epidendrum tridactylum Lindl. of the Laeliinae, and *Bletia catenulata* Ruiz & Pav. of the Bletiinae. Voucher information and GenBank accession numbers are provided in Table S1.

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

DNA extraction procedure followed the 2x CTAB protocol (Doyle and Doyle 1987), scaled to 2 mL microtubes and RNAse free. All total DNA samples are stored in a -80ºC ultra-freezer in the Laboratório de Sistemática e Ecologia Molecular de Plantas (LSEMP) of Universidade Federal do Paraná (UFPR).

DNA samples were amplified through polymerase chain reactions (PCRs) for fragments of interest. The nuclear ribosomal internal transcribed spacer (nrITS) was targeted with primer pairs 17SE (ACGAATTCATGGTCCGGTGAAGTGTTCG) and 26SE (TAGAATTCCCCGGTTCGCTCGCCGTTAC) (Sun *et al.*, 1994) and 75 (TATGCTTAAACTCAGCGGG) and 92 (AAGGTTTCCGTAGGTGAA) (Desfeux *et al.*, 1996). The two chloroplast markers, the intergenic spacer region *trnH-psbA* and the partial *matK* exon, were amplified with the respective primer pairs: trnHf_05 (CGCGCATGGTGGATTCACAATCC) (Tate & Simpson, 2003) and psbA3_f (GGTATGCATGAACGTAATGCTC) (Sang, Crawford & Stuessy, 1997), and matK 19F (5' CGT TCT GAC CAT ATT GCA CTA TG 3') and 881R (5' TMT TCA TCA GAA TAA GAG T 3') (Gravendeel *et al.*, 2001).

For nrITS region, the reactions were performed with Invitrogen Platinum Taq DNA Polymerase (Thermo Fisher Scientific) PCR kit following manufacturer's recommendations, adding 0.08 mg/mL BSA, 0.08% DMSO, 1M betaine, 0.2µM each primer and 20−50 ng of total DNA for a final volume of 20 µL. For cpDNA intergenic spacers the Top Taq Master Mix (Qiagen) PCR kit was used following manufacturer's recommendations, adding 0.2µM from each primer and 20–50 ng of total DNA for a final volume of 20 µL. For both PCR methods, the thermocycling program was: 1 min pre-melt at 94ºC, 40 cycles of 30 sec denaturation at 94ºC, 40 sec annealing at 51ºC (nrITS)/ 53ºC (cpDNA) and 30 sec extension at 72ºC, ending with a 5 min final extension at 72ºC.

PCR products were visualised with electrophoresis in agarose gel with GelRed™ (Crisafuli *et al.*, 2015), and then purified with polyethylene glycol (PEG 10%) and 80% ethanol (Paithankar & Prasad, 1991). After purification, the samples were sequenced with Big Dye Terminator kit v.3.1 (Applied Biosystems, California, USA) under Sanger method by Macrogen Inc. (http://dna.macrogen.com).

DNA SEQUENCE ALIGNMENT

Reads were trimmed on extremities when the error probability limit per base was >0.05, and were *de novo* assembled into consensus sequences in Geneious Prime® 2019.2.1 program (Kearse *et al.*, 2012, https://www.geneious.com). The sequences with quality threshold >60% were aligned with MAFFT v.7.388 implemented in Geneious Prime®, using the automatic algorithm and 1PAM scoring matrix (Katoh & Standley, 2013). The alignments were concatenated using SequenceMatrix v1.8 (Vaidya *et al.*, 2011).

PHYLOGENETIC ANALYSES

The alignment of nrITS, the two cpDNA regions (*matK* gene and *trnH-psbA* intergenic spacer), and the three molecular regions combined were used for all subsequent phylogenetic analyses. The indels (insertion/deletion markers) were treated as missing data. Phylogenetic analyses were performed using maximum likelihood, Bayesian inference, and maximum parsimony in order to explore the results under different methods. All resulting phylogenetic trees were edited in FigTree v.1.4.3 (Rambaut, 2009) and in CorelDRAW v.18.0.0.448 (http://www.coreldraw.com).

The maximum-likelihood phylogenetic tree was estimated using IQ-tree 1.6.12 (Nguyen *et al.*, 2014) with tree search with model selection and bootstrap replicates in a single run (Chernomor *et al.*, 2016; Kalyaanamoorthy *et al.*, 2017). The best-fit models of substitution inferred in the IQ-tree analyses by AIC were GTR+F+I+G4 for ITS, TVM+F+R3 for *matK*, and K3Pu+F+R3 for *trnH-psbA*. For the support, we used the 1,000 ultrafast bootstrap approximation (UFBoot, Minh *et al.*, 2013), with the argument "–bnni" to reduce the risk of overestimating branch supports.

Maximum parsimony analyses were performed with Fitch (1971) parsimony using the software PAUP 4.0a166 (Swofford, 2002). Analyses included 10,000 random taxon-addition replicates using TBR swapping algorithm, holding ten trees per replication, followed by a second search to explore all topologies from the previous search, limited to 10,000 trees. Support was estimated in 1,000 bootstrap replicates using TBR (Felsenstein, 1985), simple addition, and holding ten trees per replicate.

Bayesian phylogenetic inference was performed using MrBayes 3.2.6 (Ronquist *et al.*, 2012) with GTR+I+G model for ITS and *matK* and K81uf+I+G for *trnH-psbA*, according to jModelTest 2 (Darriba *et al.*, 2012) model search results under corrected Akaike's Information Criterion (AICc, Akaike, 2011). The analyses started from random trees and employed four chains of Markov chain Monte Carlo (MCMC) runs, over 10 million generations, sampling trees and parameters every 1,000 generations. We used a burn-in of 25% of the first generations and checked the stabilisation of the log-likelihood of the trees, as measured by the standard deviation and potential scale reduction factor (PSRF) values (Gelman & Rubin, 1992) in MrBayes and ESS>300 in Tracer 1.7 (Rambaut *et al.*, 2018). The remaining trees were used to produce a 50% majority-rule consensus tree providing the posterior probabilities of the clades using TreeAnnotator 1.8.4 (Drummond *et al.*, 2012).

ESTIMATION OF THE DIVERGENCE TIME

Divergence times of *Pabstiella* were estimated using the concatenated alignment in BEAST 1.8.4 (Drummond *et al.*, 2012) via the CIPRES server (Miller, Pfeiffer & Schwartz, 2010). We set an uncorrelated relaxed clock model with lognormal distribution, and GTR+I+Γ substitution model with empirical base frequencies. The dating analysis was constrained by fixing the topology to the ML tree topology. We tested two speciation models, Yule Process (Yule, 1925; Gernhard, 2008) and Birth-Death Process (Gernhard, 2008), that were compared on Tracer 1.6 (Rambaut *et al.*, 2014) by tree likelihood under AIC estimated by method-of-moments (AICM; Baele *et al.*, 2012), as disposed in Table S5 of supplementary material. The tree was secondarily-calibrated using Givnish *et al.* (2015) estimates for: 1) crown age of Pleurothallidinae (mean 14.2 Mya, 95% highest posterior density interval (HPD): 9.7±19.21 Mya); 2) stem age of Laeliinae (mean 19.82 Mya, 95% HPD: 14.66±25.32 Mya); and 3) the stem age of Bletiinae (mean 25.51 Mya, 95% HPD: 18.35±31.45 Mya), assuming a normal distribution. MCMC chains ran for 50 million generations, with sampling every 10,000 steps. Convergence and effective sample sizes (ESS) were assessed in Tracer 1.6, and the maximum clade credibility tree was produced in TreeAnnotator 1.8.4, with a burn-in of 25%. Visualisation and basic tree editing were performed in FigTree 1.4.3.

BIOGEOGRAPHICAL ANALYSES

In order to infer the biogeographic history of *Pabstiella*, we gathered information on the current distribution of the species provided in GBIF (www.gbif.org [10 April 2019]) and then encoded the occurrence areas according to the provinces proposed by Morrone (2014; - Table S1). The codes used were: A to Mexican transition zone, B to Mesoamerican dominion, C to Pacific dominion, D to Boreal Brazilian dominion, E to South Brazilian dominion, F to Parana dominion, G to South American transition zone, and H to the Antillean subregion. We used the package BioGeoBEARS (Matzke, 2013) implemented in RASP 4.0 (Yu *et al.*, 2015) to test several biogeographic models in order to estimate the contribution of evolutionary processes (*e.g.* range expansion, vicariance, founder-event speciation) in the present distribution of *Pabstiella*. The biogeographic model with the best-fit to our data was selected using AICc value comparison (Table S5).

MORPHOLOGICAL CHARACTER RECONSTRUCTION

After a preliminary evaluation of morphological characters of the *Pabstiella* species used in the present analysis, we selected 14 characters that are useful for defining infrageneric groups in a phylogenetic framework (Table S2). To reduce homoplasies, we scored these characters only for *Pabstiella* and two species of the *Pleurothallis* affinity, using *Dryadella edwallii* (Cogn.) Luer as outgroup. The selection of characters and states were based on diagnostic features used in taxonomic descriptions and published illustrations (Barbosa Rodrigues, 1877, 1882; Brieger & Senghas, 1976; Campacci, 2019; Chiron, 2012; Chiron & Sanson, 2012; Chiron, Sanson & Bolsanello, 2011; Cogniaux, 1896; Fraga & Kollmann, 2010; Handro & Pabst, 1971; Kollmann, 2010, 2018; Kollmann & Couto, 2014; Luer, 1976, 1986, 2000, 2002, 2006, 2007; Luer & Toscano de Brito, 2002, 2011, 2013; Pabst, 1956; Pabst & Dungs, 1976; Seehawer, 1998; Sprunger, Cribb & Toscano, 1996; Toscano de Brito & Luer, 2013, 2016) and on available preserved specimens. All morphological characters were treated as multistate and unordered. The homology between character states was established following the proposal of de Pinna (1991). The character states were coded according to Sereno (2007) into a morphological matrix using Mesquite v.3.04 (Maddison & Maddison, 2015), and is provided in Table S3. All terminologies used for morphological characters and character states were based on Rizzini (1977) and Hickey & King (2004). Apomorphies and homoplasies were mapped on molecular maximum likelihood tree using Winclada v.1.00.08 program (Nixon, 2002) with ACCTRAN optimisation (Swofford & Maddison, 1987).

RESULTS

PHYLOGENETIC RELATIONSHIPS

We produced 278 new sequences in this study (Table S1). Only two *matK* and five *trnH-psbA* sequences were missing. The maximum parsimony information of each region and the combined matrix are summarised in Table S4. The resolution provided by plastid data is low, however, no high incongruences (i.e. incompatible clades with high bootstrap support) between nuclear and plastid data were detected (Figure S1). The results of combined molecular matrix revealed a well-resolved tree, with only 13% of nodes unresolved in maximum parsimony analysis (Table S4).

The subtribe is monophyletic with high bootstrap support in maximum likelihood (BSML), maximum parsimony (BSMP) and high posterior probability (PP) in Bayesian inference (86 BSML, 79 BSMP, 0.96 PP). The taxonomic delimitations or affinities proposed by Karremans (2016) are recovered, except for the polyphyletic *Phloeophila* Hoehne & Schltr., whose type species, *P. nummularia* (Rchb.f.) Pridgeon & M.W.Chase, is placed as a sister of *Trisetella* Luer, in the *Masdevallia* affinity (60 BSML), while *P. pleurothallopsis* (Kraenzl.) Pridgeon & M.W.Chase is sister to the

Specklinia affinity (60 BSML). The *Pleurothallis* affinity is highly supported (100 BSML, 85 BSMP, 1 PP), and so is *Pabstiella* (99 BSML, 83 BSMP, 1 PP). Ten infrageneric clades can be recognised with moderate to high support, although the relationships between some of them are not well-supported (Fig. 2). These clades are discussed below in the infrageneric classification for the genus.

DIVERGENCE TIMES AND BIOGEOGRAPHY

There were few differences among the ancestral area estimations using BAYAREALIKE, DEC, and DIVALIKE models, with or without the founder-event speciation (J) parameter. We report here the estimates using Dispersal Extinction Cladogenesis (DEC; Ree & Smith, 2008), which had the highest likelihood (Table S5). DEC was implemented allowing a maximum number of either two or three biogeographic units per ancestral range (Figure S2), but this parameter did not impact the analysis and we present only the results from the former analysis (Fig. 3).

As shown in the time-calibrated tree with a Birth-Death speciation model (Fig. 3, Figure S2) the subtribe Pleurothallidinae (including the *Dilomilis* affinity) probably originated in the Parana dominion (Atlantic Rainforest) [F (0.83); FH (0.17)], in the early Miocene, with an estimated stem age of 18.67 Mya (95% HPD: 15.5±23.57). The *Pleurothallis* affinity originated in the South American transition zone (Andes) [G (0.60); FG (0.26); F (0.14)], in the late Miocene, with an estimated crown age of 8.05 Mya (95% HPD: 6.62±11.66). Our analyses inferred the crown age of *Pabstiella* in 7.93 Mya (95% HPD: 6.24±11.14; Figure S2 and Table S6 shows in detail all ages for all nodes), in the late Miocene, in a possible geographic range that includes the Andes and the Atlantic Rainforest [FG (0.39); G (0.34); F (0.27)].

