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

**ESTUDOS TAXONÔMICOS E BIOSISTEMÁTICOS DO GÊNERO *Dryadella* LUER
(ORCHIDACEAE: PLEUROTHALLIDINAE)**

DANIELA CRISTINA IMIG

**Rio Claro – SP
2022**

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LUER (ORCHIDACEAE: PLEUROTHALLIDINAE)**

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

Orientador: Dr. Eric de Camargo Smidt

Co-orientadora: Dr^a. Viviane da Silva-Pereira

**Rio Claro – SP
2022**

I32e

Imig, Daniela Cristina

Estudos taxonômicos e biossistemáticos do gênero *Dryadella* Luer (Orchidaceae: Pleurothallidinae) / Daniela Cristina Imig. -- Rio Claro, 2022

214 f. : il., tabs., fotos, mapas

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências, Rio Claro

Orientador: Eric de Camargo Smidt

Coorientador: Viviane da Silva-Pereira

1. Filogenia. 2. Taxonomia. 3. Botânica. 4. Morfometria. 5. Anatomia. I. Título.

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TÍTULO DA TESE: **ESTUDOS TAXONÔMICOS E BIOSSISTEMÁTICOS DO GÊNERO *Dryadella* LUER (ORCHIDACEAE: PLEUROTHALLIDINAE)**

AUTORA: DANIELA CRISTINA IMIG

ORIENTADOR: ERIC DE CAMARGO SMIDT

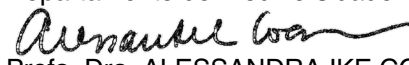
Aprovada como parte das exigências para obtenção do Título de Doutora em CIÊNCIAS BIOLÓGICAS (BIOLOGIA VEGETAL), área: Biologia Vegetal pela Comissão Examinadora:



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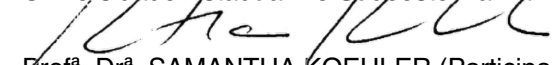
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Rio Claro, 04 de abril de 2022

AGRADECIMENTOS

Estes agradecimentos poderiam render um livro com muitas e muitas páginas, e ainda assim eu não me cansaria de citar nomes e nomes, pois muitos são aqueles que me ajudaram, direta ou indiretamente nesse processo. Foi um momento histórico, uma pandemia que chegou de repente e nos obrigou à resiliência a cada perda de amigos, parentes e conhecidos e até desconhecidos, eram números que cresciam em segundos, mas graças à CIÊNCIA, hoje aos poucos voltamos ao “novo normal” e eu, ao final de uma etapa muito importante da minha vida, viva!

Por isso, agradeço em especial ao meu orientador, Prof. Dr. Eric de Camargo Smidt, por me apadrinhar quando fiquei órfã na ciência, por toda a orientação e por estar sempre disposto a me ajudar, além da parceria e do respeito cultivados durante nosso tempo de convívio, sou grata pela oportunidade a essa viagem incrível na ciência das plantas de flores tão pequenas, e digo, ela ainda não acabou.

Sou também profundamente grata ao Dr. Antonio L.V. Toscano de Brito que em muitos aspectos representou o papel de co-orientador e um colaborador fundamental, sempre pronto a me ajudar e disponibilizar bibliografias valiosas. Que momentos difíceis hein Toscano? Sou grata por tudo!

À Prof. Dr^a. Viviane da Silva-Pereira, aquela que me fez estabelecer um domínio de pequenos movimentos, ah, logo eu? Sempre desajeitada com tudo, mas no laboratório consegui dar conta de algo que jamais imaginei conseguir, os tais tubinhos intactos! Obrigada por me desafiar!

Ah, e falando em laboratório e mil tubinhos de números minúsculos, Anna Victória S. R. Mauad, muito obrigada, você nunca me deixou sozinha (ela me conhece bem), e nem fora do lab. Obrigada Anna, por toda força e por ser minha inspiração.

À Prof. Dr^a. Erika Amano pelo grande auxílio e orientação com o trabalho de anatomia vegetativa e pela nossa amizade.

Sou grata ao suporte proporcionado pelo Programa de Pós-Graduação em Ciências Biológicas (Biologia Vegetal) do Instituto de Biociências da UNESP, Campus Rio Claro e a todos os docentes. Estendo este agradecimento aos amigos e colegas que fiz em Rio Claro por tornarem meus dias mais leves e felizes enquanto estive fora de casa cursando as disciplinas. Apesar da minha ausência física na UNESP, tenho um carinho enorme por todos.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela bolsa de estudos concedida.

Ao IBAMA/SISO e ICMBio O pelo fornecimento das licenças de coleta e aos vários funcionários das unidades de conservação visitadas. Agradeço também a todos os curadores e funcionários dos herbários visitados que permitiram o acesso ao acervo e os empréstimos das exsicatas.

Agradeço aos professores, técnicos, amigos e colegas do Departamento de Botânica da UFPR, que me acompanharam desde o mestrado, pelas instruções ao longo destes tantos anos.

Agradeço aos orquidófilos que disponibilizaram materiais e fotos de Pleurothallidinae, principalmente a Marcos L. klingelfuss e José Vanderlei Alves Neves pela grande ajuda com materiais, pela amizade e parceria durante os campos.

Aos amigos e colegas do Laboratório de Sistemática e Ecologia Molecular de Plantas (LSEMP) da UFPR, de imenso valor foi a ajuda e o convívio por parte dessas pessoas.

Sou imensamente grata aos amigos que a Botânica me proporcionou (são muitos e cada um sabe sua importância na minha vida!). Cada um a seu modo, ajudou e ainda ajuda a construir a mim mesma, nossas discussões de horas, nossos debates sociais, políticos e econômicos e claro, nossas discussões botânicas me lapidam e me tornam cada dia melhor. Obrigada a todos!!

Aos amigos da vida (alguns também são da Botânica, risos), pelos abraços que me aliviaram por muitas vezes o cansaço e principalmente por entenderem minha ausência.

Aos colegas do trabalho e à toda(s) a(s) equipe(s).

Sou especialmente grata ao meu amigo João Bento, obrigada por ter me acompanhado nos campos em MG, e por ser meu grande amigo!

Aos meus pais, que sempre marejaram os olhos quando eu não podia estar em momentos de família, mas sempre vibraram com minhas conquistas e compreenderam meus momentos de ausência. Essa conquista é de vocês e por vocês. Saibam que meu coração sempre esteve presente! Eu amo vocês!

À toda a minha família, meus alunos, meus amigos pelo incentivo e suporte imensurável em toda essa caminhada, foram muitas as mensagens que me lembravam de que eu era forte e conseguiria, mesmo muitas vezes, sem nem terem ideia do que é taxonomia e muito menos o que é *Dryadella*. Em especial, agradeço minhas primas (irmãs) Deise e Iraci que são essenciais para minha vida e aos meus pequenos Giovana, Giordana, Fabrício, Pedro Zion e Geovane...!

Um agradecimento ao Dr. Armando Carlos Cervi (*in memoriam*) por ter me inspirado a seguir na taxonomia e por ter me fortalecido na Botânica! Obrigada por ter deixado a Raphaella e a família em minha vida!

E por último, justamente por saber que quando chegasse aqui meus olhos seriam tomados de lágrimas e eu teria de ser breve, agradeço à minha Joanina que escolheu o meu ventre para evoluir, sua passagem por aqui foi breve, mas meu amor por você não! Aos irmãos dela (Chris e Sofia) e ao pai deles Nelson Fernando H. Selesu, meu parceiro e acima de tudo meu grande amigo, que esteve em um dos momentos mais difíceis que já vivi e suportou a barra, muitas vezes sozinho. Obrigada por segurar a minha mão naquele corredor onde o choro ecoava em dor! Obrigada por tanto! Amo-te!

Mais um ciclo se encerra e sou muito grata por todas as histórias vividas, amizades construídas e experiências adquiridas.

Obrigada!

“Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no mar.

Mas o mar seria menor se lhe faltasse uma gota”.

(Madre Teresa de Calcuta)

RESUMO

Dryadella é um gênero pertencente à subtribo Pleurothallidinae que contém 61 espécies. As espécies de *Dryadella* são epífitas ou raramente rupícolas, cespitosas, raramente reptantes ou pendentes. As inflorescências podem apresentar de 1 a 4 flores sucessivas dispostas em um racemo congesto. Nas flores, as sépalas laterais apresentam um calo espesso próximo à base, pétalas multiangulares e o labelo longo unguiculado com lâmina provida de dois lobos basais, geralmente retrorsos. A distribuição desse gênero é neotropical e disjunta, desde a América Central através das florestas úmidas dos Andes, até o norte da Argentina e do sul ao nordeste do Brasil na Floresta Atlântica. As relações filogenéticas de *Dryadella* foram investigadas usando o espaçador nuclear ITS e a região plastidial matK, analisadas por máxima verossimilhança e máxima parcimônia para inferir as relações evolutivas entre as espécies. Datações moleculares e análises biogeográficas também foram realizadas. Os resultados obtidos indicam que *Dryadella* é monofilético, com alto suporte, inserido na Afinidade *Specklinia*, com provável origem no noroeste da América do Sul, no final do Mioceno (~7 Ma). A primeira diversificação de *Dryadella* ocorreu no início do Plioceno (~5Ma), envolvendo um evento de dispersão e três clados foram identificados. O clado 1 tem origem no noroeste da América do Sul e em seguida dispersão para o Sul da Amazônia e para a Floresta Atlântica. O clado 2 tem origem no noroeste da América do Sul com dispersão para América Central e em seguida dispersão para o sul dos Andes. O clado 3 tem origem no noroeste da América do Sul com dispersão para o Cerrado e para a Mata Atlântica, diversificado em torno de 3 Ma. A elaboração da monografia das espécies brasileiras de *Dryadella* resultou em uma nova espécie, novos sinônimos e novas ocorrências, resultando em 14 espécies reconhecidas para o país. O tratamento taxonômico é apresentado para as espécies brasileiras, incluindo descrições morfológicas, ilustrações, fotografias, chave de identificação e distribuição geográfica. Devido a dificuldade taxonômica encontrada em *D. zebrina*, *D. edwallii* e *D. wuerstlei* durante a revisão taxonômica, dedicamos nossos esforços para explorar morfometricamente populações destes táxons, levantamos as variáveis minuciosamente e tratamos a partir de análises multivariadas a fim de clarear a taxonomia e apresentar uma nova circunscrição para estes táxons. Investigações da micromorfologia dos órgãos vegetativos a partir da anatomia da raiz, rizoma, ramicaule e folha de representantes de *Dryadella* foram descritas com achados inéditos, como ráfides de cristal de oxalato,

ausência de tricomas ou cicatrizes destes na superfície foliar e superfície cuticular verrucosa em algumas espécies.

Palavras-chave: análise filogenética, anatomia vegetativa, taxonomia, monocotiledôneas, nomenclatura, morfometria.

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ABSTRACT

Dryadella is a genus belonging to the subtribe Pleurothallidinae, comprising 61 species. They are epiphytic or rarely rupicolous, cespitose, rarely repent or pending; inflorescence always a single or 2-4 successive flowers in a congested raceme; lateral sepals with thick callus near the base, multi-angular petals and long unguiculated lip with two basal lobes. Exclusively neotropical with disjunct distribution, from Central America through the humid forests of the Andes, to northern Argentina and southern Brazil in the Atlantic Rainforest. *Dryadella* phylogenetic relationships were investigated using nrITS and the *matK* plastid region, analysed for Maximum likelihood and Maximum parsimony to infer evolutionary relationships. Molecular dating and biogeographic analyses were also performed. The results obtained provide high support for *Dryadella* monophyly, inserted in the *Specklinia* Affinity. with probable origin in northwestern South America, at the end of the Miocene (~7Ma). The first diversification of *Dryadella* occurred in the early Pliocene (~5Ma), involving a dispersion event and three clades were identified. Clade 1 originated in northwestern South America and later dispersal to southern Amazonia and a posterior dispersion event for the Atlantic Rainforest. Clade 2 originates from northwest South America with a dispersion event to Central America and subsequent dispersion to the south of the Andes. Clade 3 originates from northwest South America to the Cerrado and the Atlantic Forest, diversified around 3 My. The elaboration of the monograph of the Brazilian species of *Dryadella* resulted in a new species, in addition to new synonyms and new occurrences, a taxonomic treatment is presented for the Brazilian species, including morphological descriptions, illustrations, photographs, identification key and geographic distribution. Due to the taxonomic difficulty found in *D. zebrina*, *D. edwallii* and *D. wuerstlei* found during the taxonomic review, we dedicated our efforts to morphometrically explore populations of these taxa, survey the variables in detail and treat from multivariate analyzes in order to clarify the taxonomy and present a new circumscription for these taxa. Investigations of the micro morphology of vegetative organs from the anatomy of the root, rhizome, ramicaule and leaf of representatives of *Dryadella* were described with unpublished findings.

Keywords: phylogenetic analysis, vegetative anatomy, taxonomy, monocots, nomenclature, morphometry.

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INTRODUÇÃO GERAL

Orchidaceae é uma das maiores e mais diversas famílias de plantas com cerca de 27.800 espécies e cerca de 740 gêneros (POWO, 2022). As orquídeas estão distribuídas por quase todo o mundo, com exceção das regiões desérticas e polares (DRESSLER, 1993), com maior abundância e diversidade nas regiões tropicais e subtropicais. No Neotrópico, a região dos Andes é a área mais rica em orquídeas, seguida da Floresta Atlântica brasileira. O Brasil é um dos centros de diversidade das orquídeas, abrigando 251 gêneros, sendo 24 endêmicos e cerca de 2.680 espécies, das quais 1.490 são endêmicas para o país (FLORA DO BRASIL, 2020).

A família é monofilética, sustentada tanto por dados morfológicos quanto moleculares (DRESSLER, 1981, 1993; FREUDENSTEIN & RASMUSSEN, 1999; FREUDENSTEIN et al., 2000, 2004; PRIDGEON et al., 1999, 2001, 2003, 2005a, 2009, 2014). Apresenta alta diversidade vegetativa e ainda que a estrutura da flor seja uniforme em número e disposição de peças, há uma grande diversidade de tamanho e detalhes estruturais. Com base em dados moleculares, Orchidaceae é dividida em cinco subfamílias: Apostasioideae, Cyripedioideae, Vanilloideae, Orchidoideae e Epidendroideae, sendo essa última a mais representativa, com aproximadamente 18.000 espécies em 650 gêneros, com distribuição cosmopolita (PRIDGEON et al., 1999, 2001, 2003, 2005a, 2009). Epidendroideae, por sua vez, é subdividida em 16 tribos, entre elas Epidendreae, constituída de 6 subtribos: Bletiinae Benth, Chysinae Schlechter, Coeliinae Dressler, Laeliinae Benth, Poneriinae Pfitzer e Pleurothallidinae Lindley (PRIDGEON et al., 2005a; CHASE et al., 2015).

A subtribo Pleurothallidinae é exclusivamente Neotropical, com aproximadamente 5.100 espécies distribuídas em 44 gêneros e representa cerca de 20% das espécies de toda a família Orchidaceae (HIGGINS & WILLIAMS, 2009; KARREMANS, 2016). É um grupo monofilético com alto suporte e posicionado na tribo Epidendreae (CHASE et al., 2015), caracterizada por ervas de crescimento simpodial, unifoliadas com o caule secundário (ramicaule) não pseudobulboso, e a inflorescência apresenta uma articulação associada a uma zona de abscisão entre o ovário e o pedicelo (LUER, 1986; DRESSLER, 1993; PRIDGEON, 2005a). Suas flores possuem características de miofilia, como por exemplo, o tamanho reduzido e a forma radial, e ainda o labelo que pode conter manchas, pequenas projeções, fendas e lóbulos fusionados (van der PIJL;

DODSON, 1966; LUER, 1986; NEYLAND et al., 1995; PRIDGEON et al., 2005a; KARREMANS et al., 2015). Muitas espécies de Pleurothallidinae apresentam estruturas com função de atração de polinizadores através de secreção de fragrâncias, que podem ser encontradas no labelo, pétalas e/ ou sépalas (DRESSLER, 1993; PRIDGEON; STERN, 1985; SCHIESTL et al., 2003; SCHIESTL & JOHNSON, 2013; CARDOSO-GUSTAVSON et al., 2017) e nectários também já foram identificados dentro da subtribo como atrativos para os polinizadores oferecendo recompensa (BARBOSA et al., 2009; MELO et al., 2010).

Em Pleurothallidinae esses estudos demonstraram que a subtribo tem uma das maiores taxas de diversificação de espécies dentro de Orchidaceae, e que se originou no Mioceno Inferior (~20 Ma) (GIVNISH et al., 2015; PÉREZ-ESCOBAR et al., 2017). Uma recente atualização sobre as relações filogenéticas de Pleurothallidinae foi realizada por Karremans (2016), através de um compilado de estudos baseados em filogenia molecular que apontam para a existência de nove grupos ou afinidades.

Dryadella Luer, um dos gêneros reconhecidos em Pleurothallidinae e foco deste estudo, encontra-se posicionado na afinidade *Specklinia*, que inclui ainda os gêneros *Andinia* Luer, *Muscarella* Luer, *Platystele* Schltr., *Scaphosepalum* Pfitzer, *Specklinia* Lindl. e *Teagueia* Luer (Karremans, 2016; Karremans et al., 2016).

Dryadella foi estabelecido por Luer (1978a), a partir de algumas espécies desmembradas do gênero *Masdevallia* Ruiz & Pav., porém as primeiras espécies haviam sido descritas no gênero *Pleurothallis* R. Br. e, em seguida, alocadas no gênero *Masdevallia* seção *Saltatrices* Luer, (2005) e posteriormente em *Masdevallia* subg. *Trigonanthe* Schltr., seção *Rhombopelalae* Schltr., exceto *M. lilliputiana* inserida na seção *Floribundae* Kraenzl (Luer, 2005). Após o estabelecimento do novo gênero, as espécies de *Masdevallia* correspondentes foram transferidas por Luer (1978a, 1978b, 2005) e ao longo destes anos, novos táxons foram descritos e novos sinônimos foram propostos, somando atualmente 61 nomes aceitos (IMIG et al., 2021; POWO, 2022) (Tabela 01).

A distribuição geográfica de *Dryadella* é disjunta com duas grandes áreas de ocorrência, a Floresta Atlântica do Brasil e a região Andina (PRIDGEON et al., 2005b). No Brasil, o gênero até o momento está representado por 18 espécies, destas, 15 são endêmicas do país e 14 restritas à Floresta Atlântica. Os outros representantes são encontrados na

Amazônia (*Dryadella osmariniana* e *D. cardosoi*) e Cerrado (*Dryadella ana-paulae*) (Pridgeon, 2005b; IMIG et al. 2020; POWO 2022).

Tabela 1. Listagem e os países de ocorrência das espécies de *Dryadella* aceitas antes dos nossos estudos.

Espécies	Ocorrência
<i>Dryadella albicans</i> (Luer) Luer	Ecuador
<i>Dryadella ana-paulae</i> V.P.Castro, BPFaria & ADSantana	Brasil e Bolívia
<i>Dryadella ataleiensis</i> Campacci	Brasil
<i>Dryadella aurea</i> Luer e Hirtz	Peru e Ecuador
<i>Dryadella auriculigera</i> (Rchb.f.) Luer	Brasil
<i>Dryadella aviceps</i> (Rchb.f.) Luer	Brasil e Paraguai
<i>Dryadella barrowii</i> Luer	Ecuador
<i>Dryadella butcheri</i> Luer	Paraná
<i>Dryadella cardosoi</i> Campacci e JBFSilva	Brasil
<i>Dryadella clavellata</i> Luer e Hirtz	Ecuador
<i>Dryadella crassicaudata</i> Luer	Peru e Colômbia
<i>Dryadella crenulata</i> (Pabst) Luer	Brasil
<i>Dryadella cristata</i> Luer & R.Escobar	Colômbia
<i>Dryadella cuspidata</i> Luer e Hirtz	Colômbia e Ecuador
<i>Dryadella dodsonii</i> Luer	Colômbia e Ecuador
<i>Dryadella dressleri</i> Luer	Panamá
<i>Dryadella edwallii</i> (Cogn.) Luer	Brasil
<i>Dryadella elata</i> (Luer) Luer	Ecuador
<i>Dryadella espirito-santensis</i> (Pabst) Luer	Brasil
<i>Dryadella fuchsii</i> Luer	Costa Rica, El Salvador, Honduras e Nicarágua
<i>Dryadella gnoma</i> (Luer) Luer	Colômbia, Costa Rica, Ecuador, Panamá, Peru
<i>Dryadella gomes-ferreirae</i> (Pabst) Luer	Brasil
<i>Dryadella greenwoodiana</i> Soto Arenas, Salazar e Solano	México e Guatemala
<i>Dryadella guatemalensis</i> (Schltr.) Luer	Colômbia, Costa Rica, Guatemala, México, Panamá
<i>Dryadella hirtzii</i> Luer	Ecuador
<i>Dryadella kautskyi</i> (Pabst) Luer	Brasil
<i>Dryadella krenakiana</i> Campacci	Brasil
<i>Dryadella lilliputiana</i> (Cogn.) Luer	Bolívia e Brasil
<i>Dryadella linearifolia</i> (Ames) Luer	Belize, Costa Rica, Guatemala, Honduras, México e Nicarágua

<i>Dryadella litoralis</i> Campacci	Brasil
<i>Dryadella lueriana</i> Carnevali e G.A.Romero	Venezuela
<i>Dryadella marilyniana</i> Luer	Equador
<i>Dryadella marsupiat</i> Luer	Equador e Peru
<i>Dryadella meiracyllium</i> (Rchb.f.) Luer	Equador
<i>Dryadella minuscula</i> Luer & R.Escobar	Colômbia e Peru
<i>Dryadella mocoana</i> Luer & R.Escobar	Colômbia
<i>Dryadella nasuta</i> Luer e Hirtz	Equador
<i>Dryadella nortonii</i> Luer	Bolívia
<i>Dryadella odontostele</i> Luer	Colômbia, Costa Rica e Panamá
<i>Dryadella osmariniana</i> (Braga) Garay & Dunst.	Brasil
<i>Dryadella pachyrhiza</i> Luer e Hirtz	Equador
<i>Dryadella perpusilla</i> (Kraenzl.) Luer	Peru
<i>Dryadella pusiola</i> (Rchb.f.) Luer	Colômbia e Equador
<i>Dryadella rodrigoii</i> Luer	Colômbia
<i>Dryadella sapucaiensis</i> Campacci & S.L.X.Tobias	Brasil
<i>Dryadella simula</i> (Rchb.f.) Luer	Colômbia e Peru
<i>Dryadella sororcula</i> Luer	Panamá
<i>Dryadella speculifera</i> Vierling	Desconhecido
<i>Dryadella sublata</i> Luer & J.Portilla	Peru
<i>Dryadella summersii</i> (L.O.Williams) Luer	Equador
<i>Dryadella susanae</i> (Pabst) Luer	Brasil
<i>Dryadella toscanoi</i> Luer	Brasil
<i>Dryadella vasquezii</i> Luer	Bolívia
<i>Dryadella verrucosa</i> Luer & R.Escobar	Colômbia
<i>Dryadella vitorinoi</i> Luer e Toscano	Brasil
<i>Dryadella wernerii</i> Luer	Equador
<i>Dryadella wuerstlei</i> Luer	Brasil
<i>Dryadella xavieriana</i> Campacci e C.R.M.Silva	Brasil
<i>Dryadella yupanki</i> (Luer & R.Vásquez) Karremans	Bolívia
<i>Dryadella zebrina</i> (Porsch) Luer	Bolívia, Brasil, Colômbia e Peru

Dryadella são ervas epifíticas ou raramente litófitas, cespitosas, raramente curtas reptantes, com rizoma muito abreviado ou inconspícuo. Possuem ramicaule abreviado, geralmente ereto e sempre unifoliolados, coberto total ou parcialmente por 2(3) bainhas paleáceas; folhas eretas, raramente adpressas, planas, semi-teretes ou teretes. As inflorescências que emergem lateralmente ao ramicaule, logo abaixo do ponto de abscisão, são racemosas de flor única ou até 4 flores sucessivas, pedúnculos curtos e envoltos por 2(3) brácteas paleáceas, com pedicelos inconspicuos. As flores são ressupinadas com ovário tri-alado; a sépala a dorsal é livre, inflexa ou raramente

deflexa; as sépalas laterais são parcialmente conectadas na base formando tubo sepalínico com ápice caudado; as pétalas são geralmente inconspícuas e inseridas no tubo sepalínico, geralmente em formato sagitado; labelo é unguiculado, bilobado na base e articulado com o ápice do pé da coluna, geralmente com 2 calos próximos a base; a coluna é alada a partir da metade distal com 2 políneas (LUER, 1978a; LUER, 2005) (Figura 1 e 2).

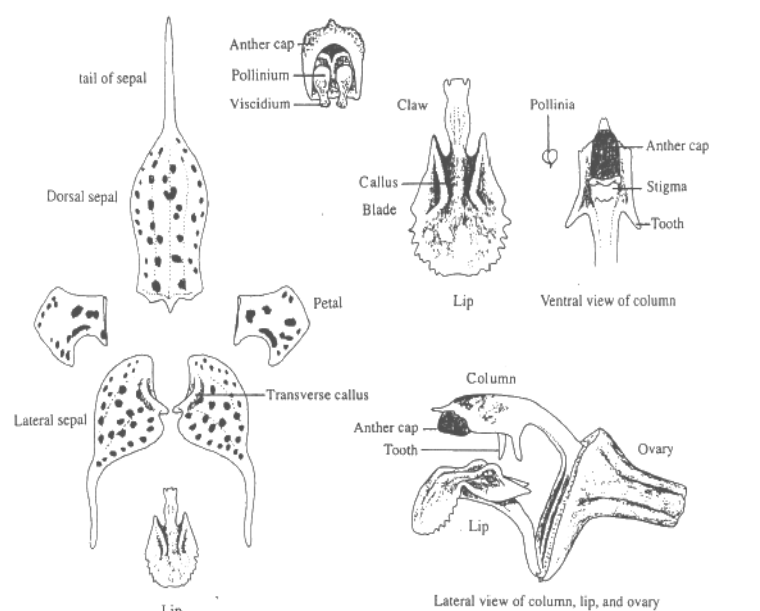


Figura 1– Terminologias morfológicas gerais da flor de *Dryadella*. Fonte: LUER (2005).

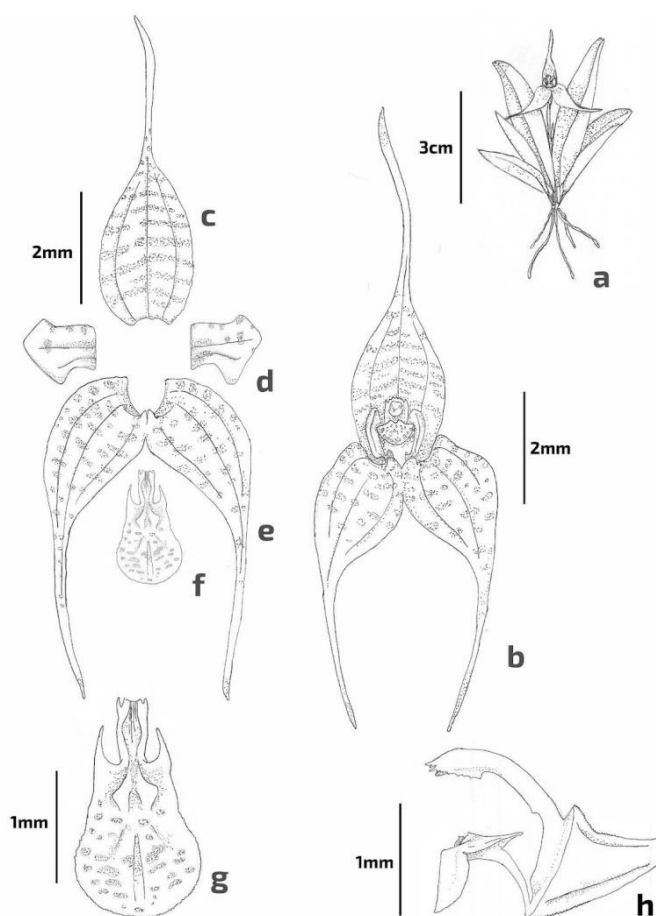


Figura 2: Aspecto morfológico geral de *Dryadella*. a. Hábito. b-f. Aspectos gerais da flor. g. Aspectos do labelo. h. Aspectos gerais do labelo.

Embora uma revisão taxonômica completa para *Dryadella* tenha sido realizada por Luer (2005), poucas espécies e amostras brasileiras foram analisadas e algumas lacunas na taxonomia do gênero permanecem o que reflete em errôneas identificações ou na falta de identificações em herbários e coleções científicas, em coleções particulares e entre os orquidófilos. Apesar de apresentar distribuição disjunta, as espécies do gênero apresentam elevada similaridade entre os táxons e por vezes, sutil diferenças no tamanho e forma das pétalas, sépalas, labelo e no tamanho das folhas são utilizadas em Luer (2005) para diagnóstico das espécies, no entanto, isso parece não contribuir suficientemente na delimitação de algumas espécies. Por outro lado, nenhuma abordagem filogenética para entender as relações entre as espécies do gênero ou abordagens biosistemáticas que possibilitem a melhor delimitação até o momento foi empregada para gênero.

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

Ferramentas estatísticas e consolidação da sistemática filogenética como um método para estabelecer relações de parentesco entre organismos, os estudos em sistemática têm apresentado cada vez mais a abordagem filogenética e evolutiva na interpretação dos padrões morfológicos, de distribuição geográfica e delimitações taxonômicas (Avice, 2000; Felsenstein, 2004) e, quando aliado aos métodos biogeográficos, permite inferir padrões de diversificação das linhagens de plantas atuais e a origem desses padrões (Morrone, 2006; Pennington et al., 2006).

Neste sentido, a Floresta Atlântica brasileira apresenta três espécies de alta complexidade taxonômica: *D. zebrina* (Porsch) Luer, *D. edwallii* (Cogn.) Luer e *D. wuerstlei* Luer. A morfometria é uma abordagem que pode indicar variação fenotípica potencial dentro de complexos de espécies (Pinheiro et al., 2018) por meio de medidas de diferenças ou semelhanças morfológicas entre as unidades amostradas (Sokal & Michener 1958). Análises morfométricas multivariadas são frequentemente associadas a estudos populacionais como auxílio na delimitação de espécies ou morfotipos, pois podem revelar a ocorrência de discontinuidades em padrões de variação, a princípio, aparentemente contínuos (Oliveira et al., 2008).

Considerando a distribuição disjunta e a similaridade morfológica entre as espécies do gênero, uma abordagem anatômica pode evidenciar caracteres de interesse taxonômico, auxiliando a delimitação das espécies ou grupos disjuntos, e na caracterização anatômica do gênero. Estas características anatômicas podem ser úteis para o reconhecimento de grupos com histórias evolutivas distintas (Aybeke, 2012), corroborando ou não a classificação infragenérica proposta por Luer (2005).

Com a finalidade de esclarecer lacunas taxonômicas, as hipóteses filogenéticas do gênero e a identidade de morfotipos endêmicos da Floresta Atlântica, propomos aqui diferentes abordagens biosistemáticas.

Para tanto, este trabalho tem como objetivo geral investigar as relações filogenéticas e identificar os padrões biogeográficos do gênero baseado em caracteres moleculares e o estudo da distribuição geográfica e taxonomia do gênero.

Precisamente: (i) testar o monofiletismo de *Dryadella*, utilizando o maior número de espécies possíveis, empregando análises com base em dados moleculares; (ii) dada a distribuição disjunta do gênero entre a região andina e a Floresta Atlântica, caracterizar os padrões de distribuição geográfica e datação molecular de *Dryadella*; (iii) realizar um levantamento florístico e tratamento taxonômico das espécies do gênero para o Brasil; (iv) propor sinônimos e/ou novos táxons e novos registros de ocorrência para o Brasil; (v) Abordar morfometricamente táxons de complexidade taxonômica ocorrentes na Floresta Atlântica; (vi) investigar caracteres morfológicos e anatômicos vegetativos e sua importância para a sistemática do gênero.

Dessa forma, esta tese está disposta em capítulos e cada um deles segue as normas do periódico em que foi ou será publicado e estão dispostos da seguinte forma:

CAPÍTULO I: Evidências filogenéticas e biogeográficas de origem Mioceno no norte da América do Sul e diversificação recente de *Dryadella* (Orchidaceae) na Floresta Atlântica

CAPÍTULO II: O gênero *Dryadella* (Orchidaceae, Pleurothallidinae) no Brasil

CAPÍTULO III: Atualização taxonômica em *Dryadella* (Orchidaceae: Pleurothallidinae) baseada em análises morfométricas de três espécies endêmicas da Floresta Atlântica

CAPÍTULO IV: Uma nova *Dryadella* Luer (Orchidaceae: Pleurothallidinae) da Floresta Atlântica Sul

CAPÍTULO V: Anatomia vegetativa e sua importância sistemática na *Dryadella* Luer (Orchidaceae: Pleurothallidinae)

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CAPÍTULO I / CHAPTER I

Evidências filogenéticas e biogeográficas para origem de *Dryadella* (Orchidaceae) no Mioceno no norte da América do Sul e diversificação recente na Floresta Atlântica

(Artigo formatado seguindo as normas do periódico *Botanical Journal of the Linnean Society*)

Phylogenetic and biogeographic evidence for a Miocene origin in north South-America and recent diversification of *Dryadella* (Orchidaceae) in Atlantic Rainforest

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Running title: Phylogenetics of *Dryadella*

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Abstract

Dryadella, a small orchids genus in the subtribe Pleurothallidinae with 61 species, are usually epiphytes, with long-repent or caespitose growth; inflorescence always a single or 2-4 successive flowered in congest raceme. Flowers with a thickened callus close to the base of the lateral sepals, multi-angled petals, and a long unguiculate lip with a blade provided with two basal, usually retrorse, lobes. It is distributed from Central America through the Andes to southeastern Brazil, with a disjunct distribution between coastal forests. Thirty species of *Dryadella* plus related genera and outgroup taxa were investigated using molecular nrITS and plastid matK, analysed using maximum likelihood and maximum parsimony to infer evolutionary relationships. Molecular dating and biogeographical analyses were also performed. Results obtained provide strong support for a monophyletic *Dryadella* and origin through the end of the Miocene (~7 Ma) in the Pacific domain, northwest of South America and Central America. The three clades identified by the analysis diversified at the beginning of the Pliocene (~5Ma). The first clade has a Pacific dominion origin with posterior dispersion to South Brazilian dominion and to Paraná dominion (Atlantic Rainforest) in the Quaternary period (~2.2Ma). The second clade also has a Pacific dominion origin, but with a dispersion event to Mexican and Mesoamerica dominion and to the South America Transition zone (south Andes). The last clade has a Pacific dominion origin with posterior dispersion by Chacoan dominion to Atlantic Rainforest. The Atlantic clade is the youngest and diversified around 3 Ma with one recent dispersion event to the Pacific dominion (~1Ma). The findings from this study contribute to a better understanding of the evolution of *Dryadella*, the non-monophyly of the proposed sections, based mainly on plant size, and the evidence of the historical connection of the coastal forests of South America. Also, for Atlantic Rainforest, two independent lineages of *Dryadella* diversified in the Quaternary period.

ADDITIONAL KEYWORDS: Atlantic Rainforest –Andes – biogeography – dispersion – Neotropical flora – orchid molecular phylogenetics

INTRODUCTION

Orchidaceae are one of the largest angiosperm families, arranged in five subfamilies and placed in 736 genera with over 27,000 species described (Chase *et al.*, 2015). Occur on all continents except Antarctica and in all biomes, except for true deserts (Givnish *et al.*, 2016). Most species are found in tropical forests, and over 80% are epiphytes (Givnish *et al.*, 2015). For the Americas, Orchidaceae are the most diverse family, including 12,983 species, and are the most diverse epiphyte plants in the tropical Andean countries (Ulloa Ulloa *et al.*, 2017).

Adaptation to epiphytic habit, the evolution of pollinaria and the development of different pollination mechanisms are the main factors that promoted the rapid diversification of the family, especially in tropical mountain regions (Tremblay *et al.*, 2004; Givnish *et al.*, 2015). In the American continent, this diversification is related to the uplift of the Andes 15 Mya and subsequent speciation events in Central America and the Atlantic Forest (ARF) (Givnish *et al.*, 2016; Pérez-Escobar *et al.*, 2017; Rodrigues *et al.*, 2017; Salazar *et al.*, 2018; Smidt *et al.*, 2018).

The subtribe Pleurothallidinae is the most diverse Neotropical group in all orchid family, including about 20% of all known species (Higgins and Williams, 2009; Karremans *et al.*, 2016). With ca 5100 species currently accepted in 44 genera, the subtribe is the most representative element of the Andean orchid flora (Pridgeon *et al.*, 2009; Kolanowska, 2014). Has about 20 My (millions of years) of age and one of the highest species diversification rates in the Orchidaceae (Givnish *et al.*, 2015; Pérez-Escobar *et al.*, 2017).

