Jéssica Nayara Carvalho Francisco

# Sistemática e biogeografia de *Pachyptera* DC. ex Meisn. (Bignonieae, Bignoniaceae)

# Systematic and biogeography of *Pachyptera* DC. ex Meisn. (Bignonieae, Bignoniaceae)

Instituto de Biociências

Universidade de São Paulo

São Paulo

2017

Jéssica Nayara Carvalho Francisco

# Sistemática e biogeografia de *Pachyptera* DC. ex Meisn. (Bignonieae, Bignoniaceae)

# Systematic and biogeography of *Pachyptera* DC. ex Meisn. (Bignonieae, Bignoniaceae)

Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Mestre em Ciências, na Área de Botânica.

Orientador(a): Dra. Lúcia Garcez Lohmann

São Paulo

2017

Francisco, J. N. C.
Sistemática e biogeografia de *Pachyptera* DC. ex Meisn (Bignonieae, Bignoniaceae)
Número de páginas

Dissertação (Mestrado) - Instituto de Biociências da Universidade de São Paulo. Departamento de Botânica.

1. Biogeografia neotropical 2. Delimitação de espécies 3. Flora Amazônica 4. Microssatélites
I. Universidade de São Paulo. Instituto de Biociências. Departamento de Botânica

## Comissão Julgadora:

_____ _____

Prof(a). Dr(a). Prof(a). Dr(a).

_____

Prof(a). Dr.(a). Lúcia Garcez Lohmann

Orientador(a)

# Dedicatória

À Prof. Amélia Cristina Elias da Ponte,
cujas aulas mudaram minha visão sobre a Botânica.

Ao Prof. Ronaldo Bastos Francini,
por despertar minha curiosidade sobre as lianas.

# Agradecimentos

À Dra. Lúcia. L. Lohmann por todas as oportunidades e experiências que tenho vivido após ter aberto as portas do Laboratório de Sistemática e Biogeografia. Agradeço em especial, pela orientação, paciência e empolgação durante as fases desse projeto. Tenho muita sorte de tê-la como orientadora e incentivadora nessa área da ciência.

Ao Alison G. Nazareno pela colaboração em um dos artigos e ensinamentos pessoais e profissionais.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e à Fundação de Amparo à Pesquisa do Estado de São Paulo pelo suporte financeiro fundamental para o desenvolvimento desse projeto.

Aos membros da banca de qualificação, Dr. José Rubens Pirani, Dr. Ricardo Pinto da Rocha e Dr. Marcos Maldonado Coelho pelos comentários e sugestões que contribuíram para o melhor andamento desse trabalho.

Aos colegas da expedição Amazonas-Roraima: Augusto Giaretta, Beatriz M. Gomes, Jenifer C. Lopes, Luis H. Fonseca, Marcelo F. Devecchi e Thais Vasconcelos pela agradável companhia, parceria e excelente trabalho em grupo. Esta foi minha primeira expedição de campo na Amazônia, gratidão por tudo que aprendi e vivenciei com vocês.

Aos colegas da expedição Pará: Annelise N. Frazão, Augusto Giaretta e Hélcio Honorato de Souza (técnico do INPA) pelos 15 dias produtivos de trabalho de campo, consultas em herbários e trocas de experiências. Graças a esse campo encontrei uma espécie rara e uma variedade que foram essenciais para essa dissertação.

Aos gestores e funcionários do Parque Nacional de Anavilhanas, Parque Nacional do Viruá, Floresta Nacional do Tapajós e Floresta Nacional Saracá-Taquera pela logística, estrutura e atenção que facilitaram os trabalhos de campo.

Às queridas Adriana Marchioni e Mirian Kaehler por toda aprendizagem e descoberta nesse novo mundo molecular! Até então, extração, amplificação, clonagem e sequenciamento, era um mundo à parte, o que aprendi com vocês foi de suma importância. Por isso, não posso deixar de agradecer pela paciência e dedicação em ensina o bê-a-bá de um laboratório molecular, além de me tranquilizar em momentos frustrantes quando surgiam problemas com amostras antigas.

Aos Alexandre R. Zuntini e Luis H. Fonseca pela amizade, dedicação e disposição em discutir pontos desse estudo, além da valiosa ajuda em realizar as análises filogenéticas e de datação. Sou extremamente grata por tudo que aprendi com vocês.

À Maila Beyer, pela amizade, conversas, pipocas e parceria durante a epopéia dos microssatélites.

Ao prof. Dr. Diego Demarco por me ensinar a preparar materiais para análises em microscópio eletrônico de varredura.

Aos Bignonietes (Alison, Anne, Bia, Dri, Eric, Juan, Luis, Maila, Mirian, Pamela, Verônica e Zuntini) pelo agradável ambiente de trabalho e pelas ajudas inestimáveis em questões teóricas, filosóficas e metodológicas. Obrigada por também dedicarem um tempo para fotografar e coletar amostras em sílica de *Pachyptera*.

Aos funcionários do Laboratório de Sistemática Vegetal, Abel, Fabrício, Homelhan, Verônica, Vivi, Norberto e Robertinha, que facilitam o dia a dia no Sobre as Ondas, além de serem pessoas adoráveis, aos colegas e amigos, Adriana Marcchioni, Annelise Frazão, Anselmo Nogueira, Augusto Giaretta, Benoit Loeullie, Camila Dussán, Carolina Sinischalchi, Daniela Costa, Eduardo Leal, Eric Kataoka, Euder Martins, Guilherme Antar, Juliana Lovo, Leonardo Borges, Marcelo Kubo, Matheus Fortes, Matheus Martins Cota, Maurício Watanabe, Mayla Beyer, Miriam Kaehler, Pamela Santana, Paulo Gonella, Renato Ramos, Verônica Thode pelo apoio e agradável convivência, em especial, Alexandre Zuntini, Beatriz M. Gomes, Gisele Alves, Jenifer C. Lopes, Juan Pablo, Juliana H. L. El Ottra, Marcelo Devecchi e Natali G. Bordon.

Aos professores Glauco Machado e Paulo Inácio que exerceram um importante papel na minha formação e escolhas na vida. Cursar a disciplina Ecologia de Campo em 2012 mudou a maneira como eu enxergo a ciência e o potencial que todos nós temos.

Por fim, à minha amada mãe, Hilda de Fatima, que sempre me incentivou a estudar e correr atrás dos meus objetivos e ao meu querido companheiro Jorge pelo apoio, cumplicidade e coragem em descobrir São Paulo comigo.

# Índice

# Resumo

A Amazônia inclui uma grande proporção da biodiversidade encontrada atualmente na Terra. Apesar disso, nosso conhecimento sobre a biodiversidade Amazônica ainda é limitado, dificultando nosso entendimento dos padrões de diversidade nesta região. Entender os processos que levaram à diversidade encontrada na Amazônia representa um grande desafio para a biologia evolutiva. Este estudo foca em *Pachyptera* (Bignonieae, Bignoniaceae), um pequeno gênero de lianas neotropicais, centrado na Amazônia. *Pachyptera* tem uma história taxonômica complicada, incluindo problemas na circunscrição genérica e específica. Este estudo visa: (i) reconstruir o parentesco filogenético entre espécies do gênero, (ii) produzir uma revisão taxonômica, incluindo nova circunscrição genérica e específica, (iii) entender a história biogeográfica do grupo e, (iv) desenvolver marcadores microssatélites (SSRs) para futuros estudos filogeográficos. Em primeiro lugar, reconstruímos a filogenia do gênero usando uma ampla amostragem de taxa e uma combinação de marcadores de cpDNA (*ndh*F and *rpl*32-*trn*L) e nDNA (*Pep*C). Em segundo lugar, analisamos a filogenia de *Pachyptera* utilizando análises de coalescência (GMYC e *BEAST) e morfologia para esclarecer limites específicos dentro do complexo *P. kerere.* Em terceiro lugar, produzimos uma filogenia datada de *Pachyptera*, a qual foi utilizada como base para reconstruir a história biogeográfica do gênero utilizando BSSVS e RASP. Por fim, desenvolvemos SSRs utilizando sequenciamento de próxima geração, os quais serão utilizados para guiar estudos filogeográficos futuros com o grupo. Nosso estudo indica que *P. ventricosa* é mais proximamente relacionada à *Mansoa* do que *Pachyptera*, levando ao reestabelecimento de *M. ventricosa*. Além disso, nossos estudos moleculares e morfológicos sustentam o reconhecimento de *P. kerere* var. *incarnata* como uma espécie separada e a descrição de uma espécie nova (*P. linearis*). Desta forma, reconhecemos um gênero com cinco espécies: (i) *P. aromatica*, (ii) *P. erythraea*, (iii) *P. incarnata*, (iv) *P. kerere*, e (v) *P. linearis*. Estas espécies são tratadas em uma revisão taxonômica do gênero. As análises biogeográficas indicam que *Pachyptera* surgiu durante o Eoceno Tardio, e diversificou durante o Mioceno, um período de intensas perturbações provocadas na América do Sul (i.e., soerguimento dos Andes, eventos de incursões marinhas, e formação de sistemas florestais secos e úmidos). Vinte-e-um

SSRs foram desenvolvidos para *Pachptera* e servirão como base para estudos filogeográficos futuros com este grupo. Esta dissertação faz parte de um projeto multi-disciplinar que visa compreender a evolução da biota amazônica e seu ambiente (FAPESP 2012/50260-6).

**Palavras chaves:** biogeografia neotropical, delimitação de espécies, flora Amazônica, microssatélites, *Pachyptera kerere*, sequenciamento de próxima geração.

# Abstract

The Amazon houses a large proportion of the overall biodiversity currently available on Earth. Despite that, our knowledge of Amazonian biodiversity is still limited, complicating our understanding of diversity patterns within this region. Understanding the drivers of Amazonian biodiversity represents a major challenge in evolutionary biology. This study focuses on *Pachyptera* (Bignonieae, Bignoniaceae), a small genus of neotropical lianas centered in the Amazon. *Pachyptera* has a complicated taxonomic history, including problematic generic and species circumscriptions. This study aims to: (i) reconstruct phylogenetic relationships among species of the genus (ii) produce a taxonomic revision, including clear generic and species circumscriptions, (iii) understand the biogeographic history of the group, and (iv) develop microsatellite markers (SSRs) for future phylogeographic and population genetic studies. First, we inferred phylogenetic relationships within a broad sampling of taxa and a combination of cpDNA (*ndh*F and *rpl*32-*trn*L) and nuclear (*Pep*C) markers. Second, we analyzed the phylogeny of *Pachyptera* using coalescent approaches (GMYC and *BEAST) and morphology to clarify species limits within the *P. kerere* species complex. Third, we produced a time-calibrated phylogeny of *Pachyptera* that was used as basis to reconstruct the biogeographical history of the genus using BSSVS and RASP. Lastly, we developed SSRs using next-generation sequencing (NGS) that will be used to guide future phylogeographic studies within this group. Our study indicates that *P. ventricosa* is more closely related to *Mansoa* than *Pachyptera*, leading to the reestablishment of *Mansoa vetricosa*. Furthermore, our molecular and morphological analyses support the recognition of *P. kerere* var. *incarnata* as a separate species, and the description of a new taxon (*P. linearis*). As such, we here recognize a genus with five species: (i) *P. aromatica*, (ii) *P. erythraea*, (iii) *P. incarnata*, (iv) *P. kerere*, and (v) *P. linearis*. These species are treated in a taxonomic revision of *Pachyptera*. The biogeographical analyses indicate that *Pachyptera* originated during the Late Eocene, and diversified during the Miocene, a time of intense perturbations in South America (e.g., uplift of the Andes, marine incursions, and formation of dry and wetland systems). Twenty-one SSRs were developed for *P. kerere* and will serve as basis for future phylogeographic studies. This

dissertation is part of a multidisciplinary project that aims to understand the evolution of the Amazonian biota and its environment (FAPESP 2012/50260-6).

**Key words:** Amazonian flora, microssatélites, neotropical biogeography, next-generation sequencing, *Pachyptera kerere*, species delimitation.

# Introdução Geral

***A Biota Amazônica***

A floresta amazônica retém ca. de 9,5% da diversidade de espécies global (Lewinsohn & Prado, 2005). Este bioma abriga uma ampla gama de grupos taxonômicos que contribuem de forma muito significativa com bens e serviços para a humanidade (Hendry *et al.*, 2010). Por exemplo, estas espécies são de suma importância para a regulação das condições climáticas, produção de oxigênio e sequestro de carbono (Malhi *et al.*, 2008), bem como prestam importantes serviços culturais e estéticos (Sponsel, 1995). Além disso, espécies Amazônicas são geradoras de matéria-prima para a indústria farmacêutica e alimentícia (Fearnside, 1999). Entender quais foram os processos evolutivos que deram origem à atual diversidade biológica encontrada na Amazônia representa um grande desafio para a biologia evolutiva (e.g., Gentry, 1982; Moritz *et al.*, 2000; Hoorn *et al.*, 2010). Diversas hipóteses foram propostas para explicar essa expressiva diversidade, várias das quais estão associadas ao isolamento de populações causadas por: (i) barreiras fluviais, (ii) refúgios florestais, (iii) incursões marinhas e/ou (iv) mudanças geológicas.

A teoria de barreiras fluviais foi a primeira hipótese desenvolvida para explicar a origem da biodiversidade amazônica (Wallace, 1852). Esta hipótese foi proposta com base na observação de que diferentes espécies de primatas apresentam distribuição restrita a certas margens dos rios Solimões, Negro e Amazonas, sugerindo que populações ancestrais teriam se fragmentado em subpopulações devido a formação dos rios e subsequente fragmentação da floresta (1997; Leite & Rogers, 2013). A interrupção do fluxo gênico entre populações ocorrentes nas diferentes margens do rio teria levado à diferenciação entre populações isoladas e posterior especiação (Leite & Rogers, 2013). Inicialmente, Wallace (1852) ressaltou a efetividade das barreiras para primatas, aves e insetos. Evidências adicionais foram obtidas para o modelo de alopatria associado à formação do Rio Amazonas em primatas (Silva & Oren, 1996), aves (Ribas *et al.*, 2011), e borboletas (Hall & Harvey, 2002). Além disso, o modelo também mostrou-se aplicável a pequenos mamíferos (Patton *et al.*, 2000), anfíbios e répteis (Ron, 2000; Funk *et al.*, 2007). Apenas dois estudos até o momento buscaram avaliar o impacto dos rios como barreira para o fluxo gênico de plantas (Collevati, 2009;

Nazareno et al., 2017). Estes estudos indicaram que a eficácia dos rios como barreiras para plantas depende da largura do rio separando populações e de características das espécies.

Uma hipótese alternativa à de barreiras fluviais foi postulada por Haffer (1969), que propôs um modelo baseando-se na sobreposição de endemismos de espécies de aves. Para Haffer (1969) as barreiras fluviais não constituíam impedimento para o fluxo gênico entre aves, uma vez que as aves seriam capazes de atravessar para a outra margem do rio. Haffer (1969) postulou que refúgios teriam originado como resultado às mudanças climáticas decorrentes do máximo glacial do Quaternário, os quais teriam levado à fragmentação da floresta amazônica e formação de refúgios de floresta, onde mais tarde se concentraria uma alta diversidade de espécies. Os refúgios hipotéticos localizavam-se especialmente nas áreas de encosta em porções periféricas da Amazônia (Brown, 1987) e estariam isolados por uma matriz de savanas e cerrados. Neste contexto, os fragmentos de floresta atuariam como barreiras contra a dispersão de plantas e animais florestais, levando a especiação alopátrica. Contudo, as oscilações climáticas durante os períodos interglaciais úmidos teriam levado a expansão dos fragmentos florestais, os quais atuariam como "zonas de sutura" entre fragmentos, permitindo o fluxo migratório entre diferentes populações. Neste contexto, populações que tivessem sofrido especiação completa poderiam apresentar sobreposição geográfica enquanto populações que ainda estivessem em processo de especiação poderiam sofrer exclusão geográfica ou hibridização (Haffer, 1969, 1979).

No entanto, evidências paleobotânicas sugerem que a vegetação amazônica não sofreu nenhum tipo de fragmentação durante o Quaternário (Colinvaux *et al.*, 2000; Anhuf *et al.*, 2006; Bush & Oliveira, 2006), refutando a hipótese de refúgio. Mais especificamente, a elevada concentração de pólen de espécies arbóreas em combinação com a ausência de pólens de elementos típicos de vegetação de savana (e.g., Gramineae e ervas) ao longo de toda a floresta amazônica (Colinvaux *et al.*, 2001), sugerem que talvez os propostos refúgios não tenham existido. Além disso, dados recentes provenientes de filogenias datadas de aves (Ribas *et al.*, 2011) e mamíferos (Patton *et al.*, 2000) sugerem que diversos eventos de especiação na Amazônia não ocorreram no início das glaciações do Quaternário como postulado pela teoria dos refúgios. Por outro lado, evidências resultantes de diversos grupos vegetais (Prance, 1982; Scotti-Saintagne

*et al.*, 2013), aves (Ribas & MiyakI, 2004), anuros (Duellman, 1982) e artrópodes (Lourenço & Florez, 1991; Brower & Egan,1997) corroboram esta teoria.

A teoria das incursões marinhas defende a ideia de que incursões marinhas associadas às flutuações de elevação do nível do mar no Cenozóico e às movimentações tectônicas de soerguimento da cordilheira dos Andes promoveram grande impacto sobre o paleo-ambiente e padrão de drenagem na região amazônica (Hoorn, 1993; Hoorn *et al.*, 2010), levando à diversificação de linhagens. Segundo esta teoria, o soerguimento andino teria provocado um rebaixamento gradual da Amazônia Ocidental, o que teria levado a incursões marinhas formando o conhecido sistema Pebas (i.e., um extenso lago e uma série de pântanos de influência marinha e fluvial) durante o Mioceno médio (Hoorn *et al.*, 1995; Antonelli *et al.*, 2009; Hoorn *et al.*, 2010). O sistema Pebas teria então atuado como barreira de dispersão para os organismos terrestres entre os Andes, Amazônia oriental e regiões da Guiana (Antonelli *et al.*, 2009). Como resultado, uma ampla gama de grupos de animais e plantas não teria conseguido realizar dispersão ou sobreviver sob as novas condições, levando a fragmentação da biota e consequente especiação *in situ*. Ocasionalmente, conexões formadas entre savanas sazonalmente inundadas poderiam ter fornecido vias de dispersão para a biota terrestre (Wesselingh & Salo, 2006).

O fato do período das incursões marinhas coincidir com a diversificação das primeiras plantas e diversos grupos de animais nesta região corrobora esta teoria (Hoorn *et al.*, 2010). Evidências paleobotânicas indicam que numerosos gêneros atribuídos às modernas famílias de angiospermas formavam florestas que se mantinham à margem do lago Pebas (Pons & De Franceschi, 2007; Hoorn *et al.*, 2010). Além disso, a alta diversidade de registros fósseis da fauna artrópode e abundantes inclusões de microfósseis de cianobactérias, fungos e algas de água doce encontrados nos afloramentos ao longo do Rio Amazonas no Médio Mioceno também corroboram esta hipótese (Antoine *et al.*, 2006). Os corredores formados pelo sistema Pebas teriam facilitado a transição evolutiva de peixes (Lovejoy *et al.*, 2006) e moluscos (Wesselingh, 2006) para hábitats lacustres ou fluviais. Dados geológicos indicam que processos neotectônicos na planície amazônica causaram o soerguimento de depósitos do Neogeno, levando a reconfiguração hidrológica e o influxo de sedimentos proveniente dos Andes no desenvolvimento de um mosaico edáfico rico em nutrientes (Räsänen *et*

*al.*, 1998) e espécies (Hoorn *et al.*, 2010). Entretanto, poucos trabalhos conseguiram de fato relacionar os eventos geológicos de vicariância provocados pelas incursões e a especiação de taxa (e.g., Antonelli *et al.*, 2009, com Rubiaceae; Cooke *et al.*, 2012, com peixes).

Por fim, há evidências de que diversos eventos geológicos foram críticos para a formação da atual Biota Amazônica. Em particular, períodos de elevação andina durante o Cretáceo, do sul para o norte e do oeste para o leste foram acompanhados pela diversificação de várias linhagens biológicas (Hoorn *et al.*, 1995; Antonelli *et al.*, 2009; Hoorn *et al.*, 2010), enfatizando a importância do soerguimento dos Andes para a diversificação da biota. Alterações passadas na paisagem amazônica, teriam promovido especiação na Amazônia através de: (a) aumento da heterogeneidade de habitats no norte da América do Sul favorecendo a radiação adaptativa em habitats de montanha; (b) criação de corredores bióticos para taxa pré-adaptados às condições de montanha que ganharam maior amplitude de distribuição; (c) favorecimento de especiação alopátrica em taxa de montanha separados por vales e cumes intransitáveis; (d) vicariância geográfica e consequente isolamento genético entre populações de terra baixa sobre ambos os lados das montanhas emergentes; (e) formação de linhagens através da dispersão e maior radiação para outros biomas, atuando como uma "bomba de espécies" (do inglês "*species pump*"); e (f) aumento da deposição de nutrientes na Amazônia ocidental seguido da desnudação das montanhas pela precipitação (Antonelli & Sanmartin, 2011).

Avanços filogenéticos e geográficos nos Andes e Amazônia têm contribuído para um melhor entendimento da origem dos ecossistemas e diversidade amazônica. Análises recentes têm explorado o uso integrado de dados filogenéticos e cenários geológicos complexos para obter uma construção mais realista do panorama evolutivo (Hoorn *et al.*, 2010). Estes dados sugerem que a origem da diversidade Amazônica não deve ser atribuída há apenas um evento durante um intervalo específico no tempo, dado que tal diversidade parece ter resultado de processos ecológicos e tendências evolutivas iniciadas pelos eventos tectônicos do Neogeno, incluindo reorganizações paleogeográficas mantidas pela ação das mudanças climáticas (Hoorn, 2010; Wesselingh *et al.*, 2010; Rull, 2011). No entanto, novas evidências provenientes de organismos diferentes são de suma importância para elaboração de modelos integrados

que expliquem melhor o cenário que deu origem a alta diversidade biológica em algumas regiões do globo terrestre, especialmente em ambientes mega-diversos como a Amazônia (Bush, 1994; Aleixo, 2004).

Neste sentido, essa dissertação focou no gênero *Pachyptera* DC. ex Meisner, um grupo amplamente distribuído pela região Amazônica (Fig. 1), o que torna este clado um excelente modelo para fornecer novos subsídios no entendimento dos processos associados à origem e diversificação da biodiversidade amazônica.

***Objeto de estudo***

A família Bignoniaceae possui distribuição Pantropical, predominantemente Neotropical e é centrada no Brasil (Gentry, 1980). A família é composta por 82 gêneros e ca. de 827 espécies (Lohmann & Ulloa Ulloa, 2016), com hábito arbóreo, arbustivo, ou lianescente (Gentry, 1980; Olmstead *et al.*, 2009). A família tem grande importância econômica por conta da madeira com alta durabilidade, produtos farmacológicos e apelo paisagístico (Gentry, 1992). Indígenas também utilizam representantes de Bignoniaceae na alimentação, medicina e rituais religiosos (Gentry, 1992). A família é caracterizada pelas folhas compostas com filotaxia oposta, flores gamossépalas e gamopétalas, com corola tubular, androceu epipétalo formado por quatro estames didínamos com um estaminódio, fruto do tipo cápsula com deiscência ao longo de duas suturas e sementes aladas (Gentry, 1980). Trabalhos com a filogenia do grupo reconhecem oito clados centrais: Bignonieae, Catalpeae, Oroxyleae, Aliança *Tabebuia*, Clado Paleotropical, Tecomeae, Jacarandeae, e Tourrettieae (Olmstead e*t al.*, 2009; Spangler & Olmstead 1999).

A tribo Bignonieae é o maior clado da família, incluindo lianas e arbustos da região neotropical (Gentry, 1989; Lohmann, 2006; Olmstead e*t al.*, 2009). Apenas *Bignonia capreolata* L. é encontrada naturalmente nos EUA (Lohmann, 2006). As principais sinapomorfias morfológicas do grupo são as folhas 2-3-folioladas ou 2-3-pinadas com o folíolo terminal modificado em gavinha e anatomia da madeira com crescimento anômalo resultante da interrupção do xilema secundário, com contínua produção de floema formando 4-32 cunhas (Gentry, 1980; Lohmann, 2006). Bignonieae também é reconhecida pelo fruto cápsula, com deiscência paralela ao septo (Gentry, 1980). A delimitação genérica da tribo permaneceu problemática por muitos anos,

incluindo poucos gêneros com numerosas espécies e muitos gêneros monotípicos (Lohmann 2006; Lohmann & Taylor, 2014). Uma filogenia molecular da tribo baseada em uma ampla amostragem de taxa e dois marcadores moleculares (*ndh*F e *Pep*C) reconstruiu 21 clados principais (Fig. 2, Lohmann 2006), que foram tratados como gêneros numa nova classificação genérica proposta para o grupo (Lohmann & Taylor, 2014). Este trabalho apenas reconheceu gêneros mornofiléticos, caracterizados por sinapomorfias morfológicas e forneceu o primeiro tratamento compreensivo da tribo. Contudo, estudos detalhados ao nível específico ainda são necessários para esclarecer problemas associados à delimitação de espécies e posicionamento dentro da tribo.

*Pachyptera* é um pequeno gênero de lianas neotropicais centrado na Amazônia. *Pachyptera* significa "com alas espessas" (do latim: *pach* = espesso; *aptera* = sem alas), uma característica encontrada na espécie tipo, *Pachyptera kerere* (Aubl.) Sandwith. O gênero é facilmente reconhecido pelos profilos achatados e ensiformes (minutos e triangulares), organizados em 3(-5) séries na axila do nó, numerosos e conspícuos nectários extraflorais distribuídos na região interpeciolar e na junção entre o ápice do pecíolo e pecíololo, inflorescência do tipo racemo, flores hipocrateliformes e infundibiliformes, com glândulas pateliformes arranjadas em linhas ou agrupadas no ápice do cálice e região mediana dos lobos da corola. A coloração das flores varia de branco à creme, rosa à roxo claro e laranja à vermelho (Fig. 3). O gênero difere de qualquer outra espécie da tribo por apresentar anteras vilosas, também encontradas em *Lundia* (Lohmann & Taylor, 2014).

*Pachyptera* tem uma história taxonômica confusa, com problemas de delimitação genérica e específica. Na recente classificação proposta por Lohmann & Taylor (2014), *Pachyptera* inclui quatro espécies: *Pachyptera aromatica* (Barb. Rodr.) L.G. Lohmann, *Pachyptera erythraea* (Dugand) A.H. Gentry, *Pachyptera kerere* e *Pachyptera ventricosa* (A.H. Gentry) L.G. Lohmann. *Pachyptera aromatica* é encontrada em florestas úmidas dos estados brasileiros Amazonas, Amapá e Rondônia. *Pachyptera erythraea* é endêmica do vale do rio Magdalena na Colômbia. *Pachyptera kerere* é tipicamente encontrada nas florestas úmidas e de igapós distribuída desde Belize na América Central até Amazônia Central no Brasil. *Pachyptera ventricosa* é uma espécie rara endêmica do Pará, Maranhão (Lohmann & Taylor, 2014) (Fig. 1). Essa classificação foi baseada em informações moleculares para toda a tribo Bignonieae e

sustentada por sinapomorfias morfológicas (por exemplo, ritidoma escamante quando velho e linhas de glândulas na corola) (Lohmann, 2006). No entanto, a filogenia de Lohmann (2006) visava reconstruir o parentesco ao nível genérico em toda a tribo Bignonieae, de forma que a amostragem dentro de cada um dos 21 gêneros amostrados não foi completa. No caso de *Pachyptera*, 50% da diversidade foi amostrada, com a inclusão de duas das quatro espécies reconhecidas (i.e., *P. aromatica* e *P. kerere*).

Espécies de *Pachyptera* apresentam ampla diversidade morfológica, especialmente em caracteres florais. Por exemplo, *P. aromatica* apresenta inflorescências racemosas, com flores brancas, hipocrateriformes, e antese noturna (Barbosa Rodrigues, 1891). Esta espécie apresenta o tipo floral *Tanaecium* e se encaixa na síndrome de polinização por mariposas (Gentry, 1974). *Pachyptera ventricosa*, por outro lado, apresenta inflorescências tirsóides e exibe flores rosa à roxo claras, campanuladas, com anteras glabras. O tipo floral desta espécie é classificado como um variante de *Martinella*, provavelmente associado a síndrome de polinização por morcegos (Gentry, 1974; Alcantara & Lohmann, 2010; Machado & Vogel, 2004). Por fim, *P. erythraea* e *P. kerere* exibem inflorescências racemosas e congestas. A coloração das flores varia de laranja à vermelha em *P. erythraea*, com beija-flores representando potenciais vetores (Gentry, 1974). A corola de *P. kerere* é creme ou branca, rosa à lilás em *Pachyptera kerere* var. *incarnata* (Aubl.) A.H. Gentry (Gentry, 1973). Flores de *P. kerere* correspondem ao tipo floral *Anemopaegma*, associado a síndrome de polinização por abelhas de médio e grande tamanho. Há indícios de que *P. kerere* represente um complexo de espécies (Sprague & Sandwith, 1932). Além disso, *P. erythraea* e *P. kerere* são vegetativamente idênticas e vários autores classificaram sua diversidade floral como variação intraespecífica (Dugand, 1955; Gentry, 1973, 1979). Um melhor entendimento do parentesco entre essas linhagens é necessário para uma delimitação de espécies mais precisa.

***Microssatélites moleculares***

Marcadores microssatélites, contribuíram de forma muito positiva para a compreensão dos processos que determinam a estrutura e variação dentro e entre populações naturais (Provan *et al*., 2001). Microssatélites (SSRs, do inglês, *Simple Sequence repeats*) são pequenas sequências de DNA compostas por repetições *in*

*tandem* (em fila) de unidades formadas por um até seis nucleotídeos (Egan *et al.*, 2012). Os SSRs são encontrados no genoma de procariotos e eucariotos sendo mais abundantes nas regiões não codificantes (Ebert & Peakall, 2009; Egan *et al*., 2012; Li *et al.*, 2002). Quanto ao tipo de repetição, SSRs são classificados em: perfeitos, quando as repetições não são interrompidas, por examplo $(AT)_{20}$; imperfeitos, quando a sequência de DNA é interrompida por diferentes nucleotídeos que não se repetem, por exemplo $(AT)_{12}GC(AT)_{8;}$ e composto, quando existe dois ou mais motivos de repetição *in tandem*, por exemplo $(AT)_7(GC)_6$ (Egan *et al*., 2012).

Os SSRs possuem alta taxa de mutação por geração e, consequente alto polimorfismo, podendo variar entre genótipos individuais (Hoshinoe e*t al*., 2012; Zalapa *et al.*, 2012). *Crossing over* desigual e *slippage* durante a replicação do DNA são os principais mecanismos sugeridos aos processos de mutação responsáveis pela alteração no número de cópias das unidades repetidas. Normalmente, assume-se que os microssatélites evoluem de forma neutra, permitindo que alguns modelos de evolução sejam aplicáveis (Li *et al.*, 2002; Ellegren, 2004). Além disso, como as regiões flanqueadoras (*primers*) dos SSRs são conservadas, geralmente os SSRs são também aplicáveis entre espécies relacionadas ou até mesmo entre gêneros (Provan *et al.*, 2001; Hoshino *et al*., 2012). Assim, sua ampla distribuição em todo o genoma, o alto polimorfismo e a fácil transferência entre espécies proporcionam uma ampla e bem sucedida aplicação desses marcadores em estudos sobre processos ecológicos e evolutivos que dão forma às populações de plantas (Ellegren, 2004; Provan e*t al.*, 2011; Ebert & Peakall, 2009).

O emprego de sequenciamento de próxima geração (do inglês, *next-generation sequencing* NGS) permite isolar e desenvolver facilmente marcadores SSR de genomas nucleares e plastidiais (Egan *et al*., 2012). Tal método é mais rápido e barato em comparação às abordagens tradicionais (Egan *et al*., 2012; Zalapa *et al*,. 2012) e, uma vez desenvolvidos, os marcadores de SSRs são rápidos e fáceis de usar. Nesse contexto, um dos objetivos do nosso trabalho foi contribuir com o desenvolvimento de SSR’s para facilitar futuros estudos filogeográficos que busquem resolver questões ecológicas e evolutivas de *Pachyptera*.

## OBJETIVOS

O presente estudo possui quatro objetivos centrais:

1. Reconstruir a filogenia do gênero *Pachyptera* (Capítulos 1 e 2);

2. Reconstruir a história biogeográfica do gênero (Capítulo 2);

3. Elaborar uma revisão taxonômica do grupo (Capítulo 3);

4. Desenvolver microssatélites de cloroplasto para serem utilizados em futuros estudos filogeográficos e de genética de população em *Pachyptera* (Capítulo 4).

## ESTRUTURA DA DISSERTAÇÃO

A dissertação é composta por quatro capítulos cuja formatação segue à revista para onde o manuscrito foi submetido. Em linhas gerais, os capítulos contêm os seguintes estudos:

***Capítulo 1.*** Este capítulo incluiu um primeiro estudo filogenético do gênero contendo todas as espécies circunscritas em *Pachyptera* segundo a classificação mais recente do gênero (Lohmann & Taylor, 2014). Nesse estudo, reconstruímos o parentesco entre as espécies e gêneros da tribo Bignonieae utilizando dois marcadores moleculares (*Pep*C e *ndh*F) de forma a testar o monofiletismo do grupo. Neste trabalho uma espécie de *Pachyptera* aparece como mais proximamente relacionada à *Mansoa* e o reestabelecimento de *M. ventricosa* é proposto. Além disso, apresentamos uma descrição detalhada dessa espécie, mapa de distribuição e sua primeira ilustração. Esse manuscrito foi aceito para publicação na revista *Phytotaxa*.

***Capítulo 2.*** Este capítulo inclui uma filogenia de *Pachyptera* reconstruída com base em uma ampla amostragem de indivíduos. A filogenia é analisada à luz de dados morfológicos e análises de coalescência de modo a avaliar a circunscrição de espécies dentro do complexo de espécies *P. kerere*. Com base nestas análises, cinco espécies são reconhecidas: (i) *P. aromatica*, (ii) *P. erythraea*, (iii) *P. incarnata*, (iv) *P. kerere*, e (iv) *P. linearis*. Além disso, este capítulo também inclui um estudo biogeográfico, que visa avaliar os principais fatores e rotas de diversificação do grupo na região amazônica

gerando hipóteses a serem testadas em estudos futuros. Este manuscrito foi submetido à revista *Systematic Botany*.

***Capítulo 3.*** Este capítulo inclui a revisão taxonômica de *Pachyptera* contendo uma chave de identificação para todas as espécies, descrições morfológicas, lista de sinônimos, informações sobre habitat, fenologia e mapas de distribuição para todos os taxa tratados. Adicionalmente, designamos dois lectótipos, propomos a elevação de uma variedade à nível específico e descrevemos uma nova espécie. Esse manuscrito foi submetido para publicação na revista *Phytokeys.*

***Capítulo 4.*** Este capítulo consiste na caracterização e desenvolvimento de microssatélites (SSRs), elaborados com base nas sequências do genoma de cloroplasto de *P. kerere* reconstruído através de sequenciamento de próxima geração. Neste trabalho realizamos testes de validação dos primers sintetizados e do grau de polimorfismo dos SSRs, bem como realizamos testes bem sucedidos de transferabilidade para outras espécies do gênero utilizando os microssatélites polimórficos. Esse manuscrito foi publicado na revista *Applications in Plant Science*.

## REFERÊNCIAS BIBLIOGRÁFICAS

Alcantara, S. & L.G. Lohmann. 2010. Evolution of floral morphology and pollination system in Bignonieae (Bignoniaceae). *American Journal of Botany* 97: 782–96.

Aleixo, A. 2004. Historical diversification of a terra-firme forest bird superspecies: A phylogeographic perspective on the role of different hypotheses of Amazonian diversification. *Evolution* 58: 1303–1317.

Anhuf, D., M.P. Ledru & H. Behling. 2006. Paleo–environmental change in Amazonian and African rainforest during the LGM. *Palaeogeography, Palaeoclimatology, Palaeoecology* 239: 510–527.

Antoine, P.O., D. De Franceschi, J.J. Flynn, A. Nel, P. Baby, M. Benammi & R. Salas–Gismondi. 2006. Amber from western Amazonia reveals Neotropical diversity during the middle Miocene. *Proceedings of the National Academy of Sciences* 103: 13595–13600.

Antonelli, A. & I. Sanmartín. 2011. Why are there so many plant species in the Neotropics? *Taxon* 60: 403–414.

Antonelli, A., J.A. Nylander, C. Persson & I. Sanmartín. 2009. Tracing the impact of the Andean uplift on neotropical plant evolution. *Proceedings of the National Academy of Sciences* 106: 9749–9754.

Barbosa Rodrigues, J. 1891. *Eclogae plantarum novarum*. Vellosia, Rio de Janeiro, 1: 1−133.

Brower, A.V. & M.G. Egan.1997. Cladistic analysis of *Heliconius* butterflies and relatives (Nymphalidae: *Heliconiiti*): A revised phylogenetic position for *Eueides* based on sequences from mtDNA and a nuclear gene. *Proceedings of the Royal Society of London* 264: 969–977.

Brown, K.S. Jr. 1987. *Biogeography and evolution of neotropical butterflies*. In: Biogeography and Quaternary history in tropical America (Eds. By T.C. Whitmore & G.T. Prance). Oxford Science Publications, Oxford, 66–104.

Bush, M.B. & P.E.D. Oliveira. 2006. The rise and fall of the refugial hypothesis of Amazonian speciation: A paleoecological perspective. *Biota Neotropica* 6.

Bush, M.B. 1994. Amazonian speciation: A necessarily complex model. *Journal of Biogeography* 21: 5–17.

Candolle, A.P. 1845. *Prodromus systematis naturalis regni vegetablis*. Paris. 9:175–176.

Colinvaux, P.A. & P.E. de Oliveira.2001. Amazon plant diversity and climate through the Cenozoic. *Palaeogeography, Palaeoclimatology, Palaeoecology* 166.1: 51–63.

Colinvaux, P.A., P.E. de Oliveira & M.B. Bush. 2000. Amazonian and neotropical plant communities on glacial time–scales: The failure of the aridity and refuge hypotheses. *Quaternary Science Reviews* 19: 141–169.

Cooke, G.M., N.L. Chao & L.B. Beheregaray. 2012. Marine incursions, cryptic species and ecological diversification in Amazonia: The biogeographic history of the croaker genus *Plagioscion* (Sciaenidae). *Journal of Biogeography* 39: 724–738.

Duellman, W.E. 1982. *Quaternary climatic–ecological fluctuations in the lowland tropics: Frogs and forests*. In: Biological Diversification in the Tropics (Ed. Prance, G.T.). Columbia University Press, New York, 389–402.

Dugand, A. 1955. *Bignoniaceas nuevas o notables de Colombia*. Caldasia 7: 16.

Ebert, D., & R.O.D. Peakall. 2009. Chloroplast simple sequence repeats (*cp*SSRs): Technical resources and recommendations for expanding *cp*SSR discovery and applications to a wide array of plant species. *Molecular Ecology Resources* 9: 673–690.

Egan, A.N., Schlueter, J. & D.M. Spooner. 2012. Applications of next–generation sequencing in plant biology. *American Journal of Botany* 99: 175–185.

Ellegren, H. 2004. Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics* 5**:**435–445.

Fearnside, P.M. 1999. Biodiversity as an environmental service in Brazil's Amazonian forests: Risks, value and conservation. *Environmental conservation* 26: 305–321.

Funk, W.C., J.P. Caldwell, C.E. Peden, J.M. Padial, I. de la Riva & D.C. Cannatella. 2007. Tests of biogeographic hypotheses for diversification in the Amazonian forest frog (*Physalaemus petersii*). *Molecular Phylogenetics and Evolution* 44: 825–837.

Gentry, A.H. 1974. Coevolutionary patterns in Central American Bignoniaceae. *Annals of the Missouri Botanical Garden* 61: 728–759.

Gentry, A.H. 1980. Bignoniaceae Part I – Tribes Crescentieae e Tourretieae. *Flora Neotropica* 25: 1–130.

Gentry, A.H. 1982. Neotropical floristic diversity: Phytogeographical connections between Central and South America, Pleistocene climatic fluctuations, or an accident of the Andean orogeny? *Annals of the Missouri Botanical Garden* 69: 557–593.

Gentry, A.H. 1973. Generic delimitations of Central American Bignoniaceae. *Brittonia* 25: 226–242.

Gentry, A.H. 1979. Additional generic mergers in Bignoniaceae. *Annals of the Missouri Botanical Garden* 66: 778–787.

Gentry, A.H. 1992. A synopsis of Bignoniaceae ethnobotany and economic botany. *Annals Missouri Botanical Garden* 79: 53–64.

Gentry, A.H. 1992. Tropical forest biodiversity: Distributional patterns and their conservational significance. *Oikos* 63: 19–28.

Haffer, J. 1969. Speciation in amazonian forest birds. *Science* 165:131–137.

Haffer, J. 1979. *Quaternary biogeography of tropical lowland South America*. In: The South American herpetofauna: its origin, evolution, and dispersal (Ed. Duellmann, W.E.). University of Kansas/Museum of Natural History, Kansas, 107–140.

Haffer, J.R. 1997. Alternative models of vertebrate speciation in Amazonia: an overview. *Biodiversity and Conservation* 6: 451–476.

Hall, J.P. & D.J. Harvey. 2002. The phylogeography of Amazonia revisited: New evidence from riodinid butterflies. *Evolution* 56: 1489–1497.

Hoorn, C. *et al*. 1993. Marine incursions and the influence of Andean tectonics on the Miocene depositional history of northwestern Amazonia: Results of a palynostratigraphic study. *Palaeogeography, Palaeoclimatology, Palaeoecology* 105: 267–309.

Hoorn, C., *et al*. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* 330: 927–931.

Hoorn, C., *et al.* 1995. Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. *Geology* 23: 237–240.

Hoshino, A.A, J.P. Bravo, P.M. Nobile & K.A. Morelli. 2012. *Microsatellites as tools for genetic diversity analysis*. In: Genetic diversity in microorganisms (Ed. Caliskan, M). Tech, Rijeka, 149–170.

Leite, R.N.& D.S. Rogers. 2013. Revisiting Amazonian phylogeography: Insights into diversification hypotheses and novel perspectives. *Organisms Diversity and Evolution* 13: 639–664.

Lewinsohn, T. M. & P. I. Prado .2005. How many species are there in Brazil? *Conservation Biology* 19: 619–624.

Li, Y.C., A.B. Korol, T. Fahima, A. Beiles & E. Nevo. 2002. Microsatellites: Genomic distribution, putative functions and mutational mechanisms: A review. *Molecular Ecology* 11: 2453–2465.

Lohmann, L.G. & C.M. Taylor. 2014. A new generic classification of tribe Bignonieae (Bignoniaceae). *Annals of the Missouri Botanical Garden* 99: 348–489.

Lohmann, L.G. 2006. Untangling the phylogeny of neotropical lianas (Bignonieae, Bignoniaceae). *American Journal of Botany* 93: 304–318.

Lohmann, L.G. & C. Ulloa Ulloa. *Bignoniaceae*. Disponível em: <www.iplants.org>. Acesso em: 12 jan. 2016.

Lourenço, W.R. & E. Florez, 1991. Scorpions (Chelicerata) from Colombia: III. The scorpion fauna of Pacific region (Choco), with some biogeographic considerations. *Amazoniana* 11: 119–134

Lovejoy, N.R., J.S. Albert & W.G. Crampton. 2006. Miocene marine incursions and marine/freshwater transitions: Evidence from Neotropical fishes. *Journal of South American Earth Sciences* 2: 5–13.

Machado, I.C. & S. Vogel. 2004 The North–East–Brazilian liana, *Adenocalymna dichilum* (Bignoniaceae) pollinated by bats. *Annals of Botany* 93: 609–613.

Malhi, Y., J.T. Roberts, R.A. Betts, T.J. Killeen, W.H. Li & C.A. Nobre. 2008. Climate change, deforestation, and the fate of the Amazon. *Science* 319: 169–172.

Moritz, C., J. L. Patton, C. J. Schneider & T. B. Smith. 2000. Diversification of rainforest faunas: An integrated molecular approach. *Annual Review of Ecology and Systematics* 31: 533–563.

Olmstead, R.G., M.L. Zjhra, L.G. Lohmann, S.O. Grose & A.J. Eckert. 2009. A molecular phylogeny and classification of Bignoniaceae. *American Journal of Botany* 96: 1731–1743.

Patton, J.L., M.N.F. da Silva & J.R. Malcolm. 2000. Mammals of the Rio Juruá and the evolutionary and ecological diversification of Amazonia. *Bulletin of the American Museum of Natural History* 244: 1–306.

Pons, D. & D. Franceschi. 2007. Neogene woods from western Peruvian Amazon and palaeoenvironmental interpretation. *Bulletin of Geosciences* 82: 343–354.

Prance, G.P. 1982. A review of the phytogeographic evidences for Pleistocene climate changes in the neotropics. *Annals of the Missouri Botanical Garden* 69: 594–624

Provan, J., W. Powell & P.M. Hollingsworth. 2001. Chloroplast microsatellites: New tools for studies in plant ecology and evolution. *Trends in Ecology & Evolution* 16: 142–147.

Räsänen, M., A. Linna, G. Irion, L. Rebata Hernani, R. Vargas Huaman & F. Wesselingh 1998. *Geología y geoformas de la zona de Iquitos*. In: Geoecología y desarollo Amazónico: estudio integrado en la zona de Iquito (Eds. Kalliola, R. & S. Flores Paitán). Annales Turkuensis, Peru, 114: 59–137

Ribas C.C., A. Aleixo, A.C.R. Nogueira, C.Y. Miyaki & J. Cracraft. 2011. A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years *Proceedings of the Royal Society of London* 279: 681–689.

Ribas, C.C.& C.Y. Miyaki. 2004. Molecular systematics in *Aratinga* parakeets: Species limits and historical biogeography in the 'solstitialis' group, and the systematic position of *Nandayus nenday*. *Molecular Phylogenetics and Evolution* 30: 663–675.

Ron, S.R. 2000. Biogeographic area relationships of lowland Neotropical rainforest based on raw distributions of vertebrate groups. *Biological Journal of the Linnean Society* 71: 379–402.

Rull, V. 2011. Neotropical biodiversity: Timing and potential drivers. *Trends in Ecology and Evolution* 26: 508–513

Scotti–Saintagne C. *et al.*. 2013. Amazon diversification and cross– Andean dispersal of the widespread Neotropical tree species *Jacaranda copaia* (Bignoniaceae). *Journal of Biogeography* 40: 707–719.

Silva, J.M.C.& D.C. Oren. 1996. Application of parcimony analysis of endemicity (PAE) in Amazon biogeography: An example with primates. *Biological Journal of the Linnean Society* 59: 427–437.

Spangler, R.E. & R.G. Olmstead. 1999. Phylogenetic analysis of Bignoniaceae based on the cpDNA gene sequences *rbcL* and *ndhF*. *Annals Missouri Botanical Garden* 86: 33–46.

Sponsel, L.E. 1995. Indigenous peoples and the future of Amazonia: An ecological anthropology of an endangered world. University of Arizona Press.

Sprague, T.A. & N.Y. Sandwith. 1932. Contributions to the flora of tropical America: X. New and noteworthy Bignoniaceae from British Guiana, mainly collected by the Oxford University Expedition, 1929. *Bulletin of Miscellaneous Information Kew* 1932: 81–93.

Wallace, A.R. 1852. On the monkeys of the Amazon. *Proceedings of the Zoological Society of London* 20: 107–110.

Wesselingh, F.P. & J.A. Salo. 2006. A Miocene perspective on the evolution of the Amazonian biota. *Scripta Geologica* 133: 439–458.

Wesselingh, F.P. 2006. Miocene long–lived Lake Pebas as a stage of mollusc radiations, with implications for landscape evolution in Western Amazonia. *Scripta Geologica* 133:1–14.

Wesselingh, F.P., C. Hoorn, S.B. Kroonenberg, A. Antonelli, J.G. Lund berg, H.B. Vonhof & H. Hooghiemstra. 2010. *On the origin of Amazonian landscapes and biodiversity: A synthesis.* In: Amazonia: Landscape and species evolution (Eds. Hoorn, C. & F. Wesselingh). Wiley-Blackwell, Oxford, 421–431

Zalapa , J.E. *et al*. . 2012 . Using next–generation sequencing approaches for the isolation of simple sequence repeat (SSR) loci in the plant sciences. *American Journal of Botany* 99: 193–208 .

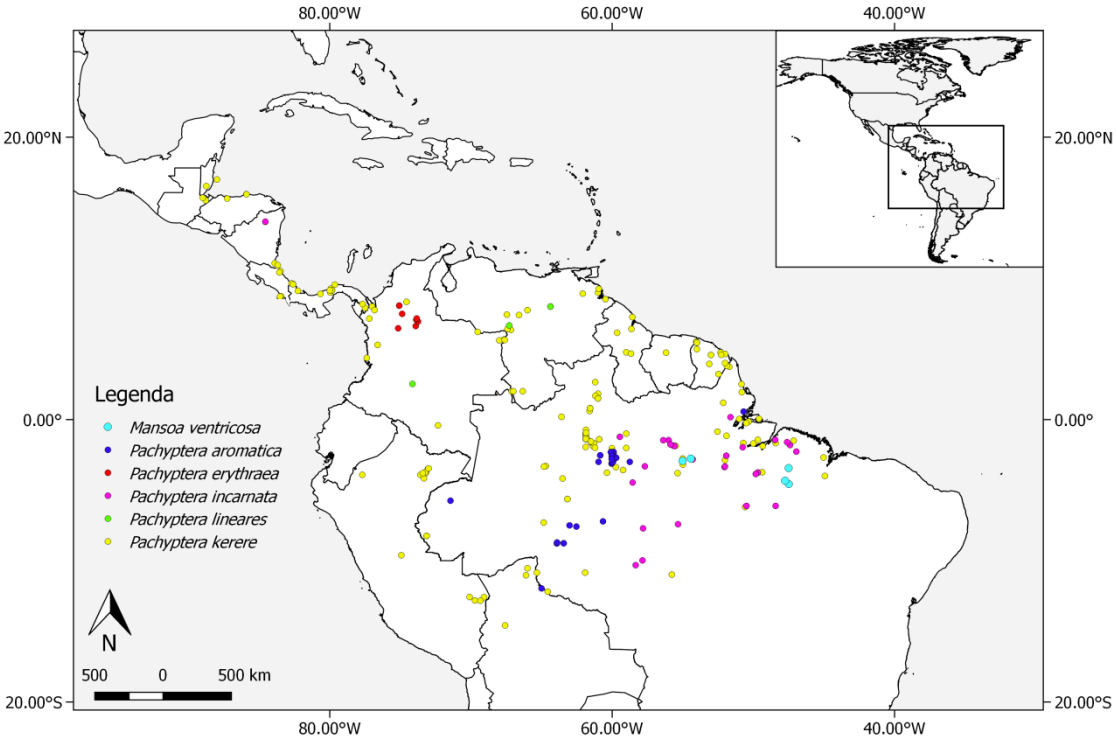


**Figura 3.** Distribuição de *Pachyptera* e *Mansoa ventricosa*, indicando as localidades de ocorrência das espécies tratadas neste trabalho.

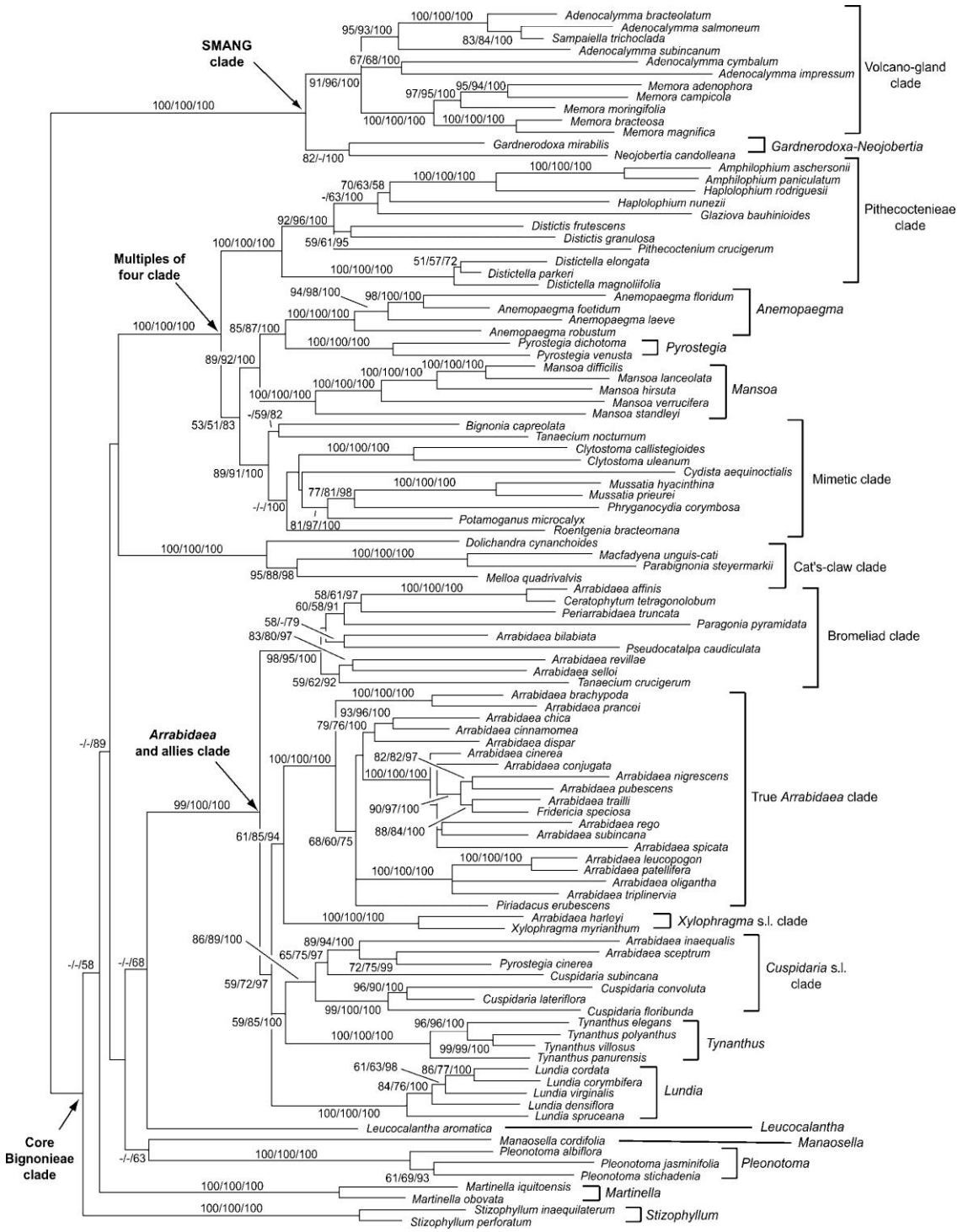


**Figura 2.** Árvore de consenso estrito oriunda da análise de máxima verossimilhança do conjunto de dados combinado (*ndh*F e *Pep*C). Esta filogenia foi utilizada como base para a atual classificação genérica da tribo Bignonieae (Lohmann & Taylor, 2014). *Leucocalantha* = *Pachyptera*. Árvore retirada de Lohmann (2006).