The main vicariance event was identified in the biogeographic history of the genus about 6.59 Mya (95% HPD: 5.27±9.46). It was responsible for the separation of the early-diverging lineage of *Pabstiella,* currently composed by *P. triperantha*, the most widely distributed species, and other Andean and Amazonian taxa, from all other species of the genus, that are mostly endemic to the Atlantic Rainforest. A second vicariance event occurred within the first diverging lineage, about 2.19 Mya, between *P. aryter* (Luer) F.Barros, distributed from the northern Andes up to Costa Rica, and *P. determannii*, present in the Amazon basin. All other biogeographic events in *Pabstiella* refer to in situ speciation in the Atlantic Rainforest that occurred during the transition from Miocene to Pliocene until the middle Pleistocene (5.4–0.5 Mya). Two species, *P. diffusiflora* e *P. ephemera*, dispersed from the Atlantic Rainforest to the Amazon and northwestern South America about 2 Mya.

MORPHOLOGICAL CHARACTER RECONSTRUCTION

The morphological matrix had a consistency index of 0.18 and a retention index of 0.60, indicating high levels of homoplasy. Only three of the 14 characters correspond to synapomorphies for the ten infrageneric clades (Figure S3, Table S2). These are the presence of whitish tubular sheaths at the ramicaul (character 3) for the clade formed by *P. lingua* (Lindl.) Luer to *P. rubrolineata* (Hoehne) Luer, and the petals with thickened apex (character 9) for the clade containing *P. fusca* Chiron & Xim.Bols to *P. villosisepala* L.Kollmann & Fraga. The latter clade, however, presents two reversion events to petals with no thickened apex (*P. calimanii* Toscano & Luer and *P. gracilicaulis* (Seehawer) Luer. A third synapomorphy is the presence of a ventral callus in the column (character 14) for a subclade of four species (*P. bradei* (Schltr.) Luer to *P. versicolor* (Porsch) Luer) within the largest clade of the genus.

Although the other selected characters do not constitute synapomorphies, some of them are not randomly distributed throughout the *Pabstiella* lineages. Thus, the sub-repent habit (character 1) is restricted to the first two branches, and the longer stem length related to the leaf (character 2) and the pseudo-lepanthiform sheaths of the stems (character 4) are restricted to more derived clades.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS

Based on nuclear and plastid genes, and with support of morphological evidence, this study confirms the monophyly of *Pabstiella*, and complements the analysis of Chiron *et al*. (2012), who for the first time attempted to understand the phylogenetic relationships among *Pabstiella* species. Our sampling includes about half of the species of the genus and represents most of the morphological variability within the genus, enabling the recognition of 10 clades, which are herein assigned sectional rank. Our infrageneric classification only accounts for the species used in the present analysis.

Although the relationships between some of the sections here proposed are still uncertain, we believe, based on morphological evidence, that the addition of more taxa in future research will not significantly affect the framework proposed here. We envisage an expansion of the sectional circumscriptions and possible establishment of new ones. We acknowledge that a more thorough study should also include a greater variety of molecular markers, together with an extensive morphological codification to establish homologies and diagnostic characters for those sections that were poorly sampled in this study.

Our analysis confirms the subtribal affinities proposed by Karremans (2016) except for *Phloeophila* Hoehne & Schtlr. In our analysis, *Phloeophila nummularia* and *P. pleurothallopsis* are not closely related, a result in line with their lack of morphological similarity. This genus has been treated as monophyletic (type species: *Phloeophila paulensis* Hoehne & Schltr. = *P. nummularia*) based only on nrITS (Chiron *et al*., 2016) or nrITS plus some samples sequenced for *matK* plastid gene (Karremans *et al*., 2016; Perez-Escobar *et al*., 2017). Although our sampling of this genus is reduced, our result thus challenges the previously assumed monophyly of this genus, which should further be evaluated with a more extensive taxonomic sampling.

ORIGIN OF THE SUBTRIBE PLEUROTHALLIDINAE

The origin of the Pleurothallidinae has been estimated around 19 Mya, in the early Miocene (Givinsh *et al*., 2016; Perez-Escobar *et al*., 2017), but the ancestral area remains in debate. Perez-Escobar *et al.* (2017) inferred the origin of the subtribe in Central America or the Antilles, with a posterior migration to the Northern Andes, considering the Caribbean *Dilomilis* affinity as the first lineage to diverge within the subtribe, following the circumscription proposed by Pridgeon *et al.* (2001, 2005). Nevertheless, the placement of this group within the subtribe, as stated by Karremans (2016), is merely interpretative. The same DNA evidence permits the placement of *Dilomilis* affinity as a sister subtribe, which in fact does not share the general morphology of the Pleurothallidinae and is bird-pollinated (Borba *et al*., 2011).

Therefore, the assumption that the ancestral lineages of the Pleurothallidinae are restricted to the Caribbean, as advocated by Perez-Escobar *et al.* (2017), is arguable. We believe that two facts might have influenced this inference. In their analysis, these authors did not include outgroups from other subtribes of the Epidendreae tribe such as

Laeliinae, Poneriinae, or Bletiinae. Moreover, when compared to other derived and well-sampled lineages of the Pleurothallidinae, few representatives of the early diverging lineages of the subtribe in its strict sense (*Octomeria* affinity), were sampled. Thus, only three species of the large genus *Octomeria* R.Br. (159 species), a single species of *Brachiodium* Lindl. (79 species), and no members of the Brazilian endemic *Sansonia* Chiron (2 species) were sampled.

Contrary to what Perez-Escobar *et al.* (2017) hypothesised, our results suggest the Atlantic Rainforest as the ancestral area of the subtribe Pleurothallidinae. Recently, two biogeographical analyses of other orchid groups, not related to the Pleurothallidinae, produced similar results. Smidt *et al.* (2018) hypothesised the Paraná Province (Atlantic Rainforest) as the ancestral area for the *Ornitocephalus* clade (Orchidaceae: Oncidiinae) around 10 Mya, and Salazar *et al.* (2018) for the Spiranthinae (Orchidoideae: Cranichideae), the latter study without a dating analysis. A more extensive biogeographical analysis, including representative species from all biomes and all affinities, focusing on the first diverging lineages of the subtribe, would be required to determine the putative origin of the subtribe.

ORIGIN AND BIOGEOGRAPHY OF *PABSTIELLA*

The reconstruction of the ancestral area of *Pabstiella* is uncertain as it is based on the present distribution of the main South American biomes. Currently, the Atlantic Rainforest is separated from the Andes and the Amazon by the Chaco and the Cerrado biomes (South American Dry Diagonal). Nevertheless, the biogeographic history of these regions reveals multiple expansions and contractions of the forests present in these biomes, related with partial inundations and drainages in South America during the mid-

Miocene to early Pliocene (Cabanne *et al.*, 2019; Kessous *et al.*, 2020, Peres *et al.*, 2020). Thereby, the past connections between the Atlantic Rainforest, the Amazon and the Andes, that occurred in different periods, and through different regions, have been inferred and recognised as fundamental for understanding the patterns of the Atlantic Rainforest biota (Kessous *et al.*, 2020, Peres *et al.*, 2020).

Pabstiella originated in the Miocene, around 7.93 Mya, and although its ancestral distribution cannot be inferred confidently, both the Andes and the Atlantic Rainforest are suggested as ancestral area, at an epoch where these two regions were probably connected (Cabanne *et al.*, 2019; Kessous *et al.*, 2020). Eventually, in the late Miocene to early Pliocene (5.27±9.46 Mya), a first vicariant event occurred between the Andean and Atlantic Rainforest lineages of the genus, probably related to the drainage of the Paranaean Sea, the contraction of the forests that connected these two biomes and the formation of the Dry Diagonal (Kessous *et al.*, 2020).

Thus, an early-diverging lineage (*Pabstiella* section *Mentosae* (Luer) Gutiérrez, Toscano & E.C.Smidt) diversified mainly in the Andes, with a posterior dispersion to the Amazon basin and Central America, and the remaining lineages established and diversified in the Atlantic Rainforest. As seen in Fig 2, the majority of the sections from this Atlantic lineage diverged during the Pleistocene and eventually diversified in the Pliocene. Several phylogeographic evidences in animals and plants support the idea that both climatic oscillations during the Pleistocene, and tectonic events in the Pliocene, shaped the diversity patterns in the Neotropics (Rull, 2020).

Similar biogeographic patterns were inferred in the bromeliad Tillandsioideae, where a main vicariant event divided the Tillandsieae plus Cipuropsidinae lineages in the Andes and the Vrieseinae in the Atlantic Rainforest. Pleistocene events were also probably responsible for the diversification of the most recent clades of the Vrieseinae subtribe in the Atlantic Rainforest (Kessous *et al.*, 2020).

As a result of these diversification events, *Pabstiella* became the genus of Pleurothallidinae with a major percentage of endemism for Brazil (more than 95%), surpassing by far the number of species present in the Atlantic Rainforest of the other two mainly Andean genera of the *Pleurothallis* affinity, *Pleurothallis* and *Stelis* (Karremans, 2016).

Recently, Peres *et al.* (2020) presented a comprehensive multi-taxon review of diversity patterns along the Atlantic Rainforest and indicated five main areas of endemism based on the distribution of biological communities: Pernambuco, Coastal Bahia, Central Bahia, Serra do Mar, and Paraná/Araucaria. A rapid assessment resulting from this study, based on the distribution of the nearly 125 species of *Pabstiella* in GBIF (www.gbif.org [10 June 2019]) that inhabits these areas, shows that richness and endemism are concentrated in the Serra do Mar (106 spp, 71 endemics), followed by Paraná/Araucária (40 spp, eight endemics) and Coastal Bahia (14 spp, seven endemics), three species are registered in the Pernambuco area, and no species are recorded in Central Bahia.

These numbers could be biased by the greater amount of data available from the Serra do Mar, particularly from the states of Sao Paulo and Rio de Janeiro, compared to other areas, nevertheless, the tendency is a highest richness and endemism of *Pabstiella* species in the southern and subtropical block of the Atlantic Rainforest (Serra do Mar and Paraná/Araucária), compared to the northern and tropical block (Central and Coastal Bahia, Pernambuco).

There is no clear relation between these patterns of richness and endemism with the current distribution of the *Pabstiella* sections herein proposed that allows a further discussion regarding a finer-scale biogeographic history. However, the highest levels of richness and endemism of the southern block of the Atlantic Rainforest may be related to the greater variety of vegetation types of this region compared to the northern block (Peres *et al.*, 2020).

Some species such as *Pabstiella ephemera* and *P. diffusiflora* (the latter usually confused with the Brazilian endemic *P. seriata*) that belong to a lineage which originated in the Atlantic Rainforest (Fig 3), dispersed outside this biome (Amazon, Guianas), indicating that not all derived clades remained endemic in the Atlantic Rainforest. Moreover, the existence of the Bolivian endemics *Pabstiella ignota* (Luer) Karremans and *P. glandulipetala* (Luer & R.Vásquez) Karremans suggests that another dispersal event, from the Atlantic Rainforest to the Andes, occurred, although there is no phylogenetic evidence to support this hypothesis.

CHARACTER EVOLUTION

In such a large and variable genus such as *Pabstiella*, it is expected that unrelated species may share morphological features. Selective pressures imposed by pollinators may derive in morphological convergence in floral characters (Karremans, 2016). This seems to be the case of the mentum, a chinlike protuberance formed by the base of the synsepal and the column foot in various *Pabstiella* species. Brieger & Senghas (1976) used the presence of a pronounced mentum or spur as a distinguishing feature for proposing the genus *Pabstiella*, and other authors (Pabst & Dungs, 1975 and Luer, 1986) also resorted to it for grouping species that share this unusual feature. Our results show, however, that this character is homoplasious, as it evolved independently in three sections within the genus. This convergence is remarkable in *Pabstiella calcarata* (Cogn.) Luer, *P. rupicola* L.Kollmann and *P. mirabilis* (Schltr.) Brieger & Senghas, three species that differ vegetatively, but share several floral features, such as the few-flowered inflorescences that usually surpass the leaves, a conspicuous spur, and a sub-entire, obcuneate lip, instead of a distinctively three-lobed lip with erect lateral lobes, as it usually occurs in *Pabstiella.* Such similar floral traits in unrelated sections suggest a possible floral specialisation (Tripp & Manos, 2008) that deserves attention for future pollination studies in the genus.

Furthermore, the presence of white, fragrant, simultaneous flowers in unrelated species like *P. carinifera* (Barb.Rodr.) Luer (Fig. 1M) and *P. leucopyramis* (Rchb.f.) Luer (Fig. 1F) also suggests an adaptation to a similar pollinator. We lack hypothesis regarding which pollinators could be responsible for such floral convergences since no pollination events have ever been documented in *Pabstiella*.

Characters related to the length and direction of the inflorescence (characters 5 and 6), the presence of calli (character 10), three-lobed lip (character 11) and minute lobules at the lip base (character 12) are homoplasious and uninformative when analysed separately. Nevertheless, we were able to define the following infrageneric groups within *Pabstiella* using combinations of homoplasious characters to support the phylogenetic results.

INFRAGENERIC CLASSIFICATION FOR *PABSTIELLA*

Pabstiella Brieger & Senghas, Orchidee (Hamburg) 27(5): 195 (1976).

Type: *Pleurothallis mirabilis* Schltr. [= *Pabstiella mirabilis* (Schltr.) Brieger & Senghas].

***Pabstiella* sect. *Mentosae* (Luer) Gutiérrez, Toscano & E.C.Smidt,** *comb. nov.*

Basionym: *Pleurothallis* subgen. *Specklinia* (Lindl.) Garay sect. *Mentosae* Luer., Monogr. Syst. Bot. Missouri Bot. Gard 20: 86. 1986. Type: *Pleurothallis mentosa* Cogn. [= *Pabstiella yauaperyensis* (Barb.Rodr.) F.Barros].

Synonyms*:*

Pleurothallis subgen. *Specklinia* (Lindl.) Garay sect. *Tripteranthae* Luer., Monogr. Syst. Bot. Missouri Bot. Gard 20: 94. 1986. Type: *Pleurothallis tripterantha* Rchb.f.