Several recent phylogenetic studies focused on the Pleurothallidinae have confirmed its monophyly (e.g. Pridgeon *et al.*, 2001; Pridgeon & Chase, 2001; Pridgeon & Chase, 2003; Karremans, 2016; Pérez-Escobar *et al.*, 2017) and informally nine genera “affinities” were proposed by Karremans (2016). However, there is still a large gap of studies to clarify the systematic placement of some lineages within this huge subtribe (e.g. Gutiérrez Morales *et al.*, 2020; Smidt *et al.*, 2020). *Dryadella* Luer belongs to the Affinity *Specklinia* together with *Andinia* Luer, *Muscarella* Luer, *Platystele* Schltr., *Scaphosepalum* Pfitzer, *Specklinia* Lindl. and *Teagueia* Luer (Karremans, 2016; Karremans *et al.*, 2016). The genus is characterised by the presence of a callus near the base of the lateral sepals and the unguiculated lip with retroverted basal lobules. These were the main characteristics used by Luer (1978) to propose the genus of some

dismembered species from the genus *Masdevallia* Ruiz & Pav., and some of which were previously treated in *Pleurothallis* R.Br. Currently *Dryadella* is composed of about 61 species (Luer, 2005; POWO 2022; Imig *et al.*, 2021) occurring from Mexico to South Brazil. The genus presents distribution is disjunct, especially between the Andean region and the ARF, except *D. ana-paulae* V.P. Castro, B.P. Faria & A. D. Santana, found in gallery forests of the South American Dry Diagonal, and *D. gnoma* (Luer) Luer, which occurs in the Amazon Rainforest (Pridgeon, 2005; Imig *et al.*, 2020). The ARF hosts 14 species of the genus, of which only two are not endemic to the biome: *D. zebrina* (Porsch) Luer and *D. lilliputiana* (Cogn.) Luer, those also occur in the Andes, where other South American species are restricted (Pridgeon, 2005; Luer, 1978, 2005; Imig *et al.*, 2020).

The species delimitation in *Dryadella* is difficult due to the remarkable morphological similarity between the species, even between groups of disjunct areas (Luer, 1978; Pridgeon, 2005). Luer (2005) proposed the division of the genus into three sections, joining the species with linear leaves “The needle leaves”, the species with diminutive habit “The tiny mites”, and the other species “All the rest”. Recent studies on the leaf anatomy of several species from different biomes showed that the variation found does not support the delimitation of sections (Imig *et al.*, 2020b).

Neotropical rainforests are currently not continuous, actually are separated by open vegetation with a diagonal distribution and a drier climate zone (Werneck, 2011). The Seasonally Dry Tropical Forests or Caatinga (northeastern Brazil) and the Cerrado savanna (central Brazil) separate the Atlantic Forest from Amazon; whereas the Chaco (northeastern Argentina, western Paraguay and south-eastern Bolivia) divides the Atlantic Forest from the Andean Forest (Werneck, 2011; Turchetto-Zolet *et al.*, 2012), and this disjunction may contribute as a barrier to gene flow between these rainforest regions. One of the fundamental questions about the biodiversity of a region is how this is distributed across major vegetation types. For this reason, identifying where and how orchid species are distributed could improve our understanding to elucidate the evolutionary history of the family (Hou *et al.*, 2017). For this, the ages available for the subtribe can be used to analyse molecular data on a smaller scale, allowing the study of the evolutionary dynamics of a group of species or genus, hypothesising events that allowed its diversification (Forest, 2009).

In this sense, our study mainly focuses on testing the monophyly and phylogenetic relationships of the genus *Dryadella*, the relationships within *Specklinia* affinity and whether molecular analyses support the proposed infrageneric relationships based on morphological data. Reconstructing the phylogeny, we seek to apply biogeographic principles and analysis to summarise geographic distribution patterns, their times of diversification, and arrivals to the Atlantic Rainforest. To do this, we collected DNA sequence data from nrITS (ITS1, 5.8S, ITS2) and plastid *matK* from a large number of taxa of the genus and related groups.

MATERIAL AND METHODS

TAXON SAMPLING

Based on previous phylogenetic studies in the subtribe Pleurothallidinae (Pridgeon *et al.*, 2001; Karremans, 2016; Karremans *et al.*, 2016; Pérez-Escobar *et al.*, 2017), we included all accepted genera of *Specklinia* affinity: *Andinia*, *Platystele*, *Scaphosepalum*, *Specklinia*, *Phloeophila* and *Muscarella* (Pérez-Escobar *et al.*, 2017), as ingroup to investigate the monophyly of the genus. A sampling of 16 other genera of subtribe Pleurothallidinae, in addition to species from closely related subtribes Bletiinae and Laeliinae [according to the phylogenetic proposal of Chase *et al.*, (2015)], were used to accommodate molecular dating calibrations better. Vouchers were deposited in the Herbarium of the Botany Department at the Federal University of Paraná (UPCB), Herbarium Rioclarense (HRCB) and Marie Selby Botanical Gardens Herbarium (SEL) (acronyms following Thiers 2022). Voucher information and GenBank accessions for the species are given in Table 1. Some species of *Dryadella* that were used in this study are shown in figure 1.

Table 1. Species sampled, GenBank (NCBI) accession numbers and geographical distribution (sensu Morrone 2014): A, Mexican transition zone; B, Mesoamerican dominion; C, Pacific Ocean dominion; D, Boreal Brazilian dominion; E, South Brazilian dominion; F, Paraná dominion; G, South American transition zone and H, Chacoan dominion (Cerrado – Caatinga) and I, Antillean subregion. *indicates new sequences generated for this study.

Species	ITS	matK	Biome
<i>Anathallis gert-</i>	MN3323458	MN332524	F

hatschbachii (Hoehne) Pridgeon & M.W. Chase

<i>Anathallis obovata</i> (Lindl.) Pridgeon & M.W. Chase	MN332358	MN332534	ABCD FG
<i>Andinia pensilis</i> (Schltr.) Luer	KP012344	KP012517	C
<i>Barbosella australis</i> (Cogn.) Schltr.	MF669943.1	MF669949	F
<i>Bletia catenulata</i> Ruiz & Pav.	MN332374	MN332549	CDEFG
<i>Brachionidium restrepioides</i> (Hoehne) Pabst	MN332375	KX686526	F
<i>Brachionidium valerioi</i> Ames & C. Schweinf.	AF262913	AF265488	B
<i>Cattleya coccinea</i> Lindl.	AY008646	MN332552	
<i>Cattleya forbesii</i> Lindl.	MN332376	MN332552	F
<i>Dilomilis montana</i> (Sw.) Sumemrh.	AF262915	AF263765	H
<i>Dryadella albicans</i> (Luer) Luer	KC425742	KC425863	G
<i>Dryadella anapaulae</i> V. P. Castro, B.P. Faria & A. D. Santana	D.C. Imig 666*	D.C. Imig 666*	H
<i>Dryadella aurea</i> Luer & Hirtz	A.L.V. Toscano de Brito 3817*	—	C
<i>Dryadella auriculigera</i> (Rchb.f.) Luer	D.C. Imig 471*	D.C. Imig 471*	F
<i>Dryadella aviceps</i> (Rchb.f.) Luer	D.C. Imig 397*	D.C. Imig 397*	F
<i>Dryadella barrowii</i> Luer	D.C. Imig 635*	—	G
<i>Dryadella catharinensis</i> Imig, Mancinelli & E.C. Smidt	D.C. Imig 656 *	D.C. Imig 656*	F
<i>Dryadella crenulata</i> (Pabst) Luer	D.C. Imig 667*	D.C. Imig 667 *	F
<i>Dryadella cristata</i> Luer & R. Escobar	D.C. Imig 399*	D.C. Imig 399*	C
<i>Dryadella elata</i> (Luer) Luer	A. L. V. Toscano de Brito 3022 (SEL)	—	C
<i>Dryadella fuchsii</i> Luer	KY988820	KY988636	AB
<i>Dryadella gnoma</i> (Luer) Luer	A.Krahl 1477*	A.Krahl 1477*	CD
<i>Dryadella guatemalensis</i> (Schltr.) Luer	KC425743	—	ABCD
<i>Dryadella hirtzii</i> Luer	D.C. Imig 505*	D.C. Imig 505*	G
<i>Dryadella kautskyi</i> (Pabst) Luer	A.L.V. Toscano de Brito 3476*	A.L.V. Toscano de Brito 3476*	F
<i>Dryadella krenakiana</i> Campacci	D.C. Imig 662*	D.C. Imig 662*	F
<i>Dryadella linearifolia</i> (Ames) Luer	A.L.V. Toscano de Brito 3435*	A.L.V. Toscano de Brito 3435*	AB

<i>Draydella lilliputiana</i> (Cogn.) Luer	D.C. Imig 395*	D.C. Imig 395*	F
<i>Dryadella odontostele</i> Luer	-	JQ771574.1	C
<i>Dryadella pachyrhiza</i> Luer & Hirtz	D.C. Imig 507*	D.C. Imig 507*	C
<i>Dryadella pusiola</i> (Rchb.f.) Luer	D.C. Imig 360*	D.C. Imig 360*	CG
<i>Dryadella simula</i> (Rchb.f.) Luer	D.C. Imig 430*	D.C. Imig 430*	CG
<i>Dryadella summersii</i> (L.O. Williams) Luer	D.C. Imig 481*	D.C. Imig 481*	G
<i>Dryadella susanae</i> (Pabst) Luer	D.C. Imig 491*	D.C. Imig 491*	F
<i>Dryadella verrucosa</i> Luer & Escobar	RinconGonzales 954*	RinconGonzales 954*	C
<i>Dryadella vitorinoi</i> Luer	D.C. Imig 506*	D.C. Imig 506*	F
<i>Dryadella toscanoi</i> Luer	D.C. Imig 661*	D.C. Imig 661*	F
<i>Dryadella zebrina</i> (Porsch) Luer	E.C. Smidt 1011*	E.C. Smidt 1011*	F
<i>Dryadella wuerstley</i> Luer	D.C. Imig 408*	D.C. Imig 408*	F
<i>Dryadella yupanki</i> (Luer & R. Vásquez) Karremans	KF747776	KP012498	E
<i>Epidendrum armeniacum</i> Lindl.	MN332381	MN332557	CEFG
<i>Epidendrum tridactylum</i> Lindl.	MN332382	MN332558	CEFG
<i>Lepanthes helicocephala</i> Rchb.f.	MN332387	MN332560	CDE
<i>Lepanthopsis floripecten</i> (Rchb.f.) Ames	MN332388	MN332561	BCDFG
<i>Madisonia bradei</i> (Schltr.) Toscano & E.C. Smidt	M. Bolson 565	M. Bolson 565	F
<i>Madisonia carrisii</i> (Brade) Toscano & ECSmidt	M. Bolson 572	M. Bolson 572	F
<i>Madisonia kerri</i> (Braga) Luer	MN332389	MN332562	D
<i>Myoxanthus lonchophyllus</i> (Barb.Rodr.) Luer	MN332390	MK642625	F
<i>Myoxanthus punctatus</i> (Barb. Rodr.) Luer	KX686538	MK642626	F
<i>Muscarella catoxys</i> (Luer & Hirtz) Luer	KY988912	KY988728	C
<i>Muscarella schudelii</i> (Luer & Hirtz) Luer	KY988914	KY988730	C
<i>Neocogniauxia hexaptera</i> Schltr.	AF260148	AF263766	A
<i>Octomeria gracilis</i> Lodd. ex Lindl.	MN332392	KX686527	F
<i>Octomeria grandiflora</i> Lindl.	WSM1372	KX686528	F
<i>Phloeophila pleurothallopsis</i> (Kraenzl.) Pridgeon & M.W. Chase	KC425747	KP012496	C
<i>Pabstiella mirabilis</i> (Schltr.) Brieger &	MN332394	MN332565	F

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<i>Pabstiella versicolor</i> (Porsh) Luer	MN551470	MN577779	F
<i>Pabstiella villosisepala</i> L. Kollmann & Fraga	MN551471	MN577780	F
<i>Pabstiella yauaperyensis</i> (Barb. Rodr.) F. Barros	MN332395	MN332566	DE
<i>Platystele misera</i> (Lindl.) Garay	KF747784	KP012389	CDG
<i>Platystele oxyglossa</i> (Schltr.) Garay	WSM1333	WSM1333	BCDEFG
<i>Pleurothallis isthmica</i> Luer	MN332398	MN332569	BC
<i>Pleurothallopsis nemorosa</i> (Barb. Rodr.) Porto & Brade	MN332400	KX686529	F
<i>Platystele stenostachya</i> (Rchb.f.) Garay	KC425758	JQ771571	ABCDEG
<i>Restrepiella ophioccephala</i> H. Karst	MN332403	MN332574	BC
<i>Scaphosepalum bicristatum</i> Luer	D.C. Imig 523*	D.C. Imig 523*	C
<i>Scaphosepalum microdactylum</i> Rolfe	A.L.V. Toscano de Brito 2957*	A.L.V. Toscano de Brito 2957*	BC
<i>Specklinia corniculata</i> (Sw.) Mutel	KF747803	KP012402	ABCI
<i>Specklinia grobyi</i> (Bateman ex Lindl.) Pridgeon & M.W.Chase	MN332406	MN332577	ABCDEFG
<i>Stelis argentata</i> Lindl.	MN332408	MN332579	BCDEFG
<i>Stelis ciliaris</i> Lindl.	MN332409	MN332580	BCEF
<i>Stelis grandiflora</i> Lindl.	MN332410	MN332581	DF
<i>Stelis ruprechtiana</i> Rchb.f.	MN332412	MN332583	F

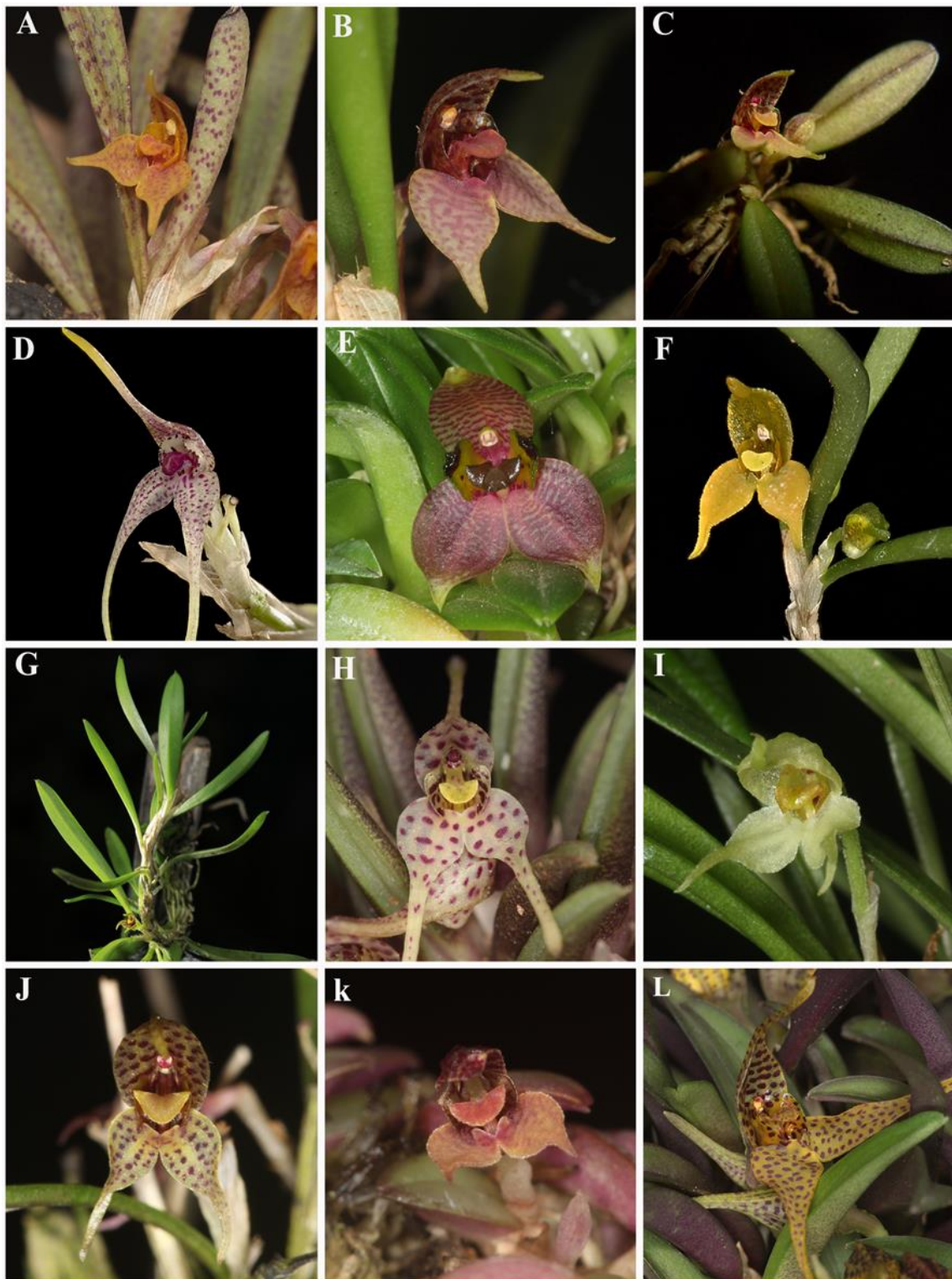


Figure 1. Species of *Dryadella* in this study. A, *Dryadella auriculigera*. B, *Dryadella aviceps*. C, *Dryadella catharinensis*. D, *Dryadella crenulata*. E, *Dryadella cristata*. F, *Dryadella kautskyi*. G, *Dryadella krenakiana*. H, *Dryadella lilliputiana*. I, *Dryadella pachyrhyza*. J, *Dryadella summersii*. K, *Dryadella susanae*. L, *Dryadella zebrina*. Photographs by Eric C. Smidt, except C by Werner Mancinelli and D and F by A.L.V. Toscano de Brito.

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Total genomic DNA was extracted from fresh-frozen leaves or leaves stored in CTAB solution (Rogstad, 1992, adapted) using the 2×CTAB protocols (Doyle & Doyle, 1987), without the addition of RNase A and scaled to 2 mL microtubes. All total DNA samples were stored in a –80 °C ultra-freezer and amplified through polymerase chain reaction (PCR) for fragments of interest. The nuclear ribosomal internal transcribed spacer (nrITS) was targeted with primer pairs 17SE (ACGAATTCATGGTCCGGTGAAGTGTTTCG) + 26SE (TAGAATTCCCCGGTTCGCTCGCCGTTAC) (Sun et al., 1994) and 75 (TATGCTTAAACTCAGCGGG) + 92 (AAGGTTTCCGTAGGTGAA) (Desfeux & Lejeune, 1996). Amplification of nrITS were performed with the Invitrogen Platinum Taq DNA polymerase (Thermo Fisher Scientific) PCR kit following the manufacturer's recommendations, adding 0.08 mg/mL of bovine serum-albumin (BSA), 0.08% dimethyl sulphoxide (DMSO), 1M betaine, 0.2 µM of each primer and 20–50 ng of total DNA for a final volume of 20 µL. For plastid DNA regions, the Top Taq Master Mix (Qiagen) PCR kit was used following the manufacturer's recommendations, adding 0.2 µM each primer and 20–50 ng of total DNA for a final volume of 20 µL. For both PCR methods, the thermocycling program was: 1 min pre-melt at 94 °C, 40 cycles of 30 s of denaturation at 94 °C, 40 s of annealing at 51 °C (nrITS)/53 °C (plastid DNA) and 30 s of extension at 72 °C, ending with a 5-min final extension at 72 °C.

The plastid markers, matK exon, were amplified with the respective primer pairs: 19F (5' CGTTCT GAC CAT ATT GCA CTA TG 3') + 881R (5' TMTTCA TCA GAA TAA GAG Tn3') (Gravendeel et al., 2001) and 2.1f (5' CCTATCCATCTGGAAATCTTAG 3') and matK 5R (5' GTTCTAGCACAAGAAAGTCG 3'; Ford et al., 2009). The Amplification was performed in 20 µL mixtures containing 1× CoralLoad buffer, 0.2 µM each primer, 1× TopTaq Master Mix and 20–50 ng of genomic DNA using the TopTaq Master Mix kit (QIAGEN Biotechnology). The thermocycler was programmed for an initial pre-melt at 94 °C for 1 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 51 °C (nrITS)/53 °C (matK) for 40 s and extension at 72 °C for 40 s, followed by a final extension for 5 min at 72 °C. All the PCR products were visualised with electrophoresis in agarose gel with GelRed (Crisafuli et al., 2015), purified with polyethylene glycol (PEG 20%) and 80% ethanol (Paithankar & Prasad,

1991), and sequenced with Big Dye Terminator version 3.1 (Applied Biosystems, Foster City, CA, USA).

DNA SEQUENCES ALIGNMENT AND PHYLOGENETIC ANALYSES

Reads were trimmed at their extremities when the error probability limit per base was > 0.05 and were de novo assembled into consensus sequences in Geneious Prime 2019.2.1 program (Kearse *et al.*, 2012, <https://www.geneious.com>). The sequences with a quality threshold > 60% were aligned with MAFFT v.7.388 implemented in Geneious Prime, using the automatic algorithm and 1PAM scoring matrix (Katoh & Standley, 2013). The alignments were concatenated using SequenceMatrix v.1.8 (Vaidya *et al.*, 2011).

The alignment of nrITS and plastid *matK* combined were used for all subsequent phylogenetic analyses. The indels (insertion/deletion markers) were treated as missing data. Maximum Likelihood (ML), and Maximum Parsimony (MP) were used to explore the results under different methods. All resulting phylogenetic trees were edited in FigTree v.1.4.3 (Rambaut, 2009) and in CorelDRAW v.18.0.0.448 (<http://www.coreldraw.com>).

The maximum-likelihood phylogenetic tree was estimated using IQ-tree v.1.6.12 (Nguyen *et al.*, 2014) with tree search with model selection and bootstrap replicates in a single run (Chernomor *et al.*, 2016; Kalyaanamoorthy *et al.*, 2017). The best-fit models of substitution inferred in the IQ-tree analyses by AICc were TIM3e+I+G4 for ITS and TIM+F+R3 for *matK*. For the support, we used the 1000 ultrafast bootstrap approximation (Nguyen *et al.*, 2014) with the argument ‘-bnni’ to reduce the risk of overestimating branch support.

Maximum parsimony analyses were performed with Fitch's (1971) parsimony using the software PAUPv.4.0a166 (Swofford, 2002). Analyses included 10 000 random taxon-addition replicates and TBR swapping algorithm, holding ten trees per replication, followed by a second search to explore all topologies from the previous search, limited to 10 000 trees. The support was estimated in 1000 bootstrap replications (BP) (Felsenstein, 1985), simple addition, and TBR algorithm, holding 20 trees per replicate in PAUP, and bootstrap percentages ≥ 50 (BS) were considered. Trees were visualised and edited with FigTree v1.4.3 (Rambaut, 2009).

ESTIMATION OF DIVERGENCE TIMES

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

GEOGRAPHICAL DATA AND ANALYSES

To infer the biogeographic history of *Dryadella*, we gathered information on the current distribution of the species provided in GBIF (www.gbif.org; Jan 2022); POWO (2022), literature and herbarium specimens. Nine biogeographical areas were defined based on literature and distribution patterns observed in other plant studies grounded on the Neotropical regionalisation proposed by Morrone (2014), Table 1: A, Mexican transition zone; B, Mesoamerican dominion; C, Pacific Ocean dominion; D, Boreal Brazilian dominion; E, South Brazilian dominion; F, Paraná dominion; G, South American transition zone and H, Chacoan dominion (Cerrado – Caatinga) and I, Antillean subregion. We used the package BioGeoBEARS (Matzke, 2013) as implemented in RASP v.4.0 (Yu, *et al.*, 2015) to evaluate several biogeographic models to infer the contribution of various evolutionary processes (i.e. range expansion, range extinctions, vicariance, founder-event speciation, speciation despite sympatry) in explaining the current species distribution of *Dryadella*. The highest AICc_{wt} value was used to select the biogeographic model best fitting the data.

RESULTS

PHYLOGENETIC RELATIONSHIPS

We analysed 144 DNA sequences, of which 51 (35,42%) are specifically produced for this study (Table 1). Both phylogenetic inferences produced similar topologies for the nrITS and *cpmatK* datasets and are available in the supplementary material (S1). Only the results from combined datasets are presented with the bootstrap percentages from both ML (MLBP) and MP (MPBP) analyses (Fig. 2), and parsimony statistics for all datasets are given in Table 2.

Table 2. Statistics of the individual and combined parsimony analyses of *Dryadella* plus outgroups.

	nrITS	matK	combined
Number of taxa	72	72	75
Number of characters	760	766	1526
Constant characters	327 (43%)	539 (70%)	866 (57%)
Potentially parsimony informative sites	304	109	413
Number of most parsimonious trees	672	10000	10000
Tree steps	1802	414	2245
Consistency index	0.40	0.66	0.44
Retention index	0.63	0.70	0.63
Percentage of resolved nodes	0.84	0.42	0.75

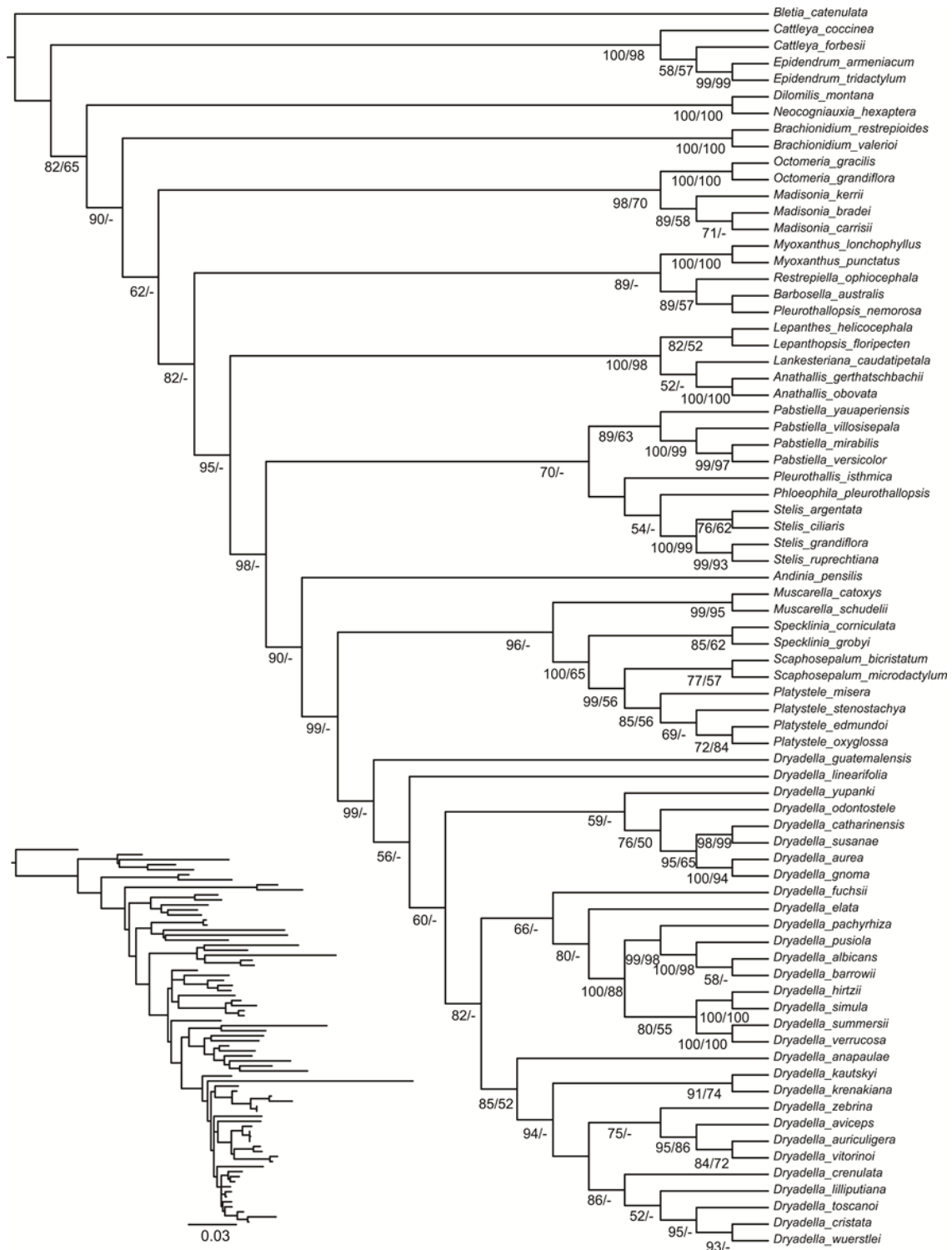


Figure 2. Maximum-likelihood (ML) tree of *Dryadella* plus outgroups. Numbers on nodes represent ML bootstrap percentages (≥ 50) and maximum parsimony (MP) bootstrap percentages (≥ 50). See the Supporting Information for trees with support resulting from the separate analyses. In detail, the ML tree is shown with proportional branch lengths.

Disagreements in the phylogenetic positions of some taxa are highlighted in the discussion. The aligned combined matrix for 75 species consisted of 2,245 characters. Of these, 413 (18%) were potentially parsimony informative. The ML topology from the analysis of the combined dataset is presented with ML bootstrap percentages and MP bootstrap percentages above the branches (Fig. 2).

The Pleurothallidinae (ML-BP 82, MP-BP 65), and the affinity *Specklinia* (ML-BP 90, MP-BP <50) are monophyletic. *Andinia* is strongly supported as a sister of the *Specklinia* affinity in ML analysis, but in MP is in an unresolved clade with *Pleurothallis*, *Stelis* and *Pabstiella* [the *Pleurothallis* affinity sensu Karremans (2016)]. *Dryadella* is monophyletic (ML-BP 99, MP-BP <50) with a grade at the base with successive *D. guatemalensis* (ML-BP 56, MP-BP <50) that occurs in Central America (AB), Pacific dominion (C) and Boreal Brazilian dominion (D) followed by *D. linearifolia* (ML-BP 60, MP-BP <50), that occurs Central America (AB), the first two early divergences in the first clade, and as a sister of three clades in ML and only *D. guatemalensis* in MP analysis as a sister of the remained species.

The first clade presents the support of 50 ML-BP/MP-BP <50 and the following two sister clades (clades 1 and 2) with moderate support 66 ML-BP/MP-BP <50 and 85 ML-BP/MP-BP <50.

The second clade has early divergent *D. fuchsii*, occurring in Central America (AB) with (ML-BP 66, MP-BP <50), sister of the other species of this clade, high supports, except in *D. barrowii* (ML-BP 58, MP-BP <50), this clade is predominantly formed by species from the Pacific dominion (C) and South American Transition Zone (G).

The last clade (clade 3) is highly supported (85 ML-BP/MP-BP <50) and is formed almost all by ARF species, except *D. ana-paulae*, the early divergent and sister of the remained species in this clade (ML-BP 94, MP-BP <50), and *D. cristata*, endemic to Colombia, in lowlands, (ML-BP 93, MP-BP <50) closely related with *D. wuerstlei*, endemic from the south of the ARF.

None of the clades formed showed support for infrageneric sections sensu Luer (2005).

DIVERGENCE TIMES AND BIOGEOGRAPHY

The birth-death speciation model was chosen based on the AIC results (Table S1).

Slight differences were found between the ancestral area estimations using BAYAREALIKE, DEC and DIVALIKE models, with or without the jump speciation parameter j . We report the estimates using DEC (Ree & Smith, 2008), which had the highest likelihood. DEC was implemented allowing a maximum number of either two or three biogeographic units per ancestral range, but this parameter did not impact the analysis and we present only the results from the three biogeographic units analysis (Fig. 3).

As shown in the time-calibrated tree with a birth-death speciation model (Fig. 3), Pleurothallidinae (node 147) probably originated in the Parana dominion (Atlantic Rainforest) [F (0.77); AF (0.22)] in the early Miocene, with an estimated crown age of 16.04 My (95% HPD: 13.17 ± 19.76). The *Pleurothallis* affinity and *Specklinia* affinity (Fig. 3; node 142) diversified in the Pacific dominion [C 58.83; CF 41.17], in the late Miocene, with an estimated crown age of 10.07 My (95% HPD: 7.43 ± 12.46).

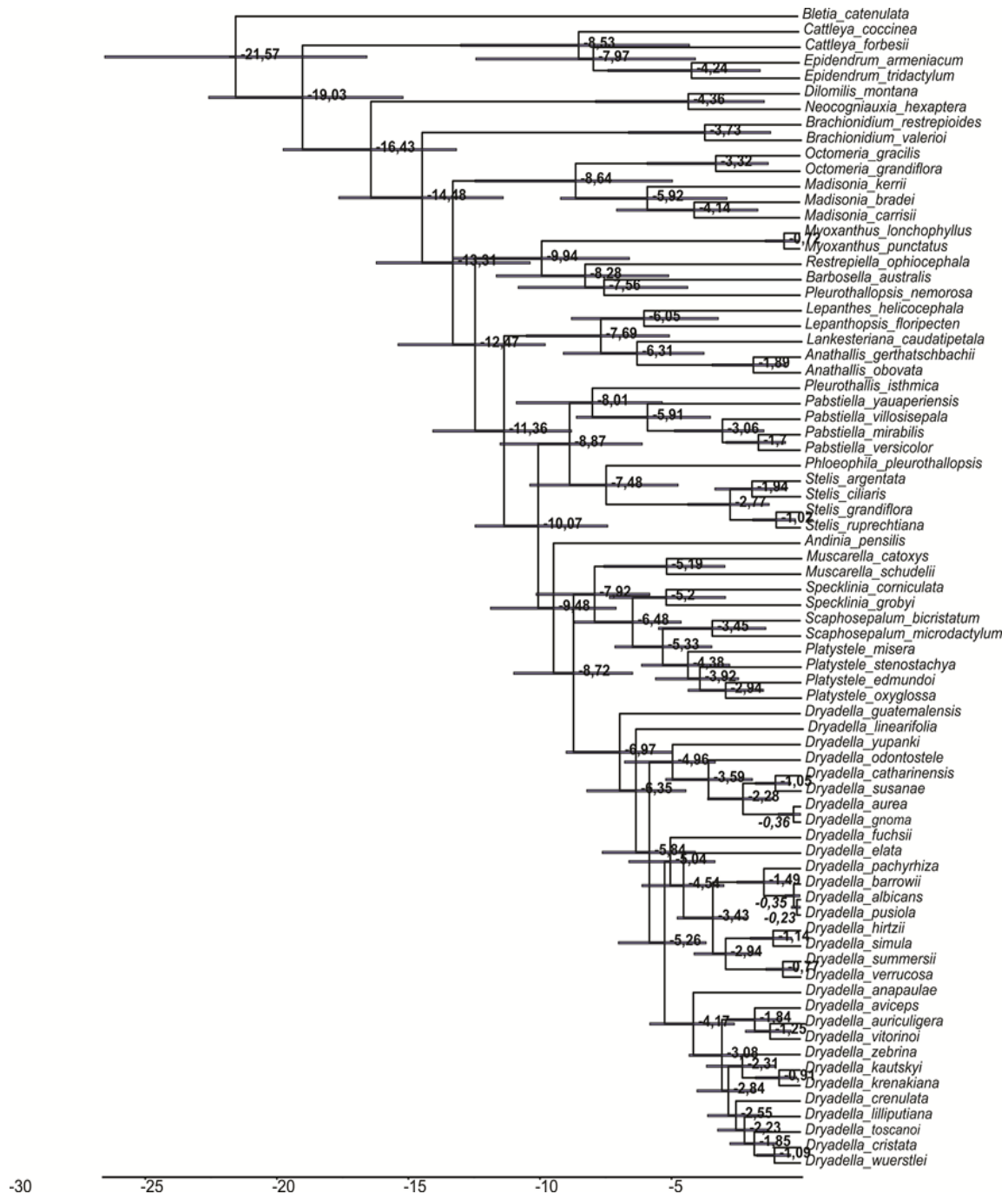


Figure 3. Time-calibrated tree for the *Dryadella* plus outgroups inferred under uncorrelated lognormal clock model in BEAST. Greybar at nodes indicates 95% highest posterior density intervals.