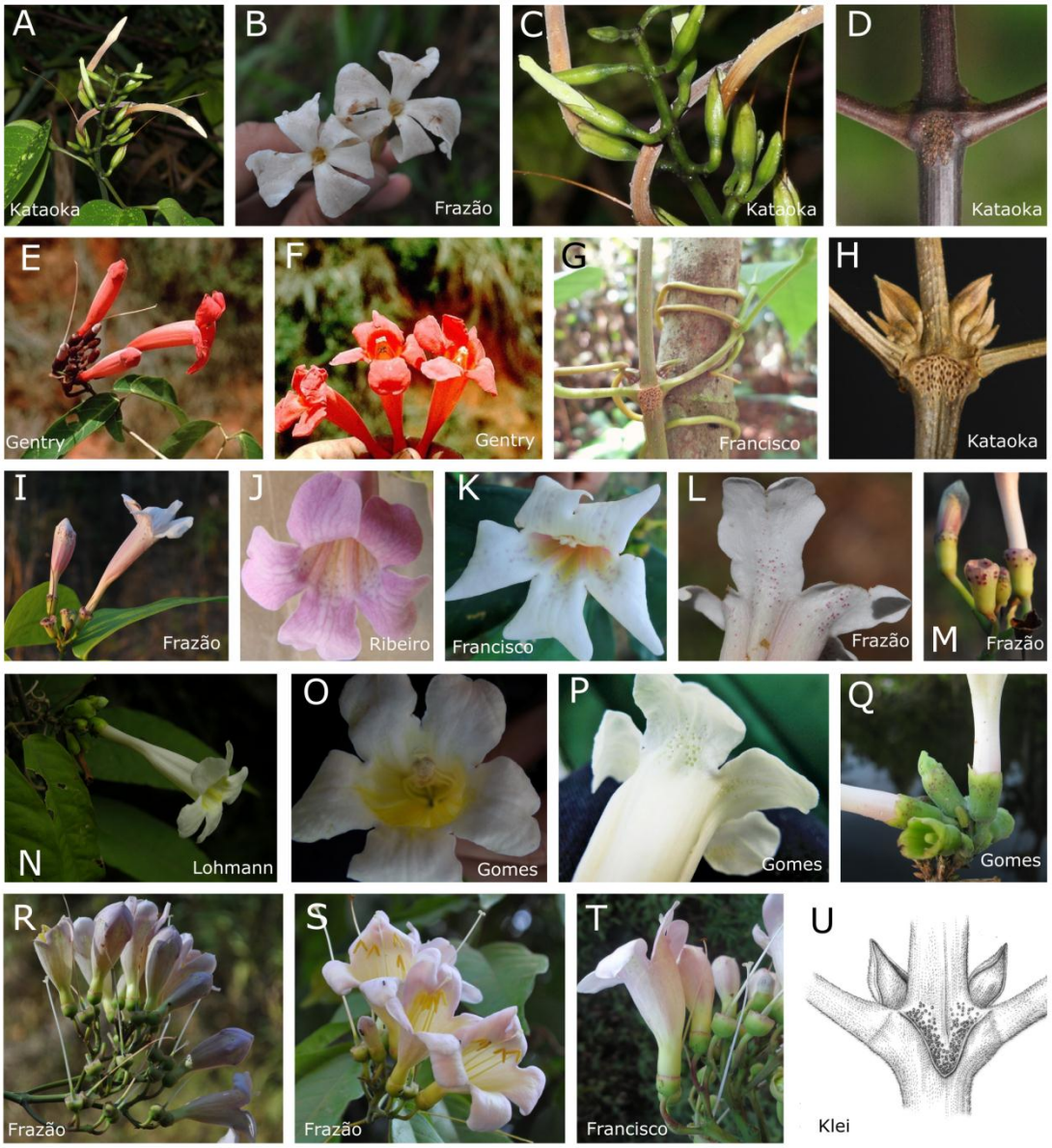


**Figura 3.** Características morfológicas de *Pachyptera aromatica* (A-D), *Pachyptera erythraea* (E-F), *Pachyptera incarnata* (G-M), *Pachyptera kerere* (N-Q) e *Mansoa ventricosa* (R-U).

# Capítulo 1

---

**Reestablishment of *Mansoa ventricosa* (Bignonieae, Bignoniaceae) based on molecular and morphological data**

Jessica Nayara Carvalho Francisco and Lúcia G. Lohmann

**Abstract**

*Pachyptera ventricosa* is a rare and poorly known species of liana that occurs in wet forests from the state of Pará, in Brazil. This species is characterized by features that are diagnostic of four genera within tribe Bignonieae: *Mansoa*, *Martinella*, *Pachyptera*, and *Tanaecium*. The currently recognized *P. ventricosa* was originally described in *Mansoa*, but subsequently transferred to *Pachyptera* based on morphological similarities. In this study, we use a combination of molecular and morphological data to evaluate the current placement of *P. ventricosa*. We conduct a broad scale molecular phylogenetic study based on 114 sequences of *ndh*F and *Pep*C representing 112 taxa and members of all genera recognized in Bignonieae. In addition, we also conduct detailed morphological studies of selected characters. Our molecular phylogenetic study indicates that the currently recognized *Pachyptera ventricosa* is more closely related to members of *Mansoa* than to *Pachyptera*. New morphological data corroborates the molecular phylogenetic placement indicating that this species is indeed best placed within *Mansoa*. We here propose the reestablishment of *Mansoa ventricosa*, and show the detailed description for this species, along with new distribution information, and the first illustration for this taxon.

**Key words:** Amazonian biota, Brazilian flora, generic circumscription.

**Introduction**

Bignonieae is the largest tribe in the plant family Bignoniaceae. It is composed mostly by neotropical lianas, except from *Bignonia capreolata* L. (1753: 624) that occurs in southeastern United States (Gentry 1979). The broad morphological diversity of members of the tribe, coupled with a lack of phylogenetic information has led to a problematic generic delimitation within the tribe in the past (Gentry 1973). The first phylogeny of Bignonieae recovered 21 generic-level clades (Lohmann 2006), that were subsequently recognized as genera in the first comprehensive generic-level classification for the whole tribe (Lohmann & Taylor 2014). While current generic limits (Lohmann & Taylor 2014) are quite stable, there are still a few genera whose delimitation may need adjustments. For instance, the circumscription of *Mansoa* de Candolle (1838: 128) and *Pachyptera* de Candolle (1845: 175 299) has been problematic historically and may still need some refinement. These genera have several overlapping morphological features (Figure 1) that has led to multiple nomenclatural changes during the last Century (Table 1).

When *Mansoa* was first described, it included two species, *M. hirsuta* de Candolle (1845: 182) and *M. laevis* de Candolle (1845: 182) [= *M. difficilis* (Chamisso 1832: 714) Bureau & Schumann (1896 [1897]: 201)]. The genus was then characterized by a bilabiate calyx with five subulate denticles, an infundibuliform corolla, inserted stamens, thick nectariferous disc, and an oval-oblong ovary. Under the most recent treatment of the tribe (Lohmann & Taylor 2014), *Mansoa* includes 12 species. Five additional species were subsequently described (Silva-Castro & Queiroz 2016), expanding the genus to 17 species. Species of *Mansoa* are characterized by angular stems, prophylls of the axillary buds minute and triangular or bromeliad-like, garlic smell in the foliage, leaflets with basal actinodromous venation and nectaries at the

base, trifid tendrils, pink to purple corollas that are pubescent outside, and linear fruits. The genus is strongly supported as monophyletic by molecular characters and is positioned within the "multiples-of four" clade (Lohmann 2006).

On the other hand, when *Pachyptera* was first described it included six species characterized by seeds with coriaceous wings (de Candolle 1845): *P. umbelliformis* de Candolle (1845: 175), *P. striata* de Candolle (1845: 176), *P. dasyantha* de Candolle (1845: 176), *P. perrottetii* de Candolle (1845: 176) [all synonyms of *Tanaecium pyramidatum* (Richard 1792: 110) L.G. Lohmann (2008: 274)], *P. puberula* de Candolle (1845: 175) [= *Dolichandra uncata* (Andrews 1808: tab. 530) L.G. Lohmann (2008: 273)], and *P. foveolata* de Candolle (1845: 175) [= *Pachyptera kerere* (Aublet 1775: 644) Sandwith (1937: 219)]. Currently, *Pachyptera* includes only four species: *P. aromatica* (Barbosa Rodrigues 1891: 47) L.G. Lohmann (2014: 456), *P. erythraea* (Dugand 1955: 16) A.H. Gentry (1977: 186), *P. kerere* (Aublet 1775: 644) Sandwith (1937: 219), and *P. ventricosa* (A.H.Gentry 1979 [1980]: 783) L.G. Lohmann (2014: 456). In its current circumscription, species in the genus are characterized by stems with four phloem wedges, bark papery that peels off as the branchlets age, interpetiolar glands, trifid tendrils, prophylls of the axillary buds flattened and ensiform or minute and triangular, tubular truncate calyces, white tubular corollas with glands arranged in lines in the upper portion, and linear fruits with glands scattered throughout the surface (Lohmann & Taylor 2014). Circumscription of *Pachyptera* has also been supported by information from a broad molecular phylogeny of the whole tribe Bignonieae that sampled half of the species currently recognized in *Pachyptera*. More specifically, Lohmann (2006) sampled *P. kerere* and *P. aromatica*, but did not include *P. erythraea* and *P. ventricosa*.

The generic placement of *P. ventricosa* is particularly complicated as this species possess morphological features that are shared among multiple genera. For instance, this species was originally described within *Mansoa* due to the trifid tendrils, interpetiolar gland fields, striate branchlets, and corolla densely pubescent on lobes (Gentry 1979 [1980]). However, these traits are also found in *Pachyptera*, with which *P. ventricosa* also shares tricolpate pollen. Furthermore, *P. ventricosa* shares subulate prophylls of the axillary buds with *Tanaecium* Swartz (1788: 91) emend L.G. Lohmann in L.G. Lohmann & Taylor (2014: 463), and a campanulate corolla and tricolpate coarse reticulate pollen with *Martinella* Baillon (1891 [1888]: 30). This broad morphological diversity has led to an unclear position for this taxon since its description. Indeed, when this species was first described, Gentry (1979 [1980]: 783) noted that: "the species has a combination of features of so many genera making it difficult to establish its clear position, especially because the fruit is unknown."

In this study, we use molecular phylogenetic data and new morphological information to investigate the generic placement of *Pachyptera ventricosa.* We paid particular attention to pollen characters, which are known to aid generic or specific delimitations within Bignoniaceae (e.g., Gentry & Tomb 1979, Zuntini *et al.* 2015). We propose moving *P. ventricosa* to the genus *Mansoa* and the reestablishment of *Mansoa ventricosa*, as well as a detailed description for this species, along with new information on its distribution, and the first illustration for this taxon.

**Material and methods**

*Sampling, DNA extraction, PCR amplification and sequencing*:—We extracted total genomic DNA from herbarium and silica-dried leaflets of the four species of

*Pachyptera* recognized by Lohmann & Taylor (2014): *P. aromatica*, *P. erythraea*, *P. kerere*, and *P. ventricosa*. Extractions were conducted using the Invisorb Plant Mini Kit (Invitek, Berlin, Germany), following the manufacturer's protocol. We amplified the chloroplast marker *ndh*F (NADH dehydrogenase) following Zuntini *et al.* (2013) and the nuclear marker *Pep*C (Phosphoenolpyruvate carboxylase) using a nested PCR approach with the external primers (4F and 5R) from Lohmann (2006) for the first round, and internal primers (IV-119F and V-25R) from Zuntini *et al.* (2013) for the second round. The first-round PCR contained 8.5 μL of $H_2O$, 1 μL dimethyl sulfoxide (DMSO), 12.5 μL GoTaq Promega Master Mix, 1 μL 10mM each primer and 1 μL 10 ng of template DNA. Cycling conditions were as follows: 94°C for 3 min, 20 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 5 min. Whenever PCR amplifications were unsuccessful, we added 5 μL 5 M of betaine and adjusted the volume of water to a reaction of 25 μL. We used 1 μL of the first round PCR from 9:1 dilution in water as template for the second PCR, which followed the same conditions as the first PCR. We then loaded 2 μL of the second round PCR product onto a 1% agarose gel to verify the amplification and size of the amplified product. Products were purified and sequenced by Macrogen (Seoul, South Korea). Sequences were deposited on GenBank under the accessions KY983570 to KY983578.

*Molecular datasets and phylogenetic analyses*:—We combined the five *ndh*F and *Pep*C newly generated sequences of *Pachyptera* with the combined molecular dataset of Lohmann (2006) that consists of *ndh*F and *Pep*C sequences for 104 taxa. In addition, we included three additional *ndh*F sequences of *Mansoa* and one of *Perianthomega* from Lohmann (2006) (i.e., *Mansoa alliacea*, *Mansoa parvifolia*, and *Perianthomega vellozoi*), plus *ndh*F and *Pep*C sequences of *Mansoa ononhualcoides* from Fonseca & Lohmann (2015). Following Lohmann (2006) we used *Tecoma*

*capensis* Lindley (1827 [1828]: 13) as outgroup. The final dataset included 114 sequences of *ndh*F and 110 sequences of *Pep*C representing 111 species (Appendix 1). Individuals not sampled for *Pep*C were coded as missing data in the final matrix. Sequences were aligned with MAFFT (Katoh *et al.* 2002) using default parameters (Auto algorithm, Scoring matrix: 200PAM/k=2, Gap open penalty: 1.53, Offset value 0.123) in Geneious 9.0.2 (Kearse *et al.* 2012). Alignments were subsequently analyzed visually and adjusted manually. We used jModelTest 2.0 (Guindon & Gascuel 2003, Darriba *et al.* 2012) and the Akaike information criterion (AIC) to select the best-fit model of nucleotide substitution for each dataset. The TVM+I+G was selected as the best model for the *ndh*F dataset whereas the TVM+G was selected as the best model for *Pep*C. Bayesian Inference (BI) analyses were conducted using MrBayes 3.1 (Ronquist & Huelsenbeck 2003) with four Markov Chain Monte Carlo (MCMC) runs using a random starting tree, and 10 million generations, with a sampling frequency of one every 1000 generations. We used Tracer 1.5 (Rambaut & Drummond 2014) to check for convergence of the MCMC chain and to check for stationarity. We discarded 25% of the trees as burn-in.

*Morphological studies*:—We prepared a detailed description for *P. ventricosa* based on voucher specimens deposited at COL, MG, MO, SPF, NY, US, and UB (acronyms following Thiers 2017) and fresh material collected during field expeditions to the Brazilian state of Pará. Morphological descriptions follow the terminology of Lohmann & Taylor (2014), with additional terms from Radford *et al.* (1974) and Hickey (1973) for leaf morphology and venation, Nogueira *et al.* (2013) for trichomes, and Weberling (1992) for inflorescences.

We further analyzed calyx, corolla, stamen, and pollen morphology using a Zeiss DSM 970 Scanning Electron Microscope (SEM). We studied selected morphological structures from a representative specimen of *P. ventricosa* (*J.N.C. Francisco 84*, SPF). These structures were mounted on stubs and sputter-coated with gold. Pollen terminology follows Hesse *et al.* (2009) and Gentry & Tomb (1979).

We compared the new morphological data obtained for *P. ventricosa* in this study to the description of *Pachyptera* and *Mansoa* available in the most recent generic treatment of the tribe (Lohmann & Taylor 2014).

**Results**

*Phylogenetic placement of Pachyptera ventricosa*

We obtained high quality *ndh*F and *Pep*C sequences from five accessions of *Pachyptera* (Appendix 1). The new *ndh*F sequences of *P. ventricosa* were 2066 bp long, which is the same length as other *Pachyptera* sequences in our data set. The length of *Pep*C sequences of *P. ventricosa* ranged from 315 to 441 bp, while the other sequences of *Pachyptera* in our data set ranged from 341 to 402 bp. The final combined Bignonieae dataset contains 2977 bp and included 114 terminals, one of which was the outgroup belonging to tribe Tecomeae. The *ndhF* alignment was 2108 bp long, whereas the *Pep*C alignment was 869 bp long. In the final alignment we found an insertion of 3 bp in the *ndh*F dataset supporting a clade with five species of *Mansoa* (i.e., *M. verrucifera*, *M. hirsuta*, *M. laceolata*, *M. difficilis* and *M. alliacea*). Multiple indels were recovered in the *Pep*C dataset. Among those indels there was 7 bp insertion (GACGTAT) unique to *M. standleyi*.

Both accessions of *P. ventricosa* formed a clade that was distantly related to the other three species of *Pachyptera* sampled. The *P. ventricosa* clade was nested within *Mansoa* and strongly supported (PP=0.97) as sister to a clade containing *M. alliacea*, *M. difficilis*, *M. hirsuta*, *M. lanceolata*, and *M. verrucifera*. This whole clade is sister to a smaller clade composed by *M. onohualcoides* and *M. standleyi*. *Mansoa parvifolia* emerges as sister to the remaining species sampled. On the other hand, *Pachyptera* (excluding *P. ventricosa*) is poorly supported as monophyletic (PP=0.54). The genus as a whole includes a *P. aromatica* clade that is sister to a strongly supported clade (PP=1.0) that included *P. erythraea* and *P. kerere* (Figure 2). Overall, other relationships recovered in the BI analyses were identical to those recovered by Lohmann (2006).

*Morphological studies of Pachyptera ventricosa*

*Pachyptera ventricosa* has a number of vegetative and reproductive characters in common with species of *Mansoa*. For instance, extra-floral nectaries (EFN) grouped on the abaxial leaflet surface and actinodromous venation are found exclusively in *P. ventricosa* and *Mansoa*. In addition, *P. ventricosa* and *Mansoa* share uniseriate prophylls of the axillary buds, unlike other *Pachyptera* species, all of which have 3-seriated prophylls (Figures 1B, F). The thyrsoid inflorescence and light purple flower color are also shared between *P. ventricosa* and *Mansoa*, whereas remaining species of *Pachypera* have a racemose inflorescence with flowers that vary in color from white, light pink to red (Table 2).

Our SEM studies showed that *P. ventricosa* has a densely puberulous calyx that bears well-developed peltate and patelliform glands. This same type of glands is also

found on the corolla lobes, which includes a pair of clustered glands arranged in lines similar to those found in *Pachyptera*. The anther connective bears short simple trichomes similar to some species of *Mansoa*. The pollen is tricolpate and coarse reticulate showing many bacula into the lumen (Figure 3) that are more similar to those found in members of *Mansoa* than members of *Pachyptera* (Table 2).

**Discussion**

Our phylogenetic and morphological study has demonstrated that *P. ventricosa* is more closely related to species in the genus *Mansoa* than any other species of *Pachyptera*. Moreover, this phylogeny also recovers a monophyletic *Pachyptera*, excluding *P. ventricosa*. However, *Pachyptera* is only poorly supported as monophyletic, indicating the need for additional studies (Francisco & Lohmann in prep.). In addition, the placement of *P. erythraea* within *Pachyptera* corroborates the recent inclusion of this species by Lohmann & Taylor (2014) based only in morphology.

Our study highlights the importance of combining molecular phylogenetic data with morphological studies while assessing the placement of taxonomically complicated species (e.g., Pace *et al.* 2016). More specifically, our morphological study indicates that *P. ventricosa* shares thyrsoid inflorescences with purple flowers, glandular stipitate trichomes on the upper portions of the corolla tube, pubescent anther connective, extra-floral nectaries grouped on the abaxial surface of leaflets, and actinodromous venation with members of *Mansoa* (Figures 3, 4). However, *P. ventricosa* also shares a number of other features with *Pachyptera*, namely the trifid tendrils, glands near the calyx margin and upper portion of the corolla tube, and glands at the interpetiolar region, all of which led Lohmann & Taylor (2014) to place this species in *Pachyptera*.

Nevertheless, *P. ventricosa* lacks the supra-numerary prophylls and the white tubular corolla, that are so typical of *Pachyptera*, only lacking in *P. erythraea* (red corolla) and a couple populations of *P. kerere* that have pink corollas.

Pollen morphology also supports the inclusion of *P. ventricosa* within *Mansoa*. More specifically, while species of *Pachyptera* have tetracolpate pollen, that is psilate, foveolate (*P. aromatica*) or tricolpate, microrreticulate (*P. kerere* and *P. erythraea;* Francisco and Lohmann in prep.), species of *Mansoa* have pollen that is tricolpate and reticulate (Gentry & Tomb 1979). The combination of tricolpate pollen and reticulate ornamentation is exclusive of *Mansoa* (Silva-Castro 2010), and has never been found within *Pachyptera*. The pollen type of *P. ventricosa* (Figures 5G, H) is tricolpate and coarse reticulate, similar to that of other *Mansoa* (i.e., tricolpate and reticulate), further supporting the placement of *P. ventricosa* within *Mansoa*. The tricolpate and reticulate pollen is also found in *M. parvifolia* (A.H. Gentry 1973: 447) A.H. Gentry (1979 [1980]: 783), and *M. standleyi* (Steyermark 1947: 235) A.H. Gentry (1979 [1980]: 783), corroborating our molecular phylogenetic findings, and indicating that *P. ventricosa* is best placed within *Mansoa*.

In light of the novel molecular phylogenetic and morphological data gathered here, we propose the reestablishment of *Mansoa ventricosa*, bringing the number of species of *Mansoa* to 18.

**Taxonomy treatment**

***Mansoa ventricosa*** A.H. Gentry (1979 [1980]: 783). *Pachyptera ventricosa* (A.H. Gentry) L.G. Lohmann (2014: 456).

Type:—BRAZIL. Pará: Along the Belém-Brasilia highway, km 345, 9 August 1956, *B. Maguire et al. 56083* (holotype, MO-2232816!; isotypes, COL-110166 not seen, MG-136673, NY-328882!, US-3189002 image!).

*Liana*; branchlets cylindrical, striated, swollen at nodes, without lenticels, sparsely to moderately puberulous, with simple and glandular peltate trichomes, without onion smell, with "V" shaped interpetiolar glands fields, with a continuous interpetiolar ridge; pith solid, with four phloem wedges in cross-section; prophylls of the axillary buds persistent, subulate, paired, 3.94–4.85 × 1.98–2.07 mm, sparsely to densely puberulous, with simple and glandular peltate trichomes. *Leaves* 2-3-foliolated, with the terminal leaflet often replaced by a trifid tendril; petiole cylindrical, 0.8–3.2 cm, sparsely to densely puberulous, with simple and glandular peltate trichomes; petiolule cylindrical, 0.5–1.6 cm, sparsely to densely puberulous, with simple and glandular peltate trichomes; blade concolor, chartaceous, elliptic, apex caudate, sometimes mucronulate, base cuneate, obtuse or rounded, margin entire, flat or sub-revolute; lateral leaflets with 8.0–14.5 × 3.0–7.9 cm, abaxial surface glabrous or very sparsely puberulous, with simple trichomes distributed only on veins, glandular peltate trichomes distributed throughout the surface, with patelliform glandular trichomes grouped at base, adaxial surface sparsely puberulous, with simple trichomes distributed on veins, and with peltate glandular trichomes distributed over the lamina; venation basal, actinodromous, secondary venation festooned-brochidromous, tertiary venation random-reticulate. *Inflorescence* terminal, thyrse, congested, 6–9 cm long, densely puberulous, with simple and glandular peltate trichomes, many-flowered, ca. 15–34 flowers; pedicel with 1.0–1.9 cm long, moderately to densely puberulous, with simple and glandular peltate trichomes; bracts caducous; bracteole triangular or rhombic 0.04–1.68 mm, densely

puberulous, with simple and glandular peltate trichomes. *Calyx* green with apex light purple, cupular, minutely 5-denticulate, coriaceous, smooth, 0.4–0.6 × 0.4–0.6 cm, densely puberulous, with simple and glandular peltate trichomes externally, often with clustered patelliform glands near the margin, glabrous inside. *Corolla* cream or greenish at base, tube and lobes light purple, with yellowish mouth, campanulate, rounded, membranous, 4.2–5.1 cm long, 1.9–2.0 cm of diameter at the distal end (mouth), 0.4–0.5 cm diameter at the base, tube densely puberulous externally, with simple, dendritic, and glandular peltate trichomes, glabrous at base and internally, except from the point of staminode insertion, which is villose with stipitate glandular trichomes; lobes oblong, 1.3–1.5 × 0.7–1.2 cm, imbricate, densely puberulous outside, with simple and peltate glandular trichomes and patelliform glands arranged at the base of lobes, glabrous inside. *Androecium* didynamous; stamens glabrous, subexseted, longer 2.85–3.09 mm long, shorter 2.69–2.78 mm long; anthers yellow, glabrous, basifixed, connective thick, acute, round; thecae straight, 5.85–6.43 × 0.51–0.87 mm, with longitudinal slits; pollen tricolpate and coarse reticulate. *Gynoecium* 4.4–4.9 cm long, exserted, sparsely puberulous, with simple and glandular peltate trichomes; stigma ovate, 1.69× 3.52 mm, with irregularly toothed margin, glabrescent, with simple trichomes; ovary greenish, linear-oblong, 4.74–4.78 × 1.29–1.62 mm, smooth, densely puberulous, with simple, glandular, peltate and patelliform trichomes; ovules arranged in two series per locule, placentation axillar; nectary disc well-developed, 2.70–3.14 × 3.34–4.78 mm, pubescent with glandular peltate trichome. *Fruit* unknown.

**Phenology:**—Flowers from August to October; fruiting season is unknown.

**Pollination:**—The corolla morphology is classified as a variant of the *Martinella* type (Gentry 1974), and is likely associated with bat pollination (Alcantara & Lohmann

2010, Machado & Vogel 2004). This species shares tricolpate coarse reticulate pollen grains with *Martinella obovata* (Gentry & Tomb 1979) suggesting convergent evolution of pollen type. Such convergence has also been observed in other floral traits such as the purple colored flower, thick corolla texture, open mouth, and subexserted anthers.

**Distribution and habitat:**—*Mansoa ventricosa* is endemic to Northeastern Brazil (Maranhão, Pará), where it occurs in wet evergreen forests (Figure 5). Prior to this work, this species was only known from the holotype collected in the Brazilian state of Pará, a paratype collected in the Brazilian state of Maranhão (*G.T. Prance 58978*; UB and NY), and one collection from 1980 (*D.C. Daly 774*; MG, MO, and NY). Two additional specimens were collected during our fieldwork in Pará, (Brazil), expanding the distribution of this species to Santarém and Belterra.

**Conservation status:**—The species is only known from one locality of Maranhão and four localities from the state of Pará (i.e., Belterra, Itinga do Pará, Paragominas, and Santarém), and is categorized as Data Deficient (DD) according to IUCN Standards and Petitions Committee (2014). Further field studies are needed to evaluate its conservation status more accurately.

**Additional specimens examined:**—BRAZIL. Maranhão: 15 Km S of Para-Maranhão border on Belém-Brasília highway, in forest, 31 August 1963, *G.T. Prance* 58978 (UB, NY). Pará: Belterra, Floresta Nacional do Tapajós. Beira da estrada para Jamaraquá, km 74, 194 m, 02°55'50.2"S, 55°00'44.6"W, 164 m, 16 September 2015, *J.N.C. Francisco et al. 84* (SPF). Itinga do Pará, Fazenda Santa Rosa, W of Belém-Brasilia Hwy, 26 October 1980, *D.C. Daly 774* (MG, MO). Santarém, Beira da PA-370, 164 m, 02°46'10.1"S, 54°25'42.6"W, 164m, 19 September 2015, *J.N.C. Francisco et al. 102* (SPF).

**Taxonomic notes:**—*Mansoa ventricosa* is easily recognized by a combination of cylindrical and striated branchlets with "V" shaped interpetiolar clusters of glands, subulate prophylls of the axillary buds, and leaflets with nectaries grouped on the base of the abaxial surface. Reproductive characters that can help in its identification are the thyrsoid inflorescences, calyx with patelliform glands clustered next to the margin, corolla campanulate, with tube light purple, densely puberulous outside, with patelliform glands at the upper portion of the apex tube, androecium subexserted with yellow anthers, and exserted gynoecium.

**Acknowledgements**

The authors thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for a graduate fellowships to J.N.F. (163990/2014-0) and a Pq-1C grant to L.G.L. (307781/2013-5), and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for a regular research grant (2011/50859-2) and a collaborative FAPESP-NSF-NASA grant (2012/50260-6) to L.G.L. We are also grateful for the curators of the herbaria listed herein for specimen loans; Annelise F. Nunez, Augusto Giaretta, and Helcio H. Souza for field assistance; Thais E. Almeida and Leandro L. Giacomin for lodging in their lovely home in Santarém (Pará) and for field assistance; FLONA dos Tapajós, INPA/LBA, FLONA Saracá-Taquera, and EMBRAPA for collection permits and field support. We also thank Beatriz M. Gomes and Alexandre Zuntini for valuable comments on the manuscript; Klei Souza for preparing the illustrations; and, Annelise F. Nunez for providing us with a beautiful photo.

**References**

Alcantara, S. & L.G. Lohmann. (2010) Evolution of floral morphology and pollination system in Bignonieae (Bignoniaceae). *American Journal of Botany* 97: 782–96.

Andrews, H.C.(1808) *Botanist's Repository, for new, and rare plants*. t. 530.

Aublet, J.B.C.F. (1775) *Histoire des plantes de la Guiane Française v2*. F. Didot jeune, London & Paris, 622 pp.

Baillon, H.E. (1891) Bignoniaceae *In*: Histoire des plantes. Libraire Hachette & Co., Paris, pp. 1–58.

Barbosa Rodrigues, J. (1891) *Eclogae plantarum novarum*. Vellosia, Rio de Janeiro, 1: 133 pp.

Bureau, E. & Schumann, K.M. (1896 [1897]) Bignoniaceae. *In*: von Martius, C.F.P., Eichler, A.G. & Urban, I. (Eds.) *Flora Brasiliensis*. Lipsiae apud Frid. Fleischer in Comm. Monachii, Leipzig, 8(2): 1–452 pp.

Candolle, A.P. (1838) *Revue sommaire de la famile des Bignoniacées*. Bibliotheque Universelle de Genève, Genève, 2: 24 pp.

Candolle, A.P. de. (1845) *Prodromus systematis naturalis regni vegetabilis*. Lipsiae, Paris, 9: 573 pp.

Chamisso, A. (1832) Bignoniaceae in de Plantis in Expeditione Romanzoffiana. *Linnaea* 7: 689–723.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9: 772.

Dugand, G.A. (1955) Bignoniaceas nuevas o notables de Colombia. *Caldasia* 7: 7–32.

Fonseca, L.H.M., & Lohmann, L.G. (2015) Biogeography and evolution of *Dolichandra* (Bignonieae, Bignoniaceae). *Botanical Journal of the Linnean Society* 179: 403–420.

Gentry, A.H. (1974). Coevolutionary patterns in Central American Bignoniaceae. *Annals of the Missouri Botanical Garden* 61: 728–759.

Gentry, A.H. (1973) Generic delimitations of Central American Bignoniaceae. *Brittonia* 25: 226–242.

Gentry, A.H. (1973). Studies in Bigniaceae IX: New species of *Dendrosicus* and *Pachyptera*. *Phytologia* 26: 447–450.

Gentry, A.H. (1977) Studies in Bignoniaceae: New taxa and combinations in northwestern South American Bignoniaceae. *Phytologia* 35: 183–198.

Gentry, A.H. (1979 [1980]) Additional generic mergers in Bignoniaceae. *Annals of the Missouri Botanical Garden* 66: 778–787.

Gentry, A.H. & Tomb A.S. (1979) Taxonomic implications of Bignoniaceae palynology. *Annals of the Missouri Botanical Garden* 66: 756–855.

Guindon, S. & Gascuel, O. (2003) A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52: 696–704.

Hesse, M., Halbritter, H., Zetter, R., Weber, M., Buchner, R., Frosch-Radivo, A. & Ulrich, S. (2009) *Pollen terminology: An illustrated handbook*. SpringerWein, New York, 264 pp.

Hickey, L.J. (1973) Classification of the architecture of dicotyledonous leaves. *American Journal of Botany* 60: 17–33.

IUCN (2014) *Guidelines for using the IUCN red list categories and criteria*, version 11. Prepared by the Standards and Petitions Subcommittee. Available from: http://www.iucnredlist.org/documents/RedListGuidelines.pdf (accessed: 29 November 2016)

Katoh, K., Misawa, K., Kuma, K.I., & Miyata, T. (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P. & Drummond, A.(2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.

Lindley, J. (1827 [1828]) *Tecoma capensis*. *Botanical Register* 2: pl. 1117.

Linnaeus, C. (1753) *Species Plantarum*. Imprensis Laurentii Salvii, Stockholm, 2: 561–1200 pp.

Lohmann, L.G. (2006) Untangling the phylogeny of Neotropical lianas (Bignonieae, Bignoniaceae). *American Journal of Botany* 93: 304–318.

Lohmann, L.G. (2008) Bignoniaceae. *In:* Hokche, O., Berry, P.E., & Huber, O. (Eds.) *Nuevo Catálogo de la Flora Vascular de Venezuela*. Fundación Instituto Botánico de Venezuela, Caracas, pp. 270–278.

Lohmann, L.G., & Taylor, C.M. (2014) A new generic classification of tribe Bignonieae (Bignoniaceae). *Annals of the Missouri Botanical Garden* 99: 348–489.

Machado, I.C., & Vogel, S. (2004) The North-East-Brazilian liana, *Adenocalymna dichilum* (Bignoniaceae) pollinated by bats. *Annals of Botany* 93: 609–613.

Nogueira, A., El-Ottra, J.H.L., Guimarães, E., Machado, S.R. & Lohmann, L.G. (2013) Trichome structure and evolution in Neotropical lianas. *Annals of Botany* 112: 1331–1350.

Pace, M.R., Zuntini, A.R., Lohmann, L.G., & Angyalossy, V. (2016) Phylogenetic relationships of enigmatic *Sphingiphila* (Bignoniaceae) based on molecular and wood anatomical data. *Taxon* 65: 1050–1063.

Rambaut, A., & Drummond, A.J. (2014). Tracer 1.5. Edinburgh: Institute of Evolutionary Biology, University of Edinburgh.

Radford, A.E., Dickison, W.C., Massey, J.R., & Bell, C.R. (1974) *Vascular plant systematics*. Harper Collins, New York, 891 pp.

Richard, L.C.M. (1792). Catalogus Plantarum ad Societatem, ineunte anno 1792, e Cayenna missarum à Dominole Blond. *Actes de la Société d'Histoire Naturelle de Paris* 1: 105–114.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Sampaio, A. J. & Kulhmann, J.G. (1934) *Pseudocalymma* A. Samp. et Kuhlm. *n. gen.* (Bignoniaceas). *Boletim do Museu Nacional* 99–101.

Sandwith, N.Y. (1937) Notes on tropical American Bignoniaceae. *Mededelingen van het Botanisch Museum en Herbarium van de Rijksuniversiteit te Utrecht* 40: 205–232.

Silva-Castro, M.M. (2010) *Estudos taxonômicos, filogenéticos e biossistemáticos em Mansoa DC. (Bignonieae, Bignoniaceae).* Ph.D. thesis, Programa de Pós-graduação em Botânica, Universidade Estadual de Feira de Santana, Feira de Santana, Bahia, Brasil. 289 pp.

Silva-Castro, M.M., & Queiroz, L.P. (2016) Five new species of *Mansoa* DC. (Bignoniaceae) from South America. *Phytotaxa* 258: 49–62.

Sprague, T.A., & Sandwith, N.Y. (1932) Contributions to the flora of tropical America: X. *Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew)* 1932: 81–93.

Steyermark, J.A. (1947) *Studies of Central American Plants- VII*. Botanical Series, Field Museum of Natural History, Chicago, 23(5): pp.1-235

Swartz, O. (1788) Nova Genera et Species Plantarum seu Prodromus. *Prodromus* 6: 91–92.

Thiers, B. (2016) *Index Herbariorum: A global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. Available from: http://sweetgum.nybg.org/ih/ (accessed: 15 November 2016).

Weberling, F. (1992) *Morphology of flowers and inflorescences*. Cambridge University Press, Cambridge, 344 pp.

Zuntini, A.R., Fonseca, L.H.M., & Lohmann, L.G. (2013) Primers for phylogeny reconstruction in Bignonieae (Bignoniaceae) using herbarium samples. *Applications in plant sciences* 1(9): 1300018.

Zuntini, A.R., Taylor, C.M., & Lohmann, L.G. (2015). Deciphering the Neotropical *Bignonia binata* species complex (Bignoniaceae). *Phytotaxa* 219: 69–77.

**FIGURE CAPTIONS**

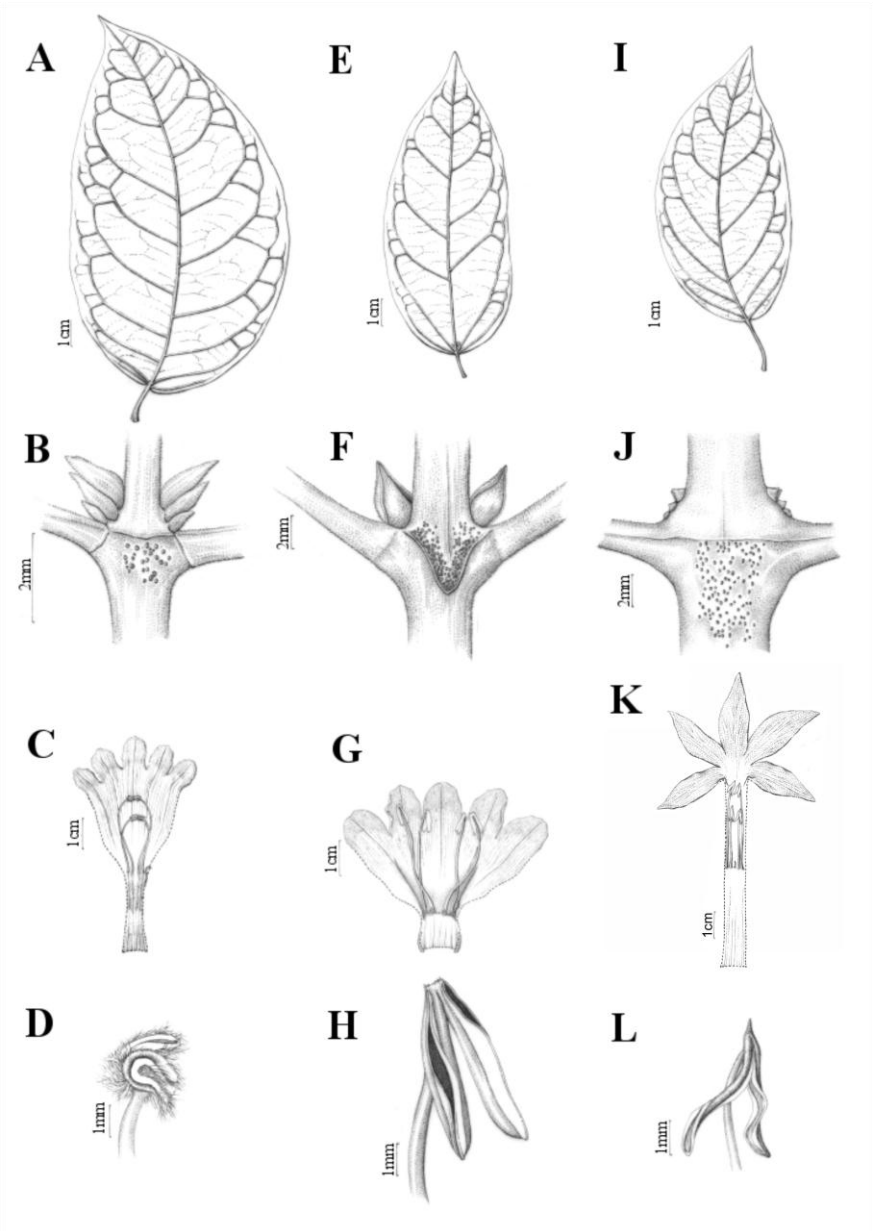


**FIGURE 1.** Morphological comparison among leaves, the interpetiolar region, flowers and anthers among species of *Pachyptera* and *Mansoa*. A–D. *Pachyptera kerere* (*J.N.C. Francisco 41*, SPF); E–H. *Mansoa ventricosa* (*J.N.C. Francisco 102*, SPF); and I–L. *Pachyptera aromatica* (*L.H. Fonseca 327*, SPF). Illustrations by Klei Souza.

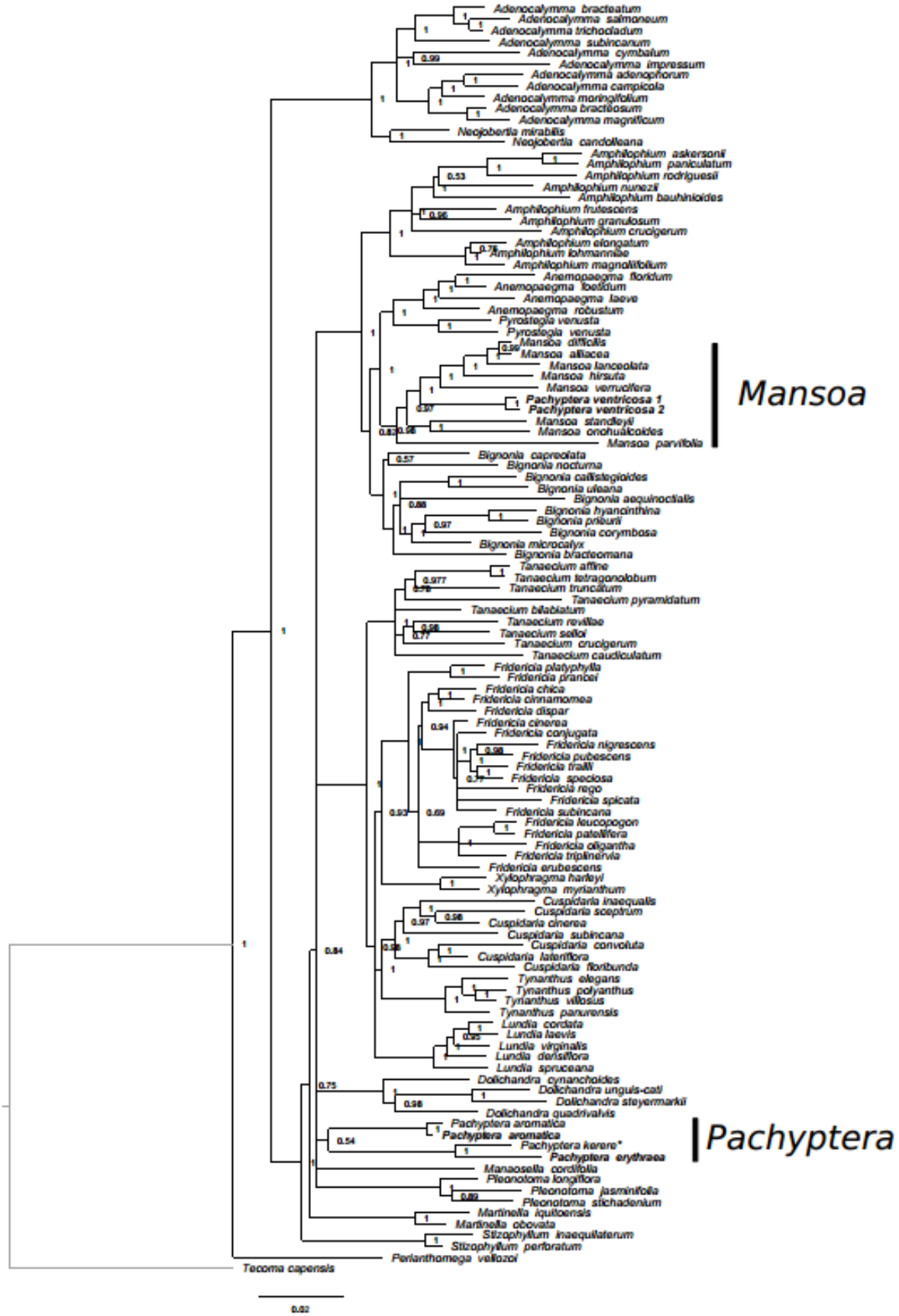


**FIGURE 2.** Phylogenetic placement of *Pachyptera ventricosa* and other species of

*Pachyptera* recognized by Lohmann & Taylor (2014) within Tribe Bignonieae. Majority-rule consensus tree derived from the Bayesian analyses of the combined *ndh*F and *Pep*C dataset. Posterior probabilities are shown above nodes. The outgroup is shown in grey. New sequences generated in this study are shown in bold and marked with asterisks (*).

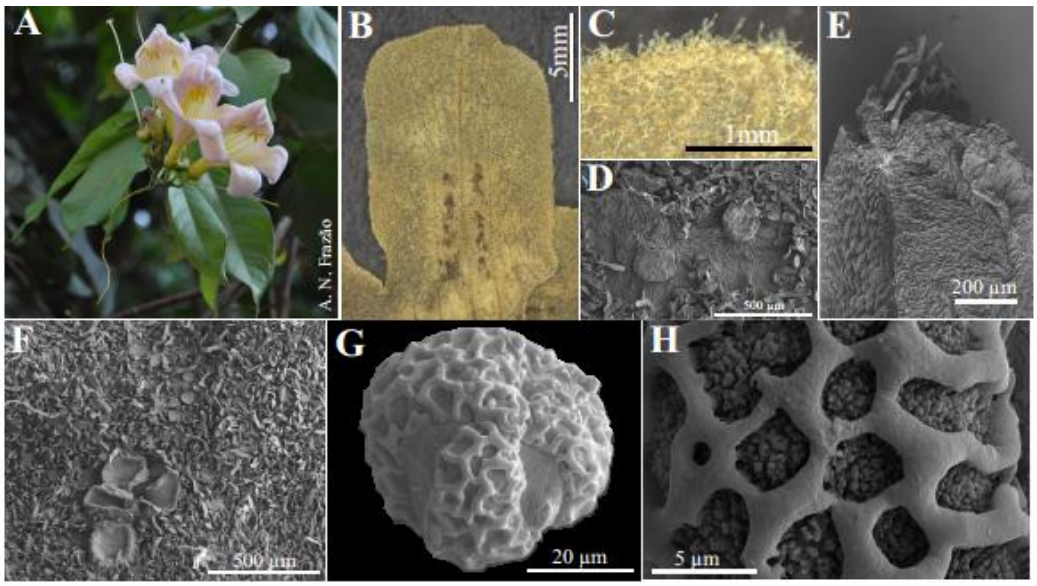


**FIGURE 3.** Morphological features of *Mansoa ventricosa*. A. Flowering branch; B. Interpetiolar region with extra-floral nectaries and subulate prophylls of the axillary buds; C. Abaxial leaflet surface showing the extra-floral nectaries; D. Trifid tentril; E. Calyx; F. Opened flower showing the androecium; G. Stamen with straight thecae; H. Gynoecium; I. Detail of ovary surface with simple and glandular patelliform trichomes (*J.N.C. Francisco 102*, SPF). Illustration by Klei Souza.

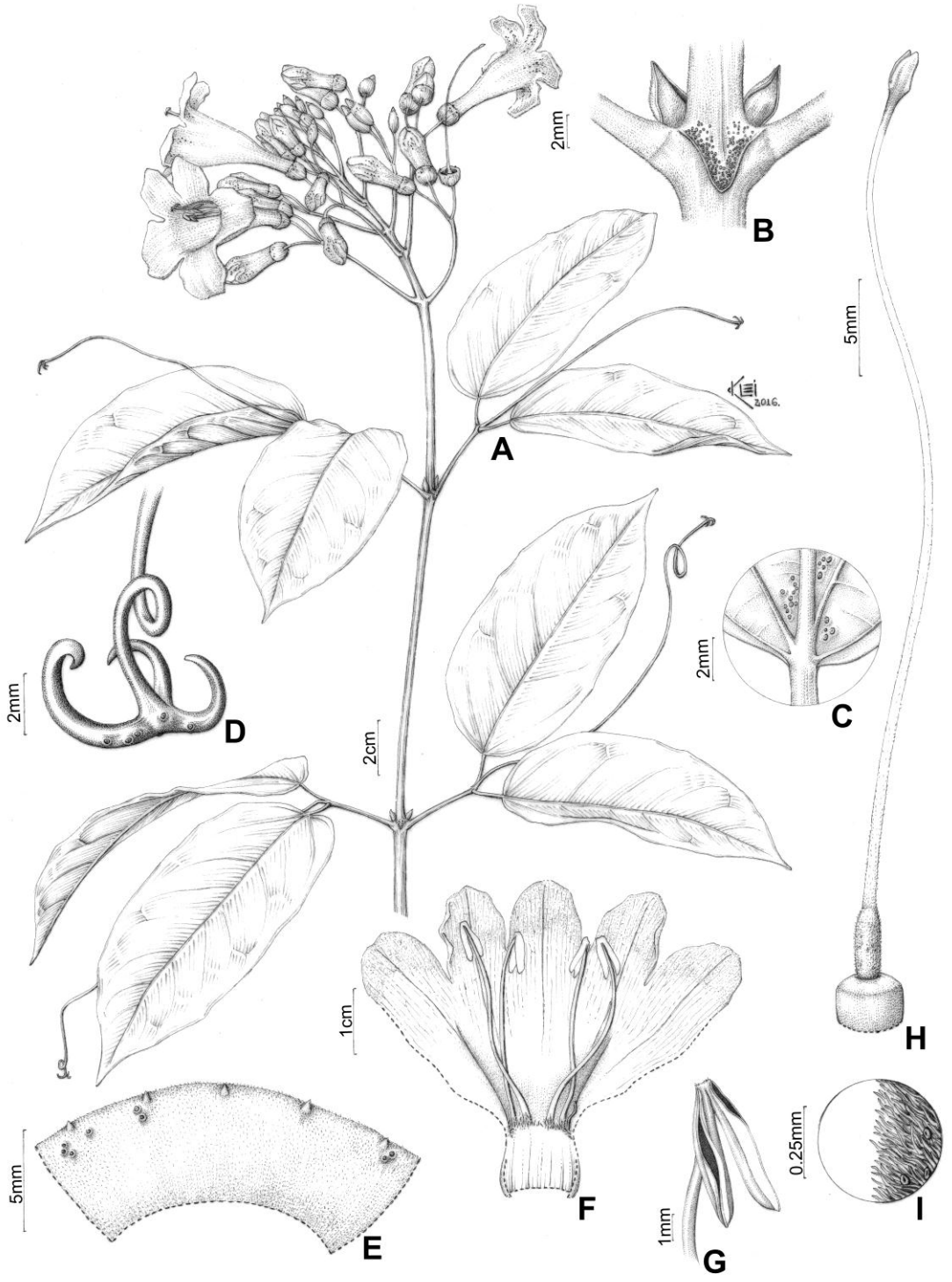


**FIGURE 4.** Morphological features of *Mansoa ventricosa*. A. Inflorescence; B. Corolla lobes puberulous, with patelliform glands; C. Glandular stipitate trichomes on the corolla; D. Detail of patelliform glands on the corolla lobes; E. Anther connective; F.

Calyx puberulous, with peltate and patelliform glandular trichomes; G. Tricolpate and coarse reticulate pollen grain; H. Pollen detail showing the bacula into the lumen (*J.N.C. Francisco 84*, SPF). Photo A by Annelise F. Nunez.