Anthereon Pridgeon & MW. Chase., Lindleyana 16: 252. 2001. Type: *Pleurothallis tripterantha* Rchb.f.

Ronaldella Luer., Monogr. Syst. Bot. Missouri Bot. Gard 105: 195. 2006. Type: *Pleurothallis determannii* Luer.

Species

Pabstiella aryter (Luer) F.Barros., Bradea. 8(43): 296. 2002.

Basionym: *Pleurothallis aryter* Luer., Selbyana 3: 54. 1976.

Pabstiella determannii (Luer) F.Barros., Bradea 8(43): 296. 2002.

Basionym: *Pleurothallis determannii* Luer., Selbyana 7: 117. 1982.

Pabstiella tripterantha (Rchb.f.) F.Barros., Bradea 8(43): 296. 2002.

Basionym: *Pleurothallis tripterantha* Rchb.f., Bonplandia 2: 24. 1854.

Pabstiella yauaperyensis (Barb.Rodr.) F.Barros., Bradea 8(43): 296. 2002.

Basionym: *Lepanthes yauaperyensis* Barb.Rodr., Vellosia 2(1): 117. 1891.

Pabstiella section *Mentosae* represents the early divergent clade within *Pabstiella* and consists of four species distributed across Central and South America, with two of them restricted to the Amazon basin. Except for one caespitose species, *P. yauaperiensis,* all others have a shortly creeping habit. The lip in these species has a pair of longitudinal calli on the disc, more or less in the middle. *Pabstiella aryter*, *P. determanii* (Fig. 1B), and *P. yauaperienesis* were formerly included in *Pleurothallis* section *Mentosae* (Luer, 1986). They all share several morphological features, such as the completely connate lateral sepals, the entire or sometimes obscurely three-lobed, obcuneate lip, and the longitudinally winged column with a cucullate apex. *Pabstiella tripterantha* (Fig. 1A), differs from all others vegetatively and florally. In this species, the flowers are often cleistogamous, which is unique in the genus, the lateral sepals are connate just above the middle, the lip is slightly three-lobed, the lateral lobes erect, and the column is dentate at the apex. Luer (1986) created a monotypic section in *Pleurothallis* to accommodate this species, *Pleurothallis* sect. *Tripteranthae.* In our analysis, *P. tripterantha* is sister to all others in this section (without phylogenetic support), which seems to corroborate the differences in vegetative and floral morphology.

***Pabstiella* sect. *Carinatae* Gutiérrez, Toscano & E.C.Smidt**, *sect. nov.*

Type: *Pleurothallis crenata* Lindl. [= *Pabstiella crenata* (Lindl.) Luer]

Etymology: The name derives from the Latin adjective, *carinatus*, "keeled," referring to the carinate sepals.

Plants caespitose or slightly repent; ramicauls covered by loose tubular sheaths; inflorescence exceeding the leaves; flowers successive, long-pedicellate; sepals dorsally carinate; petals spathulate; lip three-lobed.

Species

Pabstiella crenata (Lindl.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 119. 2007.

Basionym: *Pleurothallis crenata* Lindl., Gard. Chron 6: 207. 1846.

Pabstiella alligatorifera (Rchb. F.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 119. 2007.

Basionym: *Pleurothallis alligatorifera* Rchb.f., Hamburger Garten- Blumenzeitung 21: 299. 1865.

This currently small section consists of two species endemic to the Brazilian Atlantic Rainforest: *Pabstiella alligatorifera*, a caespitose species, and *Pabstiella crenata* (Fig. 1C), an obscurely creeping species. *Pabstiella crenata* was first described from Mexico, undoubtedly in error. Lindley received the specimen from Loddiges, in 1846, who maintained an extensive collection of Brazilian species. This error led Williams (1946) to include the name *Pleurothallis crenata* Lindl in the Flora of Panama, citing three Central American epithets in synonymy. The two species in this section are endemic to southeastern Brazil (Toscano de Brito & Luer, unpubl.), and share several features in common, among them the stout ramicaul covered by loose tubular sheaths, the long-pedicellate, successively flowered raceme far exceeding the leaves, relatively large flowers with sepals that are dorsally carinate along the mid-vein, the spathulate petals, and the three-lobed lip.

***Pabstiella* sect. *Linguiformes* Gutiérrez, Toscano & E.C.Smidt,** *sect. nov.*

Type: *Pleurothallis lingua* Lindl. [=*Pabstiella lingua* (Lindl.) Luer].

Etymology: The name derives from the Latin adjective *linguiformis*, "tongue-shaped", and alludes to the shape of the lip in several species of this section.

Plants caespitose, ramicauls enclosed by whitish tubular sheaths, inflorescence shorter than (rarely surpassing) the leaves, flowers successive, lip three-lobed in various degrees, oblong, usually linguiform.

Species

Pabstiella bowmanii (Rchb. F.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 119. 2007.

Basionym: *Pleurothallis bowmanni* Rchb.f., Gard. Chron 1869: 989.

Pabstiella calcarata (Cogn.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 119. 2007.

Basionym: *Pleurothallis calcarata* Cogn., Bull. Soc. Roy. Bot. Belgique 43: 311. 1907.

Pabstiella colorata (Pabst) Luer & Toscano., Harvard Pap. Bot 16(2): 381. 2011.

Basionym: *Pleurothallis colorata* Pabst., Arch. Jard. Bot. Rio de Janeiro 14: 8. 1956.

Pabstiella lingua (Lindl.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis lingua* Lindl., Edwards's Bot. Reg 28: 80. 1842.

Pabstiella pseudotrifida L.Kollmann & D.R.Couto., Candollea 69(1): 22. 2014.

Pabstiella rubrolineata (Hoehne) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis rubrolineata* Hoehne., Bol. Agric. (Sao Paulo) 34: 609. 1934.

Pabstiella trifida (Lindl.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis trifida* Lindl., Edwards's Bot. Reg 28(Misc.): 82. 1842.

The nine Brazilian endemics that comprise this section are recognised by the small, caespitose habit (Fig. 1E), ramicauls enclosed by pale tubular sheaths that become whitish when dried, linear-elliptic leaves, few successive flowers in racemes that rarely surpass the leaves (e.g. in some populations of *P. calcarata*), and the slightly or distinctly three-lobed, and usually linguiform lip. The group of *P. colorata* to *P. rubrolineata* has a marked 3-dentate column apex. Vegetatively, species in this section are very similar to species in *Pabstiella* section *Brachystachyae* Gutiérrez, Toscano & E.C.Smidt, which is newly proposed below, but in the specimens investigated, they differ in having whitish, instead of brownish sheaths of ramicauls.

Pabstiella **sect.** ***Pyramidales*** **Gutiérrez, Toscano & E.C. Smidt,** *sect. nov.*

Type: *Pleurothallis leucopyramis* Rchb.f., [= *Pabstiella leucopyramis* (Rchb.f.) Luer.].

Etymology: The section name derives from the Latin *pyramidalis*, "pyramid-shaped," in reference to the flowers of the type species, *P. leucopyramis*.

Plants caespitose, ramicauls enclosed by tubular sheaths, inflorescence surpassing the leaves, sepals carinate, margins of the dorsal sepal slightly recurved toward the apex.

Species

Pabstiella fragae (L.Kollmann & A.P.Fontana) L.Kollmann., Candollea 65(1): 96. 2010.

Basionym: *Specklinia fragae* L.Kollmann & A.P.Fontana., Bol. Mus. Biol. Prof. Mello-Leitão 23: 16. 2008.

Pabstiella leucopyramis (Rchb.f.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis leucopyramis* Rchb.f., Linnaea 41: 47. 1876.

The two species that comprise this section possess ramicauls enclosed by tubular, acute, paleaceous sheaths, that become brownish when dried. In *P. leucopyramis* the sheaths are trumpet-like, with thick, dilated ostia, and are often deciduous. The leaves of the two species are elliptic, the inflorescence is erect and surpasses the leaves, the sepals are carinate abaxially, the margins of the dorsal sepal are slightly recurved on the apical third.

***Pabstiella* sect. *Brevicaules* Gutiérrez, Toscano & E.C.Smidt,** *sect. nov.*

Type: *Pleurothallis ezechiasi* Hoehne. [= *Pabstiella ezechiasi* (Hoehne) Luer].

Etymology: From the Latin brevicaulis, "with short stems" referring to the short ramicauls of members of this section.

Plant caespitose, ramicauls shorter than the leaves, inflorescence elongate, flexuous, surpassing the leaves, flowers successive, sepals pubescent on the apical third, lip with a pair of lobules at the corners.

Species

Pabstiella ezechiasi (Hoehne) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis ezechiasi* Hoehne., Arq. Bot. Estado São Paulo 2: 21. 1946.

Pabstiella muricatifolia Fraga & L.Kollmann., Harvard Pap. Bot 15: 174. 2010.

Pabstiella section *Brevicaules* consists of two species that are recognised by the shorter ramicauls related to the leaves and enclosed by tubular, paleaceous sheaths. The leaves are obovate to oblong, and the inflorescence is erect, subflexuous, and exceeds the leaves (Fig. 1H). The flowers are successive, the sepals pubescent on the apical third, the dorsal sepal convex with the apical margins recurved, the petals slightly asymmetrical and acute, and the lip is three-lobed, oblong, truncate at base with a pair of lobules at the corners; the disc is provided with a pair of longitudinal calli above the middle, and the apex of the lip is densely papillose.

***Pabstiella* sect. *Brachystachyae* Gutiérrez, Toscano & E.C.Smidt,** *sect. nov.*

Type: *Pleurothallis uniflora* Lindl. [= *Pabstiella uniflora* (Lindl.) Luer].

Etymology: The name derives from the Greek *Brachystachyum*, the words *brachys* meaning "short" and *stachys* meaning "a spike," and refers to the inflorescences, which are shorter than the leaves in most species.

Plants caespitose, leaves narrowly elliptic, inflorescence shorter than the leaves, flowers successive, sepals glabrous, petals asymmetrical, acute, lip three-lobed.

Species

Pabstiella conceicionensis Chiron & N.Sanson., Richardiana 12(2): 93. 2012.

Pabstiella lineolata (Barb.Rodr.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Lepanthes lineolata* Barb.Rodr., Gen. Sp. Orchid 2: 53. 1882.

Pabstiella* aff. *ramphastorhyncha (Barb.Rodr.) L.Kollmann., Candollea 65(1): 96. 2010.

Pabstiella* aff. *uniflora (Lindl.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Pabstiella section *Brachystachyae* is very homogeneous, both florally and vegetatively. The slender ramicauls bear elliptic-linear leaves, and the inflorescences, with successive small flowers, are usually shorter than the leaves (Fig. 1G). The sepals are glabrous and the petals are slightly asymmetrical, obovate, acute to acuminate. The lip is three-lobed, often papillose and usually without callosities. This section constitutes one of the youngest groups within *Pabstiella*.

***Pabstiella* sect. *Ornithoides* Gutiérrez, Toscano & E.C.Smidt,** *sect. nov.*

Type: *Pleurothallis ephemera* Lindl. [= *Pabstiella ephemera* (Lindl.) Luer.].

Etymology: From the Latin adjective of Greek origin *ornithoides*, "resembling a bird," referring to the beak-like appearance of the flowers of the members of this section.

Plants caespitose, inflorescence few-flowered, usually shorter than the leaves, flowers successive with thickly tri-alate ovary and thick, carinate sepals, petals obovate, asymmetrical, acuminate, the lip three-lobed, callose in the middle.

Species

Pabstiella crassicaulis (Barb.Rodr.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Lepanthes crassicaulis* Barb.Rodr., Gen. Spec. Orchid 2: 60. 1881.

Pabstiella diffusiflora Luer & Toscano., Harvard Pap. Bot 16: 381. 2011.

Basionym: *Pleurothallis diffusiflora* C.Schweinf., Bot. Mus. Leafl 3: 80. 1935.

Pabstiella decurva Luer & Toscano., Harvard Pap. Bot 16: 371. 2011.

Pabstiella ephemera (Lindl.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis ephemera* Lindl., Companion Bot. Mag 2: 356. 1837.

Pabstiella quasi Luer & Toscano., Harvard Pap. Bot 16(2): 377. 2011.

Pabstiella rhombilabia Chiron & N.Sanson., Richardiana 11(3): 134. 2011.

This section comprises six species, three of them, *P. crassicaulis*, *P. ephemera*, *P. quasi* are distinct and can be recognised by the appearance of their flowers, which resemble the beak of a bird (Fig. 1I). The stout ramicauls are enclosed by two to three tubular sheaths; the leaves are coriaceous; the inflorescences are short and few-flowered; the lateral sepals are fully connate and the lip is channelled above the middle between a pair of parallel calli.

The other three species do not share the diagnostic characters previously mentioned, but the phylogenetic evidence allows the placement of these species within this section. Further studies must sample similar species to define a better morphological delimitation for this group of species.

***Pabstiella* sect. *Longicaules* Gutiérrez, Toscano & E.C. Smidt,** *sect. nov*

Type: *Pleurothallis piraquarensis* Hoehne. [= *Pabstiella piraquarensis* (Hoehne) Luer].

Plants caespitose; ramicauls longer than the leaves; inflorescence several-flowered; sepals pubescent, lip three-lobed, with minute lobules at the base, callose more or less in the middle, and papillose at the apex.

Species

Pabstiella piraquarensis (Hoehne) Luer., Novon 18(1): 79. 2008.

Basionym: *Pleurothallis piraquarensis* Hoehne, Arq. Bot. Estado São Paulo 2: 123. 1952.