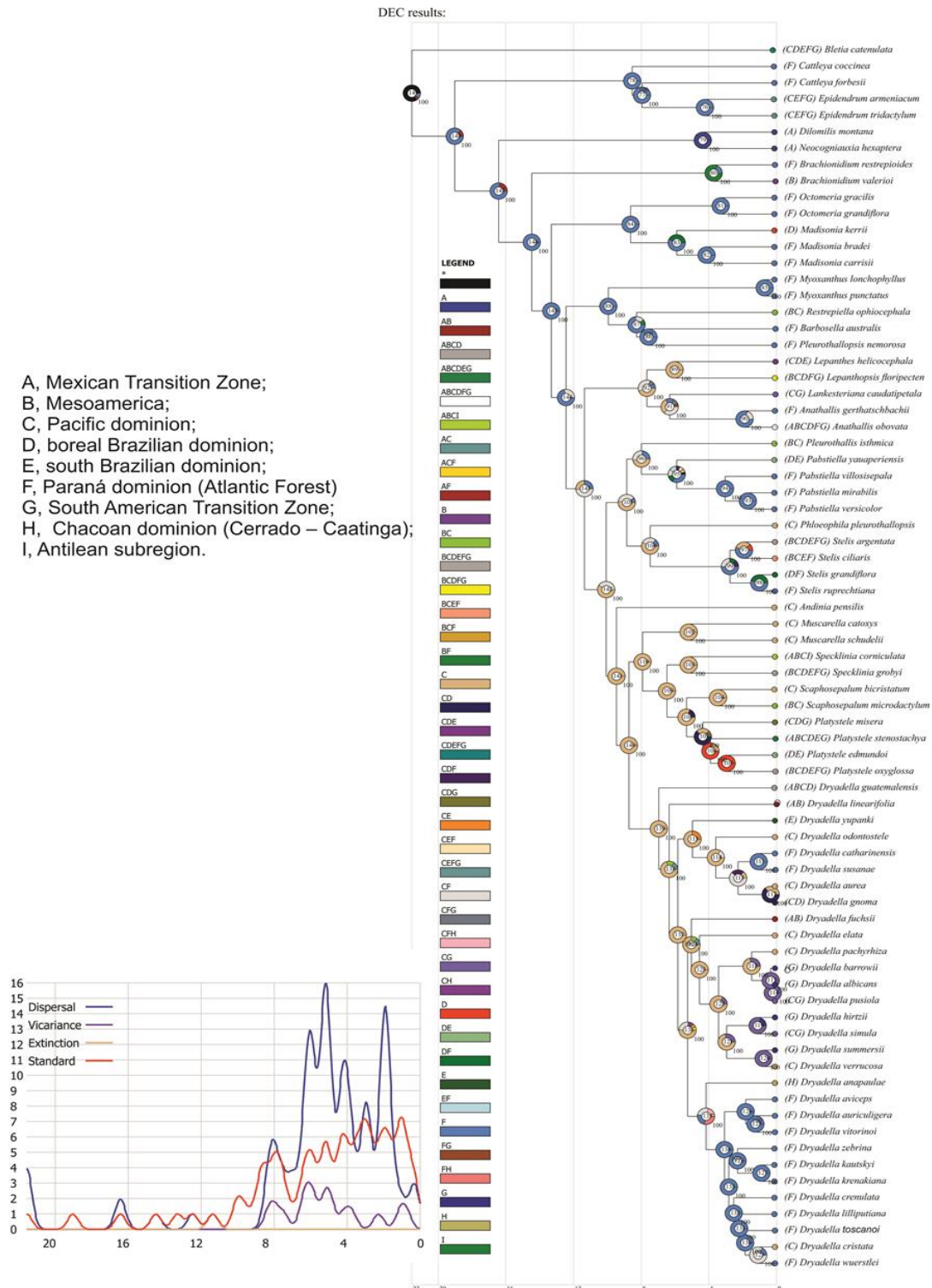


Figure 4. Maximum-likelihood tree of *Dryadella* plus outgroups with ancestral area optimisations. Pie charts at nodes indicate the most plausible geographic origin according to the DEC model. Coloured circles represent the occurrence of that species in geographical areas, following Morrone (2014) A, Mexican Transition Zone; B, Mesoamerica; C, Pacific dominion; D, Boreal Brazilian dominion; E, South Brazilian dominion; F, Paraná dominion (Atlantic Rainforest); G, South American Transition Zone; H, Chacoan dominion I, Antillean subregion.

The crown age estimated by our analyses of *Dryadella* (Fig. 3; node 139) is 6.96 My (95% HPD: 4.97 ± 7.9 - Fig. 3 shows in detail all ages for all nodes), between late Miocene and early Pliocene, in the Pacific domain as the ancestral area, northwest of South America [C (100)], further dispersion to Central America (AB) (Fig. 4).

The biogeographic history of *Dryadella* is marked by some events of long dispersion, among the main ones we identified that the first important event is c. 6.35 My (95% HPD: 4.45 ± 8.22 - Fig. 3; node 138) [C 70.1; BC 14.9; AC 14.9] and was responsible for the divergence of the lineage of clade 1, in two other lineages (clades 2 e 3). Clade 1 is composed of species occurring in the Mexican Transition Zone and Mesoamerica (A, B), up to Pacific dominion, Boreal Brazilian dominion and South Brazilian dominion (C, D, E respectively), except *D. susanae* and *D. catharinensis*, endemic to Paraná dominion (F-Atlantic Rainforest) and that have diverged more recently to 2.28 My, (CF 62.36; CDF 23.81; C 13.83) [(95% HPD: 1.16 ± 3.63] (Fig. 4).

A second event estimated at 5.84 My, (95% HPD: 4.09 ± 7.63 - Fig. 03, node 137) [C 100] was responsible for the divergence between lineages of the second and third clades. Then another important dispersion event (Fig. 3, node 136) estimated in c. 5.26 Mya (95% HPD: 3.69 ± 7.02), [C 50.87; CF 25.29; CH 9.98; BCF 6.93; ACF 6.93], was responsible for the formation of predominantly Andean lineages in the Pacific Ocean dominion (C) and Andes South American transition (G) of the species occurring in the Paraná dominion (F-Atlantic Forest) and later c. 5.04 My (95% HPD: 3.35 ± 6.62), *D. fuchsii* (AB) [C 74.09; BC 12.95; AC 12.95] diverged from the Pacific Dominion species (C) e South American Transition Zone (G) (Fig. 3, node 124). In c. 4.17 My (95% HPD: 2.61 ± 5.83) [FH 27.79; F 25.65; CF 25.57; CFH 15.43; C 5.56], another important dispersion event was responsible for the divergence between *D. anapaulae*, the only representative of the Cerrado (H) and the Atlantic Rainforest lineage (Paraná dominion F), and *D. cristata* (C) (Fig. 4).

Other dispersal events in *Dryadella* were frequent from Pacific dominion to Mexican Central America (northern Andes) and South America Transition zone (south Andes) and also to Amazon (Boreal Brazilian dominion) and Cerrado (H), and more recently spread to Atlantic Rainforest (F), the youngest lineage of the genus (Fig. 4).

DISCUSSION

PHYLOGENETIC ANALYSES

The genus *Dryadella* is, for the first time, a focus of phylogenetic study with a dense sample, with 50% of the species of the genus, including the type species, species from all proposed infrageneric classification (ex. Pabst & Dungs, 1977; Luer, 2005) as well including all morphological variations and geographic distribution.

The results based on nuclear and plastid sequences present evidence of a natural origin of the affinity *Specklinia*, with *Andinia* as a sister of the other genera and *Dryadella* sister of the *Muscarella*, *Specklinia*, *Scaphosepalum* and *Platystele*, with generally strong support. These results agree with the proposed affinities by Karremans (2016), Karremans *et al.* (2016), and Pérez-Escobar *et al.* (2017), except for *Phloeophila pleurothallopsis* Hoehne & Schltr. which appears in the *Pleurothallis* affinity, with low support (BPML 54), related to *Pleurothallis* and *Stelis* and not as a sister group to the *Specklinia* and *Pabstiella* affinities. Previous studies treat *Phloeophila* as monophyletic based on molecular data Chiron *et al.*, (2016), with nrITS and some samples of *cpmatK* (Karremans *et al.*, 2016; Pérez-Escobar *et al.*, 2017). However, our results seem to corroborate with Gutiérrez Morales *et al.* (2020); although the sampling of this genus is reduced to one species, the positioning and monophyly still seem questionable, deserving future studies.

Besides the well-supported monophyly of *Dryadella*, the results show three clades moderated supported with two species forming a grade at the base of the phylogeny. Both early divergent species (*D. guatemalensis* and *D. linearifolia*) are from northwest South America and Central America.

The monophyly of the genus based on molecular data (nrITS and *matK*) with only two species included had already been proposed by Pridgeon *et al.* (2001). Karremans *et al.* (2016) sampled ten species, recognising *Phloeophila yupanki* in *Dryadella*, and Pérez-Escobar *et al.* (2017) include nine species. In the last two studies, *D. guatemalensis* is an early divergent species of the genus, a position corroborated in our study

Three clades are formed, the first clade includes *Dryadella yupanki*, a Bolivian species, the sister of the remaining species with predominantly South-America northwest distribution, except by *D. catharinensis* and *D. susanae*, both endemic to the Brazilian Atlantic Rainforest. This clade brings representatives of the three sections proposed by Luer (2005): *D. catharinensis*, *D. susanae* and *D. yupanki*, “the tiny mites”; *D.*

odontostele in “The needle leaves”, and the others of the “All the rest” section. We did not find morphological synapomorphies that could characterise this clade.

The following two clades include the most species recognised in the “All the rest” section and have a strong geographical component. The clade of *D. fuchsii* and relatives are predominant from the Pacific dominion and South American Transition Zone (southern Andes), including *D. elata* (type of the genus). The exception is the sister species of the clade, *D. fuchsii*, from Central America (northern Andes).

This clade includes all the Andean species that have white flowers (*D. barrowii*, *D. albicans*, *D. pusiola* and *D. pachyrhiza*), caespitose habit, larger than 5 cm tall, with narrow oblong or obovate leaves with long petiole, long peduncles which in most cases raise the flowers to the height of the plant, except for *D. fuchsii* which is a smaller plant with flowers between the leaves. Although we did not find morphological synapomorphies that could characterise this clade, these characteristics seem to be common for most species of *Dryadella* from the Andes.

The next clade includes *D. ana-paulae*, the only species from Cerrado, as a sister species of all the Atlantic Rainforest clade, except by *D. cristata*, endemic of Colombia. These species are morphologically very variable, making it difficult to characterise this clade by morphological synapomorphies. The representatives of this clade are distributed among the three sections according to Luer (2005), although we disagree that *D. wuerstlei* belongs to the group of “The needle leaves”.

ORIGIN AND BIOGEOGRAPHY OF *DRYADELLA*

Our analyses estimated the origin of Pleurothallidinae in c. 19 Mya, early Miocene, which agrees with Givnish *et al.*, (2016), Pérez-Escobaret *et al.* (2017) and Gutiérrez Morales *et al.* (2020). Like Gutiérrez Morales *et al.* (2020), our results suggest the Paraná dominion (Atlantic Rainforest) as an ancestral area. This Atlantic Rainforest domain was also recognised as an ancestral area for other orchid groups, such as the *Ornithocephalus* clade (Oncidiinae, Smidt *et al.*, (2018)) and Spiranthinae (Cranichideae, Salazar *et al.*, (2018)).

The results obtained here suggest recent diversification in *Dryadella*, estimated for the late Miocene to early Pliocene (~7 My), in the Pacific dominion, after the warm period of the Middle Miocene climatic optimum, which coincides with radiation of several plants in the Neotropics (Taylor, 1991; Antonelli *et al.*, 2009; Gustafsson *et al.*, 2010;

Pérez-Escobar *et al.*, 2017), at the same time with the intensification of the uplift of the Andes mountains, estimated between 7-11 My (Taylor, 1991; Hoorn *et al.*, 2010) and the beginning of the formation of open vegetation biomes such as the Cerrado that partially separated the forests of South America (Simon *et al.*, 2009).

Currently, the Andean Forest/Amazonian and Atlantic Rainforest are separated by the 'Dry diagonal' including the biomes Chaco, Cerrado and Caatinga (Werneck, 2011; Werneck *et al.*, 2012), causing a disjunct pattern of distribution between many groups of Neotropical forested biomes (Costa 2003; Turchetto-Zolet *et al.*, 2012), this disjunction takes place in *Dryadella* (Pridgeon *et al.*, 2001, Luer, 2005). However, the forests present in these Biomes showed multiple expansions and contractions throughout their history, related to partial floods and drainage in South America from the mid-Miocene to the beginning of the Pliocene (Cabanne *et al.*, 2019; Kessous *et al.*, 2020; Peres *et al.*, 2020). Therefore, previous links between the Atlantic Rainforest, the Amazon and the Andes, which occurred in different periods and across different regions, are reflected in the patterns of Atlantic Rainforest Biota (Kessous *et al.*, 2020, Peres *et al.*, 2020).

Dispersion migration in Pleurothallidinae and other orchids between the Andes and Amazonia has been reported in the extensive biogeographic analyses presented by Pérez-Escobar *et al.* (2017). These authors concluded that the uplift of the Andes did not act as a major barrier to dispersal of orchids from the subtribes Cymbidieae and Pleurothallidinae. In *Dryadella*, dispersals were recognized as the most frequent biogeographic events in their historical distribution, and the Andes' uplift did not act as a dispersion barrier for the Northern Andes (Central America) and the Andes of South America.

Notably, *D. guatemalensis* and *D. linearifolia* and *D. fuchsii* dispersed to Central America, possibly at the beginning of the formation of the Isthmus of Panama, which was only fully established around 3.5 Ma (Hoorn *et al.*, 2010; O'Dea *et al.*, 2016).

At the beginning of Pliocene occurred dispersion between the South American Transition Zone and Paraná dominion (Atlantic Rainforest) forming the most recent lineage of the genus, probably related to the drainage of the Paranaean Sea, the contraction of the forests that connected these two biomes and the formation of the Dry Diagonal (Chaco, Cerrado and Caatinga) (Kessous *et al.*, 2020).

In this sense, subsequent dispersions from the Amazon region to the west of the continent through the South of the Andes at the beginning of the Pliocene gave rise to exclusively Andean species and, at the same time, through the most central route of the continent to the Atlantic Rainforest, where they settled and due to the isolation by the dry diagonal, became endemic to this Biome. *D. ana-paulae* is the sister species of the Atlantic clade and the only one currently found in the Cerrado and the seasonally dry forest lowlands of Bolivia, but related to gallery forests, which reinforces the hypothesis of a connection between tropical forests and the Andes, through gallery forests, as an important route for several plant groups (Oliveira-Filho & Ratter, 1995; Costa, 2003).

The fact that *D. cristata* is close to species exclusive to the Paraná dominion (Atlantic Rainforest) may also be evidence of recent links to the south of the continent, between the Andean Cordillera and the Atlantic Forest, but we recognise that we need to investigate further, to understand the relationship between these taxa better.

Recent diversification seems to reflect on the taxonomy of the genus. *Dryadella* has many homoplastic morphological characters, making the delimitation of taxa very difficult, especially in the Atlantic Rainforest endemic taxa (Imig *et al.* Cap. III).

CONCLUSIONS

From the most robust sampling of the genus, the results confirm high support for the monophyly of the genus and its positioning in the *Specklinia* affinity.

Dryadella had its origin estimated at the end of the Miocene (~7 Ma) and the Andes emergence had a fundamental contribution to the current distribution of *Dryadella* species, which allowed the dispersion to the south of the continent, as well as the Isthmus of Panama, allowing the dispersal of species to Central America. The most recent lineage is located in the Atlantic Forest. *Dryadella ana-paulae*, which occurs in the gallery forests of the Cerrado, also recorded in the lowlands of Bolivia, is sister species of this clade, making evident the connection of gallery forests between the tropical forests of Brazil to the Andes and that the formation of the dry diagonal was important for the establishment of endemisms of the genus. The recent connection between the tropical forests of Brazil and the Andes through gallery forests can be seen through the biographical history of *D. ana-paulae*.

Taxonomically, proposed sections based on plant and leaf sizes are not supported. The difficult taxonomic delimitation due to the presence of many homoplastic characters

may be related to their recent origin, and therefore, the clades do not have strong synapomorphies, which is even more evident among the species of the ARF clade, diversified in the Quaternary period. Future work with the genus should focus on including more species and markers and the study of reproductive biology and population genetics, especially in the Atlantic clade, where the species has a recent diversification, being a good model for isolation studies between these species.

ACKNOWLEDGEMENTS

We would like to thank Sistema de Autorização e Informação em Biodiversidade/Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO/ICMBio) for the collecting permits. We are also grateful for the financial support of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) with scholarship granted to DCI (168348/2017-9) and ECS (CNPq-Nível 1D-proc. 314642/2020-0 and PDJ proc. 203304/2018-7) and to the MCTI/CNPq N° 14/2013 - Universal (proc. 405493/2021-5). ALVTB thanks to the CAPES (PVE 88881.065009/2014-0).

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Fig. S1. Strict consensus nrITS parsimony tree resulting from 672 trees from *Dryadella* plus outgroups. Numbers at nodes are bootstrap percentages >50.



Fig. S2. Plastid *matK* strict consensus parsimony tree resulting from 10,000 trees from *Dryadella* plus outgroups. Numbers at nodes are bootstrap percentages >50.

Table S1: Model comparison of molecular dating analyses by Akaike's Information Criterion for MCMC samples (AICM), with standard error estimated from 1,000 bootstrap replicates. Lower AICM indicates better model fit.

Models	AICM	Std. error
Birth-Death	28071.262	± 0.414
Yule	28072.0	± 0.201

CAPÍTULO II / CHAPTER II

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

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

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

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Short title: *Dryadella* in Brazil

DCI and ECS designed the study, DCI, ALVTB and ECS collected samples; DCI, ALVTB and ECS analysed the material, all authors wrote the manuscript.

Abstract

The present work is a taxonomic revision of the *Dryadella* species of Brazil. *Dryadella* Luer comprises 61 species, distributed in humid, or seasonally humid forests, from Mexico to southern Brazil, most in the Atlantic Rainforest and the Andean forests. We provide morphological descriptions, taxonomic discussions, illustrations, an identification key, new synonyms, new occurrences and distribution maps for all taxa. Fourteen species are recognised for Brazil, of these *D. zebrina* recorded also for Bolivia and Peru, *D. aviceps* in Paraguay, and *D. lilliputiana* in Bolivia, *D. gnoma* Ecuador, Peru, Colombia, Panama and Costa Rica and recorded here for the first time. Once for Brazil, the others are endemic to the country and, with the exception of *D. gnoma* and *D. ana-paulae*, the other species are restricted to the Atlantic Rainforest. We propose the synonymisation of *D. osmariniana* and *D. cardosoi* in *D. gnoma*, *D. espirito-santensis* and *D. gomes-ferreirae* and *D. litoralis* in *D. aviceps* and *D. xaveriana* in *D. toscanoi* and eight lectotype are designed.

Keywords: endemism, nomenclature, monocots, South America, taxonomy

Resumo

O presente trabalho é uma revisão taxonômica das espécies *Dryadella* do Brasil. *Dryadella* Luer compreende 61 espécies, distribuídas em florestas úmidas ou sazonalmente úmidas, do México ao sul do Brasil, a maioria na Floresta Atlântica e nas florestas andinas. Fornecemos descrições morfológicas, discussões taxonômicas, ilustrações, chave de identificação, novos sinônimos, novas ocorrências e mapas de distribuição de todos os táxons. Quatorze espécies são reconhecidas para o Brasil, destas *D. zebrina* registrada também para Bolívia e Peru, *D. aviceps* no Paraguai, e *D. lilliputiana* na Bolívia, *D. gnoma* Equador, Peru, Colômbia Panamá e Costa Rica e registrada aqui pela primeira vez para o Brasil, as demais são endêmicas do país e com exceção à *D. gnoma* e *D. ana-paulae*, as demais espécies são restritas à Floresta Atlântica. Nós propomos a sinonimização de *D. osmariniana*, *D. cardosoi* em *D. gnoma*, *D. espirito-santensis*, *D. gomes-ferreirae* e *D. litoralis* em *D. aviceps*, e *D. xaveriana* em *D. toscanoi* e oito lectótipos são designados.

Palavras-chave: endemismo, nomenclatura, monocotiledôneas, América do Sul, taxonomia.

Introduction

Dryadella Luer (Orchidaceae, Epidendroideae) is exclusively neotropical distributed in humid, or seasonally humid forests, from Mexico to southern Brazil, most in the Atlantic Rainforest and the Andean forests (POWO 2022; Pridgeon 2005; Luer 2005; Imig *et al.* 2020a). *Dryadella*, along with *Andinia* Luer, *Muscarella* Luer, *Platystele* Schltr., *Scaphosepalum* Pfitzer, *Specklinia* Lindl. and *Teagueia* Luer form the Affinity *Specklinia* (Karremans 2016; Karremans *et al.* 2016) of the subtribe Pleurothallidinae.

Dryadella was established by Carl A. Luer (1978a), based on some dismembered species of the genus *Masdevallia* Ruiz & Pav., having a thickened callus close to the base of the lateral sepals, multi-angled petals, and a long unguiculate lip with a blade provided with two basal, usually retrorse lobes. The first species were described in the genus *Pleurothallis* R. Br. as *P. auriculigera* Rchb.f. and *P. aviceps* Rchb.f., both from samples taken from Brazil. Next, *Masdevallia simula* Rchb.f. was then described from Colombia. *Masdevallia* was then separated into infrageneric categories, and *M. obrieniana* Rolfe and *M. simula* were allocated in *Masdevallia* section *Saltatrices* Luer (Luer 2005) and later in *Masdevallia* subg. *Trigonanthe* Schltr., section *Rhombipelalae* Schltr., except *M. lilliputiana* allocated in section *Floribundae* Kraenzl (Luer 2005). Finally, Luer (1978a) recognises these species as a new genus and establishes *Dryadella* Luer, with 37 species accepted so far and based in *Dryadella elata* (Luer) Luer. New taxa were described, and some new synonyms were proposed and currently *Dryadella* consists of 61 accepted names (Luer 2002, 2005; Campacci & Silva 2015, 2022; Castro *et al.* 2007; Imig *et al.* 2020a; Imig *et al.* 2021; POWO, 2022).

Although *Dryadella* has a wide distribution, there is a great morphological similarity between the species, making infra-generic delimitation difficult (Luer 1978a; Pridgeon 2005). Luer (2005) proposed the division of the genus into three sections, joining the species with linear leaves “the needle leaves”, the species of diminutive habit “the tiny mites”, and the other species “all the rest”. Recently Imig *et al.* (2020b) studied the leaf anatomy of several species from different biomes and concluded that the variation found does not discriminate between species of different habitats and do not collaborate with sections delimitation.

In Brazil, the first broad study of Orchidaceae that included *Dryadella* species was Flora Brasiliensis (Cogniaux 1906) with the description of *Masdevallia edwalli* Cogn., *M. lilliputiana* Cogn. and recognize *M. paulensis* B. Rodr., *M. sessilis* B. Rodr. and *M. rio-*

grandensis (Hort) Cogn. Later, in *Orchidaceae brasiliensis*, Pabst & Dungs (1977) recognised nine species for Brazil: *M. edwallii*, *M. lilliputiana*, *M. espirito-santensis* Pabst, *M. kaustskyi* Pabst, *M. obrieniana*, *M. simula*, *M. paranaensis* Schltr., *M. gomes-ferreirae* Pabst and *M. zebrina* Porsch.

Over the years, new species were described (e.g. Luer 1978,b), and Luer (2005) recognised fifteen species for the country and proposed new synonyms. The same species are cited in BFG (2015, 2018), including *D. litoralis* Campacci, described later. Until now, 18 species were recognised for Brazil: *Dryadella ana-paulae* V.P.Castro, *D. auriculigera* (Rchb.f.) Luer, *D. aviceps* (Rchb.f.) Luer, *D. cardosoi* Campacci & J.B.F.Silva, *D. crenulata* (Pabst) Luer, *D. edwallii* (Cogn) Luer, *D. espirito-santensis* (Pabst) Luer, *D. gomes-ferreirae* (Pabst) Luer, *D. kautskyi* (Pabst) Luer, *D. krenakiana* Campacci, *D. lilliputiana* (Cogn.) Luer, *D. litoralis*, *D. osmariniana* (Braga) Garay & Dunst., *D. susanae* (Pabst) Luer, *D. toscanoi* Luer, *D. vitorinoi* Luer & Toscano, *D. wuerstlei* Luer, *D. zebrina* (Porsch) Luer. Of these, 15 are endemic to the country and except for *D. osmariniana* and *D. cardosoi* found in Amazon and *D. ana-paulae* restricted to the Cerrado, the other species are restricted to the Atlantic Rainforest (Imig *et al.* 2020a).

Dryadella is challenging to find in the field, and when found, the species form very small populations and are usually restricted to a few phorophytes. Furthermore, unlike most micro-orchids, *Dryadella* is rarely cultivated in private or public collections, probably because they are very similar, having the flowers immersed between the leaves (not very showy) and is hard to grow. According to previous reports in the Red Book of Brazilian Flora (Martinelli & Moraes 2013), three species were evaluated as being endangered: *Dryadella auriculigera* and *D. susanae* were treated as Critically Endangered (CR) and *D. lilliputiana* as Vulnerable (VU). At the State level in Brazil, some Red Lists cited *Dryadella* species. *Dryadella lilliputiana* was treated as VU, and *D. auriculigera* as Extinct in Nature (EX) in São Paulo (SEMA, 2016). For the Espírito Santo state, *D. aviceps* was treated as VU and *D. espirito-santensis*, *D. kautskyi* and *D. susanae* as CR (Fraga *et al.* 2019).

The present work is a taxonomic review of the *Dryadella* species of Brazil, updating the works of *Flora Brasiliensis* (Cogniaux 1906), *Orchidaceae brasiliensis* (Pabst & Dungs 1977), and *Flora do Brasil 2020* (Imig *et al.* 2020a). We provide morphological

descriptions, taxonomic revision and discussions, illustrations, an identification key, new synonyms, new occurrences and distribution maps for all taxa.

Methods

Specimens from 31 national and international herbaria were examined (BR, CEN, CTBA, COL, ESA, FLAS, FLOR, FURB, HB, HRCB, HUEFS, HUEM, HNUP, HUEN, IAN, ICN, JAUM, JOI, K, MBM, MBML, MG, MO, NY, QCA, RB, SEL, UEC, US, UPCB, VIES (acronyms following Thiers 2022). Additionally, the specimens collected in field expeditions from 2018 to 2021 were herborised according to Fidalgo & Bononi (1989) and deposited at UPCB and MBM.

The specimens were identified at the specific level by consulting the protologues and nomenclatural types and by comparisons with descriptions in specialized studies that comprehensively cover the genus (Pabst & Dungs 1975, 1977; Luer 1978, 2005). The morphological terminology follows Stearn (2004), and the descriptions follow Luer (1978b; 2002, 2005). Heterotypic synonyms are listed in chronological order. The figures and illustrations were edited using CorelDRAW X8 (<https://www.coreldraw.com/>).

Results and discussion

A total of 290 specimens from the states of SC, RS, PR, MG, RJ, SP, ES, MG, BA, MS, PA, AM were analysed. We recognise the occurrence of fourteen species (Fig. 1-2), of which ten is endemic to Brazil. *Dryadella zebrina* is recorded for Bolivia and Peru, *D. aviceps* for Paraguay, *D. lilliputiana* for Bolivia, *D. gnoma* for Ecuador, Peru, Colombia, Panama and Costa Rica and registered here for the first time to Brazil. Except for *D. gnoma*, which occurs in the Amazon and *D. ana-paulae*, which occurs in the Cerrado, the other species are restricted to the Atlantic Rainforest. The mountain region of the state of Espírito Santo is an important point of the diversity of the genus. Here, we propose the synonymisation of *D. osmariniana* and *D. cardosoi* in *D. gnoma*, *D. espirito-santensis* and *D. gomes-ferreirae* in *D. aviceps*, *D. ataleiensis* and *D. sapucaiensis* Campacci & S.L.X. Tobias in *D. auriculigera*, *D. xaveriana* in *D. toscanoi* in addition to new distribution records for *D. ana-paulae*, *D. gnoma*, *D. vitorinoi*, *D. aviceps*, *D. wuerstlei*.

Taxonomic treatment

Dryadella Luer, Selbyana 2: 207 (1978a).

Type: *Dryadella elata* (Luer) Luer.

=*Masdevallia* Sect. *Saltatrices* Rchb. f., *Linnaea* 41: 10 (1877), Woolward, *Monogr. Masdevallia*, 1898, non Rchb.f. 1877.

=*Masdevallia* Subgen. *Trigonanthe* Schltr., *Repert. Spec. Nov. Regni Veg. Beih.* 35: 48 (1925) *nom. nudum*.

=*Masdevallia* sect. *Rhombopetalae* Krz1., *l. c.* 34: 188 (1925).

=*Trigonanthe* (Schltr.) Brieg., *Die Orchideen* 448 (1975) *nom. illegit.*

Herbs perennial, very small to medium-sized in size for the subtribe, epiphytic, rarely lithophytic, caespitose rarely short-repent or pendulous. **Roots** slender or thick, produced at the base of the rhizome. **Rhizome** short to inconspicuous. **Ramical** unifoliate, erect to suberect, partially or completely enclosed by 2–3 small, imbricating paleaceous sheaths, at the base of the inflorescence emerging laterally with an annulus from below the apex (the abscission layer), truncate, entire. **Leaf** light green, green or dark green, sometimes suffused or spotted with purple, coriaceous or thickly coriaceous, erect or suberect, flat, terete or sub-teretes; with or without or indistinct petiole, blade erect or suberect, entire. **Inflorescence** uni-flowered or a successive, single-flowered, congest raceme in a 2–4 flowered, borne by a usually short peduncle, bearing 2(3) pale imbricate basal bracts; pedicels slender, longer or shorter than the floral bract; ovary tri-lobate. **Flowers** resupinate, small (2.5–4.5 mm long of dorsal sepal), medium (more 4.5–7.5 mm long. of dorsal sepal) and large (bigger than 7.5 mm long. of dorsal sepal) including the tail; sepals sometimes translucent, albescent, green, yellow, orange, absent, diffuse or densely purple or red spotted rare absent, more or less membranaceous; dorsal sepals, erect or inflexed, more or less linear lanceolate or ovate, 3-veined, more or less carinate, apex obtuse to acute, ending abruptly or attenuated in a short or long caudate, slender, thick or clavate, minutely, connate at the base with lateral sepals, forming a short sepaline cup, entire or minutely toothed; lateral sepals erect or deflexed, more or less lanceolate or ovate, asymmetrical, with or without transverse callus above the base, 3-veined, ending abruptly or attenuated in a short or long caudate, short or long caudate, slender, thick or clavate, entire, rare minutely toothed; petals albescent, green, yellow, orange, absent, diffuse or densely purple or red spotted, rare absent, mostly confined within the sepaline cup, more or less fleshy, 1–2 veined; lip green, yellow, orange, absent, diffuse or densely purple or red spotted, rare absent, unguiculate; blade suborbicular or oblong, usually deflexed near the middle, base

usually bi-lobed, entire, crenate or toothed; column curved, albescent, green, yellow, orange, usually diffuse red spotted, curved, winged on the distal half, broadly wing on the distal half; anther yellow to albescent, apiculate; pollinarium, no see. **Fruit** light green, sometimes suffused or spotted with purple or red, usually oblong and tri-alate.

Etymology: Dryad is a tree nymph in Greek mythology.

Distribution and ecology: In Brazil, most *Dryadella* species are endemic to the Atlantic Forest, only one species from the Cerrado (*D. ana-paulae*) and one from the Amazon (*D. gnoma*) (Fig. 16a). In nature, Brazilian *Dryadella* do not form large populations; generally, only between 5-10 individuals are distributed in 1-2 phorophytes, and therefore they are difficult to find, reflecting the low number of collections in herbariums. We found rare larger populations such as *D. lilliputiana* (Imig 634) densely covering a single phorophyte of *Campomanesia* sp. (Myrtaceae) with about eight meters and a population of *D. zebrina* (Imig 644) with more than 30 individuals on a wet rock. In general, they occur in phorophytes densely covered by mosses and in humid places, rarely found on rocks or dry areas; only *D. lilliputiana* was found as an epiphyte in *Araucaria angustifolia* (Bertol.) Kuntze.

Studies of floral biology, preference for phorophytes and mycorrhizal associations were not found in the literature, and it is necessary to understand and to conserve this genus of orchids.

Identification key for the Brazilian species of *Dryadella* Luer

1. Reptant or pendent habit

2. Pendent habit, leaf blade narrow-obovate, $25-38 \times 2-3.2$ mm . ***D. krenakiana***

2'. Reptant habit

3. Leaves sessile, $4-5.7 \times 2.5-3$ mm ***D. susanae***

3'. Leaves shortly petiolate, $13-19.5 \times 2.1-3.3$ mm ***D. catharinensis***

1'. Cespitose habit

4. Terete or semi-terete leaves

5. Terete leaves

6. Blade $10-32 \times 1.8-2.1$ mm, dark-green, presence of albescent in basal portion; flowers predominantly white ***D. lilliputiana***

6'. Blade $30-35 \times 2.3-2.8$ mm; light-green, absent of albescent in basal portion; flowers predominantly yellow to dark-yellow ***D. auriculigera***

5'. Semi-teretes leaves

7. Sepal's tail with less than 2mm

8. Blade $15-30 \times 2.5-4.3$ mm long, elliptical to oblanceolate; dorsal sepal $4.3-5.5 \times 2.5-3$ mm, short caudate, tail with claved apex ***D. ana-paulae***

8'. blade $4-6(7.5) \times 1.5-1.8$ mm long, linear; dorsal sepal $5.3-6.5 \times 1.8-2.2$ mm, short caudate, tail with acute to acuminate apex..... ***D. kautskyi***

7'. Sepal's tail with more than 2mm

9. Blade $15-25(30) \times 2.5-3.3$ mm; sessile; dorsal sepal $5-6.3 \times 4.0-4.5$ mm, pale-green, tail 6–11 mm), lip with minutely toothed margin ***D. wuerstlei***

9'. Blade $30-43 \times 2.5-3.5$ mm; petiolate; dorsal sepal $8-13 \times 4.3-5.7$ mm, yellow-greenish, tail 5–6.3 mm, lip with entire margin ***D. toscanoi***

4'. Flat leaves

10. Lateral sepals with remnant or absence of transverse callus, lip with blade oblong as shovel-shaped, no reflexed ***D. gnoma***

10'. Lateral sepals with presence of transverse callus, lip with blade oblong or ovate, reflexed

11. Flowers no full opening (sepals erect)

12. Petals with superior margin entire, linear *D. vitorinoi*
- 11'. Flowers full opening (sepals patent)
- 12'. Petals with superior margin lobed
13. Sepal's tail with less than 2mm *D. aviceps*
- 13'. Sepal's tail with more than 2mm
14. Leaf blade 40–65 (9) × 3.5–7 mm; petiole 2.5–3 mm long; lip 1.8–2.1 × 0.8–1.2 mm, margin entire..... *D. zebrina*
- 14'. Leaf blade 40–57.3 × 4.5–6 mm; petiole 3–4.5 mm long; lip 1.5–2.3 × 1.3–1.8 mm, margin visibly toothed to ciliated *D. crenulata*

1. *Dryadella ana-paulae* V.P.Castro, B.P.Faria & A.D.Santana, *Richardiana* 4(4): 198 (2004). Holotype: BRAZIL. DISTRITO FEDERAL: Edge of the Córrego Taboquinha, 100 m do Condomínio Itaipú, 13.X.2002, *A.D. Santana* 3 (UB 12612, lost). Lectotype (here designated): Carl Luer original's drawing 20620, reproduced in *Richardiana* 4(4): 199 (2004).

(Fig.1a; 3a-h)

Epiphyte herb, densely cespitose, small, 15–35 mm tall. **Roots** thick, 2–4 at each rhizome node, 0.5–0.8 mm diameter. **Rhizome** 2–2.5 mm between the internodes. **Ramicaul** 2–3 mm long, erect, covered by two paleaceous, triangular sheaths, abruptly mucronate, 2–3.2 × 1.5–1.8 mm. **Leaf** green to light green on both sides, thickly coriaceous, elliptical to oblanceolate, semi-terete, 15–30 × 2.5–4.3 mm; petiole 0.8–1.3 mm long; blade erect, attenuate at the base, retuse and mucronate. **Inflorescence** a successive, 1-2-flowered, congest raceme; bearing 2(3) pale basal bracts, lanceolate, mucronate, 0.8–1.3 × 0.7–1.2 mm; peduncle 2–4 mm long; pedicel plus ovary light-green, slightly tri-alate, entire to minutely toothed, 1–1.5 mm long. **Flower** small to medium; dorsal sepal pale yellow to greenish yellow, diffused dark-purple spotted, elliptic, inflexed, 3-veined, carinate and entire, 4.3–5.5 × 2.5–3 mm, base connate 0.5 mm with lateral sepals forming a short sepaline cup, acute, short caudate, tail 1.5–2 mm

with claved apex, entire; lateral sepals pale yellow to greenish-yellow, densely dark-purple spotted, ovate, 3-veined, asymmetrical, $4.5\text{--}5.5 \times 1.5\text{--}1.8$ mm, base connate, a transverse callus 1.3mm, purple, acute, short caudate, tail 1.5–2 mm with claved apex, entire; petals yellow, densely purple-spotted, oblong, 1-veined, vein parallel, asymmetrical, $1.3\text{--}1.4 \times 1.2\text{--}1.3$ mm, base truncate, obtuse, superior margin with discreet acute lobe; inferior margin excavated, acute to obtuse lobe, entire; lip yellow, unguiculate; claw 0.5–0.7 mm, bilobed at base, caniculate; blade oblong to obovate, $1.5 \times 0.9\text{--}1$ mm, base with 2 lateral, acute, divaricated lobes, 0.2 mm; a pair of lamellar callus toothed at the base; apex reflexed, obtuse, minutely toothed; column yellow, 1–1.3 mm long, curved, winged on the distal half, bi-toothed at winged base; apex acute and minutely serrated, foot smaller long; anther yellow, apiculate; pollinarium, no seen. **Fruit** light green, oblong, $8\text{--}8.5 \times 4.2$ mm.