**FIGURE 5.** Distribution of *Mansoa ventricosa* in Northeastern Brazil (Pará).

**TABLE 1.** Summary of main taxonomic changes between *Mansoa, Pachyptera,* and related genera followed by a comparison of morphological features among these taxa.

| Author | Taxonomic changes | Shared morphological features |
|---|---|---|
| Baillon (1891) | *Pachyptera* was synonymized with *Adenocalymma* | Broad and thick capsule |
| Bureau & Schumann (1896) | Species of *Pachyptera* and *Mansoa* were transferred to *Adenocalymma* section *Hanburyophyton* | Large and pubescent capsules; terminal leaflets larger than lateral leaflets; inflorescences in racemes or panicles |
| Sprague & Sandwith (1932) | *Pachyptera* was restored to generic rank as a monotypic genus | Pollen tricolpate |
| Sampaio & Kuhlmann (1933) | *Pseudocalymma* [=*Mansoa*] was described a monospecific genus | Ovary with 2-series of eggs per locule, pollen tricolpate and absence of nectars on calyx |
| Gentry (1973) | *Pseudocalymma* was synonymized with *Pachyptera* | Trifid tendrils, interpetiolar gland-fields; white to red or purple corollas; 3-colpate pollen |
| Gentry (1979) and Gentry & Tomb (1979) | *Pachyptera* and *Hanburyphython* were merged into *Mansoa* | Palynological data |

| | | |
|---|---|---|
| | *Pachyptera* was restored to generic rank including the monotypic *Leucocalantha* | Older branchlets with papery peeling epidermis or bark; calyx with glands arranged in a line |
| Lohmann & Taylor (2014) | Circumscription of *Mansoa* similar to that adopted by Gentry (1979, 1997) except from the transfer of *M. erythraea*, *M. kerere* and *M. ventricosa* to *Pachyptera* | Angular stems, garlic smell; leaflets with basal actinodromous venation and nectaries at the base; pink to purple corollas |

**TABLE 2**. Morphological comparison between *Pachyptera s.s.*, *Mansoa* and *Pachyptera ventricosa*.

| **Character** | ***Pachyptera s.s.*** | ***Mansoa*** | ***Pachyptera ventricosa*** |
|---|---|---|---|
| EFN grouped on the abaxial surface of leaflets | absent | present (except from some *M. alliacea*) | present |
| Prophylls of the axillary buds | triangular and minute or flattened and ensiform, 3-seriate | triangular and minute or bromeliad-like | subulate, paired |
| Primary venation | pinnate | actinodromous | basal actinodromous |
| Inflorescence | raceme | thyrse or fascicle | thyrse |
| Floral color | white, light pink or red | pink to purple | light purple |
| Connective indumentum | glabrous | puberulous or glabrous | puberulous |
| Pollen aperture | 3-4-colpate | 3-colpate, pantocolpate or pantossincolpate | 3-colpate |
| Pollen ornamentation | psilate foveolate or micro-reticulate | reticulate, heterobrocade or areolate | coarse reticulate with bacula into the lumen |

**APPENDIX 1.** Species, locality, vouchers and GenBank accession numbers used in this study. Sequences obtained in this study are marked with an asterisk (*).‘-’ indicates sequence was not available/generated.

| ***Species***, locality, voucher (herbarium): *ndh*F, *Pep*C Genbank accession numbers. |
|---|
| ***Adenocalymma adenophorum*** (Sandwith) L.G.Lohmann, BRAZIL, Amazonas, Reserva Ducke, Lohmann 30, (INPA, K, MG, MO, NY, RB, SP): DQ222608, DQ222766. ***Adenocalymma bracteatum*** (Cham.) DC., BRAZIL, São Paulo, Santa Cruz da Conceição, Lohmann 719, (MO, SPF): DQ222527, DQ222649. ***Adenocalymma bracteosum*** (DC.) L.G.Lohmann , BRAZIL, Amazonas, Rio Negro, Lohmann 290, (MO, NY, SPF, UNIP): DQ222609, DQ222767. ***Adenocalymma campicola*** (Pilg.) L.G.Lohmann, BRAZIL, Minas Gerais, Uberlândia, Lohmann 266, (MO, SPF, U): DQ222610, DQ222770. ***Adenocalymma cymbalum*** (Cham.) Bureau & K.Schum. , BRAZIL, Minas Gerais, PE do Rio Doce, Lombardi 2495, (BHCB, MO): DQ222528, DQ222650. ***Adenocalymma impressum*** (Rusby) Sandwith, BRAZIL, Amazonas, Reserva Ducke, Vicentini 1155, (INPA, K, MG, MO, NY, SP, U): DQ222529, DQ222652. ***Adenocalymma magnificum*** Mart. ex DC., BRAZIL, Pará, PE Moju, Silva 30, (IAN, MO): DQ222612, DQ222771. ***Adenocalymma moringifolium*** (DC.) L.G.Lohmann, BRAZIL, Amazonas, Reserva Ducke, Lohmann 19, (INPA, K, MG, MO, NY, R, SP, SPF, U): DQ222613, DQ222773. ***Adenocalymma salmoneum*** J.C.Gomes, BRAZIL, Espírito Santo, Linhares, Lohmann 658, (CVRD, MO): DQ222530, DQ222653. ***Adenocalymma subincanum*** Huber, BRAZIL, Amazonas, Reserva Ducke, Lohmann 12, (INPA, MO): DQ222531, DQ222654. ***Adenocalymma*** |

***trichocladum*** (DC.) L.G.Lohmann, BRAZIL, Bahia, Santa Maria da Vitória, Hatschbach 50496, (MO): DQ222635, DQ222807. ***Amphilophium aschersonii*** Ule , BRAZIL, Acre, Rio Juruá, Lohmann 390, (MO, NY, SPF, UFAC): DQ222532, DQ222655. ***Amphilophium bauhinioides*** (Bureau ex Baill.) L.G.Lohmann, BRAZIL, Espírito Santo, Linhares, Lohmann 655, (CVRD, MO): DQ222586, DQ222734. ***Amphilophium crucigerum*** (L.) L.G.Lohmann , BRAZIL, Espírito Santo, Linhares, Lohmann 685, (CVRD, MO): DQ222623, DQ222789. ***Amphilophium elongatum*** (Vahl) L.G.Lohmann , BRAZIL, Minas Gerais, PE do Rio Doce, Lombardi 2433, (BHCB, MO): DQ222578, DQ222720. ***Amphilophium frutescens*** (DC.) L.G.Lohmann, BRAZIL, Paraíba, Rio Tinto, Lohmann 695, (MO, SPF): DQ222581, DQ222724. ***Amphilophium granulosum*** (Klotzsch) L.G.Lohmann , BRAZIL, Acre, Rio Juruá, Lohmann 470, (MO, NY, SPF, UFAC): DQ222582, DQ222726. ***Amphilophium lohmanniae*** (A. Pool) L.G.Lohmann , BRAZIL, Amazonas, Reserva Ducke, Lohmann 20, (INPA, MO): DQ222580, DQ222723. ***Amphilophium magnoliifolium*** (Kunth) L.G.Lohmann , SURINAME, Sipaliwini, Tafelberg Tepui, Lohmann 214, (BBS, MO): DQ222579, DQ222722. ***Amphilophium nunezii*** (A.H.Gentry) L.G.Lohmann, PERU, Madre Díos, Manu National Park, Lohmann 606, (MO, MOL): DQ222587, DQ222735. ***Amphilophium paniculatum*** (L.) Kunth , PERU, Madre Díos, Manu National Park, Lohmann 609, (MO, MOL): DQ222533, DQ222656. ***Amphilophium rodriguesii*** (A.H.Gentry) L.G.Lohmann, BRAZIL, Acre, Rio Arara, Lohmann 475, (MO, NY,SPF, UFAC): DQ222588, DQ222737. ***Anemopaegma floridum*** Mart. ex DC., BRAZIL, Amazonas, Reserva Ducke, Lohmann 121, (INPA, MO, SPF): DQ222534, DQ222658. ***Anemopaegma foetidum*** Bureau &

K.Schum. , BRAZIL, Amazonas, Reserva Ducke, Lohmann 35, (INPA, MO, SPF): DQ222535, DQ222659. ***Anemopaegma laeve*** DC., BRAZIL, Bahia, Chapada Diamantina, Lohmann 253, (MO, SPF): DQ222536, DQ222661. ***Anemopaegma robustum*** Bureau & K.Schum. , BRAZIL, Amazonas, Reserva Ducke, Apostolo 126, (INPA, MO): DQ222538, DQ222663. ***Bignonia aequinoctialis*** L., BRAZIL, Amazonas, Rio Negro, Lohmann 320, (MO, NY, SPF, UNIP): DQ222577, DQ222719. ***Bignonia bracteomana*** (K.Schum. ex Sprague) L.G.Lohmann, PERU, Madre Díos, Manu National Park, Lohmann 614, (MO, MOL): DQ222634, DQ222806. ***Bignonia callistegioides*** Cham., U.S.A., Missouri, MOBOT, Lohmann 352, (MO): DQ222569, DQ222708. ***Bignonia capreolata*** Kunth , U.S.A., Illinois, Johnson County, Lohmann 356, (MO): DQ222566, DQ222706. ***Bignonia corymbosa*** (Vent.) L.G.Lohmann, BRAZIL, Espírito Santo, Linhares, Lohmann 654, (MO): DQ222621, DQ222785. ***Bignonia hyacinthina*** (Standl.) L.G.Lohmann, PERU, Madre Díos, Manu National Park, Lohmann 642, (MO, MOL): DQ222614, DQ222775. ***Bignonia microcalyx*** G.Mey., SURINAME, Sipaliwini, Road between Blanche Marie and Paramaribo, Evans 3198, (BBS, MO): DQ222629, DQ222797. ***Bignonia nocturna*** (Barb.Rodr.) L.G.Lohmann, BRAZIL, Acre, Rio Juruá, Lohmann 451, (MO, NY, SPF, UFAC): DQ222641, DQ222813. ***Bignonia prieurii*** DC., BRAZIL, Espírito Santo, Linhares, Lohmann 651, (INPA, MO): DQ222615, DQ222776. ***Bignonia uleana*** (Kraenzl.) L.G.Lohmann, PERU, Madre Díos, Manu National Park, Lohmann 617, (MO, MOL): DQ222572, DQ222709. ***Cuspidaria cinerea*** (Bureau ex K.Schum.) L.G.Lohmann, BRAZIL, Amazonas, Reserva Ducke, Lohmann 34, (INPA, K, MG, MO, NY, SP, SPF, U, UB): DQ222631, DQ222801. ***Cuspidaria convoluta*** (Vell.)

A.H.Gentry, BRAZIL, São Paulo, Instituto Plantarum, Lohmann 713, (MO, SPF): DQ222573, DQ222711. ***Cuspidaria floribunda*** (DC.) A.H.Gentry, BRAZIL, Acre, Rio Juruá, Lohmann 418, (MO, NY, SPF, UFAC): DQ222574, DQ222713. ***Cuspidaria inaequalis*** (DC. ex Splitg.) L.G.Lohmann, SURINAME, Sipaliwini, Tafelberg Tepui, Lohmann 127, (BBS, MO): DQ222548, DQ222679. ***Cuspidaria lateriflora*** (Mart.) DC., PERU, Madre Díos, Manu National Park, Lohmann 628, (MO, MOL): DQ222575, DQ222716. ***Cuspidaria sceptrum*** (Cham.) L.G.Lohmann, BRAZIL, São Paulo, Santa Cruz da Conceição, Lohmann 717, (MO, SPF): DQ222557, DQ222698. ***Cuspidaria subincana*** A.H.Gentry, BRAZIL, Amazonas, Reserva Ducke, Lohmann s.n. (Tree # 2638-24) , (INPA, MO): DQ222576, DQ222717. ***Dolichandra cynanchoides*** Cham., ARGENTINA, Buenos Aires, , Galleto 1019, (MO): DQ222583, DQ222728. ***Dolichandra quadrivalvis*** (Jacq.) L.G.Lohmann, U.S.A., Missouri, MOBOT, Lohmann 353, (MO): DQ222607, DQ222764. ***Dolichandra steyermarkii*** (Sandwith) L.G.Lohmann, BRAZIL, Acre, Rio Arara, Lohmann 477, (MO, NY, SPF, UFAC): DQ222617, DQ222780. ***Dolichandra unguis-cati*** (L.) L.G.Lohmann, BRAZIL, Minas Gerais, PE do Rio Doce, Lombardi 2432, (BHCB, MO): DQ222595, DQ222749. ***Fridericia chica*** (Bonpl.) L.G.Lohmann, BRAZIL, Amazonas, Reserva Ducke, Lohmann s.n. (Tree # 2618-24) , (INPA): DQ222542, DQ222671. ***Fridericia cinerea*** (Bureau ex K.Schum.) L.G.Lohmann, BRAZIL, Bahia, Chapada Diamantina, Lohmann 358, (MO, SPF): DQ222543, DQ222673. ***Fridericia cinnamomea*** (DC.) L.G.Lohmann, BRAZIL, Amazonas, Reserva Ducke, Vicentini 809, (INPA, MO): DQ222544, DQ222674. ***Fridericia conjugata*** (Vell.) L.G.Lohmann, BRAZIL, Espírito Santo, Linhares, Lohmann 650, (CVRD, MO):

DQ222545, DQ222675. ***Fridericia dispar*** (Bureau ex K.Schum.) L.G.Lohmann, BRAZIL, Paraíba, Santa Rita, Lohmann 694, (MO, SPF): DQ222546, DQ222676. ***Fridericia erubescens*** (DC.) L.G.Lohmann, BRAZIL, Bahia, Chapada Diamantina, Lohmann 359, (MO, SPF): DQ222622, DQ222787. ***Fridericia leucopogon*** (Cham.) L.G.Lohmann, BRAZIL, São Paulo, Santa Cruz da Conceição, Lohmann 714, (MO, SPF): DQ222549, DQ222681. ***Fridericia nigrescens*** (Sandwith) L.G.Lohmann, BRAZIL, Amazonas, Reserva Ducke, Lohmann 78, (G, INPA, K, MG, MO, NY, RB, SP, U, UB): DQ222550, DQ222683. ***Fridericia oligantha*** (Bureau & K.Schum.) L.G.Lohmann, BRAZIL, Acre, Rio Juruá, Lohmann 483, (MO, NY, SPF, UFAC): DQ222551, DQ222685. ***Fridericia patellifera*** (Schltdl.) L.G.Lohmann, BRAZIL, Acre, Rio Juruá, Lohmann 412, (MO, NY, SPF, UFAC): DQ222552, DQ222687. ***Fridericia platyphylla*** (Cham.) L.G.Lohmann, PERU, Madre Díos, Manu National Park, Lohmann 639, (MO, MOL): DQ222554, -. ***Fridericia prancei*** (A.H.Gentry) L.G.Lohmann, BRAZIL, Amazonas, Reserva Ducke, Sothers 460, (INPA, K, MBM, MG, MO, NY, RB, SPF, U, UFMT): DQ222555, DQ222689. ***Fridericia pubescens*** (L.) L.G.Lohmann, BRAZIL, Minas Gerais, PE do Rio Doce, Lombardi 2529, (BHCB, MO): DQ222556, DQ222690. ***Fridericia rego*** (Vell.) L.G.Lohmann, BRAZIL, Espírito Santo, Linhares, Lohmann 660, (CVRD, MO): DQ222558, DQ222692. ***Fridericia speciosa*** Mart., BRAZIL, Minas Gerais, PE do Rio Doce, Lombardi 2521, (BHCB, MO): DQ222584, DQ222730. ***Fridericia spicata*** (Bureau & K.Schum.) L.G.Lohmann, PERU, Madre Díos, Manu National Park, Lohmann 607, (MO, MOL): DQ222561, DQ222699. ***Fridericia subincana*** (Mart.) L.G.Lohmann, BRAZIL, Espírito Santo, Linhares, Lohmann 659, (CVRD, MO): DQ222562, DQ222701. ***Fridericia***

***trailii*** (Sprague) L.G.Lohmann, BRAZIL, Amazonas, Reserva Ducke, Lohmann 29, (INPA, K, MG, MO, NY, SPF): DQ222563, DQ222703. ***Fridericia triplinervia*** (Mart. ex DC.) L.G.Lohmann, BRAZIL, Amazonas, Reserva Ducke, Lohmann 18, (GH, IAN, INPA, K, MO, PEUFR, S, SPF, UEC): DQ222564, DQ222705. ***Lundia cordata*** (Vell.) DC., BRAZIL, Espírito Santo, Linhares, Lohmann 652, (CVRD, MO): DQ222590, DQ222741. ***Lundia densiflora*** DC., BRAZIL, Amazonas, Reserva Ducke, Lohmann 82, (INPA, MO): DQ222592, DQ222743. ***Lundia laevis*** Kaehler, BRAZIL, Acre, Rio Abuna, Lohmann 497, (MO,NY, SPF, UFAC): DQ222591, DQ222742. ***Lundia spruceana*** Bureau, PERU, Madre Díos, Manu National Park, Lohmann 610, (MO, MOL): DQ222593, DQ222745. ***Lundia virginalis*** DC., BRAZIL, Minas Gerais, PE do Rio Doce, Lombardi 2530, (BHCB, MO): DQ222594, DQ222747. ***Manaosella cordifolia*** (DC.) A.H.Gentry, BRAZIL, Minas Gerais, PE do Rio Doce, Lombardi 2546, (BHCB, MO): DQ222596, DQ222750. ***Mansoa alliacea*** (Lam.) A.H.Gentry, BRAZIL, Amazonas, Reserva Ducke, Vicentini 672, (INPA, K, MG, MO, NY, RB, SPF): DQ222597, -. ***Mansoa difficilis*** (Cham.) Bureau & K.Schum. , BRAZIL, Espírito Santo, Linhares, Lohmann 662, (CVRD, MO): DQ222598, DQ222752. ***Mansoa hirsuta*** DC., BRAZIL, Bahia, Chapada Diamantina, Lohmann 364, (MO, SPF): DQ222599, DQ222753. ***Mansoa lanceolata*** (DC.) A.H.Gentry, BRAZIL, Espírito Santo, Linhares, Lohmann 661, (CVRD, MO): DQ222601, DQ222755. ***Mansoa onohualcoides*** A.H. Gentry, BRAZIL, Espírito Santo, Linhares, Zuntini 276, (SPF): KP691455, KP697961. ***Mansoa parvifolia*** (A.H.Gentry) A.H.Gentry, PERU, Madre Díos, Manu National Park, Lohmann 605, (MO, MOL): DQ222602, -. ***Mansoa standleyi*** (Steyerm.) A.H.Gentry, PERU,

Madre Díos, Manu National Park, Lohmann 638, (MO, MOL): DQ222603, DQ222757. ***Mansoa ventricosa*** A.H. Gentry, BRAZIL, Pará, Belém, Francisco 102, (SPF): KY983570*, KY983574*; Belterra, Francisco 84, (SPF): KY983573*, KY983578*. ***Mansoa verrucifera*** (Schltdl.) A.H.Gentry, PERU, Madre Díos, Manu National Park, Lohmann 612, (MO, MOL): DQ222604, DQ222759. ***Martinella iquitoensis*** A.Samp., PERU, Madre Díos, Manu National Park, Lohmann 616, (MO, MOL): DQ222605, DQ222760. ***Martinella obovata*** (Kunth) Bureau & K.Schum. , SURINAME, Sipaliwini, Tafelberg Tepui, Lohmann 126, (BBS, MO): DQ222606, DQ222762. ***Neojobertia candolleana*** (Mart. ex DC.) Bureau & K.Schum. , BRAZIL, Bahia, Chapada Diamantina, Lohmann 363, (MO, SPF): DQ222616, DQ222778. ***Neojobertia mirabilis*** (Sandwith) L.G.Lohmann, BRAZIL, Espírito Santo, Linhares, Lohmann 681, (CVRD, MO): DQ222585, DQ222732. ***Pachyptera aromatica*** (Barb.Rodr.) L.G.Lohmann, BRAZIL, Amazonas, Novo Airão, Lohmann 794, (SPF): KY983572*, KY983575*; Reserva Ducke, Lohmann 28, (INPA, MO, SPF): DQ222589, DQ222739. ***Pachyptera erythraea*** (Dugand) A.H. Gentry, COLOMBIA, Santander, Gentry 15372, (MO): KY983571*, KY983577*. ***Pachyptera kerere*** (Aubl.) Sandwith, BRAZIL, Amazonas, Rio Negro, Lohmann 336, (MO, NY, SPF, UNIP): DQ222600, KY983576*. ***Perianthomega vellozoi*** Bureau, BOLIVIA, Santa Cruz, Close to the Santa Cruz Botanical Garden, Nee 35808, (LPB, MO): DQ222619, -. ***Pleonotoma jasminifolia*** (Kunth) Miers, BRAZIL, Amazonas, Reserva Ducke, Lohmann 122, (INPA): DQ222625, DQ222793. ***Pleonotoma longiflora*** B.M. Gomes & Proença, BRAZIL, Amazonas, Reserva Ducke, Forzza 290, (G, INPA, K, MG, MO, NY, RB, SP, U, UB): DQ222624, DQ222791. ***Pleonotoma stichadenia*** K.Schum. ,

BRAZIL, Espírito Santo, Linhares, Lohmann 656, (CVRD, MO): DQ222627, DQ222795. ***Pyrostegia venusta*** (Ker Gawl.) Miers, BRAZIL, Acre, Plácido do Castro , Lohmann 534, (MO, NY, SPF, UFAC): DQ222632, DQ222803; São Paulo, Santa Cruz da Conceição, Lohmann 718, (MO, SPF): DQ222633, DQ222804. ***Stizophyllum inaequilaterum*** Bureau & K.Schum. , BRAZIL, Acre, Rio Juruá, Lohmann 454, (MO, NY, SPF, UFAC): DQ222638, DQ222808. ***Stizophyllum perforatum*** (Cham.) Miers, BRAZIL, Minas Gerais, PE do Rio Doce, Lombardi 2431, (BHCB, MO): DQ222639, DQ222809. ***Tanaecium affine*** (A.H.Gentry) L.G.Lohmann, PERU, Madre Díos, Manu National Park, Lohmann 633, (MO, MOL): DQ222539, DQ222665. ***Tanaecium bilabiatum*** (Sprague) L.G.Lohmann, BRAZIL, Amazonas, Rio Solimões, Lohmann 92, (MO, NY, SPF, UNIP): DQ222540, DQ222667. ***Tanaecium caudiculatum*** (Standl.) L.G.Lohmann, BELIZE, Cayo, Grano de Oro Camp, Whitefoord 9231, (BRH, MO): DQ222630, DQ222800. ***Tanaecium crucigerum*** Seem., U.S.A., Missouri, MOBOT, Lohmann 355, (MO): DQ222640, DQ222811. ***Tanaecium pyramidatum*** (Rich.) L.G.Lohmann, BRAZIL, Amazonas, Rio Solimões, Lohmann 274, (MO, NY, SPF, UNIP): DQ222618, DQ222781. ***Tanaecium revillae*** (A.H.Gentry) L.G.Lohmann, BRAZIL, Amazonas, Rio Solimões, Lohmann 265a, (MO, NY, SPF, UNIP): DQ222559, DQ222694. ***Tanaecium selloi*** (Spreng.) L.G.Lohmann, BRAZIL, Paraíba, Guarabira, Lohmann 702, (MO, SPF): DQ222560, DQ222696. ***Tanaecium tetragonolobum*** (Jacq.) L.G.Lohmann, PERU, Madre Díos, Manu National Park, Lohmann 619, (MO, MOL): DQ222568, DQ222707. ***Tanaecium truncatum*** (A.Samp.) L.G.Lohmann, BRAZIL, Amazonas, Reserva Ducke, Lohmann 33, (INPA, K, MG, MO, NY, SPF): DQ222620, DQ222783. ***Tecoma***

***capensis*** (Thunb.) Lindl., SURINAME, Paramaribo, , Lohmann 125, (BBS, MO): DQ222642. ***Tynanthus elegans*** Miers, BRAZIL, Espírito Santo, Linhares, Lohmann 663, (CVRD, MO): DQ222643, DQ222815. ***Tynanthus panurensis*** (Bureau ex Baill.) Sandwith, BRAZIL, Amazonas, Reserva Ducke, Procopio 14, (G, INPA, K, MG, MO, NY, RB, SP, U, UB): DQ222644, DQ222817. ***Tynanthus polyanthus*** (Bureau ex Baill.) Sandwith, BRAZIL, Acre, Cruzeiro do Sul, Lohmann 370, (MO, NY, SPF, UFAC): DQ222645, DQ222819. ***Tynanthus villosus*** A.H.Gentry, BRAZIL, Acre, Rio Juruá, Lohmann 413, (MO, NY, SPF, UFAC): DQ222647, DQ222820. ***Xylophragma harleyi*** (A.H.Gentry ex M.M.Silva & L.P.Queiroz) L.G.Lohmann, BRAZIL, Bahia, Chapada Diamantina, Lohmann 362, (MO, SPF): DQ222547, DQ222678. ***Xylophragma myrianthum*** (Cham.) Sprague, BRAZIL, Espírito Santo, Linhares, Lohmann 649, (CVRD, MO): DQ222648, DQ222822.

# Capítulo 2

**Phylogeny and biogeography of the Amazonian genus *Pachyptera* (Bignonieae, Bignoniaceae)**

Jessica Nayara Carvalho Francisco and Lúcia G. Lohmann

***Abstract***— The Amazon houses a large proportion of the overall biodiversity currently available on Earth. Despite that, our knowledge of Amazonian biodiversity is still limited, complicating our understanding of overall diversity patterns within this region. Understanding the drivers of Amazonian biodiversity represents a major challenge in evolutionary biology. In this study, we reconstruct the phylogeny of *Pachyptera,* a genus of neotropical lianas, centered in the Amazon. We then use this phylogenetic framework to re-evaluate species limits and study the biogeographic history of the genus. We sampled three molecular markers (i.e., *Pep*C, *ndh*F, *rpl*32-*trn*L) and 51 individuals representing the breath of morphological variation and geographical distribution of all three species recognized to date. We used this information to reconstruct phylogenetic relationships among individuals of *Pachyptera* using Bayesian and Maximum Likelihood approaches. We then used this molecular phylogenetic framework as basis to identify potential species within the *P. kerere* species complex using a cpDNA coalescent approach (GMYC). GMYC identified five potential species with the *P. kerere* species complex that were subsequently evaluated in the light of morphology. Morphological data supported the recognition of four of those species, all of which received subsequent support from a multispecies coalescent model in a Bayesian framework (*BEAST). The phylogeny of *Pachyptera*, including a single individual of the five species recognized (i.e., *P. aromatica* plus the four species recognized within the *P. kerere* species complex), was time-calibrated using BEAST and used as basis to reconstruct the biogeographical history of the genus within the Amazon using RASP, and the DEC model. We also inferred the spatial history of *Pachyptera* and identified historically important migration pathways within the genus using our comprehensive cpDNA dataset in BSSVS. Our results indicate that the genus originated during the Late Eocene, and subsequently occupied lowland Amazonia, Central America & the Andes, respectively. Most of the diversification of the genus occurred in the Late Miocene, a period of intense perturbations in South America (e.g., uplift of the Andes, marine incursions, and formation of dry and wetland systems), all of which likely played an important role in the diversification history of the genus.

***Keywords***— ancestral area reconstructions, coalescent approaches, neotropics, species delimitation.

## INTRODUCTION

The Amazon houses a large proportion of the overall biodiversity currently available on Earth (Silva et al. 2005). Despite that, Amazonian biodiversity has been relatively little studied, leaving a major gap in our understanding of global biodiversity patterns (Hopkins 2007). Understanding the drivers of Amazonian biodiversity represents a major challenge in evolutionary biology (e.g., Gentry 1982; Moritz et al. 2000; Hoorn et al. 2010). Several hypotheses have been proposed to explain the origin and maintenance of the Amazonian biota such as the riverine barrier hypothesis (Wallace 1854), the forest refugia hypothesis (Haffer 1969), the marine incursions hypothesis (Hoorn 1993), and the Andean uplift hypothesis (Hoorn et al. 2010).

Despite the multitude of hypotheses available to date, it is clear that Amazonian biodiversity has a complex history and that no single factor can explain the patterns observed (Hoorn 2010; Wesselingh et al. 2010; Rull 2011). As such, an integrative approach, involving multiple taxa, is necessary to understand such complex diversity patterns (Cracraft 1988; Bush 1994; Moritz et al. 2000; Aleixo 2004). However, integrative approaches depend on the identification of equivalent evolutionary units derived from detailed studies of individual taxa. Species are arguably the most suited evolutionary units for such comparisons. Despite that, accurate species delimitation represents one of the most challenging issues in biology (Cracraft 1983; De Queiroz 1998, 2007; Sites and Marshall 2003; Velasco 2008). As such, detailed studies based on a broad sampling of taxa are critical for a detailed understanding of the main processes shaping the Amazonian biota.

This study focuses on *Pachyptera* DC., a small genus of lianas that belongs to tribe Bignonieae, in the plant family Bignoniaceae. This family includes 82 genera and ca. 827 species (Lohmann and Ulloa Ulloa 2016 onwards) of trees, shrubs, wood lianas, vines and herbaceous plants. The tribe Bignonieae is the largest clade within the family and contains all neotropical lianas and some shrubs (Gentry 1989; Lohmann 2006; Olmstead et al. 2009). *Pachyptera* has a complicated taxonomic history and underwent various taxonomic changes during the last 150 years (see Francisco and Lohmann submitted). The genus was reduced to a single species in the past, with *P. kerere* (Aubl.) Sandwith representing a species complex, with multiple varieties (Sprague and Sandwith 1932; Dugand 1955; Gentry 1973). One infra-specific taxon was subsequently

treated as a separate species, *P. erythraea* (Dugand) A.H. Gentry (Gentry 1979; Lohmann and Taylor 2014). Despite that, it is not yet completely clear whether *P. erythraea* should indeed be recognized as a separate taxon.

The first phylogenetic study to sample *Pachyptera* (Lohmann 2006) focused on deciphering generic limits within the tribe and sampling within the genus was limited. Only two individuals of *Pachyptera* were sampled, representing two different species, *P. kerere* and *P. aromatica* (Bar. Rodr.) L.G. Lohmann. Based on those phylogenetic results, Lohmann and Taylor (2014) resurrected *Pachyptera* and recognized the genus based on the striated, cylindrical to tetragonal stems, with four phloem wedges in cross-section, papery and peeling bark, and corolla with glands arranged in lines on the upper portion.

A more recent phylogenetic study of the genus (Francisco and Lohmann submitted) indicated that *Pachyptera ventricosa* (A.H. Gentry) L.G. Lohmann is more closely related to species of *Mansoa* than to other species of *Pachypera*, transferring this species back to *Mansoa* (Francisco and Lohmann submitted). As such, *Pachyptera* currently includes three species, *P. aromatica*, *P. erythraea*, and *P. kerere*. While *Pachyptera aromatica* is found in wet forests throughout Amazonia, *Pachyptera erythraea* is restricted to wet forests from the medium Magdalena River Valley in Colombia (Lohmann and Taylor 2014). *Pachyptera kerere* is the most broadly distributed species, occurring in humid and flooded forests of Amazonia and Central America, all the way to Belize (Lohmann and Taylor 2014).

Species in the genus include broad patterns of morphological diversity, especially in reproductive traits. *Pachyptera aromatica* has white and hypocrateriform corollas, nocturnal anthesis, and hawk-moth pollination (Barbosa Rodrigues 1891; Gentry 1974). *Pachyptera erythraea* has orange to red, infundibiliform to tubular-campanulate corollas, likely pollinated by bees and hummingbirds (Gentry 1974). *Pachyptera kerere* has white to cream, infundibuliform corollas, likely pollinated by large to medium-sized bees, mainly euglossine and anthophorids (Gentry 1974). As far as fruit and seed characters are concerned, fruits of *P. aromatica* and *P. erythraea* are flattened with winged seeds that are likely wind dispersed, while *P. kerere* has corky and inflated seeds, most likely water dispersed. However, patterns of morphological variation can be variable even within individual species. For instance, individuals with

pink to purplish flowers, flattened fruits, and winged seeds were treated as *Pachyptera kerere* var. *incarnata* in the past (Gentry 1973). This infra-specific classification was not recognized in the most recent treatment of the genus (Lohmann and Taylor 2014). The overlapping distribution and patterns of morphological variation in reproductive features within the genus raise species limits to question.

Accurate species delimitation represents one of the most challenging issues in biology. Even though species are fundamental units in evolutionary biology, conflicting biological and philosophical concepts have been proposed (Cracraft 1983; De Queiroz 1998, 2007; Sites and Marshall 2003; Velasco 2008). It is more or less common sense that species are independently evolving meta-population lineages (De Queiroz 1998, 2007). As genetic data can contain the signal of historical processes involved in lineage divergence (Nielsen and Wakeley 2001), it provides primary data for lineage diagnosis. As such, DNA sequences represent a helpful tool for exploring species boundaries, especially in the case of cryptic species (Duminil and Michele 2009). The combination of multiple genetic markers in coalescent-based models provide a good framework within which to define species limits, especially when associated with morphological data (Sites and Marshall 2004; Pons et al. 2006; Knowles and Carstens 2007; Fujita et al. 2012).

In this study, we (i) reconstruct phylogenetic relationships within *Pachyptera*, (ii) clarify species limits within the genus, and (iii) assess patterns of temporal and spatial variation in this Amazonian plant genus. For that, we infer phylogenetic relationships among species of the genus using chloroplast (cpDNA) and nuclear (nDNA) markers and a broad sampling of individuals representing the breath of morphological variation and geographical distribution of taxa. We then use this framework as basis to evaluate species limits and study the biogeographical history of the genus.

## MATERIALS AND METHODS

***Taxon sampling*—** We sampled 51 accessions of all three species currently recognized in *Pachyptera*, i.e., *P. aromatica*, *P. erythraea*, and *P. kerere* (Francisco and Lohmann submitted), plus the pink flowered individuals of *P. kerere*, treated earlier as

*P. kerere* var. *incarnata* (Gentry 1973). Samples were selected in order to include the breath of morphological variation and distribution of each taxon. For that, we divided Amazonia into four main biogeographic areas, divided by important biogeographic barriers, as follows: NE = North-Eastern Amazonia, including the Guyana region; NW = North-Western Amazonia; SE = South-Eastern Amazonia; and SW = South-Western Amazonia (Fig. 1). In addition, Central America & the Andes were treated as a fifth area (CA&A). We tried to sample at least two individuals of each species per area. Our final sampling included two individuals of *P. erythraea* (restricted to NE), five individuals of *P. aromatica* (restricted to NW, NE, and SE), 31 individuals of *P. kerere* (occurring in the five biogeographical areas), 10 individuals of *P. kerere* var. *incarnata* (occurring in NE and SE), and three individuals of a *spec. nov.* (restricted to NW). In addition, we also included sequences of four additional species of Bignonieae as outgroups, i.e., *Tanaecium bilabiatum* (Sprague) L.G. Lohmann, *Fridericia speciosa* Mart., *Lundia spruceana* Bureau, and *Stizophyllum riparium* (Kunth) Sandwith. Outgroup selection was based on earlier phylogenetic studies (Lohmann 2006; Francisco and Lohmann submitted). All sequences were newly generated for this study, except from outgroup sequences and sequences from three individuals of *Pachyptera* that were retrieved from Genbank (Tab. S1).

***DNA extraction, amplification and sequencing—*** We extracted genomic DNA from silica-dried leaflets collected during fieldwork and herbarium specimens using the DNeasy Plant mini kit (Qiagen. Düsseldorf, Germany) or the Invisorb Plant Mini Kit (Invitek, Berlin, Germany) following the manufacturer's instructions. For old herbarium samples, we added 1,5 μL de β-mercaptoethanol to each reaction. We selected two chloroplast DNA markers, i.e., the *ndh*F (NADH dehydrogenase) and the *rpl*32-*trn*L (intergenic spacer region), and one nuclear marker, the *Pep*C (fosfoenolpiruvato carboxylase), all of which have been shown to provide an adequate number of informative characters to resolve phylogenetic relationships within Bignonieae (Lohmann 2006; Kaehler and Lohmann 2012; Fonseca and Lohmann 2015; Medeiros and Lohmann 2015) We amplified chloroplast markers following the protocol of Lohmann (2006), and adjustments suggested by Zuntini et al. (2013). For the nuclear markers, we used a nested PCR strategy described in Francisco and Lohmann (submitted). All samples were purified and sequenced by Macrogen Inc. (Seoul, Korea).

We inspected all chromatographs visually using Geneious 9.0.2 (Kearse et al. 2012), and evaluated sequence quality through Phred scores (Ewing and Green 1998), discarding base pairs with values below 16, corresponding to less than 97.5% of assurance. We aligned contig sequences using MAFFT 7.22, implemented in Geneious 9.0.2 (Kearse et al. 2012), using default parameters (i.e., auto algorithm, scoring matrix: 200PAM/k = 2, gap open penalty: 1.53, offset value 0.123), followed by visual inspection and manual adjustments.

***Data partitions*—** We constructed five datasets: (i) *ndh*F dataset, 50 individuals; (ii) *rpl*32-*trn*L dataset, 49 individuals; (iii) *Pep*C dataset, 16 individuals; (iv) cpDNA dataset (*ndh*F and *rpl32-trn*L), 51 individuals, and (v) combined dataset (*ndh*F, *rpl32-trn*L, and *Pep*C), 51 individuals. Given the wide distribution of taxa, we were not able to carry out fieldwork throughout the species entire ranges. Hence, more than half of the samples were obtained from herbarium specimens collected between 1950-1990, which explains the discrepancy among the number of individuals sampled for each marker.

***Phylogeny reconstruction*—** We analyzed our molecular dataset using Bayesian inference (BI) and maximum likelihood (ML). For BI and ML analyses, we selected the best-fitting model of DNA substitution for each data partition using the Akaike information criterion implemented in JModelTest 2.1.4 (Darriba et al. 2012). The best models of DNA substitution recovered were the TVM+G for the *ndh*F dataset, TPM1uf+G for the *rpl*32-*trn*L dataset, and TVM+I for the *Pep*C dataset. We performed BI analyses in MrBayes 3.2.2 (Ronquist et al. 2012), using four Markov Chain Monte Carlo (MCMC) runs for 10 million of generations. Trees were saved every 1000 generations to minimize autocorrelation among samples. Stationarity was determined by visually monitoring likelihood values in Tracer 1.5 (Rambaut and Drummond 2009). Trees were summarized into a consensus tree after discarding the burn-in (25%). We conducted ML analyses in RaxML (Stamatakis 2006) using the graphical user interface RaxMLGUI 1.3 (Silvestro and Michalak 2012). Tree support was evaluated through ML bootstrap, using 1000 rapid bootstrap replicates. Nodes with posterior probabilities (PP) $\geq 95$ and bootstrap (BS) $\geq 70$ were considered well supported.

***Species delimitation within the Pachyptera kerere species complex*—** We used a combination of approaches to investigate species limits within the *P. kerere* species complex, the most morphologically variable and broadly distributed group within the

genus. These approaches aimed at reducing investigator-driven biases so that more robust species boundaries could be established. Single-locus data derived from a broad samples of individuals and species can provide sufficient information for species delimitation methods (Fujita et al. 2012; Fujisawa and Barraclough 2013). Given our limited sampling of individuals for the nuclear marker (16 individuals vs. 51 individuals sampled in the cpDNA dataset), we used the chloroplast dataset for our species delimitation analyses.

First, we used the Generalized Mixed Yule Coalescent (GMYC) method to identify putative species (Pons et al. 2006). GYMC overcomes quantitative species delimitation methods by establishing confidence intervals while evaluating the uncertainty associated with species limits instead of defining hypothetical populations *a priori*, thus avoiding problematic population delimitation (Pons et al. 2006). As such, this method does not require previous information about species, allowing a careful evaluation of groups with uncertain taxonomy. For this analysis, we generated an ultrametric time-tree based on the Bayesian analysis of the combined cpDNA dataset using BEAST 2.4.3 (Bouckaert et al. 2014). The same models of DNA substitutions used in earlier analyses were applied here (i.e., TVM+G model to *ndh*F and TPMuf+G model to *rpl*32-*trn*L). We employed a Relaxed Clock Log Normal with clock rate estimated, and Yule tree prior. We used three secondary calibration points (Tab. S2) based on a previous estimation of the age of the genus (Lohmann et al. 2013). We ran three replicate searches of MCMC chains with 10 million generations sampled every 1000 generations. Results were mapped in Tracer 1.5 (Rambaut and Drummond 2009) to ensure that effective sample sizes (ESS) were > 200. We used LogCombiner 2.4.3 (Bouckaert et al. 2014) to combine trees derived from the three runs. We generated a summary tree in TreeAnotator 2.4.3 (Bouckaert et al. 2014) using the posterior distribution of trees with a maximum clade credibility (MCC) tree annotated with median node ages and removing 25% of samples as burn-in. The analysis of GMYC was performed in R using the 'splits' package (Species Limits by Threshold Statistics project; Ezard et al. 2009) fit for the single-threshold algorithm (Fig. S4). We also run a Bayesian Poisson Tree Processes (bPTP, Zhang et al. 2013) to detect possible errors associated with the time calibration procedures required by GMYC. This analysis verifies whether the uncertainty associated with the time-calibrated branch lengths are

affecting the ultrametric phylogeny. This analysis was run using with the same Bayesian combined cpDNA tree reconstructed with BEST 2.4.3. Analyses were run on the bPTP web server (http://species.h-its.org/ptp/) using the following parameters: MCMC = 500,000 generations; thinning = 1000; burn-in = 0.25; seed = 1234.

Because the GYMC is known to underestimate intraspecific variation and to identify the highest possible number of species in the case of broadly distributed taxa (Bergsten et al. 2012; Talavera et al 2013), we re-evaluated the results derived from GYMC based on "morphological distinctiveness" (Tab. S3) as an additional criterion to assign species limits. We then validated our species hypotheses based on GMYC and morphology, using a multispecies coalescent model implemented in a Bayesian framework, implemented in *BEAST (Bouckaert et al. 2014). While GMYC does not require *a priori* species hypotheses allowing an unbiased evaluation of groups with uncertain taxonomy (Pons et al. 2006), *BEAST required the definition of *a priori* species and is be used to validate hypotheses pre-defined based on other methods. *BEAST provides a species tree estimation assumed to not exchange genes, forming independent distinct lineages. We ran *BEAST in BEST 2.4.3 using the combined chloroplast dataset. Since *BEAST uses a clock model to estimate the roots of individual gene trees through a multispecies coalescent approach (Heled and Drummond 2009), no outgroups were included in this analysis. The following parameters were used: linked trees, unlinked substitution models, and strict clock model parameters, Yule process.

***Biogeographical analyses—*** We time-calibrated the phylogeny of *Pachyptera* and conducted ancestral area reconstructions using the five biogeographical regions selected for our sampling (Fig. 1). These regions were defined based on the distribution patterns of the various taxa, paleogeological data and geographical barriers (i.e., major rivers and Andean uplift). These areas are based on a digitized version (Löwenberg-Neto 2014) of Morrone's map (2014), with slight modifications.

We conducted a Bayesian Stochastic Search Variable Selection (BSSVS, Lemey et al. 2009) analysis of discrete-states using a diffusion model in BEAST 2.4.3 (Bouckaert et al. 2014). This approach allowed us to infer the spatial history of *Pachyptera* and to identify historically important migration pathways within the genus. This method accommodates uncertainty in dispersal and in the unknown phylogeny,

preventing overstated conclusions. For this analysis, we used the comprehensive tree derived from the combined chloroplast dataset (cpDNA) linked to the trait "location." The same models of DNA substitutions used in earlier analyses were applied here (i.e., TVM+G model to *ndh*F and TPMuf+G model to *rpl*32-*trn*L). In addition, we used a shape estimate with empirical frequencies and used a symmetric substitution model for the discrete traits in the location partition with the social network using the BSSVS procedure. We used a relaxed clock log normal with 1.0 estimate rate and coalescent constant population prior for sequences. Furthermore, a strict clock model was employed for the "location" trait, exponential prior for the discrete location state rate (locations.clock.rate), keeping the default settings for the remaining parameters. We assessed the convergence of model parameters using Tracer 1.5 (Rambaut and Drummond 2009) and discarded the first 25% of sampled generations as burn-in. A chronogram with ancestral areas with the maximum sum of clade credibility (MCC) was obtained with TreeAnnotator 2.4.3 (Bouckaert et al. 2014) and visualized in FigTree 1.4.0 (Rambaut and Drummond 2012). We assessed statistical significance for dispersal events using the Bayes factor (BF) in SPREAD 1.0.6 (Bielejec et al. 2011).

Furthermore, we also time-calibrated the phylogeny of *Pachyptera* using BEAST 2.4.3 (Bouckaert et al. 2014). For this analysis, we used our combined molecular dataset, including a single accession per species recognized. The dataset was partitioned applying a TPM1uf model to *ndh*F, TPM1uf+G model to *rpl*32-*trn*L, and TVM model to *Pep*C model as suggested by JModelTest 2.1.4 (Darriba et al. 2012). We used a relaxed-clock log-normal approach with normal distribution of rates and a Yule speciation model. We used one secondary calibration point based on the genus age estimated by Lohmann et al. (2013). We performed the analysis with four runs for $10^6$ generations, sampling every 1000 generations. We checked for convergence in Tracer 1.5 (Rambaut and Drummond 2009). We combined all trees that resulted from the three runs using LogCombiner 2.4.3 (Bouckaert et al. 2014). We built a maximum clade credibility tree with median height nodes in TreeAnnotator 2.4.3 (Bouckaert et al. 2014), after excluding 25% of samples as burn-in.

We used the phylogeny of *Pachyptera* that was time-calibrated with BEAST, as basis to reconstruct ancestral areas using our species tree derived from the analysis of the combined cpDNA and nDNA datasets (including a single individual per species) in

RASP 3.1 (Yu et al. 2015), using the Dispersal Extinction Cladogenesis model (DEC, Ree et al. 2008). For this analysis we used the time-calibrated tree estimated for *Pachyptera* using the combined dataset (i.e., *ndh*F, *rpl*32-*trn*L and *Pep*C) and a single individual per species. This analysis reconstructed uncertainty in the geographic origin of *Pachyptera*, which is likely due to the broad distribution of *P. kerere*. Indeed, broadly distributed taxa can lose biogeographic signal given that lineage ancestors are found in multiple regions (i.e., Fonseca and Lohmann 2015). In order to recuperate the ancestral area of *Pachyptera*, we constructed a haplotype network of *P. kerere*, our broadly distributed taxon, using the cpDNA dataset. For this analysis, we assumed that higher levels of genetic diversity and central positions in the haplotype network should be located at more ancient areas than newly colonized areas located at the tips of the network (Kingman 1982, Posada and Crandall 2002). We constructed a haplotype network using the cpDNA dataset of *P. kerere* (n = 31) and statistical parsimony implemented in TCS 1.21 (Clement et al. 2000). For this analysis, we used a connection limit of 95% and treated gaps as a fifth character state. We visualized the network with tcsBU (dos Santos et al. 2015). The network of chloroplast haplotypes recovered 19 haplotypes with the *ndh*F dataset and 31 haplotypes with the *rpl*32-*trn*L dataset. The network derived from the analysis of the *ndh*F dataset indicated that North-Western Amazon and Central America & Andes were the most likely ancestral areas of *P. kerere* (Fig. S6). On the other hand, the network derived from the analysis of the *rpl*32-*trn*L dataset indicated that South-Eastern Amazon might be the ancestral area. However, the network constructed with the *rpl*32-*trn*L dataset included loops suggesting recombination. As such, we used North-Western Amazon and Central America & Andes as putative areas for the distribution of *P. kerere* in our final ancestral area reconstruction with RASP 3.1 (Yu et al. 2015).

## RESULTS

***Molecular datasets—*** Our dataset comprised 51 *Pachyptera* specimens distributed as follows among the various taxa: *P. aromatica* (5 indivs.), *P. erythraea* (2 indivs.), *P. kerere* (31 indivs.), *P. kerere* var. *incarnata* (10 indivs.), and samples of a new species (3 indivs.). All vouchers and GenBank accessions are shown in Tab. S1, while details about each dataset are shown in Tab. S2.

***Phylogeny of Pachyptera*—**The analyses of the *ndh*F and *rpl*32-*trn*L datasets recovered highly congruent topologies (Figs. S1-S2, respectively). In both analyses *Pachyptera* emerged as monophyletic, and divided into three main clades: (i) a clade that includes all specimens of *P. aromatica*; (ii) a clade that includes a monophyletic *P. erythraea* sister to a monophyletic *P. kerere* var. *incarnata*; and (iii) a clade that includes all individuals of *P. kerere s.s.* sister to a clade composed of all specimens of the putative *spec. nov*. The topology derived from the analysis of the combined cpDNA dataset recovered a similar topology but more strongly resolved (Fig. S3). The topology derived from the analysis of the *Pep*C dataset also recovered a monophyletic *Pachyptera* and the same three main clades (Fig. S4).

Topologies derived from the Bayesian and Maximum Likelihood analyses of the cpDNA and nDNA datasests were highly congruent and combined into a single data matrix. The phylogeny derived from the analysis of the combined *ndh*F, *rpl*32-*trn*L, and *Pep*C dataset also recovered a monophyletic *Pachyptera* (PP = 94, BS = 84). *Pachyptera aromatica* appears as sister to the remaining species of the genus. This clade (PP = 100, BS = 100), includes two major sub-clades: a highly supported clade (PP = 100, BS = 100) composed of *P. erythraea* (PP = 100, BS = 100) and *P. kerere* var. *incarnata* (PP = 100, BS = 99). This whole clade is sister to a moderately supported clade (PP = 76, BS = 84) composed of *P. kerere* (PP = 65, BS = 76) sister to the *spec. nov.* (PP = 99, BS = 86). As such, *P. kerere* (including *P. kerere* var. *incarnata*) emerged as a polyphyletic taxon.

***Species delimitation within Pachyptera kerere***. The GMYC analysis using the single threshold approach recovered five putative species within the *P. kerere* species complex (Figs. 3, S4) with confidence intervals from 3-12 ($logL_{null}$ = 23.14685; $logL_{GMYC}$ = 26.60204; LR = 6.910382; p = 0.03158128) and threshold time (transition in branching rate occurring) of ca. 7.1 Mya. The likelihood ratio test was significant, indicating that the null model (i.e., a single population) could be rejected. The bPTP result (Fig. S5) was similar to the GMYC delimited species indicating that the species boundaries suggested by GMYC did not vary with branch length.

The five species recovered within the *P. kerere* species were evaluated using morphological data. This data only provided support for the recognition of four out of the five possible species identified with GMYC. More specifically, morphological data

provided further support for the recognition of *P. erythraea*, *P. kerere*, *P. kerere* var. *incarnata*, and the *spec. nov.*, but did not support the recognition of the earliest diverging clade within *P. kerere* as a separate species. All the morphological data is summarized in Table S3. More specifically, while *Pachyptera kerere* and the *spec. nov* share white infundibuliform corollas, included androecia, and a puberulous ovary, these taxa differ in fruit traits. Namely, members of the *spec. nov*. differ from *P. kerere* by the linear and flat fruits, with thin, coriaceous to woody and winged seeds (vs. the inflated fruits with thick, corky and wingless seeds of *P. kerere*). While the fruits of the *spec. nov.* are similar to those of *P. kerere* var. *incarnata* and *P. erythraea*, taxa can be distinguished based on other morphological features. Namely, the *spec. nov* has a puberulous ovary, while *P. kerere* var. *incarnata* and *P. erythraea* share a lepidote ovary. Furthermore, while *P. kerere* var. *incarnata* has light pink to pale purple infundibuliform corollas, and included androecium, *P. erythraea* has red infundibuliform campanulate corollas, and a sub-exserted androecium. All of these morphological features provide support for the recognition of these four species.

The four species hypothesis identified based on GYMC and morphology (i.e., *P. erythraea*, *P. kerere*, *P. kerere* var. *incarnata*, and the *spec. nov.*) was further tested with *BEAST, which provided additional support for the recognition of four taxa within the *P. kerere* species complex (PP > 95, Figs. 4). As such, the possible recognition of a sub-clade of *P. kerere* as a separate species was discarded.

***Biogeography of Pachyptera—*** Divergence times estimated based on the analysis of the combined cpDNA dataset (i.e., *ndh*F, *rpl*32-*trn*L) using BSSVS, suggested that the genus originated at ca. 40.4 Mya [95% highest posterior density (HPD): 43.5–37.3 Mya], in the Late Eocene. These analyses further suggest that the split between *P. erythraea* and *P. kerere* var. *incarnata* may have occurred during the Miocene, at ca. 14.6 Mya [95% HPD: 24.7–5.8 Mya], while the divergence between *P. kerere* and the *spec. nov.* might have occurred at ca. 12.9 Mya [95% HPD: 24.0–4.7 Mya]. On the other hand, divergence times estimated based on the analysis of the combined dataset (i.e., *ndh*F, *rpl*32-*trn*L, *Pep*C) using RASP and the DEC model and including a single individual per species, suggested that the genus may have originated at ca. 40.3 million years ago (Mya), in the Late Eocene [95% HPD: 43.5–37.2 Mya]. These analyses further suggest that the genus diversified during the Miocene, at ca. 23.5

Mya [95% HPD: 38.5–10.5 Mya]. The species diversity belonging to *P. kerere* dates back to the Late Miocene, with the split between *P. erythraea* and *P. kerere* var. i*ncarnarta* occurring at approximately 15.4 Mya [95% HPD: 34.9–3.4 Mya], and the divergence between *P. kerere* and the *spec. nov.* at ca. 9.8 Mya [95% HPD: 23.8–1.0 Mya]. Node ages were very similar regardless of the dataset used (i.e., combined cpDNA dataset and nuclear dataset) (Tab. S4).

The reconstruction of ancestral areas within *Pachyptera* using the BSSVS is summarized in Figure 4. These reconstructions showed uncertainty in the geographic origin of *Pachyptera* and its species, recovering multiple possible ancestral areas (< 31), all of which are poorly supported. BSSVS recovered three equally likely (PP = 21) biogeographical areas for the first split of *Pachyptera*, i.e., Central America & Andes, North-Western Amazon, and North Eastern Amazon. Subsequent splitting occurred in Central America & the Andes (PP = 24). Two alternatives areas, Central America & Andes and North Eastern Amazon, were recovered as equally likely (PP = 23) for the *P. erythraea* and *P. kerere* var. *incarnata* clade. Central America & Andes was recovered as the most likely ancestral area for the *P. kerere* and the *spec. nov.* clade (PP = 31; Figure 5; Tab. S5). Dispersal frequencies revealed two significant (BF > 3) routes for dispersal between: (i) North Western Amazon to South Western Amazon (BF = 131); and (ii) North Western Amazon to South Eastern Amazon (BF = 21). Furthermore, one moderately significant route (BF > 2.9) was recovered between North Eastern Amazon to South Eastern Amazon (BF = 2.9). Two additional routes were not-significant and only supported by BF < 1.4 (Tab. S6): (i) North Eastern Amazon to central America & Andes (BF = 1.4), and (ii) South Eastern Amazon to Central America & Andes (BF = 1.3).

Biogeographical analyses using RASP with the DEC model and coding the distribution of *P. kerere* using the two ancestral areas identified by the haplotype networks (i.e., North-Western Amazon, and Central America & Andes), ambiguously recuperated the ancestral area of the genus (Fig. 5, Tab. S7). In this analysis, Central America & Andes was recovered as the most likely ancestral area for the *P. erythraea* + *P. kerere* var. *incarnata* clade (RP = 0.54). On the other hand, Central America & Andes, plus North-Western Amazon were recovered as most likely ancestral areas for the *P. kerere* + *spec. nov.* clade (RP = 0.67).

## DISCUSSION

In this study, we used molecular data (i.e., *ndh*F, *rpl*32–*trn*L, and *Pep*C) to reconstruct relationships among species of *Pachyptera*, a small genus of Neotropical lianas. Our analysis included a broad sampling of taxa (i.e., 51 individuals from all species recognized), and was used as basis to evaluate species boundaries. A polyphyletic *P. kerere* was recovered, indicating the need for taxonomic changes. The phylogenetic framework produced was used as basis to evaluate species limits within the *P. kerere* species complex, estimate divergence times and reconstruct the biogeographic history of the genus. The genus originated in the Late Eocene, and diversified during the Miocene, a period of intense geological, hydrological and climatic event. Below, we summarize our major findings and discuss their implications for the systematics and biogeography of *Pachyptera*.

***Phylogeny of Pachyptera—*** The phylogeny constructed here provides further support for the monophyly of *Pachyptera* recovered in earlier studies (Lohmann 2006, Francisco and Lohmann submitted). *Pachyptera aromatica* is strongly supported as sister to the rest of the genus which, in turn, includes two main clades: (i) a clade composed of *P. erythraea* and *P. kerere* var *incarnata*, and (ii) a clade composed of *P. kerere* and a *spec. nov.* (Fig. 3). This new phylogenetic framework provides additional support for the position of *P. aromatica* within *Pachyptera*. These findings are further supported by morphological synapomorphies such as stems with four phloem wedges in cross section, corollas with glands arranged in lines in the upper portions of the tube, racemose inflorescences and 3-seriated prophylls of the axillary buds, all of which are unique in tribe Bignonieae and a potential synapomorphy of the clade (Lohmann and Taylor 2014, Francisco and Lohmann submitted). The phylogenetic framework reconstructed here also clarifies species limits within the *P. kerere* species complex. While *P. kerere* var. *incarnata* was thought to be more closely related to *P. kerere* than *P. erythraea* (Gentry 1973, Lohmann and Taylor 2014), our study shows that *P. kerere* var. *incarnata* is sister to *P. erythraea*. As such, *P. kerere* (including *P. kerere* var. *incarnata*) appears as polyphyletic as currently circumscribed. In addition, a lineage with dubious identification (i.e., *spec. nov.*), including mixed flower and fruit features between *P. kerere P. kerere* var. *incarnata* emerged strongly supported as clade sister of

*P. kerere*, providing further support for the recognition of this lineage as a separate taxon.

***Species delimitation and taxonomic implications—*** Species delimitation is a controversial topic in biology, which is partly due to the fact that speciation is a continuous process and any group of study may be in different stages of differentiation (de Queiroz 1998; de Queiroz 2007). Multiple species concepts have been proposed in the literature (see de Queiroz 1998, 2007). For this study, we treat independently evolving meta-populations as species (de Queiroz 1998; de Queiroz 2007), and integrate morphological and molecular data while defining species limits (Padial et al. 2010; Fujita et al. 2012).

Our findings provide important new insights for the delimitation of species within *Pachyptera*, especially in what concerns the taxonomically complicated *P. kerere* species complex. The *P. kerere* species complex is reconstructed as polyphyletic, with the pink flowered *P. kerere* var. *incarnata*, appearing as sister to the red flowered *P. erythraea*. On the other hand, the white flowered *P. kerere* appears as sister to the white flowered *spec. nov.* Despite the similarities in flower morphology, these taxa differ in fruit and seed morphology. More specifically, while seeds of the *spec. nov.* are thin, winged and wind dispersed, *P. kerere* has corky and wingless seeds, that are water dispersed. The cryptic *spec. nov.* was never noticed before, probably due the overlapping patterns of morphological variation with *P. kerere* and *P. kerere* var. *incarnata*, the lack of a phylogenetic framework and the need of fruits and flowers for its recognition. The recognition of these four clades as separate species within the *P. kerere* species complex is strongly supported by the molecular phylogeny of the genus (Fig. 2), the species delimitation analyses conducted with GMYC and morphology (Tab. S1, Fig. 3), and the final *BEAST validation (Fig. 3).