Pabstiella wacketii (Handro & Pabst) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis wacketii* Handro & Pabst., Bradea 1(7): 45. 1971.

This section can be distinguished from the rest of the genus by the ramicauls that are proportionally much longer than the elliptic leaves (Fig. 1J), the inflorescence of successive flowers that may or may not exceed the leaves, the sepals are pubescent, usually recurved at apex, the three-lobed lip, with a pair of obscure lobules at the corners, possesses two elongate, low callosities on the disc, and is papillose on the apical third.

***Pabstiella* sect. *Effusae* (Lindl.) Gutiérrez, Toscano & E.C.Smidt,** *comb. nov.*

Type: *Pleurothallis hypnicola* Lindl. [= *Pabstiella fusca* (Lindl.) Chiron & Xim.Bols.].

Basionym: *Pleurothallis* subgen. *Specklinia* sect. *Effusae* Lindl., Edwards's Bot. Reg. 28: misc.75.1842. Lectotype selected by Luer (1986: 81): *Pleurothallis hypnicola* Lindl.

Species

Pabstiella arcuata (Lindl.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis arcuata* Lindl., Fol. Orchid 9: 34. 1859.

Pabstiella fusca (Lindl.) Chiron & Xim.Bols., Richardiana 10(2): 56. 2010.

Basionym: *Pleurothallis fusca* Lindl., Companion Bot. Mag 2: 354. 1837.

Pabstiella hians (Lindl.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis hians* Lindl., Edwards's Bot. Reg 21: t. 1797. 1835.

Pabstiella calimanii Toscano & Luer., Lankesteriana 16(2): 154. 2016.

Pabstiella lueriana Fraga & L.Kollmann., Harvard Pap. Bot 15(1): 172. 2010.

Pabstiella nymphalis Luer & Toscano., Harvard Pap. Bot 16(2): 376. 2011.

Pabstiella silvanae Chiron & Xim.Bols., Richardiana 13: 117. 2012.

Pabstiella fasciata (Seehawer) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis fasciata* Seehawer., Orchidee (Hamburg) 56(4): 458. 2005.

Pabstiella gracilicaulis (Seehawer) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis gracilicaulis* Seehawer., Orchidee (Hamburg) 56(4): 461. 2005.

Pabstiella villosisepala L.Kollmann & Fraga., Harvard Pap. Bot 15(1): 176. 2010.

Pabstiella eunapolitana C.R.M Silva & S.L.X. Tobias., Colet. Orquídeas Brasil 15: 614. 2019

This section is one of the largest of the genus, and it has been already recognised as a morphological group by Chiron *et al*. (2010). All species so far analysed are endemic to the Brazilian Atlantic Rainforest. Luer (2000) recorded *Pabstiella arcuata* for Venezuela based on the type of *Pleurothallis rhombipetala* Rolfe, a synonym. The specimen which served as the basis for Rolfe's original description (Rolfe, 1893) came from cultivated material and its Venezuelan origin is probably an error. The species in this section can be distinguished by their shorter ramicauls related to the leaves, flexuous racemes that usually surpass the leaves, and successive flowers (Fig. 1K, L). The dorsal sepal is concave at the base and convex at the apex, the connate lateral sepals form a synsepal which is concave below the middle, with subacute apices, and both dorsal and lateral sepals are adaxially pubescent in various degrees. The petals are rhomboid, asymmetrical, and thickened at the apex, the three-lobed lip has a truncate base with two minute lobules at the corners, and two slightly verrucose or glandular-cellular, parallel, thick calli on the disc.

Pabstiella sect. *Pabstiella*

Type: *Pleurothallis mirabilis* Schltr. [= *Pabstiella mirabilis* (Schltr.) Brieger & Senghas].

Synonym*:* *Pleurothallis* subgen. *Mirabilia* Luer., Monogr. Syst. Bot. Missouri Bot. Gard 20. 47. 1986. Type: *Pleurothallis mirabilis* Schltr.

Species

Pabstiella bicolor (Barb. Rodr.) Luer & Toscano., Harvard Pap. Bot 16(2): 380. 2011.

Basionym: *Lepanthes bicolor* Barb.Rodr., Revista Engen 3(7): 110. 1881.

Pabstiella bradei (Schltr.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis bradei* Schltr., Anexos Mem. Inst. Butantan, Secc. Bot 1(4): 41. 1922.

Pabstiella carinifera (Barb.Rodr.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Lepanthes carinifera* Barb.Rodr., Revista Engen 3(8): 110. 1881.

Pabstiella ghillanyi (Pabst) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis ghillanyi* Pabst., Anais 15 Congr. Soc. Bot. Brasil 111. 1967.

Pabstiella juquitibensis Campacci & S.L.X.Tobias., Colet. Orquídeas Brasil 15: 622. 2019

Pabstiella matinhensis (Hoehne) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis matinhensis* Hoehne, Arq. Bot. Estado Sao Paulo 1: 13. 1938.

Pabstiella mirabilis (Schltr.) Brieger & Senghas., Orchidee (Hamburg) 27: 195. 1976.

Basionym: *Pleurothallis mirabilis* Schltr., Notizbl. Bot. Gart. Berlin-Dahlem 7: 274. 1918.

Pabstiella miragliae (Leite) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis miragliae* Leite., Arq. Bot. Estado São Paulo 2: 93. 1947.

Pabstiella pristeoglossa (Rchb.f. & Warm.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis pristeoglossa* Rchb.f. & Warm., Otia Bot. Hamburg 2: 92. 1881.

Pabstiella punctatifolia **(**Barb.Rodr.) Chiron, Phytotaxa 46: 55. 2012.

Basionym: *Lepanthes punctatifolia* Barb.Rodr., Gen. Spec. Orchid 2: 55. 1881.

Pabstiella purpurea (Seehawer) Luer & Toscano., Harvard Pap. Bot 16: 381. 2011.

Basionym: *Trichosalpinx purpurea* Seehawer., Orchidee (Hamburg) 49: 128. 1998.

Pabstiella recurviloba Toscano & Luer., *Lankesteriana* 16(2): 158. 2016.

Pabstiella rupicola L.Kollmann., Candollea 65: 96. 2010.

Pabstiella ruschii (Hoehne) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym*: Pleurothallis ruschii* Hoehne., Arq. Bot. Estado São Paulo 1: 44. 1939.

Pabstiella sordida (Kraenzl.) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis sordida* Kraenzl., Ark. Bot 16(8): 8. 1921.

Pabstiella varellae Toscano, Luer & J. Klein., Harvard Pap. Bot 18: 251. 2013.

Pabstiella verboonenii (Luer & Toscano) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis verboonenii* Luer & Toscano., Selbyana 23: 194. 2002.

Pabstiella versicolor (Porsch) Luer., Monogr. Syst. Bot. Missouri Bot. Gard 112: 120. 2007.

Basionym: *Pleurothallis versicolor* Porsch., Oesterr. Bot. Z 55: 155. 1905.

This large section is very variable in floral morphology, but comparatively more stable vegetatively. It is characterised by small plants with slender ramicauls that are usually longer than the leaves and enclosed by closely appressed, usually microscopically pubescent, tubular sheaths. In several members of this group, the sheaths are trumpet-like with the apex thickened and dilated, and the margins of the ostia microscopically scabrous or pubescent, resembling the sheaths of the ramicauls in the genus *Lepanthes* SW., and has been termed "pseudo-lepanthiform" (Toscano de Brito & Luer, 2013). In other representatives of this section, the sheaths are glabrous and lack a pronounced thickly margined ostia, such as in *P. ghillanyi, P. juquitibensis, P. pristeoglossa,* and *P. rupicola*. In most of the species, the inflorescence is fractiflex and well exceeds the leaves, but in others they are only slightly flexuous, and in some cases shorter than the leaves as in some individuals of *P. punctatifolia* and *P. versicolor*. The flowers are successive in most species. However, in *P. carinifera, P. ghillanyi, P. matinhensis*, and *P. rupicola*, the flowers open simultaneously. Despite all this morphological variability, some subclades within this section seem to share similar vegetative and floral traits. For example, according to our molecular analysis, *P. punctatifolia, P. purpurea* (Fig. 1O)*, P. recurviloba* and *P. verboonenii* are closely related. They all share purple-spotted leaves and a deeply hollow apex of the dorsal sepal. These features have evolved in other members of subtribe Pleurothallidinae. For example, purple-spotted leaves are present in other *Pabstiella* species and other genera (e.g. *Acianthera*), and a hollow apex of the dorsal sepal is also found in *Specklinia digitale* (Luer) Pridgeon & MW.Chase and *Muscarella segregatifolia* (Ames & C.Schweinf.) Karremans. Their simultaneous presence in these species, however, seems to be phylogenetically correlated.

Another group of closely related species is the subclade comprised of *P. bicolor, P. bradei, P. sordida,* and *P. versicolor.* They all share the presence of a pyramidal or tooth-like callosity on the abaxial surface of the column, which seems to be a pronounced, thick stigmatic margin.

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Authors' contributions

NG, ECS, and ALVTB designed research and collected samples; NG and AVSRM performed the laboratory work; ECS, AVSRM and NG performed all analyses; NG, ALVTB, ECS and AVSRM wrote the manuscript.

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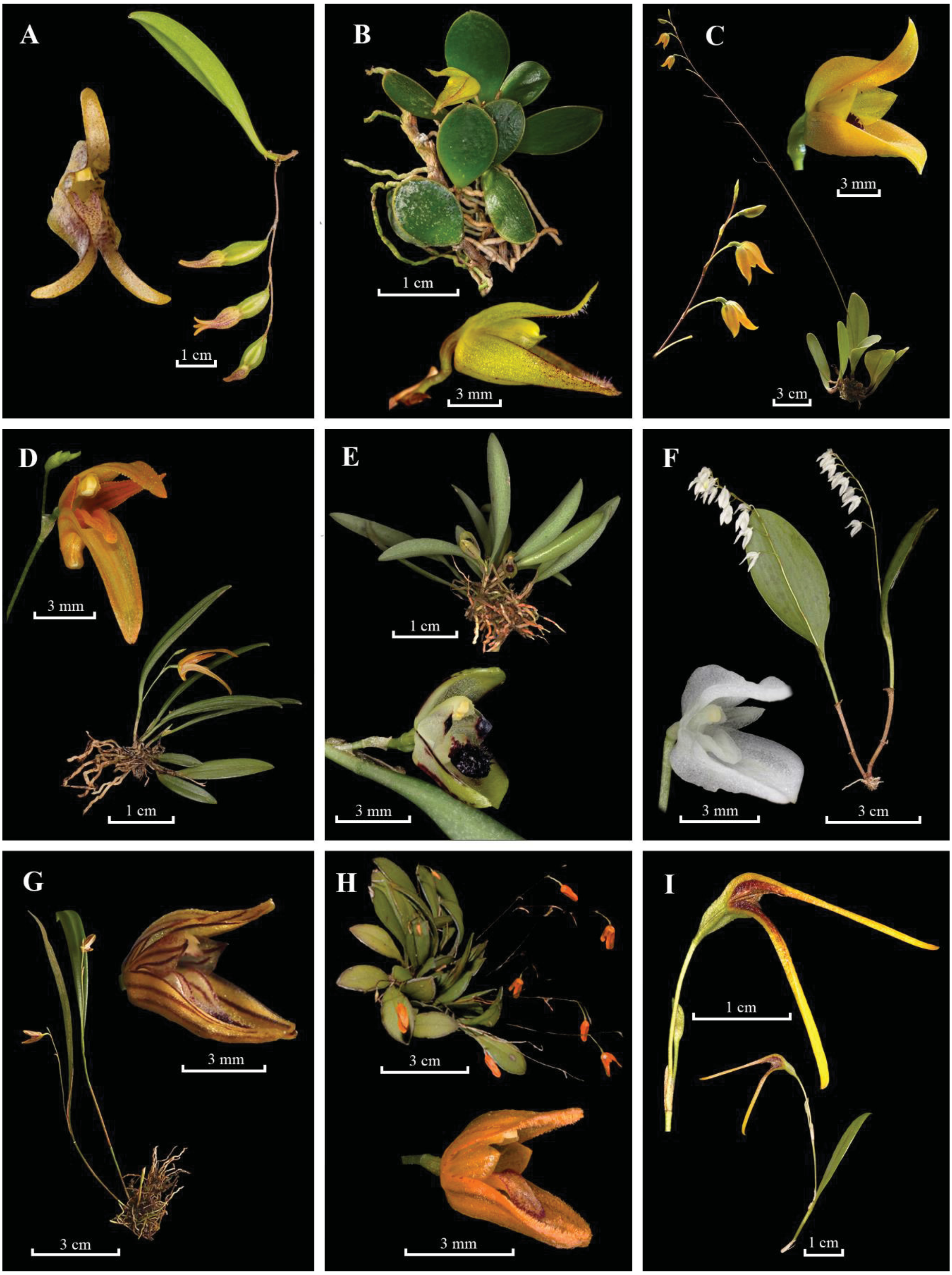
Figure legends:

Figure 1. Representative species of each section of *Pabstiella*. A, *Pabstiella tripterantha*. B, *Pabstiella determannii*. C, *Pabstiella crenata*. D, *Pabstiella calcarata*. E, *Pabstiella bowmanii*. F, *Pabstiella leucopyramis*. G, *Pabstiella* aff. *rhamphastoryncha*. H, *Pabstiella ezechiasi*. I, *Pabstiella ephemera*. J. *Pabstiella piraquarensis*. K, *Pabstiella* aff. *hians*. L, *Pabstiella eunapolitana*. M, *Pabstiella carinifera*. N. *Pabstiella mirabilis*. O, *Pabstiella pupurea*. Photographs by W. Collier, A.L.V Toscano de Brito & N. Gutiérrez-Morales, except for J (habit), made by C.R.M Silva.