Material examined: BRAZIL. GOIÁS: Anápolis, 15.XI.2000, fl., *G. Miranda* 039 (CEN). Goianápolis, 26.V.2021, fr., *D.C. Imig & D. Rodrigues* 666 (UPCB). DISTRITO FEDERAL, Edge of the Córrego Taboquinha, 100 m do Condomínio Itaipú, 13.X.2002, *A.D. Santana* 3 (UB 12612 lost); Fazenda Sucupira – EMBRAPA, 23.XI.1999, fl., *J.A.N. Batista & K. Proite* 1093 (CEN); 14.XI.1999, fl., *J.A.N. Batista & K. Proite* 956 (CEN); 18.I.2013, fl., *R.P. Oliveira* 78 (CEN).

Taxonomic notes: *Dryadella ana-paulae* is one of the smallest plants of the genus and is related to *D. lilliputiana*. They differ in light-green to green leaves on both sides, without purple punctuations (vs dark-green leaves with diffuse purple punctuations and a whitish portion at the base of the blade and petiole). Flowers are pale-yellow to greenish-yellow with purple punctuations and lip with indented margin (vs white flowers with purple punctuations and lip with entire margin) (Fig. 1 a,l).

Dryadella ana-paulae was compared by Luer (2005) to *Dryadella vasquezii* Luer. *Dryadella ana-paulae* has oblong to obovate lip with denticulated margins and oblong petals (vs suborbicular lip with entire margins and hastatus petals). *Dryadella vasquezii* is endemic to the lowland forests of central Bolivia, and *D. ana-paulae* is endemic and the only species of the genus found in the Brazilian Cerrado. *Dryadella ana-paulae* was described based on the specimen collected by Castro *et al.* s.n., from the Federal District near the Córrego Taboquinha. The protologue indicates the holotype was deposited in the herbarium UB-13430 (Castro Neto *et al.* 2004), but this material is deposited as UB-12612, and was not found during our studies. Luer (2005) erroneously cites an isotype

(MO100538865) that is not indicated in the protologue. Additionally, a paratype is mentioned in the protologue but deposited in a private herbarium of one of the authors, therefore inaccessible. The type was illustrated by Luer (2005, illustration 20620). Luer (2005) cites this species for Bolivia, in Santa Cruz (Luer 3617, SEL 048273!; Luer 15233, MO100539123 and R. Vásquez 1127, Herb. Vasqu., MO 100538867). However, when analysing the material, especially Luer 3617, we agree with Castro et al. (2004) that it is not the same species for the reasons mentioned above. The extra-Brazilian material cited by Luer (2005) must therefore be *D. vasquezii*.

2. *Dryadella auriculigera* (Rchb.f) Luer, Selbyana 2: 208 (1978a). Holotype: BRAZIL. Without locality, imported from Brazil in 1866 by F. Sanders, flowered in cultivation, *W. Bowmann* 1636 (W50984!).

≡ *Plerothallis auriculigera* Rchb.f, Gard. Chron. 1871: 1579 (1871).

≡ *Masdevallia auriculigera* (Rchb.f) Rchb.f, Otia Bot. Hamburguesia 2: 93 (1878).

= *Dryadella ataleiensis* Campacci, Coletânea de Orquídeas Brasileiras 9: 332 (2011). Holotype: BRAZIL. MINAS GERAIS: Ataléia, V.2009, *M.A. Campacci & R. Vasconcellos Leitão* MAC-1963 (ESA 114455!). *syn. nov.*

= *Dryadella sapucaiensis* Campacci & S.L.X. Tobias, Coletânea de Orquídeas brasileiras: Novas espécies e híbridos naturais 16: 662 (2020). Holotype: BRAZIL. SÃO PAULO: São Bento do Sapucaí, 950 m, XII.2018, fl., *S.L.X. Tobias* (ESA, not found in herbarium). Lectotype (here designated): M. A. Campacci original's drawing, which appeared in Novas espécies e híbridos naturais 16: 664 (2020). *syn. nov.*

(Fig. 1b; 4a-h)

Epiphyte herb, caespitose, small, 3–4.5 mm tall. **Roots** thick, 2–4 at each rhizome node, 0.5–0.8 mm diameter. **Rhizome** 2–3 mm between the internodes. **Ramical** 1–2.5 mm long, erect, covered by two paleaceous, lanceolate sheaths, abruptly mucronate, 3.9–5 × 2.4–3.8 mm. **Leaf** light green with dark-purple spotted on both sides, thickly coriaceous, linear, terete, 30–35 × 2.3–2.8 mm; petiole 1.3–1.8 mm long; blade erect, long attenuate at the base, truncated and minutely tri-toothed. **Inflorescence** a successive 1-3 flowered, congest raceme; bearing 2 pale basal bracts, lanceolate, mucronate, 1.8–2.4 × 1.3–1.7 mm; peduncle 0.4–0.5 mm long; pedicel plus ovary light green to alveolate with diffuse purple dots, slightly tri-lobate, toothed, 1.3–2 mm long.

Flower medium; dorsal sepal dark yellow to orange, diffused dotted dark-purple spotted, oval to oval lanceolate, inflexed, 3-veined, slightly carinate and entire, 5–5.5 × 2.5–3 mm, base connate 0.6 mm with lateral sepals forming a short sepaline cup, acute, short caudate, tail 1–1.5 mm with acuminate apex, entire; lateral sepals dark yellow to orange, diffused dotted dark-purple spotted, ovate, 3-veined, asymmetrical, 5–5.5 × 1.7–2.2 mm, base connate, a transverse callus 1 mm, orange, acute, short caudate, tail 0.8–1 mm with acuminate apex, entire; petals translucent orange, diffused purple spotted, oblong, 2-veined, basal veins curved, asymmetrical, 1–1.5 × 1.8–2 mm, base truncate, wide acute, superior margin with discreet obtuse lobe; inferior margin deep excavated, obtuse lobe, entire; lip dark yellow to dark orange, densely dark-purple spotted, unguiculate; claw 0.6–0.7 mm, bilobed at base, caniculate; blade oblong, 1.9–2.1 × 0.8–1 mm, base with 2 lateral, acute, retrorse lobes, 0.4 mm; a pair of lamellar callus at the base, apex reflexed, widely rounded, visibly toothed (erose); column dark yellow to orange, 1.5–1.8 mm long, curved, winged on the distal half, crenated at winged, an acute angle on the lower edge; apex apiculated, foot longer; anther red, apiculate; pollinarium, no seen. **Fruit** light green with light purple-spotted, oblong, 5–7.5 × 3.5 mm.

Material examined: BRAZIL. ESPÍRITO SANTO: Domingos Martins, III.1991, fl., A.L.V. Toscano de Brito 828 (MO). Venda Nova do Imigrante, 14.I.2017, fl., D.C. Imig & M. Bolson 471 (UPCB); 07.VI.2012, fl., A.L.V. Toscano de Brito 3091 (UPCB). MINAS GERAIS: Ataléia, XII.2009, fl. & fr., M.A. Campacci & R.V. Leitão MAC-1963 (ESA114455 - Holotype *D. ataleiensis*). Medina, Pedra de Atenas, 07.IV.2017, fl., D.C. Imig 479 (UPCB). SÃO PAULO: São Bento do Sapucaí, 950 m, XII.2018, fl., S.L.X. Tobias (ESA, not found in herbarium). Without locality, W. Bowmann 1636 (W50984).

Distribution and ecology: *Dryadella auriculigera* was described from a specimen collected from Brazil without a precise location. Our study confirms this species as endemic to Brazil and the Atlantic Rainforest vegetation with rock outcrops in the state of Espírito Santo and new records for Minas Gerais (Fig. 17b). In nature, *D. auriculigera* is rarely found, and the same happens in private or public living collections, despite being a beautiful species due to the colour of its tiny flowers, different from other Brazilian *Dryadella*. The flowering period occurs from December to June.

In a RedList of São Paulo State (SEMA 2016), *D. auriculigera* was considered Extinct in Nature (EX). However, no specimens were recorded in São Paulo in any study or any herbaria analysed to date. Since this study did not indicate any voucher and we did not find any specimen or citation of this species for the state of São Paulo, we conclude that it must be the result of some mistake.

Etymology: Do latim *auriculiger* "bearing ears", referring to the shape of the petals.

Taxonomic notes: *Dryadella auriculigera* is easily identifiable in relation to the other Brazilian species, mainly due to the colour and shape of the flowers and leaves. It has an affinity with *D. kautskyi* and *D. lilliputiana*. Vegetatively it forms small and slightly loose clumps; leaves are linear and terete, light-green with vinaceous nuances and dark-purple dots (vs *D. lilliputiana* presents well-congested clumps and leaves linear and terete, slightly furrowed on the adaxial side, light-green, and an alvescent portion evident at the base. *Dryadella kautskyi* presents linear leaves, semiterete grooved on the adaxial face, light-green in colour, with absent or inconspicuous punctuations). The flowers of *Dryadella auriculigera* are orange with diffuse purple punctuations and three well-marked veins; sepals are short caudate with attenuate apex; the lip is dark-orange with visibly denticulated margin (vs *D. lilliputiana* presents alvescent flowers without marked veins, with dark-purple punctuations; sepals long caudate with clavate apex; lip with entire margin. *Dryadella kautskyi* has light-yellow flowers, with inconspicuous or absent light-purple punctuations, without marked veins; short caudate sepals with acute apex; lip with the same colour as the sepals, with minutely denticulated margin) (Fig. 1b,j,l).

Dryadella auriculigera was initially described in the genus *Pleurothallis* by Reichenbach (1871). The species was based on material originating in Brazil, collected in 1866 by Bowmann and cultivated in the Saunders Garden by Mr Green, who initially believed it to be *Pleurothallis aviceps*. However, the plant flowered a year later, and Reichenbach realised this species would be new. In the protologue, the author does not compare the species with any other. Reichenbach (1878) transferred the species to *Masdevallia auriculigera*, and later, Luer (1978a) moved to *Dryadella*. In the review of the genus, Luer (2005) highlights the rarity of this species, which we corroborate in this work.

Dryadella ataleiensis, a synonym proposed here, was described by Campacci (2011), from a collection in the state of Minas Gerais, Ataléia in May 2009, by M.A. Campacci

and *R. de Vasconcellos Leitão* MAC- 1963 (ESA 114455). In the protologue, the author compares the species with *D. lilliputiana* (Cong.) Luer (1978b) but differs from the latter only by the smaller size and colour of the flowers. After analysing the protologues and the nomenclatural types, we noticed that *D. ataleiensis* is a synonym of *D. auriculigera*, although, in the illustration and image of the protologue, the leaves do not present purple punctuations, as characteristic of *D. auriculigera*. The other distinctive features of the flowers include the intense yellow to orange colour with an even darker lip, evident punctuations and the veins dark and well-marked, with denticulated margin, and the shape and colour of the sepals and petals represent *D. auriculigera*.

Dryadella sapucaiensis was recently described by Campacci & Silva (2020), is known only by the type and, according to the authors, is located between the region of São Bento do Sapucaí and Campos de Jordão in São Paulo State. We did not have access to any material, and the type is not deposited at the ESA, as mentioned in the protologue. The authors compare vegetatively with *D. krenakiana* (Fig. 15k) for presenting pseudo monopodial pendant growth; however, this characteristic is not evident in the images and in the original plate, where the plant is cespitose. We highlight the affinity with *D. auriculigera* in vegetative and floral aspects. However, the lack of access to the material does not allow us to make precise comparisons between the species. Comparing the descriptions of the species *D. sapucaiensis* (vs *D. auriculigera*), we note the overlapping of measurements in almost all parts, for example, ramicaule 2.5 mm (vs 1–2.5 mm), purple-spotted green leaves, linear-lanceolate, grooved 23×0.2 mm, (vs light-green with dark-purple spotted, linear, terete, $30\text{--}35 \times 2.3\text{--}2.8$ mm). The tri-alate ovary with denticulated wings, as seen in Campacci & Silva (2020, page 664) (vs ovary slightly tri-alate, toothed), dorsal sepal oval 4×3 mm (vs dorsal sepal oval to oval-lanceolate $5\text{--}5.5 \times 2.5\text{--}3$ mm), rhomboid dolabriformis petals, 1.2×1 mm (vs petals oblong, $1\text{--}1.5 \times 1.8\text{--}2$ mm), lip 2×0.7 mm, with margin slightly denticulate (although described as entire and drawn denticulate) (vs lip $1.9\text{--}2.1 \times 0.8\text{--}1$ with denticulate margin (erosis)). The column was also described without conformity with the illustration with greenish with purple spots, and approx 2mm winged on the distal half, crenated at winged (Campacci *et al.* 2020, page 664), but in fig. 05 (Campacci & Silva 2020, pp. 664–665), it is noted that the wings of the column end at an acute angle (vs column dark yellow to orange, 1.5–1.8 mm long, winged on the distal half, crenated at winged, an acute angle on the lower edge). In addition, some terminology and

measurements traditionally used for the genus have been neglected or are inaccurate, such as, the size of the sepal tail described as "short tail apex, with somewhat trapezoidal transverse callus at the base of the abaxial face", as well as like the shape that does not show the characters in detail in the lip and column, and in the photos whose colours seem to have been edited. Although the flowers of *D. auriculigera* are usually yellow-orange, this can vary according to the sun's incidence. Plants in cultivation tend to have lighter flowers, between yellow and greenish-yellow, and the same happens in the colour of the leaves. It tends to be less spotted with purple, showing green when in cultivation. Therefore, in the absence of material for consultation, especially the type and contradictions found in the protologue, we consider this species a new synonym. Future investigations allow us to make more precise decisions regarding this taxon.

3. *Dryadella aviceps* (Rchb.f.) Luer, Selbyana 2: 208 (1978). Type: BRAZIL. Without locality, *Bowmann 1633* (Holotype: W, Reichenbach: herbarium orchid. 50983 drawing 1833 [digital image!]).

≡ *Pleurothallis aviceps* Rchb.f., Gard. Chron. Bradea 1871: 1579 (1871).

≡ *Masdevallia aviceps* (Rchb.f.) Rchb.f., Otia Bot. Hamburgensia 2: 93 (1881).

= *Masdevallia obrieniana* Rolfe, Gard. Chron. 8: 524 (1890). Holotype: Without locality, flowered in cultivation in 1890 from the collection of *R.I. Measures* (K000077697!).

= *Masdevallia sessilis* Barb. Rodr., Plantas novas cultivadas no Jardim botânico do Rio de Janeiro 6: 17, t.3, fig. b (1898). Holotype: BRAZIL, RIO DE JANEIRO: Itaguay, João Barbosa Rodrigues Junior. Lost. Lectotype (here designated): Barbosa Rodrigues' original drawing which appeared in his work "Pl. Jard. Rio de Janeiro 6: 17 (1898)".

= *Masdevallia paulensis* Barb. Rodr. Contributions du Jardin Botanique de Rio de Janeiro 2: 51, t.6, fig. a1-6 (1901). (Holotype: BRAZIL, *M.G. Edwall* (Lost). Lectotype (here designated): Barbosa Rodrigues' original drawing, which appeared in his work "Contributions du Jardin Botanique de Rio de Janeiro 2: 51, t.6, fig. a1-6 (1901).

≡ *Masdevallia simula* var. *obrieniana* (Rolfe) Kraenzl. Repert. Spec. Nov. Regni Veg. Beih. 34: 192 (1925).

= *Masdevallia bradei* Schltr. ex Hoehne, Boletim do Museu Nacional de Rio de Janeiro. Botânica 12(2): 32 (1936). Holotype: BRAZIL, SÃO PAULO: Iguape, II.1917 A.C. Brade (Isotype HB 8058!).

= *Dryadella silvana* F. Barros, Rev. Bras. Bot. 18(1): 35 (1995). Holotype: BRAZIL. BAHIA: Sul do estado. 04.IV.1993. E.F. da Silva (SP267767!).

= *Dryadella espirito-santensis* (Pabst) Luer, Selbyana 2: 208 (1978). Holotype: BRAZIL. ESPÍRITO SANTO: Redentor, near Pedra Azul. Alt.1200 m, 25.IX.1972, Kautsky. R. 381b (HB:58522!). *syn. nov.*

= *Masdevallia gomesii-ferreirae* Pabst, Additamenta ad orchideologiam brasiliense 2: 10, t. 49, fig. f (1975). Holotype: BRAZIL. PERNAMBUCO: without locality, collected by Severino Parasita, cultivated by Burle Gomes-Ferreira 294 (HB, lost). Lectotype (here designated): Pabst' original drawing which appeared in Bradea 2(10): 51, t.1, fig. f. (1975) *syn. nov.*

= *Dryadella litoralis* Campacci, Coletânea de orquídeas brasileiras 5: 154 (2007). Holotype: BRAZIL. SÃO PAULO: Bertioga, 10-50m, 19.VII.2002. Cultivated by S.P. dos Santos, SPS-035, Flowered in cultivation, X.2002 (SP: 402125 [digital image!]). *syn. nov.*

(Fig. 1c-e; 5a-h)

Epiphyte herb, densely cespitose, medium to larger, 4.5–6.3 mm tall. **Roots** slender, 24–6 at each rhizome node, 0.6–0.9 mm diameter. **Rhizome** 4–6 mm between the internodes. **Ramicaul** 3–3.7 mm long, erect, covered by 2(3) paleaceous, oblong to lanceolate sheaths, acute and mucronate, entire, 1.8–2.5 × 2–2.4 mm. **Leaf** dark green on both sides, and sometimes with vinaceous nuances, coriaceous, lanceolate to oblanceolate, flat, 45–55 × 5.5–6.3 mm; petiole 1.3–2.5 mm long; blade erect, attenuate at the base, minutely tri toothed. **Inflorescence** a successive single-flowered, congest raceme; bearing 2(3) pale basal bracts, elliptical, acute and mucronate, 1.8–2.9 × 0.8–1 mm; peduncle 3–4.5(6) mm long; pedicel plus ovary light green to alvescent, slightly trialate, entire to minutely toothed, 1–1.5 mm long. **Flower** small to medium; dorsal sepal yellow to light yellow with diffused light-purple spotted or alvescent and densely dark-purple spotted, ovate, slightly inflexed, slightly 3-veined, carinate and entire, 3.5–5 (6.5) × 2.1–2.5 mm, base connate 0.4 mm with lateral sepals forming a a short sepaline cup, acute to narrow acute, short caudate, tail 0.8–1 mm with narrow acute apex, margin discreetly toothed at the base; lateral sepals yellow to light yellow with diffused

light purple spotted or densely dark-purple spotted, elliptical, slightly 3-veined, asymmetrical, $4-5(6.5) \times 2-2.5$ mm, base connate, a transverse callus 1.3 mm purple, acute, short caudate, tail 1–1.3 mm with thick apex, entire; petals yellow, densely dark-purple spotted, oblong, 2-veined, veins parallel, asymmetrical, $1.5-1.8 \times 1.2-1.5$ mm, base truncate, obtuse, superior margin with acute to obtuse lobe; inferior margin excavated, acute to obtuse lobe, discreetly designed backwards, entire; lip purple, unguiculate; claw 1–2 mm, bilobed at base, caniculate; blade oblong, $0.6-1.8 \times 1.2-1.4$ mm, base with 2 acute, retrorsos lobes, 0.3 mm; a pair of lamellar callus toothed at the base; apex reflexed, wide obtuse, entire or minutly toothed; column dark yellow, 0.9–1.2 mm, curved, winged on the distal half, dentate at winged base; apex acute and minutely serrated, foot not equally long; anther yellow, apiculate; pollinarium, no seen.

Fruit light green, oblong, 10 – 12 x 4.5 mm.

Selected examined material: BRAZIL. Cultivated in Orquidário Colibri, 02.XII.2015, fl., *D.C. Imig 397* (UPCB). BAHIA: Sul do Estado, 15.III.1982, fl., *E.F. da Silva* (SP 247586 - Holotype of *Dryadella silvana*). Guaratinga, Córrego Jacutinga, 23.IV.2009, fl., *A.P. Fontana et al. 5912* (MBML). Prado, 16.V.2018, fl., *D.C. Imig 616* (UPCB). Vitória da Conquista, fl., *A.L.V. Toscano de Brito 1844* (UPCB); Faz. Oriente, 3.III.2013, fl., *C.O. Azevedo 605* (HUESB). ESPÍRITO SANTO: Redentor, near to Pedra Azul. Alt.1200 m, 25.IX.1972, *R. Kautsky 381* (HB 58522 - Holotype of *D. espirito-santensis*). Conceição da Barra, P. E. de Porto Ferreira, 09.VIII.2000, fl., *C.N. Fraga 691* (MBML); Aracruz celulose, 12.VIII.1993, fl., *O.J. Pereira et al. 4832* (VIES). Guarapari, Setiba, 08,VIII.1992, fl., *L.V. Rosa 264* (VIES). Linhares, Restinga de Povoação, 08.VII.2000, fl., *C.N. Fraga 683* (MBML); 16.I.2018, *D.C. Imig 525* (UPCB). Venda Nova do Imigrante, Cultivated in Orquidário Caliman, 07.VII.2012, fl., *A.L.V. Toscano de Brito 3094* (UPCB); 07.VI.2012, fl., *A.L.V. Toscano de Brito 3095* (UPCB); 27.I.2018, fl., *D.C. Imig 476* (UPCB); Cultivated by Fábio Tesch, 19.II.2021. fr., *D.C. Imig 664* (UPCB). Vila Velha, Barra do Jacu, 12.XI.1998, fr., *C.S. Silva* (VIES3007). MATO GROSSO DO SUL: Jateí, 30.VI.2004, fl., *V. Tomazini 348*, (HUEM; HNUP). PARANÁ: Icaraíma, Porto Camargo, Rio Paraná, 22.I.1967, fl., *G. Hatschbach 15821* (MBM). Guaira: Sete Quedas, 24.I.1967, fl., *G. Hatschbach 1590* (MBM); 24.I.1967, fl., *G. Hatschbach 15902* (NY,V,US,K); 30.IV.1967, *H.D. Bicalho* (SP303264); 24.III.1977, *G. Hatschbach 39818* (MBM); Rio Piquiry, 2km da barra, 09.IV.1961, *G. Hatschbach 7894* (MBM); Matinha, *J.C. Lindeman 4455* (NY414926,

K940351). São Pedro do Paraná, 29.V.2001, fr., V. Tomazini 58 (HUEM; HNUP). RIO DE JANEIRO: Paty do Alferes, 18.XI.2015, fl., C.A. Royer 70 (UPCB). Petrópolis, 27.I.1997, fl., A.L.V. Toscano de Brito & C. Luer, 18297 (MO); A.L.V. Toscano de Brito & C. Luer, 18298 (MO). PERNAMBUCO: Without locality, B. Gomes-Ferreira 294 (HB lost - Holotype of *Masdevallia gomesii-ferreirae*). SÃO PAULO: Bertioxa, 10-50m, 19.VII.2002, S.P. Santos SPS-035, VII.2002 (SP 402125 - Holotype of *D. litoralis*). Botucatu, Córrego Indaiá, Faz. São Roque, 05.I.1973, fl., A. Amaral Jr. 1368 (SP). Campos de Jordão, 25.I.2017, D.C. Imig 475 (UPCB); Fazenda da Guarda, 26.VII.1967, fl., J. Mattos & N. Mattos 14999 (SP). Caraguatatuba, 14.V.1938, M. Kuhlmann & A. Gehrt (SP39467); 18.VII.2016, D.C. Imig 354 (UPCB). Guarujá, 30 june 1931, fl., A. Gehert (SP27878); 17.I.1997, C. Luer 18172 (MO). Iguape, II.1917 A.C. Brade (HB 8058! - Isotype of *Masdevallia bradei*). Itú, Reserva Florestal Washington Luiz, 07.III.1934, A. Gehrt 31587 (SP; NY). Itirapina, Morro Pelado, I.1901, G. Edwall 6015 (SP); Jundiaí, 02.II.2018, D.C. Imig 598, (UPCB); 14.III.2018, D.C. Imig 607 (UPCB); Sentido Malota, prox. à base da Serra do Japi sentido Malota, 21.I.2000, E.R. Pasarin 694 (UEC); Serra do Japi, Bairro Eloy Chaves, 22.XI.1989, E.R. Pasarin 97/89 (UEC); Sentido Bairro Eloy Chaves, 22.XI.1997, fl., E.R. Pasarin 736 (UEC); Porto Ferreira, 14.I.2014, fl., G.M. Macusso 356 (HRCB); Trilha de uso exclusivo do Rio Mogi-Guaçu, fl., R.M.G. Konopczyk (HRCB62383); P. E. de Porto Ferreira, 13.XI.2014, fl., G.M. Macusso & Monteiro 392 (HRCB); Trilha do Rio Mogi-Guaçu, 17/III/2011, fl., J.A. Lombardi & R. Oliveira 8096 (HRCB 54953). Teodoro Sampaio, R. E. do Morro do Diabo, 27.I.1986, fl., P. Martuscelli 214 (SP); 27.I.1986, fl., Martuscelli 213 (SP); 27.I.1986, fl., P. Martuscelli 212 (SP320166); 6.I.1986, P. Martuscelli 201 (SP); 04.I. 1990, fl., P. Martuscelli (SP315484); 17.III.1989, fl., P. Martuscelli (SP 315018); 15.III.1989, fl., P. Martuscelli (SP315467). São Sebastião, Praia da Baleia, 16.I.1997, C. Luer 18145 (MO). Silveiras, Serra da Bocaina, 26.II.1951, fl., (K940347). São Manuel, 16.I.2018, fl., D.C. Imig 598 (UPCB); 31.V.2016, fl., D.C. Imig 448 (UPCB). Pavuna, prox. a Botucatu, 29.IX.1972, fl., A. Amaral Jr 1141 (SP); Faz. Sta Margarida, 27.XII.2013 fl., P. Macusso & F.C. Gallerani 334 (HRCB). São Sebastião, Praia da Baleia, 06.III.1995, fl., E.L.M. Catharino 2067 (SP). Sorocaba, 11.I.2022, fl., M. Klingelfuss 340 (UPCB). Ubatuba, fl., F.C.P. Garcia, (HRCB 10410); fl., J.E.L.S. Ribeiro (HRCB8818). PARAGUAY. Paraguari, Luer 12259 (MO 100539090), Luer 10471 (MO 100539088).

Distribution and ecology: *Dryadella aviceps* is not endemic to Brazil; it also occurs in Paraguay. In Brazil, it is a species with a wide distribution, different from most *Dryadella*. The distribution limits to the northeast are Pernambuco and Bahia, also occur in the Espírito Santo (common in mountain regions), Rio de Janeiro and São Paulo States, and to the south of the Paraná State. It is mentioned here for the first time for Mato Grosso do Sul State (Fig. 17b). The extreme west distribution is in Foz do Iguaçu, on the border with Paraguay and extends to the Ybycuí National Park and Paraguari. It occurs in small and sparse populations in dry and open forests and arboreal restinga. Despite its wide distribution, it is not easily found in nature and is rarely found in living collections.

Sampaio (2016) cites this species to São Thomé das Letras-MG, but when analysing the photo, it seemed to be *D. vitorinoi*. During our studies, we did not find material in the field to confirm the identity of this specimen. Mancinelli & Esemann-Quadros (2016) cite an occurrence for Santa Catarina; however, this is *D. catharinensis*. The flowering occurs all year.

Etymology: From the Latin *aviceps* "a bird head" alluding to the similarity of a flower bud to a bird's head.

Taxonomic notes: *Dryadella aviceps* is similar to *D. vitorinoi*, but differs vegetatively by lanceolate to oblanceolate leaves, erect and dark-green and generally vinaceous (vs leaves lanceolate, suberect light-green on both sides, sometimes with vinaceous nuances when exposed to high sun intensity). In the flowers, *D. aviceps* has sepals dark-yellow with sparse or dense dark-purple punctuations, the dorsal sepals are slightly inflexed and fully open flowers (vs dorsal sepals light-yellow, absence or diffused and inconspicuous light-purple spotted, dorsal sepals inflexed and barely open flowers). Petals oblong, 2 parallel veins, $1.5\text{--}1.8 \times 1.2\text{--}1.5$ mm, superior margin with acute to obtuse lobe (vs petals yellow, oblong, 2-veined, the basal vein is curved, $5.2\text{--}5.8 \times 3.4\text{--}3.6$ mm, superior margin without lobe (Fig. 15c-e; Fig. 16d).

Dryadella aviceps was initially described as *Pleurothallis* by Reichenbach (1871). The type material is originally from Brazil, taken in 1866 by Bowmann 1633 and cultivated in the Saunders garden by Mr Green. The following year, when the plant flowered, Reichenbach realised that this species was unpublished and described it with the epithet specific "aviceps" (bird's head) due to the similarity of the flower bud to a bird's head. The same author transfers the species to the genus *Masdevallia* (Reichenbach 1878).

Rolfe (1890) published *Masdevallia obrieniana*, whose provenance of the specimen is unknown, in honour of Mr James O'Brien, English writer of the book Harrow-on-the-Hill, who drawn and coloured the plate of the species.

Barbosa Rodrigues (1898) described *Masdevallia sessilis* from material found by his son in Itaguaby, Rio de Janeiro State. The specific epithet refers to the short peduncle (sessile), uncommon in *Masdevallia*. Also, Barbosa Rodrigues describes the yellow sepals stained with dark crimson and draws attention to this, saying that it could resemble a *Pleurothallis*, but the short floral tube (lateral sepals joined) would include it in *Masdevallia*.

Barbosa Rodrigues (1901) described *Masdevallia paulensis* Barb. Rodr., whose epithet is related to São Paulo, state of origin of the material (Salto Grande, Rio Paranapanema). *Masdevallia sessilis* and *M. paulensis* were synonymised in *M. obrieniana* by Pabst (1976), justifying that the only detail that differs them from *M. sessilis* is that they do not have concolor yellow sepals without spots of carmine and *M. paulensis* also differs because they do not present the yellow lateral sepals, but they are punctuated with purple, therefore, insufficient details to keep them autonomous from *M. obrieniana*. Kraenzlin (1925) synonymises *M. obrieniana* to *M. simula* var. *obrieniana* (Rolfe) Kraenzl. Luer (1978a) makes the new combination for *Dryadella aviceps* (Rchb. f.) Luer.

Barros (1995) describes *D. silvana* with material from the south of Bahia, cultivated by E. F. da Silva. The author compares it to *D. obrieniana*, which has yellow flowers with violet punctuations, non-tailed sepals, and lip short unguiculated and obovate. In contrast, in *D. silvana* the flowers are yellowish-brown with vinaceous punctuations and a long unguiculated and 'oblate' lip.

Masdevallia espirito-santensis was described by Pabst (1973), whose epithet is related to the state of Espírito Santo, State where the type was found, later transferred to *Dryadella* by Luer (1978a). Pabst (1973) compares to *M. obrieniana*, now synonymous of *D. aviceps*, but differentiates *M. espirito-santensis* by the purple and not yellow flowers, and greater vegetative size, in addition to the different lip, but without the indication of the differences.

Analysing the descriptions, illustrations and holotype, we found it is a synonym of *D. aviceps*. There is a variation in the flowers' colour and distribution. In places with a greater sun incidence and heat, the flowers tend to have darker sepals and petals due to

the increase of purple punctuations. Luer (2005) highlights that this species is only known by the type, and believed to be lost and therefore based its description on the drawn made by Pabst in the original description.

Dryadella gomes-ferreirae was published by Pabst (1975), whose epithet is in honour of Augusto Burle Gomes-Ferreira, who received the type specimen from Severino Parasita and whose collection location is unknown. In the protologue, Pabst (1975) points out an affinity with *M. espirito-santensis* and *M. obrieniana* for having short caudate sepals but differs in the tiny red flowers, the size of the flowers and the general morphology. Analysing the descriptions and illustrations of the protologue, we found that it is a synonym of *D. aviceps*. As mentioned above, there is a variation in the colour of the flowers throughout the distribution. Luer (2005) highlights that *Dryadella gomes-ferreirae* is only known by the type, that is lost. The same author emphasises the need for further investigation, given the little knowledge about the species. Considering the lack of type, Luer (2005) based the description and drawn on a material acquired in Munich Botanical Garden and cultivated in Tarporley, England, by S. Manning (MO). This material was also not found.

Dryadella litoralis was described by Campacci (2007), whose specific epithet refers to the place where the plant was found, litoral (coastal) of São Paulo state, and cultivated by Solange Perez dos Santos. In the protologue, the author describes the affinity with *D. obrieniana*. At that time, it had already been synonymised in *D. aviceps*. In justification of this new species, Campacci declares that *D. litoralis* differs in the morphology and colours of the flowers, which presents whitish petals and sepals with purple macules and caudate sepals, a longer lip with sub-pandurate central lobe, and a long canaliculate unguiculus. *Dryadella obrieniana* has yellow petals and sepals with violet macules, short caudate sepals, lip with an obovate central lobe, and a short unguiculus. In the description of *D. litoralis*, the author describes yellow or whitish flowers, abundantly stained with purple, which contradicts the justification. He justifies that the habitat is different, as it is on the north coast of São Paulo, while *M. obrieniana* occurs on the plateau and has a larger dispersion area.

Analysing the holotype, descriptions, illustrations, and exsiccatae from various locations, we believe it is a synonym of *D. aviceps*. In addition to the holotype of *D. litoralis*, we compared the live material, both from specimens from the coast of São Paulo: Caraguatatuba (*D.C.Imig* 354), Iguape (*D.C. Imig* 452), Campos de Jordão (*D.C.*

Imig 475), Jundiaí (*D.C. Imig 598*), Espírito Santo: Venda Nova do Imigrante (*D.C. Imig 476*), Linhares (*D.C. Imig 525*), Bahia: Prado (*D.C. Imig 616*) and Vitória da Conquista (*C.O. Azevedo 605*), and we observed that there is a great variation in the colors of the sepals and petals. Throughout the wide distribution area of this species, we noticed that specimens from south of its distribution present lighter-yellow flowers with sparse purple punctuations and large and wide leaves, rarely vinaceous. In the warmer regions, like Bahia, the flowers are darker, as the purple punctuations are more juxtaposed, and the leaves are smaller, narrower, and entirely vinaceous (Fig. 1 c-e).

4. *Dryadella catharinensis* Imig, Mancinelli & E.C.Smids, *Phytotaxa*, 508(2): 206 (2021). Holotype: BRAZIL. SANTA CATARINA: Joinville, Morro do Tromba, 03.IX.2009, [fl.], W.S. Mancinelli 1007 (JOI!).

(Fig. 1f; 6a-g)

Epiphytic herb, caespitose, small, 16–23 mm tall. **Roots** thick, 2–3 at each rhizome node, 0.5–0.7 mm diameter. **Rhizome** 1–1.5 mm between the internodes. **Ramical** 1.5–2 mm long, suberect, covered by 2 paleaceous, triangular sheaths, obtuse and mucronate, entire, 0.8–1.2 × 0.6–0.8 mm. **Leaf** dark green on the adaxial side and vinaceous on the abaxial side, thickly coriaceous, narrowly elliptic, flat, 13–19.5 × 2.1–3.3 mm; petiole 1–1.5 mm long; blade suberect, cuneate at the base, retuse to truncate. **Inflorescence** a successive, single-flowered, congested raceme; bearing 2 pale basal bracts, triangular, mucronate, 1.51.8 × 1–1.3 mm; peduncle 5.5–8.5 mm long; pedicel plus ovary light green with purple dots, slightly triolate, entire, 0.8–1.3 mm long. **Flower** small to medium; dorsal sepal pale yellow, densely dark-purple spotted, ovate, inflexed, 3-veined, carinate and entire, 4.5–5.5 × 2–2.5 mm, base connate 0.5 mm with the lateral sepals forming a short sepaline cup, obtuse, inconspicuous caudate, entire; lateral sepals light pale yellow, sparse light purple spots at base, ovate, 3-veined, asymmetrical, 3.3–3.7 × 1.8–2.3 mm, base connate, a transversal callus 1mm, orange, subacute, short caudate, tail 1–1.5 mm, slightly toothed; petals dark-yellow, diffused red-spotted, rhomboid, 2-veined, basal vein is curved, asymmetrical, 1.0–1.4 × 1.3–1.5 mm, base truncate, obtuse, superior margin erect, lobe acute lobe, slightly projected forward; inferior margin excavated, lobe acute, entire; lip dark yellow to orange, not spotted, unguiculate; claw 0.7–0.8 mm, bilobed at base, caniculate, a ring callus in the median portion; blade cuneate, 1.8–2.1 × 1.5–1.6 mm, base with 2 lateral, acute retrorse

lobes, 0.3 mm; a pair of lamellar callus at the base, apex reflexed, obtuse, toothed; column dark-yellow, red in the ventral margin, 1.5–1.7 mm long, curved, winged on the distal half, acute-toothed at winged base; apex minutely toothed, foot equally long; anther red, apiculate; pollinarium not seen. **Fruit** not seen.

Material examined: BRAZIL. SANTA CATARINA: Corupá, Bairro Izabel, 01.IX.2020, [fl.], D.C. Imig 656 (UPCB). Joinville, Morro do Tromba, 03.IX.2009, [fl.], W.S. Mancinelli 1007 (JOI!); Serra Dona Francisca, next to Estrada Piraí, 29.VIII.2020, fl., W.M. Mancinelli & D.C. Imig 1591 (MBM).

Distribution and ecology: *Dryadella catharinensis* is endemic to the Atlantic Forest and, until now, known only from the type localities in the state of Santa Catarina (Fig. 17b). It was found in Joinville and Corupá, north-northeast of the Santa Catarina state, in the Dense Ombrophilous submontane rainforest. In Joinville, it occurs in the Morro da Tromba (holotype locality) and Morro Pelado, within the Serra Dona Francisca Environmental Protection Area (APA) with an extension of 401.77 km². Few individuals were found at both sites, growing at 2–3 m from the ground, next to bifurcations of the central stem of the phorophyte. The flowering period occurs from August to October.