In our study, morphological observations and DNA sequences acted in concert, allowing a clear definition of species boundaries. Species boundaries can be semi-permeable, reflecting limited gene flow between taxa (Harrison and Larson 2014). Pollination shifts are known to represent a key speciation driver within tribe Bignonieae (Alcantara and Lohmann 2010). Indeed, differences in floral structure and phenology have been hypothesized to represent a key speciation driver within this tribe (Gentry 1974; Gentry 1990; Alcantara and Lohmann 2010). Specifically in *Pachyptera*, the

white flowered *P. aromatica* is hawk-moth pollinated (Barbosa Rodrigues 1891; Gentry 1974), while the red flowered *P. erythraea* is hummingbird pollinated (Gentry 1974), and the white flowered *P. kerere* is likely pollinated by large to medium-sized bees (Gentry 1974). The pink flowered *P. kerere* var. *incarnata* and the *spec. nov*. are likely pollinated by small bees. The great diversity in pollination systems found in such a small genus of tropical lianas may be associated with the diversification of this group. However, additional studies are needed to further test this hypothesis. These morphological and ecological differences provide further support for the recognition of four separate taxa within the *P. kerere* species complex (Sites and Marshall 2004; Pons et al. 2006; Knowles and Carstens 2007; Patial et al. 2010). A new taxonomic circumscription of taxa within *Pachyptera* is greatly needed in order to accommodate those changes (Francisco and Lohmann submitted).

***Biogeography of Pachyptera—*** The biogeographic history of *Pachyptera* was reconstructed with BSSVS using the complete cpDNA dataset (population level sampling), and RASP based using the combined cpDNA and nDNA datasets (including one individual per species recognized). Dates reconstructed based on both datasets are highly consistent. Our results indicate that *Pachyptera* diverged between 40.3 Mya (95% HPD, 43.5–37.2 Mya according to RASP) and 40.4 Mya (95% HPD, 43.5–37.3 according to BSSVS), a period of intense activity in the Amazon basin (Jaramillo et al. 2006). These results support an origin of *Pachyptera* in lowland Amazonia during the Late Eocene, corroborating earlier findings (Lohmann et al. 2013).

Although our analysis could not establish the origin of *Pachyptera* with certainty, it suggests movements from North to South within the Amazon (BF >3, Tab. S6). *Pachyptera aromatica* is sister to the rest of the genus. The diversification of this taxon likely began in Guiana, and was followed by the occupation of South Eastern Amazon and North-Western Amazon, at approximately 2 Mya. Early diverging evolutionary units are often associated with more stable and geologically older terrains that may have acted as “species-pumps” of diversity (Aleixo and Rossetti 2007). The Guiana Shields remained stable since the Late Cretaceous (Rosetti et al. 2005), representing an important center of diversity for birds (Aleixo and Rossetti 2007), insects (Solomon et al. 2008), and palms (Roncal et al. 2012).

The next split within the genus gave rise to the *P. kerere* species complex. This diversification event occurred during the Miocene, sometime between 23.5 Mya (95% HPD: 38.5–10.5 Mya according to RASP) and 26.5 Mya (95% HPD: 39.7–13.2 Mya according to BSSVS), in Central America & the Andes (PP = 24, Fig. 4, Tab. S5, according to BSSVS; PP = 30, Fig. 5, Tab. S7, according to RASP). This age predates the closure of the Panama land bridge (Montes et al. 2012, 2015; Leigh et al. 2014; Bacon et al. 2015, 2016; O'Dea et al. 2016) suggesting long dispersal of *Pachyptera* from Amazonia to Central America, a pattern also suggested for other organisms (e.g., Bacon et al. 2015; Cody et al. 2010).

The split between *P. erythraea* and *P. kerere* var. i*ncarnata* occurred sometime between 15.4 Mya (95% HPD: 34.9–3.4 Mya, according to RASP) and 14.6 Mya (95% HPD: 24.7–5.8 Mya according to BSSVS), most likely in Central America & the Andes. While *P. erythraea* remained in Central America & the Andes, *P. kerere* var. *incarnata* expanded its range to the Amazon (Figs. 4, 5). *Pachyptera erythraea* is endemic to the Magdalena Valley, which separated the northeastern Cordillera into Eastern and Western Cordilleras, at ca. 11.8 Mya (Hoorn et al. 1995). The diversification of *P. kerere* and the *spec. nov.* occurred at a similar time, sometime around 9.8 Mya (95% HPD: 23.8–1.0 Mya according to RASP) and 12.9 Mya (95% HPD: 24.0–4.7 Mya according to BSSVS). Two alternative areas were reconstructed by BSSVS and RASP as possible ancestral areas for *P. kerere*. According to the BSSVS results, Central America & the Andes are the most likely areas for the ancestor of the *P. kerere*, which would have subsequently expanded into the Amazon, while its sister taxon (*spec. nov.*) would have expanded to North-Western Amazon (Fig. 4, Tab. S5). According to the RASP results, the ancestor of the *P. kerere* clade would have been broadly distributed through Central America & Andes plus North-Western Amazon and would have subsequently expanded to Southwestern and Southeastern Amazon and the Guiana, while the ancestor of the *spec. nov.* would have dispersed to a different region of North-Western Amazon (Fig. 6, Tab. S7).

Range expansion in *P. kerere* may have been facilitated by fluvio-marine dispersal via floodplains in Western Amazon, while regional differentiation of the *spec. nov.* may have been tied to geographic heterogeneity. The potential switch from wind-dispersed to water-dispersed seeds may have also helped the spread of *P. kerere*

populations across the Amazon river drainage (Gentry 1983). The influence of the paleo- and contemporaneous river drainage on the origin of *P. kerere* can be easily tested. The coalescent-analysis BSSVS of the *P. kerere* species complex was not structured geographically. Despite that, one of the first lineages to diverge is restricted to Madre de Dios and appears to be isolated from other populations. It is possible that the formation of the Fitzcarrald arch from foreland (Encarnación and Basilio 2008) may have been associated with this genetic break (e.g., Roncal et al. 2015).

The biogeographic history of *Pachyptera* suggests that switches in ecological traits (i.e., dispersal) seem to have played important roles for the diversification of this group. Furthermore, *Pachyptera* diversified substantially during the Miocene, a period of intense pertubations in South America, suggesting that important geological events (e.g., uplift Andes, marine incursions, dry and wetland systems) may have played a significant impact on the history of the genus, similar to what has been observed in other angiosperm groups (Antonelli et al. 2009; Hoorn *et al*. 2010; Antonelli and Sanmartín 2011). Indeed, global climatic changes affected plant diversity in the tropics and triggered diversification during this time (Jaramillo et al. 2006, 2010). Similarly, the Andean orogeny and sea level fluctuations prompted landscape changes by reconfiguring reliefs, regional climatic regimes, influx of sediments, and changes in the drainage pattern in the Amazon. The dynamic sedimentary history of this region revealed several episodes of marine incursions that covered western Amazonian lowlands forming a huge fluvio-lacustrine system (known as Lake Pebas), which included marshes, from early Miocene until at least the late Miocene (Hoorn 1993; Hoorn et al. 1995; Hoorn et al. 2010). This system may have acted as a dispersal barrier between Northern Andes, Eastern Amazonia and the Guyana (Wesselingh and Salo 2006; Antonelli et al. 2009), leading to the fragmentation of the biota and subsequent *in situ* speciation or extinction. At that time, the extensive Lake Pebas started to undergo a new reorganization and to acquire its modern characteristics, including the eastward flow that led to the formation of the Amazon River during the Late Pleistocene (Rossetti et al. 2005; Aleixo and Rossetti, 2007; Ribas et al. 2011). These geological events created plenty of opportunities for new colonization by others plants in this region.

***Conclusions and future directions*—** Our findings provide the basis for a series of taxonomic changes and a detailed species-level account for the whole genus

(Francisco and Lohmann in prep.). More specifically, our findings corroborate the recognition of five species within *Pachyptera*, namely the previously recognized *P. aromatica* and *P. erythraea*, the newly circumscribed *P. kerere* and *P. kerere* var. *incarnata*, and an additional *spec. nov*. Coalescence analysis indicated that the studied populations of each of these taxa are genetically and historically isolated from others lineages, indeed deserving species recognition. These molecular findings are further supported by a suit of morphological traits and differences in pollination syndrome. Our study also establishes initial hypotheses about the biogeographic history of the genus. More specifically, *Pachyptera* seems to have originated in the lowland Amazon during the Late Eocene and to have diversified during the Late Miocene, a period with intense perturbations (e.g., uplift Andes, marine incursions, dry and wetland systems). However, more detailed phylogeographic studies are still necessary to investigate the mechanisms underlying the patterns recovered here. Recently developed microsatellites markers for *Pachyptera* (Francisco et al. 2016) offer a great opportunity for future population genetic and phylogeographic studies within *Pachyptera*.

## LITERATURE CITED

Alcantara, S., and L.G. Lohmann. 2010. Evolution of floral morphology and pollination system in Bignonieae (Bignoniaceae). *American Journal of Botany* 97: 782–796.

Aleixo, A. 2004. Historical diversification of a terra firme forest bird superspecies: a phylogeographic perspective on the role of different hypotheses of Amazonian diversification. *Evolution* 58: 1303–1317.

Aleixo, A., and D. de Fátima Rossetti. 2007. Avian gene trees, landscape evolution, and geology: towards a modern synthesis of Amazonian historical biogeography? *Journal of Ornithology* 148: 443–453.

Antonelli, A., and I. Sanmartín. 2011. Why are there so many plant species in the Neotropics? *Taxon* 60:403–14.

Antonelli, A., J.A. Nylander, C. Persson, and I. Sanmartín. 2009. Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences* 106: 9749–9754.

Bacon, C. D., D. Silvestro, C. Jaramillo, B.T. Smith, P. Chakrabarty, and A. Antonelli. 2015. Biological evidence supports an early and complex emergence of the Isthmus of Panama. *Proceedings of the National Academy of Sciences* 112: 6110–6115.

Bacon, C.D., P. Molnar, A. Antonelli, A.J. Crawford, C. Montes, and M.C. Vallejo-Pareja. 2016. Quaternary glaciation and the Great American Biotic Interchange. *Geology* 44: 375–378.

Barbosa Rodrigues, J. 1891. *Eclogae plantarum novarum*. Vellosia, Rio de Janeiro, 1: 1−133.

Bergsten, J., D.T. Bilton, T. Fujisawa, M. Elliot, M. T. Monaghan, M. Balke, L. Hendrich, J. Geijer, J. Herrmann, G. N. Foster, I. Ribera, A. N. Nilsson, T. G. Barraclough and A. P. Vogler. 2012 . The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology* 61: 851−869.

Bielejec, F., A. Rambaut, M.A. Suchard, and P. Lemey. 2011. SPREAD: spatial phylogenetic reconstruction of evolutionary dynamics. *Bioinformatics* 27: 2910–2.

Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C.H. Wu, D. Xie, M.A. Suchard, A. Rambaut, and A.J. Drummond. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* 10:e1003537.

Bush, M. B. 1994. Amazonian speciation: A necessarily complex model. *Journal of Biogeography* 21:5–17.

Clement, M., D. Posada, and K.A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657–1659.

Cody, S., J.E. Richardson, V. Rull, C. Ellis, and R.T. Pennington. 2010. The great American biotic interchange revisited. *Ecography* 33: 326–32.

Cracraft, J. 1983. Species concepts and speciation analysis. *Current Ornithology* 1: 159–187.

Cracraft, J. and R. O. Prum. 1988. Patterns and processes of diversification: speciation and historical congruence in some neotropical birds. *Evolution* 42: 603–620.

Darriba, D., G.L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and high-performance computing. *Nature Methods* 9: 772.

de Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. In Endless Forms: *Species and Speciation*, ed. DJ Howard, SH Berlocher, pp. 57–75. Oxford: Oxford Univ. Press

de Queiroz, K. 2007. Species concepts and species delimitation. *Systematic Biology* 56: 879–86.

Dugand, A. 1955. Bignoniaceas nuevas o notables de Colombia. Caldasia 7: 16.

Duminil, J., and M. Di Michele. 2009. Plant species delimitation: a comparison of morphological and molecular markers. *Plant Biosystems* 143: 528–542.

Encarnación, I.B.M. 2008. *Modelado de los sistemas petroleros en las cuencas subandinas del Perú (cuencas: Madre De Dios, Ucayali, Huallaga, Santiago y Marañón)*. Dissertation. Lima, Perú: Universidad Nacional de Ingenieria.

Ewing, B., L. Hillier, M.C. Wendl, and P. Green. 1998. Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. *Genome Research* 8: 175–85.

Ezard, T., T. Fujisawa, and T.G. Barraclough. 2009. Splits: species limits by threshold statistics. R package version 1.0-11/r29. Available from: https://rdrr.io/rforge/splits/man/splits-package.html (17 March 2017).

Fonseca, L.H., and L.G. Lohmann. 2015. Biogeography and evolution of *Dolichandra* (Bignonieae, Bignoniaceae). *Botanical Journal of the Linnean Society* 179: 403–20.

Fujisawa, T., and T.G Barraclough. 2013. Delimiting species using single-locus data and the Generalized Mixed Yule Coalescent approach: a revised method and evaluation on simulated data sets. *Systematic Biology* 62: 707–724.

Fujita, M.K., A.D. Leaché, F.T. Burbrink, J.A. McGuire, and C. Moritz. 2012. Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology & Evolution* 27: 480–488.

Gentry, A. H. 1973a. Generic delimitations of Central American Bignoniaceae. *Brittonia* 25: 226-242.

Gentry, A. H. 1974. Coevolutionary patterns in Central American Bignoniaceae. *Annals of the Missouri Botanical Garden* 61: 728–759.

Gentry, A. H. 1979. Additional generic mergers in Bignoniaceae. *Annals of the Missouri Botanical Garden* 66: 778–787.

Gentry, A. H. 1982. Patterns of neotropical plant species diversity. *Evolutionary Biology* 15: 1–84.

Gentry, A. H. 1990. Evolutionary patterns in neotropical Bignoniaceae. *Memoirs of the New York Botanical Garden* 55: 118–129.

Gentry, A.H. 1983. Dispersal and distribution in Bignoniaceae. *Sonderbaende des Naturwissenschaftlichen Vereins in Hamburg* 7: 187–99

Gentry, A.H. 1989. Speciation in tropical forests. Pp. 113–134 in *Tropical forests: botanical dynamics, speciation and diversity*, ed. Holm Nielsen, L.B., Nielsen, I.C. & Balslev, H. San Diego: Academic Press.

Haffer, J. 1969. Speciation in Amazonian forest birds. *Science* 165: 131–137.

Harrison, R. G., and E.L. Larson. 2014. Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity* 105: 795–809.

Heled, J., and A.J. Drummond. 2009. Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* 27: 570–580.

Hoorn, C. 1993. Marine incursions and the influence of Andean tectonics on the Miocene depositional history of northwestern Amazonia: results of a palynostratigraphic study. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 105: 267–309.

Hoorn, C., F.P. Wesselingh, H. Ter Steege, M.A. Bermudez, A. Mora, J. Sevink, I. Sanmartín, A. Sanchez-Meseguer, C.L. Anderson, J.P. Figueiredo, and C. Jaramillo. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* 330: 927–931.

Hoorn, C., J. Guerrero, G.A. Sarmiento, and M.A. Lorente. 1995. Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. *Geology* 23: 237–240.

Hopkins, M. J. G. 2007. Modelling the known and unknown plant biodiversity of the Amazon Basin. *Journal of Biogeography* 34: 1400–1411.

Jaramillo, C., C. Hoorn, S. A. F. Silva, F. Leite, L. Herrera, F. Quiroz, R. Dino, and L. Antonioli. 2010. The origin of the modern Amazon rainforest: implications of the palynological and palaeobotanical record. Pp. 317–334. In: Hoorn C. and F.P. Wesselingh (Eds.), *Amazonia, Landscape and Species Evolution: a Look into the Past*. Oxford: Wiley-Blackwell.

Jaramillo, C, M.J. Rueda, and G. Mora. 2006. Cenozoic plant diversity in the Neotropics. *Science* 311: 1893–1896.

Kaehler, M., F.A. Michelangeli, and L.G. Lohmann. 2012. Phylogeny of *Lundia* (Bignoniaceae) based on *ndhF* and *PepC* sequences. *Taxon* 61: 368–80.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, and T. Thierer. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.

Kingman, J.F.C. 1982. The coalescent. *Stochastic Process and Their Impplications* 13: 235–248.

Knowles, L.L., and B.C. Carstens. 2007. Delimiting species without monophyletic gene trees. *Systematic Biology* 56: 887–95.

Leigh, E. G., A. O'dea, and G.J. Vermeij. 2014. Historical biogeography of the Isthmus of Panama. *Biological Reviews* 89: 148–172.

Lemey, P., A. Rambaut, A.J. Drummond, and M.A. Suchard. 2009. Bayesian phylogeography finds its roots. *PLoS Computational Biology* 5: e1000520.

Lohmann, L. G. and C.M. Taylor. 2014. A new generic classification of Bignonieae (Bignoniaceae) based on molecular phylogenetic data and morphological synapomorphies. *Annals of the Missouri Botanical Garden* 99: 348–489.

Lohmann, L.G. 2006. Untangling the phylogeny of neotropical lianas (Bignonieae, Bignoniaceae). *American Journal of Botany* 93: 304–18.

Lohmann, L.G. and Ulloa Ulloa, C. Bignoniaceae in iPlants prototype Checklist. http://iplants.org (accessed 27 Apr 2016).

Lohmann, L.G., C.D. Bell, M.F. Calió, and R.C. Winkworth. 2013. Pattern and timing of biogeographical history in the Neotropical tribe Bignonieae (Bignoniaceae). *Botanical Journal of the Linnean Society* 171:154–170.

Löwenberg–Neto, P. 2014. Neotropical region: a shapefile of Morrone's (2014) biogeographical regionalisation. *Zootaxa* 3802: 300.

Medeiros, M.C., and L.G. Lohmann. 2015. Phylogeny and biogeography of *Tynanthus* Miers (Bignonieae, Bignoniaceae). *Molecular Phylogenetics and Evolution* 85:32–40.

Montes, C., A. Cardona, C. Jaramillo, A. Pardo, J. Silva, V. Valencia, C.Ayala, L.C. Pérez–Angel, L.A. Rodriguez–Parra, V. Ramirez and H. Niño. 2015. Middle Miocene closure of the Central American seaway. *Science* 348: 226–229.

Montes, C., A. Cardona, R. McFadden, S.E. Morón, C.A. Silva, S. Restrepo–Moreno, D.A. Ramírez, N. Hoyos, J. Wilson, D. Farris, and G.A. Bayona. 2012. Evidence for middle Eocene and younger land emergence in central Panama: implications for Isthmus closure. *Geological Society of America Bulletin* 124: 780–799.

Moritz, C., J. L. Patton, C. J. Schneider and T. B. Smith. 2000. Diversification of rainforest faunas: an integrated molecular approach. *Annual Review of Ecology and Systematics* 31: 533–563.

Morrone, J.J. 2014. Biogeographical regionalisation of the neotropical region. *Zootaxa* 3782: 1–110.

Nielsen, R., and J. Wakeley. 2001. Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics* 158: 885–896.

O'Dea, A., H.A. Lessios, A.G. Coates, R.I. Eytan, S.A. Restrepo–Moreno, A.L. Cione, L.S. Collins, A. de Queiroz, D.W. Farris, R.D. Norris, and R.F. Stallard. 2016. Formation of the Isthmus of Panama. *Science Advances* 2: e1600883.

Olmstead, R.G., M.L. Zjhra, L.G. Lohmann, S.O. Grose, and A.J. Eckert. 2009. A molecular phylogeny and classification of Bignoniaceae. *American Journal of Botany* 96:1731–1743.

Padial, J. M., A. Miralles, I. De la Riva, and M. Vences. 2010. The integrative future of taxonomy. *Frontiers in Zoology* 7: 16.

Pons, J., T.G. Barraclough, J. Gomez–Zurita, A. Cardoso, D.P. Duran, S. Hazell, S. Kamoun, W.D., and A.P. Vogler. 2006. Sequence–based species delimitation for the DNA taxonomy of undescribed insects. *Systematic Biology* 55: 595–609.

Posada, D., and K.A. Crandall. 2002. The effect of recombination on the accuracy of phylogeny estimation. *Journal of molecular evolution* 54: 396–402.

Rambaut, A., and A.J. Drummond. 2009. Tracer, Version 1.5, MCMC Trace Analysis Package. Available from: http://tree.bio.ed.ac.uk/software/ (02 January 2015).

Rambaut, A., and A.J. Drummond. 2012. FigTree. Version 1.4. 0. Available from: http.tree.bio.ed.ac.uk/software/figtree (02 January 2015).

Ree, R. H.,S.A. Smith, and A. Baker. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* 57: 4–14.

Ribas, C. C., A. Aleixo, A.C. Nogueira, C.Y. Miyaki, and J. Cracraft. 2011. A palaeobiogeographic model for biotic diversification within Amazonia over the past three million years. *Proceedings of the Royal Society of London B: Biological Sciences* 279: 681–689.

Roncal, J., F. Kahn, B. Millan, T.L. Couvreur, and J.C. Pintaud. 2012. Cenozoic colonization and diversification patterns of tropical American palms: evidence from *Astrocaryum* (Arecaceae). *Botanical Journal of the Linnean Society* 171: 120–139.

Roncal, J., M. Couderc, P. Baby, F. Kahn, B. Millán, A.W. Meerow, and J.C. Pintaud. 2015. Palm diversification in two geologically contrasting regions of western Amazonia. *Journal of Biogeography* 42: 1503–1513.

Ronquist, F., P. Teslenko, D. van der Mark, A. Ayres, S.H. Darling, B. Höhna, L. Larget, M. Liu, A. Suchard, and J.P. Huelsenbeck. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.

Rossetti, D. D., P. M. de Toledo, and A. M. Goes. 2005. New geological framework for western Amazonia (Brazil) and implications for biogeography and evolution. *Quaternary research*. 63: 78–89.

Rull, V. 2011. Neotropical biodiversity: timing and potential drivers. *Trends in Ecology & Evolution* 26: 508–13.

Silva, J. M. C., A. B. Rylands, and G. A. B. da Fonseca. 2005. The fate of the Amazonian areas of endemism. *Conservation Biology* 19:689–694.

Silvestro, D., and I. Michalak. 2012. raxmlGUI: a graphical front–end for RAxML. *Organisms Diversity & Evolution* 12: 335–337.

Santos, A.M., M. P. Cabezas, A.I. Tavares, R. Xavier, and M. Branco. 2015. tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics* 32: 627–628.

Sites, J. W., and J.C. Marshall. 2003. Delimiting species: a Renaissance issue in systematic biology. *Trends in Ecology & Evolution* 18: 462–470.

Solomon, S.E., Jr M. Bacci, Jr J. Martins, G.G. Vinha, and U.G. Mueller. 2008. Paleodistributions and comparative molecular phylogeography of leafcutter ants (*Atta* spp.) provide new insight into the origins of Amazonian diversity. *PLoS One* 3:e2738.

Sprague, T.A., and N.Y. Sandwith. 1932. Contributions to the flora of tropical America: X. *Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew)* 81–93.

Stamatakis, A. 2006. RAxML–VI–HPC: maximum likelihood–based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.

Talavera, G., V. Dincă, and R. Vila. 2013. Factors affecting species delimitations with the GMYC model: insights from a butterfly survey. *Methods in Ecology and Evolution* 4: 1101–1110.

Velasco, J. D. 2008. Species concepts should not conflict with evolutionary history, but often do. *Studies in History and Philosophy of Biological and Biomedical Sciences* 39:407–414.

Wallace, A. R. 1854. On the monkeys of the Amazon. *Journal of Natural History*, 14: 451–454.

Wesselingh, F. P., and J.A. Salo. 2006. A Miocene perspective on the evolution of the Amazonian biota. *Scripta Geologica* 133: 439–458.

Wesselingh, F.P., Hoorn, C., Kroonenberg, S.B., Antonelli, A., Lund berg, J.G., Vonhof, H.B. and Hooghiemstra, H. 2010. On the origin of Amazonian landscapes and biodiversity: A synthesis. Pp. 421–431 in: Hoorn, C. & Wesselingh, F. (eds.), *Amazonia: Landscape and species evolution*, 1st ed. Oxford: Wiley-Blackwell

Yu, Y., A.J. Harris, C. Blair, and X. He. 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Molecular Phylogenetics and Evolution* 87: 46–49.

Zhang, J., P. Kapli, P. Pavlidis, and A. Stamatakis 2013. A general species delimitation method with applications to phylogenetic lacements. *Bioinformatics* 29: 2869–2876.

Zuntini, A.R, L.H. Fonseca, and L.G. Lohmann. LG. Primers for phylogeny reconstruction in Bignonieae (Bignoniaceae) using herbarium samples. *Application in Plant Science* 1:1300018.

**FIGURE CAPTIONS**

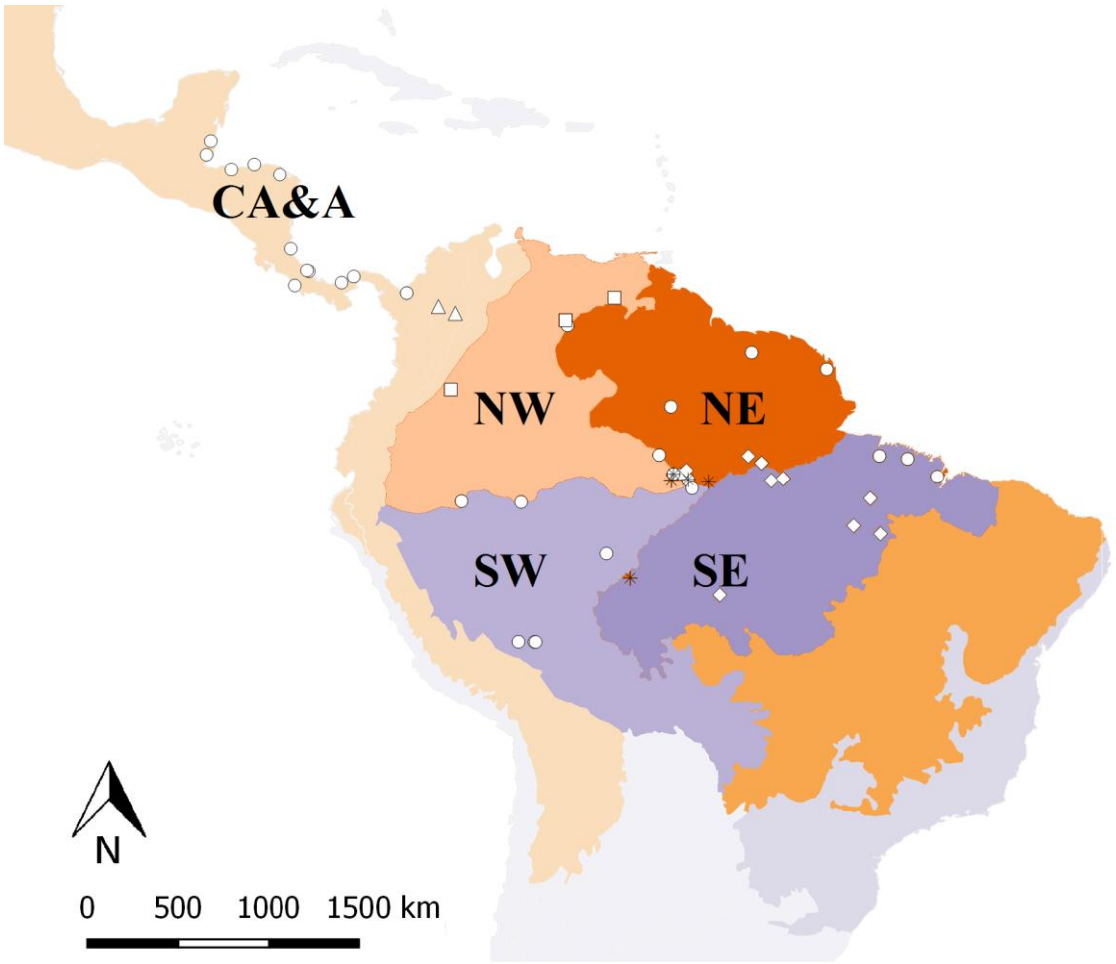


**Figure 1.** Central and South America showing the location of *Pachyptera* sampling by species. Asterisks corresponds to *P. aromatica*, diamond corresponds to *P. kerere* var. *incarnata*, triangle corresponds to *P. erythraea*, circle corresponds to *P. kerere*, and square corresponds to a *spec. nov.* Biogeographic areas color coded and correspond to Central America & Andes (CA&A), North–Western Amazonia (NW), North Eastern Amazonia (NE), South Western Amazonia (SW), and South Eastern Amazonia (SE).

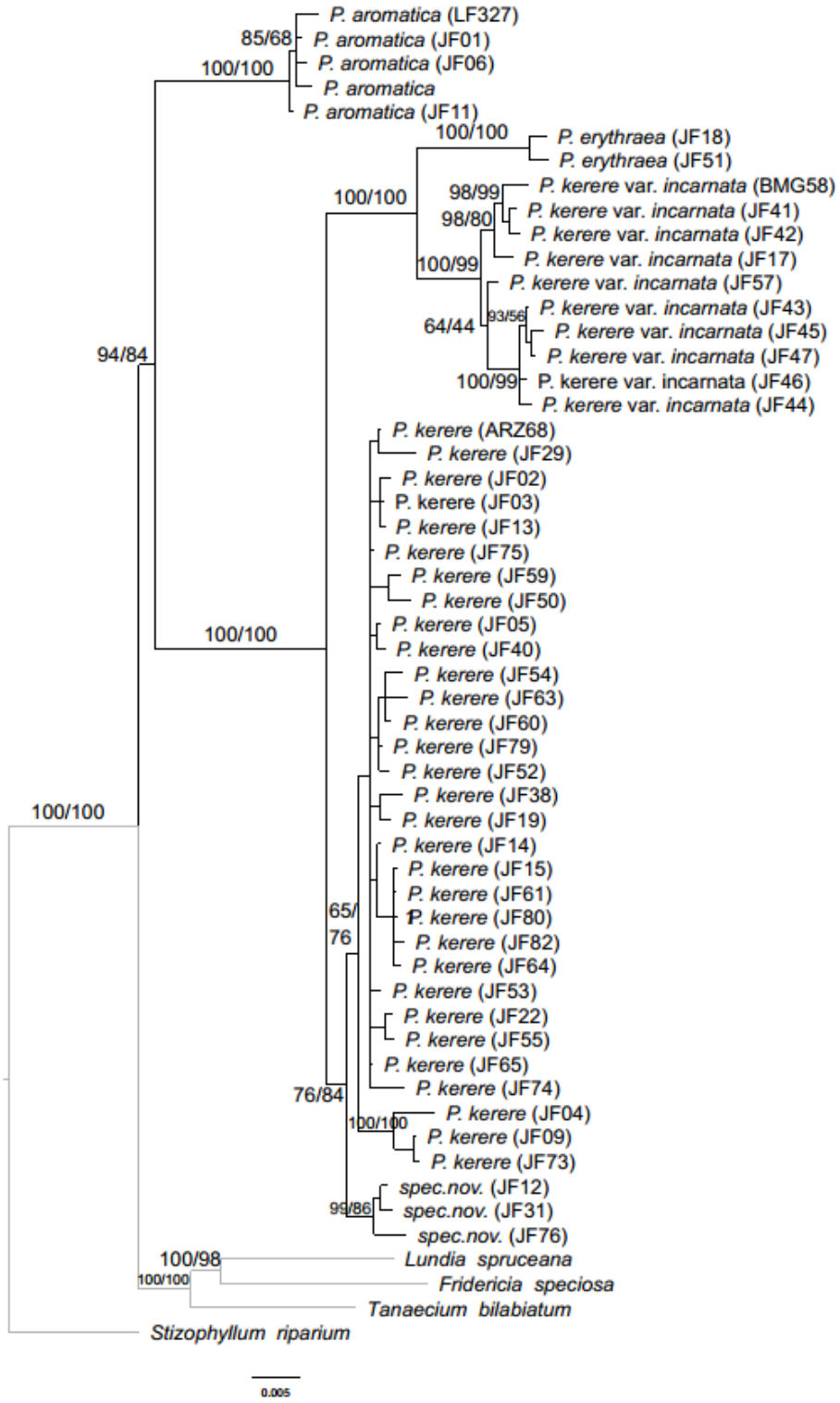


**Figure 2.** Consensus tree derived from the Bayesian analysis of the combined dataset (i.e., *ndh*F, *rpl*32–*trn*L, and *Pep*C) indicate phylogenetic relationships within *Pachyptera*. Posterior probability and bootstrap values are shown above branches. Outgroups are shown in grey.

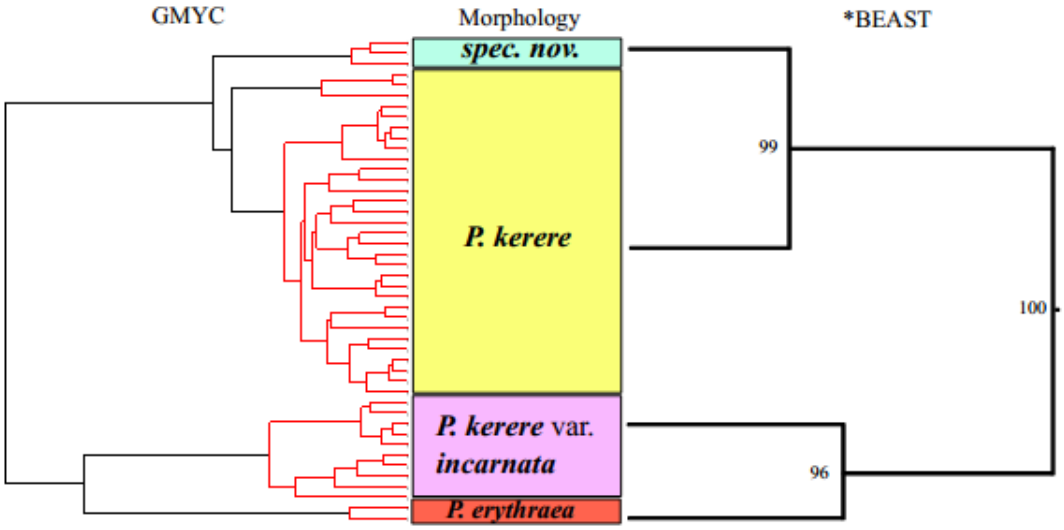


**Figure 3.** Comparison between different species delimitation approaches. A. Results derived from the GYMC analysis supporting the recognition of five species, indicated by five independent clusters (shown in red). B. Results from the analysis of morphological data supporting the recognition of four species. C. Results from the analysis of *BEAST supporting the recognition of four species.

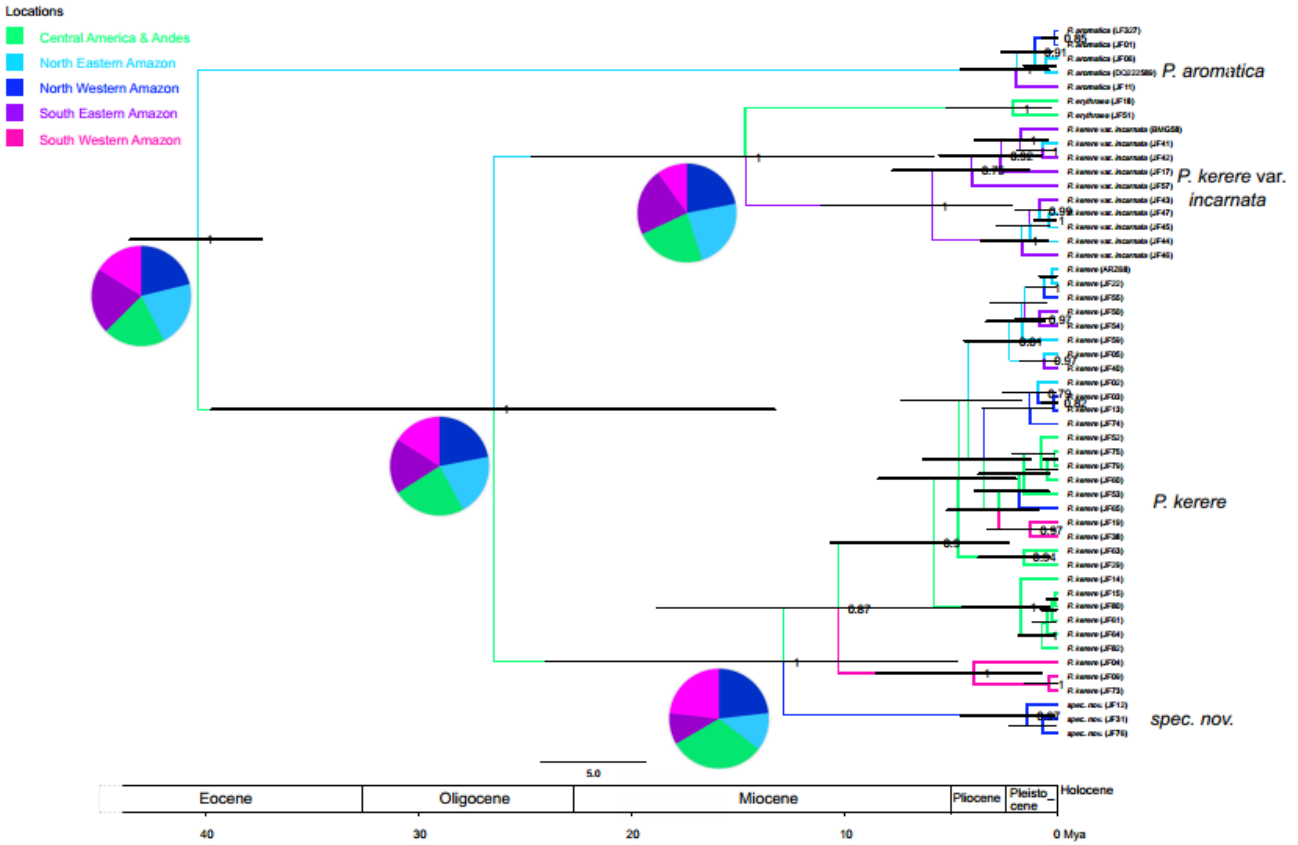


**Figure 4.** Ancestral areas reconstructed with BSSVS on the time-calibrated tree of *Pachyptera* that resulted from the BEAST analysis of the cpDNA dataset (i.e., *ndh*F + *rpl*32-*trn*L). Branch colors correspond to biogeographic areas indicated next to boxes. Posterior probabilities > 0.70 are shown next to branches. Pie charts indicating the probability of ancestral areas are shown next to nodes. A detailed description of divergence time estimates and ancestral areas for the individual nodes are shown in Tab. S4 and Tab. S5, respectively.

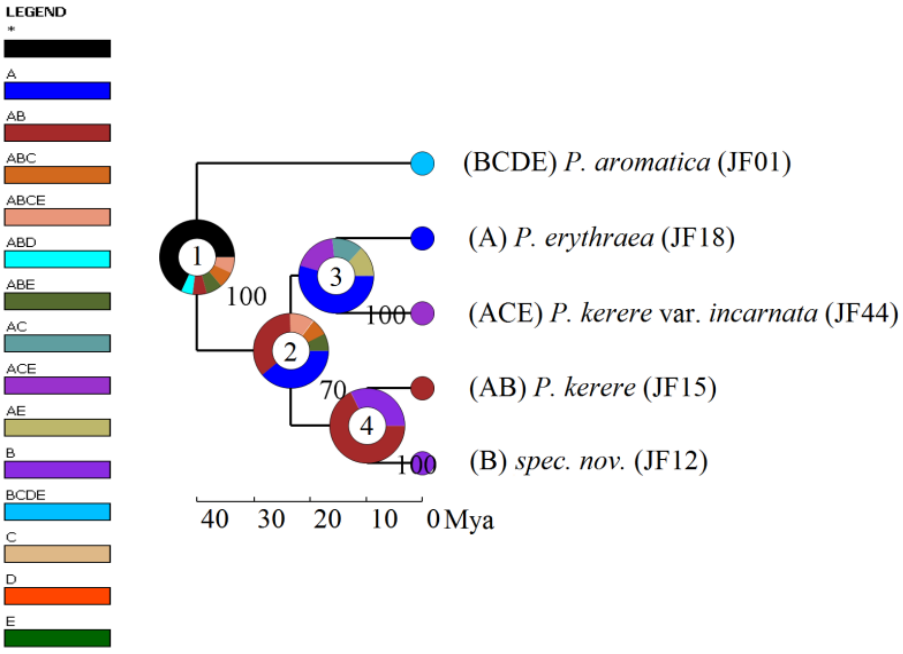


**Figure 5.** Ancestral areas of *Pachyptera* species inferred with RASP, using the dispersal, extinction and cladogenesis (DEC) model based on the analysis of the combined dataset (i.e., *ndh*F, *rpl*32-*trn*L, and *Pep*C), including a single individual per species. Branches are proportional to time. Distributions assigned to each species are indicated before tip names. Pie charts describe the relative probabilities of putative ancestral areas. Detailed divergence times and ancestral areas for nodes are presented in Tab. S4 and Tab. S7, respectively.

**SUPPLEMENTARY MATERIALS**

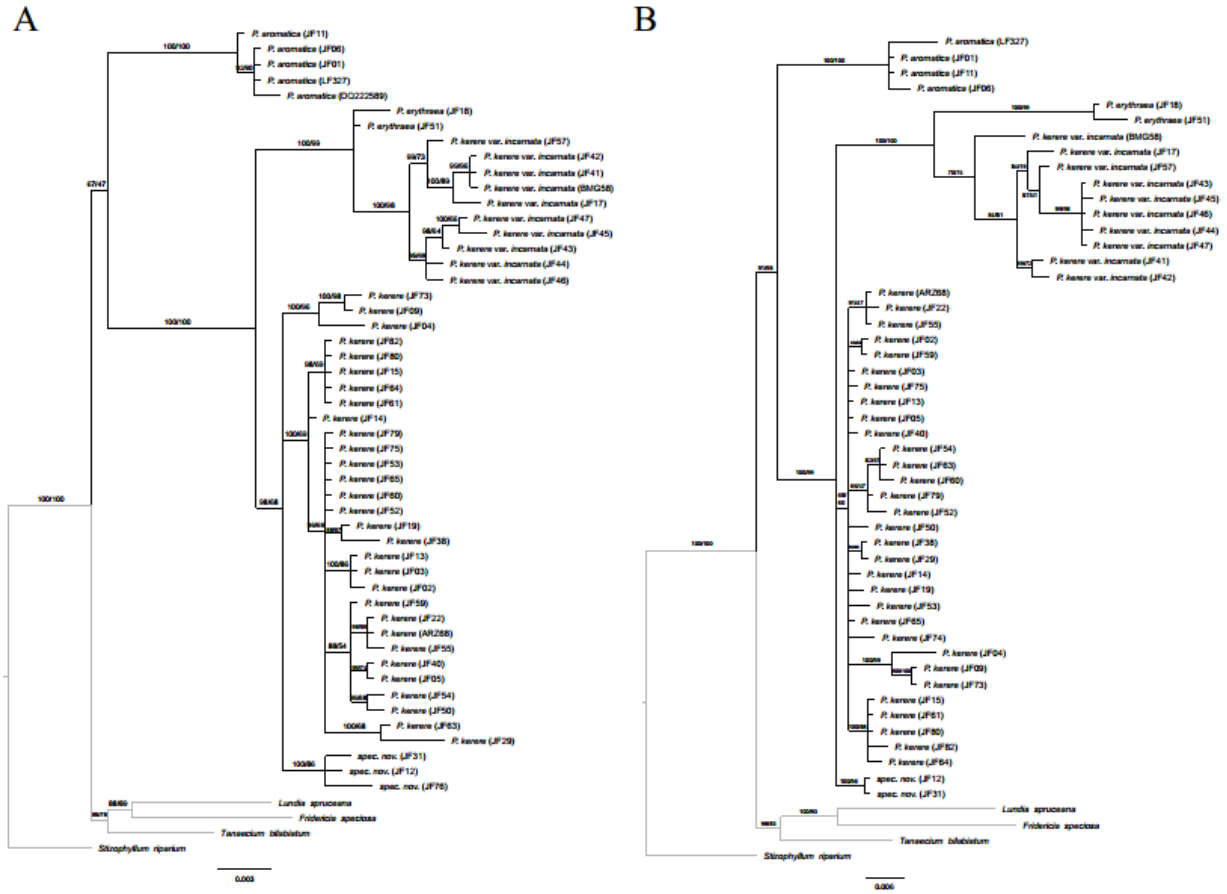


**Figure S1.** Consensus tree of *Pachyptera* inferred from the Bayesian analysis of the *ndh*F (A) and *rpl*32–*trn*L (B) datasets. Posterior probability and bootstrap values are shown above branches. The outgroups are shown in grey.

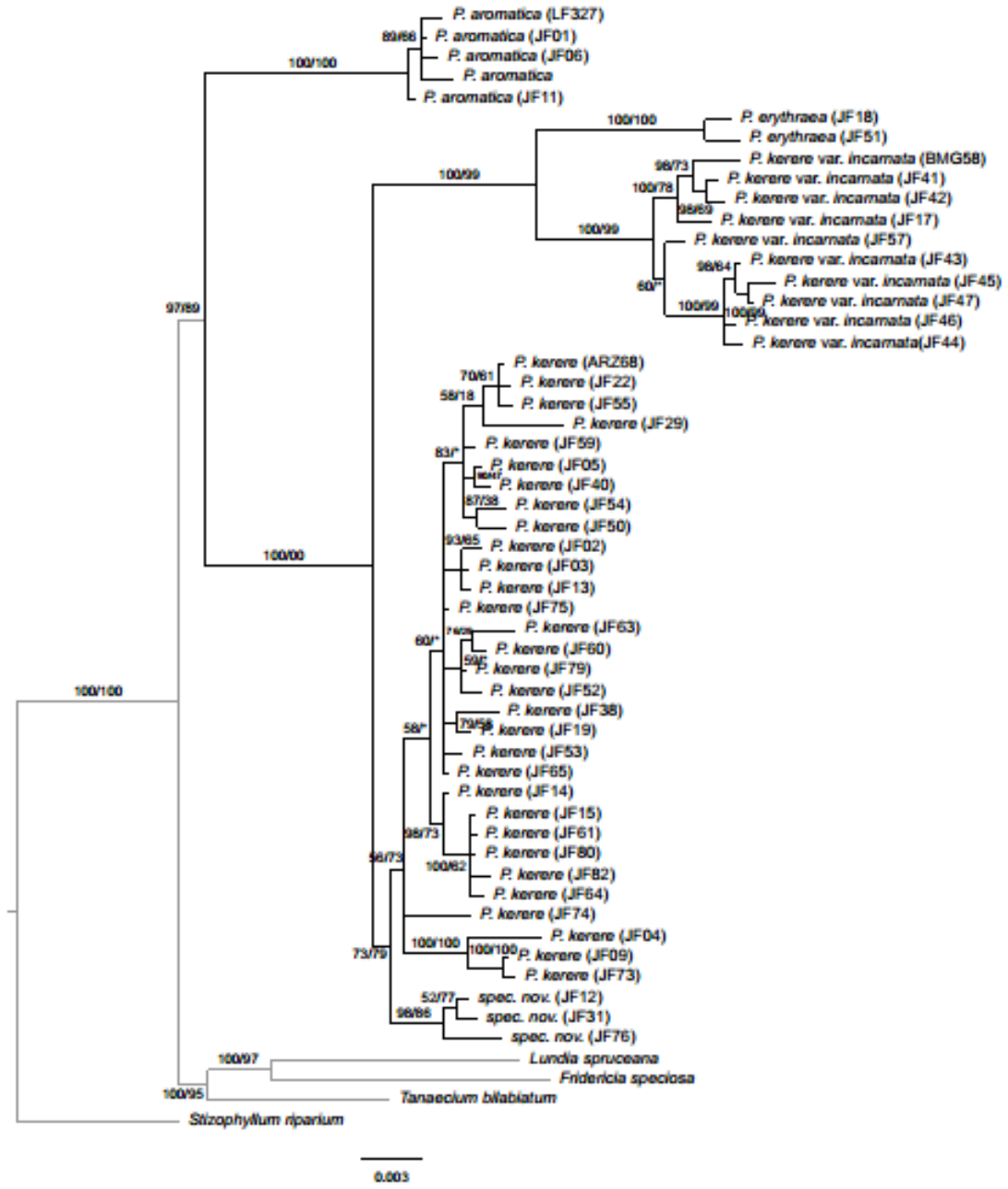


**Figure S2.** Consensus tree of *Pachyptera* inferred from the Bayesian analysis of the combined cpDNA dataset (i.e., *ndhF*, *rpl32–trnL*). Posterior probability and bootstrap values are shown above branches. The outgroups are shown in grey.

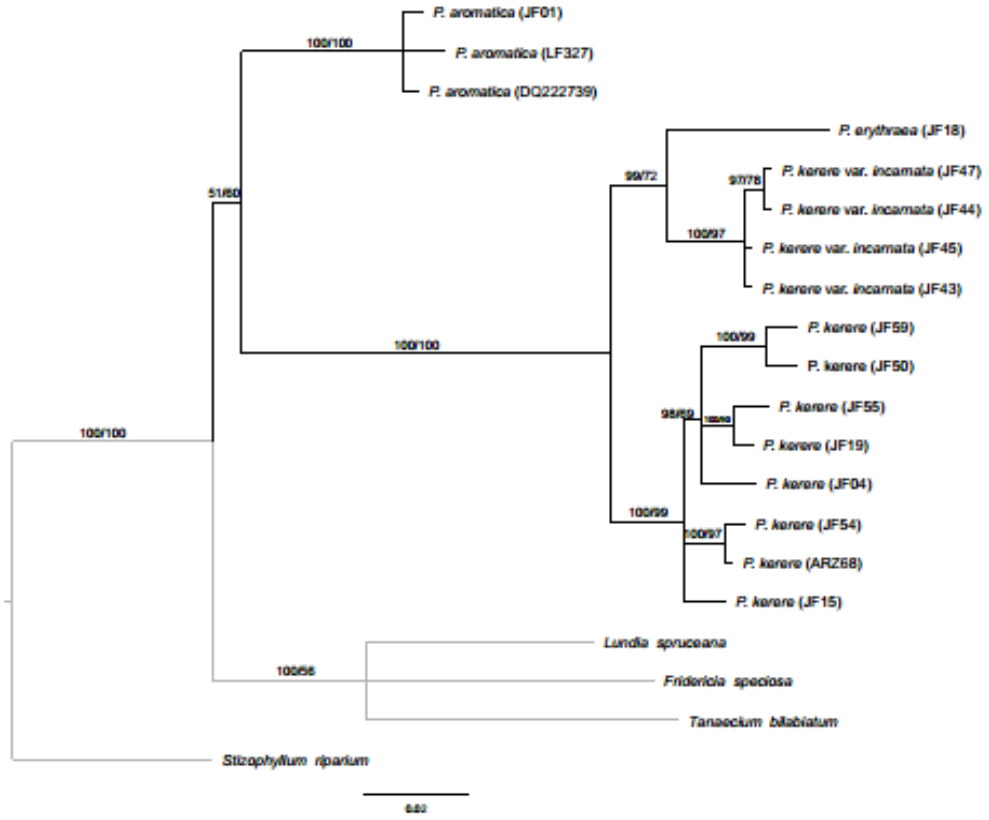


**Figure S3.** Consensus tree of *Pachyptera* inferred from the Bayesian analysis of the *Pep*C dataset. Posterior probability and bootstrap values are shown above branches. The outgroups are shown in grey.

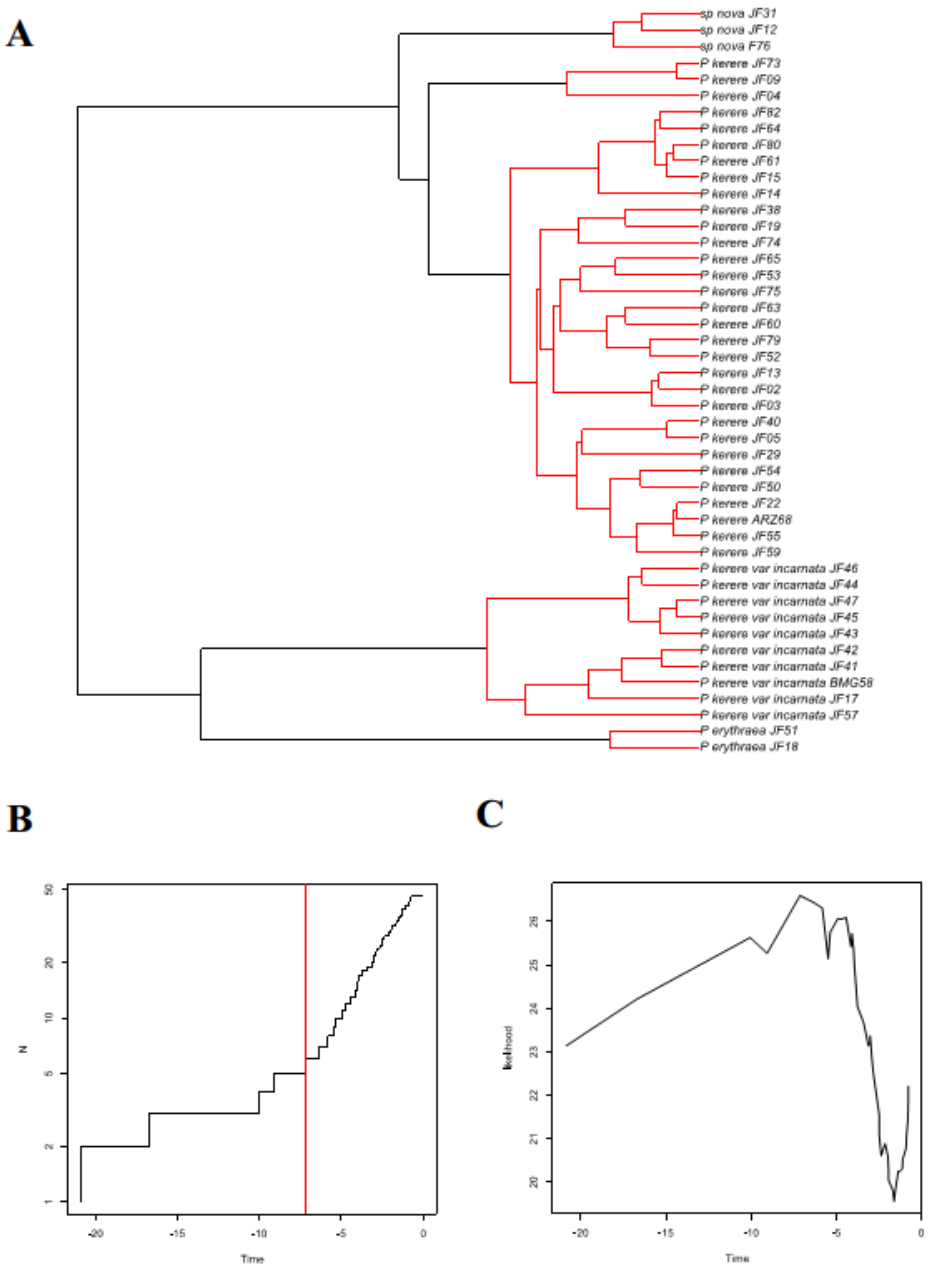


**Figure S4.** Results of the species delimitation analysis using the GMYC single–threshold model (based on cpDNA). A. Ultrametric tree obtained in BEAST with individual clusters (= putative species) highlighted in red. B. Lineage–through–time plot based on the time calibrated tree obtained from all 46 specimens. The sharp increase in branching rate, corresponding to the transition from interspecies to intraspecies branching events is indicated by the red line. C. Likelihood profile through time.