Figure 2. Maximum-likelihood tree of *Pabstiella* plus outgroups. Numbers on nodes represent ML bootstrap percentages, posterior probability from Bayesian inference and maximum parsimony bootstrap percentages (≥ 50). See the Supporting Information for trees with support resulting from all three analyses separately. In detail, the same ML tree with branch lengths. Sections proposed for *Pabstiella* are indicated.

Figure 3. Time calibrated tree for *Pabstiella* plus outgroups inferred under uncorrelated lognormal relaxed clock model in BEAST with biogeographic inference. Bar at nodes indicates 95% highest posterior density intervals. Coloured circles in tip nodes represent the actual occurrence of that species in the delimited geographical areas depicted on the map of the Neotropics. Coloured circles at nodes indicate the most likely geographic distribution of the hypothetical ancestor, according to the BioGeoBEARS analysis. Colour code follows the legend on the map.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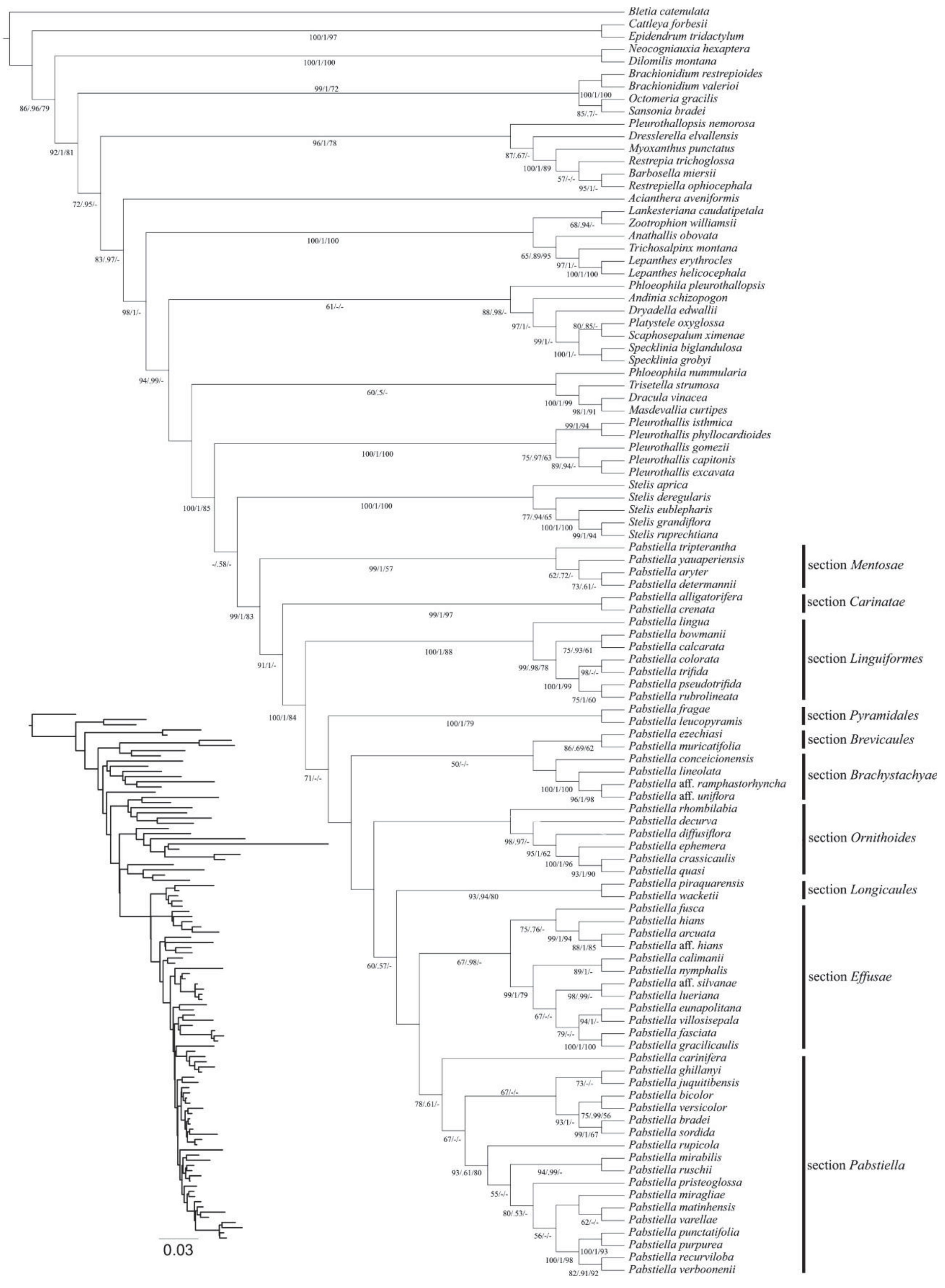

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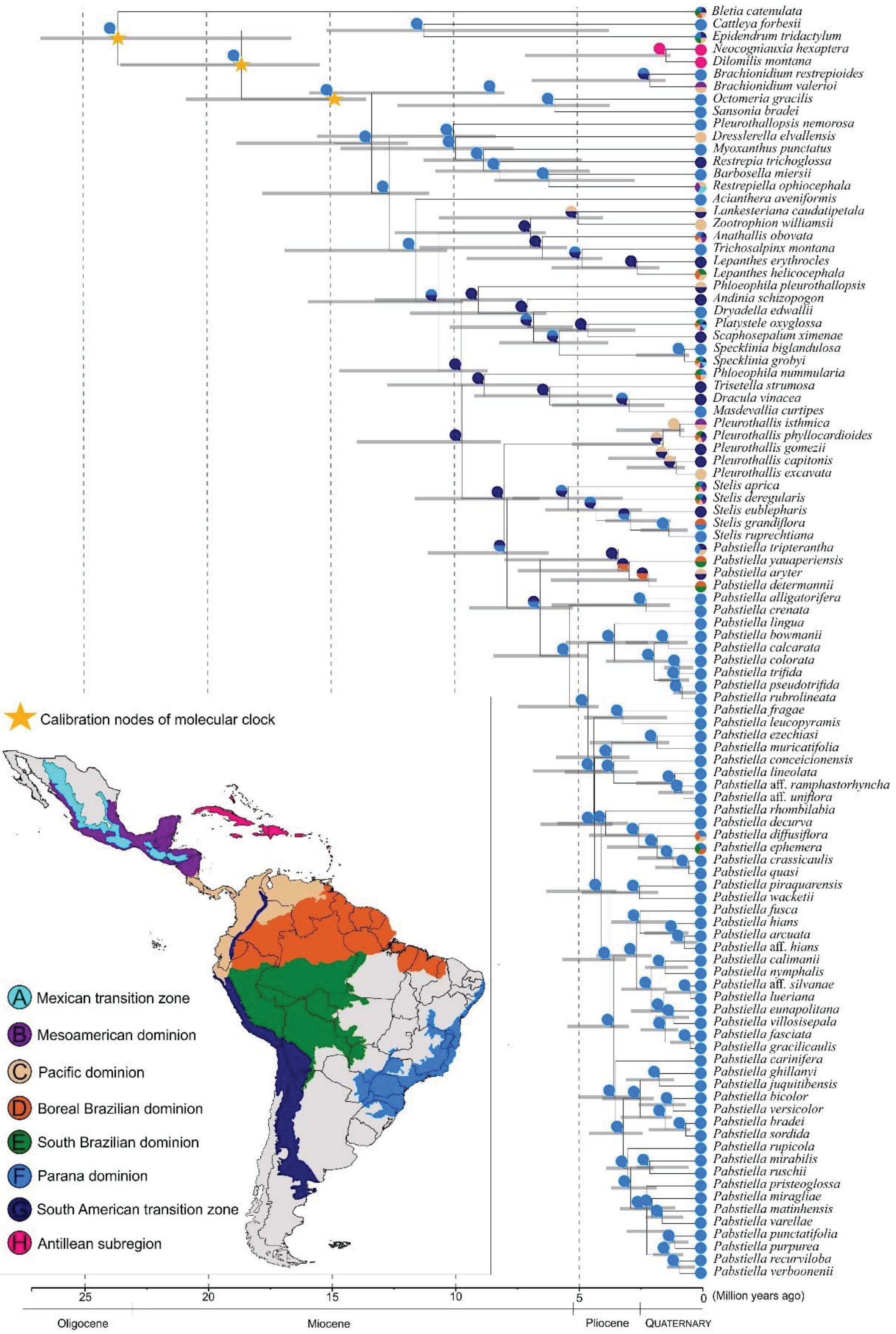

Figure 3. Time calibrated tree for *Pabstiella* plus outgroups inferred under uncorrelated lognormal clock model in BEAST with biogeographic inference. Bar at nodes indicate 95% highest posterior density intervals. Coloured circles represent the occurrence of that species in the delimited geographical areas depicted on the map of the Neotropics. Coloured circles at nodes indicate the most likely geographic distribution of the hypothetical ancestor, according to the BioGeoBEARS analysis. Colour code follows the legend on the map.

Phylogeny of *Pabstiella* (Pleurothallidinae: Orchidaceae)

NICOLÁS GUTIÉRREZ, ANTONIO L.V. TOSCANO DE BRITO, ANNA V. S. R. MAUAD, ERIC C. SMIDT

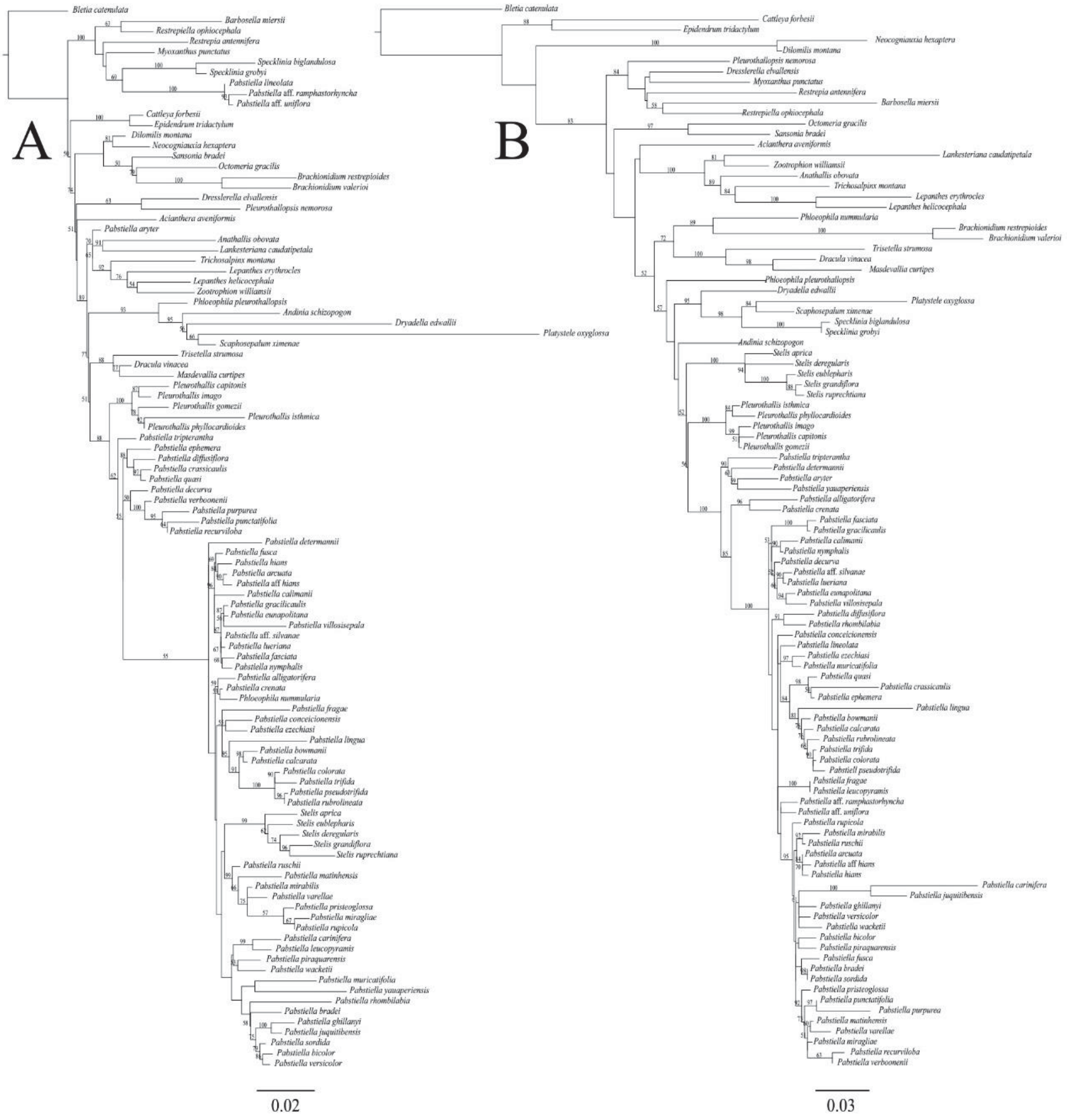

Figure S1. Maximum-likelihood tree resulting from the analysis of cpDNA (*matK*+*trnH-psbA*) (A) and nrITS (B) for *Pabstiella* plus outgroups. Numbers at nodes are bootstrap percentages. Values >50 are not shown.