Etymology: Named after Santa Catarina State, where the new species was collected.

Taxonomic notes: The tiny *Dryadella catharinensis* is similar to *D. susanae* and may be distinguished vegetatively in the large size of the plant 16–23 mm (vs 10–13.5 mm), and in the large, 13–19.5 × 2.1–3.3 mm (vs 3–5 × 1.5–3 mm), narrowly elliptical, suberect and petiolate leaves (vs leaves broadly elliptical, prostrate and sessile). In the flowers, *D. catharinensis* could be distinguished by the yellow (vs dark red) flower, the dorsal sepal 4.5–5.2 mm long (vs. 3.8–4.0 mm), ovate, obtuse and thin (vs triangular-ovate, acute, thickened), the lateral sepals with the margin slightly toothed (vs margin entire), the 1.0–1.4 mm long petals (vs petals 1.5 mm) and the larger lip blade 1.8–2.1 × 1.5–1.6 mm (vs lip blade 1.5 × 1.4 mm), cuneate, with margins toothed, and a ring callus in the median portion of the claw (vs obovate, margins entire, without callosities in the claw) (Fig. 1f; 2a).

5. *Dryadella crenulata* (Pabst) Luer, Selbyana 2: 371 (1978a)

≡ *Masdevallia crenulata* Pabst, Bradea 2: 66 (1976). Holotype: BRAZIL. ESPÍRITO SANTO: Domingos Martins, 08.X.1975, *R. Kautsky* (HB68940 probably lost).

Epiphyte herb, caespitose, large, 50–65 mm tall. **Roots** slender, 2–3 at each rhizome node, 0.3–0.4 mm diameter. **Rhizome** 1.5–2 mm between the internodes. **Ramicaul** 8–9.5 mm long, erect, covered by 2(3) paleaceous, oblanceolate sheaths, acute and mucronate, entire, 8–9.2 × 3–3.7 mm. **Leaf** green to dark green on both sides, coriaceous, oblanceolate, flat, 40–57.3 × 4.5–6 mm; petiole 3–4.5 mm long; blade erect, attenuate at the base, obtuse and minutely mucronate. **Inflorescence** successive single-flowered, congest raceme; bearing 2(3) pale basal bracts, narrow elliptical, acute and mucronate, 1.8–3.2 × 1–1.4 mm; peduncle 10–17 mm long; pedicel plus ovary light green, slightly triolate, entire, 2.3–2.5 mm long. **Flower** medium to large; dorsal sepal translucent albescent to light yellow, densely dark-purple spotted, ovate, totally inflexed, 3-veined, carinate and entire, 5.5–8 × 3–3.7 mm, base connate 1 mm with lateral sepals forming a short sepaline cup, acute, long caudate, tail 6–7(9) mm with clavate apex, entire; lateral sepals translucent yellow, diffused dark-purple spotted, ovate, 3-veined, asymmetrical, totally deflexed, 4.8–6.5 × 2.1–2.8 mm, base connate, a evident transverse callus 1.5 mm, purple, acute, long caudate, tail 5–6 (7.5) mm with clavate apex, entire; petals translucent yellow, diffused purple-spotted, rhomboid, 2-veined, basal vein bifurcated, asymmetrical, 2–2.3 × 1.9–2.1 mm base truncate, wide obtuse, superior margin with discreet obtuse lobe slightly projected forward; inferior margin excavated, wide obtuse lobe, entire; lip purple, unguiculate; claw 0.5–0.7 mm, bilobed at base, caniculate; blade ovate, 1.5–2.3 × 1.3–1.8 mm, base with 2 lateral acute, retrorse lobes, 0.3 mm, a pair of lamellar callus at the base, apex reflexed, wide obtuse, visibly toothed to ciliated; column yellow, 0.9–1.2 mm, curved, winged on the distal half, toothed at winged margins; apex apiculate, foot equally long; anther yellow, apiculate; pollinarium not seen. **Fruit** not seen.

Additional specimens examined: BRAZIL. BAHIA: Boa nova, 03.III.2019, fl., *A.L.V. Toscano de Brito* 3837 (UPCB); 25.VI.2019, fl., *A.L.V. Toscano de Brito* 3884 (UPCB). Poções, Serra do arripio, área de florestas estacional semidecidual, 25.VI.2021, fl., *D.C. Imig & D. Rodrigues* 667 (UPCB). ESPÍRITO SANTO: 7.X.1975, fl., *C. Luer* 6469 (SEL). Domingos Martins, 07.X.1975, fl., *R. Kautsky* (HB68940 probably lost).

Distribution, ecology and conservation status: *Dryadella crenulata* is endemic to the Brazilian Atlantic Forest, and until now, it was only recorded for the state of Espírito Santo. We registered here for the first time for the Bahia state. The location of the type is degraded; in our expeditions in Domingos Martins and surroundings, we could not find it. The Orchidologists and collectors of the region were visited to find the species in the living collections without success. *Dryadella crenulata* was found in seasonal semideciduous forests, forms small and sparse populations and is quite rare in nature (Fig. 17c). The flowering period occurs from March to October.

Etymology: From the Latin “crenulatus”, crenulate, referring to the margin of the blade of the lip.

Taxonomic notes: *Dryadella crenulata* differs from other species of the genus in Brazil, especially for the dorsal sepal projected forward (totally inflexed), long caudate and clavate; lateral sepals facing backwards (totally reflex), lip purple and visibly denticulated to ciliate with dark purple margins, and column with denticulated margins (Fig. 1g,h) *Dryadella crenulata* was initially described in *Masdevallia* by Pabst (1976a) and included in the *Masdevallia* section *Rhombipetala* (Bas: *Masdevallia crenulata*), whose type was collected by R. Kautsky in 1975 and deposited under (HB68940), but this material was not found in the collection. Later, Luer (1978a) transferred *M. crenulata* to *Dryadella*.

6. *Dryadella gnoma* (Luer) Luer, Selbyana 2: 208 (1978a). (Holotype: SEL!).

≡ *Pleurothallis gnoma* Luer, Selbyana 3(1): 110, fig. 158 (1976). Holotype:

EQUADOR. NAPO: 26.VII.1975, C. Luer, G. Luer & S. Wilhelm 698 (MO652922).

= *Dryadella misasii* Luer & R. Escobar, Orquideologia 13(2): 141, t. 14 (1979).

Holotype: COLÔMBIA. CHOCO: Bahia Solano, 09.I.1975, R. Escobar 14544 (JAUM050!); 29.IX.1977, C. Luer 1809 (MO, SEL).

= *Dryadella osmariniana* (Braga) Garay & Dunst. Orchid. Venez. III. Field Guide, 172: 47 (1979). Holotype: BRAZIL, AMAZONAS: Manaus, Estrada Manaus-Itacoatiara, IV. 1976, O.P. Monteiro & J. Ramos 675 (INPA56525!; Paratype INPA35875!; Isoparatypes HB59217 e 74271!). *syn. nov.*

= *Dryadella cardosoi* Campacci & J.B.F Silva. Coletânea de Orquídeas Brasileiras 11: 414 (2015). Type: BRAZIL. PARÁ: Trairão, V.1999, A. Cardoso 720 (Holotype MG,

not found in the collection.). Lectotype (here designated): original drawing which appeared in *Coletânea de Orquídeas Brasileiras* 11: 416, fig. 1 (2015). *syn. nov.*

(Fig. 1i; 8a-h)

Epiphyte herb, caespitose, medium, 45–70 mm tall. **Roots** fleshy, 2–3(4) at each rhizome node, 0.6–1.2 mm diameter. **Rhizome** 4–5 mm between the internodes. **Ramical** 3.3–6 mm long, erect, covered by 2(3) paleaceous, oblanceolate sheaths, acute minutely mucronate, $2.5\text{--}4.5 \times 1.3\text{--}2.7$ mm. **Leaf** dark green and sometimes with purple portions, coriaceous, oblanceolate to narrow-obovate, flat, $32\text{--}65 \times 3\text{--}4.5$ mm; petiole 1–1.5(2.5) mm long; blade erect, attenuate at the base, attenuate and minutely tridenticulate. **Inflorescence** a successive, 2–3 flowered, congest raceme; bearing 2(3) pale basal bracts, oval lanceolate, acute, $2.8\text{--}5.2 \times 1.2\text{--}1.8$ mm; peduncle 2.5–5 mm long; pedicel plus ovary light green to albescent, slightly tridentate, entire, 2–2.5 mm long. **Flower** medium; dorsal sepal light yellow to greenish yellow, occasionally dark yellow, no spotted, ovalate, inflexed, 3-veined, carinate and entire to slightly erose, $5.5\text{--}7.5 \times 2.3\text{--}2.5$ mm, base connate 1–1.5 mm with lateral sepals forming a short sepaline cup, attenuate, short caudate, tail 1 mm with acute apex, entire; lateral sepals light yellow to greenish yellow to occasionally dark yellow, no spotted, ovate, 3-veined, asymmetrical, (4.2) $5.5\text{--} \times 2.3\text{--}2.6$ mm, base connate, absence or rudimentary transverse callus, wide acute, short caudate, tail 0.6–0.8 mm with attenuate apex, entire; petals yellow, light purple-spotted, oblong, 2-veined, veins parallel, asymmetrical, $2.1\text{--}2.4 \times 1.7\text{--}2.1$ mm, base truncate, apex wide obtuse, superior margin with well marked acute lobe; inferior margin excavated, obtuse lobe, entire; lip dark-purple with white margins, unguiculate; claw 0.3–0.7 mm, bilobed at base, caniculate; blade oblong as shovel-shaped, $2.2\text{--}2.8 \times 1\text{--}1.5$ mm, absent or discreetly obtuse lobes; lamellar callus absent, discreetly channeled, apex not reflexed, truncate to wide obtuse, entire; column light yellow, 1.7–2 mm long, curved, winged on the distal half, crenated at winged margins ending in a sharp tooth; apex toothed, foot equally long; anther light yellow, apiculate; pollinarium, not seen. **Fruit** not seen.

Selected examined material: BRAZIL. AMAZONAS: Manaus, estrada Manaus-Itacoatiara, 04/1976, fl., O.P. Monteiro & J. Ramos 675, (INPA56525! - Holotype of *D. osmariniana*); VI/1972, O.P. Monteiro & J. Lima 53 (INPA35875! - Paratype of *D. osmariniana*), M-1, km 138, 08.IV.1972, O.J. Lima 53 (HB59217 - Isoparatype of *D. osmariniana*). Presidente Figueiredo, 25.V.2020, fl., A.H. Krahel 1477 (INPA). AM-1,

Rio Preto da Eva, 17.III.1977, fl., O.P. Monteiro 1345 (INPA). PARÁ: Trairão, V.1999, A. Cardoso 720 (MG). COLOMBIA. CHOCO: Bahia Solano, Alto de Nabugá, 9.I.1975, R. Escobar 1451 (JAUM!, SEL - Holotype *D. misasii*). CHOCO: Serrania del Baudó, VI.1988, G.M. Urreta 70 (COL!). EQUADOR. NAPO: piscinas de Laguna Azul, Rio Jatunyacu, Comunidade de Alkus, 29.XI.2010, L. Endara 1610 (QCA!, FLAS!); Cantón Tena: Rio Jatunyacu, 18.XII.2009, L. Endara 1553 (QCA, FLAS!); 27.XI.2010, L. Endara et al. 1601 (QCA, FLAS).

Distribution and ecology: *Dryadella gnoma* is a species with a relatively wide distribution and is related to the low forests of the Andes (between 500 and 900 m altitude), from Costa Rica to Colombia and Peru (Luer, 2005). Here we report the first record for the Brazilian Amazon, in the state of Pará. Material with flowers and young fruits was collected almost every month of the year (Fig 17c).

Etymology: From Latin “*gnomus*“, a dwarf, in reference to the tiny habit.

Taxonomic notes: *Dryadella gnoma* was described as *Pleurothallis gnoma* Luer (Luer, 1976), based on material from Ecuador. Latter, Luer (1978a) transferred it to *Dryadella*. Subsequently, Luer & R. Escobar (1979) describe *Dryadella misasii*, from Colombia, Department of Choco. This species is also synonymised in *D. gnoma* by Luer (2005) where it justifies that the petals present variable angles, but the morphology of the shovel-shaped lip (different from all other species of the genus), in the deflexed and absence of transversal callus at the base of the lateral sepals (characteristic of *Dryadella*) and the lack of total opening of the flowers are characteristics that justify the synonymisation.

Dryadella osmariniana was initially described in *Masdevallia* by Braga (1978) and inserted in the *M.* section Rhombipetalae; it is compared to *D. obrieniana*, differing from it by linear-lanceolate leaves and lip with denticulated margins. Dunsterville & Garay (1979) did a new combination in *Dryadella*.

Recently *Dryadella cardosoi* Campacci & J.B.F. Silva was described and published by Campacci (2015), based on material collected in Brazil, Pará, Trairão (A. Cardoso 720 (MG, not found in the herbarium)). The authors do not comment on similarities with other species in the protologue.

Analysing the type, descriptions, illustrations, in addition to materials of *D. gnoma* in the herbaria QCA, FLAS and COL, we propose synonymisation here, thus prevailing the oldest species *D. gnoma*, including *D. osmariniana* and *D. cardosoi* as synonyms,

especially considering the shape of the flowers that do not open completely. The characteristic of oblong (shovel-shaped) and non-deflected lip, in addition to the typical colouration of *D. gnoma* used to characterise *D. cardosoi*, which are greenish-yellow sepals and petals, with a purple lip, without basal lobes and absence of transverse callus at the base of the lateral sepals. The colour of the flowers is variable along with the geographic distribution; they occasionally presents with dark-yellow sepals, petals without purple punctuations and dark-yellow lip.

Luer (2005) suggested synonymisation of *D. osmariniana* in *D. guatemalensis*, but *D. osmarinana* (now *D. gnoma*) has lanceolate to narrow-obovate leaves, 3.2–65 × 3–4.5 mm (vs 100mm). The flowers have greenish-yellow sepals and absence of purple punctuations, absence of transverse callus at the base of the lateral sepals and oblong (spade-shaped) lip, non-reflex, dark-purple (vs yellow sepals and densely punctuated with dark-purple, presence of transverse callus at the base of the lateral sepals and reflex lip, obovate to orbicular, yellow to greenish-yellow and densely marked with dark purple).

7. *Dryadella kautskyi* (Pabst) Luer, Selbyana 2: 208 (1978a). Holotype: BRAZIL. ESPÍRITO SANTO: Domingo Martins, Caxixe Frio, X.1972, *R. Kautsky* 381-A (HB59136; Isotype HB59136!)

≡ *Masdevallia kautskyi* Pabst, Bradea 1: 33 (1973).

(Fig. 1j; 9a-h)

Epiphyte herb, densely cespitose-ascending, medium, 40–5.5 mm tall. **Roots** slender, 3–4(5) at each rhizome node, 0.3–0.5 mm diameter. **Rhizome** 1–2 mm between the internodes. **Ramicaul** 4.5–5 mm long, erect, covered by 2 paleaceous, oblong to lanceolate tubular sheaths, acute and mucronate, 8.3–1.3 × 2.4–2.9 mm. **Leaf** light green, sometimes suffused with purple, thickly coriaceous; linear, semiterete, grooved on the adaxial face, 4–6(7.5) × 1.5–1.8 mm; petiole 2–3 mm long; blade erect, attenuate at the base, retuse and mucronate. **Inflorescence** a successive, single flowered, bearing 3(4) pale basal bracts, narrow elliptical, attenuate and mucronate, 1.3–1.9 × 0.6–0.8 mm; peduncle 7–10 mm long; pedicel plus ovary light green to alvescent, slightly triolate, entire, 1.6–2.5 mm long. **Flower** medium; dorsal sepal yellow to greenish yellow, sparse and not evidente light-purple spotted, ovate, inflexed, 3-veined, carinate

and minutely crenate, $5.3\text{--}6.5 \times 1.8\text{--}2.2$ mm, base connate 0.5 mm with lateral sepals forming a short sepaline cup, acute, short caudate, tail 1–1.5 mm with acute to acuminate apex, minutely crenated; lateral sepals yellow to greenish yellow, sparse and not evidente light-purple spotted, ovate, 3-veined, asymmetrical, $5\text{--}5.5 \times 2\text{--}2.3$ mm, base connate, a minutely transverse callus 0.5 mm, purple, acute, short caudate, tail 1–1.3 mm with acuminate and deflex apex, minutely crenated; petals yellow to dark-yellow, sparse and not evidente light-purple spotted, oblong, 2-veined, basal veins is curved, asymmetrical, $1.3\text{--}1.5 \times 1.5\text{--}1.8$ mm, base truncate, obtuse, superior margin with acute to obtuse lobe; inferior margin excavated, wide obtuse lobe, entire to minutely crenated; lip yellow, not evidente light-purple spotted on th base, unguiculate; claw 0.5–0.8 mm, bilobed at base, caniculate; blade oblong to obovate, $1.8\text{--}2 \times 1.1\text{--}1.4$ mm, base with 2 acute, divaricated lobes, 0.5 mm; a pair of minutely lamellar callus at the base, apex reflex, widely obtuse, minutely crenated; column yellow, 1.2–1.5 mm, curved, winged on the distal half, crenated at winged base ending on a slightly protruding back tooth; apex acute and minutely serrated, foot equally long; anther ligh yellow, apiculate; pollinarium not seen. **Fruit** not seen.

Selected examined material: BRAZIL. ESPÍRITO SANTO: Cultivated in Petrópolis from ES, 30.XI.1989, *C. Luer 14501* (MO 10053937). Venda Nova do Imigrante, 21.XI.2015, fl., *A.L.V. Toscano de Brito 3476* (UPCB); 25.XI.1989, fl., *C. Luer 14478* (MO00539378). Santa Teresa, fl., *Orquidário Calimann 8831*, (MBML 42995). Goiabeiras/Tijuco Preto, 26.X.1981, *R. Kautsky 703* (SP 175570).

Distribution and ecology: *Dryadella kautskyi* is endemic to the Atlantic Forest and seems to be restricted to the mountainous region of ES, where it is rare. The type locality is degraded, and during field expedition visits to orchidologists and collectors in the area, no individuals of the species were found (Fig. 17c). According to information from the herbarium labels, the flowering period occurs from October to November.

Etymology: In honour of Roberto Kautsky, who discovered this species.

Taxonomic notes: *Dryadella kautskyi* is easily recognised among the other Brazilian species due to its light-green linear and semi-terete leaves. Solitary single-flower inflorescence arranged on a short peduncle measuring 7–10 mm, with barely apparent flowers, pale-yellow to greenish-yellow in colour and absent or inconspicuous light-purple punctuations. (Fig. 1j)

8. *Dryadella krenaquiana* Campacci, Coletânea de Orquídeas Brasileiras 11: 418 (2015). Holotype: BRAZIL. MINAS GERAIS: Conselheiro Pena, Padre Ângelo, Riacho João Pinto, 1300 m Alt. *R. Vasconcelos Leitão et al.* 178, X.2014, II.2015 (ESA 13485!).

(Fig. 1k; 10a-h,)

Epiphyte or lithophytic herb, pending, small to medium, 35–48 mm tall. **Roots** thick, 4–5(6) at each rhizome node, 0.3–0.4 mm diameter. **Rhizome** 8–11 mm between the internodes. **Ramicaul** 2–2.3 mm long, pending, covered by 2(3) paleaceous, oblanceolate sheaths, acute and minutely mucronate, 1–2.1 × 0.7–1.4 mm. **Leaf** light green on both sides or older leaves may have purplish nuances, coriaceous, narrow-obovate, flat, 25–38 × 2–3.2 mm; petiole 1–1.3 mm long; blade suberect, attenuate at the base, obtuse and mucronate. **Inflorescence** uni-flowered; bearing 2(3) pale basal bracts, narrow elliptical, acute and minutely mucronate, 1–1.5 × 0.5–0.8 mm; peduncle 0.5–0.8 mm long; pedicel plus ovary light green to albescent, slightly tri-lobate, entire, 1.2–1.4 mm long. **Flower** small; dorsal sepal yellow with purple punctuations over the veins, elliptic, inflexed, 3-veined, carinate and entire, 3.5–4.5 × 2.0–2.8 mm, base connate 0.4 mm with lateral sepals forming a short sepaline cup, acute, short caudate, tail 0.5–0.7 mm with acute apex, entire; lateral sepals yellow yellow with purple punctuations over the veins, ovate, 3-veined, asymmetrical, 3.5–4.8 × 1.8–2.5 mm, base connate, a transverse callus 0.5 mm, purple, acute, short caudate, tail 0.3–0.6 mm with acute attenuate apex, entire; petals yellow with purple punctuations over the veins, semi-lunatus, 3-veined, basal veins is curved, asymmetrical, 1–1.2 × 1.1–1.4 mm, base truncate, wide obtuse, superior margin with discreet wide acute lobe; inferior margin excavated with wide lobe, entire; lip yellow, unguiculate; claw 0.5–0.6 mm, bilobed at base, caniculate; blade obovate, 1.8–2.1 × 0.8–1.2 mm, base with 2 acute, retrorse lobes, 0.2 mm; a pair of minutely lamellar callus at the base, apex reflex, obtuse, entire to crenated; column 1–1.7 mm, curved, winged on the distal half, discreetly entire to crenated at winged base, apex acute and entire, foot longer; anther not seen; pollinarium not seen. **Fruit** not seen.

Selected examined material: BRAZIL. MINAS GERAIS: Conselheiro Pena, Riacho João Pinto, 01.X.2014, fl., *R. Vasconcelos Leitão et al.* 178 (ESA 13485); Serra do Padre Ângelo, montanha do Sossego, 10.I.2020, fl., *D.C. Imig & L. Medeiros* 662 (UPCB).

Distribution, ecology and conservation status: *Dryadella krenaquiana* is restricted to the state of Minas Gerais; it is endemic to the Brazilian Atlantic Forest. The region of occurrence suffers frequent fires putting populations at risk (Luciano Medeiros 2020, *personal communication*) (Fig 17c). The flowering period occurs from October to January.

Etymology: In reference to Krenak, an almost extinct indigenous ethnicity that dominated the region where the species was found.

Taxonomic notes: *Dryadella krenaquiana* is easily distinguishable from the other Brazilian species due to a cespitose but pendant habit. It has an affinity with *D. susanae*. It differs by vegetatively large 35–48 mm tall, light-green leaf $25\text{--}38 \times 2\text{--}3.2$ mm, oblanceolate (vs vegetatively 10–13.5 mm tall, dark-green leaves, $4\text{--}5.7 \times 2.5\text{--}3$ mm, elliptical). Also by the yellow flowers (the smallest flower among the species), with sepals and petals whose veins are evidenced by purple punctuations (vs dark red to purple flowers with sepals and petals whose veins are not stand out from the rest of the blade). (Fig. 1k; 2a)

9. *Dryadella lilliputiana* (Cogn.) Luer, Selbyana 2: 208 (1978a). Holotype: BRAZIL. São Paulo: epífita em Campo de Bocaina, G. Edwall (BR657053!; Lectotype SEL3263!).

≡ *Masdevallia lilliputiana* Cogn., Fl. Bras. 3(4): 555 (1906).

= *Masdevallia paranaensis* Schltr., Notizbl. Bot. Gart. Berlin-Dahlem 7(66): 268, t. 26, f. 102 (1918). Holotype: BRAZIL. PARANÁ, Villa Velha, P. Dusén (B, destroyed).

Lectotype (here designated): Original drawing which appeared in Notizbl. Bot. Gart. Berlin-Dahlem 7(66): 268, t.26, fig.102 (1918).

= *Dryadella paranaensis* (Schltr.) Luer, Selbyana 2: 209 (1978a).

= *Masdevallia melloi* Pasbt., Bradea 2: 169 (1977). Holotype: BRAZIL. RIO DE JANEIRO: Paty Alferes, 20.VII.1975, A.F. Mello MAS-10a (HB 1797 spirit).

= *Dryadella melloi* (Pabst) Luer, Selbyana 2: 209 (1978a).

(Fig.1l; 11a-h)

Epiphyte herb, densely cespitose, small, 2.5–3.5 mm tall. **Roots** slender, 2–3 at each rhizome node, 0.5–0.8 mm diameter. **Rhizome** 1–1.3 mm between the internodes. **Ramicaul** 2–3.5 mm long, erect, covered by 2 paleaceous, oblanceolate sheaths, acute

and minutely mucronate, entire, $3-4 \times 1.3-1.7$ mm. **Leaf** dark green and alvescente in basal portion, with diffuse purple-spotted, thickly coriaceous, linear to fusiform, terete, grooved on the adaxial face, $10-32 \times 1.8-2.1$ mm; petiole 1–1.3 mm long; blade erect, cuneated at the base, obtuse and minutely mucronate. **Inflorescence** a successive 1-3(4) flowered, congest raceme; bearing 2 pale basal bracts, narrow-elliptical, imbricated, acute and mucronate, $1.8-3.2 \times 1-1.4$ mm; peduncle 3–4.5(6) mm long; pedicel plus ovary light-green, slightly tri-alate, 2,3–3,2 mm long. **Flower** medium to large; dorsal sepal alvescente with diffused dark-purple spotted, ovate, inflexed, 3-veined, carinate and entire, $7.3-13 \times 2.5-3$ mm, base truncate, connate 0.4 mm with lateral sepals forming a short sepaline cup, attenuate, long caudate, tail 1.5–2.5 mm with claved apex, entire; lateral sepals alvescente with diffused dark-purple spotted, ovate, 3-veined, asymmetrical, $8.5-10.5 \times 2.5-4$ mm, base connate, a transverse evidente callus, 1.2 mm, purple, acute, long caudate, tail 1.5–2.5 mm with slightly claved apex, entire; petals translucent, alvescente with diffused dark-purple spotted, oblong to ovate, 3-veined, veins parallel, asymmetrical, $1.5-2 \times 1.8-2.1$ mm, base truncate, obtuse to acute, superior margin with obtuse lobe projected forward; inferior margin excavated, lobe obtuse, entire; lip yellow to greenish yellow with diffused light-purple spotted, unguiculate; claw 0.5–0.7 mm, slightly bilobed at base, caniculate; blade oblong to obovate, $1.2-1.8 \times 1.5-1.8$ mm, base with 2 acute, retrorse lobes, 0.3 mm; a pair of lamellar callus toothed at the base; apex reflexed, wide obtuse, entire or discreetly crenated; column alvescent, 1.8–2.3 mm, curved, winged on the distal half, serreateus at winged base; apex acute and minutely serrated, foot equally long; anther light yellow, apiculate; pollinarium, no seen. **Fruit** light green, oblong, 7.5–8 mm.

Selected examined material: BRAZIL. MINAS GERAIS: Santana do Riacho, Serra do Cipó, 01.XI.2017, fl., *D.C. Imig 495* (UPCB). PARANÁ: Campina Grande do Sul, 27.XII.1966, fl., *G. Hatschbach 15560* (MBM); Canguiri, 30.XII.1969, fl., *G. Hatschbach 23291* (MBM). Cascavel, 2.XII.2016, fl., *D.C. Imig 466* (UPCB). Contenda, 12.VII. 2004, fl., *R. Kersten 941* (UPCB); 14.I.2005, fl., *R. Kersten & K. Kozera 996* (MBM). Curitiba, Bosque do Trabalhador, XI.2010, fl., *E.C. Smidt 1010* (UPCB). General Carneiro, 26.II.2007, fl., *A. Bonnet 1030118* (UPCB). Pinhão, 02.XII.2014, fl., *A.C. Costa* (UPCB91621). Piraí do Sul, Serra das Furnas, 4.XI.1999, fl., *A. Carneiro 799* (UPCB). Jaguariaíva, Rio do Sabiá, 28.XI.1968, fl., *G. Hatschbach 20459* (MBM). Lapa, 23.XII.1951, fl., *G. Hatschbach 214111* (UPCB).

Piraquara, Reservatório do Carvalho, 2.XII.2016, fr., *D.C. Imig 467* (UPCB). 2.XII.2016, fl., *D.C. Imig 467* (UPCB); 5.XII.2016, fl., *D.C. Imig 469* (UPCB); 19.X.2015, fl., *D.C. Imig 381* (UPCB); XII.2015, fl., *D.C. Imig 394* (UPCB); XII.2015, fl., *D.C. Imig 394* (UPCB). Tijucas do Sul, Margem do Rio Negro, XI.2018, *T.F. Santos 268* (UPCB). RIO GRANDE DO SUL: Butiá, 02.XII.2015, fl., *D.C. Imig 396* (UPCB). Canela, J. Klein 42 (UPCB). São Francisco de Paula, fl., 9.VII.1985 *Y. Folz* (ICN061649). Villa Velha, *P. Dusén* (B, destroyed). RIO DE JANEIRO: RIO DE JANEIRO: Itatiaia, 27.XII.2017, fl., *M. Bolson 559* (UPCB). Nova Friburgo, 30.XII.2017, fl., *M. Bolson 625* (UPCB). Paty Alferes, 20.VII.1975, *A.F. Mello MAS-10a* (HB 1797 spirit - Holotype of *Masdevallia melloi*). SANTA CATARINA: Benedito Novo, Faz. do Zinco, 03.IV.2015, fl., *D.C. Imig 356* (UPCB). Joinville, 20.XII.2013, fl., *W. Mancinelli 1458* (JOI). Porto União, 22.II.2011, *W. Mancinelli 1424* (JOI). Pomerode, XII.2017, fl., *M. Klingelfuss 206* (UPCB). Praia Grande, Morro Lateral ao Itaimbezinho, trilha dos porcos, 26.XI.2018, fl., *D.C. Imig 646* (UPCB). Pomerode, XII.2017, *M. Klingelfuss 206* (UPCB). Santo Amaro da Imperatriz, 17.XI.2006, fl., *J.Z. Matos*. (FLOR44243). SÃO PAULO: São José do Barreiro, 19.XI.2004, fl., *F. Barros*. (SP401847). Campos da Bocaina, *G. Edwall* (BR657053!, SEL3263!)

Distribution and ecology: *Dryadella lilliputiana* is not endemic to Brazil. There is a record for Bolivia, whose material was collected in 1980, by C. Luer et al., 5065 (MO 3499264 and SEL). The species was registered for SC, RS, PR, SP and RJ in Brazil. For Minas Gerais, it was recorded for the first time in São Thomé das Letras by Sampaio et al. (2016). Now we have added other localities for the state (Fig. 17d). Restricted to the Atlantic Forest, it occurs in humid and open places (forest edges) of the mixed and dense ombrophilous forest, gallery forests and riparian forest. In southern Brazil, it is commonly found as an epiphyte of *Araucaria angustifolia* (Bertol.) Kuntze, a phorophyte that is not common to the other species of the genus. This species can be found in Capões and urban parks, especially those of mixed ombrophilous forests. Unlike most species, *D. lilliputiana* can form larger populations but is generally restricted to a few phorophytes. During our expeditions to São Bento do Sul-SC, we found a phorophyte about 10m in height, entirely covered by the species, being almost an exclusive epiphyte. This species is also frequent in live collections. The flowering period occurs from October to December.

Etymology: Named for Jonathan Swift's of the Lilliput Kingdom.

Taxonomic notes: *Dryadella lilliputiana* is related to *D. ana-paulae*, but differs by the white flowers with purple dots, lip with entire or slightly crenate margins, densely caespitose habit and leaves with diffuse purple dots with alveolate base (vs *D. ana-paulae* presents yellow flowers with purple punctuations, lip with denticulate margins, caespitose habit, the leaves are entirely green, and its distribution is restricted to the Cerrado) (Fig. 1a,l).

Dryadella lilliputiana was initially described in *Masdevallia* by Cogniaux (1906), whose description was based on a collection by G. Edwall, from São Paulo, Campos da Bocaina. The lectotype was indicated by Luer (1983), and it is the illustration of the obra princeps (SEL3263!). Later, Schlechter (1918) described *Masdevallia paranaensis* from material collected in Paraná by Herrn P. Dusén. Pabst (1977) described *Masdevallia melloi* Pabst, including in the section Rhombipetala, based on material collected in Rio de Janeiro, Mata de Paty Alferes, by Arthur Ferreira de Mello (Holotype HB1797 spirit). It is compared with *M. lilliputiana* due to the shape of the leaves and the size and morphology of the flowers. Luer (1978b) synonymise *M. paranaensis* and *M. melloi* into *D. lilliputiana*.

10. *Dryadella susanae* (Pabst) Luer, Selbyana 2: 371 (1978a). Holotype: BRAZIL. ESPÍRITO SANTO: Conceição do Castelo, Ribeirão do Meio, KM123 of BR-262 I.1975, R. Kautsky 459, cultivated by S. Ferreira de Mello MAS-7-A (HB66463!).

≡ *Masdevallia susanae* (Pabst), Bradea 2: 68 (1976).

(Fig. 2a; 12a-h)

Epiphyte herb, shortly repent, small, 10–13.5 mm tall. **Roots** slender, 2-3(5) at each rhizome node, 0.2–0.3 mm diameter. **Rhizome** 0.8–2 mm between the internodes. **Ramicaul** 0.8–1 mm long, repent, covered by 2 paleaceous, triangular, sheaths, obtuse and minutely mucronate, 5–6 × 1.5–2 mm. **Leaf** light green with purplish margins on the adaxial side and purplish on the abaxial face, thickly coriaceous, elliptical, semi flat, slightly grooved on the adaxial face, 4–5.7 × 2.5–3 mm; sessile; blade prostrate, base shortly attenuate at the base, apex retuse and mucronate. **Inflorescence** a successive single flowered,; bearing 2 pale basal bracts, narrow elliptical, acute and minutely mucronate, 0.8–1.3 × 0.5–0.7 mm; peduncle 0.4–0.8 mm long; pedicel plus ovary light green, slightly tripartite, minutely toothed, 1.6–2 mm long. **Flower** small;

dorsal sepal dark red to purple, no spotted, oblong, inflexed, inconspicuous 2-veined, carinate and entire, $2.5\text{--}3.5 \times 1.8\text{--}2.2$ mm, base connate 0.3 mm with lateral sepals forming a short sepaline cup, attenuate, inconspicuous caudate, entire; lateral sepals dark red to wine, no spotted, ovate, inconspicuous 3-veined, asymmetrical, $2.5\text{--}3 \times 1.8\text{--}2$ mm, base connate, a transverse callus 0.6 mm, purple, acute, short caudate, tail 2 mm with attenuate apex, entire; petals dark red to wine, no spotted, 2-veined, veins parallel, asymmetrical, $1.3\text{--}1.5 \times 1.2\text{--}1.5$ mm, base truncate, wide acute to obtuse, superior margin with discreet acute lobe, slightly backwards, inferior margin excavated, acute lobe, slightly facing forward, entire; lip wine no spotted, unguiculate; claw 0.5–0.7 mm, bilobed at base, caniculate; blade obovate, base with 2 acute, divaricated lobes, 0.3 mm; a pair of minutely lamellar callus at the base, apex reflex, entire; column 1–1.2 mm, curved, winged on the distal half, entire at winged margin ending a tooth turned back; apex acute and minutely serrated, foot not equally long; anther apiculate. **Fruit** not seen. Selected examined material: BRAZIL. ESPÍRITO SANTO: Conceição do Castelo, Ribeirão do Meio, KM123 da BR-262, I.1975, R. Kautsky 459, cultivated by Susana Ferreira de Mello MAS-7-A (HB66463). Domingos Martins em cultivo no orquidário Caliman, fl., 01.I.2017, D.C. Imig & M. Bolson 491 (UPCB). Marechal Floriano, Biriricas, 2011, fl., E. B. da Silva 11240 (MBML). Nova Almeida, km 64 of highway BR 262, 31.I.1983, fl., A.L.V. Toscano de Brito 401 (MO100539294 spirit).

Distribution, ecology and conservation status: *Dryadella susanae* is restricted to Espírito Santo and is endemic to the Atlantic Forest, with few records in herbarium and living collections (Fig. 17d). For this work, the only fresh material found was in cultivation at the Caliman nursery (D.C. Imig and M. Bolson 491) from Domingos Martins-ES. It occurs in humid places in the interior of the semi-deciduous forest. The type locality was visited, but it is degraded, and we do not find them in the municipalities of Conceição do Castelo and its surroundings. We did not find any data on the reproductive biology of the species. The material in cultivation started flowering in January until the beginning of March.

Etymology: In honor of Susan Ferreira Mello.

Taxonomic notes: *Dryadella susanae* is the smallest of all species in the genus and is related to *Dryadella catharinensis*. It differs by shortly repent cespitose habit with 10–13.5 mm tall (vs 16–23 mm tall), and by the smaller leaves $3\text{--}5 \times 1.5\text{--}3$ mm, broadly elliptical, prostrate and sessile (vs leaves $13\text{--}19.5 \times 2.1\text{--}3.3$ mm narrowly elliptical,

suberect and petiolate). *Dryadella susanae* is distinguished by the dark-red (vs yellow) flower, the dorsal sepal 3.8–4.0 mm long (vs 4.5–5.2 mm), triangular-ovate, acute, thickened ovate, obtuse and thin (vs ovate, obtuse and thin), the lateral sepals with the margin entire (vs slightly toothed), the 1.5 mm long petals (vs petals 1.0–1.4 mm) and the smaller lip blade 1.5 × 1.4 mm (vs lip blade 1.8–2.1 × 1.5–1.6 mm), obovate, margins entire, without callosities in the claw (vs cuneate, with toothed margins and a ring callus in the median portion of the claw) (Fig. 1f; 2a).