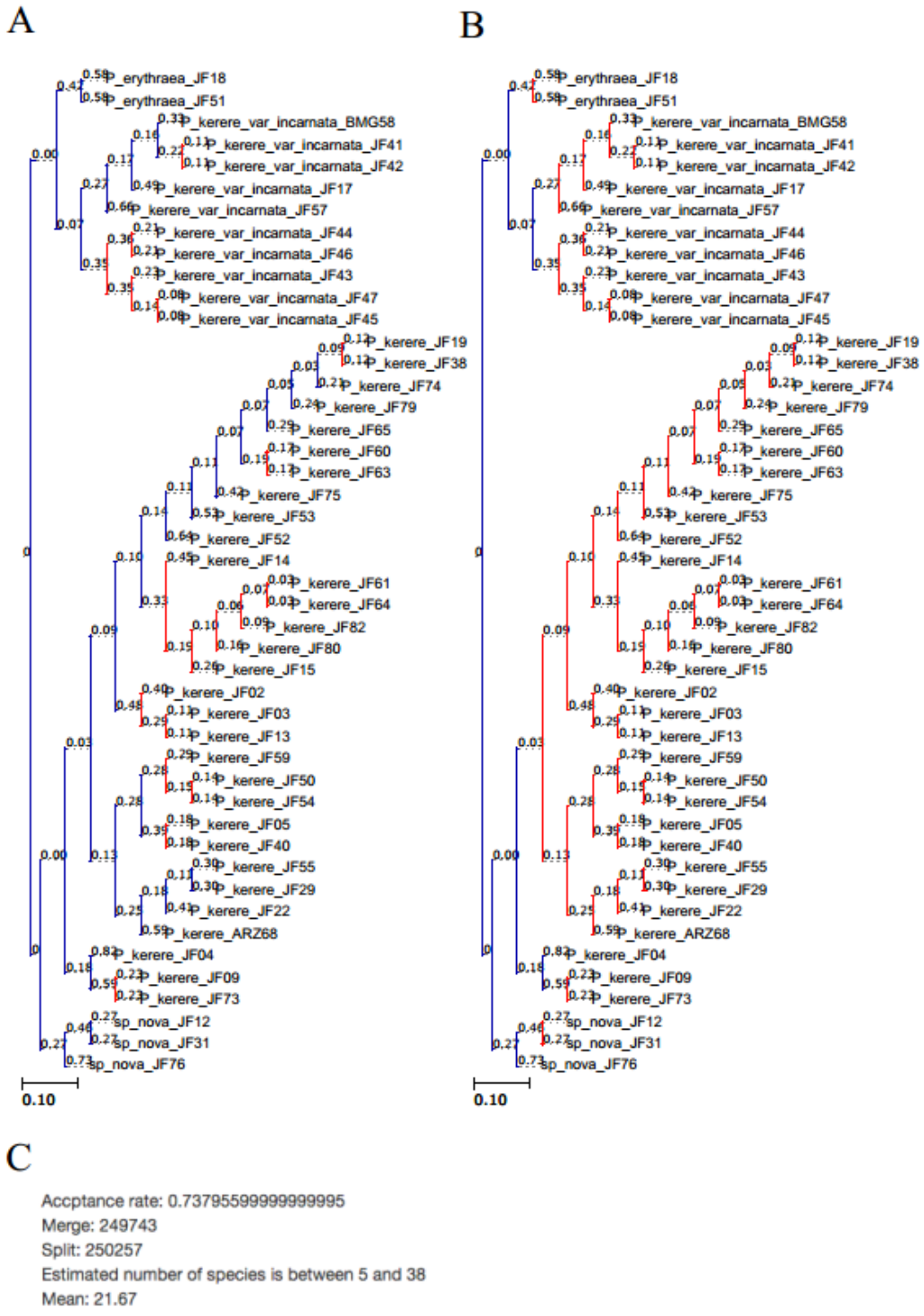


**Figure S5.** Results of the Bayesian Poisson tree processes (bPTP) model derived from the analyses of the combined cpDNA datasets. Putative molecular species are indicated using transitions between blue–colored branches to red colored branches. A. Highest Bayesian supported solution. B. Maximum likelihood solution. C. Summary results**.**

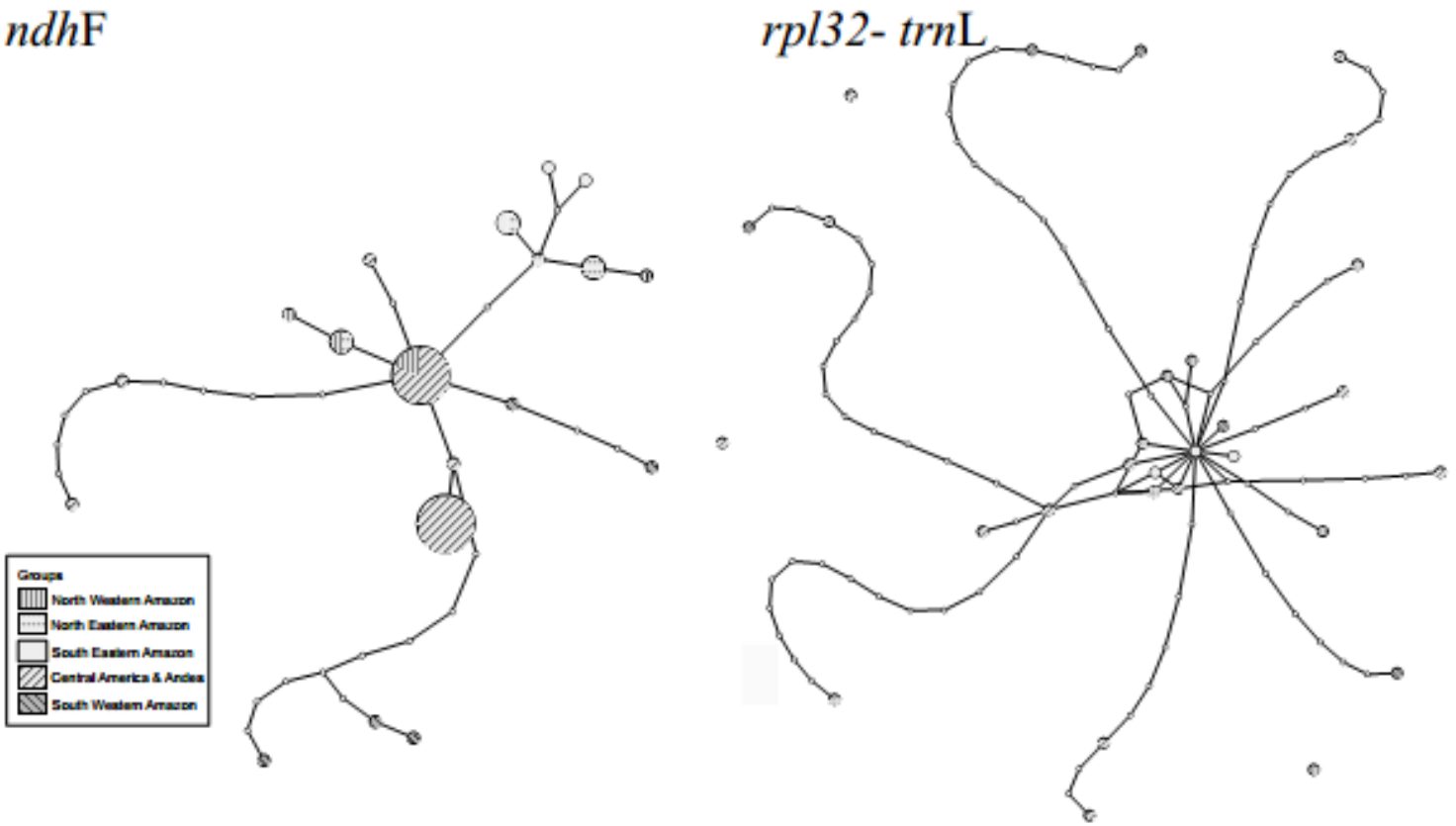


**Figure S6.** Statistical parsimony network of *Pachyptera kerere* constructed based on the analysis of the *ndh*F and *rpl*32–*trn*L datasets. Circle sizes are proportional to the frequency of each haplotype. Small empty circles represent mutational steps

**Table S1.** Specimens sampled, followed by locality, collector and GenBank accession numbers. Sampling localities are shown in Figure 1.

| Location code | Sample ID | Voucher code* | Colector | Locality | *ndhF* | rpl32-trnL | *Pep*C |
|---|---|---|---|---|---|---|---|
| NW | *spec. nov.* JF12 | SPF | Martino, 22 | VE, Bolivar, Moitaco | x | x | no |
| NW | *spec. nov.* JF31 | P | Wurdack, 41357 | VE | x | x | no |
| NW | *spec. nov.* JF76 | MO | Stevenson P.,403 | CO | p | no | no |
| NW | *P. aromatica* JF01 | SPF | Lohmann, 794 | BR, AM, Novo Airão | KY983572 | x | KY983575 |
| NW | *P. aromatica* LHF327 | SPF | Fonseca, 327 | BR, AM, Iranduba | x | p | x |
| NE | *P. aromatica* JF06 | MO | Gentry, 69107 | BR, AM, Rio Solimões | x | x | no |
| SE | *P. aromatica* JF11 | MO | Maguire, 56679 | BR, RO, Porto Velho | x | x | no |
| NE | *P. aromatica* | GenBank | Lohmann, 28 | BR, Amazonas, Ducke | DQ222589 | no | DQ222739 |
| CA&A | *P. erythraea* JF18 | MO | Gentry, 15372 | CO, Santander | KY983571 | x | KY983577 |
| CA&A | *P. erythraea* JF51 | HUA | Ramiro, 2580 | CO, ANT, Zaragoza | x | x | no |
| SE | *P. kerere* JF50 | MG | Lins, 899 | BR, PA, Santa Luzia | x | x | x |
| SE | *P. kerere* JF54 | IAN | Vieira, 72 | BR, MA, Palmeirândia | x | x | x |
| NE | *P. kerere* AZ68 | SPF | Nogueira, 162 | BR, AM, Manaus | x | x | x |
| NE | *P. kerere* JF02 | SPF | Lohmann, 805 | BR, AM, Novo Airão | x | x | no |
| NW | *P. kerere* JF03 | SPF | Lohmann, 836 | BR, AM, Novo Airão | x | x | no |
| SW | *P. kerere* JF04 | SPF | Janovec, 2606 | PE, Madre de Dios, Manu | x | x | x |
| NE | *P. kerere* JF05 | SPF | Udulutsch, 2708 | BR, PA, Altamira | x | x | no |
| SW | *P. kerere* JF09 | MO | Gentry, 66005 | PE, Madre de Dios, Tambopata | x | x | no |
| NW | *P. kerere* JF13 | MO | Gentry, 12710 | CO, AM, Leticia | x | x | no |
| CA&A | *P. kerere* JF14 | MO | Aguilar, 4824 | CR, Puntarenas, Osa | x | x | x |
| CA&A | *P. kerere* JF15 | MO | Gentry, 7540 | HN, Gracieas a Dios, Brus Laguna | x | x | x |
| SW | *P. kerere* JF19 | MO | Loureiro, s.n. | BR, AM, Autaz-Mirim | x | x | x |
| NE | *P. kerere* JF22 | SPF | Francisco, 40 | BR, RO, Caracaraí | x | x | x |
| CA&A | *P. kerere* JF29 | MO | G. Herrera, 7551 | CR | p | x | no |
| SW | *P. kerere* JF38 | INPA | Prance, 14036 | BR, AM, Rio Ituxi | x | x | no |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SE | *P. kerere* JF40 | INPA | Gentry, 13075 | BR, PA, Belém | x | p | no |
| CA&A | *P. kerere* JF52 | HUA | Castaño, 107 | CO, ANT,Necoclí | x | x | no |
| CA&A | *P. kerere* JF53 | HUA | Castaño, 93a | CO, ANT, Necoclí | x | x | no |
| NW | *P. kerere* JF55 | SPF | Lohmann, 336 | BR, AM, Rio Negro | DQ22260 | x | KY983576 |
| NE | *P. kerere* JF59 | MO | Hoffman, 5362 | SR, Sipiwalini, Voltzberg Nature Reserve | x | x | x |
| CA&A | *P. kerere* JF60 | MO | McPherson, 20983 | PA, Colon, Tailings Area | x | x | x |
| CA&A | *P. kerere* JF61 | MO | Saunders, 397 | HN, Colón, Trujillo | x | x | no |
| CA&A | *P. kerere* JF63 | MO | Grayum, 4411 | CR, Limon Puerto Viejo de Talamanca | x | x | x |
| CA&A | *P. kerere* JF64 | MO | Davidse, 32046 | BZ,Toledo, Maya | x | x | no |
| NW | *P. kerere* JF65 | MO | Vásquez, 17115 | PE, Loreto, Maynas | x | x | no |
| SW | *P. kerere* JF73 | MO | Gentry A.H.,68887 | PE, Madre de Dios | x | x | no |
| NW | *P. kerere* JF74 | MO | Gentry A.H.,14686 | VE, Bolivar, Rio Parhueña | no | x | no |
| CA&A | *P. kerere* JF75 | MO | Rueda, R.,3216 | NC | x | p | no |
| CA&A | *P. kerere* JF79 | MO | Nee M.,8948 | PA, Colón | x | x | no |
| CA&A | *P. kerere* JF80 | MO | Brant A.E.,2917 | HN, Atlantida, Tela | x | x | no |
| CA&A | *P. kerere* JF82 | MO | Dwyer J.D.,12737 | Belize | x | x | no |
| SE | *P. kerere* var. *incarnata* BMG58 | SPF | Pereira-Silva, 8765 | BR, PA, Palestina do Pará | x | x | no |
| SE | *P. kerere* var. *incarnata* JF17 | MO | Santos, 95 | BR, PA, Maraba | x | p | no |
| NE | *P. kerere* var. *incarnata* JF41 | INPA | Gentry, 13027 | BR, AM, Manaus | x | x | no |
| SE | *P. kerere* var. *incarnata* JF42 | SPF | Beyer, 293 | BR, PA, Goianésia do Pará | x | x | x |
| SE | *P. kerere* var. *incarnata* JF43 | SPF | Francisco, 89 | BR, PA, Belterra | x | x | x |
| NE | *P. kerere* var. *incarnata* JF44 | SPF | Francisco, 121 | BR, PA, Óbidos | x | x | x |
| NE | *P. kerere* var. *incarnata* JF45 | SPF | Francisco, 122 | BR, PA, Óbidos | x | x | x |
| SE | *P. kerere* var. *incarnata* JF46 | SPF | Francisco, 105 | BR, PA, Santarém | x | x | x |
| NE | *P. kerere* var. *incarnata* JF47 | SPF | Francisco, 151 | BR, PA, Oriximiná | x | x | x |
| SE | *P. kerere* var. *incarnata* JF57 | SPF | Ribeiro,78 | BR, MT, Nova Bandeirantes | x | x | x |

**Table S2.** Information on the phylogenetic, phylogeographic and calibration analyses, partitions, models and parameters.

| Dataset | Partition | Length | Model | Clock model | Rate | Tree model | Other Priors/Parameters | Runs |
|---|---|---|---|---|---|---|---|---|
| Gene (Outgroups included) | *ndh*F | 2077 | TVM+G | | | | | 4 runs, $10^6$ generations, $10^2$ sampling frequency, 25% burning |
| | *rpl*32-*trn*L | 1226 | TPM1uf+G | | | | | |
| | *Pep*C | 471 | TVM+I | | | | | |
| Concatened (Outgroups included) | *ndh*F, *rpl*32-*trn*L | 3302 | TVM+G, TPM1uf+G | | | | | |
| | *ndh*F, *rpl*32-*trn*L, *Pep*C | 3774 | TVM+G, TPM1uf+G, TVM+I | | | | | |
| Partitioned | *ndh*F | 2068 | TPM1uf | Relaxed Clock Log Normal Link Clock Normal | Estimed | Yule Model | Normal Mean 40.0, Sigma 1.6 and Offset 0.5 | 4 runs, $10^6$ generations, $10^2$ sampling frequency, 25% burning |
| | *rpl*32-*trn*L | 1084 | TPM1uf+G | | | | | |
| | *Pep*C | 469 | TVM | | | | | |
| Concatened (only members of the *P. kerere* species complex) | *ndh*F | 2068 | TPM1uF+I | Relaxed Clock Log Normal | Estimed | Yule Model | Normal Mean 20.0, Sigma 1.0 and Offset 1.0 | 3 runs, $10^6$ generations, $10^2$ sampling frequency, 25% burning |
| | *rpl*32-*trn*L | 1179 | TVM+G | | | | | |
| Partitioned cpDNA (Members of P. kererespecies complex plus P. aromatica as outgroup) | *ndh*F | 2067 | TVM+G | | | | | 4 runs, $10^6$ generations, $10^2$ sampling frequency, 25% burning |
| | *rpl*32-*trn*L | 1203 | TPM1uf+G | | | | | |
| Partitioned cpDNA (only members of *P. kerere* species complex) | *ndh*F | 2067 | TPM1uf+I | Strict clock | Estimed | Yule Model | Multi Species Coalescent with Pop Function linear with constant root | 3 runs, $10^6$ generations, $10^2$ sampling frequency, 25% burning |
| | *rpl*32-*trn*L | 1184 | TPM1uf+G | | | | | |
| cpDNA (all 51 individuals sampled) | *ndh*F | 2068 | TVM+G | Relaxed Clock Log Normal | Estimed | Coalescent Constant Population | Normal Mean 40.0, Sigma 1.6 and Offset 0.5 | 2 runs, $10^8$ generations, $10^2$ sampling frequency, 25% burning |
| | *rpl*32-*trn*L | 1203 | TPM1uf+G | | | | | |

**Table S3.** Morphological traits of taxa within the *Pachyptera kerere* species complex.

| Clade | Flower color | Corolla shape | Androecium | Ovary | Fruit | Seed | Ornamentation seed |
|---|---|---|---|---|---|---|---|
| *P. erythraea* | orange to red | tubular campanulate | subexserted | lepidote | linear and flat | thin, chartaceous to woody, winged | striated, secondary sculpture NI |
| *P. kerere* var *incarnata* | light pink to pale purple | infundibiliform | Included | lepidote | linear and flat | thin, chartaceous to sub–coriaceous, winged | striated, secondary sculpture with randomly distributed micropores |
| *P. kerere* | white to cream | infundibiliform | Included | puberulous | fusiform and inflated | thick, corky, wingless | striated, secondary sculpture with two pairs of medium micropores to each stria |
| *spec. nov.* | white | infundibiliform | Included | puberulous | linear and flat | thin, coriaceous to woody, winged | striated, secondary sculpture with strias regularly interrupted by lateral rays |

**Table S4.** Age estimates of *Pachyptera* using different parameters and markers: (i) calibration with BEAST using the combined dataset (i.e., *ndh*F, *rpl*32–*trn*L, and *Pep*C); (ii) calibration with BSSVS using the cpDNA dataset (i.e., *ndh*F and *rpl*32–*trn*L). The HPD 95% confidence interval is shown between parentheses.

| Clade/taxon | Age (Mya) | |
|---|---|---|
| | **partitioned *ndh*F, *rpl*32-*trn*L, *Pep*C** | **combined cpDNA** |
| *Pachyptera* | 40.3 (37.2–43.5) | 40.4 (37.3–43.5) |
| Complex clade | 23.5 (10.5–38.5) | 26.5 (13.2–39.7) |
| *P. erythraea-P. kerere* var *incarnata* | 15.4 (3.4–34.9) | 14.6 (5.8–24.7) |
| *P. kerere -spec. nov.* | 9.8 (1.0–23.8) | 12.9 (4.7–24.0) |

**Table S5.** Ancestral areas for nodes indicated in Figure 4. Areas with (/) suggest similar probabilities.

| Nodes | Ancestral areas | Probability |
|---|---|---|
| 1 | Central America & Andes/North- Western Amazon/ North Eastern Amazon | 0.21 |
| 2 | Central America & Andes | 0.24 |
| 3 | Central America & Andes/North Eastern Amazon | 0.23 |
| 4 | Central America & Andes | 0.31 |

**Table S6.** Dispersal routes of *Pachyptera* as inferred under BSSVS using a Bayes factor (BF) test. Well–supported rates of dispersal with BF values >3.

| Dispersal | BF | pk |
|---|---|---|
| North Western Amazon to South Western Amazon | 131 | 0.99 |
| North Western Amazon to South Eastern Amazon | 21 | 0.95 |
| North Eastern Amazon to South Eastern Amazon | 2.9 | 0.72 |
| North Eastern Amazon to Central America &Andes | 1.4 | 0.54 |
| South Eastern Amazon to Central America & Andes | 1.3 | 0.53 |

**Table S7.** Ancestral areas for nodes indicated in Figure 5.

| Nodes | Ancestral Areas | Events | Relative probability |
|---|---|---|---|
| 1 | * | * | 0.02 |
| 2 | A | A->A^A->AB^->AB/A | 0.30 |
| 3 | A | A->A^A->ACE^A->A/ACE | 0.54 |
| 4 | AB | AB->AB^B->AB/B | 0.67 |

# Capítulo 3

**Taxonomic revision of *Pachyptera* (Bignonieae, Bignoniaceae)**

Jessica Nayara Carvalho Francisco & Lúcia G. Lohmann

**Abstract**

*Pachyptera* DC. ex Meisn. is a small genus of neotropical lianas included in tribe Bignonieae (Bignoniaceae). The genus has a complicated taxonomic history but currently includes species distributed from Belize to central Brazilian Amazon. *Pachyptera* is quite distinct vegetatively and can be recognized by the papery peeling bark when old, prophylls of the axillary buds generally flattened, ensiform and superimposed in series of three, and the conspicuous extrafloral nectaries on the interpetiolar region and petiole apices. Reproductively, the genus is characterized by patelliform glands arranged in lines on the upper portions of the calyx and corolla, and villous anthers. Here, we present a taxonomic revision of *Pachyptera*, which includes an identification key for all species recognized, detailed morphological descriptions to all species, a complete list of synonyms, information on the habitat, distribution and phenology, taxonomic discussions, and illustrations. In addition, we designate one lectotype for *Pachyptera aromatica*, propose one new combination, raise one variety to species status, and describe a new species. After these adjustments in the circumscription of *Pachyptera*, we recognize a genus with five well-defined species.

**Keywords:** Amazonian flora, *Pachyptera kerere*, monograph, Neotropical flora.

**Taxonomic history**

*Pachyptera* DC. ex Meisner is a genus of Neotropical lianas that represents one of the smallest genera included in the tribe Bignonieae (Bignoniaceae). The genus is distributed from Belize to central Brazilian Amazon, with most species restricted to wet Amazonian forests (Lohmann and Taylor 2014). The genus has a complicated taxonomic history, including a difficult circumscription and several poorly defined taxa.

*Pachyptera* was originally described by de Candolle (1845), who characterized the genus by a compressed capsule and seeds with coriaceous wings. The genus originally included six species, four of which [i.e., *P. umbelliformis* DC., *P. striata* DC., *P. dasyantha* DC, and *P. perrottetii* DC.] are synonyms of *Tanaecium pyramidatum* (Rich.) L.G. Lohmann, while *P. puberula* DC. is a synonym of *Dolichandra uncata* (Andrews) L.G. Lohmann. Only *P. foveolata* DC. remains in *Pachyptera*, although as a synonym of *Pachyptera kerere* (Aubl.) Sandwith.

Nearly five decades after being described, *Pachyptera* was synonymized into *Adenocalymma* Mart. ex Meisn by Baillon (1891) based on the broad and thick capsule shared among members of these genera. Subsequently, Bureau and Schumann (1896 [1897]) transferred *P. foveolata* to *Adenocalymma* section *Pachyptera*, which was characterized by villous anthers, and plate-shaped glands arranged in lines outside the corolla tube, right below the lobes. At the same time, *P. kerere* was transferred to *Adenocalymma* section *Hanburyophyton* together with four species of *Mansoa,* i.e., *A. alliaceum* (Lam.) Miers, *A. asperulum* Bureau & K. Schum., *A. splendens* Bureau & Schum. [= *Mansoa difficilis*], and *A. lanceolatum* Miers. *Pachyptera* was subsequently segregated from *Adenocalymma* by Sprague and Sandwith (1932) and restored to generic rank, as a monotypic genus that only included *P. foveolata*.

*Pachyptera foveolata*, as circumscribed by Sprague and Sandwith (1932), consisted on a species complex that included individuals with white to crimson flowers. While the authors themselves recognized the difficulties associated with the recognition of such a diverse species, the restricted sampling prevented them from analyzing the breath of morphological variation included in this group and the

recognition of a single species. Five years later, Sandwith (1937) noted that Aublet's epithet "*kerere*" was the correct name for *P. foveolata* and proposed the new combination *Pachyptera kerere*. Dugand (1955) also noted the high variation found in flower traits of specimens of *Pachyptera kerere* and described the new variety *P. kerere* var. *erythraea* Dugand. This variety differs from *P. kerere* in the red corolla (vs. white in *P. kerere*). Gentry (1977) subsequently noted that *P. kerere* and *P. kerere* var. *erythraea* also differed in the sub-exserted to exserted anthers (vs. inserted anthers in *P. kerere*), campanulate corolla with 11-15 mm in diameter (vs. sub-bilabiate corolla with 3-7 mm in diameter in *P. kerere*), and leaf blade puberulous (vs. leaf blade glabrous in *P. kerere*), which led him to raise *P. erythraea* (Dugand) A.H. Gentry to species rank.

Although *Bignonia incarnata* Aubl. was described in the same work as *Bignonia kerere* Aubl. (1775), the close relationship between those two taxa was not noted. In fact, *Bignonia incarnata* was thought to be morphologically similar and perhaps closely related to *Cydista aequinoctialis* (L.) Miers by various authors (see Sandwith 1937). Nearly two decades later, Gentry (1973) noted the similarity between individuals of *B. incarnata* and *P. kerere*, which led him to treat *B. incarnata* as a variety of *P. kerere*, i.e., *Pachyptera kerere* var. *incarnata* (Aubl.) A.H. Gentry. At the same time, Gentry (1973) reduced *Pseudocalymma* [= *Mansoa*] into *Pachyptera* due to the shared trifid tendrils, white to red or purple flowers, interpetiolar gland-fields, 3-colpate pollen and deciduous bracts. In this work, three species of *Pseudocalymma* were transferred to *Pachyptera* [*P. alliacea* (Lam.) A.H. Gentry, *P. hymenaea* (DC.) A.H. Gentry, and *P. standleyi* (Steyerm.) A.H. Gentry], all of which are currently placed in *Mansoa*.

Gentry (1979) and Gentry and Tomb (1979) used new palynological data as basis to merge *Pachyptera* and *Hanburyphython* Bureau ex Warm. into *Mansoa* DC., resulting in seven new combinations: *M. alliacea* (Lam.) A.H. Gentry, *M. erythraea* (Dugand) A.H. Gentry, *M. hymenaea* (DC.) A.H. Gentry, *M. kerere* (Aubl.) A.H. Gentry, *M. kerere* var. *incarnata* (Aubl.) A.H. Gentry, *M. parvifolia* (A.H. Gentry) A.H. Gentry, and *M. standleyi* (Steyerm.) A.H. Gentry. In addition, a new species was described, *Mansoa ventricosa* A.H. Gentry, a taxon known from the type specimen plus one additional material.

While the taxonomic confusion between *Mansoa* and *Pachyptera* remained for several years, molecular phylogenetic data (Lohmann 2006) indicated that *Mansoa* and *Pachyptera* are distantly related, while the monotypic *Leucocalantha* Barbosa Rodrigues is closely related to *Pachyptera*. *Leucocalantha* was described based on the long and white corollas that resembled the Asian genus *Millingtonia* L.f. (Oroxyleae, Bignoniaceae). The genus only included *Leucocalantha aromatica* Barb. Rodr., which is characterized by white, pubescent and hypocrateriform corolla tubes, included stamens, and glands at the apices of petioles and corollas. While the close relationship between *Leucocalantha* and *Pachyptera* was initially surprising, a careful morphological study recovered multiple morphological features shared among these taxa (e.g., stems with four phloem wedges in cross section, tubular corollas with glands arranged in lines in the upper portions of the tube, and racemose inflorescences). This observation led to the reestablishment of *Pachyptera* and the inclusion of *Leucocalantha* into *Pachyptera* in a revised generic classification of the whole tribe Bignonieae (Lohmann and Taylor 2014).

As currently circumscribed (Lohmann and Taylor 2014), *Pachyptera* includes four species, i.e., *P. aromatica* (Barb. Rodr.) L.G. Lohmann, *P. erythraea* (Dugand) A.H. Gentry, *P. kerere*, and *P. ventricosa* (A.H. Gentry) L.G. Lohmann. The genus is characterized by several morphological synapomorphies such as bark papery and peeling, ensiform and rigid prophylls arranged in three series (triangular and minute in *P. aromatica*), and glands patelliform arranged in lines on the upper portions of the calyx and corolla (Lohmann and Taylor 2014). In addition, stems with four phloem wedges in cross-section, gland fields on the interpetiolar region and petiole apices, and villous anthers also help to identify members of the genus (Lohmann and Taylor 2014). This circumscription of *Pachyptera* was based on new morphological observations and a molecular phylogeny for tribe Bignonieae that sampled two of the four species recognized (Lohmann 2006). In this study, two morphologically complicated species (i.e., *P. erythraea* and *P. ventricosa*) were not sampled, raising their generic placement to question.

A phylogenetic study of *Pachyptera* (Francisco and Lohmann submitted) sampled all species recognized by Lohmann and Taylor (2014). In this phylogeny, *Pachyptera ventricosa* was shown to be more closely related to *Mansoa* than to other

species of *Pachyptera*, which led to the reestablishment of *Mansoa ventricosa* (Francisco and Lohmann submitted). In addition, this phylogeny also provided additional support for the inclusion of *P. aromatica* and *P. erythraea* into *Pachyptera.* A more comprehensive phylogenetic study of the genus (Francisco and Lohmann in prep.) sampled multiple individuals of all species of *Pachyptera* and recovered a polyphyletic *Pachyptera kerere*. More specifically, this phylogenetic study indicated that the light pink form of *P. kerere*, previously treated as *P. kerere* var. *incarnata*, corresponds to a separate lineage and is best treated as a separate species. Furthermore, a cryptic species, with mixed morphology between *P. kerere* and *P. incarnata* was also recovered indicating that a new species should be recognized.

**Morphology**

*Habit.* All species of *Pachyptera* are lianas, although seedlings are initially herbaceous and free-standing until ca. 80 cm (grow vertically).

*Stems.* The stem of *Pachyptera* exhibits four phloem wedges in cross-section, a type of cambial variation also found in *Adenocalymma*, *Martinella*, *Cuspidaria*, *Fridericia* and *Tanaecium* (Lohmann 2006, Lohmann and Taylor 2014). Moreover, the pith of the stem of *Pachyptera* is solid although a few specimens of *P. aromatica* also showed slightly hollow pith. This state is unusual in Bignonieae and only previously documented in *Stizophyllum* and *Pleonotoma* (Lohmann and Taylor 2014). Cylindrical to tetragonal stems are found in *Pachyptera*, sometimes within a single individual. Young stems are usually cylindrical, becoming tetragonal in more advanced stages of development. Tetragonal stems are only found in stems with $\geq 6$ $cm^2$ of *P. aromatica* but also found in stems with smaller diameter in other species of the genus (Francisco personal observation). Stem surface is striated and frequently bears lenticels (except from *P. aromatica*). The bark peeling in older branches is a morphological synapomorphy of the genus (Lohmann 2006, Lohmann and Taylor 2014).

*Prophylls of the axillary buds.* Prophylls of the axillary buds, sometimes referred as "pseudostipules" in the literature (e.g., Gentry 1980), exhibit several shapes and are useful generic characters within Bignonieae (see Lohmann and Taylor

2014). Species of *Pachyptera* usually have multiple flattened and ensiform prophylls of the axillary buds (triangular and minute in *P. aromatica*, Fig. 4B), arranged in 3(–5) series (Fig. 1H). Sometimes the prophylls are so minute in *P. aromatica* that only the larger prophyll series is visible to the naked eye (Fig. 1D). The supra-numerary prophylls are a morphological synapomorphy of *Pachyptera* (Lohmann and Taylor 2014).

*Extrafloral nectaries.* Extrafloral nectaries (i.e., EFN's) are useful generic and species level markers within Bignonieae, aiding the identification of sterile materials (Seibert 1948, Lohmann and Taylor 2014). EFN's produce sugar that attracts ants that, in turn, have an important protective role against herbivores (Gentry 1974). In *Pachyptera*, EFN's are composed of large groups of patelliform glands located between the petioles (Figs. 1D, G, H), and at the petiole apex, right below the junction with the petiolules. Interpetiolar gland fields are also found in other Bignonieae genera (e.g., *Fridericia, Lundia, Tanaecium*) and have evolved multiple times within the tribe (Lohmann and Taylor 2014, Nogueira et al. 2013). EFN's are particularly abundant and conspicuous in *Pachyptera*. On the other hand, clusters of patelliform glands located on petioles and petiolules are rare in Bignonieae and only known from a few species (e.g., *Tanaecium pyramidatum* and *Mansoa standleyi*).

*Leaves and tendrils.* As most representatives of Bignonieae, leaves of *Pachyptera* are 2-3-foliolated, with the terminal leaflet replaced by a trifid tendril. Tendrils are often deciduous, leaving a tiny scar in the position of tendril detachment. Leaflets can be quite variable in shape, varying even within a single species. Leaflet asymmetry is striking in the group. While cuneate, obtuse or rounded leaflet bases are diagnostic of *P. aromatica*, cordate and oblique leaflet bases are found on all other species of *Pachyptera*.

*Inflorescences.* The inflorescence of *Pachypera* is a simple raceme. Racemes can be lax, with a well-developed central axis, ca. 6-24 cm in *P. aromatica* (Figs. 1A, C) or reduced, with a short central axis (< 4.8 cm long) in all others species of the genus (Figs. 1E, I, N). In *Pachyptera,* even though the inflorescence bears ca. 6-30 flowers, only 1-2 flowers open at a time.

*Calyx.* The calyx of *Pachyptera* is tubular (cupular in *P. erythraea*) with grouped patelliform glands on the upper half (Figs. 1M-N, 2A, F, K, P, U), a synamoporphy of the genus (Lohmann 2006; Lohmann and Taylor 2014). These patteliform glands are conspicuous, sometimes wine-colored (Fig. 1M), and thought to play an important role against nectar robbers (Gentry 1974).

*Corollas.* Most species of *Pachyptera* have infundibuliform and dorso-ventrally compressed corollas, with internal yellow nectar guides (Figs. 1F, K, O), and a villose portion where stamens and staminodes are inserted (Figs. 2G, L, Q, V). The corolla of *P. aromatica*, on the other hand, is hypocrateriform, not compressed (Figs. 1A-B), without nectar guides, and glabrous internally (Fig. 2B). The corolla tube of *P. erythraea* expands above the short basal constriction and becomes tubular-campanulate from the base (Figs. 1 E-F, 7B), while the corolla tubes of other species are infundibuliform (except *P. aromatica*), only becoming tubular-campanulate towards the middle portion of the tube (Figs. 1I, N; Gentry 1977). Corolla color is useful for species identification, ranging from white or cream to light pink, pale purple, orange or red. The upper portion of the corolla tube and base of the corolla lobes are covered by nectaries (Figs. 1L, P) that exude large globules of colorless and viscous liquid, likely associated with ant-plant interactions. This feature is a morphological synapomorphy of the genus (Lohmann 2006; Lohmann and Taylor 2014), although also found in some species of *Adenocalymma*, *Pleonotoma*, and *Anemopaegma*.

*Androecium.* As most members of Bignonieae, *Pachyptera* has four didynamous stamens and one staminode. Filaments are usually glabrous but puberulous in *P. erythraea*. The anthers are generally inserted, but subexserted in *P. aromatica* (Fig. 1B) and *P. erythraea* (Fig. 1F). The anther connective helps to differentiate *P. aromatica*, which shows acute connectives instead of the round connective found in all other species of *Pachyptera*. The densely villous anthers, with curved thecae, are diagnostic of *Pachyptera* (Figs. 1F, K, O). Villous anthers are also found in *Lundia.*

*Pollen.* Pollen has been shown to be useful for generic delimitation within the Bignoniaceae (Gentry and Tomb 1979). Members of *Pachyptera* generally have

microrreticulate 3-colpate pollen (Figs. 2H, M, R, W), a condition also found in *Lundia*, *Pleonotoma*, and *Tanaecium* (Gentry and Tomb 1979). However, *P. aromatica* has psilate-foveolate to microrreticulate 4-colpate pollen grains (Figs. 2C-D). This pollen type appears to be unique within the tribe.

*Gynoecium.* Members of *Pachyptera* have capitate, elliptic, and ovate stigmas. While the style and stigma are always glabrous, the ovary can be puberulous (Figs. 2E, T, Y) or lepidote (Figs. 2J, O). As most representatives of Bignoniaceae, *Pachyptera* has bilocular ovaries, with two ovules per locule and axillary placentation. A well-developed nectar disc is also found.

*Fruits.* Fruits are coriaceous to woody septicidal capsules, with two valves. The capsule is linear and flattened in most species (fusiform and inflated in *P. kerere*), puberulous, sparsely lepidote, covered with patelliform glandular trichomes, without lenticels (Figs. 3A, C, E, G). Each valve has an inconspicuous longitudinal midline (conspicuous and raised in *P. kerere*).

*Seeds.* The seeds of *Pachyptera* are mostly oblong, thin, chartaceous to coriaceous, with membranaceous and hyaline wings, except from *P. kerere* in which seeds are irregulary circular and obcordate, thick, corky and wingless. The secondary sculpture of the seed surface is useful for species identification (Figs. 3B, D, F, H).

**Material and methods**

*Species delimitation.* Molecular phylogenetic data (Francisco and Lohmann submitted, in prep.) was used to help delimit all taxa recognized. While we understand that not all species need to be monophyletic, species are evolutionary lineages that reach a status of reciprocal monophyly in advanced stages of the speciation process (de Queiroz 2007; Funk and Olmland 2003). As such, we here treat independent evolutionary units that share a unique combination of features as separate species (Cracraft 1983).

*Morphological descriptions.* Morphological descriptions of all species of *Pachyptera* were based on extensive fieldwork, and on the analysis of multiple

herbarium specimens. More specifically, we examined 389 specimens deposited in the following herbaria: A, B, COL, ESA, F, G, HB, HERBAM, HRCB, HUA, IAN, INPA, K, LINN, MBM, MG, MICH, MO, NY, P, R, RB, RBR, S, SPF, SP SPSF, UEC, UFACPZ, UNEMAT, US, VEN and WU (acronyms following Thiers 2015). We also analyzed images or the actual specimens of all type materials. Fieldwork was conducted between 2014 and 2015, in the Brazilian states of Amazonas, Pará, and Roraima, the center of diversity of *Pachyptera*. Voucher specimens collected during field expeditions were deposited at SPF and MO. All accepted names are listed alphabetically, with nomenclatural discussions and citations following McNeill et al. (2012).

Morphological descriptions and measurements were conducted on dried specimens and fresh materials following the terminology of Lohmann and Taylor (2014), with additional terms from Radford (1974), Gentry and Tomb (1979), Hesse et al. (2009), Hickey (1979), Nogueira et al. (2013), and Weberling (1992). Rare conditions are presented within parentheses. Calyx, corolla, and ovary surface, fruit coat, pollen surface and seed coat were analyzed from representative specimens of each taxon using scanning electron microscopy (SEM) (Appendix 1). The selected structures were mounted on stubs and sputter-coated with gold. Micrographs were obtained on a Zeiss DSM 970 scanning electron microscope.

*Distribution maps and list of examined specimens.* Distribution maps were prepared using QGIS 2.16.3 (QGIS Development Team 2016), while the list of examined specimens was prepared and listed alphabetically using the R package monographaR (Reginato 2016) implemented in R (R Development Core Team 2017).

## Taxonomic treatment

***Pachyptera* DC.** ex Meisn., Pl. Vasc. Gen. 1: 299. 1840. Type: *Pachyptera foveolata* DC. (lectotype, designated by Sandwith [1932: 84]) [= *Pachyptera kerere* (Aubl.) Sandwith]

*Sererea* Raf., Sylva Tellur. 107. 1838. Type: *Sererea heterophyla* Raf., Sylva Tellur. 107. 1838. nom. illeg. superfl. [= *Pachyptera kerere* (Aubl.) Sandwith]

*Leucocalantha* Barb. Rodr., Vellosia, ed. 2. 1: 46, tab. 7. 1891. Type: *Leucocalantha aromatica* Barb. Rodr., Vellosia. ed. 2. 1: 47, tab. 7. 1891.

Description. *Liana*; stems with four phloem wedges in cross-section, solid (hollow in some specimens of *P. aromatica*), cylindrical to tetragonal, green or brown, sometimes reddish in *P. incarnata* (vinaceous in *P. aromatica*), with lighter striations (grayish in *P. aromatica*), with lenticels (without in *P. aromatica*), with interpetiolar gland fields, with a continuous (discontinuous) and transversal interpetiolar ridge, bark peeling when older, puberulous, with simple trichomes, lepidote, with glandular peltate trichomes, becoming glabrescent with age; prophylls of the axillary buds flattened and ensiform (triangular and minute in *P. aromatica*), 3(–5) seriated (a single series visible to the naked eye in some specimens of *P. aromatica*), sparsely to densely puberulous (glabrous). *Leaves* 3-foliolated or 2-foliolated with the terminal leaflet replaced by a trifid tendril; petioles semi-cylindrical to cylindrical, striated, apices articulated, sparsely to densely puberulous, with simple trichomes, lepidote scales, and patelliform glands grouped at the apical portions; petiolules with unequal lengths, striated, apices not-pulvinate (pulvinated in some specimens of *P. aromatica*), sparsely to densely puberulous, with simple trichomes, lepidote scales, and glandular peltate trichomes, petiolules not puvinated, lateral petiolules shorter than the apical ones; blades discolor (concolor), chartaceous or coriaceous, elliptic, obovate or ovate-lanceolate, usually asymmetric, apex acute, acuminate, caudate, or mucronulate (retuse), base cordate, oblique, cuneate, obtuse, or rounded, glabrous to puberulous, with simple trichomes covering the veins (throughout surface), sparsely lepidote, with glandular peltate and patelliform trichomes distributed over the lamina, venation pinnate, secondary venation brochidromous, tertiary venation percurrent, margin entire, flat or sub-revolute. *Inflorescence* axillary or terminal, a few-flowered raceme, congested (lax in *P. aromatica*); axis moderately to densely puberulous, with simple trichomes, sparsely to densely lepidote, with glandular peltate trichomes, patelliform glands grouped at the axis; pedicel moderately puberulous, with simple trichomes, lepidote scales sparsely to moderately distributed; bracts and bracteoles caducous, scarcely evident, brittle, cymbiform, triangular or lanceolate, densely puberulous, with simples trichomes, lepidote scales sparsely distributed. *Calyx* green, reddish-wine, sometimes with

purplish to pink apex, tubular (cupular in *P. erythraea*), sub-bilabiate, truncate, 5-denticulate, shortly 5-lobed, coriaceous, smooth, glabrous internally, puberulous externally, with simple and/or dentritic trichomes, lepidote scales sparsely distributed, patelliform glands grouped at the upper portion. *Corolla* white, cream, light pink, pale purple, orange or red, with yellow nectar guides, hypocrateriform, infundibuliform or tubular campanulate, straight, dorso-ventrally compressed or not compressed, membranaceous, tube moderately to densely puberulous externally, with simple and dendritic trichomes, sparsely lepidote, glabrous internally, but villose at the region of insertion of stamens and staminode (glabrous in *P. aromatica*), with stipitate glandular trichomes; lobes elliptic, obovate, rounded (sub-circular), imbricate, with a pair of patelliform glands arranged in line externally, densely lepidote internally. *Androecium* didynamous, inserted in two heights, with one staminode, glabrous (puberulous in *P. erythraea*); anthers white, becoming darkish with age, included or subexserted, villose (glabrous in *P. aromatica*), basifixed, connective thick, acute, round, with thecae divergent, curved forward or straight; pollen 3 or 4 colpate, psilate-foveolate-microrreticulate or microrreticulate. *Gynoecium* glabrous; stigma capitate, elliptic, ovate, glabrous; ovary cylindrical, not-sulcate (bisulcate in *P. erythraea*), smooth, sparsely to densely pubescent, with simple and dentritic trichomes, sparsely to densely lepidote, with glandular peltate trichomes, without patelliform glandular trichomes (with patelliform glandular trichomes in *P. erythraea*); ovules arranged in two series per locule, placentation axial; nectar disc well developed, glabrous. *Capsule* linear (fusiform in *P. kerere*), flattened (inflated in *P. kerere*), coriaceous to woody, smooth, sparsely to moderately puberulous, with simple trichomes, sparsely lepidote, with glandular peltate and patelliform glandular trichomes throughout, in higher densities at the margins of valves, without lenticels, each valve with an inconspicuous longitudinal midline (conspicuous and raised in *P. kerere*), calyx caducous; seeds oblong, thin, not-corky, (irregulary circular and obcordate, thick, and corky in *P. kerere*), chartaceous to coriaceous, glabrous, smooth, striated, winged (wingless in *P. kerere*), with membranaceous (chartaceous) and hyaline wings.

*Pachyptera* comprises five species, distributed from Belize to Bolivia and Brazil. The main features that distinguish each species are described below and

summarized in the key to species.

**Key to species of *Pachyptera***

1. Stems cylindrical (tetragonal when ≥ 6 $cm^2$ diameter), without lenticels; prophylls of the axillary buds triangular and minute, corolla hypocrateriform ..... *P. aromatica*

1'. Stems sub-tetragonal or tetragonal (if cylindrical, then only on younger portions), with lenticels; prophylls of the axillary buds ensiform and flattened; corolla infundibuliform or tubular campanulate ..... 2

2. Corolla white to cream throughout; ovary puberulous ..... 3

2'. Corolla light pink, pale purple, orange or red; ovary lepidote ……..…….… 4

3. Ovary densely puberulous; capsule fusiform, inflated, each valve with a conspicuous midline; seeds thick, corky, and wingless ……... *P. kerere*

3'. Ovary sparsely to moderately puberulous; capsule linear, flat, each valve with an inconspicuous midline; seeds thin, coriaceous, and winged ……. *P. linearis*

4. Calyx green, light pink at the apex, tubular; corolla light pink to pale-purple, infundibuliform; stamens included; capsule linear, 10.5–42.6 cm long, < 2.6 cm wide ……...… *P. incarnata*

4'. Calyx reddish-wine throughout, cupular; corolla orange to red, tubular campanulate; stamens subexserted; capsule linear, 34.0–41.0 cm long, > 2.7 cm wide ..... *P. erythraea*

1. ***Pachyptera aromatica*** (Barb. Rodr.) L.G. Lohmann, Ann. Missouri Bot. Gard. 99(3): 456. 2014. *Leucocalantha aromatica* Barb. Rodr., Vellosia. ed. 2. 1: 47, tab. 7. 1891. *Pachyptera aromatica* (Barb. Rodr.) L.G. Lohmann, Cat. Pl. Fung. Brasil 1: 770. 2010, *nom. nud.* Type: Brazil. Amazonas: in capoeiras prope Manáos, in Rio Negro, July, fl., B. Rodrigues 633. Lectotype (designated here): tab. 7, in Vellosia. 1891, excluding the pollen image.

Fig. 4

**Description.** *Liana*; stems solid or hollow, cylindrical, vinaceous, with grayish striations, without lenticels, sparsely to densely puberulous; prophylls of axillary buds triangular and minute, 3-seriated (a single series visible to the naked eye in some specimens), moderately to densely puberulous. *Leaves* 3-foliolated or 2-foliolated with the terminal leaflet replaced by a trifid tendril; petioles cylindrical, (0.3-) 1.0–6.0 cm long, sparsely to densely puberulous; petiolules often pulvinated, lateral petiolules 0.5–6.0 cm long, apical petiolules 3.0–8.5 cm long; blades discolor, chartaceous or coriaceous, elliptic, obovate or ovate-lanceolate, symmetric (assymetric), apex acuminate or caudate, mucronulate, base cuneate, obtuse or rounded, lateral blades 5.3–19.8 × 2.0–8.0 cm, apical blades 9.0–19 × 3.8–9.0 cm, glabrous to puberulous abaxially, glabrous to sparsely puberulous adaxially. *Inflorescence* a lax raceme, 6–24 cm long; pedicel 0.5–1.8 cm long, moderately to densely puberulous; bracts 0.4–2.1 mm long; bracteoles cymbiform or lanceolate, 0.7–0.8 mm. *Calyx* green, tubular, sub-bilabiate, 5-denticulate (truncate), 1.0–1.8 × 0.3–0.6 cm, densely puberulous externally, with simple and dendritic trichomes, clustered patelliform glands, sometimes arranged in lines, next to the margin. *Corolla* white, hypocrateriform, 6.3–12.2 cm long, 0.4–1.0 cm of diameter at the tube mouth, tube moderately to densely puberulous externally; lobes elliptic or obovate, 1.9–4.0 × 1.1–1.9 cm. *Androecium* with the longer stamens 14.0–23.1 mm long, the shorter stamens 11.0–19.7 mm long, glabrous; anthers glabrous, sub-exserted, with thecae straight, 3.8–6.2 × 0.3–0.6 mm; pollen 4-colpate, psilate-foveolate-microrreticulate. *Gynoecium* 5.4 cm long; stigma ovate, 2.3× 1.6 mm; ovary 2.7–4.8 × 1.0–1.2 mm, cylindrical, not-sulcate, smooth, moderately to densely pubescent, with simple and dentritic trichomes, sparsely lepidote, with glandular peltate trichomes, without patelliform glandular trichomes; nectar disc 0.3–1.4 × 1.3–2.1 mm. *Capsule* linear, flattened, 33.1–95.0 × 1.0–2.0 cm, each valve with an inconspicuous longitudinal midline; seeds oblong, 4.0–5.5 × 1.0–1.6 cm, thin, not-corky, chartaceous, striated, secondary sculpture smooth, winged, with membranaceous (chartaceous) and hyaline wings.

**Distribution and habitat.** *Pachyptera aromatica* grows in wet forest vegetation in the Brazilian Amazon (Amapá, Amazonas, Rondônia). Fig. 5.

**Phenology.** This species flowers from June to January. Fruits were collected in January, March and July through November.

**Ecology.** *Pachyptera aromatica* has white and hypocrateriform corollas, with a few flowers blooming at a time. This species flowers at dawn (Barbosa Rodrigues 1891), providing further support to the hypothesis of hawkmoth pollination (Gentry 1974).

**Nomenclatural notes.** Like Lohmann and Taylor (2014), we were unable to locate the holotype of *P. aromatica* during multiple visits to RB, where the holotype was thought to be deposited. We were also unable to locate any isotypes in any of the collections visited, indicating that the types of *P. aromatica* were likely lost, just like several other type materials collected by Barbora Rodrigues in the Amazon (Mori and Ferreira 1987). As such, we here designate the illustration used in the original description of this species as the lectotype.

**Taxonomic comments.** *P. aromatica* is characterized by cylindrical and vinaceous stems, with grayish striations and bark papery when old, by the triangular and minute 3-seriated prophylls of the axillary buds, extrafloral nectaries on the interpetiolar region and petiole apices, and white and hypocrateliform corollas, with conspicuous glands arranged in lines on the upper portion (Figs. 1A-D, 4A). This species was originally described within a monotypic genus due to its unusual morphology (Barbosa Rodrigues 1891), but was later transferred into *Pachyptera* based on a combination of morphological and molecular phylogenetic data (Lohmann and Taylor 2014). In a compreensive phylogeny of *Pachyptera, P. aromatica* appeared as the earliest diverging lineage within the genus (Francisco and Lohmann in prep.). The phylogenetic placement of *P. aromatica* corroborates its placement within *Pachyptera* and helps to explain the unusal morphology of this taxon. More specifically, *P. aromatica* has poorly developed, triangular and minute prophylls of the axillary buds (vs. well developed, flattened and ensiform prophylls of all other species of *Pachyptera*), glabrous region of stamen and staminode insertion (vs. villose region of stamen and staminode insertion of all other species of *Pachyptera*), glabrous anthers

with straight thecae (vs. villose anthers with curved thecae of all other species of *Pachyptera*), and white hypocrateriform corollas (vs. white to red infundibuliform corollas of all other species of *Pachyptera*) (Figs. 4 A-B, E-F).

**Specimens examined. BRAZIL. Amapá:** Macapá, Margem de campo, 31 Oct 1980, fl., B. Rabele 1003 (MG). **Amazonas:** Humaitá, Basin of Rio Madeira, on Rio Livramento, 6 Oct 1934, fr., B.A. Krukoff 6845 (NY, K); *Ibid.*, 1 Jan 1982, fl., B.A. Krukoff 12511 (INPA); Iranduba, Estrada entre Novo Airão e Manacapurú, 2º54'46.6"S, 60º57'58.8"W, fl., L.H. Fonseca 327 (SPF); Itacoatiara, Rio Solimões, West of Itacoatiara, brazilnut plantattion.EPILOC360, 3º00'S, 58º45'W, 100 m, 15 Jan 1990, fr., A.H. Gentry 69107 (MO); Manaus, Mar 1907, fr., M. Labroy 1906 (P); 30 July 1929, fl., A. Sucre s.n. (R, RB); 30 July 1929, fl., fr., W. A. Ducke s.n. (MO); 31 July 1929, fl., A. Ducke 22698a (P, R); 8 Aug 1931, fr., A. Ducke s.n. (R); 8 Nov 1931, fr., A. Ducke 22698b (P); *Ibid.*, BR-17, km 3, 30 Aug 1955, fl., F.C. Mello s.n. (INPA); Manaus, ca. 80 km N de Manaus, Distrito Agropecuário da SUFRAMA, Rodovia BR 174, km 64, despois 21 km leste na ZF3, Fazenda Porto Alegre, 1 Jan 1962, st., M.H. Nee s.n. (INPA); Manaus, Campos Sales, margem do Igarapé do Buião, 28 Sept 1954, fl., J.C. Almeida INPA137 (INPA); Manaus, Estrada BR-17, 30 Aug 1955, fl., C.M. Francisco s.n. (MG), Luís s.n. (MG); Manaus, Estrada do Aleixo, near Manaus, km 11 past INPA, 2 Dec 1974, fl., A.H. Gentry 13022 (INPA, MO); Manaus, Estrada do igarapé do Tabatinga, 17 Sept 1963, fl., W.A. Rodrigues 5476 (INPA); Manaus, Estrada do Passarinho, 6 Aug 1962, fl., W.A. Rodrigues 4578 (INPA, SPF); Manaus, Estrada Manaus-Itacoatiara, Reserva Florestal Adolpho Ducke, 1 Jan 1976, st., J.A. Souza s.n. (INPA); *Ibid.*, 6 Aug 1976, J.A. Souza INPA71827 (INPA); Manaus, Groundof INPA at Manaus, 5 Apr 1974, st., A.H. Gentry 11201 (MO), A.H. Gentry 11207 (MO); Manaus, Igarapé do Buião, 15 Oct 1962, st., W.A. Rodrigues 4693 (MO, US); Manaus, Igarapé do Franco, 29 Aug 1957, fl., J.C. Almeida INPA5722 (INPA); Manaus, Industrial development, Mauazinho, 2º18'57.6"S, 60º04'58.8"W , 50-60 m, 4 Aug 1987, fl., S. Tsugaru B-690 (NY); Manaus, Km 3, BR-17, 26 Aug 1955, fl., L.F. Coelho INPA1731 (INPA); Manaus, Loco Cachaeira Grande,4 July 1943, fl., W. A. Ducke 239 (NY, K); Manaus, Manaus/Itacoatiara Road, Km 16, 3 Dec 1974, fl., A.H. Gentry 13056 (MO); Manaus, Reserva Florestal Adolpho Ducke, Estrada Manaus-Itacoatiara, 15 July 1976, st., J.A.