Phylogeny of *Pabstiella* (Pleurothallidinae: Orchidaceae)

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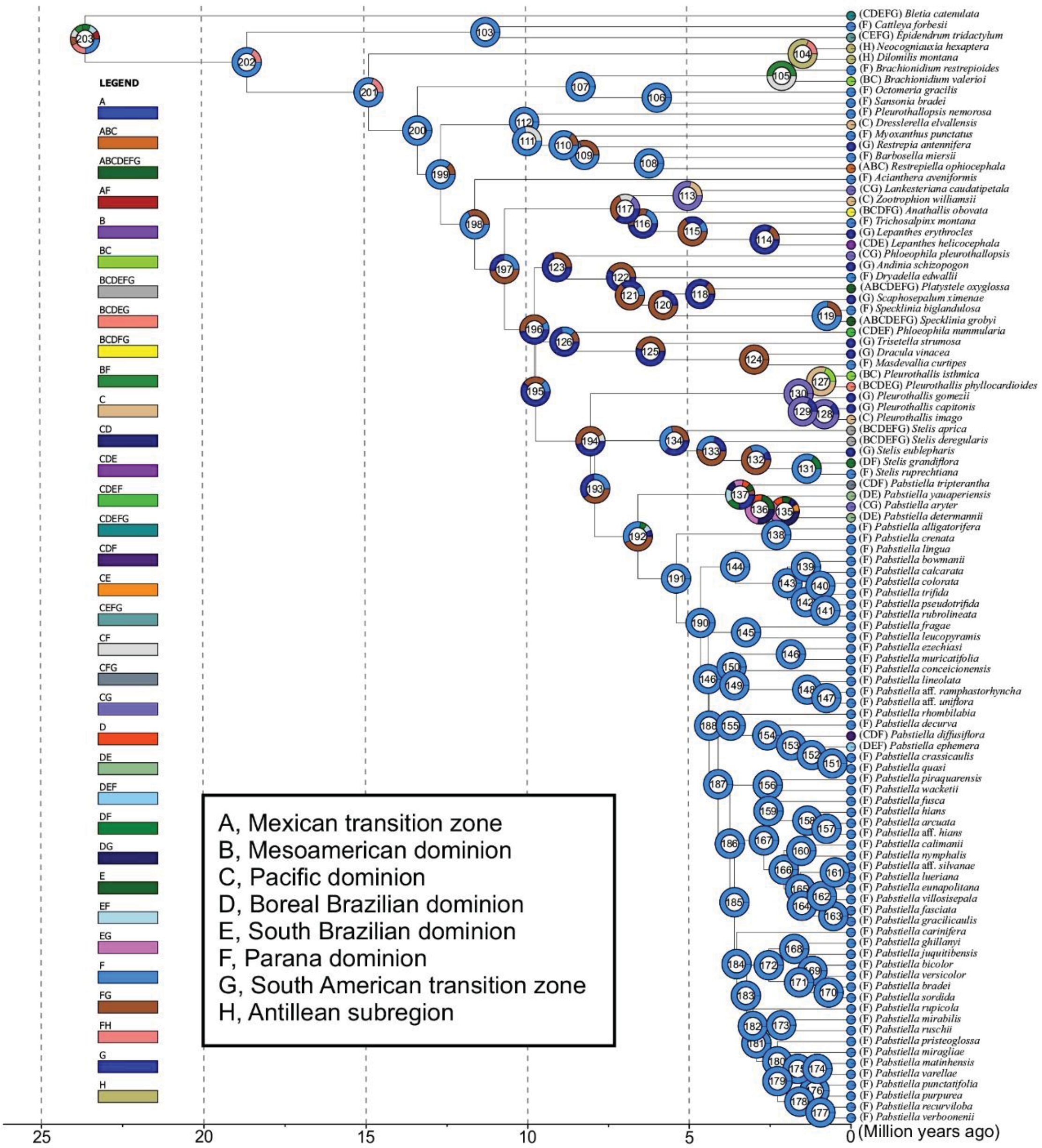

Figure S2. Biogeographic reconstruction under DEC model for the *Pabstiella* plus outgroups based on Bayesian maximum clade credibility tree. Nodes are numbered and colored according to RASP results. Node informations are available at Table S6. Delimitation and nomenclature of biogeographical regions followed Morrone (2014).

Phylogeny of *Pabstiella* (Pleurothallidinae: Orchidaceae)

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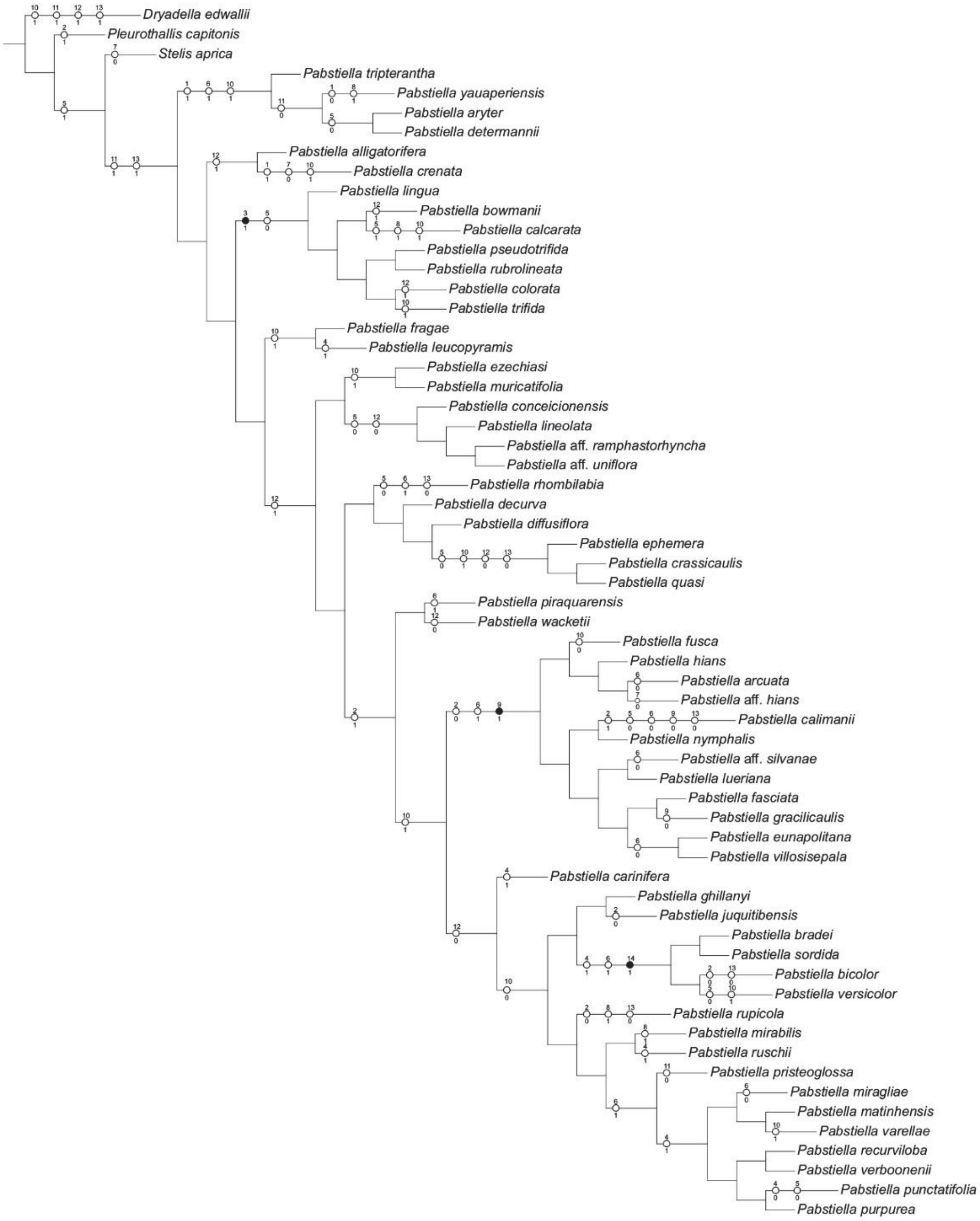

Figure S3. Historical character reconstruction with ACCTRAN optimization calculated in Winclada, based on molecular ML tree. Black circles indicate character states present only in the given clade (hypothetical synapomorphies); white circles indicate homoplasic character states. Numbers above circles refers to characters number, and those below circles are character states of absence (0) or presence (1).

Table S1. List of sampled species with voucher information, GenBank accession number for each molecular marker used and the Province used in the biogeographical analyses. Access numbers marked with an asterisk (*) refer to sequences extracted from the Genbank-NCBI database. Biogeographical Provinces follow Morrone (2014): A, Mexican Transition Zone; B, Mesoamerica; C, Pacific; D, northern Brazilian; E, southern Brazilian; F, Paraná (Atlantic Forest) and G, South American Transition Zone, H, Anthillean Zone.

Species	**Voucher**	**nrITS**	**matK**	**trnH-psbA**	**Distribution**
Acianthera aveniformis (Hoehne) C.N. Gonç. & Waechter	W.S. Mancinelli 1274 (UPCB)	MN551414	MN577721	MN577791	F
Anathallis obovata (Lindl.) Pridgeon & M.W. Chase	A.L.V. Toscano de Brito 3240 (SEL)	MN332358	MN332534	MN331597	BCDFG
Andinia shizopogon (Luer) Pridgeon & M. W. Chase	D.C. Imig 610 (UPCB)	MN551415	MN577722	MN577792	G
Barbosella miersii Schltr	M. Bolson 498 (UPCB)	KX686535*	MN332548	MN331612	F
Bletia catenulata Ruiz & Pav	A.V.S.R. Mauad 9 (UPCB)	MN332374	MN332549	MN331613	CDEFG
Brachionidium restrepioides (Hoehne) Pabst	W.S. Mancinelli 1249 (UPCB)	MN332375	KX686526*	MN331614	F
Brachionidium valerioi Ames & C. Schweinf	M.W. Chase 1459 (K)	AF262913*	AF265488*	---	BC
Cattleya forbesii Lindl	E.C. Smidt 967 (UPCB)	MN332376	MN332552	MN331616	F
Dilomilis montana (Sw.) Sumemrh	M.W. Chase 206 (K)	AF262915*	AF263765*	---	H
Dracula vinacea Luer & Escobar	N. Gutierrez 40 (JBB)	MN551416	MN577723	MN577793	G
Dresslerella elvallensis Luer	M.W. Chase 1279 (K)	AF262901*	AF265477*	---	C
Dryadella edwallii (Cogn.) Luer	E.C. Smidt 1011 (UPCB)	MN332379	MN332555	MN331619	F
Epidendrum tridactylum Lindl	G.F. Gonçalves 49 (SP)	MN332382	MN332558	MN331622	CEFG
Lankesteriana caudatipetala (C. Schweinf.) Karremans	A.L.V. Toscano de Brito 3277 (UPCB)	MN332384	MN332559	MN331624	CG

Lepanthes erythrocles Luer & Escobar	N. Gutierrez 9 (JBB)	MN551417	MN577724	MN577794	G
Lepanthes helicocephala Rchb.f	M.R. Cabral5 (UPCB)	MN332387	MN332560	MN331627	CDE
Masdevallia curtipes Barb. Rodr	A.L.V. Toscano de Brito 3475 (UPCB)	MN551418	MN577725	MN577795	F
Myoxanthus punctatus (Barb. Rodr.) Luer	A.L.V. Toscano de Brito 2871 (UPCB)	KX686538*	MK642626	MN331630	F
Neocogniauxia hexaptera Schltr	C244 (K)	AF260148*	AF263766*	---	H
Octomeria gracilis Lodd. ex Lindl	E.C. Smidt 947 (UPCB)	MN332392	KX686527*	MN331631	F
Pabstiella alligatorifera (Rchb. F.) Luer	A.L.V. Toscano de Brito 2742 (UPCB)	MN551422	MN577729	MN577799	F
Pabstiella arcuata (Lindl.) Luer	E.C. Smidt 985 (UPCB)	MN551423	MN577730	MN577800	F
Pabstiella aryter (Luer) F. Barros	D. Bogarín 6501 (JBL)	JF934816*	JF934876*	---	CG
Pabstiella bicolor (Barb. Rodr.) Luer & Toscano	A.L.V. Toscano de Brito 3099 (UPCB), nrITS and trnH-psbA / A.L.V. Toscano de Brito 2900 (UPCB), matK	MN551424	MN577731	MN577801	F
Pabstiella bowmanii (Rchb. F.) Luer	A.L.V. Toscano de Brito 3076 (UPCB)	MN551425	MN577732	MN577802	F
Pabstiella bradei (Schltr.) Luer	W.S. Mancinelli 1177 (UPCB)	MN551426	MN577733	MN577803	F
Pabstiella calcarata (Cogn.) Luer	W.S. Mancinelli 1338 (UPCB)	MN551427	MN577734	MN577804	F
Pabstiella calimanii Toscano & Luer	A.L.V. Toscano de Brito 3073 (UPCB)	MN551428	MN577735	MN577805	F
Pabstiella carinifera (Barb. Rodr.) Luer	M. Bolson 600 (UPCB)	MN551429	MN577736	MN577806	F
Pabstiella colorata (Pabst) Luer & Toscano	M. Rodrigues 61 (UPCB)	MN551431	MN577738	MN577808	F
Pabstiella conceicionensis Chiron & N. Sanson	C. Van den Berg 2128 (HUEFS), nrITS / C.R.M Silva 11 (UPCB), matK and trnH-psbA	JQ306499*	MN577739	MN577809	F
Pabstiella crassicaulis (Barb.Rodr.) Luer	S.L.X. Tobias 22 (UPCB)	MN551432	MN577740	MN577810	F
Pabstiella crenata (Lindl.) Luer	A.L.V. Toscano de Brito 2877 (UPCB)	MN551433	MN577741	MN577811	F
Pabstiella decurva Luer & Toscano	A.L.V. Toscano de Brito 3072 (UPCB)	MN551434	MN577742	MN577812	F
Pabstiella determannii (Luer) F. Barros	A.L.V. Toscano de Brito 3139 (SEL)	MN551435	MN577743	MN577813	DE