Dryadella susanae was initially described in *Masdevallia* by Pabst (1976) and inserted in the section *Rhombipetala*. Roberto Kautsky, n.459, collected the type in January of 1975 from material in cultivation by Susana Ferreira de Mello (MAS-7A), which was named after the species. The origin of this plant was from the municipality of Conceição do Castelo-ES and was deposited at (HB66463!).

11. *Dryadella toscanoi* Luer, *Icones Pleurothallidarum* XXVII: 37 (2005). Holotype: BRAZIL. MINAS GERAIS: South of Minas Gerais, Serra da Mantiqueira, near the border with São Paulo and Rio de Janeiro, 1200m, 25.XII.1981 (*spirit* 277), A.L.V. Toscano de Brito 144 (MO100539296)

= *Dryadella xaveriana* Campacci & C.R.M. Silva, *Coletânea de Orquídeas brasileiras: Novas espécies e híbridos naturais*, vol. 16, 2020. Holotype: BRAZIL. SÃO PAULO: Pilar do Sul, XI, 2019, *B. Xavier s.n.* (*ESA not found in herbarium*). Lectotype (here designated): M. A. Campacci original's drawing, which appeared in *Novas espécies e híbridos naturais*, vol. 16, page 668. *syn. nov.*

(Fig. 2b,c; 13a-h)

Epiphyte herb, caespitose, medium, 30–45 mm tall. **Roots** slender 3–4 at each rhizome node, 0.3–0.4 mm diameter. **Rhizome** 1.5–2 mm between the internodes. **Ramicaul** 10–13.2 mm long, erect, covered by (2)3 paleaceous, lanceolate sheaths, acute em minutely mucronate, 5–8.5 × 2.8–3.4 mm. **Leaf** green to dark green on both sides, sometimes suffused with purple, narrow-elliptical to narrow-obovate, slightly semiterete, 30–43 × 2.5–3.5 mm; petiole 2–2.5 mm long; blade erect, base attenuate at the base, retuse and mucronate. **Inflorescence** a successive 1-2 flowered, congest raceme; 2 pale basal bracts, oblong, acute and minutely mucronate, 1–2.2 × 0.8–1.0 mm; peduncle 0.4–0.5 mm long; pedicel plus ovary light green, slightly tri-alate, minutely toothed, 1.5–2 mm long. **Flower** medium to largest; dorsal sepal translucent, light yellow to yellow

greenish, diffused dark-purple spotted, ovalate, inflexed, 3-veined, carinate and entire, $7.3\text{--}13.5 \times 4.3\text{--}5.7$ mm, base connate 0.5 mm with lateral sepals forming a short a short sepaline cup, acute, long caudate, tail 4.5–6.2 mm with slender apex, minutely crenated; lateral sepals translucent, light yellow to yellow greenish, diffused dark-purple spotted, ovate, 3-veined, asymmetrical, $7.5\text{--}13 \times 4\text{--}4.3$ mm, base connate, a transverse callus 1 mm, yellow, acute attenuate, long caudate, tail 4.5–5 mm with slender apex, a transverse callus 1 mm, minutely crenated; petals translucent, translucent, light yellow to yellow greenish, diffused dark-purple spotted, oblong, 2-veined, basal veins is curved, asymmetrical, $3.5\text{--}4 \times 3.7\text{--}4$ mm, base truncate, acute, superior margin with evident little acute lobe; inferior margin deep excavated, with evident wide acute lobe, entire; lip dark yellow densely dark-purple spotted, unguiculate; claw 1–1.7 mm, bilobed at base, caniculate; blade oblong to obovate, $3.2\text{--}3.5 \times 2.8\text{--}3$ mm, base with long acute lobe, retrorse, 1.2 mm; a pair of lamellar callus toothed at the base, apex reflex, wide obtuse, entire; column light yellow, 1.8–2.7 mm long, curved, winged on the distal half, minutely crenated at winged base; apex acute acuminate, foot longer; anther not seen; pollinarium, not seen. **Fruit** not seen.

Selected examined material: BRAZIL. MINAS GERAIS: South of Minas Gerais, Serra da Mantiqueira, near the border with São Paulo and Rio de Janeiro, 1200m, 25.XII.1981, (*spirit* 277), A.L.V. Toscano de Brito 144 (MO100539296!); 08.VII.2019, M. Klingelfuss 272 (UPCB). SÃO PAULO: Campos de Jordão, VI.2018, fl., S. L. X. Tobias 026 (UPCB); 19.X. 2016, fl., D.C. Imig 461 (UPCB). 11.XI.2016, fl., D.C. Imig 464 (UPCB); 07.XI.2020, fl., M. Klingelfuss 270 (UPCB); 13.VIII.2018, fl., M. Klingelfuss 228 (UPCB). Sorocaba, 07.XI.2020, fl., M. Klingelfuss 301 (UPCB). Pilar do Sul, 06.X.2020, fl., D.C. Imig 661 (UPCB).

Distribution and ecology: *Dryadella toscanoi* is endemic to Brazil, described for Minas Gerais, and so far known only from the type material. We record here for the first time this occurrence for São Paulo (Fig. 17 d). It is restricted to the Atlantic forest and occurs in transition areas between rainforest and mixed rainforest, in humid places, close to rivers or streams and does not form large populations. The flowering period occurs from June to December (Fig. 17d).

Etymology: named in honour of Antônio L.V. Toscano de Brito, who collected this species.

Taxonomic notes: *Dryadella toscanoi* is similar to *D. wuerstlei*, but differs in larger vegetative size 30–45 mm (vs 20–35 mm), leaf 30–43 × 2.5–3.5 mm, narrow-elliptical to narrow-obovate, slightly semiterete, petiole 2–2.5 mm (vs leaf 15–25(30) × 2.5–3.3 mm, narrow-elliptical, semiterete and sulcate, sessile); dorsal sepal 8–13 × 4.3–5.7 mm, translucent, light-yellow to yellow-greenish, diffused dark-purple spotted, ovate, slightly carinate, smooth carinate and tail 5–6.3 mm) (vs dorsal sepal 5.0–6.3 × 4.0–4.5 mm, pale-green, diffused light-purple spotted, oval to elliptical, evidently carinate, toothed carina and tail 6–11 mm). Lip dark-yellow densely dark-purple spotted with blade 3.2–3.5 × 3–3.5 mm, margin entire (vs lip light-yellow, thickly purple-spotted, unguiculate with blade 4.0–4.7 × 1.8–2.1 mm and margins toothed) (Fig. 2b,c,e,f; 13a–h).

Recently *Dryadella xaveriana* Campacci & C.R.M. Silva was described and published based on material collected in Brazil, São Paulo, Pilar do Sul by Benedito Xavier s.n. on 11.2019 (ESA). In the protologue, the authors compare with *D. zebrina*, especially in the colour and size of the flowers, and emphasise that *D. xaveriana* has shorter and fleshier leaves and floral segments with a different structure. The type was not found at ESA, but we received a fragment of the type individual for cultivation. When analysing the descriptions, illustrations, and the *D. xaveriana* sample, we noticed the overlapping of characters, both in the leaves and in the floral characters of the *D. toscanoi*, as can be seen too in the drawing by Campacci & Silva (2020). *Dryadella xaveriana* presents sepals with tail 5.0–6.0 mm with slender apex (vs. tail 4.5–6.2 mm with slender apex) and the shape of the petals oblong, 3.5–4.1 × 3.6–4.2 mm, with 2-veined, basal veins is curved (vs. oblong, 3–4 × 3.7–4 mm, 2-veined, basal veins is curved), superior margin with evident little acute lobe; inferior margin deep excavated, with evident wide acute lobe, entire (superior margin with evident little acute lobe; inferior margin deep excavated, with evident wide acute lobe, entire). The lip blade oblong with 3.5–3.6 × 2.6–2.9 mm, base with long acute lobe, retrorse (vs. blade oblong to obovate, 3.2–3.5 × 2.8–3 mm, base with long acute lobe, retrorse).

We recognise here *D. xaveriana* as a new synonym of *D. toscanoi*, and we emphasise that *D. toscanoi* presents a variation in leaf thickness, especially in cultivation. In addition, the leaves can vary between light-green to dark-green with purple punctuations when exposed to the sun. The colour of the flowers can vary from light-yellow to greenish-yellow and translucent, diffused or densely dark-purple spotted.

12. *Dryadella vitorinoi* Luer & Toscano, Selbyana 23(2): 181 (2002). Holotype: BRAZIL. Without locality, collected by Vitorino Castro, from *G. Carnevali* 1991, A.L.V. Toscano de Brito s.n. (HUEFS 187213!), C. Luer illustr. 15540.

(Fig. 2d; 14a-h)

Epiphyte herb, caespitose, large, 65–80.5 mm tall. **Roots** fleshy, 2–3 at each rhizome node, 0.7–1.2 mm diameter. **Rhizome** 2–2.5 mm between the internodes. **Ramicaul** 2–4.2 mm long, erect to suberect, covered by 2(3), paleaceous, oblong sheaths, acute and abruptly mucronate, $3.0\text{--}3.3 \times 1.6\text{--}1.9$ mm. **Leaf** light green on both sides, sometimes with vinaceous nuances when exposed to high sun intensity, coriaceous, lanceolate, flat, $40\text{--}70 \times 3\text{--}8.2$ mm; petiole 1.5–2.5 mm long; blade erect, attenuate at the base, apex retuse and minutely mucronate. **Inflorescence** a successive 1-2 flowered, congest raceme; bearing 2 pale basal bracts, narrow-elliptical, obtuse and minutely mucronate, $1.5\text{--}1.8 \times 0.4\text{--}0.5$ mm; peduncle 1.0–1.7 mm long; pedicel plus ovary light green, evident tri-lobate, minutely toothed, 1.6–2.5 mm long. **Flower** largest; dorsal sepal light yellow, diffused and dark-purple spotted, oval lanceolate, inflexed, 3-veined, slightly carinate on entire, $8\text{--}9.5 \times 3.2\text{--}3.5$ mm, base connate 1–1.2 mm with lateral sepals forming a short sepaline cup, acute, inconspicuous tail, tail 1 mm with attenuate apex, entire; lateral sepals light yellow, diffused and inconspicuous light-purple spotted, ovate, inflexed, 3-veined, asymmetrical, $7.5\text{--}8.5 \times 3.9\text{--}4.2$ mm; base connate, a evident transverse callus 1–1.2 mm, purple, attenuate, short caudate, tail 1.5–2.5 mm with attenuate apex, entire; petals yellow, densely dark-purple spotted, oblong, 2-veined, the base vein is curved, asymmetrical, $5.2\text{--}5.8 \times 3.4\text{--}3.6$ mm, base truncate, attenuate, superior margin without lobe; inferior margin excavated, obtuse lobe, entire; lip yellow bordered with light purple spotted, unguiculate; claw 0.8–1 mm, bilobed at base, caniculate; blade oblong, $2.5\text{--}3 \times 1.5\text{--}1.7$ mm, base without or inconspicuous lobes; a pair of inconspicuous lamellar callus at the base, apex slightly reflex, wide obtuse, entire; column yellow, 1.5–1.8 mm long, curved, winged on the distal half, crenated at winged base; apex acute and serrated, foot equally long; anther yellow, apiculate; pollinarium, no seen. **Fruit** light green, oblong, minutely tri-lobate, $5\text{--}8 \times 4\text{--}6$ mm.

Selected examined material: BRAZIL. BAHIA: collected by Vitorino Castro, from *G. Carnevali* 1991, A.L.V. Toscano de Brito (HUEFS 187213). Poções, Serra do Arrepio,

3.III.201, fl., A.L.V. Toscano de Brito 3885 (UPCB); A.L.V. Toscano de Brito 3839 (UPCB). Vitória da Conquista, A.L.V. Toscano de Brito 1846 (UPCB). ESPÍRITO SANTO: Domingos Martins, São Bento de Urânia, 7.VII.2019, fl., A.L.V. Toscano de Brito & W. Collier 3900 (UPCB). MINAS GERAIS: Boa Esperança, 11.XII.2017, fl., D.C. Imig 506 (UPCB). Nova Era, 19.XII.2008, fl., A.L.V. Toscano de Brito 2568 (UPCB).

Distribution and ecology: *Dryadella vitorinoi* is endemic to Brazil; it was described without locality data and has only been recorded for Espírito Santo until now. We record the occurrence for the first time for the state of Bahia and Minas Gerais (Fig. 17d). We believe that *D. aviceps* cited by Sampaio (2016) for São Thomé das Letras-MG is *D. vitorinoi*, but during our studies and expeditions, we did not find material to confirm the identity of this specimen. *Dryadella vitorinoi* occurs in Seasonal Deciduous Forest (liana forests) and does not form large populations. It is found in illuminated sites on trunks and apex of tree branches, shrubs and even the most robust vines. The flowering period occurs from December to March.

Etymology: In honour of Vitorino Castro, collector of the species.

Taxonomic notes: *Dryadella vitorinoi* is similar to *D. aviceps* but differs vegetatively by the leaves lanceolate, sub-erect, light-green on both sides, sometimes with vinaceous colour when exposed to the high sun (vs leaves lanceolate to oblanceolate, erect, dark-green and usually vinaceous on both sides). *D. vitorinoi* present flowers with sepals light-yellow, diffused and inconspicuous light-purple spotted, ovate to oval-lanceolate and inflexed, which gives the flowers no full opening (vs sepals dark-yellow with dense dark-purple punctuations - rarely the lateral sepals have clear and few evident punctuations - dorsal sepal is erect or slightly inflexed and bearing full opening flowers; petals two-veined, the base vein is curved, superior margin without lobe (vs two-veined, veins parallel, superior margin with acute to obtuse lobe). (Fig. 1c-e; 2d).

D. vitorinoi was described by Luer & Toscano (2002), based on a specimen collected in 1991, A. L.V. Toscano de Brito (HUEFS 187213), Luer's Illustration nr. 15540, fig. 01 of the original description. The paratype is indicated for Domingos Martins-ES, locality of Caxixe Frio, 03.III.1991, A. L.V. Toscano de Brito 889 (MO 100539280). Luer (2005) in the review of the genus, cites the type of *D. vitorinoi* from Brazil, without locality, collected in 1991, C. Luer 15540 (Holotype: MO); however, the holotype is in (HUEFS 187213) and the paratype (A.L.V. Toscano de Brito 889) in MO. In Flora do

Brasil (2020) and specieslink (2020), this material is cited as holotype, not paratype, and needs to be corrected.

13. *Dryadella wuerstlei* Luer, Icones Pleurathallidinarum XXVII: 39 (2005). Holotype: BRAZIL. Without collecting data purchased by B. Würstle from Ghillany, Flowered in cultivation at Spielber, Germany, 23.V.1980, C. Luer 5269 (SEL!).

(Fig. 2 e-f; 15a-h)

Epiphyte herb, densely caespitose, small, 20–35 mm tall. **Roots** slender 2–3(4) at each rhizome node, 0.4–0.6 mm diameter. **Rhizome** 1–2 mm between the internodes. **Ramicaul** 4–6 mm long, erect to suberect, covered by 2(3) paleaceous, oblanceolate sheaths, obtuse and mucronate, 1–1.2 × 0.5–1 mm. **Leaf** light green on both sides, sometimes spotted with purple, thickly coriaceous, narrow-elliptical, semiteres and sulcate, 15–25(30) × 2.5–3.3 mm; sessile; blade suberect, attenuate at the base, apex retuse and mucronate. **Inflorescence** a successive 1-2 flowered, congest raceme; bearing 2(3) pale basal bracts, narrow-elliptical, acute and mucronate, 1.8–3.2 × 1–1.4 mm; peduncle 7–15 mm long; pedicel plus ovary light green, tri-alate, minutely toothed, 3.3–4.5 mm long. **Flower** medium; dorsal sepal pale green, diffused light-purple spotted, oval to elliptic, inflexed, 3-veined, asymmetrical, evidently carinate, toothed, 5.0–6.3 × 4.0–4.5 mm, base truncate, connate 0.8 mm with lateral sepals forming a short sepaline cup, attenuate, long caudate, tail 6–11 mm with slender apex, entire to discreetly toothed; lateral sepals pale green, diffused dark-purple spotted, ovate, 3-veined, asymmetrical, 4.5–5.3 × 3.5–3.8 mm, base connate, a transverse callus 1 mm, pale green, attenuate, long caudate, tail 6–10 mm with thick apex, entire to discreetly toothed; petals translucent light yellow, thickly purple-spotted, oval to oblong, 2-veined, veins parallel, asymmetrical, 2.3–3.0 × 2.3–2.7 mm, base truncate, acute apex, superior margin with wide obtuse lobe; inferior margin excavated, acute lobe, minutely toothed; lip light yellow, thickly purple-spotted, unguiculate; claw 0.6–0.8 mm, minutely bilobed at base, caniculate; blade oblong to obovate, 4.0–4.7 × 1.8–2.1 mm, base with 2 acute, retroses lobes, 0.3 mm; a pair of lamellar callus at the base, apex reflexed, obtuse, minutely toothed; column light green, 1.2–1.5 mm, curved, winged on the distal half, serrate at winged base; apex acute and minutely serrated, longer foot; anther light

green, apiculate; pollinarium, no seen. **Fruit** light green with light purple spotted, oblong, 5–7.5 x 3.5 mm.

Selected examined material: BRAZIL. Flowered in cultivation at Spielber, Germany, 23.V.1980, C. Luer 5269 (SEL!). RIO GRANDE DO SUL: Capão do Leão, 09 XI.1986, fl., J.A. Jarenkow 496 (FLOR). Iraí, 07.X.2016, fl., D.C. Imig 462 (UPCB); Pelotas, Distrito de Rincão da Cruz, Morro Quilongongo, 17.II.2008, fl., T. Perleberg 233 (ECT). São Francisco de Paula, 20.X.2009, fl., A.L.V. Toscano de Brito 2646 (UPCB); 17.XII.2008, fl., A.L.V. Toscano de Brito 2563 (UPCB). SANTA CATARINA: Calmon, Pinhelão, 08.III.2019, fl., A. Kassner-Filho et al. 5277 (FURB); Rio do Sul, 03.X.2015, fl., D.C. Imig, M. Bolson & J. Caetano 408 (UPCB). Idem, 23.XI. 2016, fl., D.C. Imig 465 (UPCB); Vitor Meireles, Pratinha, 18.I.2012, fl., E. Caglioni et al. 132 (FURB).

Distribution and ecology: The geographic distribution of *D. wuerstlei* was unknown until now, the type material comes from cultivation, and there was no specified provenance. From our collections, we found it to be endemic to the Brazilian Atlantic Forest, recorded here for the states of Santa Catarina and Rio Grande do Sul. *Dryadella wuerstlei* is found in less dense and slightly humid forests (away from rivers) and forms small populations (1-3 individuals). During our expeditions, we did not find it in living collections. The flowering period occurs from April to November. (Fig. 17e)

Etymology: Named after Berthold Würstle, who cultivated the species.

Taxonomic notes: *Dryadella wuerstlei* was described by Luer (2005) and appeared on plate 1096 in *Orchidaceae Brasilienses* by Pasbt & Dungs (1979) as *D. edwalli*.

Dryadella wuerstlei has an affinity with *D. zebrina* and *D. toscanoi*. Differing from *D. zebrina* in that it is vegetatively dense cespitose, leaves 15–25(30) × 2.5–3.3 mm, narrow-elliptical, semi-terete and sulcate, sessile and sub-erect (vs cespitose, leaves 40–65(9) × 3.5–7 mm, elliptical to narrow-elliptical and flat, petiolate and erect). The flowers are proportionally large in relation to the habit and stand out from the leaves, pale-green in colour and sepals with attenuated apex, long caudate, whose tail exceeds the size of the blade (vs flowers that are medium in relation to the vegetative and generally located at the base, not protruding leaves, yellow to dark yellow, sepals with an acute apex, abruptly long caudate, slender tail that does not exceed the size of the blade). Dorsal sepal keeled with denticulated keels (vs less keeled and entire keels). The lip is light-green, with congested purple punctuations, obovate, margins minutely denticulate; blade 4.0–4.7 × 1.8–2.1 mm, claw 0.6–0.8 mm (vs lip yellow densely dark-

purple spotted, oblong to obovate, entire margins; blade 3–3.5 × 2.8–3.3 mm, claw 1.8–2.3 mm) (Fig. 2 e-l).

While it differs from *D. toscanoi* in vegetative size 20–35 mm (vs 30–45 mm), leaf 15–25(30) × 2.5–3.3 mm, narrow-elliptical, semi-terete and sulcate, sessile (vs leaf 30–43 × 2.5–3.5 mm, narrow-elliptical to narrow-obovate, slightly semi-terete, petiolate, petiole with 2–2.5 mm); dorsal sepal 5.0–6.3 × 4.0–4.5 mm, pale green, diffused light-purple spotted, oval to elliptical, evidently carinate, toothed carina and tail 6–11 mm (vs dorsal sepal 8–13 × 4.3–5.7 mm, translucent, light yellow to yellow-greenish, diffused dark-purple spotted, ovate, slightly carinate, smooth carinate and tail 4.5–6.2 mm); lip light-yellow, thickly purple-spotted, unguiculate with blade 4.0–4.7 × 1.8–2.1 mm and margins toothed (Lip dark-yellow densely dark-purple spotted with blade 3.2–3.5 × 3–3.5 mm, margin entire (Fig. 2 e-l).

14. *Dryadella zebrina* (Porsch) Luer, Sebyana 2: 209 (1978a). Neotype: BRAZIL. 30.XI.1989, C. Luer 14500 (MO5489062!).

≡ *Masdevallia zebrina* Porsch, Oesterr. Bot. Z. 55: 154 (1905).

= *Masdevallia carinata* Cogn., Bull. Soc. Roy. Belgique 43: 305 (1905). Holotype: BRAZIL. *Brasilia austro-orientali*, C. Glaziov (BR997389!).

= *Masdevallia edwalli* Cogn., Flora Brasiliensis 3(4): 553 (1906). Holotype: BRAZIL. SÃO PAULO, Campos da Bocaína, A. Loefgren & G. Edwall 2302, 26.III.1894, G. Edwall 2302 (BR657020!, K).

= *Dryadella edwallii* (Cogn.) Luer, Selbyana 2: 208 (1978a).

(Fig. 2 g-l; 16 a-h)

Epiphyte herb, densely cespitose, lager, 40–85 (130) mm tall. **Roots** thick, 3–4 at each rhizome node, 0.7–1 mm diameter. **Rhizome** 1–1.5(2) mm between the internodes. **Ramicaul** 7–12 mm long, erect, covered by 2 paleaceous, lanceolate sheaths, abruptly acute and mucronate, entire, 3–5 × 0.7–1.2 mm. **Leaf** dark green on both sides, sometimes spotted with purple, coriaceous, elliptical to narrow-elliptical, flat, 40–65 (9) × 3.5–7 mm; petiole 2.5–3 mm long; blade erect, retuse and mucronate. **Inflorescence** a successive, 1–3 flowered, congested raceme; bearing 2 pale basal bracts, narrow-elliptical, acute and mucronate, 8–12 × 0.8–1.3 mm; peduncle 45–80 (100–120 mm) long; pedicel plus ovary green, sometimes suffused with purple, slightly

trialate, erose, 4–6.5 mm long. **Flower** large; dorsal sepal yellow to dark yellow, diffused or densely dark-purple spotted, the spots more or less in transverse bars, ovate, inflexed, 3-veined, carinate, entire, $5.5\text{--}8.5(9.5) \times 5\text{--}5.5$ mm, base connate 1.5–2.3 mm with lateral sepals forming a short sepaline cup, acute, long caudate, tail 5–7.3 mm with usually clavate apex, greenish yellow or densely covered with purple dots, entire; lateral sepals yellow to light yellow, diffused or densely dark-purple spotted, ovate, 3-veined, asymmetrical, as veins deflexed, $4.8\text{--}6.5 \times 4\text{--}5.3$ mm, base connate, a transversal callus 0.8–1.2 mm purple, long caudate, tail (4)5–6.7 mm with slender or minutely clavate apex, entire; petals translucent yellow, densely purple-spotted, oblong, 2-veined, veins parallel, asymmetrical, $3.5\text{--}3.8 \times 2.9\text{--}3.5$ mm base truncate, wide obtuse, superior margin with obtuse lobe; inferior margin excavated, wide obtuse lobe, entire; lip yellow densely dark-purple spotted, unguiculate; claw 1.8–2.3 mm, bilobed at base, caniculate; blade oblong to obovate, $3\text{--}3.8 \times 2.8\text{--}3.3$ mm, base with 2 acute, divaricate lobes, 0.3 mm; a pair of lamellar callus obtuse, minutely erose, apex reflex, wide obtuse, entire; column yellow 2.5–3.8 mm long, curved, winged on the distal half, toothed at winged base ending in a acute lobe directed backwards; apex acute and minutely serrated, foot equally long; anther yellow, apiculate; pollinarium, no seen. **Fruit** light green, oblong, $10\text{--}12 \times 4.2\text{--}5$ mm.

Selected examined material: BRAZIL. *C. Glaziou* (BR997389! - Holotype of *Masdevallia carinata*). BAHIA: Camacã, 02.IX.2016, fl., D.C. Imig 453 (UPCB); 18.IX.2016, fl., D.C. Imig 455 (UPCB); 19.IX.2016, fl., D.C. Imig 457 (UPCB). MINAS GERAIS: Camanducaia, Distrito de Monte Verde, Trilha da Pedra Redonda 28.VII.2017, fl., D.C. Imig 484 (UPCB); 29.VII.2017, fl., D.C. Imig 482 (UPCB). Santana do Riacho, Serra do Cipó, 18.X.2018, fl., D.C. Imig 485 (UPCB). ESPÍRITO SANTO: Venda Nova do Imigrante, em cultivo no Orquidário Caliman, 21.XI.2015, fl., A.L.V. Toscano de Brito 3478 (UPCB). PARANÁ: Guaratuba, Serra do Araçatuba, Morro dos perdidos, 20.XI.1998, E.P. Santos et al. 641 (UPCB); 01.XII.1998, G. Hatschbach et al. 2639 (MBM); 27.XI.1998, fl., E.P. Santos et al. 661 (UPCB). Quatro Barras, Morro Sete, 17.XI.1994, fl., O. Ribas et al. 723 (MBM); Serra da Graciosa, 13.IV.2016, fl., D.C. Imig 406 (UPCB); 02.XII.2015, D.C. Imig 405 (UPCB); 22.XII.2016, fl., D.C. Imig 468 (UPCB); 28.XI.2014, fl., A.L.V. Toscano de Brito et al. 3311 (UPCB); 28.XI.2014, fl., A.L.V. Toscano de Brito 3453 (UPCB); 2.XII.2015, fl., A.L.V. Toscano de Brito 3312 (UPCB). Morretes, 19.X.2015, D.C. Imig 378 (UPCB);

Cabeceiras do Arraial, 11.XI.1965, *G. Hatschbach* 13116 (V, US); Trilha da Estação Engenheiro, 19.X.2015, *D.C. Imig* 379 (UPCB); P.E. Marumbi, 11.X.1998, fl., *C. Giongo et al.* 64 (UPCB); 25.XI.1999, *C. Giongo* (UPCB); Morro Facãozinho, 08.XI.1998, fl., *C. Giongo* 75 (UPCB); Mãe Catira, 11.XI.2016, fl., *D.C. Imig* 463 (UPCB); 12.XII.1985, fl., *R. Kummrow* 2677 (MBM); XI.2010, fl., *E.C. Smidt* 1011 (UPCB). Piraquara, Queimada, Morro Albino Souza, 27.XII.1947, fl., *G. Hatschbach* 819 (SP); Caiguava, 19.XI.1909, fl., *K. Dusén* 8987 (NY, V); Mananciais da Serra, XII.2005, fl., *M. Reginato* 636 (UPCB); X.2004, fl., *M. Reginato* 95 (UPCB); Morro do Canal, 30.X.2009, fl., *R. Kersten* 1387 (HUCP); P.E. Marumbi, 09.XI.2015, fl., *D.C. Imig* 389 (UPCB); Trilha do Olimpo, 09.XI.2015, fl., *D.C. Imig* 384 (UPCB); Trilha do Vigia, 03.XI.2017, fl., *T.F. Santos* 298 (UPCB); 06.XI.2017, fl., *T.F. Santos* 327 (UPCB); 27.XI.2003 fl., *J. Silva et al.* 3916 (MBM); Morro do Bruninho, 02.X.2017, fl., *D.C. Imig* 488 (UPCB); 10.XI. 2015, fl., *D.C. Imig* 393 (UPCB); Reservatório do Carvalho, 15.X.2015, fl., *D.C. Imig* 374 (UPCB); 15.X.2015, fl., *D.C. Imig* 376 (UPCB); Serra do Emboque; 03.XII.1970, fl., *G. Hatschbach* 25749 (MBM, K). São José dos Pinhais, Borda da Campo, 04.XII.2019, fl., *D.C. Imig, T. Imig & F. Imig* 654 (UPCB; CTBA); Morros Perdidos, 17.XI.2009, fl., *R. Kersten* 005 (HUCP); Nhandara Guaricana, 01.XI.2017, *D.C. Imig et al.* 493 (UPCB; CTBA); Nhandara Guaricana, 15.X.2015, fl., *D.C. Imig* 377 (UPCB); Rio Pequeno, 05.XI.1969, fl., *G. Hatschbach* 22850 (MBM). Tijucas do Sul, 15.IX.2015, fl., *D.C. Imig* 375 (UPCB); Represa Vossoroca, km160 09.XI.2017, fl., *M. klingelfuss* 157 (UPCB); 10.XI.2017, fl., *M. klingelfuss* 159 (UPCB); *M. klingelfuss* 160 (UPCB); *M. klingelfuss* 161 (UPCB); Serra de Papanduva, 15.XII.1997, fl., *O. Ribas et al.* 2121 (MBM). Telêmaco Borba, Monte Alegre, fl., 16.XI.1915, *P. Dusén* 13334 (K940348!). RIO GRANDE DO SUL: Canoas, 06.XI.2017, fl., *D.C. Imig* 483 (UPCB). Porto Alegre, Morro da Glória, 5.XI.1932 (SP50515). Torres, 07.IX.2020, fl., *D.C. Imig* 657 (UPCB); RIO DE JANEIRO: Angra dos Reis, arredores do P. N. Serra da Bocaina, 02.IX.2016, fl., *D.C. Imig* 454 (UPCB). Alto Macahé, 05.XI.1888, fl., *A. Glaziou* 17802 (K; BR; P493105!). Próximo a Petrópolis, 30.XI.1989, fl., *C. Luer* 14500 (Neotypus MO5489062!). SANTA CATARINA: Angelina, 28.X.2009, fl., *A. Cadorin et al.*, 316 (FURB). Antônio Carlos, 09.XI.2009, fl., *A. Cadorin et al.*, 482 (FURB). Benedito Novo, Trilha da Cruz de Pedra, 13.IX. 2018, fl., *L.A. Funez* 7742 (FURB); Fragmento florestal ao lado da cachoeira Campo do Zinco, 22.VII.2012 fl., (FURB); Bom Retiro, 17.X.1973

fl., A. Bresolin 877 (FLOR); Campo Alegre, Rio dos Bugres, 26.X.2019, fl., P. Schwirkowski & C.R. Hantschel 3555 (FURB; FPS); Serra do Quiriri, 27.XII.2004, fl., O. Ribas et al. 6580 (MBM); Rio Manso, 02.II.2010, fl., T. Cadorin et al. 1182 (FURB); Corupá, 07.IX.2020, fl., D.C. Imig 659 (UPCB); Palmeiras II, 24.VIII.2010, fl., A. Korte & A. Kniess 4197 (FURB). Doutor Pedrinho, 25.V.2010, fl., T. Cadorin et al. 2423 (FURB). Florianópolis, Residencial dos Araças, 04.I.2010, Siqueira 210 (FLOR); 28.IX.1967, fl., R.M. Klein & A. Bresolin 7587 (FLOR); Morro do Ribeirão, 24.X.1967, fl., R.M. Klein & A. Bresolin 7609 (FLOR). Garuva, Serra do Quiriri, 24.XI.2010, fl., A. Korte 5101 (FURB). Governador Celso Ramos, 11.VIII.1971, R.M. Klein 9649 (FLOR); Vargem do Macário, 20.III.1972, A. Bresolin 527 (FLOR). Ilhota, Morro do Baú, 16.IX.2016, fl., Gagliioni & Junckes 501 (UPCB). Imaruí, Morro D'una, 18.X.1973, fl., A. Bresolin 900 (FLOR). Joinville, Cubatão, 06.VII.2010, fl., T. Cadorin et al., 2879 (FURB); Serra Dona Francisca, 06.VI.2010, fl., T. Cadorin et al. 1975 (FURB); 10.XII.2009, fl., T. Cadorin et al., 870 (FURB); Serra do Piraí, 18.X.2010, fl., A. Korte & A. Kniess 4090 (FURB); Serra Queimada, 03.IX.2010, fl., W. Mancinelli & A. Mancinelli 1301 (JOI); Morro do Tromba, 20.III.2006, fl., W. Mancinelli 557 (JOI). Lages, Encruzilhada, 30.X.1962, fl., R.M. R. Reitz & R.M. Klein 13924 (MBM, US). Monte Castelo, Serra da Garganta, 17.IX.2010, fl., A. Korte & A. Kniess 4381 (FURB). Nova Trento, 9.IX.2017, fl., A. Kassner-Filho et al. 1031 (FURB); Monte Barão, RPPN Prima Luna, 16.VI.2006, T. Cadorin et al., 2573 (FURB). Palhoça, Morro Cambirela, 20.VIII.1971, A. Bresolin 349 (FLOR); 20.VIII.1971, fl., A. Bresolin 342 (FLOR). Praia Grande, Morro em frente ao Canyon Itaimbezinho, Trilha dos porcos, 24.XII.2018, fl., D.C. Imig & G.A. Mantovani 637 (UPCB); 26.XII.2018, fl., D.C. Imig 644 (UPCB); Serra do Faxinal, 13. V.2010, fl., J.L. Schmitt et al. 2216 (FURB). São Bento do Sul, 08.X.2018, fl., D.C. Imig 632 (UPCB); fl., D.C. Imig 630 (UPCB); fl., D.C. Imig 632 (UPCB); fl., D.C. Imig 629 (UPCB). Ponte Alta, Morro do Funil, 06.X.2009, fl., J.L. Schmitt et al. 160 (FURB). Rancho Queimado, BR 282, 10.X.1993, fl., F.F. Neves 82 (FLOR). Rio dos Cedros, 27.VII.2017, fl., A. Kassner-Filho et al., 604 (FURB). Rodeio, Eremitério Frei Egídio, 24.IX.2016, L.A. Funez et al. 5552 (FURB). São Martinho, 26.I.2010, fl., J.L. Schmitt et al. 995 (FURB). Vargem do Cedro/P. E. da Serra do Tabuleiro, 15.VI.2010, fl., Verdi et al., 5023 (FURB). São José, Serra da Boa Vista, 14.X.1962, fl., R. Reitz & R.M. Klein 10217 (FLOR, US); 24.X.1957, fl., R. Reitz & R.M. Klein 5397 (US). Santo Amaro da

Imperatriz, 13.X.2006, J.Z. Matos 24 (FLOR). Taió, Passo Manso, 09.X.2009, fl., T. Cadorin *et al.* 201 (FURB); Gramado, 15.IX.2010, fl., A. Korte & A. Kniess 4347 (FURB). Treviso, Brasília, 24.XI.2009, J.L. Schmitt *et al.* 594 (FURB). Vidal Ramos, Águas Frias, 21.X.2009, A. Korte & A. Kniess 686 (FURB). SÃO PAULO: Bananal, 10.IX.2020, FL., M. Klingelfuss 309 (UPCB). Capão Bonito, 15.IX.2020, fl., D.C. Imig 658 (UPCB). São José do Barreiro, Campos da Bocaina, Invernada Pinhal, 26.III.1894, fl., Loefgren & Edwall, 2302 (SP 29265!). Campos da Bocaina, 26.III.1894, fl., A. Loefgren & G. Edwall 2302 (K, BR657020! - Holotype of *D. edwallii*). Serra do Mar Monte Alegre, fl., XI.1914, P. Dusén 15842 (MO-1109578).

Distribution and ecology: *Dryadella zebrina* has a wide geographic distribution, especially when compared to other species of the genus. In Brazil, it is endemic to the Atlantic Forest. It occurs in the states of the South (SC, RS and PR), Southeast (ES, SP, RJ and MG) and Bahia in the Northeast. Also, it occurs in La Paz, Bolivia, and Amazonas in Peru (Luer, 2005). (Fig 17e).

Dryadella zebrina is found in dense and humid forests (near rivers) and mountain tops; unlike other species, it can form dense populations (up to 15/20 individuals). The flowering period occurs from April to November.

Etymology: From Latin *zebrinis* "like the zebra", referring to the stripes on the dorsal sepal.