Souza INPA71829 (INPA); *Ibid.*, 21 July 1976, st., J.A. Souza INPA71832 (INPA); *Ibid.*, 25 May 1976, st., J.A. Souza INPA71839 (INPA); *Ibid.*, 3 Aug 1976, st., J.A. Souza INPA71828 (INPA); *Ibid.*, 02º53'S, 59º58'W,15 July 1995, fl., M.J.G. Hopkins 1570 (SPF); *Ibid.*, 15 July 1995, fl., M.J.G. Hopkins 1574 (NYBG); *Ibid.*, 2 Sept 1962, st., A.P. Duarte 7048 (RB); *Ibid.*, 15 Feb 1995, st., M.J.G. Hopkins 1543 (INPA, SPF); *Ibid.*, Nova Prainha, SB-20-ZA, Ponto 02, 10 Sept 1976, st., J.A. Souza INPA61048 (INPA); *Ibid.*, km 26, 30 Sept 1976, st., J.A. Souza INPA61920 (INPA); *Ibid.*, 2º18'S, 59º54'W, 80 m, 19 Jan 1990, fl., A.H. Gentry 69308 (MO); *Ibid.*, 23 Nov 1974, st., A.H. Gentry 12815 (INPA, MO); Manaus, Manaus/Itacoatiara, km 55, 24 Oct 1963, fr., E. Oliveira 2790 (IAN); Manaus, Margem da estrada do Paredão, 3 Aug 1955, fl., J.C. Almeida INPA1540 (INPA); Manaus, Margem do igarapé da cachoeira Alta, Estrada da Forquilha, 22 Aug 1955, fl., J.C. Chagas INPA1701 (INPA); Manaus, Margem do Igarapé do Buião, 19 Aug 1955, fl., J.C. Almeida INPA1687 (INPA); Manaus, Margem do igarapé do Parque 10, 5 Aug 1958, fr., J. Chagas s.n. (MG); Manaus, Outskirts of Manaus, road to INPA, boat landing, behind airport, 26 Nov 1974, fr., A.H. Gentry 12862 (INPA, MO, R); Manaus, Reserva Florestal Adolpho Ducke. Área interna da Reserva Ducke, planta na trilha LO2 entre 1050 e 1100 metros, no interior do gride PPBIO, 15 Oct 2012, st., A. Nogueira 190 (SPF); Manaus, Reserva Florestal Adolpho Ducke. Planta fichada: 2966-24, 15 July 1995, fl., L.G. Lohmann 28 (INPA, SPF); Manaus, Reserva Florestal Adolpho Ducke. Próxima a sede da reserva, na área de platô, 02º55'49.0"S, 59º58'23.2"W, 100 m, 5 May 2015, st., C.S. Gerolamo 9 (SPF); Manaus, Rio Negro, Aug 1900, fl., U. Ule 5217 (K); Manaus, 2-5 km N of Manaus-Itacoatiara Road at km 79 near Río Preto da Eva, 24 Nov 1974, fl., A.H. Gentry 12832 (MO); Manaus, Road toward Río Negro, 10 km N from Manaus on Estrada Aleixo, 21 Nov 1974, st., A.H. Gentry 12778 (MO, R); Manaus, Rodovia BR-174 Manaus-Presidente Figueiredo, sentido norte., 02º47'53.97"S, 60º02'13.12"W, 87 m, 23 Sept 2016, fl., E. Kataoka 349 (SPF); Manaus, Rodovia Manaus-Presidente Figueiredo (BR-174), próximo a entrada da Reserva de Campinarana do INPA, 2º35'23.76"S, 60º02'2.26"W, 88 m, 23 Sept 2016, fr., A. Frazão 313 (SPF); Manaus, s.d. fl., Ule U. 4217 (HB, MO); Manaus, Sede do Inpa, Aleixo, depostio do Oficina, 10 July 1972, fl., M. Silva 1024 (INPA, MO); Manaus, Wedge of Rio Negro, a few km N of Ma, 26 Nov 1974, st., A.H. Gentry 12888 (INPA, MO); Novo Airão, Estação Ecológica Anavilhanas, 2º32'08.0"S,

60º50’49.0"W, 9 Oct 2006, fl., L.G. Lohmann 794 (SPF); Porto Velho, Rio Madeira, Aug 1936, fl., A. Sucre s.n. (RB); Rio Cuieras, 2 km below mouth of Rio Brancinho, 11 Sept 1973, fl., G.T. Prance 17773 (INPA, MO, NY). **Rondônia:** Porto Velho, 11 Sept 1963, fl., B. Maguire 56679 (MO); Porto Velho, Guaporé, 1 June 1952, fl., G.A. Black 52-14674 (IAN); Porto Velho, Rio Madeira, Aug 1936, fl., W. A. Ducke 35624 (MO, RB, US); Porto Velho, Sub-base do Projeto RADAM, aeroporto internacional local, 3 Sept 1975, fl., C.D.A. Mota 18 (INPA); Porto Velho, Sub-base do Projeto RADAM, aeroporto internacional local, 3 Sept 1975, fr., C.D.A. Mota 26 (INPA); s.loc., 1 Jan 1972, fr., B. Maguire s.n. (INPA).

2. ***Pachyptera erythraea*** (Dugand) A.H. Gentry, Phytologia 35(3): 186, fig. 2A. 1977. *Pachyptera kerere* var. *erythraea* Dugand, Caldasia 7(31): 16. 1955. *Mansoa erythraea* (Dugand) A. Gentry, Ann. Missouri Bot. Gard. 66(4): 782. 1979 [1980]. Type: Colombia. Santander: 10 leguas al S.E. de Barranca Bermeja, a 9 km de la margen izquierda del Río Opón, 200 m., 26 Aug. 1954, fl., R. Romero-Castañeda 4727 (holotype, COL-47478!).

Fig. 6

**Description.** *Liana*; stem solid, subtetragonal or tetragonal, green or brown, with lighter striations, sparsely lenticeled, glabrescent to moderately puberulous; prophylls of axillary buds flattened and ensiform, 3(-4)-seriated, glabrous or sparsely to densely puberulous. *Leaves* 3-foliolated or 2-foliolated with the terminal leaflet replaced by a trifid tendril; petioles semi-cylindrical, 3.8–6.5 cm long, sparsely to moderately puberulous; petiolules not pulvinated, lateral petiolules 1.3–2.4 cm long, apical petiolules 2.5–4.1 cm long; blades discolor, chartaceous, elliptic, ovate-lanceolate, asymmetric, apex caudate, mucronulate, base cordate, oblique, lateral blades 11.0–19.2 × 4.7–8 cm, apical blades 12.0–12.3 × 5.7–6.2 cm, glabrous to puberulous abaxially, puberulous adaxially. *Inflorescence* a congested raceme, 2.2–4.5 cm long; pedicel 1.8–2.6 cm long, moderately puberulous; bracts 1.2–1.3 × 0.5–0.9 mm; bracteoles cymbiform or triangular, 0.4–0.8 mm. *Calyx* reddish-wine, cupular, truncate, minutely 5-lobed, 0.9 × 0.7–0.9 cm, densely puberulous externally, with simple and dentritic trichomes, clustered patelliform glands, sometimes arranged

in lines, next to the margin. *Corolla* orange to red, tubular campanulate, 8.5–8.7 cm long, 2.2 cm of diameter at the tube mouth, tube moderately puberulous externally; lobes rounded (sub-circular), 1.8 × 1.4–1.6 cm. *Androecium* with the longer stamens 44.8–49.3 mm long, the shortest stamens 34.6–39.3 mm long, sparsely puberulous at the dorsal portion of the apex; anthers villose, sub-exserted, with thecae curved forward, 2.0–2.6 × 0.6–1.4 mm; pollen 3-colpate, microrreticulate. *Gynoecium* 6.6 cm long; stigma elliptic, 3.8 × 1.9–2.1 mm; ovary 3.1–3.9 ×1.3–1.6, cylindrical, bisulcate, smooth, sparsely puberulous at grooves, with simples trichomes, moderately lepidote, with patelliform glandular trichomes distributed in two vertical lines parallel to the grooves, glandular peltate trichomes forming a vertical line of transition between internal and external grooves, and mixing with simples trichomes into grooves; nectar disc ca. 2.4 × 3.8 mm. *Capsule* linear, flattened, 34.0–41.0 × 2.7–3.0 cm, each valve with an inconspicuous longitudinal midline; seeds oblong, 7.5–9.8 × 2.0–2.1 cm, thin, not-corky, chartaceous to woody, striated, secondary sculpture not seen, winged, with membranaceous and hyaline wings.

**Distribution and habitat.** *Pachyptera erythraea* grows in wet forest vegetation in northern Colombia (Antioquia, Santander), endemic of medium Magdalena River Valley. Fig. 7.

**Phenology.** Flowers collected in January, March, and July to December. Two fruiting collections are known, one collected in July and the other in September.

**Ecology.** *Pachyptera erythraea* has orange to red corollas, with long tubular campanulate shape and sub-exserted anthers. These flower traits are expected to support the hypothesis of hummingbird pollination (Gentry 1974) besides bees.

**Taxonomic comments.** *Pachyptera erythraea* is distinguished by the orange to red corollas (vs. white, light pink or pale purple in all other species), cupular calyces (vs. tubular in all other species), and subexserted anthers (vs. included anthers in all other species). Moreover, the corolla tube of *P. erythraea* expands right above the short basal constriction leading to a tubular-campanulate corolla, while the corolla tube of *P. incarnata, P. kerere,* and *P. linearis* starts at the middle portion of the corolla tube, leading to an infundibiliform corolla. The fruit of *P. erythraea* is flat, without a

visible longitudinal midline, while the seeds are thin and winged, similar to those of *P. incarnata* and *P. linearis*. *Pachyptera eryhtraea* is closely related to *P. incarnata*, with which it shares a moderately to densely lepidote ovary. However, these species are separated by the bi-sulcate ovary, with glandular peltate and patelliform trichomes arranged in vertical lines in *P. erythraea* vs. the non-sulcate ovary, fully covered by glandular peltate trichomes of *P. incarnata*.

**Specimens examined. COLOMBIA. Antioquia:** Caucasia, along road to Nechi 24 km from Caucasia-Planeta Rica road, Hacienda Costarica, margin of primary forest and trees remaining in cleared pasture, 8º03'36.0"N, 75º04'48.0"W, 60 m, 21 Mar 1987, fl., J.L. Zarucchi 4887 (HUA, K, MO); *Ibid.*, 21 Mar 1987, fl., J.L. Zarucchi 4862A (MO); Zaragoza, Carretera a Zaragoza entre Carralao y Angostura, 70 m, 13 Jan 1989, fl., G. Ramiro Fonnegra 2580 (HUA); Rio Magdalena, July 1868, fl., M. Weir 72 (K).**Santander:** Barranca Bermeja, 10 leguas al SE de Barranca Bermeja,7º03'36.0"N, 73º51'36.0"W, 200 m, 26 Aug 1954, fl., R. Romero-Castañeda 4727 (COL, MO); Barranca Bermeja, 12 leguas al SE de Barranca Bermeja, orilla derecha del rio Opón, 200 m, 4 Oct 1954, fl., R. Romero-Castañeda 4979 (COL); Barranca Bermeja, 2 km S. of Llanitas, 19 km N. of Barranca Bermeja, 7º03'36.0"N, 73º51'36.0"W, 160 m, 24 July 1975, fl., A.H. Gentry 15369 (MO); 11-13 km N of Barranca Bermeja on road to Puerto, 07°09'19"N, 73°50'28"W, 160m, 24 July 1975, fl., A.H. Gentry 15372 (MO); Campo Capote, Magdalena Valley, campo Capote, 30 km E of Carare, 6.61, -73.91, 300 m, 29 Sept 1977, fr., A.H. Gentry 20050 (MO); El Centro, 3 km S. of El Centro on road to Yarima, 200 m, 25 July 1975, fl., fr., A.H. Gentry 15402 (MO).

3. ***Pachyptera incarnata*** (Aubl.) J.N.C. Francisco & L.G. Lohmann, *comb. nov.* *Bignonia incarnata* Aubl., Hist. Pl. Guiane. 2: 645, tab. 261, 262, fig. 1–8. 1775. *Bignonia incarnata* Aublet sec. Splitg., Tijdschr. Nat. Geschied 9: 7. 1842. *nom. nud.* *Cydista incarnata* Miers Proc. Roy. Hort. Soc. London. 3: 192. 1863. *nom. nud.* *Pachyptera kerere* var. *incarnata* (Aubl.) A.H. Gentry, Brittonia 25(3): 235. 1973. *Mansoa kerere* var. *incarnata* (Aubl.) A.H. Gentry, Ann. Missouri Bot. Gard. 66(4): 783. 1979 [1980]. Type: French Guiana. s.loc., s.d., (fl., fr), J.B.C.F. Aublet s.n. Lectotype (designated here): tab. 261 and 262, in Hist. Pl. Guiane 1775.

Fig. 8

**Description.** *Liana*; stems solid, cylindrical to tetragonal, green or brown (reddish), with lighter striations, moderately lenticeled, glabrous to moderately puberulous; prophylls of axillary buds flattened and ensiform, 3(–5)-seriated, sparsely to densely puberulous. *Leaves* 3-foliolated or 2-foliolated with the terminal leafltes replaced by a trifid tendril; petioles semi-cylindrical, 0.8–5.5 cm long, sparsely to densely puberulous (adaxialy glabrous), petiolules not puvinated, lateral petiolules 0.8–5.5 cm long, apical petiolules 1.4–4.4 cm long; blades discolor (concolor), chartaceous or coriaceous (membranaceous), elliptic, obovate or ovate-lanceolate, asymmetric, apex acute, acuminate or caudate (retuse), mucronulate, base cordate, oblique, lateral blades 2.6–21.1 × 1.9–9.2 cm, apical bades 5.7–20 × 2.6–7.6 cm, glabrous to sparsely puberulous abaxially, glabrous to sparsely puberulous adaxially. *Inflorescence* a congested raceme, 0.8–3.5 cm long; pedicel 0.5–1.3 (7.5) cm long, moderately to densely puberulous; bracts 0.8–2.5 × 0.7–0.9 mm long; bracteoles cymbiform or filiform, 0.2–0.7 mm. *Calyx* green, light pink at apex, tubular, bilabiate, minutely 5-lobed or truncate, 0.4–1 × 0.4–0.6 cm, densely puberulous externally, with simple and dentritic trichomes, clustered patelliform glands, sometimes arranged in lines, next to margin. *Corolla* light pink to pale purple, infundibuliform, 2.9–7.6 cm long, 0.7–1.9 cm of diameter at the tube mouth, tube moderately to densely puberulous externally; lobes rounded (sub-circular), 0.3–1.5 × 0.4–1.3 cm. *Androecium* with the longer stamens 23.4–17.0 mm long, the shorter stamens 10.0–15.7 mm long, glabrous; anthers villose, included, with thecae curved forward, 2.6–3.1 × 0.5–0.5 mm; pollen 3-colpate, microrreticulate. *Gynoecium* 3.0–5.0 cm long; stigma capitate or ovate, 0.8–3.5 × 0.5 mm; ovary 1.8–2.9× 0.7–0.91, cylindrical, not-sulcate, smooth, glabrous, densely lepidote, with glandular peltate trichomes, rarely with some patelliform glandular trichomes; nectar disc 0.8– 1.0 × 1.7–1.8 mm. *Capsule* linear, flattened, 10.5–42.6 × 1.4–2.6 cm, each valve with an inconspicuous longitudinal midline; seeds oblong, 4.0–7.0 × 1.3–2.8 cm, thin, not-corky, chartaceous to sub-coriaceous, striated, secondary sculpture with randomly distributed micropores, winged, with membranaceous and hyaline wings.

**Distribution and habitat.** This species is found in wet forest vegetation in Brazil (Amazonas, Mato Grosso, Pará, Rondônia), Nicaragua (Zelaya) and French Guiana. Fig. 9.

**Phenology.** *Pachyptera incarnata* flowers in February to May and July to December. Fruiting material has been colected in April, May, July to October and December.

**Ecology.** *Pachyptera incarnata* is found in dry forests, igapó and riverbanks. Their seeds are thin and winged suggesting anemocoric dispersion.

**Nomenclatural notes and taxonomic comments.** *Pachyptera incarnata* is characterized by the infundibuliform, light pink to pale purple corolla, often greenish-cream at the base. The capsule is linear, flattened, and coriaceous (almost woody), with pink patelliform glandular trichomes throughout the surface, and an inconspicuous longitudinal midline, bearing seeds that are linear, oblong or sub-oblong, chartaceous to subcoriaceous, thin, winged, with membranaceous and hyaline wings.

This species was first described by Aublet (1775) as *Bignonia incarnata*. Gentry (1973) treated *B. incarnata* as a variety of *P. kerere* due to the shared racemose inflorescences, corolla infundibuliform, and villose anther, and 3-seriated prophylls of the axillary buds. He distinguished the two varieties based on differences in the fruit and seed morphology. More specifically, *P. kerere* var. *kerere* included the individuals with inflated fruits, corky and wingless seeds, while *P. kerere* var. *incarnata* included the individuals with flattened fruits, thin and winged seeds. Despite the floral similarity between these two species (see taxonomic comments under *P. kerere*), *P. incarnata* is phylogenetic more closely related to *P. erythraea*, with which it shares flattened and linear fruits and a lepidote ovary (see discussion under *P. erythraea*). Based on our new molecular phylogeny (Francisco and Lohmann submitted) and morphological data, we raise this taxon back to species-level, following Aublet (1775). We were unable to located original material, and the original illustration is here designated as the lectotype.

**Specimens examined. BRAZIL. Amapá:** Campaipi, Embrapa reserve and vicinity, 0°10'N, 51°37'W, 3 Sept 1983, fl., S.A. Mori 15783 (MG, MO). **Amazonas:** Manaus,

31 Aug 1931, fl., A. Ducke 24091 (R); *Ibid.*, estrada do Aleixo, near Manaus, turn off to Río Negro at km 11 past INPA, 2 Dec 1974, fl., A.H. Gentry 13027 (MO); *Ibid.*, INPA boat landing behind Manaus airport, Río Negro, 15 Dec 1974, fr., A.H. Gentry 13323 (MO); Maués, across from Guarara factory, 20 Apr 1974, fl., D.G. Campbell P22008 (MO); Presidente Figueiredo, Balbina, Rebio Uatumã, grade do PPBio, 6 Oct 2006, fr., J.R. Carvalho-Sobrinho 1078 (INPA). **Mato Grosso:** Aripuanã, MT-420, beira do rio, 10º15'00.0"S, 59º07'12.0"W, 11 July 1997, fl., G.F. Árbocz 4256 (ESA); Juruena, beira do Rio Juruena, floresta aluvial, 10º18'36.0"S, 58º19'48.0"W, 10 July 1997, fl., V.C. Souza 18583 (ESA); Nova Bandeirantes, estrada Iporã, 255 m, 22 July 2015, fl., fr., R.S. Ribeiro 78 (SPF). **Pará:** Belterra, Floresta Nacional do Tapajós, estrada para comunidade de Jamaraguá, km 72, 02°55'15.9"S, 55°01'39.4"W, 114 m, 16 Sept 2015, fl., J.N.C. Francisco 89 (SPF); estrada do Mocambo, IPEAN, 02 May 1969, fl., J.M. Pires 12075 (IAN, MO); Irituia, Rio Irituia, varzea S. Miguel do Guamá, 29 Oct 1948, fl., G.A. Black 48-3355 (IAN); Itaituba, estrada Santarém-Cuiabá, BR 163, km 794, 7°25'S, 55°20'W, 12 May 1983, fl., I.L. Amaral 1248 (MO); Marabá, Marabu, Serra Norte, Carajás, 7º42'36.0"S, 57º48'36.0"W, 01 Aug 1983, fl., M. Silva 1604 (MO, UEC); Óbidos, beira do Lago Curumu, floresta de várzea, 01°51'37.3"S, 55°38'47.3"W, 24 m, 23 Sept 2015, fl., J.N.C. Francisco 130 (SPF); Óbidos, lago Maria Teresa, floresta de várzea, 01°52'37.7"S, 55°35'28.7"W, 14 m, 23 Sept 2015, fl., J.N.C. Francisco 121 (SPF); *Ibid.*, 01°52'38.2"S, 55°35'27.4" W, 14 m, 23 Sept 2015, fr., J.N.C. Francisco 122 (SPF); Oriximiná, Floresta Nacional de Saracá-Taquera, próximo ao alojamento Pioneiros de pesquisadores, floresta de terra firme, 01°27'56.6"S, 56°22'43.8"W, 71 m, 27 Sept 2015, fl., J.N.C. Francisco 151 (SPF); Oriximinã, Porto Trombetas, rejeitos, Linha 69, beira de floresta, 1º45'36.0"S, 55º51'36.0"W, 09 Dec 1987, fl., O.H. Knowles 1120 (INPA); Oriximinã, Porto Trombetas, Serra Assas, descampado, 21 Oct 1987, fl., O.H. Knowles 1106 (INPA); Palestina do Pará, fazenda Andorinha sede 2, início da mata do rio Gameleira, 6º06'36.0"S, 48º24'36.0"W, 160 m, 18 Apr 2004, fr., G. Pereira-Silva 8765 (CEN); Parauapebas, Serra dos Carajás, Platô N2, vegetação de canga, 7 Mar 2010, fl., L.C.B. Lobato 3870 (MG); Paraupebas, Serra dos Carajás, à margem da estrada Raymundo Mascarenhas, 8 Feb 1990, fl., J.B.P. Rocha 701 (IAN); Portel, 1º57'36.0"S, 50º45'00.0"W, 21 Oct 1955, fl., L. Williams 18222 (IAN, MO); Porto Trombetas, Mineração Rio do Norte, 1991, fr., Evando 542 (INPA); Santarém, beira da PA-370,

próxima à guarita da Usina Hidrelétrica Curuá-Uma, floresta de terra firme, 02°49'21"S, 54°17'58.9"W, 49 m, 19 Sept 2015, fl., J.N.C. Francisco 103 (SPF); Santarém, ramal próximo à Usina Hidrelétrica Curuá-Uma, solo areno argiloso, floresta de terra firme, 02°48'45.2"S, 54°18'08.8"W, 47 m, 19 Sept 2015, fl., J.N.C. Francisco 105 (SPF); São Miguel do Guamá, Rio Guamá, beira do rio, igapó, 21 Aug 1948, fl., fr., Dardano 48-3092 (IAN); Senador José Porfirio, margem direita do Rio Xingu, capoeira de terra firme, 02°34'00"S, 51°55'00"W, 3 Dec 1991, fr., G. Santos 282 (MG); Tucuruí, área de desmatamento, 1 Sept 1983, fl., F.E. Miranda 362 (NY); Tucuruí, BR-422, Km 45, Breu Branco, margem do rio Tocantins, 5 Nov 1983, fl., J. Ramos 1011 (INPA); Tucuruí, desmatamento na margem direita, estrada para o lago 31 de março, 30 Aug 1983, fl., J. Revilla 8397 (NY); Tucuruí, margens da PA-149 até ca. Km 50, 22 Aug 1983, fr., J. Revilla 8326 (NY); Viseu, Serra do Piriá, à 13km de Açaiteua, 4 Dec 1993, fl., J. Sales 1539 (MG); Vitória do Xingu, 3°19'32"S, 52°00'16"W, 1 Aug 2015, fl., R.V. Pyramo PSACF_EX06147 (RB); V *Ibid.*, 3°22'4"S, 52°02'23"W, 12 Aug 2015, fl., B.R. Silva PSACF_EX06201 (RB). **NICARAGUA. Zelaya:** along Río Sucio, ca. 0.5 km E of first suspension bridge E of Bonanza, gravel bars and gallery forest, 14º00'36.0"N, 84º33'36.0"W, 140 m, 24 Apr 1978, fl., fr., W.D. Stevens 8082 (MO).

4. ***Pachyptera kerere*** (Aubl.) Sandwith, Recueil Trav. Bot. Néerl. 34: 219. 1937. *Bignonia kerere* Aubl., Hist. Pl. Guiane 2: 644, tab. 260. 1775, excluding the fruit description and tab. 263. *Bignonia heterophylla* Willdenow, Sp. Pl. 3: 298. 1800 [1801]. *nom. superfl. illeg. Sererea heterophyla* Rafinesque, Sylva Tellur. 107. 1838. *nom. superfl. illeg. Adenocalymma kerere* (Aubl.) Bureau & K. Schum. Fl. Bras. 8(2): 119. 1891. *Petastoma kerere* (Aubl.) Schnee in. Pittier, Cat. Fl. Venez. 2: 404.1947. *Mansoa kerere* (Aubl.) A. Gentry, Ann. Missouri Bot. Gard. 66(4): 783. 1979 [1980]. Type: French Guiana. Cayenne, s.d., fl., J.B.C.F. Aublet s.n. (holotype, BM-992379!).

Fig. 10

*Adenocalymma stridula* Miers, Ann. Mag. Nat. Hist. ser. 3 7: 392. 1861. *nom. superfl. illeg.* Type: Guiana, s. loc., s.d., J. Miers s.n. (BM ?, not located).

*Adenocalymma foveolatum* (DC.) Baillon, Hist. Pl. 10: 7, fig. 9-16. 1891. *Adenocalymma foveolatum* (DC.) K. Schumann, Nat. Pflanzenfam 4(3b): 214, fig. 89 F-G. 1894. *nom. illeg. Adenocalymma foveolatum* (Bureau) Bureau & K. Schumann, Fl. Bras. 8(2): 109. 1896. *nom. superfl. illeg. Pachyptera foveolata* DC., Prodr. 9: 175. 1845. Type: French Guiana, s.loc., 1819-1821, fr., M. Poiteau s.n. (lectotype, designated by Sprague and Sandwith 1929, p. 84: G-DC [G-1405]!).

*Adenocalymma brachybotrys* DC., Prod. 9: 202. 184. Type: French Guiana. s.loc., 1821, fl., G.S. Perrottet s.n. (holotype, P-3578200!).

*Adenocalymma symmetrica* Rusby, Descr. S. Amer. Pl. 122. 1920. Type: Venezuela. Lower Orinoco, 1896, fl., H.H. Rusby s.n. (holotype, NY-313053!).

*Bignonia benensis* Britton ex Rusby, Bull. Torrey Bot. Club 27: 70. 1900. Type: Bolivia. Junction of Beni and Madre de Dios rivers, Aug. 1886, fl, H.H. Rusby 1143. (holotype, NY-313133!; isotype, MICH-1115822!, NY-313132!, US-603898!, US-125816!).

*Tanaecium zetekii* Standley, Contr. Arnold Arbor 5:140. 1933. Type: Panama. Canal zone: Barro Colorado Island, 3 Feb 1932, fl., R.H. Woodworth 363. (holotype, F-651874!; isotype, A-93244!, MO-807829!, US-125783!).

**Description.** *Liana*; stems solid, cylindrical to tetragonal, green or brown, with lighter striations, moderately lenticeled, glabrescent to moderately puberulous; prophylls of axillary buds flattened and ensiform, 3(–5)-seriated, sparsely to densely puberulous. *Leaves* 3-foliolated or 2-foliolated with the terminal leaflet replaced by a trifid tendril; petiole semi-cylindrical, 0.3–6.9 cm long, sparsely to densely puberulous throughout the surface or very sparsely puberulous on the abaxial side and densely puberulous on the adaxial side, petiolules not puvinated, lateral petiolules 0.3–6.0 cm long, apical petiolules 0.8–6.0 cm long; blades discolor (concolor), membranaceous or chartaceous (coriaceous), elliptic, obovate or ovate-lanceolate, asymmetric, apex acute, acuminate or caudate, mucronulate, base cordate, oblique, lateral blades 4.4–22.5 × 2.1–14.3 cm, apical blades 5.2–22.5 × 2.0–11.5 cm, sparsely to moderately (densely) puberulous abaxially, (glabrous) sparsely to moderately puberulous adaxially. *Inflorescence* a congested raceme, 0.6–4.8 cm long; pedicel (0.2-)0.5–5.7(-7.5) cm long, moderately puberulous; bracts 1.1–2.4 mm long;

bracteoles cymbiform or filiform, 0.4–2.3 × 0.5–0.8 mm. *Calyx* green, sometimes with purplish apex, tubular, bilabiate or sub-bilabiate, truncate, minutely 5-lobed, 0.5–1.2 × 0.4–0.9 cm, moderately to densely puberulous and sparsely lepidote externally, with simpe and dentritic trichomes, with clusters of patteliform glands, sometimes arranged in lines, next to the margin. *Corolla* white or cream, infundibuliform, 4.0–9.5 cm long, 0.9–2.5 cm of diameter at the tube mouth, tube moderately to densely puberulous externally; lobes rounded (sub-circular), 0.6–1.9 × 0.5–1.8 cm. *Androecium* with the longer stamens 18.0–29.1 mm long, the shorter stamens 11.9–20.3 mm long, glabrous; anthers villose, included, thecae curved forward, 1.9–3.1 × 0.3–1.0 mm; pollen 3-colpate, microrreticulate. *Gynoecium* 3.2–6.0 cm long; stigma capitate or ovate, 1.6–3.1 × 0.9–3.5 mm; ovary 1.8–3.6 × 0.8–1.6, cylindrical, not-sulcate, smooth, densely pubescent, with simple and dentritic trichomes, sparsely lepidote, with glandular peltate trichomes, without patelliform glandular trichomes; nectar disc 0.4–1.9 × 0.5–4.0 mm. *Capsule* fusiform, inflated (slightly flattened), 8.0–26.0 × 1.5–3.6 cm, each valve with a conspicuous and raised longitudinal midline; seeds irregularly circular and obcordate, 2.8–4.4 × 1.4–3.0 cm, thick, corky, striated, secondary sculpture with two pairs of medium micropores on each striation, wingless.

**Distribution and habitat.** This species is typically found in wet and often flooded forest vegetation in Belize (Toledo), Bolivia (Beni), Brazil (Acre, Amapá, Amazonas, Maranhão, Mato Grosso, Pará, Rondônia, Roraima), Colombia (Amazonas, Antioquia, Bolivar, Choco), Costa Rica (Limon, Puntarenas), French Guiana (Cayenne), Guatemala (Izabal), Guyana (Demerara Superior-Berbice, Berbice), Honduras (Atlantida, Cólon), Nicaragua, Panama (Barro do Colorado, Bocas del Toro, Colon, Darien), Peru (Amazonas, Huanuco, Loreto, Madre de Dios), Suriname (Sipiwalini), and Venezuela (Amazonas, Apure, Bolivar, Delta Amacuro). Fig. 11.

**Phenology.** This species flowers and fruits throughout the year.

**Ecology.** *Pachyptera kerere* is restricted to igapó and riverbanks, with corky and wingless seeds that are dispersed by water. This feature likely contributed for the broad distribution of this taxon throughout Amazonian flooded forests (Gentry 1974, Francisco and Lohmann submitted).

Etymology. The specific epithet is derived from vernacular name “kérérê” or “térérê” adopted by the indigenous group Galibis, from French Guiana, who use this plant as rope material.

Nomenclatural notes. The original description of this species by Aublet (1775) included a mistake in the fruit description, which actually consists on the description of *Amphilophium magnollifolium* (Kunth) L.G. Lohmann instead.

**Taxonomic comments.** *Pachyptera kerere* is easily recognized by the infundibuliform, white to cream corollas, with densely pubescent ovary. The fusiform, woody, inflated (sometimes slightly flattened) and smooth fruit, with a conspicuous and raised longitudinal midline, is very distinctive. The seeds are irregulary circular and obcordate, corky, and wingless.

*Pachyptera kerere* shares infundibuliform corollas with *P. linearis* and *P. incarnata*. However, these species can be differentiated by the corolla color and ovary indumentum. While *P. kerere* has white corollas and densely pubescent ovaries, *P. linearis* has white corollas and sparsely pubescent ovaries. *Pachyptera incarnata* has light pink to pale purple corollas and densely lepidote ovaries.

**Specimens examined. BELIZE. Belize:** Mile 5 3/4, Northen Highway, 7 June 1974, fl., J.D. Dwyer 12737 (MO). **Stann Creek:** 16°50'N, 88°30'W, May 1901, fl., fr., E.J.F Campbell 95 (K). **Toledo:** Maya Mts, Solomon Camp, vicinity of the junction of Richardson Creek and Bladen Branch, foothillof the Maya Mountains, 16º31'48.0"N, 88º45'00.0"W, 80 m, 5 Mar 1987, fl., G. Davidse 32046 (MO). **BOLIVIA. Beni:** junction of Beni and Madre de Dios rivers, Aug 1886, fl., H.H. Rurby 1143 (MICH, NY, US); Riberlata, ca. 3 km SW of Riberalta on road to Hamburgo (crossing of Río Beni), várzea forest, heavily disturbed, 11º01'48.0"S, 66º06'00.0"W, 230 m, 20 Sept 1981, fl., J.C. Solomon 6349 (MO); Vaca Diez, Cachuela Esperanza Río Beni, 12 Sept 1985, fl., M. Moraes 550 (MO). **BRAZIL.** s.loc., s.d., fl., s.inf. s.n. (B). **Acre:** Porto Walter, Along Rio Juruá-Mirim, ca. 3 hrs by boat, above its mouth at Rio Juruá, 11 Nov 2001, fl., P. G. Delprete 7688 (NY). **Amapá:** Macapá, canteiro do Museu Macapá, 30 June 1981, fl., Verônica 17 (RBR); Rio Araguari, 1º10'48.0"N, 52º07'48.0"W, 22 July 1951, fr., R.L. Fróes 27540 (IAN,

MO); Rio Calcoene, 16 Nov 1901, fl., W.A. Ducke s.n. (K, MO); Rio Puchacá afluente do Vila Nova, 15 Feb 1961, fl., A.G. Andrade 855 (R). **Amazonas:** Anavilhanas, Rio Negro, May 1980, fl., M. Goulding 66a (MG, MO); *Ibid.*, s.d., fl., M. Goulding 2120 (MG, MO); Autaz-Mirim, igapó do Curi, 20 Mar 1973, fl., fr., A.A. Loureiro INPA37554 (INPA); Autaz-Mirim, Lago do Purupuru, igapó, 17 Mar 1973, fl., fr., A.A. Loureiro s.n. (INPA); Careiro, Lago do Castanho, igapó, 7 June 1972, fr., M. Honda INPA36004 (INPA); Itaubarana, Rio Purus region, Rio Ipixuna, 15 km downstream from Itaubarana (30 km from Tapaua), igapo, 5°38'0"S, 63°10'0"W, 19 Jan 1986, fl., G. Gottsberger 115-19186 (MO); *Ibid.*, 23 Jan 1986, fl., G. Gottsberger 16-23186 (MO); Janauacá, Lago do Castanho, 7 June 1972, fr., M. Honda INPA36004 (INPA); Manaus, bank of Rio Negro and Rio Amazonas near Manaus, 4 Apr 1974, fl., fr., A.H. Gentry 11191 (MO); Manaus, Campus do INPA, Séde do INPA, estrada do Aleixo, km 3, capoeira, solo argiloso, 5 Aug 1973, fl., P.N. Conceição 5 (INPA); Manaus, estrada do Igarapé do Mariano, capoeira fechada, 23 Apr 1958, fl., fr., J.C. Almeida INPA6383 (INPA); Manaus, Igarapé do Binda, 2º18'36.0"S, 60º04'48.0"W, 19 Jan 1955, fl., J.C. Chagas 606 (K); *Ibid.*, 19 Jan 1955, fl., J.C. Almeida INPA606 (INPA); Nova Airão, margem do Rio Negro, mata de igapó, 01°54'21.0"S, 61°20'08.9"W, 21 m, 12 May 2015, fl., M. Beyer 324 (SPF); *Ibid.*, 1°40'07.0"S, 61°25'00.1"W, 13 m, 12 May 2015, fl., M. Beyer 332 (SPF); Novo Airão, Estação Ecológica Anavilhanas, 2º31’48.0”S, 60º50’24.0”W, 5 Feb 2007, fl., L.G. Lohmann 836 (SPF); Presidente Figueiredo (entorno), beira do Rio Uatumã, abaixo do Ramal da Morena, 1°00'S, 59°00'W, 24 Feb 2007, fl., C.E. Zartman 6349 (INPA, SPF); *Ibid.*, 24 Feb 2007, fr., C.E. Zartman 6333 (INPA); Presidente Figueiredo, no entorno do Lago da Rebio Uatumã, Balbina, 2º01'48.0"S, 60º01'12.0"W, 13 Aug 2008, fl., F.F. Melo 532 (INPA); Rio Ituxi, Boca du Curuquete, Rio Purus, 10 July 1971, fl., G.T. Prance 14036 (INPA); Rio Negro between Rio Quinini and Moreira, sandy river bank, 13 Oct 1971, fl., G.T. Prance 15178 (INPA); Rio Negro, Parana do Jacaré, 2º01'48.0"S, 60º01'12.0"W, 24 June 1992, fl., S.A. Mori 22470 (MO); São Francisco, Rio Negro, Paraná do Camanaú até Ponta do Canta Galo, 1º41'24.0"S, 61º16'12.0"W, 25 Apr 1973, fl., fr., M.F. Silva 1092 (INPA); Solimões, boca do Tefé, capoeira, 27 Sept 1904, fl., A. Ducke MG6821 (INPA); Tefé, Lago Tefé, northwest shore, igapó habitat, sandy, flooded lakeshor, 11 Dec 1982, fl., T. Plowman INPA126243 (INPA); Xiborema, solo argiloso, mata,

várzea, 1 Jan 1957, fl., L.F. Coêlho s.n. (INPA). **Maranhão:** Lago Verde, fazenda São Francisco, 11Km N of Km 337 of BR 316, forest with Orbignya Palm, 4°0'S, 44°56'W, 25 Sept 1980, fr., D.C. Daly D264 (MG); Palmeirândia, 17 Dec 2006, fl., C.M. Vieira 72 (IAN). **Mato Grosso:** Colíder, canteiro de obras da UHE Colíder, terra firme, 10º58'48.0"S, 55º46'12.0"W, 258 m, 6 June 2011, fl., C.R.A. Soares 3594 (HERBAM, UNEMAT); near Tabajara, upper Machado River region, Nov 1931, fl., B.A. Krukoff 1517 (K, NY, P). **Pará:** Aguá, Rio Irucú, mata de várzea, 1992, fl., fr., U.R. Maciel 1971 (MG); Aguá, Rio Marajozinho, mata de várzea, 1992, fl., U.R. Maciel 1793 (MG), U.R. Maciel 1823 (MG); Almeirim, Distrito de Monte Dourado, coletas ao longo do Rio Jari, 0°51'00"S, 52°32'00"W, 68 m, 4 July 2010, fl., R.C. Forzza 5994 (RB); Altamira, Rodovia Transamazônica (BR-230), margem do Rio Xingú, antes da travessia da Balsa, lado esquerdo da rodovia, sentido Altamira - Marabá, 03°07'34.4"S, 51°42'03.2" W, 5 m, 30 Nov 2005, fl., R.G. Udulutsch 2708 (HRCB, MBM, SPF); Aveiro, Flona do Tapajós, Rio Cupari, 12 May 2011, fl., M.A. Braga 77 (RB); Belém, Campus of IPEAN, 6 Dec 1974, fl., A.H. Gentry 13075 (MO); Belterra, caminho para Pindobal, 29 Oct 1947, fl., G.A. Black 47-1861 (IAN, K); Chaves, Ilha Mexiana, Faz. Nazareth, 18 Sept 1901, fl., M. Guedes s.n. (MO); estrada entre S. Miguel e Río Caracuru, varsea, capoeira, 17 Jan 1969, fl., N.T. Silva 1654 (K, MO); Furo Macujubirm, 30 Aug 1901, fl., M. Guedes s.n. (MO); Ilha de Marajó, Rio Gipuru, 00°15'S, 50°30'W, 24 Oct 1987, fl., H.T. Beck 178 (F, MO); Melgaco, na Ilha do Marajó, Río Mapari, afluente do Río Tajapuru, 29 Nov 1991, NA, G. Santos 226 (MO); Óbidos, Aug 1902, st., W.A. Ducke s.n. (K, MO); Ourém, capoeira, 4 Dec 1903, fl., R.S. Rorb s.n. (MG); Paraupebas, Reserva Biologica da Serra dos Carajás, Companhia Vale do Río Doce, área da planta piloto, mina de exploracao de ferro-N4, 500 m, 20 Nov 1991, fr., G. Santos 183 (G, MO); Piriá, Bankof Rio Piria, N of km 90, 28 Oct 1965, fl., G.T. Prance 1736 (IAN, K, MO); Rio Mojú, 1 June 1954, fl., G.A. Black 54-16283 (K); Río Tocentius, reg. de S. Joazuim de Itaquara, 18 Dec 1960, fr., E. Oliveira 1243 (IAN, MO); Santarém, 1877-78, fl., M. Jobert 857 (P); São Sebastião da Boa Vista, estrada de acesso a Vila Cocal, 2 Sept 1992, fl., C.A. Santos 31 (MG); Senador Jose Porfirio (Sozel), margem direita do Rio Xingu, 02°34'00"S, 51°55'00"W, 3 Dec 1991, fr., G. Santos 287 (MO); Tucuruí, Breu Branco, igapó às margens do rio Tocantis, 14 Oct 1983, fl., J. Revilla 8681 (NY, SPF); *Ibid.*, 12 Sept 1983, fl., F.E. Miranda 576 (NY); Vitória do Xingu, Rio Xingu, Sítio

Pimental,52º00’36.0”S, 2º52’48.0”W, 15 Jan 2012, fl., C.S. Rosario s.n. (MBM). **Rondônia:** Costa Marques, às margens do rio Caltário, 28 Oct 1996, fr., L.C.B. Lobato 2398 (MG); Pacáas Novos, Rio Pacaas Novos, 3 Aug 1968, fl., G.T. Prance 6759 (INPA, K, MO, R). **Roraima:** Caracaraí, margem do Rio Branco, 0°56'46.4"S, 61°52'32"W, 36 m, 15 May 2015, fl., A. Frazão 149 (SPF); Caracaraí, Parque Nacional do Viruá, margem do Rio Branco, igapó, 01°40'29.0"N, 61°11'24.6"W, 42 m, 26 Sept 2014, fl., J.N.C. Francisco 41 (SPF); Caracaraí, Parque Nacional do Viruá, próximo da sede do Parque, floresta de terra firme, 01°29'23.3"N, 61°00'09.1"W, 68 m, 24 Sept 2014, fl., J.N.C. Francisco 40 (SPF); *Ibid.*, 01°29'24.9"N, 61°00'11.4"W, 67 m, 24 Sept 2014, fl., J.N.C. Francisco 39 (SPF); Caracaraí, Rio Branco, 0°36'14"N, 61°35'43"W, 50 m, 20 Mar 2012, fl., G. Martinelli 17395 (RB); *Ibid.*, 0°56'49"N, 0°58'14"W, 34 m, 27 Mar 2012, fl., fr., M. Nadruz 2647 (RB); Caracaraí, Rio Branco, próximo ao encontro com o Rio Negro, 1°22'24.5'S, 61°51'59.7" W, 34 m, 16 May 2015, fl., A. Frazão 153 (SPF); Igarape Agua Boa, Río Mucajai between Pratinha and Río Apiau, 22 Jan 1967, fl., G.T. Prance 4012 (INPA, K, MO, R, US); Rio Branco, 00°43'46.3"S, 61°51'24.0"W, 32 m, 14 May 2015, fl., V. Thode 424 (SPF); Rorainópolis, boca do Rio Branco com o Rio Negro, 1°23'8"S, 61°52'46"W, 40 m, 25 Apr 2014, fl., R.C. Forzza 8094 (RB); *Ibid.*, 40 m, 25 July 2014, fl., R.C. Forzza 8113 (RB); Rorainópolis, foz do rio Branco no rio Negro, mata de igapó com interferência da água branca do rio Branco, 1°23'0.2"S, 61°51'6"W, 35 m, 13 May 2015, fl., B.M. Gomes 648 (SPF); Rorainópolis, margem do rio Branco, em direção à Caracaraí, mata de igapó, 1°12'12.7"S, 61°50'37.3"W, 34 m, 13 May 2015, fr., B.M. Gomes 651 (SPF); Rorainópolis, margem do rio Branco, em direção ao encontro dos rio Branco com o Negro, mata de igapó com influência das águas do rio Negro, 1°5'46.5"S, 61°52'53"W, 28 m, 15 May 2015, fl., B.M. Gomes 659 (SPF); Rorainópolis, margem do rio Branco, mata de igapó, 1°14'42.7"S, 61°50'56.2"W, 29 m, 16 May 2015, fl., B.M. Gomes 662 (SPF); Rorainópolis, margem do Rio Negro, mata de igapó, 01°33'14.2"S, 61°30'27.8"W, 13 m, 12 May 2015, fl., M. Beyer 336 (SPF); *Ibid.*, 01°33'14.2"S, 62°30'27.8"W, 13 m, 12 May 2015, fl., M. Beyer 337 (SPF); *Ibid.*, 1°22'5.2"S, 61°45'55.3"W, 18 m, 13 May 2015, fl., B.M. Gomes 639 (SPF); Rorainópolis, Rio Branco, Ponto 11, 0°56'24.0"S, 61°50'24.0"W, 22 m, 14 May 2015, fl., fr., A. Frazão 136 (SPF); Rorainópolis, Rio Xixuaú, floresta beirando pequenos igarapés, 0°48'22"S, 61°33'32"W, 5 Mar 2010, fl., M.J.G. Hopkins 1961 (INPA);

Rorainópolis, Rio Xixuaú, Ilha da casa do Chris, 0°48'01"N, 61°33'29"W, 25 m, 3 Feb 2011, fl., T. Marinho 208 (INPA). **COLOMBIA.** Llamos de Cumaral ad vedem Andim bogosensim orinocum versus, 386 m, Jan 1876, fl., L. Aruz 1035 (P). **Amazonas:** Araracuara, rocks along Rio Caqueta, Araracuara, 0, 200 m, 17 Jan 1989, fr., A.H. Gentry 64809 (MO). **Antioquia:** Chigorodo, Rio Leon 15 km W of Chigorodo, 07° 45'N , 76° 50'W, 100 m, 19 Mar 1962, fl., C. Feddema 1954 (MICH, MO, US); Necoclí, Reserva Indígena Cainán Nuevo, 76° 46'W, 8° 16' 36" N, 2 m, Aug 1992, fl., L. Castaño 93a (HUA). **Bolivar:** La Raya, Achi, Inspeccion de la Raya, 8°19'48.0"N, 74°33'36.0"W, 30 m, 5 May 1987, fl., H.V. Cuadros 3601 (MO). **Choco:** Boca del Togoroma, Bank of Quebrada Togoroma, 13 June 1944, fl., fr., E.P. Killip 39122 (COL, F, MO, US); Las Animas, Jequedo, 42 km W of Las Animas, E of Rio Pato on Pan American (under construction) W of Las Animas, 5º16'48.0"N, 76º36'36.0"W, 250 m, 11 Jan 1979, fl., A.H. Gentry 23990 (MO); Truando, cativo swamps along Rio Truando, 7º09'N, 77º12'W, 18 Jan 1974, fl., A.H. Gentry 9313 (MO). **COSTA RICA. Limon:** Parque Nacional Tortuguero, Estación Agua Fría, alrededores de la casa-estación, vegetación secundaria y relictos de vegetación primaria, 10°24'36"N, 83°33'36"W, 40 m, 24 Oct 1987, fl., R. Robles 1121 (MO); Parque Nacional Tortuguero, Estación Cuatro Esquinas, 800 m al Sur de la casa-estación, a orillas de la Laguna de Tortuguero, 10º30'36.0"N, 83º30'00.0"W, 2 m, 29 Nov 1987, fr., R. Robles 1391 (MO); Puerto Viejo de Talamanca, along road in vicinity of beach between Punta Cocles and Punta Uva, E of Puerto Viejo de Talamanca, 9°37'48"N, 82°42'36"W, 0–5 m, 6 Nov 1984, fl., M.H. Grayum 4411 (MO); Rio Gandoca, Refugio Gandoca-Manzanillo Low-lying coastal swamps and forests, Gandoca (slightly to N of trail from Mata de Limón), 9º36'N, 82º36'W, 0 m, 27 Jan 1987, fl., M.H. Grayum 8032 (MO); Talamanca, Sixaola, Gandoca, finca Cangrejo, Anai, 9°34'45"N, 82°36'20"W, 10 m, 24 Mar 1995, fr., G. Herrera 7551 (K). **Puntarenas:** Cantón de Osa, cuenca Térraba-Sierpe, Chocuaco, 8°43'50"N, 83°27'17"W, 150 m, 29 Dec 1996, fl., R. Aguilar 4824 (MO); Golfo Dulce, Reserva Forestal Golfo Dulce Aguabuena, sector sur, 08°42'00"N, 83°31'12"W, 50 m, 15 Jan 1992, fl., R. Aguilar 818 (MO). **FRENCH GUIANA.** Javanes de Mana, 1855, fl., Gusllet s.n. (P); s.loc., 15 Dec 1956, fl., fr., A. Lemée 11 (P); s.loc., 1819-1821, fr., M. Poiteau s.n. (G); s.loc., 1821, fl., G.S. Perrottet s.n. (P); s.loc., 1845, fl., fr., M. Melinón 64 (P); s.loc., 1856, fl., s.inf. s.n. (P); s.loc., 8 May 1874, fl., M. Melinón

121 (P); s.loc., Jan 1900, fl., F. Geay 1861 (P). **Cayenne:** Camopi, Camopi River, env. 12 km en amout de Camopi, 16 Dec 1965, fl., R.A.A. Oldeman 1796 (P, MO); Mahury River, Crique Gabrielle, tributary of the Mahury River, across from Stoupan, 4º45'00.0"N, 52º18'36.0"W, 10 m, 18 Oct 1991, fl., S.A. Mori 22137 (MO, NY); Mana River, Awara, village Galibi sur la reiver S de l'estucire de la Mana, env. à 18 km de Mana, 26 Jan 1978, fl., A. Raynal-Roques 19920 (MO, P); Maroni River, 1861, fl., M. Melinón 201 (P); *Ibid.*, 1982, fl., M. Melinón 205 (K, P); Montagne de Kaw, Montagnes de Kaw, Auberge de Brousse des Cascades, savanna and forest edges at end of road, 4º34'48.0"N, 52º16'48.0"W, 140 m, 12 Sept 1987, fl., A. Weitzman 287 (MO); Rives de l'Oyapock, entre St George at Maripa, Mar 1968, fr., R.A.A. Oldeman B-1449 (MO); Rivière Counana, affluent de l'Orapu, Dégrad Counana, 23 Dec 1966, fr., R.A.A. Oldeman B-778 (P). **Regina:** pont sur la crique Kourouaie, RN2, 04°06'53"N, 52°03'37"W, 4 m, 21 Mar 2009, fl., O. Tostain 2664 (P, NY, US). **GUATEMALA. Izabal:** Dartmouth, between Dartmouth and Morales toward Lago Izabel, Montana del Mico, 15º30'36.0"N, 88º46'48.0"W, 35 m, 7 Apr 1940, fl., fr., J.A. Steyermark 39022 (F, MO). **Puerto Barrios:** Dept. Izabal, near Rio Pargueña, 38-40 km N of Puerto Ayacucho, 25 May 1939, fl., P.C. Standley 73082 (F). **GUYANA.** Beryen de L'orenoque, 1864, fl., R. Grosourdy 13 (P); s.loc., s.d. , fl., Senudeas s.n. (P). **British Guyana:** Madoony Creek, Jan 1889, fl., G.S. Jenman 20968 (K). **Cuyuni-Mazaruni:** Mazaruni Station, 29 Oct 1943, fl., Fanshawe 4155 (K). **Demerara Superior-Berbice:** Moraballi Creek near Bartica, Essequibo River, 15 Nov 1929, fl., N.Y. Sandwith 617 (K, MO). **East Berbice:** Mazaruni River, low creeper on wall promenante by river, 28 Aug 1937, fl., N.Y. Sandwith 1226 (K); Pomeroon river, Mora Island, Wakapoa, 27 Dec 1958, fl., V. Graham P232 (K). **HONDURAS. Atlantida:** Esparta, 41.5 km E of Tela on the Tela-Ceiba Hwy then ca. 6 km N along old timber road. In remaining patches of primary forest, 15°39'N, 87°16'W, 100 m, 24 Apr 1994, fl., fr., A.E. Brant 2917 (MO). **Colon:** 1.8 mi strip on the north bank of rio Guaimoreto between old bridge and opening of Laguna Guaimoreto 4.5 NE of Trujillo on old road to Castilla, 15°57'30"N, 85°54'30"W, 0 m, 10 July 1980, fl., J.G. Saunders 453 (MO); Trujillo, 1.8.mi strip on the north bank of rio Guaimoreto between old bridge and opening of Laguna Guaimoreto 4.5 mim, NE of Trijillo on old road to Castilla, 15°57'N, 85°54'W, 0 m, 19 June 1980, fl., J.