Pabstiella diffusiflora (C.Schweinf.) Luer & Toscano	S.L.X. Tobias 15 (UPCB)	MN551436	MN577744	MN577814	CDF
Pabstiella ephemera (Lindl.) Luer	A.L.V. Toscano de Brito 2867 (UPCB)	MN551437	MN577745	MN577815	DEF
Pabstiella eunapolitana C.R.M Silva & S.L.X.Tobias	C.R.M Silva 6 (UPCB)	MN551438	MN577746	MN577816	F
Pabstiella ezechiasi (Hoehne) Luer	A.L.V. Toscano de Brito 3766 (UPCB)	MN551439	MN577747	MN577817	F
Pabstiella fasciata (Seehawer) Luer	F.J. de Jesus 4 (UPCB)	MN551440	MN577748	MN577818	F
Pabstiella fragae (L. Kollmann & A.P. Fontana) L. Kollmann	A.L.V. Toscano de Brito 3372 (UPCB)	MN551441	MN577749	MN577819	F
Pabstiella fusca (Lindl.) Chiron & Xim. Bols	E.C. Smidt 986 (UPBC), nrITS and matK / J. Klein. 157 (UPCB), trnH-psbA	MN551442	MN577750	MN577820	F
Pabstiella ghillanyi (Pabst) Luer	F.J. de Jesus 7 (UPBC)	MN551443	MN577751	MN577821	F
Pabstiella gracilicaulis (Seehawer) Luer	A.L.V. Toscano de Brito 2901 (UPCB)	MN551444	MN577752	MN577822	F
Pabstiella hians (Lindl.) Luer	A.L.V. Toscano de Brito 2868 (UPBC), nrITS and matK / E.C. Smidt 935 (UPCB), trnH-psbA	MN551445	MN577753	MN577823	F
Pabstiella aff. hians (Lindl.) Luer	N. Gutierrez 96 (UPCB)	MN551430	MN577737	MN577807	F
Pabstiella juquitibensis Campacci & S.L.X.Tobias	S.L.X. Tobias 24 (UPCB)	MN551446	MN577754	MN577824	F
Pabstiella leucopyramis (Rchb. f.) Luer	A.L.V. Toscano de Brito 3500 (UPCB)	MN551447	MN577755	MN577825	F
Pabstiella lineolata (Barb. Rodr.) Luer	A.L.V. Toscano de Brito 3630 (UPCB)	MN551448	MN577756	MN577826	F
Pabstiella lingua (Lindl.) Luer	M. Bolson 656 (UPCB)	MN551449	MN577757	MN577827	F
Pabstiella lueriana Fraga & L. Kollmann	A.L.V. Toscano de Brito 3374 (UPCB)	MN551450	MN577758	MN577828	F
Pabstiella matinhensis (Hoehne) Luer	A.L.V. Toscano de Brito 2906 (UPCB)	MN551451	MN577759	MN577829	F
Pabstiella mirabilis (Schltr.) Brieger & Senghas	E.C. Smidt 921 (UPCB)	MN332394	MN332565	MN331633	F
Pabstiella miragliae (Leite) Luer	A.L.V. Toscano de Brito 2908 (UPCB)	MN551452	MN577760	MN577830	F

Pabstiella muricatifolia Fraga & L. Kollmann	A.L.V. Toscano de Brito 2864 (UPCB)	MN551453	MN577761	MN577831	F
Pabstiella nymphalis Luer & Toscano	A.L.V. Toscano de Brito 2874 (UPCB)	MN551454	MN577762	MN577832	F
Pabstiella piraquarensis (Hoehne) Luer	A.L.V. Toscano de Brito 3666 (UPCB)	MN551455	MN577763	MN577833	F
Pabstiella pristeoglossa (Rchb.f. & Warm.) Luer	S.L.X. Tobias 17 (UPCB)	MN551456	MN577764	MN577834	F
Pabstiella pseudotrifida L. Kollmann & D.R. Couto	A.L.V. Toscano de Brito 3663 (UPCB)	MN551457	MN577765	MN577835	F
Pabstiella punctatifolia (Barb. Rodr.) Luer	A.L.V. Toscano de Brito 2894 (UPCB)	MN551458	MN577766	MN577836	F
Pabstiella purpurea (Seehawer) Luer & Toscano	A.L.V. Toscano de Brito 3768 (UPCB)	MN551459	MN577767	MN577837	F
Pabstiella quasi Luer & Toscano	A.L.V. Toscano de Brito 3074 (UPCB)	MN551460	MN577768	MN577838	F
Pabstiella recurviloba Luer & Toscano	A.L.V. Toscano de Brito 3567 (UPCB)	MN551461	MN577769	MN577839	F
Pabstiella aff ramphastorhyncha (Barb. Rodr.) L. Kollmann	N. Gutierrez 91 (UPCB)	MN551419	MN577726	MN577796	F
Pabstiella rhombilabia Chiron & Xim. Bols.	G. Chiron 11081 (HUEFS), nrITS / A.L.V. Toscano de Brito 3778 (UPCB), matK and trnH-psbA	JQ306488*	MN577770	MN577840	F
Pabstiella rubrolineata (Hoehne) Luer	A.L.V. Toscano de Brito 2896 (UPCB)	MN551462	MN577771	MN577841	F
Pabstiella rupicola L. Kollmann	A.L.V. Toscano de Brito 2865 (UPCB)	MN551463	MN577772	MN577842	F
Pabstiella ruschii (Hoehne) Luer	A.L.V. Toscano de Brito 2872 (UPCB)	MN551464	MN577773	MN577843	F
Pabstiella aff silvanae Chiron & Xim. Bols	N. Gutierrez 97 (UPCB)	MN551420	MN577727	MN577797	F
Pabstiella sordida (Kraenzl.) Luer	A.L.V. Toscano de Brito 2630 (UPCB)	MN551465	MN577774	MN577844	F
Pabstiella trífida (Lindl.) Luer	A.L.V. Toscano de Brito 2878 (UPCB)	MN551466	MN577775	MN577845	F
Pabstiella tripterantha (Rchb.f.) F. Barros	A.L.V. Toscano de Brito 2897 (UPCB)	MN551467	MN577776	MN577846	CGF

Pabstiella aff. uniflora (Lindl.) Luer	N. Gutierrez 87 (UPCB)	MN551421	MN577728	MN577798	F
Pabstiella varellae Toscano, Luer & J. Klein	J. Klein 172 (UPCB)	MN551468	MN577777	MN577847	F
Pabstiella verboonenii (Luer & Toscano) Luer	A.L.V. Toscano de Brito 3467 (UPCB), nrITS and matK / A.L.V. Toscano de Brito 3345 (UPCB), trnH-psbA	MN551469	MN577778	MN577848	F
Pabstiella versicolor (Porsh) Luer	J. Klein 69 (UPCB)	MN551470	MN577779	MN577849	F
Pabstiella villosisepala L. Kollmann & Fraga	A.L.V. Toscano de Brito 2860 (UPCB)	MN551471	MN577780	MN577850	F
Pabstiella wacketii (Handro & Pabst) Luer	F.J. de Jesus 10 (UPCB)	MN551472	MN577781	MN577851	F
Pabstiella yauaperyensis (Barb.Rodr.) F. Barros	A.L.V. Toscano de Brito 3054 (UPCB)	MN332395	MN332566	MN331634	DE
Phloeophila nummularia (Rchb.f.) Pridgeon & M.W. Chase	A. Karremans 5959	KF747839*	KP012380*	---	CDEF
Phloeophila pleurothallopsis (Kraenzl.) Pridgeon & M.W.Chase	A. Karremans 4818 (L), nrITS / D.C. Imig 521 (UPCB), matK and trnH-psbA	KC425746*	MN577782	MN577852	CG
Platystele oxyglossa (Schltr.) Garay	W.S. Mancinelli 1333 (UPCB)	MN332397	MN332568	MN331636	ABCDEFG
Pleurothallis capitonis Luer & Escobar	N. Gutierrez 53 (JBB)	MN551473	MN577783	MN577853	G
Pleurothallis excavata Schltr	A.L.V. Toscano de Brito 2969 (SEL)	MN551474	MN577784	MN577854	C
Pleurothallis gomezii Luer & Escobar	N. Gutierrez 20 (JBB)	MN551475	MN577785	MN577855	G
Pleurothallis isthmica Luer	A.L.V. Toscano de Brito 2945 (SEL)	MN332398	MN332569	MN331637	BC
Pleurothallis phyllocardioides Schltr	A.L.V. Toscano de Brito 2943 (SEL)	MN551476	MN577786	MN577856	BCDEG
Pleurothallopsis nemorosa (Barb. Rodr.) Porto & Brade	A.L.V. Toscano de Brito 3414 (UPCB)	MN332400	KX686529*	MN331638	F
Restrepia trichoglossa Sander	M.L. Klingelfus 117 (UPCB)	MN332402	MN332573	MN331640	G
Restrepiella ophiocephala H. Karst	A.L.V. Toscano de Brito 3126 (SEL)	MN332403	MN332574	MN331641	ABC
Sansonia bradei (Schltr.) Chiron	M. Bolson 565 (UPCB)	MN332404	MN332575	MN331642	F
Scaphosepalum ximenae Luer & Hirtz	D.C. Imig 599 (UPCB)	MN551477	MN577787	MN577857	G
Specklinia biglandulosa (Schltr) Pridgeon & M.W. Chase	N. Gutierrez 95 (UPCB)	MN551478	MN577788	MN577858	F

Specklinia grobyi (Bateman ex Lindl.) Pridgeon & M.W. Chase	J. Klein 94 (UPCB)	MN332406	MN332577	MN331644	ABCDEFG
Stelis aprica Lindl	M.E. Engels 1599 (UPCB)	MN332407	MN332578	MN331645	BCDEFG
Stelis deregularis Barb. Rodr	M.C. Santos *et al.* 1 (UPCB)	MN332377	MN332553	MN331617	BCDEFG
Stelis grandiflora Lindl	E. Caglioni and C. Signoelli 288 (UPCB)	MN332410	MN332581	MN331648	DF
Stelis eublepharis Rchb. f	N. Gutierrez 10 (JBB)	MN551479	MN577789	MN577859	G
Stelis ruprechtiana Rcbh.f	M.C. Santos *et al.* 14 (UPCB)	MN332412	MN332583	MN331650	F
Trichosalpinx montana (Barb. Rodr.) Luer	A.L.V. Toscano de Brito 2909 (UPCB)	MN551480	MN577790	MN577860	F
Trisetella strumosa Luer & Andreetta	D.C. Imig 609 (UPCB)	MN551481	---	MN577861	G
Zootrophion williamsii Luer	A.L.V. Toscano de Brito 2952 (SEL)	MN332416	---	MN331654	C

Table S2. Character list and their corresponding states; consistency (CI), retention (RI) index, number of steps, and ancestral states under maximum parsimony for *Pabstiella* over the maximum likelihood molecular tree.

Character/State	Consistence index	Retention index	Steps	Ancestral State
1. Habit 0 Caespitose/1 Shortly creeping	0.333	0.333	3	0
2. Stem length related to the leaf 0 Shorter/1 Longer	0.167	0.706	6	0
3. Stem, tubular whitish sheats 0 Absent/1 Present	1.000	1.000	1	0
4. Stem, pseudo-lepanthiform sheaths 0 Absent/1 Present	0.167	0.583	6	0
5. Inflorescence, axis, length related to the leaf 0 Shorter/1 Longer	0.111	0.556	9	1
6. Inflorescence, raceme, rachis, direction 0 Strict_or_sinuose/1 Fractiflex	0.091	0.565	11	0
7. Flower, lateral sepals, connation 0 Connate at base or to about the middle/ ……1 Forming a synsepal	0.500	0.000	2	1
8. Flower, lateral sepals, spur 0 Absent/1 Present	0.250	0.000	4	0
9. Flower, petals, apex thickened 0 Absent/1 Present	0.333	0.778	3	0
10. Flower, lip, calli 0 Absent/1 Present	0.083	0.593	12	0
11. Flower, lip, lateral lobes 0 Absent/1 Present	0.500	0.667	2	1
12. Flower, lip, base, lobules 0 Absent/1 Present	0.125	0.667	8	0
13. Flower, column, wings 0 Absent/1 Present	0.200	0.333	5	1
14. Flower column, ventral surface, callus 0 Absent/1 Present	1.000	1.000	1	0
TOTAL	0.18	0.60	73	-

Table S3. Matrix of morphological characters for *Pabstiella* and outgroups.