Taxonomic notes: *Dryadella zebrina* is among the largest species in Brazil and has the widest distribution, and is commonly found in herbaria and living collections. The flowers are proportionately large in relation to the other species of the genus and stand out from the leaves due to the larger peduncle size, which can reach 4.5–8(100–120) mm (ex: Hatschbach 13116 and Ribas *et al.* 6580). We highlight the high vegetative variability of this species, even within the same or close populations. The size range is 40–85(130) mm tall, with leaves ranging from dark-green to fully purplish on both sides (e.g. Santos *et al.* 641 and Santos *et al.* 661) collected in the same location. (Fig. 16g-l) This wide variation is also present in the colour and characteristics of the flowers. Within the same or close populations, they may vary from light-yellow to dark-yellow with diffuse or very congested dark-purple punctuations. The punctuations may be transversely distributed in a "zebra" shape, particularly on the dorsal sepal. The clavate tail apex of the dorsal sepal may be greenish or covered by purple punctuations. The

floral structures are sometimes so densely punctuated with purple that sometimes they cover almost the entire lamina. (see Fig. 16g-l)

Dryadella zebrina is closely related to *D. wuerstlei* but vegetatively larger, 40–80 (130) mm tall and with petiolate leaves with flat blades (vs 20–35 mm tall with sessile, semi-terete and sulcate leaves). Flowers have yellow to dark-yellow sepals, diffused or densely dark-purple spotted. The spots are more or less in transverse bars on dorsal sepals, tapering to a 5–7.3 mm tail with usually clavate apex, smaller in relation to the blade, discreetly carinated with entire keels and margins (vs pale-green sepals, diffused light-purple spotted, ending abruptly in a slender tail, 6–11 mm, exceeding blade size, evidently carinated and toothed, margin entire to discreetly toothed). Lip oblong to obovate with the same colour of the sepals and petals and entire margins (vs lip obovate, green in colour and congested dark purple punctuations highlighting the structure between the petals and margins, which are minutely denticulated). (Fig. 15g-l)

Dryadella zebrina was described by Porsch (1905) in *Masdevallia* from a material of origin cited only as Raiz da Serra and possibly referred to as the neighbourhood and district of the Municipality of Magé in the state of Rio de Janeiro. It was collected in August 1901 by Wettstein & Schiffner s.n. and deposited in herbarium B, possibly destroyed. Later neotyped by Luer (2005), with material from RJ, near Petrópolis, collected by Luer 14500 (MO!).

Masdevallia carinata, was published in Bulletin de la Société Royale de Botanique de Belgique 43: 305. 1907, from material from Brazil, Brasília austro-orientali, without locality, collected by C. Glaziov s.n. deposited at (BR997389!) and synonymised by Luer, in 1978a.

Dryadella edwallii (as *Masdevallia edwallii* Cogn.), was described by Cogniaux (1906), in Flora Brasiliensis, whose type material is from Campos da Bocaina, São Paulo, by Comm. Geography & Geol. 2302 (BR657020! and K). In its original description, the measurements of the floral parts were not presented, and the shapes overlap the description of *D. zebrina* (Cogniaux 1906; Luer 2005). A more detailed description was presented in the revision of the genus (Luer 2005), which highlights the great similarity with *D. zebrina*, differentiating them only by the lip size, 3x3 mm, while in *D. zebrina* this structure would have 2x2mm. Due to this taxonomic difficulty, these taxa were studied from a morphometric point of view, whose results led to the synonymisation of *D. edwallii* in *D. zebrina* (Imig et al. *in preparation*).

Acknowledgment

The authors would like to thank the curators of the herbaria mentioned here for loaning the herborized material, sending digital images and providing access to their collections; to the orchidologists Marcos Klingelfus, José Vanderlei Alves Neves, Daniel de Assis Rodrigues, Sandro Lucas Xavier Tobias, Amauri Herber Krah, Roberto Martins, Marcelo Rodrigues and Maria Rita Cabral to send live plants for study. We are also grateful for the financial support of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) with scholarship granted to DCI (168348/2017-9) and ECS (CNPq-Nível 1D-proc. 314642/2020-0 and PDJ proc. 203304/2018-7) and to the MCTI/CNPq N° 14/2013 - Universal (proc. 405493/2021-5). ALVTB thanks to the CAPES (PVE 88881.065009/2014-0).

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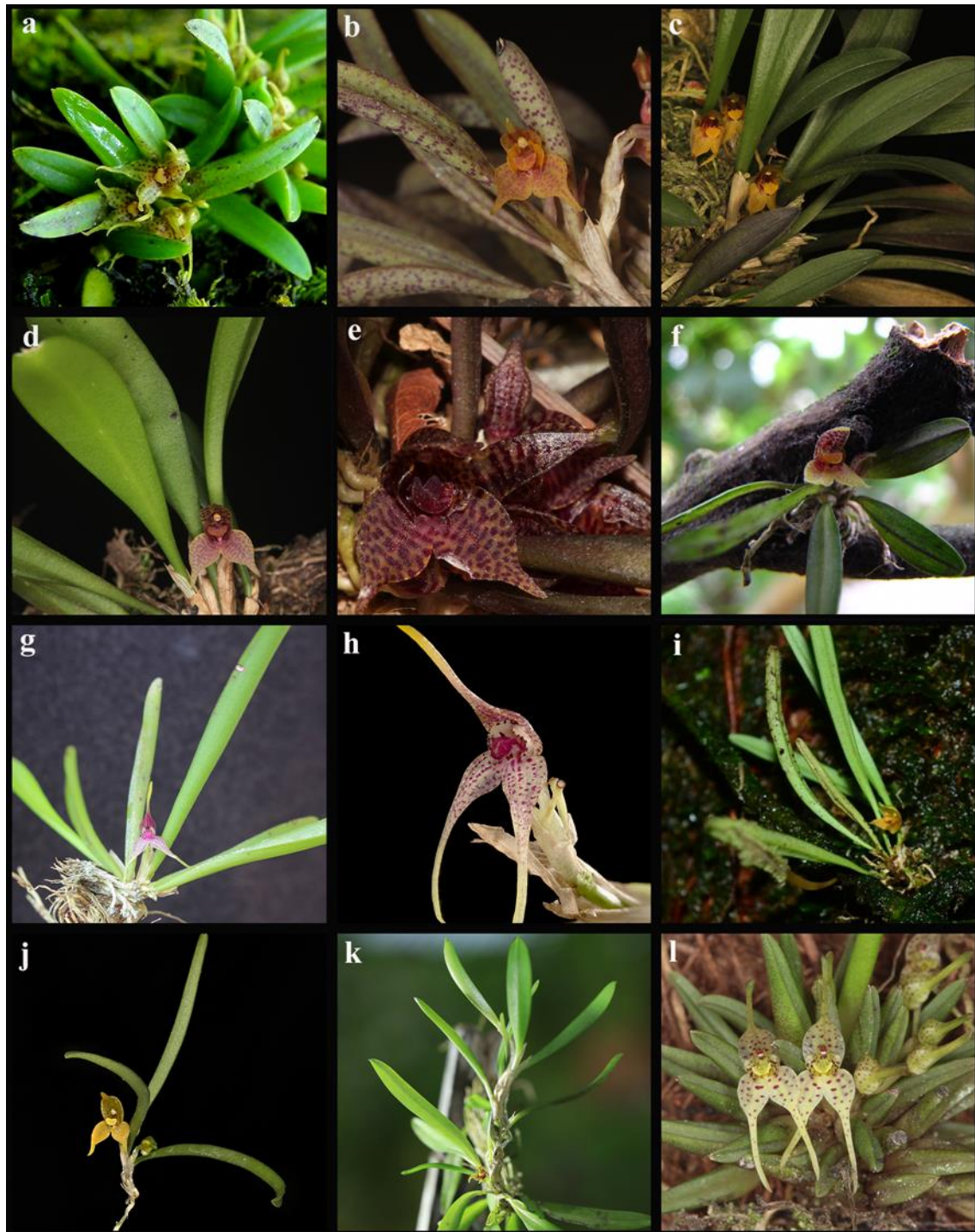


Figure 1— a-l. *Dryadella* species recognised for Brazil—a. *Dryadella ana-paulae*; b. *Dryadella auriculigera*; c-e. *Dryadella aviceps*; f. *Dryadella catharinensis*. g-h. *Dryadella crenulata*; i. *Dryadella gnoma*. j. *Dryadella kautskyi*; k. *Dryadella krenakiana*; l. *Dryadella lilliputiana*. Photographs: a. Daniel Oliveira; e. Cecília O. de Azevedo. f. Werner Mancinelli; b, c, d, l. Eric de Camargo Smidt; g-j. A. L. V. Toscano de Brito; k. Luciano Medeiros; i. Ana H. Krahll; l. Daniela Imig.



Figure 2– a-l *Dryadella* species recognised for Brazil –a. *Dryadella susanae*; b-c. *D. toscanoi*; d. *Dryadella vitorinoi*; e-f. *Dryadella wuerstlei*; g-l. Variações em *Dryadella zebrina*. Photographs: a, b, c, e, f, g, h, i. Eric de Camargo Smidt; d. Jesulino Namba.

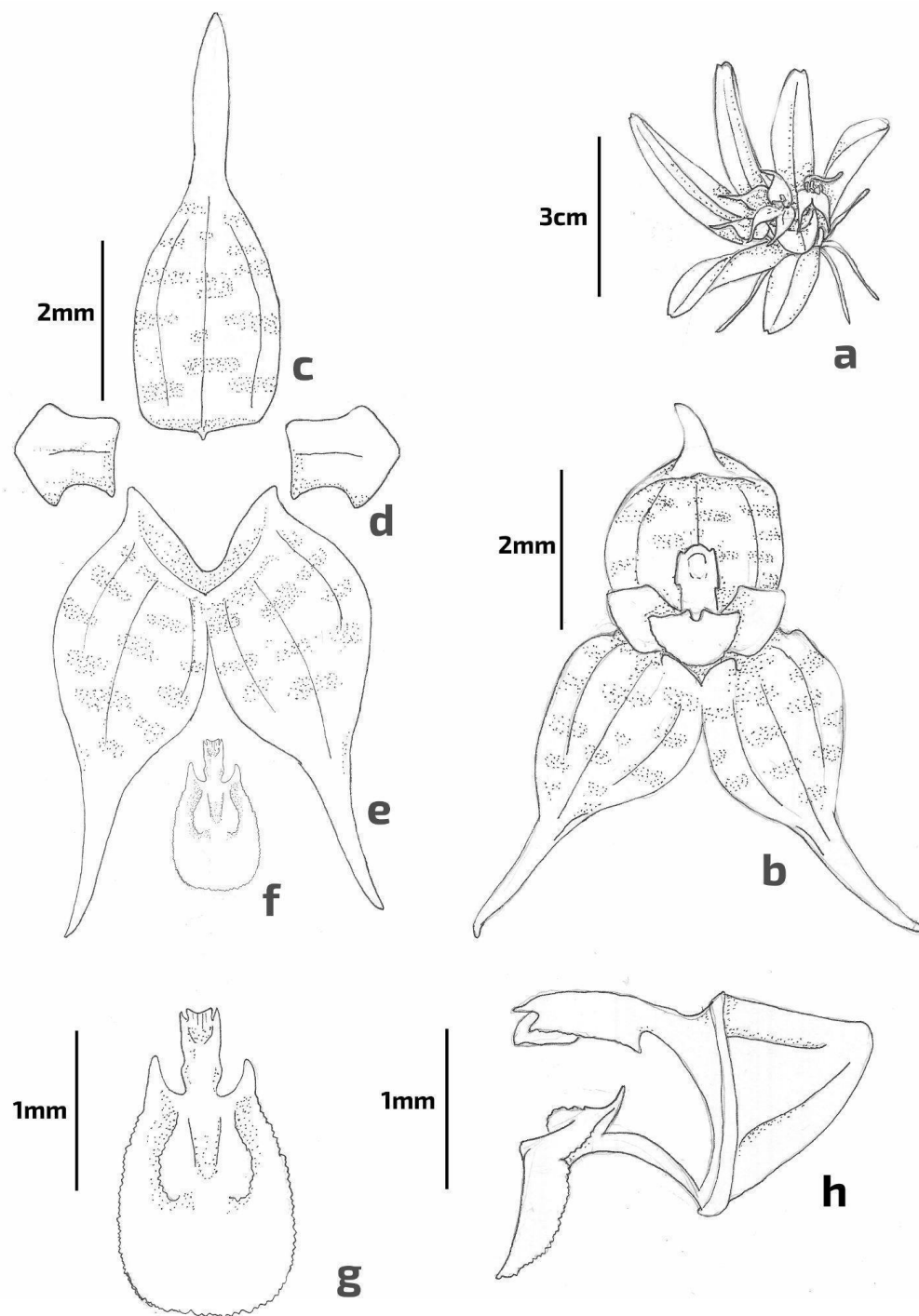


Figure 3 – a-h. *Dryadella ana-paulae* – a. habit; b. Front view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f-g. lip; h. column, lip and ovary. (a-h. D.C. Imig D. Rodrigues 666).

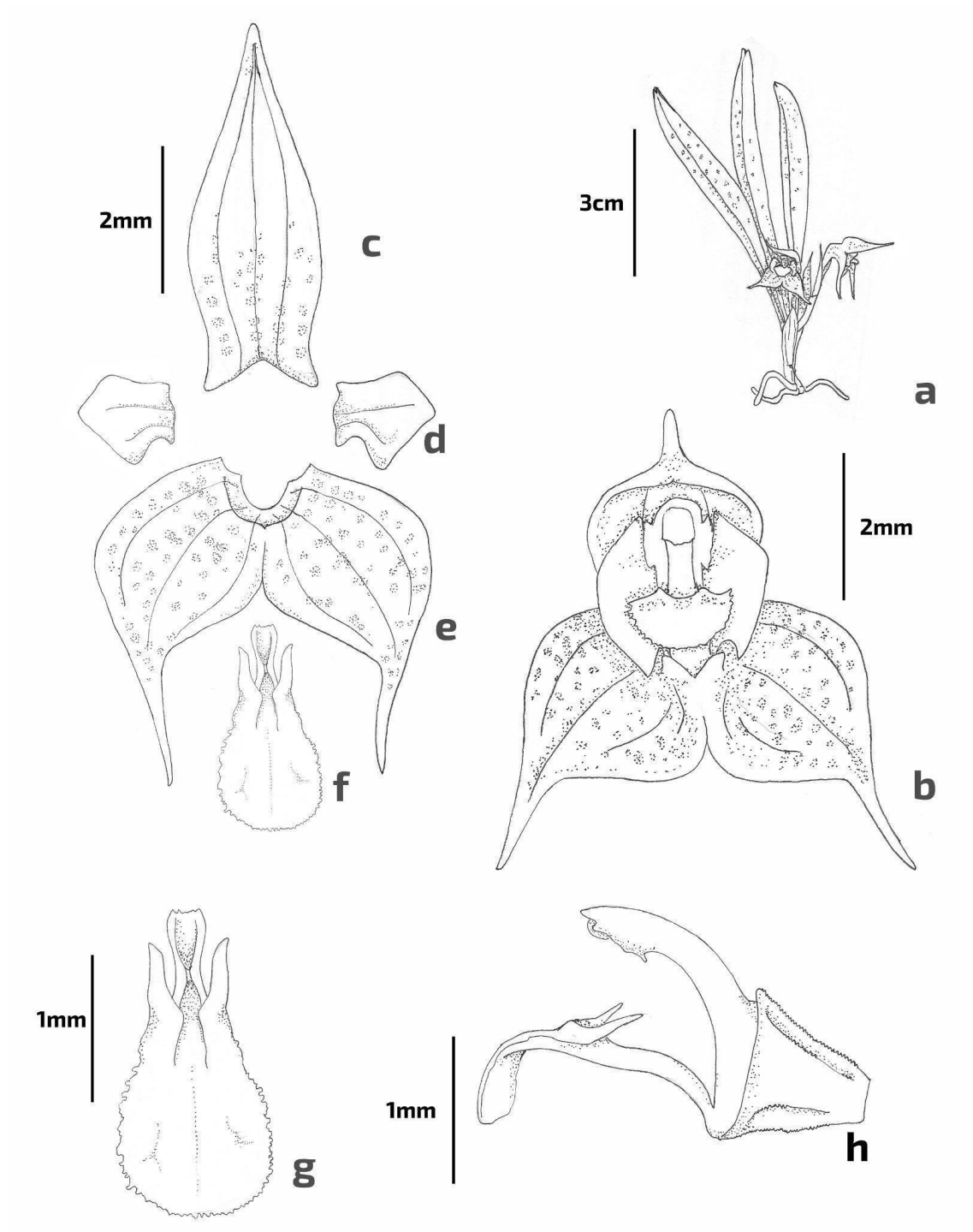


Figure 4 – a-h. *Dryadella auriculigera* – a. habit; b. Front view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f-g. lip; h. column, lip and ovary. (a-h. D.C. Imig & M. Bolson 471).

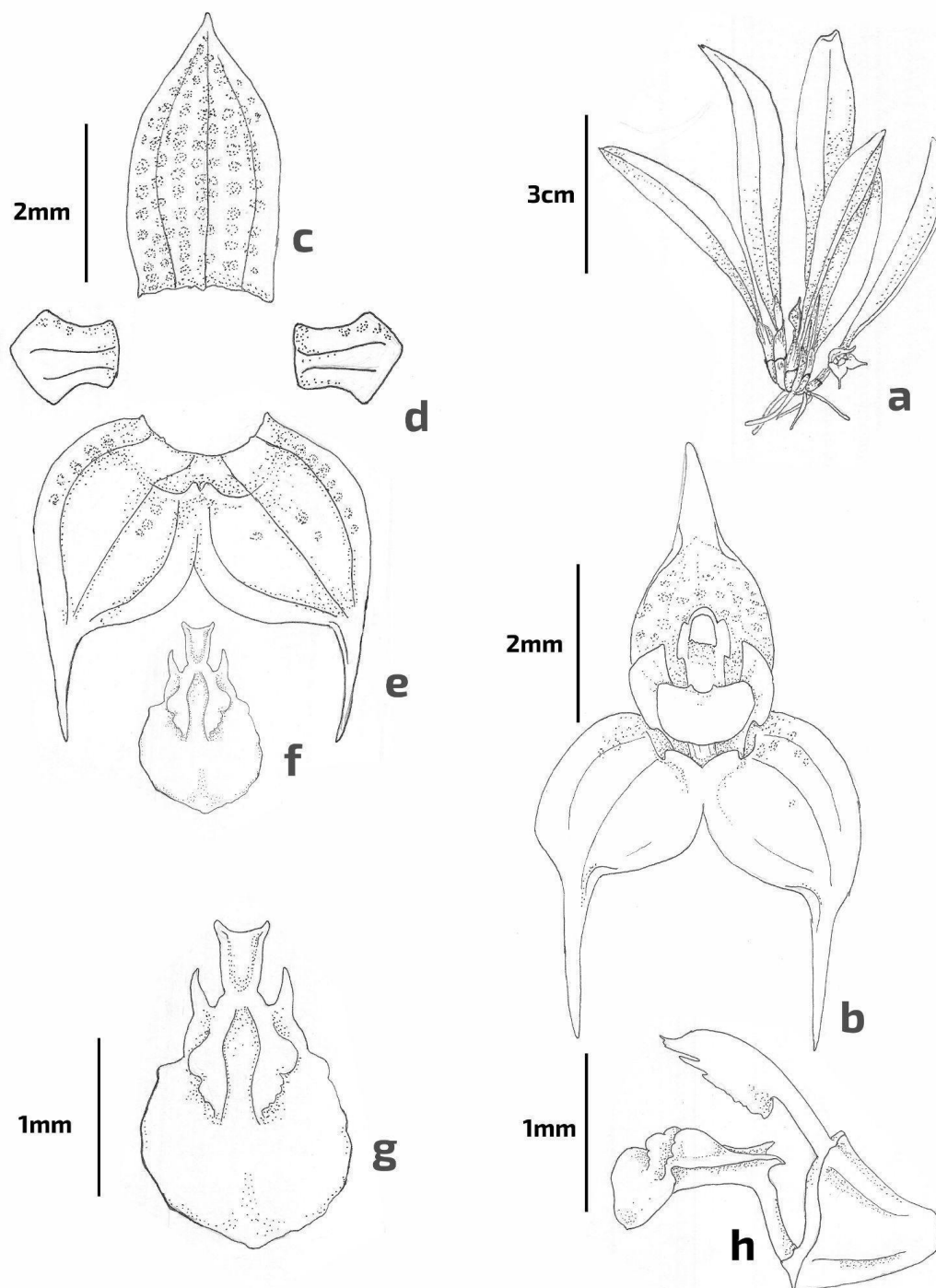


Figure 5 – a-h. *Dryadella aviceps* – a. habit; b. Front view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f-g. lip; h. column, lip and ovary. (a-h. D.C. Imig 397).

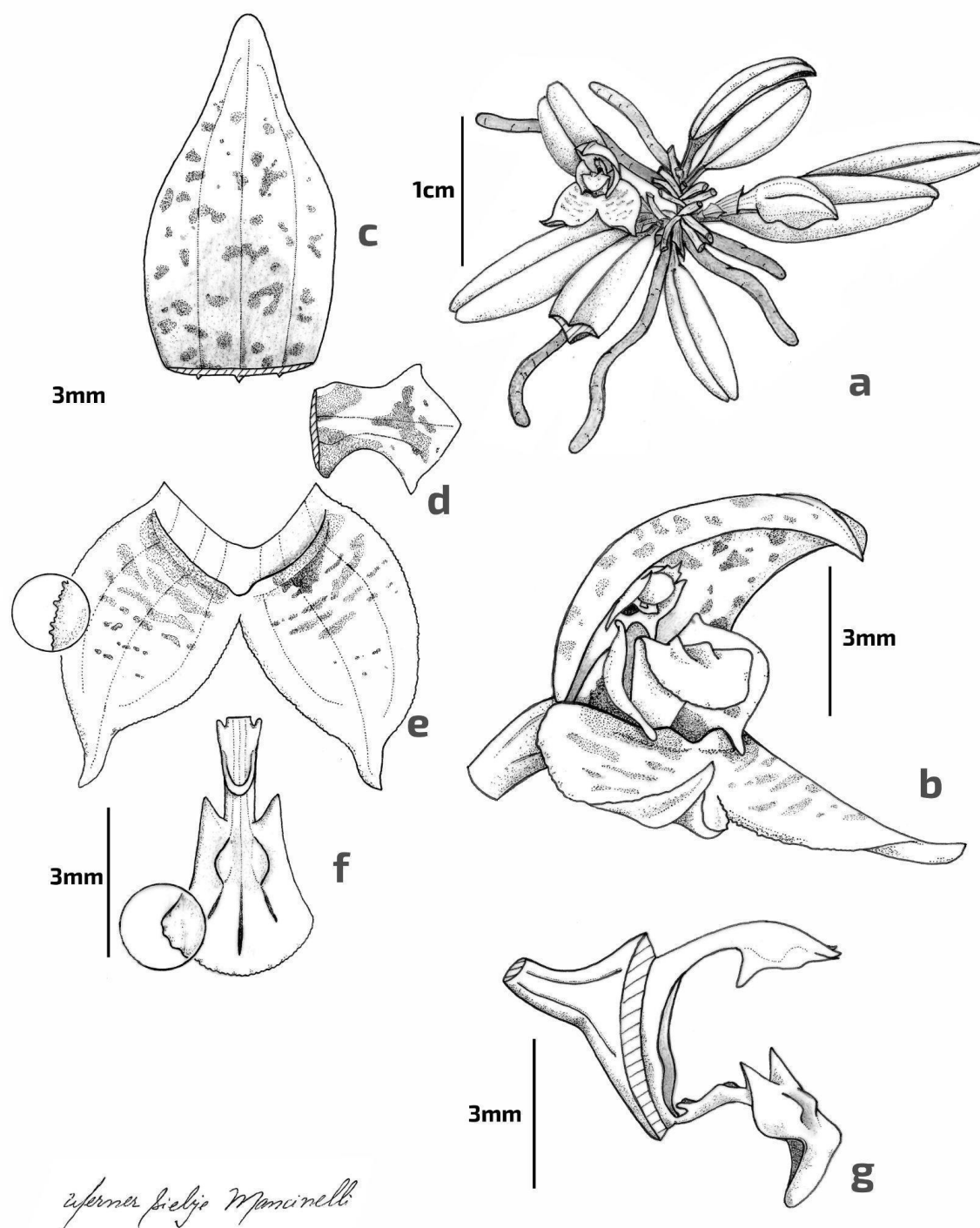


Figure 6 – a-g. *Dryadella catharinensis*– a. habit; b. Side view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f. lip; g. column, lip and ovary. (a-g. D.C. Imig 656).

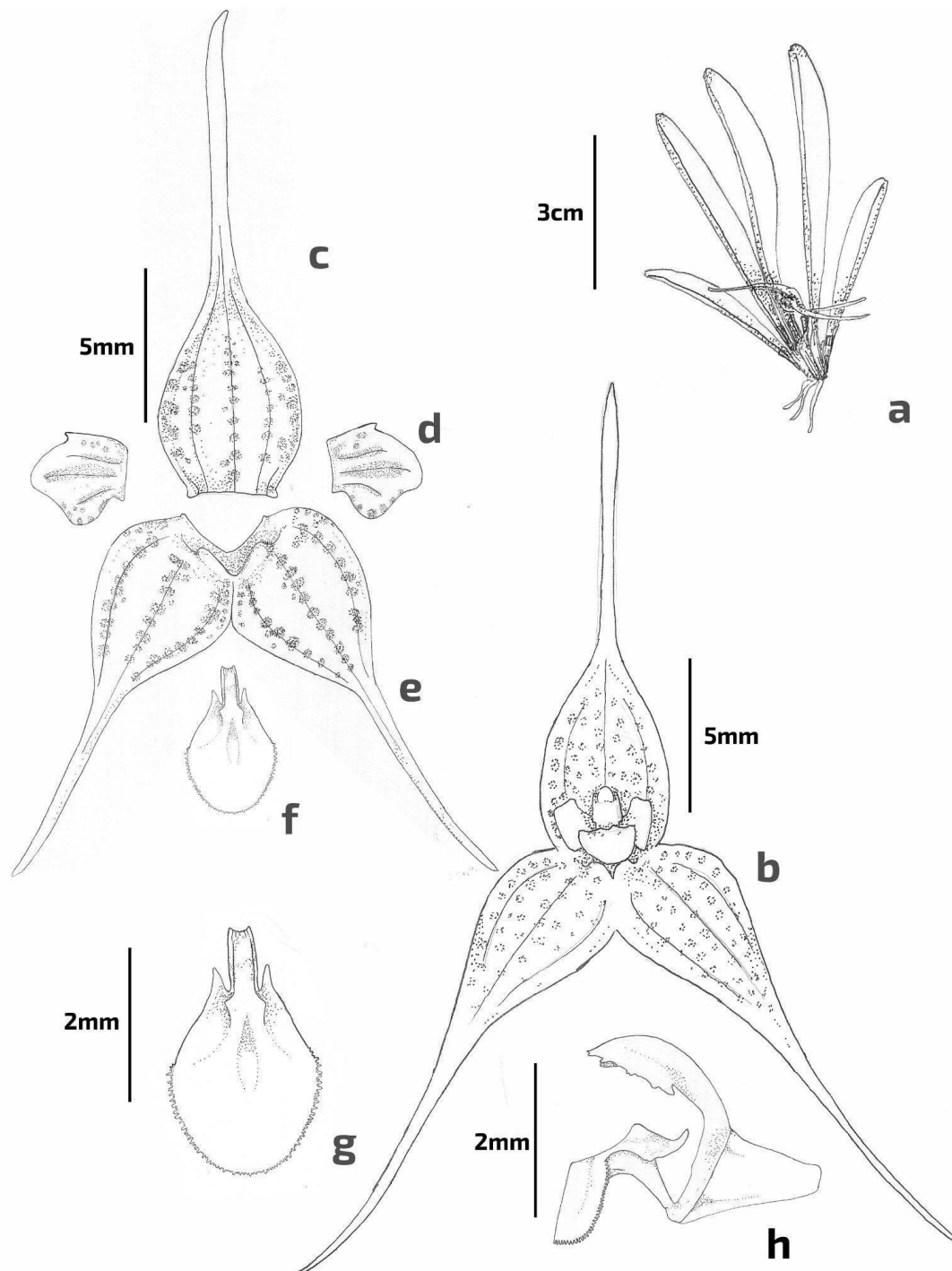


Figure 7 – a-h. *Dryadella crenulata*– a. habit; b. Front view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f. lip; g. column, lip and ovary. (a-h. D.C. Imig & D. Rodrigues 667).

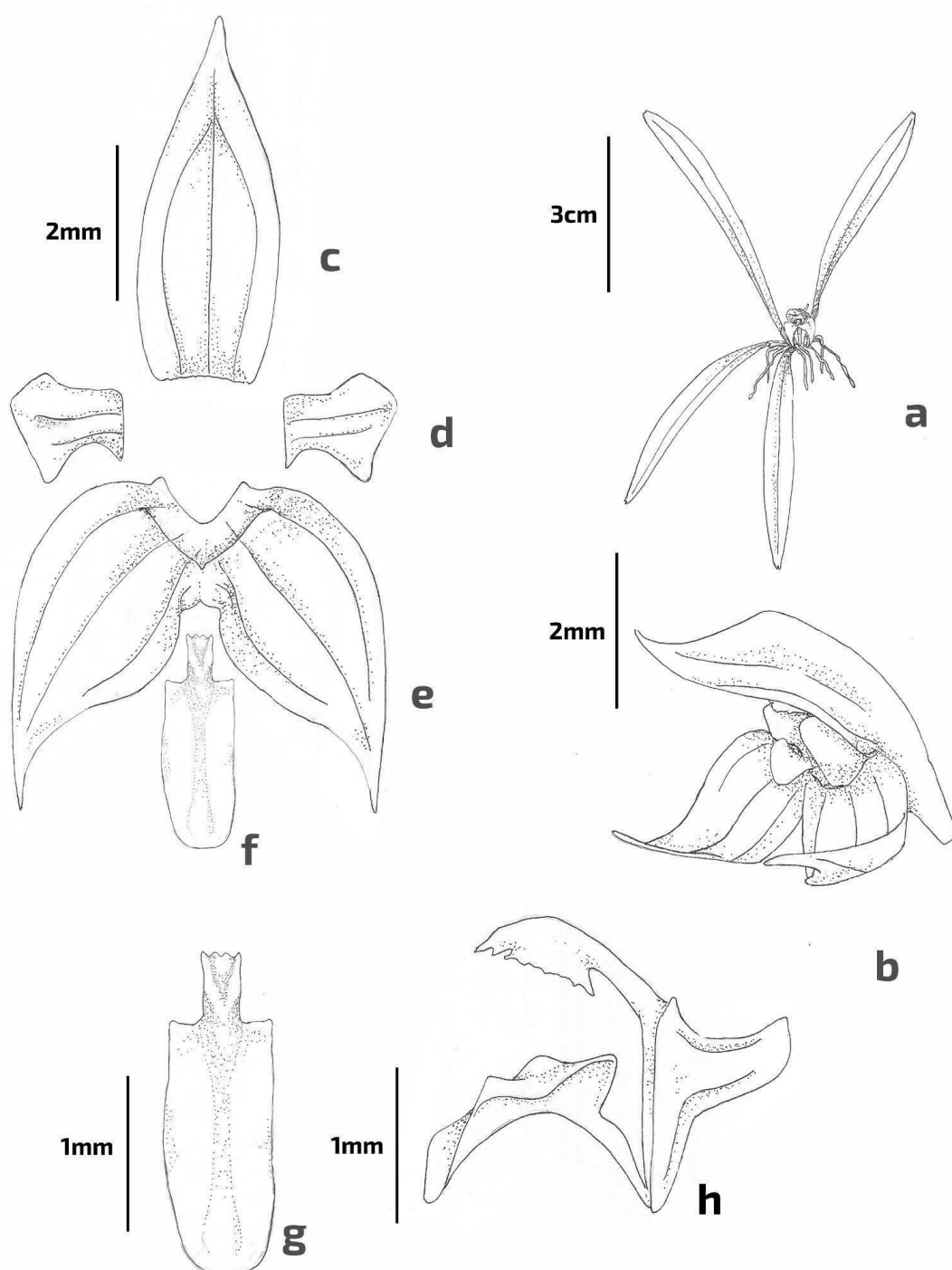


Figure 8 – a-h. *Dryadella gnoma*– a. habit; b. Side view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f-g. lip; h. column, lip and ovary. (a-h. A.H. Krah1 1477).

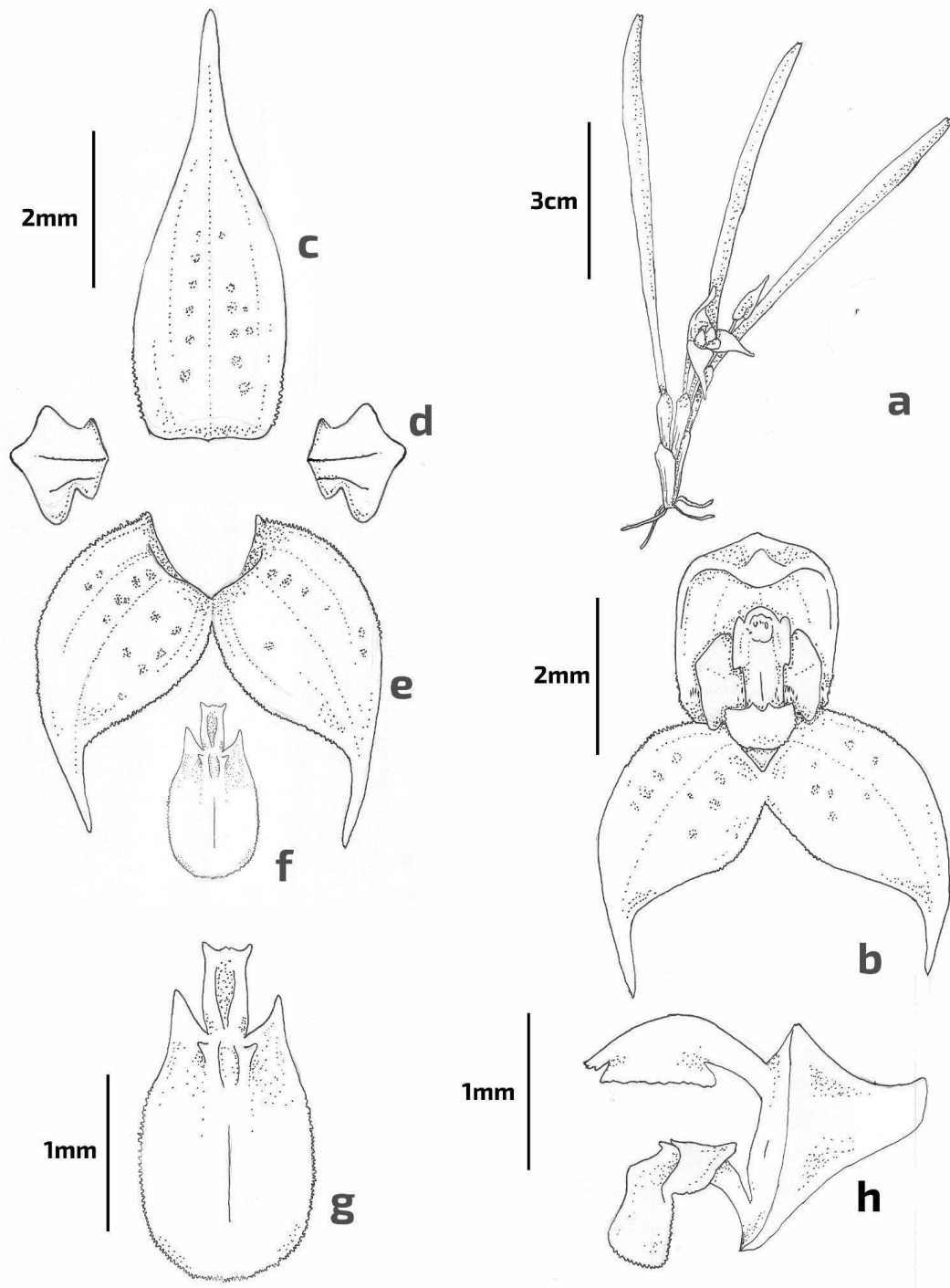


Figure 9 – a-h. *Dryadella kautskyi*– a. habit; b. Side view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f-g. lip; h. column, lip and ovary. (a-h. A.L.V. Toscano de Brito 3476).