Saunders 397 (MO). **NICARAGUA. Atlantico Sur:** Rio Pijibaye, 11°27'N, 83°54'W, 10- 20 m, 18 Feb 1995, fl., fr., R. Rueda 3216 (MO). **San Juan del Norte:** Reserva Indio-Maíz, entre San Juan del Norte y la Finca de Chepelión, Rio San Juan, 50 m, 8 July 2002, fl., R. Rueda 16901 (MO). **PANAMA. Barro Colorado Island:** Canal Zone, shore east of laboratory, 3 Feb 1932, fl., R.H. Woodworth 363 (A,F, US). **Barro do Colorado Island:** Canal Zone, shore line N of Smithsonian Laboratory Habour towards Salud Point, 9. 16°, menos 79. 84°, 28 Feb 1964, fl., F. Ehrendorfer 6400-22 (WU); Shoreline, 24 Jan 1968, fl., fr., J.D. Dwyer 8450 (F). **Bocas del Toro:** Water Valley, vicinity of Chiriqui Lagoon, 23 Nov 1940, fl., H. von Wedel 1754 (MO). **Colon:** Chagres, Isthmus of Panama, 26 Mar 1850, fl., A. Fendler 206 (K); Portobello, ridge top, 1-3 miles W of Portobello, 7 Sept 1971, fl., A.H. Gentry 1766 (MO); premontane wet forest along Road S1 as it climbis the hill 1 Km SE of Camp Pina, 6 km WNW of Gatun Dam, 125 m, 21 Dec 1973, fl., M. Nee 8948 (MO); Western most part of province, site of proposed copper mine (INMET), Tailings Area, lowland forest on steep slopes, 8°53'50"N, 80°39'44"W, 40 m, 15 Apr 2009, fl., G. McPherson 20983 (MO). **Darien:** Rio Cupe, Rio Tuira between Boca de Cupe and mouth of Rio Pucro, 7º54'N, 77º30'W, 0 m, 12 Jan 1975, fl., A.H. Gentry 13528 (MO); Yavisa, Rio Chucunaque, 0–1 hour above Yaviza, near sea level, 8º10'48.0"N, 77º40'48.0"W, 0 m, 8 Jan 1975, fl., A.H. Gentry 13477 (MO). **Panama:** Barro Colorado Island, Canal Area, cove north of dock, 2 July 1970, fr., T.B. Croat 11085 (MO); Canal Area, Barro Colorado Island, tip of Pearson Trail Peninsula S & W to 3rd large cove, 09°10'07"N, 79°51'31"W, 0–5 m, 7 May 1968, fr., T.B. Croat 5406 (MO). **PERU. Amazonas:** Condorcanqui, Monte virgin, 1 km atras de la comunidad de Caterpiza, trocha de metayar, banda este de la Quebrada Caterpiza, Rio Santiago, 3º54'36.0"S, 77º42'00.0"W, 180 m, 30 Oct 1979, fl., V. Huashikat 1145 (MO). **Huanuco:** Pachitea, region of Pucallpa, western part of the Sira mountains and adjacent lowland, c 26 km of Puerto Inca, next to the junction of the Rio Pachitea and Rio Yuyapichis, biological field station Panguana, primary lowland rain forest with some xer, 9º36'36.0"S, 74º55'48.0"W, 260 m, 21 Sept 1988, fl., fr., W. Morawetz 11-21988 (MO). **Loreto:** Boca del Rio Itaya, above Iquitos, 110 m, 17 Sept 1929, fl., E.P. Killip 29401 (F); Indiana, trail from Indiana on Rio Amazonas to Rio Napo, well drained upland forest on clay, 3º27'36.0"S, 73º00'00.0"W, 200 m, s.d. , fl., A.H.

Gentry 22205 (MO); Iquitos, Carretera de Picuruyacu, en terreno arenoso, 3º44’24.0’’S, 73º14’24.0’’W, 160 m, 23 Sept 1981, fl., Y.M. Rimachi 5716 (MO); Maynas, explorer's inn tourist camp near Indiana on Rio Amazonas, seasonally inundated tahuampa forest, 3º30'S, 73º00'W, 120 m, 21 Feb 1988, fl., A.H. Gentry 61828 (MO); Maynas, Mishana, (Rio Nanay), bosque secundario de mas de 20 años, 03°55'S, 73°35'W, 130 m, 25 July 1984, fl., R. Vásquez 5404 (MO); Maynas, Pto. Almendras (Rio Nanay), bosque inundable estacional (tahuampa), 3º48'00.0"S, 73º24'36.0'W, 122 m, 7 Sept 1984, fl., R. Vásquez 5540 (MO); Requena, Sanangal, bosque secundario inundable (Tahuampa), 04°10'S , 73°20'W, 120 m, 8 Aug 1980, fl., R. Vásquez 347 (MO). **Madre de Dios:** Laguna Cocacocha, edge of Laguna Cocacocha 39 km SW of Pto Moldanado near confl of Rios La Torre & Tambopata, 17 Oct 1968, fl., fr., S.F. Smith 408 (MO, US); Manu, Puerto Maldonado, Los Amigos Biological Station, ca. 7km upriver from mouth of Rio Los Amigos, between Cocha Llena and Cocha Lobo, 12º34'12.0"S, 70º06'00.0"W, 270 m, 4 Nov 2001, fr., J.P. Janovec 2606 (SPF); Tambopata, Cusco Amazonico, 15 km ENE of Puerto Maldonado, 12º24'48.0"S, 69º04'48.0"W, 200 m, 17 Dec 1989, fr., A.H. Gentry 68887 (MO); Tambopata, explorer's inn tourist camp at junction of Rios La Torre and Tambopata, swampy forest, 12º48'36.0"S, 69º42'36.0"W, 270 m, 28 July 1985, fl., A.H. Gentry 51536 (MO). **SURINAME. Île Portal:** 1888, fl., P. Sagot s.n. (P). **Sipiwalini:** Voltzberg Nature Reserve, Coppename River, 1-2 Km north of Foengoe Island, 4°44'N, 56°11'W, 40 m, 21 Feb 1999, fl., B. Hoffman 5362 (MO). **VENEZUELA.** Des bords de l'Orinoque, 27 Sept 1886, fl., M. Chaffanjon 336 (P); lower Orinoco, s.d., fl., H.H. Rurby s.n. (NY). **Amazonas:** Boca Casiquiare, selvas pluviales en y los alrededores de la orilla del Rio Casiquiare, entre la boca y Isla de la Paloma, 18 Feb 1986, fl., B. Stergios 9001 (MO); Carinagua, Dept Atures, alrededores de Puerto Ayacucho (ca. 9 Km al S), bosque de galeria del Caño Carinagua, alrededor del puente de la carretera Pto. Ayacucho-Samariapo, 10 Jan 1978, fl., O. Huber 1406 (MO); Dept. Átures, Puerto Ayacucho, end of road from airport to Rio Orinoco, gallery forest along river, 4 Apr 1984, fl., T. Plowman 13473 (F); Puerto Ayacucho, seasonally inundated forest at edge of Raudales del Orinoco, behind Pto. Ayacucho airport, sandy beach and adjacent laja, 5º39'36.0"N, 67º39'36.0"W, 100 m, 3 Apr 1984, fl., A.H. Gentry 46267 (MO); Rio Casiquiare, entre Piedra Guachapita y Curimacare, 2º00'00.0"N, 66º19'48.0"W, 150 m, 16 Jan

1987, fl., B. Stergios 9778 (MO); Rio Orinoco, caño Morocoto below San Fernando de Atabapo, 03°40'41"N, 67°14'15"W, 26 Mar 1974, fl., A.H. Gentry 10943 (MO). Apure: La Ceiba, between Rio Borgue and El Jordan, 7 km E de la Ceiba, 16 km E del Jordan, 350 m, 6 Apr 1968, fl., J.A. Steyermark 101948 (K); locally frequent along Rio Cinaruco for 20 km above las Galeras de Cinaruco, 24 Jan 1956, fl., J.J. Wurdack s.n. (RB); San Fernando, mouth of Rio Arauca at Rio Orinoco, 7º24'N, 66º36'W, 35 m, 14 May 1977, fl., G. Davidse 13198 (MO). **Bolivar:** Dpto. de Atures, Territorio Federal Amazonas, bosque humedo del rio Cataniapo, cercano a la desembocadura con el rio Orinoco, 6º24'36.0"N, 67º24'36.0"W, 37 m, 15 Feb 1983, fl., A. Castillo 1604 (MO); Moitaco, Distrito Sucre, rebalse del Orinoco, Hato Curumutopo, 11 Sept 1963, fl., fr., G. Martino 18 (MO, NY); Rio Orinoco, frequent on rocky outcrops on Isla Sta. Elena, opposite mouth of Rio Pargueni, 80 m, 13 Dec 1955, fl., J.J. Wurdack 39860 (K); Rio Parhueña, 38-40 km N of Puerto Ayacucho, 6º21'00.0"N, 67º09'36.0"W, 100 m, 30 June 1975, fr., A.H. Gentry 14686 (US, MO). **Delta Amacuro:** Depto. Antonio Diaz, Cano Atoiba, 9º15'N, 60º57'W, 50 m, 19 Oct 1977, fl., J.A. Steyermark 114985 (MO); Depto. Antonio Diaz, Cano Joba-Suburu, 8°59'N, 61°00'W, 50 m, 21 Oct 1977, fl., fr., J.A. Steyermark 115147 (MO); Rio Amacuro, between Amacuro and mouth of Deadwater Creak Moat, 8º31'12.0"N, 60º28'12.0"W, 65 m, 7 Nov 1960, fr., J.A. Steyermark 87341 (MO); Rio Amacuro, between Amacuro and mouth of Deadwater Creek Moat, 8º31'12.0"N, 60º28'12.0"W, 65 m, 7 Nov 1960, fl., J.A. Steyermark 87347 (MO).

**5.** ***Pachyptera linearis*** J.N.C Francisco & L.G. Lohmann, *sp. nov.* Type: Colombia. Meta: Parque Nacional Natural Tinigua, Serrania Chamusa Centro de Investigación Primatologicas La Macarena, 120 m, Apr 1992, fr., P. Stevenson 403 (holotype: MO-088944!; isotype, COL-000349706!, COAH-21210, not seen).

Fig. 12

**Diagnosis.** *Pachyptera linearis* is similar to *Pachyptera kerere*, but can be distinguished by the **linear and flattened capsule (vs. the** fusiform and inflated capsule of *P. kerere*)**,** inconspicuous longitudinal midline **on each valve (vs.** conspicuous and raised longitudinal midline on each valve of *P. kerere*)**, and thin,**

**oblong and wingless seeds** (vs. corky, irregularly circular, obcordate and wingless seeds in *P. kerere*).

**Description.** *Liana*; stems solid, cylindrical to tetragonal, with lighter striations, moderately lenticeled, sparsely to moderately puberulous; prophylls of axillary buds flattened and ensiform, 3-seriated, moderatly to densely puberulous. *Leaves* 3-foliolated or 2-foliolated with the terminal leafltes replaced by a trifid tendril; petiole semi-cylindrical, 1.0–4.2 cm long, moderately puberulous, petiolules not puvinated, lateral petiolules 0.5–1.6 cm long, apical petiolules 1.5–3.5 cm long; blades discolor or concolor, membranaceous or chartaceous, elliptic, obovate-lanceolate, asymmetric, apex acute, acuminate, or mucronulate, base cordate, lateral blades 6.0–12.4 × 3.0–4.9 cm, apical blades 8.0–14.7 × 3.4–6.0 cm, densely puberulous abaxially, sparsely to moderately puberulous adaxially. *Inflorescence* a congested raceme, 0.6–1.2 cm long; pedicel ca. 0.8 cm long, moderately puberulous; bracts ca. 0.7 mm long; bracteoles cymbiform, ca. 0.72 mm. *Calyx* green, tubular, minutely 5-lobed, 0.9 × 0.8 cm, moderately to densely puberulous, with simple and dentritic trichomes, with clusters of patelliform glands, sometimes arranged in lines, next to margin. *Corolla* white, infundibuliform, ca. 6.7 cm long, ca. 1.4 cm of diameter at the tube mouth, tube moderately to densely puberulous externally; lobes rounded, 0.8 × 0.9 cm. *Androecium* with the longer stamens ca. 32.0 mm long, the shorter stamens ca. 10.9 mm long, glabrous; anthers villose, included, with thecae curved forward, 2.5 × 0.9 mm; pollen 3-colpate, microrreticulate. *Gynoecium* 5.0–5.2 cm long; stigma capitate or ovate, 1.7–2.8 × 1.7–2.8 mm; ovary 2.9–4.0 × 1.2 mm, cylindrical, not-sulcate, smooth, sparsely to moderately pubescent, with simple trichomes, sparsely lepidote, with glandular peltate trichomes, without patelliform glandular trichomes; nectar disc 1.8 × 2.0 mm. *Capsule* linear, flattened, 19.0–35.0 × 2.2–2.4 cm, each valve with an inconspicuous longitudinal midline; seeds oblong, 4.0–7.0 × 1.5–1.8 cm, thin, not-corky, coriaceous to woody, striated, secondary sculpture regularly interrupted by lateral rays, wingless, with short membranaceous or chartaceous and hyaline wings.

**Distribution and habitat.** *Pachyptera linearis* is only known from wet forests of Venezuela (Apure, Bolivar) and Colombia (Meta). Fig. 13.

**Phenology.** *Pachyptera linearis* flowers in January and fruits in April.

**Etymology.** The epithet *linearis* makes reference to the linear fruit.

**Nomenclatural notes.** *Pachyptera linearis* is a new species described here based on new morphological and molecular phylogenetic data (Francisco and Lohmann submitted). The best quality material was selected as the holotype of this species.

**Taxonomic comments.** *Pachyptera linearis* is characterized by the linear and flattened capsule (19.0–35.0 × 2.2–2.4 cm), coriaceous to woody, with an inconspicuous longitudinal midline on the valves. The seeds are oblong (4.0–7.0 × 1.5–1.8 cm), thin, coriaceous to woody, smooth, glabrous, winged, with short membranaceous or coriaceous and hyaline wings. The corolla is white and infundibuliform, with sparsely puberulous and sparsely lepidote ovary. *Pachyptera linearis* shares the white infundibuliform flowers with its sister species *P. kerere* (Francisco and Lohmann submitted). Nevertheless, *P. linearis* is distinguible from *P. kerere* by a series of fruit traits (see taxonomic comments under *P. kerere*). Seed surface has provided excellent information for the systematics of various plant groups (Barthlott 1981). In *Pachyptera*, the seed coat is useful to separate species. The seed surface of *Pachyptera* species are striated with a distinctive secondary sculpture in each species (except in *P. erythraea* that is unknown). More specifically, the seed surface of *P. aromatica* is striated and smooth, while the seed surface of *P. incarnata* is striated with randomly distributed micropores, and the seed surface of *P. kerere* is striated with two pairs of medium micropores on each striation. In *P. linearis*, seed surface is striated with the striations being regularly interrupted by lateral rays (Figs. 3B, D, F, H).

**Paratypes. COLOMBIA. Meta:** Parque Nacional Natural Tinigua, Serrania Chamusa Centro de Invest, Primatologicas La Macarena, 120 m, Apr 1992, fr., P. Stevenson 403 (MO). **VENEZUELA. Apure**: galeras del Cinaruco, Rio Cinaruco, 29 km above Las Galeras de Cinaruco, 80 m, 24 Jan 1956, fl., J.J. Wurdack 41357 (K, MO, RB, S, VEN). **Bolivar:** Moitaco, rebalse del Orinoco, Hato Curumutopo, 8º00’00.0”N, 64º21’36.0”W, 24 Apr 1991, fr., G. Martino 22 (MO).

**Doubtful and excluded names**

*Bignonia incarnata* var. *caribaea* DC., Prodr. 9: 154. 1845. Type: Guadeloupe. s.loc., s.d., fl., F.L. L'Herminier s.n. (holotype, G-DC [G-133268]!) = *Bignonia aequinoctialis* L.

*Pachyptera alliacea* (Lam.) A.H. Gentry Brittonia 25(1): 236. 1973. Type: French Guiana. s.loc., s.d., fl., J.B.C.F. Aublet s.n. (holotype, P-AD [P-307351]!) = *Mansoa alliacea* (Lam.) A.H. Gentry

*Pachyptera dasyantha* DC. Prodr. 9: 176. 1845. Type: Brazil. Bahia: Rio São Francisco, s.d., fl., J.S. Blanchet 2903 (holotype, G-DC [G-133367]!, K not seen) = *Tanaecium pyramidatum* (Rich.) L.G. Lohmann

*Pachyptera hymenaea* (DC.) A.H. Gentry Brittonia 25(3): 236. 1973. Type: Brazil. Bahia, s.d., fl., J.S. Blanchet 1434 (holotype, G-DC [G-133196]!; isotype, P-481498!) = *Mansoa hymanaea* (DC.) A.H. Gentry

*Pachyptera parvifolia* A.H. Gentry Phytologia 26(6):447–450. 1973. Type: Colombia, Sur de Santander, vicinity of Puerto BerRío between carare and Magdalena Rivers, raizudo, large liana, flowers light purple, forest at about 200m, 22 Apr 1937, fl., O. Haught 2179 (holotype, MO-100091, not seen) = *Mansoa parvifolia* (A.H.Gentry) A.H. Gentry

*Pachyptera perrottetii* DC. Prodr. 9: 176. 1845. Type: French Guiana, s.loc., s.d., fl., G.S. Perrottet 2851 (holotype, G-DC [G-133301]!) = *Tanaecium pyramidatum* (Rich.) L.G. Lohmann

*Pachyptera puberula* DC. Prodr. 9: 175. 1845. Type: Brazil: Mato Grosso: close to Cuiabá, s.d., fr., S. Manso 105a (holotype, G-DC [G-133299]!) = *Dolichandra uncata* (Adrews) L.G. Lohmann

*Pachyptera standleyi* (Steyerm.) A.H. Gentry Brittonia 25(3): 236.1973. Type: Guatemala, Quetzaltenango, between Finca Pirineos and Finca Soledad, lower southern slopes of Volcán de Santa María, between Santa María de Jesús and Calahuaché, 1300-1400 m, 5 Jan 1940, J.A. Steyermark 33533 (holotype, F-1054546!; isotype, F-1054531!, F-1054543! , US-00125753!) = *Mansoa standleyi* (Steyerm.) A.H. Gentry

*Pachyptera striata* DC. Prodr. 9: 176. 1845. Type: Brazil, São Paulo, s.d., P.W. Lund 783 (holotype, G-DC [G-133363]!) = *Tanaecium pyramidatum* (Rich.) L.G. Lohmann

*Pachyptera umbelliformis* DC. Prodr. 9: 175. 1845. Type: Brazil, São Paulo, s.d., fl., C.F.P. von Martius (syntype, M not seen; isosyntype, G-DC [G-133346]!), Brazil, Rio da Paraiba, Neuwied (syntype, M not seen) = *Tanaecium pyramidatum* (Rich.) L.G. Lohmann

*Pachyptera ventricosa* (A.H. Gentry) L.G. Lohmann Ann. Missouri Bot. Gard. 99(3): 456. 2014. Type: Brazil, Pará: along Belém-Brasilia hwy., Km 345, 9 Aug 1963, fl., B. Maguire et al. 56083 (holotype, MO-2232816!; isotypes, COL-110166 not seen, MG-136673!, NY-328882!, US-3189002!) = *Mansoa ventricosa* A.H. Gentry

**Acknowledgements**

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for a graduate fellowships to J.N.F. (163990/2014-0) and a Pq-1C grant to L.G.L. (307781/2013-5), and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for a regular research grant (2011/50859-2) and a collaborative FAPESP-NSF-NASA grant (2012/50260-6) to L.G.L. We are grateful for the curators of the herbaria listed herein for specimen loans; Klei Souza for preparing the illustrations; and, Annelise F. Nunez, Beatriz M. Gomes, Erik Kataoka, and Ricardo S. Ribeiro for providing us with beautiful photos.

**References**

Aublet JBCF (1775) Histoire des plantes de la Guiane Française. Pierre-François Didot. Paris, 2: 1−4563.

Baillon HE (1891) Histoire des Plantes. Libraire Hachette & Co, 1−112.

Barbosa Rodrigues J. (1891) Eclogae plantarum novarum. Vellosia, Rio de Janeiro, 1: 1−133.

Bureau E, Schumann KM (1896 [1897]) Bignoniaceae. In: von Martius CFP, Eichler AG, Urban I (Eds.) Flora Brasiliensis. Lipsiae apud Frid. Fleischer in Comm. Monachii, Leipzig, 8 (2): 1−452.

Candolle AP (1845) Bignoniaceae. In: A.P. de Candolle (Eds). Prodromus systematis naturalis regni vegetabilis. Fortin, Masson. Paris, 9: 142−248.

Cracraft J (1983) Species concepts and speciation analysis. Current Ornithology 1: 159−187.

Dugand A (1955) Bignoniaceas nuevas o notables de Colombia. Caldasia 7: 7−32.

Gentry AH (2009) Flora de Colombia No. 25: Bignoniaceae. Bogotá, DC: Universidad Nacional de Colombia, 1−462.

Funk DJ, Omland KE (2003) Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology, Evolution, and Systematics 34: 397−423.

Gentry AH (1973) Generic delimitations of Central American Bignoniaceae. Brittonia 25: 226−242.

Gentry AH (1974) Coevolutionary patterns in Central American Bignoniaceae. Annals of the Missouri Botanical Garden 61: 728−759.

Gentry AH (1977) Studies in Bignoniaceae: New taxa and combinations in northwestern South American Bignoniaceae. Phytologia 35: 183−198.

Gentry AH (1979) Additional generic mergers in Bignoniaceae. Annals of the Missouri Botanical Garden 66: 778−787.

Gentry AH (1980) Bignoniaceae. Part I (tribes Crescentieae and Tourretieae). Flora Neotropical. Monografy 25: 1−131.

Gentry AH, Tomb AS (1979) Taxonomic implications of Bignoniaceae palynology. Annals of the Missouri Botanical Garden 66: 756−855.

Hesse M, Halbritter H, Zetter R, Weber M, Buchner R, Frosch-Radivo A, Ulrich S. (2009) Pollen terminology: An illustrated handbook. SpringerWein, 1−264.

Hickey LJ (1973). Classification of the architecture of dicotyledonous leaves. American Journal of Botany 60: 17−33.

Lohmann LG (2006) Untangling the phylogeny of Neotropical lianas (Bignonieae, Bignoniaceae). American Journal of Botany 93: 304−318.

Lohmann LG, Taylor, CM (2014) A new generic classification of Tribe Bignonieae (Bignoniaceae). Annals of the Missouri Botanical Garden 99: 348−489.

McNeill J, Barrie F R., Buck WR., Demoulin V, Greuter W, Hawksworth DL, Prud'homme Van Reine WF (2012) International Code of Nomenclature for algae, fungi and plants. Regnum vegetabile 1−154.

Mori SA,Ferreira FC (1987). A distinguished Brazilian botanist, João Barbosa Rodrigues (1842–1909). Brittonia 39: 73−85.

Nogueira A, El-Ottra JHL, Guimarães E, Machado SR, Lohmann LG (2013) Trichome structure and evolution in Neotropical lianas. Annals of Botany 112: 1331−1350.

QGIS Development Team (2016) QGIS Geographic Information System. Open Source Geospatial Foundation Project.

De Queiroz K (2007) Species concepts and species delimitation. Systematic Biology 56: 879−886.

Radford AE, Dickison WC, Massey JR, Bell C.R. (1974) Vascular plant systematics. Harper Collins,1−891.

Reginato M. (2016) MonographaR: An R package to facilitate the production of plant taxonomic monographs. Brittonia 68: 212−216.

Sprague TA. Sandwith NY (1932) Contributions to the flora of tropical America: X. Bulletin of Miscellaneous Information (Royal Botanic Gardens, Kew) 2: 81−93.

Sandwith NY (1937) Notes on tropical American Bignoniaceae. Mededelingen van het Botanisch Museum en Herbarium van de Rijksuniversiteit te Utrecht 40: 205–232.

Weberling F (1992) Morphology of flowers and inflorescences. Cambridge University Press, 1−344.

**APPENDIX 1.** Vouchers used on the scanning electron microscopy study.

*Pachyptera aromatica* (Barb. Rodr.) L.G. Lohmann: BRAZIL. Amazonas: 2-5 km N of Manaus-Itacoatiara, road at km 79 near Río Preto da Eva, 24 Nov 1974, fl., A.H. Gentry 12832 (MO); Iranduba, estrada entre Novo Airão e Manacapurú, 2°54 46.6' S, 60°57'58.8"W, fl., L.H. Fonseca 327 (SPF); Manaus, outskirts of Manaus, road to INPA, boat landing, behind airport, 26 Nov 1974, fr., A.H. Gentry 12862 (MG).

*Pachyptera erythraea* (Dugand) A.H. Gentry: COLOMBIA. Antioquia: Caucasia, along road to Nechi 24 km from Caucasia-Planeta Rica road, hacienda Costarica, margin of primary forest and trees remaining in cleared pasture, 8.06, -75.08, 60 m, 21 Mar 1987, fl., J.L. Zarucchi 4887 (K).

*Pachyptera incarnata* (Aubl.) J.N.C. Francisco & L.G. Lohmann: BRAZIL. Amazonas: Presidente Figueiredo, Balbina, Rebio Uatumã, grade do PPBio, 6 Oct 2006, fr., J.R. Carvalho-Sobrinho 1078 (INPA). Pará: Belterra, Floresta Nacional do Tapajós, estrada para comunidade de Jamaraguá, km 72, 02°55'15.9"S, 55°01'39.4"W, 114 m, 16 Sept 2015, fl., J.N.C. Francisco 89 (SPF); Óbidos, lago Maria Teresa, floresta de várzea, 01°52'38.2"S, 55°35'27.4"W, 14 m, 23 Sept 2015, fr., J.N.C. Francisco 122 (SPF); Santarém, ramal próximo à Usina Hidrelétrica Curuá-Uma, solo areno argiloso, floresta de terra firme, 02°48'45.2"S, 54°18'08.8"W, 47 m, 19 Sept 2015, fl., J.N.C. Francisco 105 (SPF).

*Pachyptera kerere* (Aubl.) Sandwith: BRAZIL. Roraima: Caracaraí, Parque Nacional do Viruá, margem do Rio Branco, igapó, 01°40'29.0"N, 61°11'24.6"W, 42 m, 26 Sept 2014, fl., J.N.C. Francisco 41 (SPF); Caracaraí, Parque Nacional do Viruá, próximo da sede do Parque, floresta de terra firme, 01°29'23.3"N, 61°00'09.1"W, 68 m, 24 Sept 2014, fl., J.N.C. Francisco 40 (SPF); Rorainópolis, Rio Branco, Ponto 11, 0°56'24.0"S, 61°50'24.0"W, 22 m, 14 May 2015, fl., fr., A. Frazão 136 (SPF).

*Pachyptera linearis* J.N.C Francisco & L.G. Lohmann *new sp.*: VENEZUELA. Apure: galeras del Cinaruco, Rio Cinaruco, 29 km above Las Galeras de Cinaruco, 80 m, 24 Jan 1956, fl., J.J. Wurdack 41357 (K). Bolivar: Moitaco, rebalse del Orinoco, Hato Curumutopo, 8º00’00.0”N, 64º21’36.0”W, 24 Apr 1991, fr., G. Martino 22 (MO).

**APPENDIX 2.** Index to Numbered Collections

Specimens are listed by collector in alphabetical order, followed by collector's number. Collections by anonymous collectors without date or other identifying features are not listed here. Type specimens are in bold.

Aguilar, R. 818 (kerere); 4824 (kerere).
Almeida, J.C. INPA137, INPA1540, INPA1687, INPA5722 (aromatica); INPA606, INPA6383 (kerere).
Amaral, I.L. 1248 (incarnata).
Andrade, A.G. 855 (kerere).
Árbocz, G.F. 4256 (incarnata).
Aruz, L. 1035 (kerere).
**Aublet, J.B.C.F. s.n. (BM-992379)(kerere)**
Beck, H.T. 178 (kerere).
Beyer, M. 324, 332, 336, 337 (kerere).
Black, G.A. 47-1861, 54-16283 (kerere); 48-3355 (incarnata); 52-14674 (aromatica).
Braga, M.A. 77 (kerere).
Brant, A.E. 2917 (kerere).
Campbell, D.G. P22008 (incarnata).
Campbell, E.J.F 95 (kerere).
Carvalho-Sobrinho, J.R. 1078 (incarnata).
Castaño, L. 93a (kerere).
Castillo, A. 1604 (kerere).
Chaffanjon, M. 336 (kerere).
Chagas, J.C. 606 (kerere); INPA1701 (aromatica).
Coêlho, L.F. INPA1731 (aromatica); s.n.(kerere).
Conceição, P.N. 5 (kerere).
Croat, T.B. 5406, 11085 (kerere).
Cuadros, H.V. 3601 (kerere).
Daly, D.C. D264 (kerere).
Dardano 48-3092 (incarnata).
Davidse, G. 13198, 32046 (kerere).

Delprete, P.G. 7688 (kerere).

Duarte, A.P. 7048 (aromatica).

Ducke, A.W. 239, 22698a, 22698b, 35624, s.n. (R), s.n. (MO) (aromatica); s.n. (K, MO), MG6821, (kerere); 24091 (incarnata).

Dwyer, J.D. 8450, 12737 (kerere).

Ehrendorfer, F. 6400-22 (kerere).

Evando 542 (incarnata).

Fanshawe 4155 (kerere).

Feddema, C. 1954 (kerere).

Fendler, A. 206 (kerere).

Fonseca, L.H. 327 (aromatica).

Forzza, R.C. 5994, 8094, 8113 (kerere).

Francisco, C.M. s.n. (MG)(aromatica)

Francisco, J.N.C. 39, 40, 41 (kerere); 89, 103, 105, 121, 122, 130, 151 (incarnata).

Frazão, A. 136, 149, 153 (kerere); 313 (aromatica).

Fróes, R.L. 27540 (kerere).

Geay, F. 1861 (kerere).

Gentry, A.H. 1766, 9313, 10943, 11191, 13075, 13477, 13528, 14686, 22205, 23990, 46267, 51536, 61828, 64809, 68887 (kerere); 13027, 13323 (incarnata); 15369, 15372, 15402, 20050 (erythraea); 11201, 11207, 12778, 12815, 12832, 12862, 12888, 13022, 13056, 69107, 69308 (aromatica).

Gerolamo, C.S. 9 (aromatica).

Gomes, B.M. 639, 648, 651, 659, 662 (kerere).

Gottsberger, G. 16-23186, 115-19186 (kerere).

Goulding, M. 66a, 2120 (kerere).

Graham, V. P232 (kerere).

Grayum, M.H. 4411, 8032 (kerere).

Grosourdy, R. 13 (kerere).

Guedes, M. s.n. (MO)(kerere).

Gusllet s.n. (P)(kerere).

Herrera, G. 7551 (kerere).

Hoffman, B. 5362 (kerere).

Honda, M. INPA36004 (kerere).

Hopkins, M.J.G. 1570, 1543, 1574 (aromatica); 1961 (kerere).

Huashikat, V. 1145 (kerere).

Huber, O. 1406 (kerere).

Janovec, J.P. 2606 (kerere).

Jenman, G.S. 20968 (kerere).

Jobert, M. 857 (kerere).

Kataoka, E. 349 (aromatica).

Killip, E.P. 29401, 39122 (kerere).

Knowles, O.H. 1106, 1120 (incarnata).

Krukoff, B.A. 1517 (kerere); 6845, 12511 (aromatica).

Labroy, M. 1906 (aromatica).

Lemée, A. 11 (kerere).

Lobato, L.C.B. 2398 (kerere); 3870 (incarnata).

Lohmann, L.G. 28, 794 (aromatica); 836 (kerere).

Loureiro, A.A. s.n. (INPA), INPA37554 (kerere).

Luís, s.n. (MG)(aromatica).

Maciel, U.R. 1793, 1823, 1971 (kerere).

Maguire, B. s.n. (INPA), 56679 (aromatica).

Marinho, T. 208 (kerere).

Martinelli, G. 17395 (kerere).

**Martino, G. 18 (kerere); 22 (linearis).**

McPherson, G. 20983 (kerere).

Melinón, M. 64 (kerere); 121, 201, 205 (kerere).

Mello, F.C. s.n. (INPA)(aromatica).

Melo, F.F. 532 (kerere).

Miranda, F.E. 362 (incarnata); 576 (kerere).

Moraes, M. 550 (kerere).

Morawetz, W. 11-21988 (kerere).

Mori, S.A. 15783 (incarnata); 22137, 22470 (kerere).

Mota, C.D.A. 18, 26 (aromatica).

Nadruz, M. 2647 (kerere).

Nee, M.H. s.n. (INPA) (aromatica); 8948 (kerere).

Nogueira, A. 190 (aromatica).

Oldeman, R.A.A. B-778, B-1449, 1796 (kerere).

Oliveira, E. 1243 (kerere); 2790 (aromatica).

Pereira-Silva, G. 8765 (incarnata).

**Perrottet, G.S. s.n. (P-3578200)(kerere)**.

Pires, J.M. 12075 (incarnata).

Plowman, T. 13473, INPA126243 (kerere).

**Poiteau, M. s.n. (G-1405)(kerere).**

Prance, G.T. 1736, 4012, 6759, 14036, 15178 (kerere); 17773 (aromatica).

Pyramo, R.V. PSACF_EX06147 (incarnata).

Rabele, B. 1003 (aromatica).

Ramiro Fonnegra, G. 2580 (erythraea).

Ramos, J. 1011 (incarnata).

Raynal-Roques, A. 19920 (kerere).

Revilla, J. 8326, 8397 (incarnata); 8681 (kerere).

Ribeiro, R.S. 78 (incarnata).

Rimachi, Y.M. 5716 (kerere).

Robles, R. 1121, 1391 (kerere).

Rocha, J.B.P. 701 (incarnata).

Rodrigues, W.A. 4578, 4693, 5476 (aromatica).

**Romero-Castañeda, R. 4727**, 4979 (erythraea).

**Rusby, H.H. s.n. (NY-313053), 1143 (kerere).**

Rosario, C.S. s.n. (MBM)(kerere).

Rueda, R. 3216, 16901 (kerere).

Rurby, H.H. s.n. (NY),1143 (kerere).

Sales, J. 1539 (incarnata).

Sandwith, N.Y. 617; 1226 (kerere).

Santos, C.A. 31 (kerere).

Santos, G. 183, 226, 287 (kerere); 282 (incarnata).

Sagot, P. (P)(kerere).

Saunders, J.G. 397, 453 (kerere).

Senudeas, s.n. (P)(kerere).

Silva, B.R. PSACF_EX06201 (incarnata).

Silva, M. 1024 (aromatica); 1604 (incarnata).

Silva, M.F. 1092 (kerere).

Silva, N.T. 1654 (kerere).

Smith, S.F. 408 (kerere).

Soares, C.R.A. 3594 (kerere).

Solomon, J.C. 6349 (kerere).

Souza, J.A. s.n. (INPA), INPA61048, INPA61920, INPA71827, INPA71828, INPA71829, INPA71832, INPA71839 (aromatica).

Souza, V.C. 18583 (incarnata).

Sucre, A. s.n. (R, RB), (RB)(aromatica).

Standley, P.C. 73082 (kerere).

Stergios, B. 9001, 9778 (kerere).

**Stevens, W.D. 8082 (linearis).**

**Stevenson, P. 403 (linearis).**

Steyermark, J.A. 39022, 87341, 87347, 101948, 114985, 115147 (kerere).

Thode, V. 17, 424 (kerere).

Tostain, O. 2664 (kerere).

Tsugaru, S. B-690 (aromatica).

Udulutsch, R.G. 2708 (kerere).

Ule, U. 4217, 5217 (aromatica).

Vásquez, R. 347, 5404, 5540 (kerere).

Verônica 17 (kerere).

Vieira, C.M. 72 (kerere).

Wedel, H. von 1754 (kerere).

Weir, M. 72 (erythraea).

Weitzman, A. 287 (kerere).

Williams, L. 18222 (incarnata).

**Woodworth, R.H. 363 (kerere).**

Wurdack, J.J. s.n. (JBRJ, RB), 39860 (kerere); **41357 (linearis)**.

Zartman, C.E. 6333, 6349 (kerere).

Zarucchi, J.L. 4862A, 4887 (erythraea).

**FIGURE CAPTIONS**

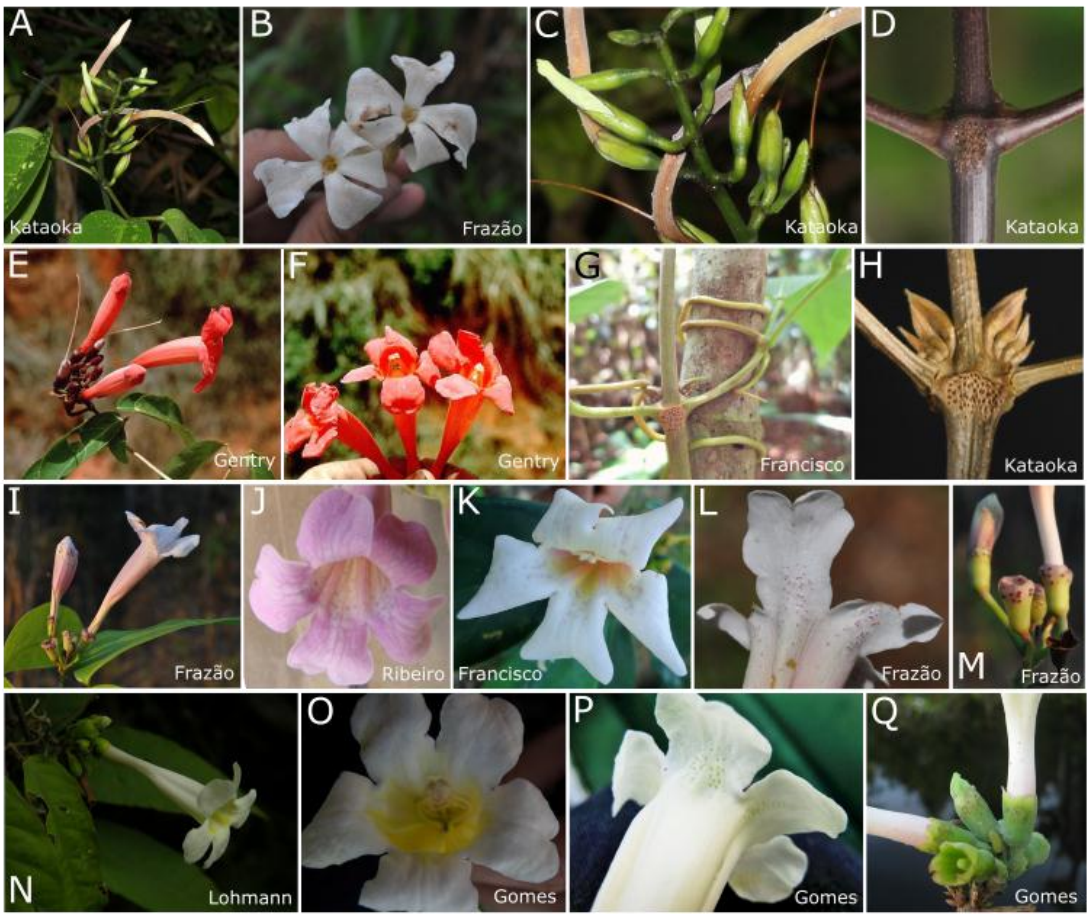


**Figure 1.** Sample of morphological features of *Pachyptera*. A-D. *Pachyptera aromatica*: A. Inflorescence; B. Frontal view of flowers; C. Detail of inflorescence and flowers, showing calyx partition; D. Interpetiolar region of stem with extra floral nectaries (NEF's) and prophylls of the axillary buds triangular, minute, and 3-seriated. E-F. *P. erythraea*: E. Inflorescence; F. Frontal view of flowers. G-M. *P. incarnata*: G. Stem with tendril surrounding a tree; H. Interpetiolar region of stem with NEF's and prophylls of the axillary buds flattened, ensiform and seriated; I. Inflorescence; J-K. Frontal view of corollas showing color variation within the species; L. Pink patteliform glands on flower lobes; M. Detail of calyx. N-Q. *P. kerere*: N. Inflorescence; O. Frontal view of flower; P. White patteliform glands on flower lobes; Q. Detail of calyx.

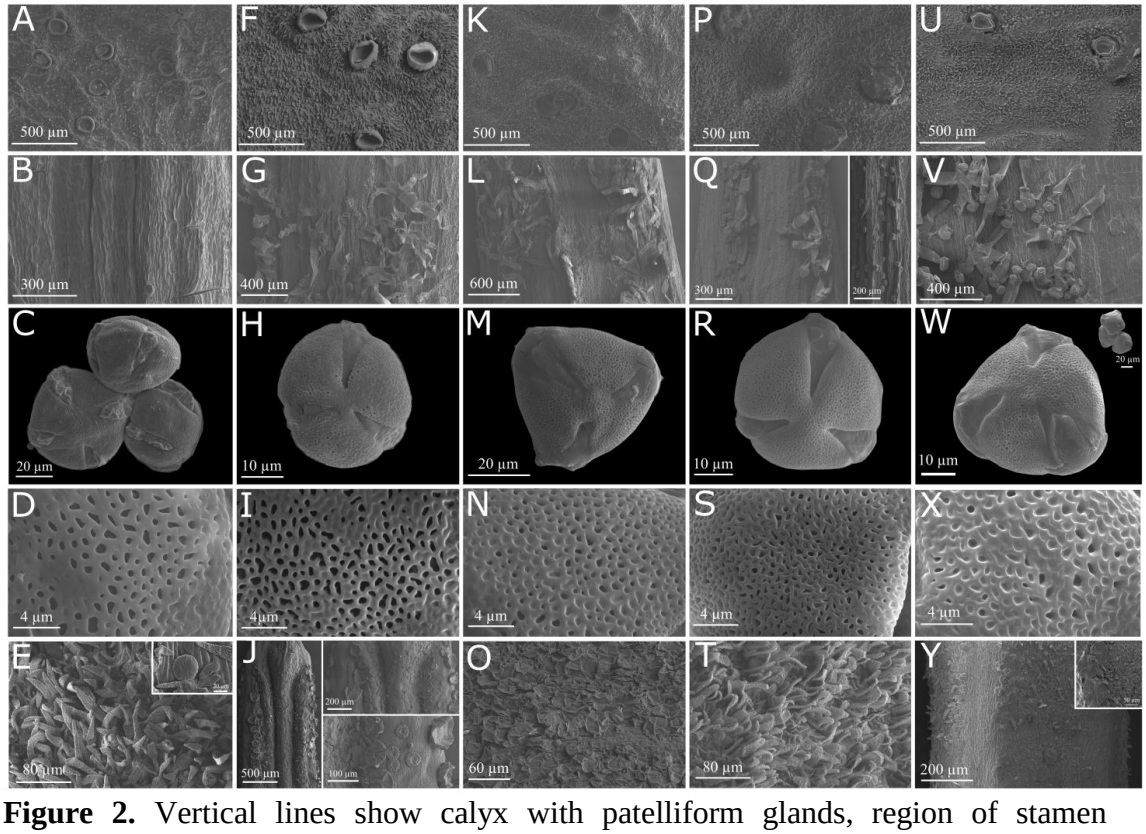


**Figure 2.** Vertical lines show calyx with patelliform glands, region of stamen insertion, pollen grains, detail of pollen exine, and ovary surface variation in all species of *Pachyptera*, respectively. A-E. *P. aromatica*; F-J. *P. erythraea*; K-O. *P. incarnata*; P-T. *P. kerere*; U-Y. *P. linearis*.

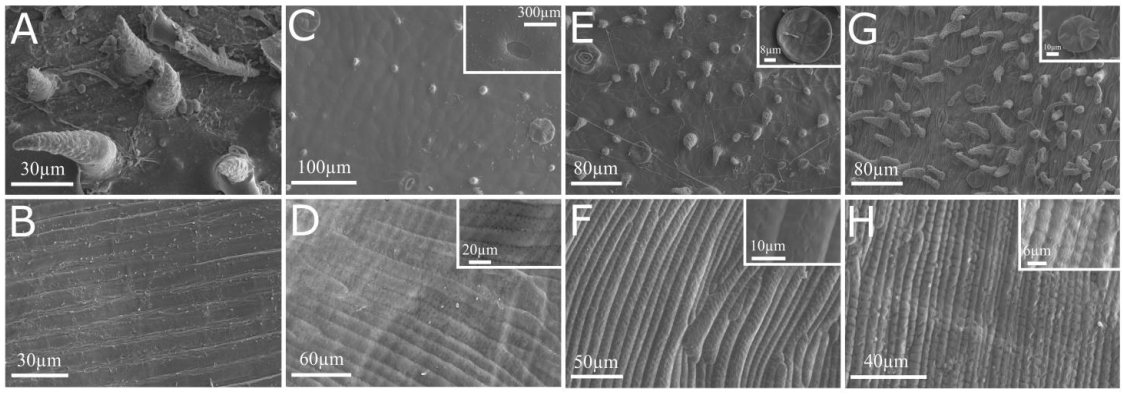


**Figure 3.** Vertical lines show fruit and seed surface, respectively. A-B. *P. aromatica*; C-D. *P. incarnata*; E-F. *P. kerere*; G-H. *P. linearis*.

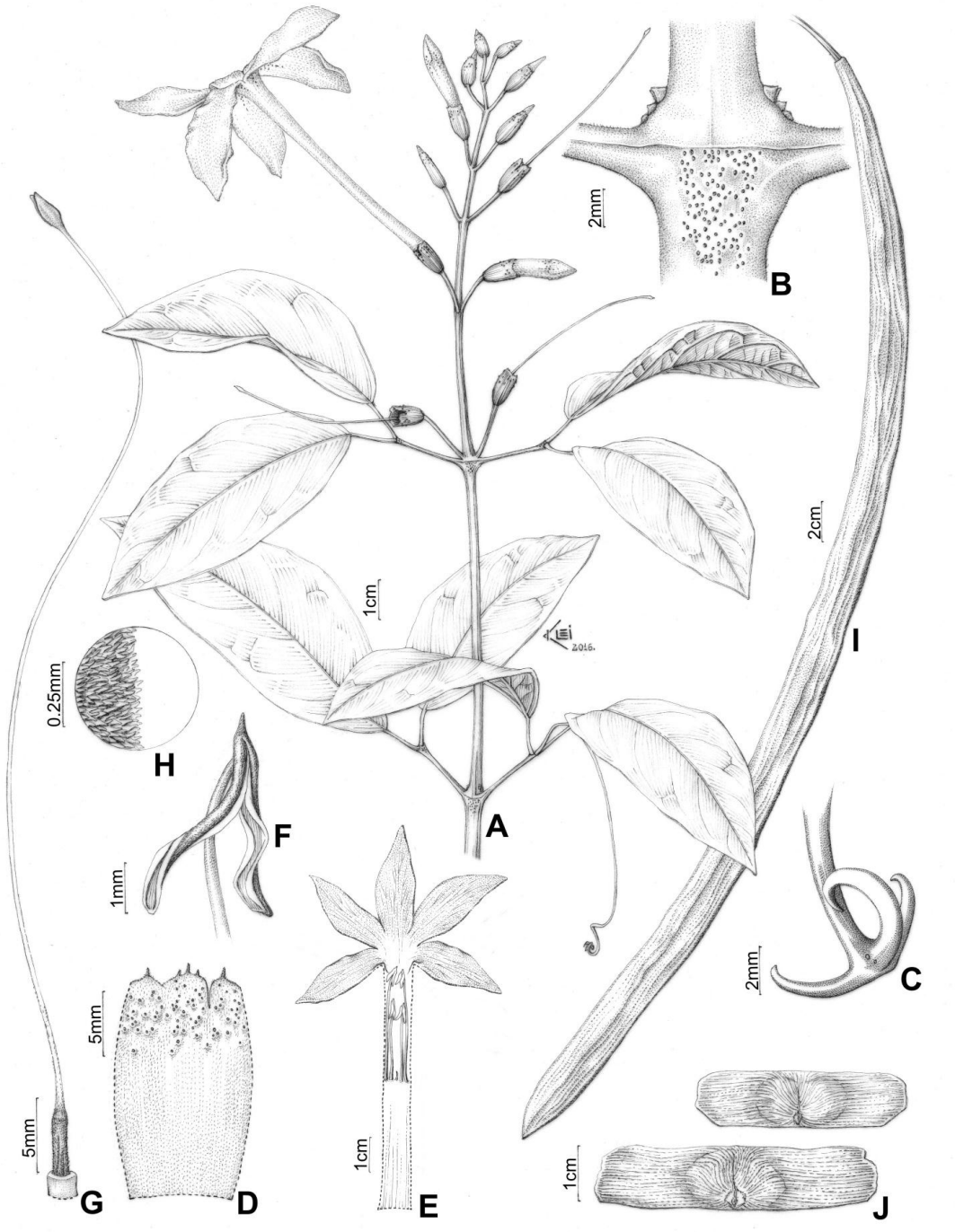


**Figure 4.** *Pachyptera aromatica*: A. Flowering branch; B. Interpetiolar region with extra-floral nectaries and prophylls of the axillary buds triangular, minute, and 3-seriated; C. Trifid tentril; D. Open calyx (external view); E. Open flower showing the androecium; F. Upper portion of stamen showing glabrous filament, glabrous anther and acute connective; G. Gynoecium; H. Detail of ovary surface showing pubescent indument (L.H. Fonseca 327, SPF); I. Fruit linear and flattened; J. Seeds wings (T.B. Croat 11085, MO).

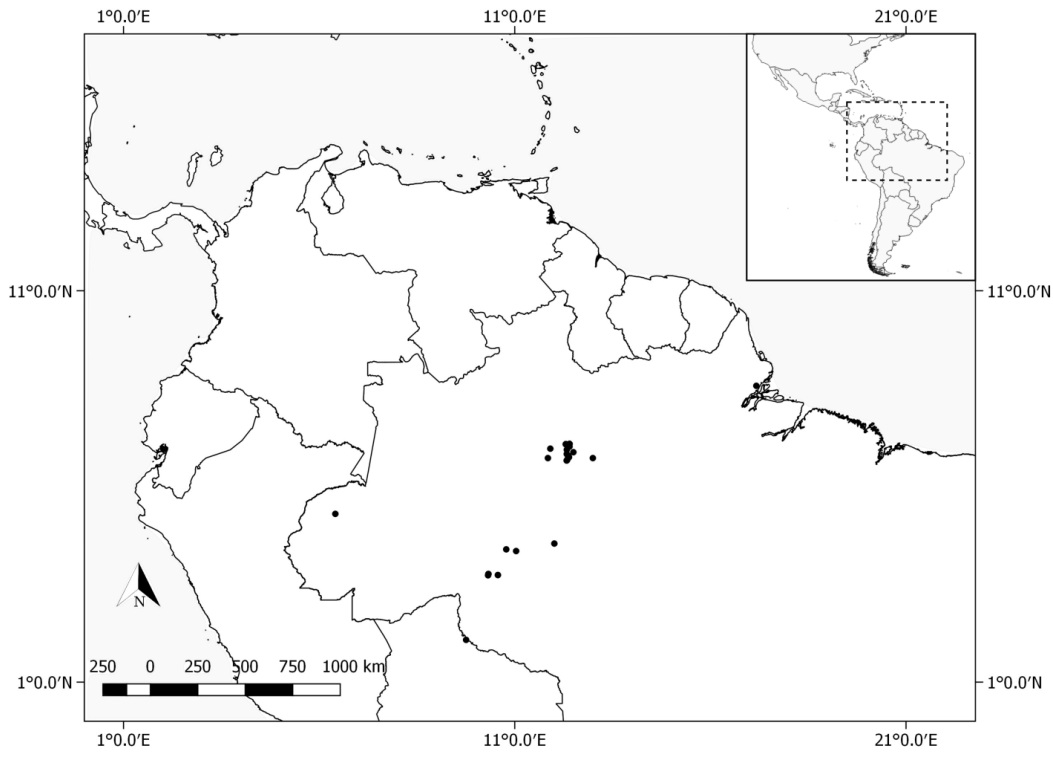


**Figure 5.** Distribution of *Pachyptera aromatica*.

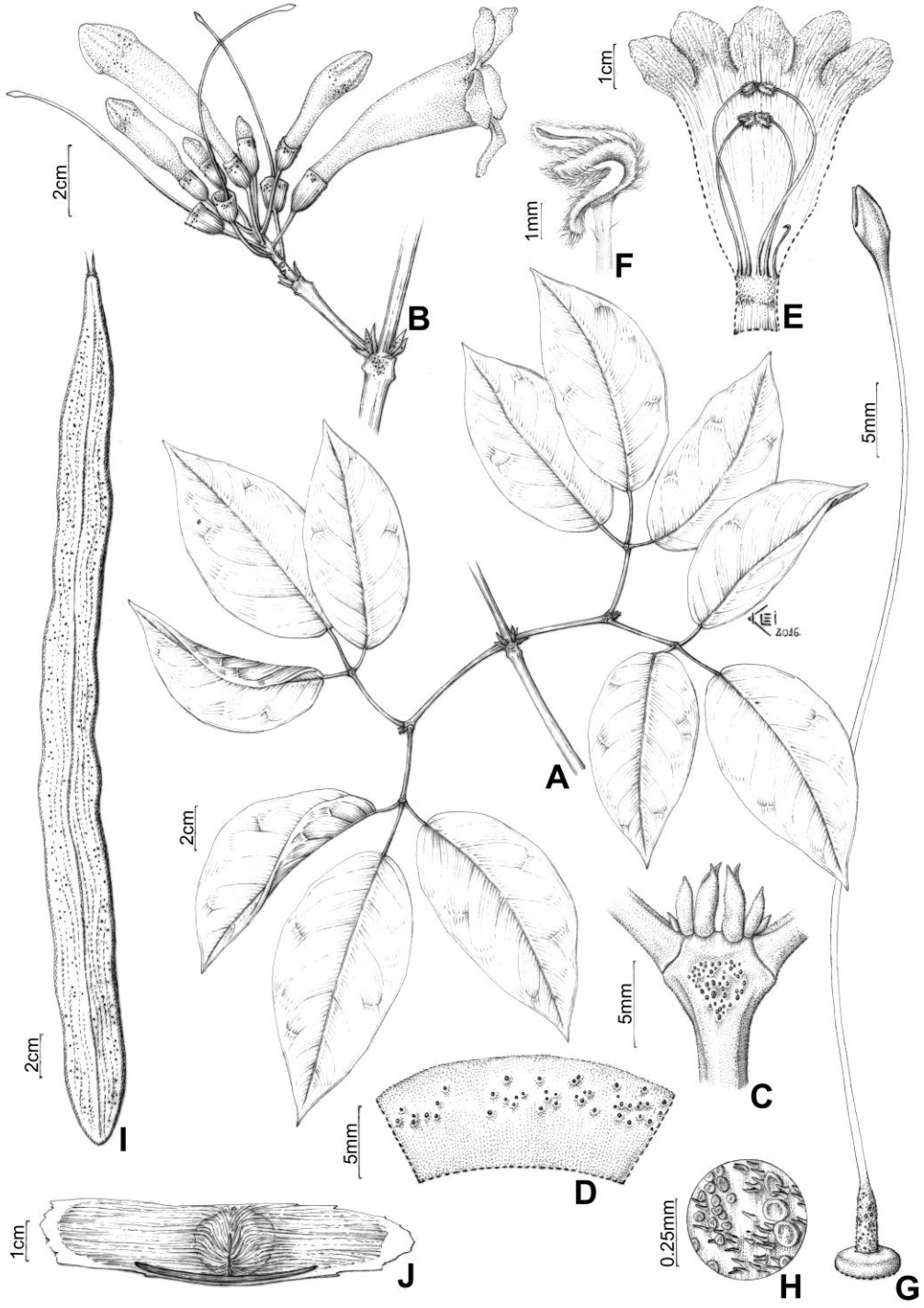


**Figure 6.** *Pachyptera erythraea*: A. Branchlets with four leaves; B. Inflorescence; C. Interpetiolar region showing extra floral nectaries and prophylls of axillary buds flattened, ensiform and 3-seriated; D. Opened calyx (external view); E. Open flower showing the androecium with anthers united by the villose indument; F. Upper portion of stamen, showing villose and curved thecae; G. Gynoecium; H. Detail of the lepidote ovary indument, with simples trichomes and glandular peltate, and

patelliform trichomes (M. Weir 72, K); I. Linear and flattened fruit; J. Seed wings (A.H. Gentry 20050, MO).