Dryadella edwallii	00000010011110
Pabstiella alligatorifera	00001010001110
Pabstiella arcuata	00001010111110
Pabstiella aryter	10000110010010
Pabstiella bicolor	00011110001001
Pabstiella bowmanii	00100010001110
Pabstiella bradei	01011110001011
Pabstiellacalcarata	00101011011010
Pabstiella calimanii	01000010011100
Pabstiella carinifera	01011010011010
Pabstiella colorata	00100010001110
Pabstiella conceicionensis	00000010011010
Pabstiella crassicaulis	00000010011000
Pabstiella crenata	10001000011110
Pabstiella decurva	00001010001110
Pabstiella determannii	10000110010010
Pabstiella diffusiflora	00001010001110
Pabstiella eunapolitana	00001010111110
Pabstiella ephemera	00000010011000
Pabstiella ezechiasi	00001010011110
Pabstiella fasciata	00001110111110
Pabstiella fragae	00001010011010
Pabstiella fusca	00001110111110
Pabstiella ghillanyi	01001010001010
Pabstiella gracilicaulis	00001110011110
Pabstiella hians	00001110111110
Pabstiella aff. *hians*	00001100111110
Pabstiella *juquitibensis*	00001010001010
Pabstiella *leucopyramis*	00011010011010
Pabstiella *lineolata*	00000010001010
Pabstiella *lingua*	00100010001010
Pabstiella lueriana	00001110111110
Pabstiella matinhensis	01011110001010
Pabstiella mirabilis	01011011001010
Pabstiella miragliae	01011010001010
Pabstiella muricatifolia	00001010011110
Pabstiella nymphalis	00001110111110
Pabstiella piraquarensis	01001110011110
Pabstiella pristeoglossa	01001110000010
Pabstiella pseudotrifida	00100010001010
Pabstiella punctatifolia	01010110001010
Pabstiella purpurea	01011110001010
Pabstiella quasi	00000010011000
Pabstiella aff. r*amphastorhyncha*	00000010001010
Pabstiella recurviloba	01011110001010
Pabstiella rhombilabia	00000110011100
Pabstiella rubrolineata	00100010001010
Pabstiella rupicola	00001011001000
Pabstiella ruschii	010110100010-0
Pabstiella aff. *silvanae*	00001010111110
Pabstiella sordida	01011110001011
Pabstiella trifida	00100010011010
Pabstiella tripterantha	10001110011010
Pabstiella aff. *uniflora*	00000010001010
Pabstiella varellae	01011110011010
Pabstiella verboonenii	01011110001010
Pabstiella versicolor	01010110011011
Pabstiella villosisepala	00001010111110
Pabstiella wacketii	01001010011110
Pabstiella yauaperiensis	00001111010010
Pleurothallis capitonis	01000-10000000
Stelis aprica	00001000000000

Table S4. Maximum parsimony statistics for each partition used in the analyses of the genus *Pabstiella* and outgroups.

Matrix	Taxons	Characters	Variable characters	Informative characters	Trees retained	Tree length	Consistence index (IC)	Retention index (IR)	Co inf
nrITS	102	760	439 (58%)	318 (42%)	10000	1916	0.40	0.64	
matK	100	1095	384 (35%)	193 (18%)	10000	783	0.61	0.71	
trnH-psbA	96	1273	411 (32%)	193 (15%)	10000	886	0.63	0.63	
cpDNA	102	2368	795 (33%)	386 (16%)	10000	1787	0.58	0.63	
nrITS+cpDNA	102	3128	1234 (39%)	704 (22%)	4320	3856	0.47	0.60	
Morphology	62	14	14	14	-	79	0.18	0.60	

Table S5. Model comparison table from BioGeoBEARS ancestral area reconstruction, and from BEAST molecular dating analysis. For the former is included log-likelihood, number of parameters, d (dispersion), e (extinction) and j (founder speciation event), also corrected Akaike Criterion (AICc), and AICc weights (AICc_wt). For the latter is included likelihood mean (LM) and ESS (LESS), standard error (S.E.) and Akaike Criterion estimated under method-of-moments estimator (AICM). In bold the preferred models.

BioGeoBEARS	LnL	Num params	d	e	j	AICc	AICc_wt
DEC	**-257.8**	**2**	**0.018**	**2.0e-08**	**0**	**519.7**	**0.55**
DEC+J	-256.9	3	0.018	1.0e-12	0.0040	520.1	0.45
DIVALIKE	-264.6	2	0.021	1.0e-12	0	533.3	0.0006
DIVALIKE+J	-263.5	3	0.020	4.3e-09	0.0045	533.3	0.0006
BAYAREALIKE	-276.6	2	0.013	0.090	0	557.4	3.6e-09
BAYAREALIKE+J	-276.6	3	0.013	0.090	1.0e-05	559.5	1.2e-09
BEAST	LM	LESS	S.E			AICM	
Yule Process	-26291.884	4307	0.231			52784.464	
Birth-Death Process	**-26291.893**	**4209**	**0.371**			**52776.944**	

Table S6: Node information from molecular dating analysis and biogeographical reconstruction. Node numbers and geographic regions' letters code are assigned in Figure S2. Ancestral area probabilities are rounded down to two decimal places. *p= 0.001.

Node	**Mean age (Mya)**	**95% HPD minimum**	**95% HPD maximum**	**RASP result: ancestral area (probability)**
103	11.3	3.8	15.24	F (1.00)
104	1.5	1.31	7.2	H (0.82); FH (0.18)
105	2.14	1.51	6.93	CF (0.50); BF (0.50)
106	6.0	3.76	12.36	F (1.00)
107	8.36	8.02	15.93	F (1.00)
108	6.24	2.76	8.45	F (1.00)
109	8.24	4.57	10.82	F (0.69); FG (0.31)
110	8.87	4.89	11.3	F (0.85); FG (0.15)
111	10.01	7.66	14.66	F (0.72); CF (0.28)
112	10.1	8.38	15.61	F (1.00)
113	5.05	4.05	10.69	CG (0.79); C (0.21)
114	2.67	1.76	6.13	G (0.83); FG (0.17)
115	4.9	4.06	9.56	FG (0.66); G (0.22); F (0.11)
116	6.5	5.5	11.47	G (0.48); FG (0.32); F (0.20)
117	6.98	6.37	12.47	G (0.36); FG (0.32); CF (0.17); CG (0.15)
118	4.66	2.73	6.84	G (0.85); FG (0.14)
119	0.75	0.56	2.7	F (0.76); FG (0.24)
120	5.8	3.83	8.24	FG (0.74); G (0.26)
121	6.85	5.26	10.25	FG (0.65); G (0.24); F (0.11)
122	7.1	6.33	11.86	G (0.59); FG (0.41)
123	9.09	7.3	13.29	G (0.71); FG (0.29)
124	2.99	1.56	6.1	FG (1.00)
125	6.19	3.65	9.24	G (0.53); FG (0.47)
126	8.86	6.56	12.78	G (0.72); F (0.16); FG (0.12)
127	0.92	0.75	3.5	C (0.81); BC (0.19)
128	1.09	0.73	3.08	CG (0.86); G (0.14)
129	1.42	1.1	3.82	CG (0.83); G (0.17)
130	1.62	1.8	5.29	CG (1.00)
131	1.37	0.62	2.51	F (0.83); DF (0.17)
132	2.93	1.3	3.95	FG (0.68); F (0.21); G (0.10)
133	4.31	2.47	6.38	FG (0.51); F (0.27); G (0.21)
134	5.46	3.24	7.69	G (0.36); F (0.35); FG (0.28)
135	2.19	1.86	6.16	DG (0.31); EG (0.31); D (0.11); E (0.10); CD (0.09); CE (0.09)
136	2.98	2.97	7.49	DG (0.27); EG (0.27); D (0.23); E (0.22)

137	3.42	3.47	8.03	G (0.26); DF (0.15); EF (0.15); DG (0.11); EG (0.11); D (0.08); E (0.08); FG (0.05)
138	2.31	1.34	6.12	F (1.00)
139	1.39	0.61	3.11	F (1.00)
140	0.94	0.39	1.56	F (1.00)
141	0.85	0.27	1.2	F (1.00)
142	0.95	0.55	1.81	F (1.00)
143	1.96	1.35	3.92	F (1.00)
144	3.58	2.23	5.55	F (1.00)
145	3.24	1.45	4.8	F (1.00)
146	1.86	1.35	4.57	F (1.00)
147	0.8	0.37	1.8	F (1.00)
148	1.14	0.83	2.71	F (1.00)
149	3.6	2.63	5.58	F (1.00)
150	3.69	2.96	5.94	F (1.00)
151	0.58	0.49	1.92	F (1.00)
152	1.21	0.87	2.65	F (1.00)
153	1.85	1.49	3.88	F (1.00)
154	2.59	1.99	4.62	F (1.00)
155	3.94	3.03	5.88	F (1.00)
156	2.57	1.81	4.9	F (1.00)
157	0.76	0.21	1.31	F (1.00)
158	1.03	0.58	2.38	F (1.00)
159	2.55	1.2	3.7	F (1.00)
160	1.53	0.6	2.35	F (1.00)
161	0.5	0.19	1.55	F (1.00)
162	1.18	0.55	1.92	F (1.00)
163	0.52	0.35	1.33	F (1.00)
164	1.52	1.02	2.53	F (1.00)
165	1.57	1.26	2.9	F (1.00)
166	2.08	1.47	3.29	F (1.00)
167	2.69	2.07	4.32	F (1.00)
168	1.75	1.16	3.12	F (1.00)
169	1.21	0.69	2.61	F (1.00)
170	0.69	0.49	2.19	F (1.00)
171	1.51	1.26	3.32	F (1.00)
172	2.54	1.98	4.08	F (1.00)
173	2.15	0.57	2.69	F (1.00)
174	1.65	0.79	2.33	F (1.00)
175	2.06	1.13	2.76	F (1.00)
176	1.13	0.58	1.62	F (1.00)
177	0.94	0.32	1.45	F (1.00)
178	1.34	0.8	2.01	F (1.00)
179	2.27	1.45	3.08	F (1.00)

180	2.28	1.64	3.36	F (1.00)
181	2.92	1.87	3.71	F (1.00)
182	3.03	2.0	3.93	F (1.00)
183	3.22	2.44	4.6	F (1.00)
184	3.54	2.72	5.02	F (1.00)
185	3.6	3.0	5.48	F (1.00)
186	3.74	3.11	5.69	F (1.00)
187	4.1	3.5	6.34	F (1.00)
188	4.38	3.65	6.58	F (1.00)
189	4.42	3.83	6.87	F (1.00)
190	4.65	4.22	7.48	F (1.00)
191	5.4	4.65	8.47	F (1.00)
192	6.59	5.27	9.46	FG (0.43); F (0.35); DF (0.08); EF (0.07); G (0.07) *
193	7.93	6.24	11.14	FG (0.39); G (0.34); F (0.27)
194	8.05	6.62	11.66	G (0.46); FG (0.45); CF (0.09) *
195	9.75	8.18	14.0	G (0.60); FG (0.26); F (0.14)
196	9.78	8.7	14.72	G (0.48); FG (0.44); F (0.09)
197	10.71	9.71	15.99	FG (0.48); G (0.26); F (0.26)
198	11.63	10.34	16.94	F (0.67); FG (0.33)
199	12.68	11.08	17.83	F (0.86); FG (0.14)
200	13.4	11.94	18.88	F (1.00)
201	14.91	13.64	20.93	F (0.80); FH (0.20)
202	18.67	15.5	23.57	F (0.83); FH (0.17)
203	23.67	16.65	26.81	F (0.24); FH (0.18); FG (0.10); CF (0.10); BF (0.09); DF (0.09); EF (0.09); AF (0.09)

CONCLUSÃO FINAL

Esta proposta filogenética permite, de uma forma consistente e clara, delimitar e nomear dez seções infragenéricas que compõem o gênero *Pabstiella*, e definir os caracteres morfológicos, tanto vegetativos quanto reprodutivos, que as distinguem. No entanto, o relacionamento entre algumas dessas seções é discutível e pode mudar em futuras análises, e ainda especificamente, a resolução dos nós dentro de algumas seções como a seção tipo do gênero é baixa.

Evolutivamente, é frequente que caracteres reprodutivos similares aconteçam em espécies distantes filogeneticamente devido a adaptações a polinizadores semelhantes. Por exemplo, o mento, o caractere usado inicialmente para diferenciar à espécie tipo do gênero, apareceu independentemente em três das dez seções propostas. A função do mento deve ser avaliada em futuros estudos de polinização, pois poderia estar relacionada com algum tipo específico de polinizador. Para saber quais possíveis síndromes de polinização acontecem no gênero é imprescindível registrar eventos de polinização, assim como analisar evidencias indiretas como secreções de néctar no calcar de algumas espécies como *P. mirabilis*, ou a fragrância das flores de algumas espécies como *P. carinifera* ou *P. leucopyramis*.

Por outro lado, os caracteres vegetativos foram mais estáveis, as bainhas do ramicaule são muito importantes para definir morfologicamente as seções propostas, e deveriam ser estudadas a profundidade. Da mesma maneira, as "alianças" propostas por Pabst & Dungs (1976) coincidem em maior parte quando baseadas em caracteres vegetativos e da inflorescência, aquelas definidas por caracteres florais só levaram a agrupamentos artificiais de espécies.

Com respeito a sua história biogeográfica, *Pabstiella* possivelmente originou-se em uma época em que as florestas dos Andes e da Mata Atlântica estavam conectadas, e o que propiciou a divergência das suas linhagens principais foi a desconexão entre estes biomas, relacionada a eventos climáticos no período entre o final do Mioceno e o Plioceno. Isto representa a primeira evidência para Pleurothallidinae da importância que estas conexões e desconexões tiveram na diversificação de um gênero em América do sul e poderia ajudar a entender a história biogeográfica de outros gêneros da subtribo que possuem padrões de distribuição disjunta entre estes dois biomas mega diversos.

Embora a reconstrução da área ancestral da subtribo Pleurothallidinae não tenha sido um dos objetivos primários do presente trabalho, nossos resultados não concordam com o estudo prévio de Perez-Escobar *et al.*, (2017), e sugerem no futuro uma maior abordagem, que inclua temáticas como paleoclima durante o Mioceno, conexões passadas entre a Mata Atlântica e os Andes, e uma maior amostragem nos clados mais basais da subtribo (afinidade *Octomeria*), para uma hipótese biogeográfica da subtribo mais robusta.

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