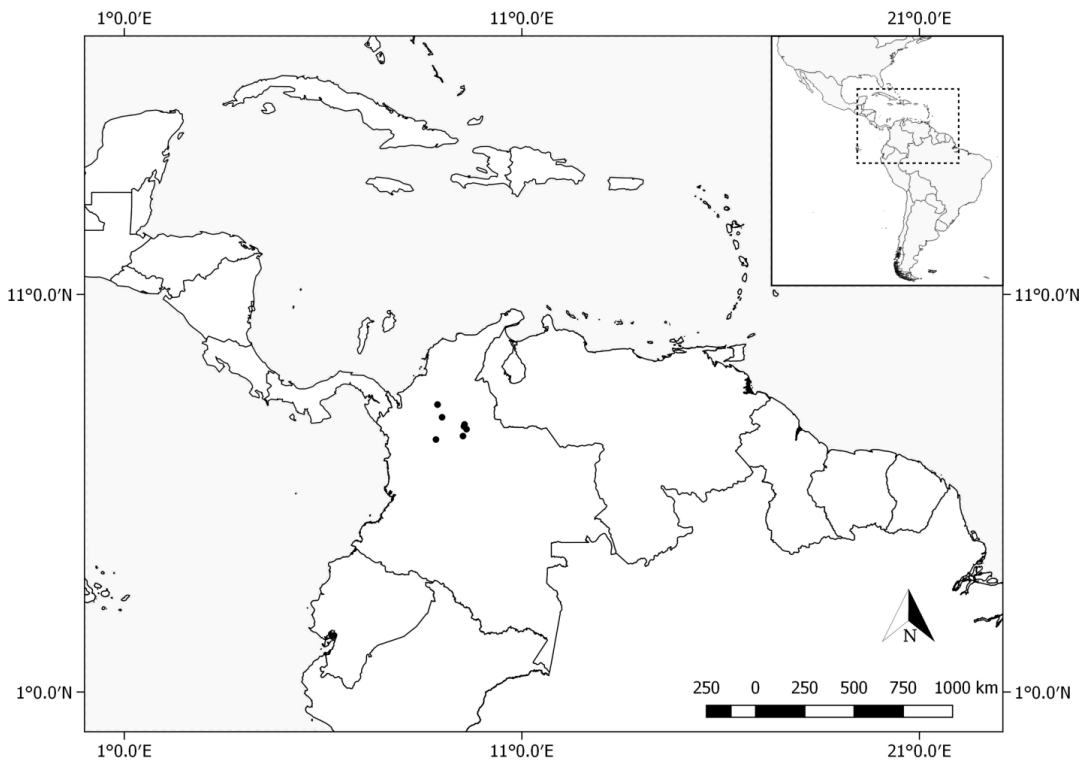


**Figure 7.** Distribution of *Pachyptera erythraea*.

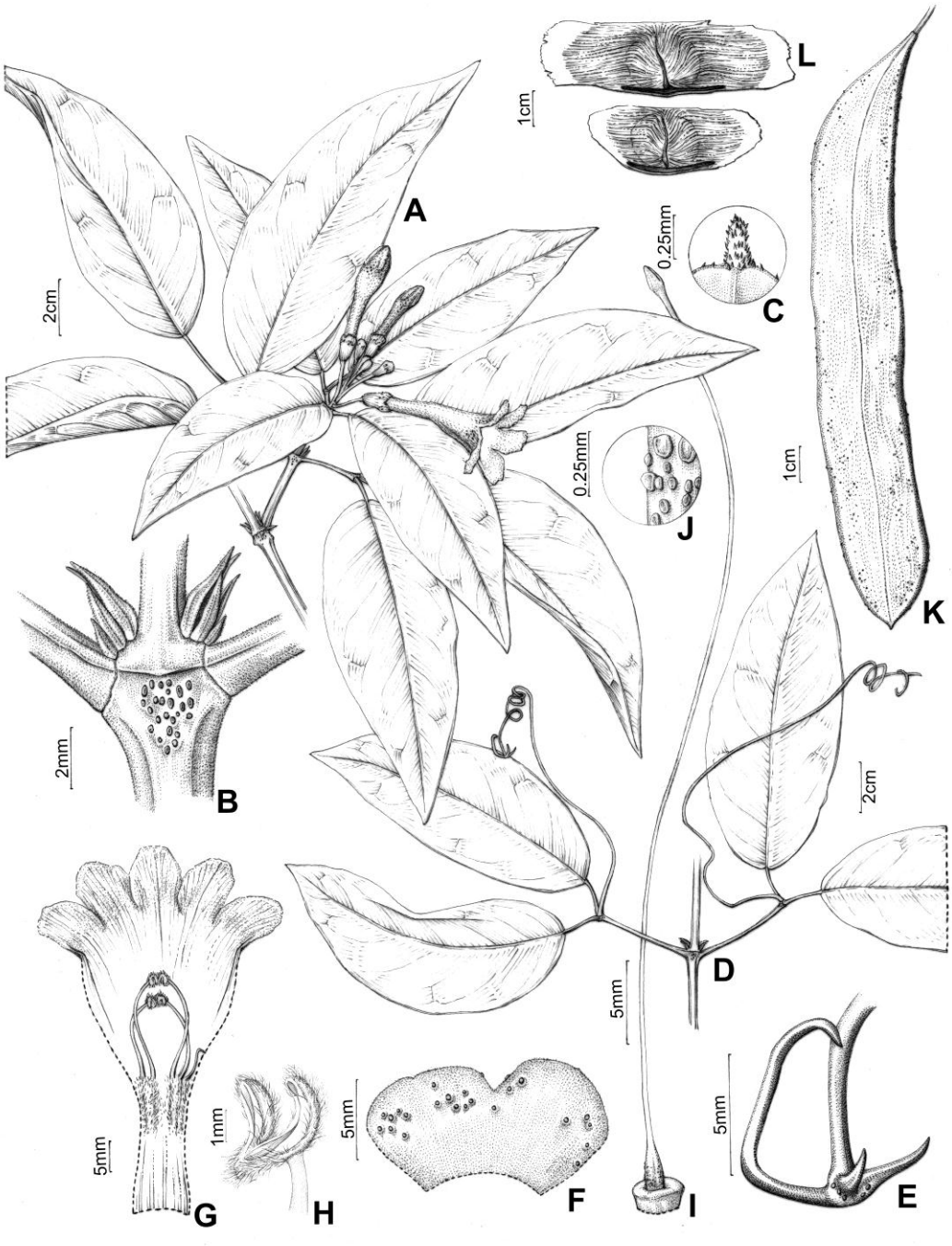


**Figure 8.** *Pachyptera incarnata*: A. Flowering branch; B. Interpetiolar region with NEF's and prophylls of axillary buds flattened, ensiform and 3-seriated; C. Mucronulate leaflet apex; D. Branchlets trifoliolated with terminal leaflet replaced by trifid tendril; E. Trifid tentril; F. Calyx external view; G. Open flower showing the androecium with anthers united; H. Stamen with villose and curved thecae; I. Gynoecium; J. Ovary surface lepidote, with glandular peltate trichomes (J.N.C.

Francisco 103, SPF); K. Fruit linear flattened capsule; L. Seeds wings (J.N.C. Francisco 122, SPF).

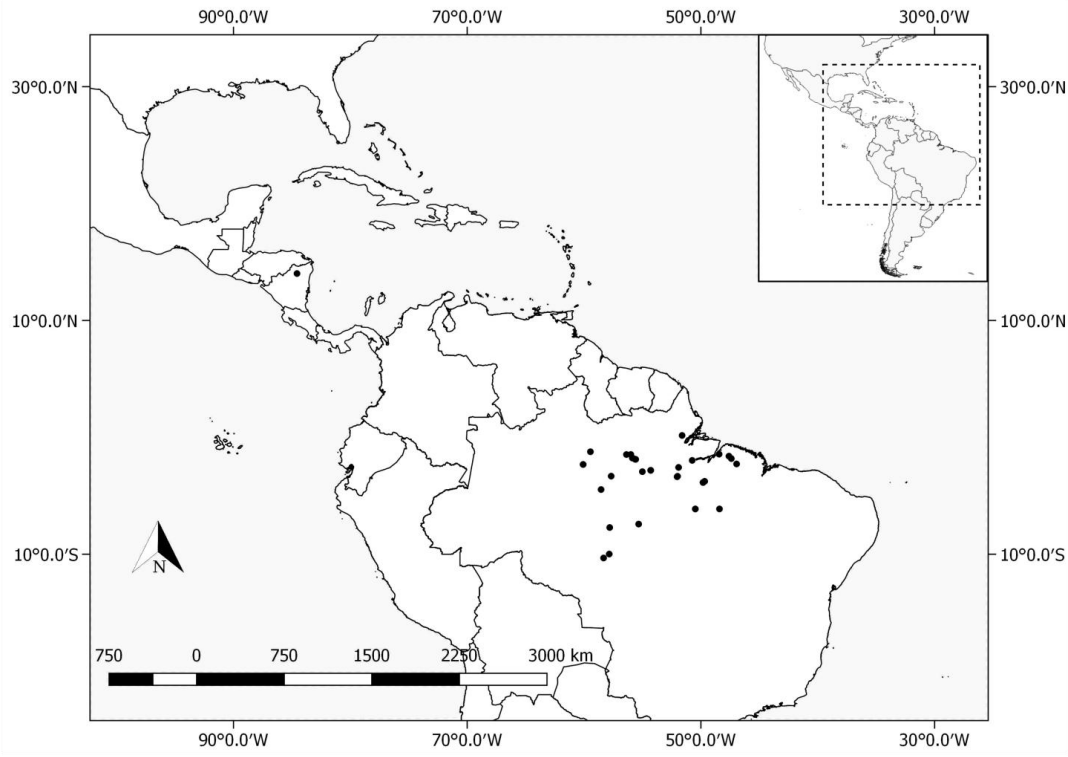


**Figure 9.** Distribution of *Pachyptera incarnata*.

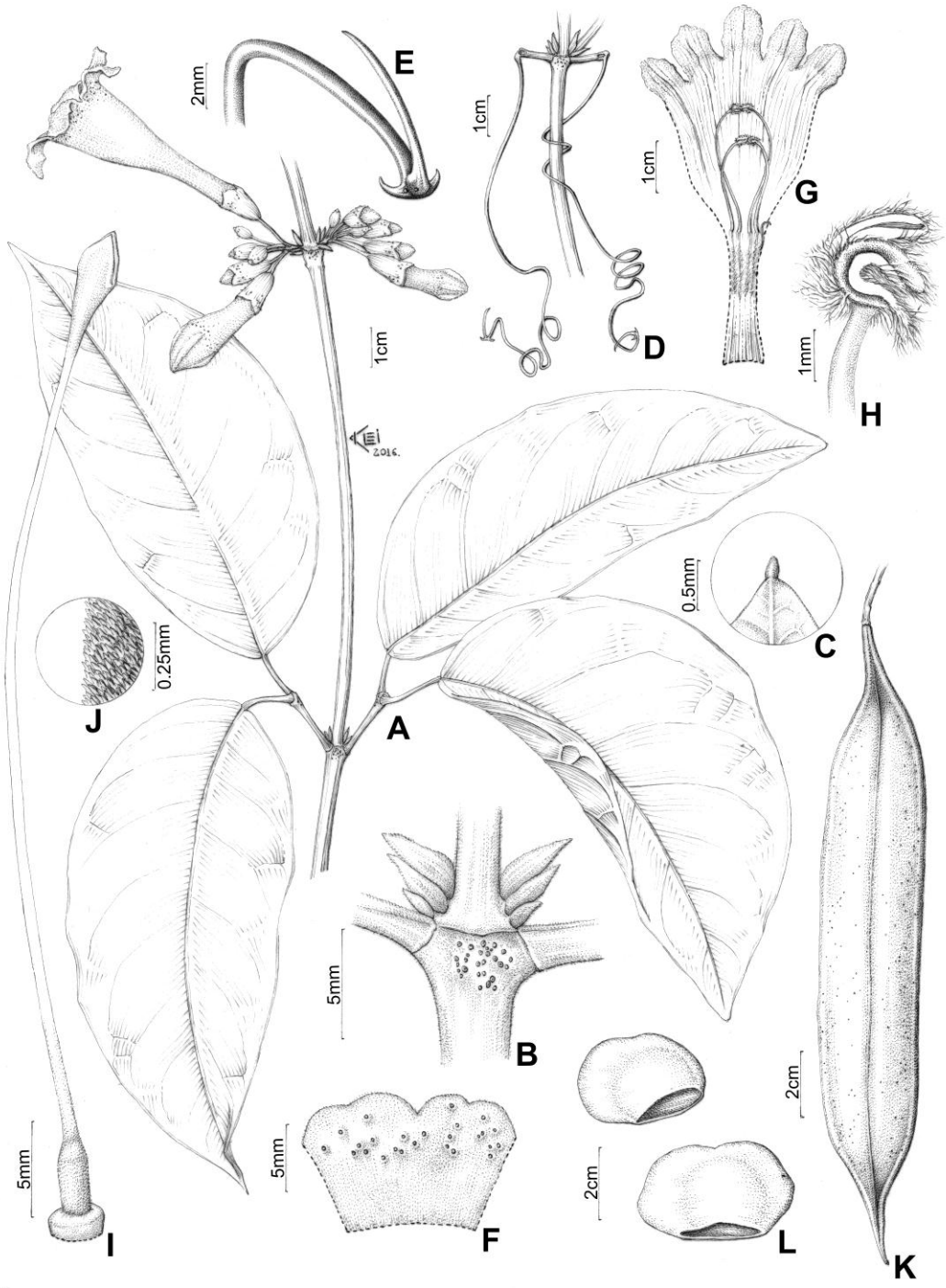


**Figure 10.** *Pachyptera kerere*: A. Flowering branch; B. Interpetiolar region with NEF's and prophylls of axillary buds flattened, ensiform and 3-seriated; C. Apice of the leaflet mucronulate; D. Branchlets with terminal leaflet replaced by trifid tendril; E. Trifid tentril; F. Calyx external view; G. Open flower showing the androecium with anthers united; H. Stamen with villose and curved thecae; I. Gynoecium; J. Ovary surface pubescent (J.N.C. Francisco 41, SPF); K. Fruit fusiform and

inflattened, with a conspicuous and raised longitudinal midline on valve (T.B. Croat 11085, MO); L. Seeds corky and wingless (R.A.A. Oldeman B-1449, MO).

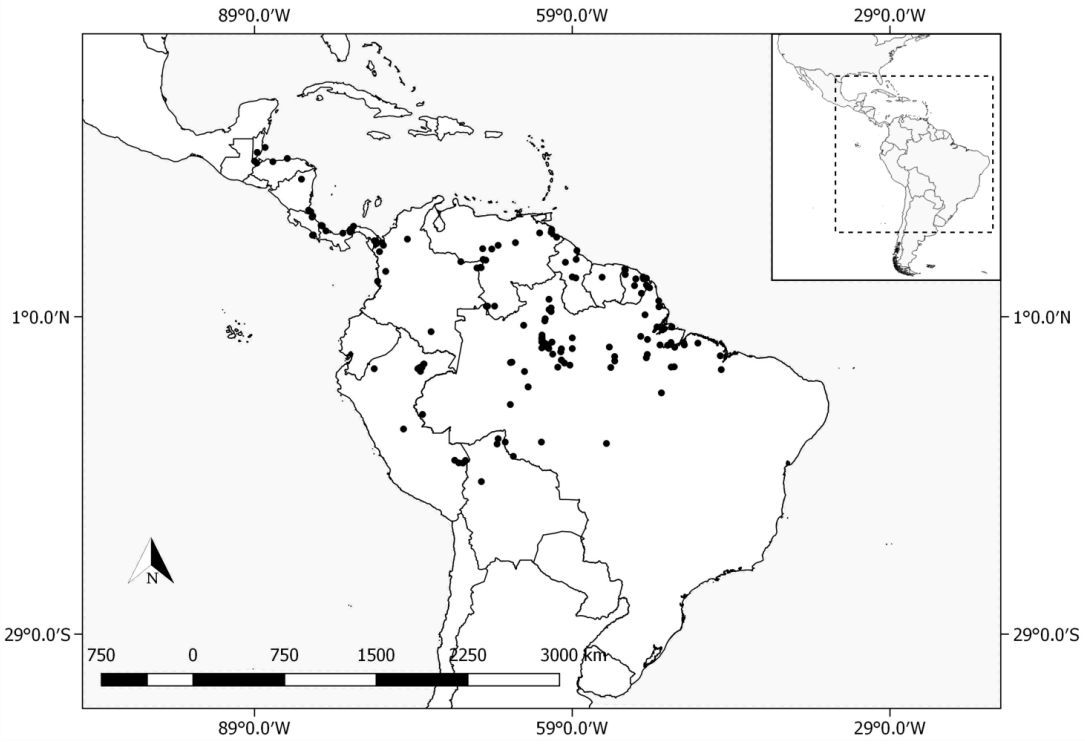


**Figure 11.** Distribution of *Pachyptera kerere.*

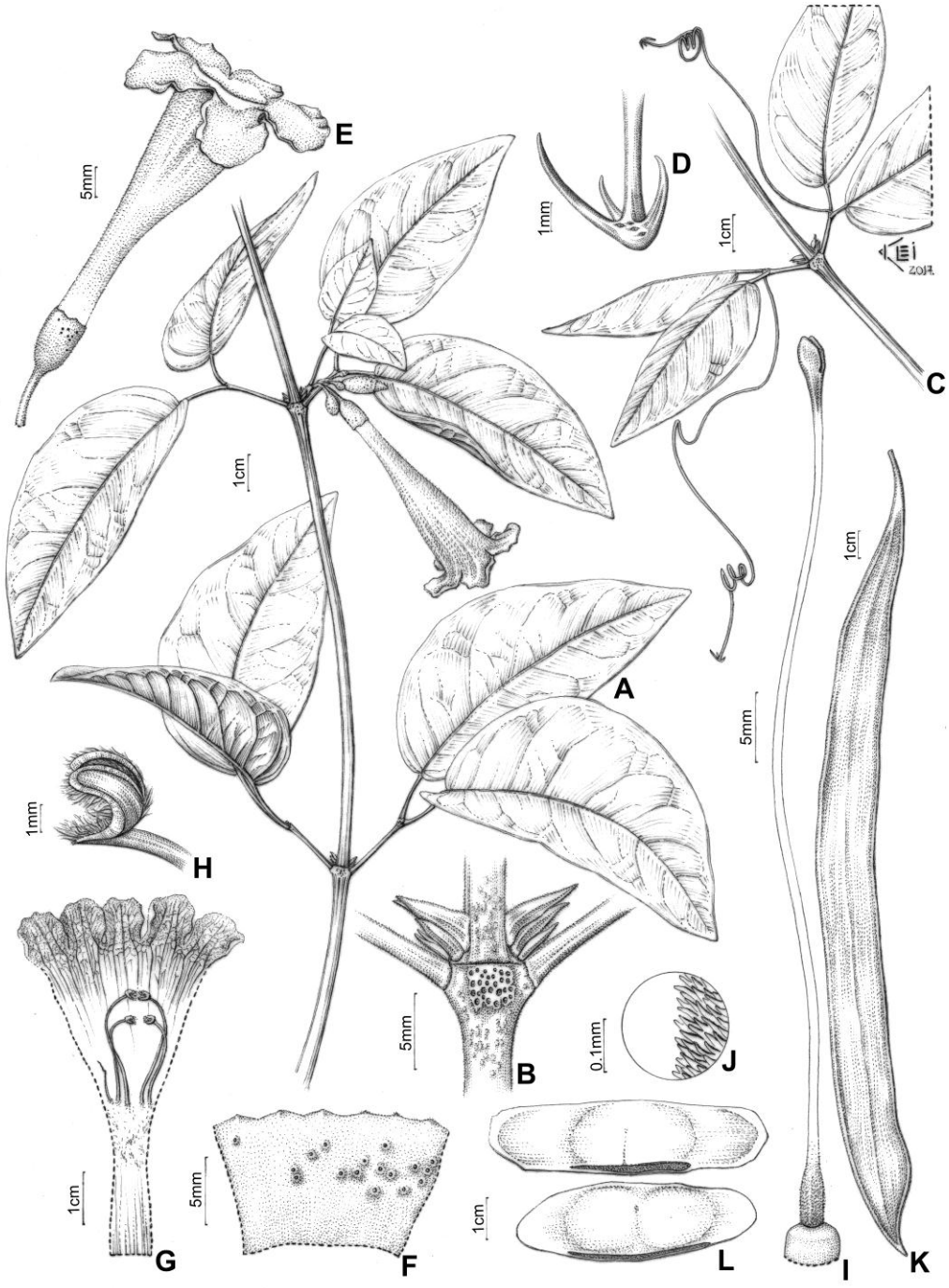


**Figure 12.** *Pachyptera linearis.* A. Flowering branch; B. Interpetiolar region with NEF's and prophylls of axillary buds flattened, ensiform and 3-seriated; C. Branchlets with terminal leaflet replaced by trifid tendril (leaflet fells); D. Trifid tentril; E. Flower; F. Calyx external view; G. Open flower showing the androecium with anthers united; H. Stamen with villose and curved thecae; I. Gynoecium; J. Ovary surface

pubescent (J.J. Wurdack 41357, K); K. Fruit flattened, with a inconspicuous longitudinal midline; L. Seeds wingless (P. Stevenson 403, MO).

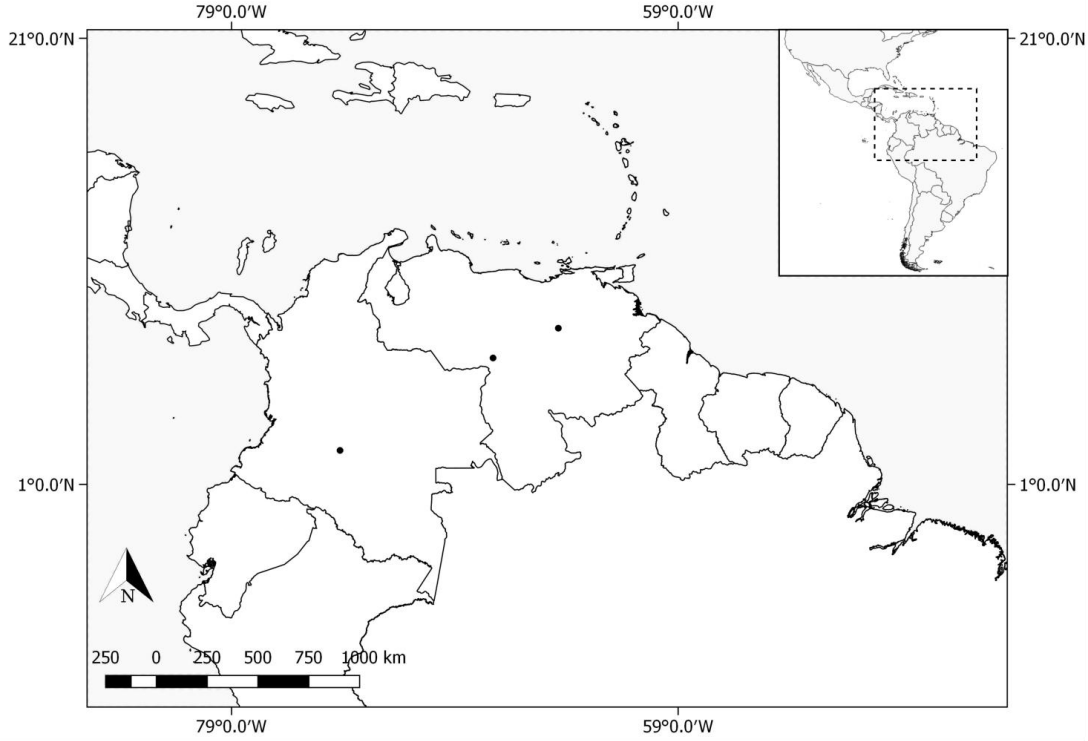


**Figure 13.** Distribution of *Pachyptera linearis.*

# Capítulo 4

**A genomic approach for isolating chloroplast microsatellite markers for *Pachyptera kerere* (Bignoniaceae)**

Jessica N. C. Francisco, Alison G. Nazareno, and Lúcia G. Lohmann

Applications in Plant Sciences

Applications in Plant Sciences 2016 4(9): 1600055

Primer Note

# A genomic approach for isolating chloroplast microsatellite markers for *Pachyptera kerere* (Bignoniaceae)[1]

Jessica N. C. Francisco[2,3], Alison G. Nazareno[2], and Lúcia G. Lohmann[2,3]

[2]Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo (USP), Rua do Matão 277, 05508-090 São Paulo, São Paulo, Brazil

- *Premise of the study:* In this study, we developed chloroplast microsatellite markers (cpSSRs) for *Pachyptera kerere* (Bignoniaceae) to investigate the population structure and genetic diversity of this species.
- *Methods and Results:* We used Illumina HiSeq data to reconstruct the chloroplast genome of *P. kerere* by a combination of de novo and reference-guided assembly. We then used the chloroplast genome to develop a set of cpSSRs from intergenic regions. Overall, 24 primer pairs were designed, 21 of which amplified successfully and were polymorphic, presenting three to nine alleles per locus. The unbiased haploid diversity per locus varied from 0.207 (Pac28) to 0.817 (Pac04). All but one locus amplified for all other taxa of *Pachyptera*.
- *Conclusions:* The markers reported here will serve as a basis for studies to assess the genetic structure and phylogeographic history of *Pachyptera*.

**Key words:** Bignoniaceae; Bignonieae; chloroplast genome; microsatellite; *Pachyptera kerere*; transferability.

*Pachyptera kerere* (Aubl.) Sandwith (Bignoniaceae) is a Neotropical liana that is widely distributed from Belize to central Amazon in Brazil (Lohmann and Taylor, 2014). This species occurs in humid and often flooded forest vegetation almost entirely along stream banks and rivers, where it is found in low densities. The flowers of *P. kerere* are white and infundibuliform and bloom throughout the year, providing a constant nectar source for different species of *Euglossa*, which are the most likely pollinators (Gentry, 1974, 1976). This species falls within the *Anemopaegma* flower type and steady-state phenology proposed by Gentry (1974). Specialized secretory glands are concentrated near the calyx margin and on the upper portion of the corolla tube. In addition, glands are also present at the interpetiolar region and the petiole apex, and play an important role in ant–plant interactions (Lohmann and Taylor, 2014). The seeds of *P. kerere* are corky and most likely water dispersed (Gentry, 1979). The broad distribution of *P. kerere*, combined with its habitat specificity and morphology, make it an interesting model to study the biological processes that determine the patterns of intra- and interpopulation variation of plant species in the Amazon.

[1]Manuscript received 22 April 2016; revision accepted 14 June 2016.

The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for a scholarship to J.N.C.F. and for a Pq-1C grant to L.G.L. We also thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for a scholarship to A.G.N. (2013/12633-8), a regular research grant to L.G.L. (2011/50859-2), and a collaborative Dimensions of Biodiversity Grant supported by FAPESP (2012/50260-6), the U.S. National Science Foundation, and the National Aeronautics and Space Administration.

[3]Authors for correspondence: jnc_francisco@yahoo.com.br, llohmann@usp.br

doi:10.3732/apps.1600055

Microsatellites (simple sequence repeats [SSRs]) constitute an important genomic resource for botanical studies (Ellegren, 2004) and have been widely used to study the ecological and evolutionary processes that shape plant populations (Ebert and Peakall, 2009). Next-generation sequencing (NGS) technologies now allow us to easily isolate and develop SSR markers from nuclear and plastid genomes (Egan et al., 2012). In this study, we reconstructed the chloroplast genome of *P. kerere* and used this genome to develop a set of chloroplast microsatellite markers (cpSSRs) for population genetic studies of *P. kerere*. We also tested the transferability of these markers to *P. kerere* var. *incarnata* (Aubl.) A. H. Gentry and the three other recognized species of *Pachyptera* DC. ex Meisn. (Lohmann and Taylor, 2014): *P. aromatica* (Barb. Rodr.) L. G. Lohmann, *P. erythraea* (Dugand) A. H. Gentry, and *P. ventricosa* (A. H. Gentry) L. G. Lohmann.

## METHODS AND RESULTS

Whole genomic DNA was extracted from silica-dried leaf tissue of one individual of *P. kerere* (collection *A. Nogueira 162*) using a mini-scale cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). An aliquot of 5 µg of total DNA was fragmented using a Covaris S-series sonicator (Covaris, Woburn, Massachusetts, USA) and used to construct short-insert libraries (300 bp) using the NEBNext DNA Library Prep Master Mix Set and the NEBNext Multiplex Oligos for Illumina (New England BioLabs, Ipswich, Massachusetts, USA) following the manufacturer's instructions. The *P. kerere* library was diluted to a concentration of 10 mM, indexed by tags, and sequenced on an Illumina HiSeq 2000 system (Illumina, San Diego, California, USA) at the Universidade de São Paulo (Escola Superior de Agricultura Luiz de Queiroz [ESALQ], Piracicaba, Brazil). Clean reads (100-bp single-end) were filtered for quality using a Perl script that trimmed reads from the ends until there were three consecutive bases with a Phred quality score of 20 or more. Reads with more than three uncalled bases or fewer than 40 bp in length were removed from the data set. The chloroplast genome of *P. kerere* was reconstructed using a combination of de novo and reference-guided assembly following Nazareno et al. (2015).

 

Applications in Plant Sciences 2016 4(9): 1600055
doi:10.3732/apps.1600055



The chloroplast genome for *P. kerere* was annotated using the software Geneious version 4.7.5 (Biomatters Ltd., Auckland, New Zealand). Start and stop codons were inspected and adjusted manually.

We used the Imperfect Microsatellite Extractor (IMEx) interface (Mudunuri and Nagarajaram, 2007) to detect perfect and imperfect microsatellites, with minimum thresholds of four repeat units for tri-, tetra-, penta-, and hexa-; six for di-; and 10 for mononucleotide repeats, respectively. Chloroplast microsatellite–flanking primers for cpSSRs found only on intergenic regions were designed using the software Primer3 (Rozen and Skaletsky, 1999) and the following settings: (i) length ranging from 20 to 23 nucleotides, (ii) annealing temperature from 50°C to 62°C, and (iii) minimum GC content of 50%.

In total, 24 primer pairs were designed. To validate those primer pairs, PCR amplifications were performed in 8.5-µL reactions containing 10 ng of template DNA, 0.5 µL 10 mM of each primer with forward primers labeled with 6-FAM or JOE fluorescent dyes (Macrogen, Seoul, South Korea), 5 µL 1× of Kapa2G Fast ReadyMix (Kapa Biosystems, Wilmington, Massachusetts, USA), and 0.6 µL 25 mM $MgCl_2$ (Promega Corporation, Madison, Wisconsin, USA). PCR conditions were as follows: 94°C for 3 min; 20 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, 72°C for 1 min; and a final elongation step at 72°C for 5 min. Initial screens were performed with three *P. kerere* individuals, and their amplicons were visualized on an agarose gel (0.8%) with a 100-bp ladder (Promega Corporation).

Twenty-one of the 24 primer pairs produced a single band with strong amplification and were selected for polymorphism assessment in 65 *P. kerere* samples. These samples were grouped in three populations (11–39 individuals per population; Appendix 1). For these samples, genomic DNA was extracted from silica-dried leaves using an Invisorb Plant Mini Kit (Invitek, Berlin, Germany) following the manufacturer's protocol. Fluorescently labeled amplicons were resolved to genotype on an automated sequencer (ABI 3730XL) with GeneScan 500 ROX Size Standard (Applied Biosystems, Foster City, California, USA). Chloroplast microsatellite profiles were analyzed with GeneMarker (Holland and Parson, 2011). Each cpSSR was considered a locus at a specific site and the length variants were considered alleles. For each polymorphic locus, we obtained the number of alleles (*A*) and unbiased haploid diversity index (*h*) using the program GenAlEx version 6.41 (Peakall and Smouse, 2006). Transferability of polymorphic cpSSRs was tested in five individuals of each of the following taxa: *P. aromatica*, *P. erythraea*, *P. ventricosa*, and *P. kerere* var. *incarnata*. The PCR amplification profile followed the same conditions described above.

We obtained a partial chloroplast genome (149,076 bp) and used it to develop a set of 21 polymorphic chloroplast microsatellite markers (Table 1). Considering all samples ($n = 65$), *A* ranged from three to nine and *h* ranged from 0.207 (Pac28) to 0.817 (Pac04) (Table 2). Most of the polymorphic primers (96%) successfully amplified for *P. kerere* var. *incarnata* and for all species of *Pachyptera* (Table 3).

Table 1. Characteristics of 21 intergenic chloroplast microsatellite primers developed for *Pachyptera kerere*.[a]

| Locus | Primer sequences (5′–3′) | Repeat motif | Allele size range (bp) | Fluorescent dye | Position | GenBank accession no. |
|---|---|---|---|---|---|---|
| Pac03 | F: TCGTTCTAGACCATCGGATT<br>R: GGAACTTCCGTCTAATCAAATG | $(A)_6(G)_{13}$ | 179–190 | JOE | *trnkUUU/rps16* | KP867116 |
| Pac04 | F: GGATTCGACGTAAACAATGA<br>R: GGAACTTCCGTCTAATCAAA | $(C)_{11}$ | 164–174 | 6-FAM | *trnkUUU/rps16* | KP867117 |
| Pac05 | F: TCTAATGATCCGGGGCGTAA<br>R: CCCTCTCTTTCCCTTTCCGT | $(A)_{14}$ | 166–173 | 6-FAM | *psbK/psbI* | KP867118 |
| Pac06 | F: ACTCCTGCCTTCATCATCTCT<br>R: ACGGTAGAAGAGAAGGTTCCA | $(T)_{10}C(A)_{10}$ | 145–153 | JOE | *rps2/rpoC2* | KP867119 |
| Pac08 | F: GTTTGATAAAGATGAGGCCGGT<br>R: ACTAGTAAAGGGTGTCGGGG | $(A)_{10}$ | 173–180 | JOE | *psbM/trnD-GU* | KP867120 |
| Pac09 | F: CGCCTCTTGAATCACCAAAGAT<br>R: TGGGTCAGGTCCACTTACTTT | $(A)_{10}$ | 91–96 | JOE | *trnlGRU/psbD* | KP867121 |
| Pac11 | F: GCGCGGTTGGTTTCTAAGAT<br>R: ACTTCAGCAAACTTCGCTTCA | $(T)_{13}$ | 220–230 | JOE | *trnSGGA/rps4* | KP867122 |
| Pac12 | F: CAAGATTGTTTAGATCTGAGGGG<br>R: CCCATAGATCATTTTCTGCAGG | $(T)_{11}$ | 157–176 | JOE | *accD/psaI* | KP867123 |
| Pac13 | F: GGAAATCCTTCTGTGAGATT<br>R: GGAATTAGACCTAACACGAT | $(T)_{10}$ | 184–199 | JOE | *psbE/petL* | KP867124 |
| Pac15 | F: GTGACGCTGAATTGGACTCC<br>R: CACGTACAGCATTCCCTCAC | $(A)_{10}$ | 228–241 | 6-FAM | *rps12/psi-psbT* | KP867125 |
| Pac16 | F: AGATGGTTCTACTTCGTCGGA<br>R: TCCCTGAGTAAGAACCATTGGA | $(A)_{11}$ | 207–220 | JOE | *psbH/petB* | KP867126 |
| Pac17 | F: AGACAACTCACCCTCTTTCT<br>R: CTTCTCGAGGTATAATGACAGAC | $(T)_{11}$ | 144–151 | JOE | *rpl36/infA* | KP867127 |
| Pac18 | F: GTAGATGCTATGCGAACAAC<br>R: GTGTCTCACGCATATACCT | $(T)_{11}$ | 187–199 | 6-FAM | *rps8/rpl14* | KP867128 |
| Pac19 | F: GTCCTTTATCCAAGTTTACC<br>R: ATTCACTAATCGGGATGG | $(A)_{11}$ | 155–162 | 6-FAM | *rpl16/rp53* | KP867129 |
| Pac20 | F: TGACTGCTTCTTTAGATCCAGA<br>R: TTGCTATGCTTAGTGTGTGAC | $(A)_{10}$ | 119–124 | JOE | *rpl16/rp53* | KP867130 |
| Pac21 | F: CTGGGTTCTTCTACTTCATT<br>R: CAATGGTCAAATTCTACAGG | $(T)_{10}$ | 104–110 | JOE | *rps12_end/trnV-GAC* | KP867131 |
| Pac23 | F: AGGAACCCGCAAATATTGGC<br>R: ACTCGCAGTATGGGTCTAGC | $(A)_{10}$ | 199–215 | JOE | *ndhD/psaC* | KP867132 |
| Pac24 | F: TCCTTTGTGTATCTTGGTCTTCC<br>R: TCGAGACTGTTTACCCCAAGA | $(T)_{11}$ | 161–171 | 6-FAM | *ndhA/orf188* | KP867133 |
| Pac25 | F: TTCCTGTCTTGTTTTCCACA<br>R: TCTTAGCGAGTAGTTCCGAA | $(TA)_7$ | 185–193 | JOE | *trnP-GGG/psaJ* | KP867134 |
| Pac27 | F: CCCCTTGTCCCTTTAATTCACA<br>R: CAGGAACCAGGAACCAGACT | $(TAA)_4$ | 146–155 | JOE | *trnL-UAA/trnF-GAA* | KP867136 |
| Pac28 | F: AGGTCTTCTGAACCGCTTCC<br>R: TTGACCTACGCCTGTTTGAAC | $(GGA)_4$ | 181–187 | 6-FAM | *rbcL/psal* | KU867864 |

[a]The annealing temperature for all loci was 58°C.

Applications in Plant Sciences 2016 4(9): 1600055
doi:10.3732/apps.1600055



Table 2. Characteristics of 21 polymorphic chloroplast microsatellite loci in three populations of *Pachyptera kerere*.[a]

| | Amazon ($n$ = 15) | | Caracaraí ($n$ = 39) | | Rorainópolis ($n$ = 11) | | All ($n$ = 65) | |
|---|---|---|---|---|---|---|---|---|
| Locus | *A* | *h* | *A* | *h* | *A* | *h* | *A* | *h* |
| Pac03 | 5 | 0.725 | 4 | 0.693 | 3 | 0.678 | 7 | 0.784 |
| Pac04 | 5 | 0.755 | 6 | 0.737 | 5 | 0.854 | 9 | 0.817 |
| Pac05 | 3 | 0.533 | 3 | 0.234 | 2 | 0.555 | 4 | 0.369 |
| Pac06 | 4 | 0.782 | 4 | 0.596 | 3 | 0.654 | 5 | 0.687 |
| Pac08 | 3 | 0.560 | 2 | 0.229 | 2 | 0.545 | 3 | 0.377 |
| Pac09 | 3 | 0.604 | 4 | 0.310 | 4 | 0.818 | 4 | 0.535 |
| Pac11 | 4 | 0.782 | 5 | 0.253 | 3 | 0.714 | 6 | 0.531 |
| Pac12 | 4 | 0.525 | 4 | 0.279 | 4 | 0.694 | 6 | 0.395 |
| Pac13 | 5 | 0.787 | 3 | 0.374 | 3 | 0.638 | 6 | 0.523 |
| Pac15 | 5 | 0.757 | 4 | 0.331 | 7 | 0.909 | 9 | 0.628 |
| Pac16 | 4 | 0.712 | 5 | 0.477 | 5 | 0.818 | 7 | 0.615 |
| Pac17 | 3 | 0.530 | 3 | 0.237 | 3 | 0.709 | 4 | 0.410 |
| Pac18 | 4 | 0.679 | 4 | 0.211 | 5 | 0.833 | 5 | 0.462 |
| Pac19 | 3 | 0.703 | 3 | 0.316 | 5 | 0.892 | 5 | 0.599 |
| Pac20 | 4 | 0.714 | 4 | 0.571 | 3 | 0.666 | 4 | 0.693 |
| Pac21 | 2 | 0.527 | 3 | 0.243 | 4 | 0.709 | 5 | 0.429 |
| Pac23 | 6 | 0.802 | 7 | 0.369 | 7 | 0.890 | 9 | 0.597 |
| Pac24 | 4 | 0.638 | 2 | 0.051 | 3 | 0.644 | 6 | 0.493 |
| Pac25 | 4 | 0.756 | 4 | 0.252 | 5 | 0.866 | 6 | 0.538 |
| Pac27 | 4 | 0.742 | 4 | 0.475 | 4 | 0.777 | 6 | 0.629 |
| Pac28 | 3 | 0.500 | 2 | 0.057 | 2 | 0.333 | 4 | 0.207 |
| Mean | 3.9 | 0.672 | 3.8 | 0.347 | 3.9 | 0.724 | 5.7 | 0.539 |

*Note*: *A* = number of alleles; *h* = unbiased haplotype diversity.
[a]Voucher and locality information are provided in Appendix 1.

Table 3. Transferability of 21 microsatellite markers developed for *Pachyptera kerere* across four different taxa of *Pachyptera*.

| Locus | Repeat motif | *P. aromatica* | *P. erythraea* | *P. ventricosa* | *P. kerere* var. *incarnata* |
|---|---|---|---|---|---|
| Pac03 | $(A)_6(G)_{13}$ | + | + | + | + |
| Pac04 | $(C)_{11}$ | + | + | + | + |
| Pac05 | $(A)_{14}$ | + | + | + | + |
| Pac06 | $(T)_{10}C(A)_{10}$ | + | + | + | + |
| Pac08 | $(A)_{10}$ | + | + | + | + |
| Pac09 | $(A)_{10}$ | + | + | + | + |
| Pac11 | $(T)_{13}$ | + | + | + | + |
| Pac12 | $(T)_{11}$ | + | + | + | + |
| Pac13 | $(T)_{10}$ | + | + | + | + |
| Pac15 | $(A)_{10}$ | + | + | + | + |
| Pac16 | $(A)_{11}$ | + | + | + | + |
| Pac17 | $(T)_{11}$ | + | + | + | + |
| Pac18 | $(T)_{11}$ | + | + | + | + |
| Pac19 | $(A)_{11}$ | + | + | + | + |
| Pac20 | $(A)_{10}$ | — | + | + | + |
| Pac21 | $(T)_{10}$ | + | + | + | + |
| Pac23 | $(A)_{10}$ | + | + | + | + |
| Pac24 | $(T)_{11}$ | + | + | + | + |
| Pac25 | $(TA)_7$ | + | + | + | + |
| Pac27 | $(TAA)_4$ | + | + | + | + |
| Pac28 | $(GGA)_4$ | + | + | + | + |

*Note*: + = successful amplification as evidenced by the occurrence of distinct single bands on sequencing gels; — = no amplification.

## CONCLUSIONS

We developed and amplified a set of polymorphic chloroplast microsatellite markers for *P. kerere*. These markers will be useful for evolutionary and phylogeographic studies. The applicability of these microsatellite loci in *Pachyptera* congeneric species was confirmed by successful transferability. We plan to use these markers to assess patterns of genetic structure of *Pachyptera* species in the Amazon rainforest.

## LITERATURE CITED

Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Ebert, D., and R. Peakall. 2009. Chloroplast simple sequence repeats (cpSSRs): Technical resources and recommendations for expanding cpSSR discovery and applications to a wide array of plant species. *Molecular Ecology Resources* 9: 673–690.

Egan, A. N., J. Schlueter, and D. M. Spooner. 2012. Applications of next-generation sequencing in plant biology. *American Journal of Botany* 99: 175–185.

Ellegren, H. 2004. Microsatellites: Simple sequences with complex evolution. *Nature Reviews. Genetics* 5: 435–445.

Gentry, A. H. 1974. Coevolutionary patterns in Central American Bignoniaceae. *Annals of the Missouri Botanical Garden* 61: 728–759.

Gentry, A. H. 1976. Bignoniaceae of southern Central America: Distribution and ecological specificity. *Biotropica* 8: 117–131.

Gentry, A. H. 1979. Additional generic mergers in Bignoniaceae. *Annals of the Missouri Botanical Garden* 66: 778–787.

Holland, M. M., and W. Parson. 2011. GeneMarker® HID: A reliable software tool for the analysis of forensic STR data. *Journal of Forensic Sciences* 56: 29–35.

Lohmann, L. G., and C. M. Taylor. 2014. A new generic classification of tribe Bignonieae (Bignoniaceae). *Annals of the Missouri Botanical Garden* 99: 348–489.

Mudunuri, S. B., and H. A. Nagarajaram. 2007. IMEx: Imperfect Microsatellite Extractor. *Bioinformatics (Oxford, England)* 23: 1181–1187.

Nazareno, A. G., M. C. Carlsen, and L. G. Lohmann. 2015. Complete chloroplast genome of *Tanaecium tetragonolobum*: The first Bignoniaceae plastome. *PLoS ONE* 10: e0129930.

Peakall, R., and P. E. Smouse. 2006. GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

Rozen, S., and H. Skaletsky. 1999. Primer3 on the WWW for general users and for biologist programmers. *In* S. Misener and S. A. Krawetz [eds.], Methods in molecular biology, vol. 132: Bioinformatics methods and protocols, 365–386. Humana Press, Totowa, New Jersey, USA.

Applications in Plant Sciences 2016 4(9): 1600055
doi:10.3732/apps.1600055



Appendix 1. Voucher and locality information for the individuals of *Pachyptera* sampled.

| Species | Population code | Locality | Geographic coordinates | Voucher no. |
|---|---|---|---|---|
| *Pachyptera kerere* (Aubl.) Sandwith | AM | Brazil, Amazonas, Novo Airão | 1°54′21.0″S, 61°20′08.9″W | Beyer 324 |
| | AM | Brazil, Amazonas, Novo Airão | 1°54′21.0″S, 61°20′08.9″W | Beyer 324 |
| | AM | Brazil, Amazonas, Novo Airão | 2°43′12.2″S, 60°45′16.7″W | Francisco 28 |
| | AM | Brazil, Amazonas, Novo Airão | 2°43′12.7″S, 60°45′16.7″W | Francisco 29 |
| | AM | Brazil, Amazonas, Novo Airão | 2°43′12.9″S, 60°45′16.6″W | Francisco 29 |
| | AM | Brazil, Amazonas, Novo Airão | 2°43′11.9″S, 60°45′16.6″W | Francisco 29 |
| | AM | Brazil, Amazonas, Novo Airão | 2°43′11.7″S, 60°45′16.6″W | Francisco 29 |
| | AM | Brazil, Amazonas, Novo Airão | 2°43′11.4″S, 60°45′16.8″W | Francisco 29 |
| | AM | Brazil, Amazonas, Novo Airão | 2°43′11.8″S, 60°45′17.4″W | Francisco 29 |
| | AM | Brazil, Amazonas, Novo Airão | 2°43′12.9″S, 60°45′17″W | Francisco 29 |
| | AM | Brazil, Amazonas, Novo Airão | 2°43′12.4″S, 60°45′16.8″W | Francisco 30 |
| | AM | Brazil, Amazonas, Novo Airão | 2°43′12.3″S, 60°45′16.4″W | Francisco 31 |
| | AM | Brazil, Amazonas, Novo Airão | 2°32′09″S, 60°50′20″W | Lohmann 805 |
| | AM | Brazil, Amazonas, Novo Airão | 2°32′09″S, 60°50′49″W | Lohmann 836 |
| | AM | Brazil, Amazonas, Manaus | 2°57′42″S, 59°55′40″W | Nogueira 162 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′26.1″N, 61°0′13.3″W | Francisco 29 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′26.3″N, 61°0′16.8″W | Francisco 36 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′10.9″N, 61°0′41.3″W | Francisco 37 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′12.6″N, 61°0′39″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′11.9″N, 61°0′39″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′11.1″N, 61°0′39″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′10.9″N, 61°0′39″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′10.4″N, 61°0′42.1″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′8.4″N, 61°0′42.1″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′5.4″N, 61°0′42.1″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′0.4″N, 61°0′41.9″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°28′36.9″N, 61°0′54.5″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°28′38″N, 61°0′57.6″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°17′1.5″N, 61°18′50.7″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°17′1.3″N, 61°18′50.7″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°17′1″N, 61°18′50.5″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′10.9″N, 61°0′41.3″W | Francisco 38 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′24.9″N, 61°0′11.4″W | Francisco 39 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′23.3″N, 61°0′09.1″W | Francisco 40 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′23.3″N, 61°0′09.1″W | Francisco 40 |
| | CA | Brazil, Roraima, Caracaraí | 1°40′29.0″N, 61°11′24.6″W | Francisco 41 |
| | CA | Brazil, Roraima, Caracaraí | 1°40′29.0″N, 61°11′24.6″W | Francisco 41 |
| | CA | Brazil, Roraima, Caracaraí | 1°33′11.9″N, 61°13′58.3″W | Francisco 43 |
| | CA | Brazil, Roraima, Caracaraí | 1°39′45.2″N, 61°11′43.6″W | Francisco 43 |
| | CA | Brazil, Roraima, Caracaraí | 1°39′45.3″N, 61°11′43.7″W | Francisco 43 |
| | CA | Brazil, Roraima, Caracaraí | 1°34′16.3″N, 61°13′45.6″W | Francisco 43 |
| | CA | Brazil, Roraima, Caracaraí | 1°34′10.9″N, 61°13′36.4″W | Francisco 43 |
| | CA | Brazil, Roraima, Caracaraí | 1°34′7.1″N, 61°13′24.5″W | Francisco 43 |
| | CA | Brazil, Roraima, Caracaraí | 1°31′16.9″N, 61°14′25.8″W | Francisco 43 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′24.2″N, 61°0′3.5″W | Francisco 47 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′24.1″N, 61°0′2.1″W | Francisco 47 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′18″N, 60°59′56.8″W | Francisco 47 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′15″N, 60°59′51.6″W | Francisco 47 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′24.6″N, 61°0′11.4″W | Francisco 47 |
| | CA | Brazil, Roraima, Caracaraí | 1°29′23.4″N, 61°0′8.8″W | Francisco 47 |
| | CA | Brazil, Roraima, Caracaraí | 1°25′21.8″N, 60°50′34.2″W | Francisco 47 |
| | CA | Brazil, Roraima, Caracaraí | 1°25′21.3″N, 60°50′38.3″W | Francisco 47 |
| | CA | Brazil, Roraima, Caracaraí | 1°25′20″N, 60°50′42.1″W | Francisco 57 |
| | CA | Brazil, Roraima, Caracaraí | 1°5′46.5″N, 61°52′53″W | Gomes 659 |
| | RR | Brazil, Roraima, Rorainópolis | 1°33′14.2″S, 61°30′27.8″W | Beyer 337 |
| | RR | Brazil, Roraima, Rorainópolis | 1°33′14.2″S, 61°30′27.8″W | Beyer 337 |
| | RR | Brazil, Roraima, Rorainópolis | 1°22′5.2″S, 61°45′55.3″W | Gomes 639 |
| | RR | Brazil, Roraima, Rorainópolis | 1°22′5.2″S, 61°45′55.3″W | Gomes 639 |
| | RR | Brazil, Roraima, Rorainópolis | 1°22′5.2″S, 61°45′55.3″W | Gomes 639 |
| | RR | Brazil, Roraima, Rorainópolis | 1°22′5.2″S, 61°45′55.3″W | Gomes 639 |
| | RR | Brazil, Roraima, Rorainópolis | 1°22′5.2″S, 61°45′55.3″W | Gomes 640 |
| | RR | Brazil, Roraima, Rorainópolis | 1°23′0.2″S, 61°51′6″W | Gomes 648 |
| | RR | Brazil, Roraima, Rorainópolis | 1°12′12.7″S, 61°50′37.3″W | Gomes 651 |
| | RR | Brazil, Roraima, Rorainópolis | 1°23′42.0″S, 61°41′45.0″W | Lohmann 336 |
| | RR | Brazil, Roraima, Rorainópolis | 0°43′46″S, 61°51′24″W | Thode 424 |

Applications in Plant Sciences 2016 4(9): 1600055
doi:10.3732/apps.1600055



Appendix 1. Continued.

| Species | Population code | Locality | Geographic coordinates | Voucher no. |
|---|---|---|---|---|
| *Pachyptera aromatica* (Barb. Rodr.) L. G. Lohmann | Individual | Brazil, Amazonas, Novo Airão | 2°32′08″S, 60°50′49″W | Lohmann 794 |
| *Pachyptera erythraea* (Dugand) A. H. Gentry | Individual | Colombia, Santander | 7°09′19″N, 73°50′28″W | Gentry 15372* |
| *Pachyptera kerere* var. *incarnata* (Aubl.) A. H. Gentry | Individual | Brazil, Pará, Óbidos | 1°52′38.2″S, 55°35′27.4″W | Francisco 122 |
| *Pachyptera ventricosa* (A. H. Gentry) L. G. Lohmann | Individual | Brazil, Pará, Belterra | 2°55′50.2″S, 55°0′44.6″W | Francisco 84 |

*Note*: All specimens are deposited at the University of São Paulo Herbarium (SPF), São Paulo, Brazil, except one sample (*) which is deposited at the Missouri Botanical Garden (MO), St. Louis, Missouri, USA.

# Considerações Finais

Essa dissertação reconstruiu a filogenia de *Pachyptera* utilizando uma ampla amostragem de taxa e marcadores moleculares. A filogenia do grupo indicou que *P. ventricosa* é mais proximamente relacionada à *Mansoa* do que *Pachyptera*. Embora a espécie compartilhe várias características morfológicas com ambos os gêneros, encontramos novas características morfológicas que corroboram a posição de *P. ventricosa* em *Mansoa*. Assim, propusemos o restabelecimento de *Mansoa ventricosa* (Capítulo 1). Com esta modificação, *Pachyptera* passou a consituir um grupo monofilético.

Utilizando uma abordagem integrativa que incluiu a interpretação da filogenia molecular do grupo à luz e de dados morfológicos e resultados de análises de coalescência, esclarecemos as relações infra-genéricas do confuso complexo de espécies *P. kerere* (Capítulo 2). A clara definição dos limites entre espécies serviu como base para importantes decisões taxonômicas, incluindo o reconhecimento de *P. kerere* var. *incarnata* como uma espécie e a descrição de uma espécie nova (*P. linearis*). Ao todo reconhecemos cinco espécies de *Pachyptera*, as quais são tratadas em uma monografia do gênero. Esta monografia inclui descrições morfológicas detalhadas, lista completa de sinônimos, dados de ecologia, fenologia, distribuição e comentários taxonômicos para cada espécie. Uma chave de identificação e ilustrações para cada espécie também são fornecidas permitindo a rápida identificação dos taxa tratados (Capítulo 3).

A filogenia de *Pachyptera* foi utilizada como base em um estudo da história biogeográfica do gênero. Este estudo indicou que o grupo teve sua origem no Eoceno Tardio, e diversificou-se durante o Mioceno, um período com perturbações intensas que parecem estar associadas à diversificação do grupo (Capítulo 2). Este estudo levanta diversas hipóteses sobre a história de diversificação do gênero para serem testadas em estudos futuros. Para tal, desenvolvemos SSR's com base em dados de sequenciamento de próxima geração. Os testes de transferabilidade demonstraram que a aplicabilidade desses SSRs não se restringe apenas as espécies de *Pachyptera*, mas também se aplica a outros gêneros de Bignonieae, especificamente *Mansoa*. Portanto, o alto polimorfismo dos SSR's indica o alto potencial desses marcadores em outros grupos

taxonômicos. Estes marcadores serão de grande utilidade para futuros estudos filogeográficos com o grupo.