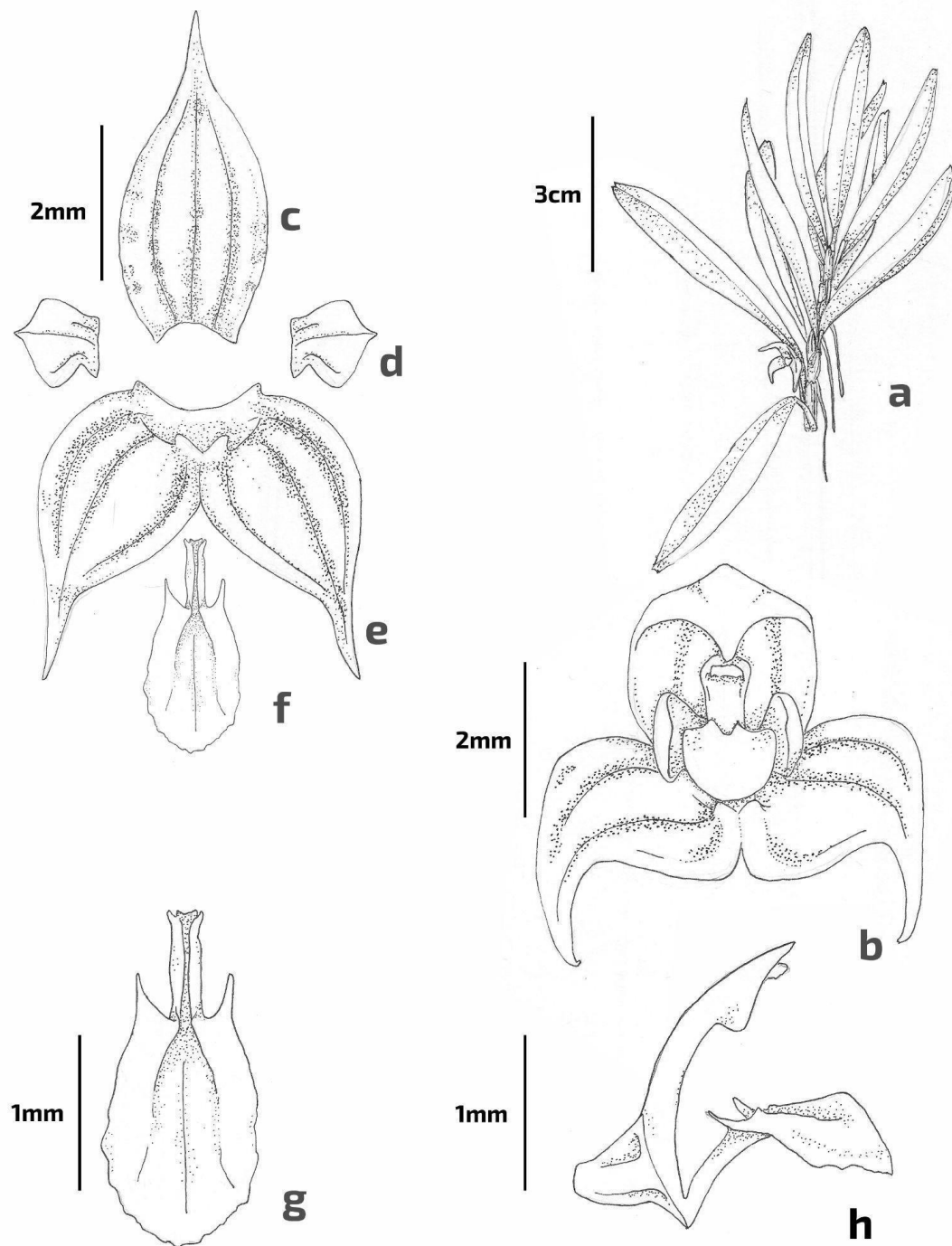


Figure 10 – a-h. *Dryadella krenakiana*– a. habit; b. Front view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f-g. lip; h. column, lip and ovary. (a-h. D.C. Imig & L. Medeiros 662).

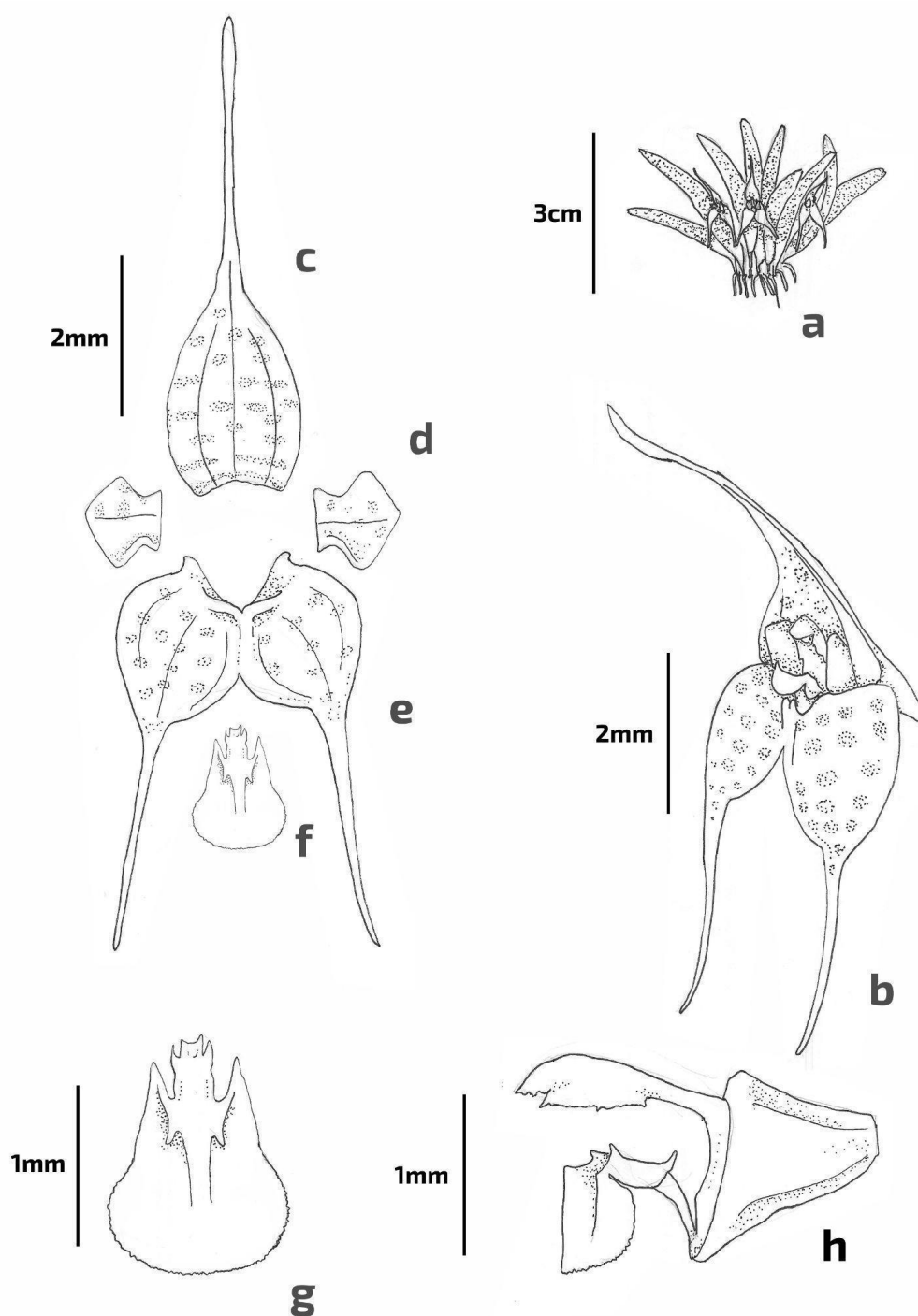


Figure 11 – a-h. *Dryadella lilliputiana*– a. habit; b. Side view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f-g. lip; h. column, lip and ovary. (a-h. D.C. Imig 634).

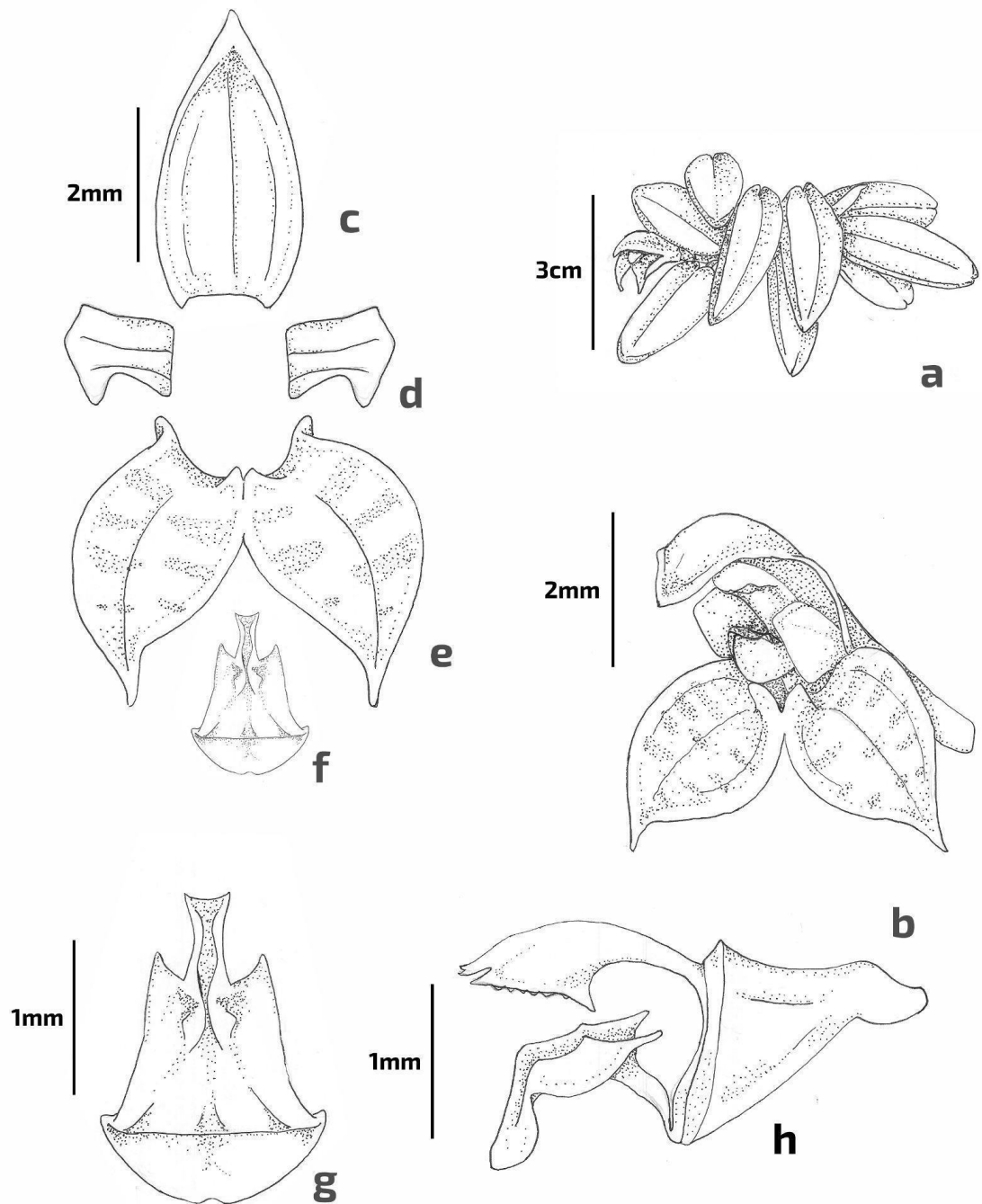


Figure 12 – a-h. *Dryadella susanae*– a. habit; b. Side view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f-g. lip; h. column, lip and ovary. (a-h. D.C. Imig & M. Bolson 491).

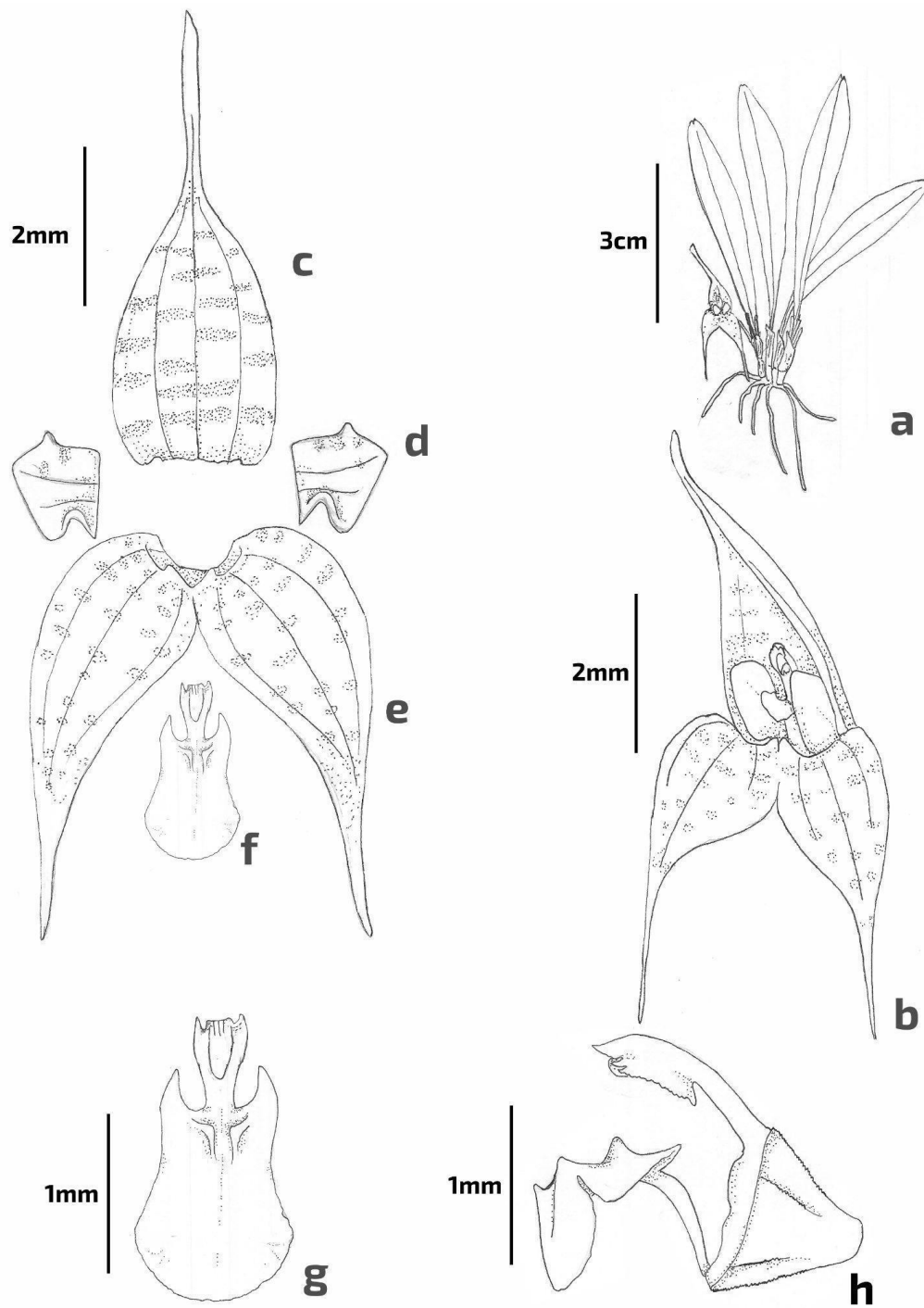


Figure 13 – a-h. *Dryadella toscanoi*– a. habit; b. Side view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f-g. lip; h. column, lip and ovary. (a-h. D.C. Imig 661).

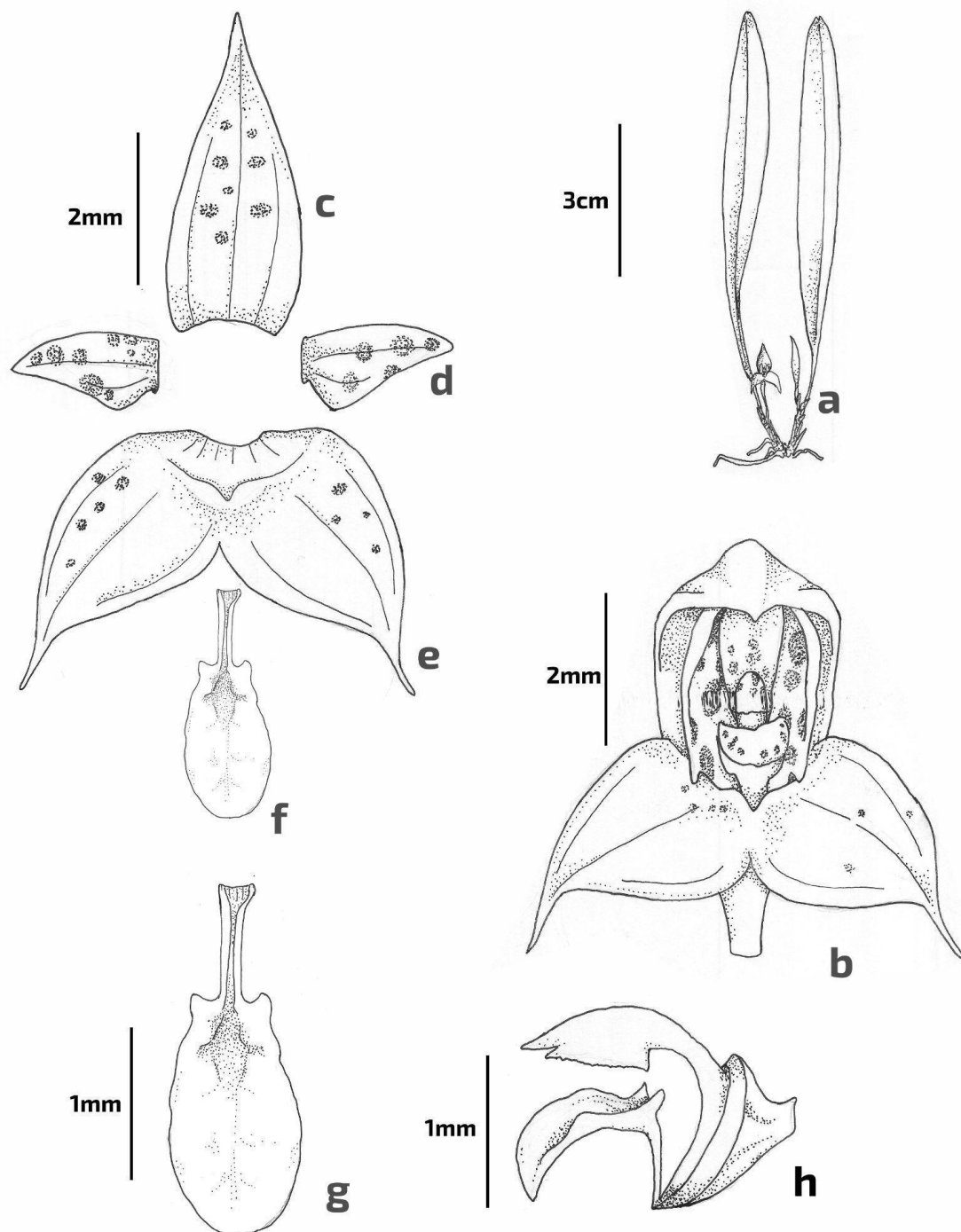


Figure 14 – a-h. *Dryadella vitorinoi* – a. habit; b. Side view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f. lip; g. column, lip and ovary. (a-h. D.C. Imig 506).

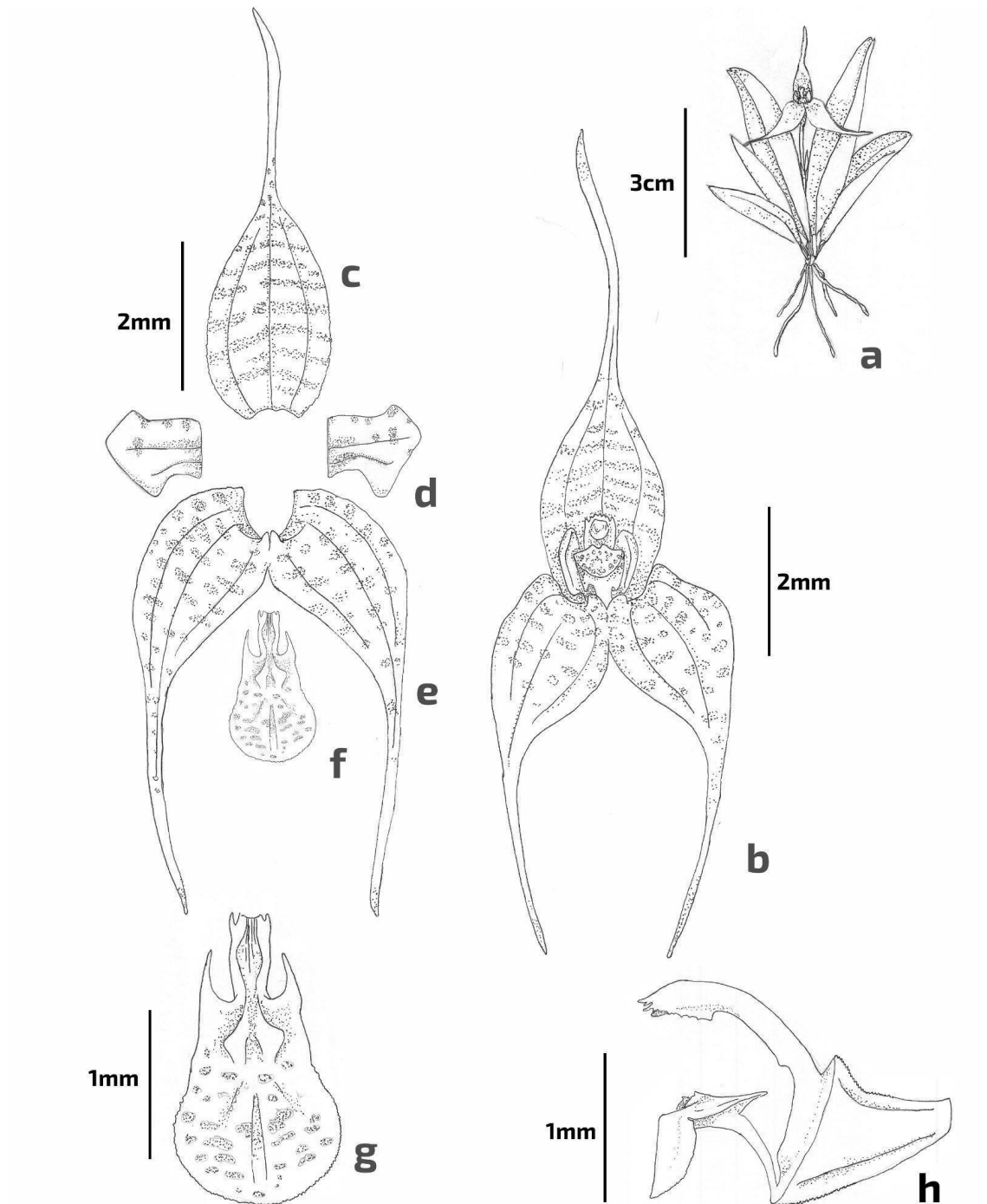


Figure 15 – a-h. *Dryadella wuerstlei* – a. habit; b. Side view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f-g. lip; h. column, lip and ovary. (a-h. D.C. Imig 462).

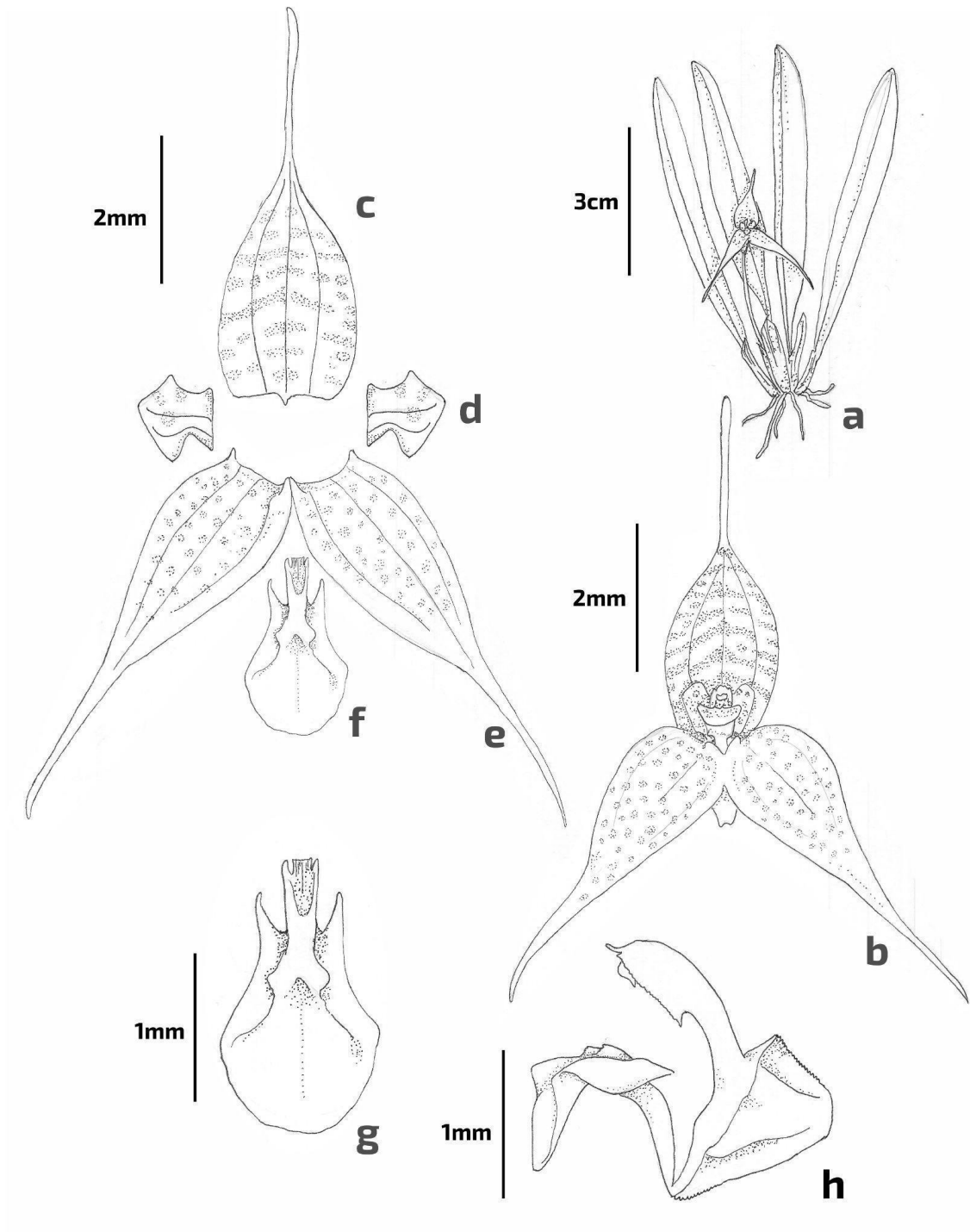


Figure 16 – a-h. *Dryadella zebrina*. a. habit; b. Side view of the flower; c. Dorsal sepal; d. petal; e. lateral sepal; f-g. lip; h. column, lip and ovary. (a-h. D.C. Imig 457).

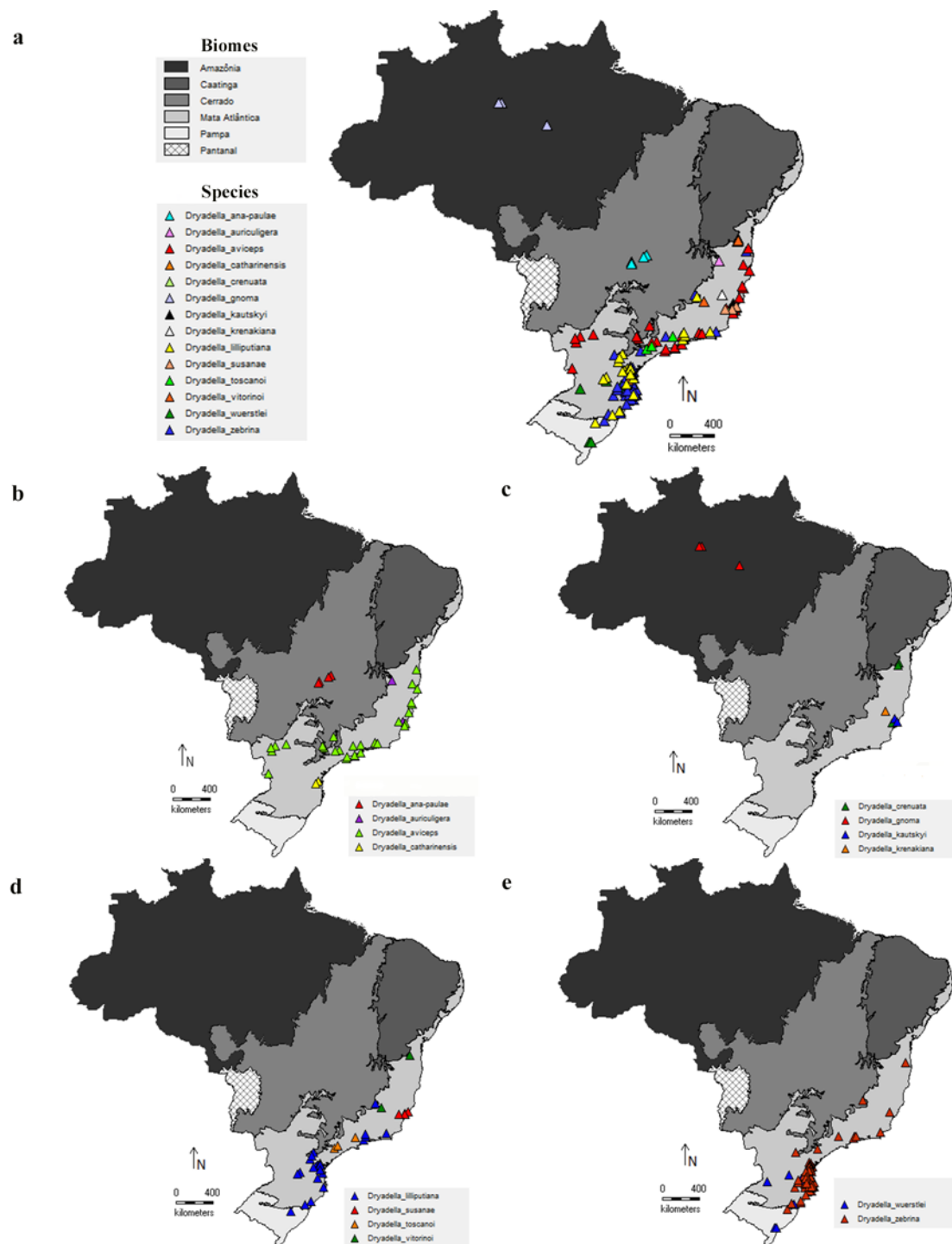


Figure 17 – a-e. Distribution map of *Dryadella* in Brazil. –a. General distribution of the genus in different Biomes.; b. *Dryadella ana-paulae*, *Dryadella auriculigera*, *Dryadella aviceps*, *Dryadella catharinensis*; c. *Dryadella crenulata*, *Dryadella gnoma*, *Dryadella kautskyi*, *Dryadella krenakiana*; d. *Dryadella lilliputiana*, *Dryadella susanae*, *Dryadella toscanoi*, *Dryadella vitorinoi*; e. *Dryadella wuerstlei*, *Dryadella zebrina*.

CAPÍTULO III / CHAPTER III

Atualização taxonômica em *Dryadella* (Orchidaceae: Pleurothallidinae) baseada em análises morfométricas de três espécies endêmicas da Floresta Atlântica

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

Taxonomic update in *Dryadella* (Orchidaceae: Pleurothallidinae) based on morphometric analyses of three species endemic to the Atlantic Rainforest

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Short title: Flower morphometry of *Dryadella zebrina* complex

DCI and ECS designed the study, DCI, ALVTB and ECS collected samples; DCI, AVSRM and ECS analysed the data, all authors wrote the manuscript.

Abstract

Dryadella (Orchidaceae, Epidendreae) is a Neotropical genus, predominantly distributed in the Andean region and the Atlantic Rainforest (ARF). Three species occurring in North-East to Southern Brazil in the ARF, *Dryadella edwallii*, *D. wuerstlei* and *D. zebrina*, have high taxonomic complexity and therefore were considered here as morphotypes. To evaluate current taxonomic proposals and identifying suitable characters for the delimitation of these morphotypes, we analysed the morphological patterns of 40 flower characters from 145 individuals from 23 populations distributed throughout Brazil, representing all the morphological diversity of these taxa with complex delimitation. We performed a Principal Component Analysis (PCA) to summarise all the morphological variation found, and also a Permutational Multivariate Analysis of Variance (PERMANOVA) to verify whether the morphological variation between the groups formed by the PCA is statistically significant. To assess the phenetic relationships among individuals, we generated an Unweighted Pair Group Method with Arithmetic Mean (UPGMA). As a result, it was possible to clearly differentiate *D. wuerstlei* from the other morphotypes. The variables that contributed most were: perimeter and area of the sepals (SD1, SD2, SL10); area and width of the apex, mid-portion, and base of the petals (PT19, PT21, PT22 and PT2); and the measurements of the base and widest portion of the lip blade (LB36, LB38). The second group was formed by all individuals of *D. edwallii* and *D. zebrina* morphotypes, with no clear separation between their populations based on PCA and UPGMA results. Both morphotypes present highly overlapping characters, making the morphological definition of these two taxa not possible. Based on our results, we suggest that *D. edwallii* and *D. zebrina* correspond to a single species, while *D. wuerstlei* is an independent taxon. Hence, we present a taxonomic update of *D. wuerstlei* and *D. zebrina* to clarify the taxonomic delimitation of these two taxa and present their new geographical distribution.

Key-words: Atlantic Rainforest; geographical distribution; morphometric analyses; Taxonomy

Resumo

Dryadella (Orchidaceae, Epidendreae) é um gênero Neotropical, com distribuição predominante na região Andina e na Floresta Atlântica (ARF). Três espécies que ocorrem do Nordeste ao Sul do Brasil na ARF, *Dryadella edwallii*, *D. wuerstlei* e *D. zebrina*, apresentam alta complexidade taxonômica e portanto foram consideradas aqui como morfotipos. Com o objetivo de avaliar as propostas taxonômicas atuais e identificar caracteres adequados para a delimitação desses morfotipos, nós analisamos os padrões morfológicos de 40 caracteres florais de 145 indivíduos de 23 populações distribuídas pelo Brasil, representando toda a diversidade morfológica desses táxons com delimitação complexa. Realizamos uma análise de componentes principais (PCA) para resumir toda a variação morfológica encontrada, bem como uma análise de variância multivariada permutacional (PERMANOVA) para verificar se a variação morfológica entre os grupos formados pela PCA é significativa estatisticamente. Para avaliar as relações fenéticas entre os indivíduos, geramos um dendrograma a partir do método de agrupamento de pares não ponderado com média aritmética (UPGMA). Como resultado, foi possível diferenciar claramente *D. wuerstlei* dos demais morfotipos cujas variáveis que mais contribuíram para essa separação foram o perímetro e a área das sépalas (SD1, SD2, SL10); a área e a largura do ápice, da porção mediana e da base das pétalas (PT19, PT21, PT22 and PT2); e as medidas da base e da maior largura do labelo (LB36, LB38). O segundo grupo foi formado por todos os indivíduos de *D. edwallii* e *D. zebrina*, não havendo separação clara entre as populações com base nos resultados da PCA e do UPGMA. Ambos os morfotipos possuem caracteres altamente sobrepostos, impossibilitando a definição morfológica desses dois táxons. Com base nesses resultados, nós sugerimos que *D. edwallii* e *D. zebrina* correspondem a uma única espécie, enquanto que *D. wuerstlei* é uma espécie distinta. Portanto, nós apresentamos a atualização taxonômica para *D. wuerstlei* e *D. zebrina* para esclarecer a delimitação taxonômica desses táxons, juntamente com a nova distribuição geográfica dessas espécies.

Palavras-chave: Floresta Atlântica; Análises morfométricas, Orchidaceae; Taxonomia.

Introduction

Dryadella Luer (Orchidaceae, Epidendreae) is a Neotropical genus of the Pleurothallidinae that comprises 61 species predominantly distributed in the Andean region and the Atlantic Rainforest (ARF) (Pridgeon 2005; POWO 2022; Imig *et al.* 2020a; Imig *et al.* 2021). Brazil harbours 17 species, of which 13 are endemic to the country. Almost all species that occur in Brazil are exclusive to ARF, with only two occurring in other ecosystems: *D. ana-paulae* V.P.Castro in the Cerrado (tropical savanna) and *D. osmariniana* (Braga) Garay & Dunst. at the Amazon Forest (Pridgeon 2005; Luer 1978, 2005; Imig *et al.* 2020a).

Despite the wide geographic distribution of *Dryadella*, with a disjunction in South American Dry Diagonal, the species are morphologically very similar and the existence of intermediate forms between populations of related species hinder the taxonomy of the species (Luer 2005). The species are characterised by small, caespitose plants, with a transversal callus in lateral sepals, asymmetrical petals, and unguiculate lip with a pair of retrorse lobes at the base. The leaves, sepals, petals, and lip differ subtly in size and shape among species (Luer 2005; Pridgeon 2005). Also, leaf anatomy does not discriminate between species of different habitats and sections (Imig *et al.* 2020b).

During our studies for the elaboration of the flora of the *Dryadella* from Brazil (Imig *et al.* in prep.) we detected the high taxonomic complexity of three species occurring in South and Southeastern Brazil in the ARF: *D. edwallii* (Cogn.) Luer, *D. wuerstlei* Luer, and *D. zebrina* (Porsch) Luer. Flower morphological characters traditionally used in the taxonomy of *Dryadella* are poorly diagnosable among the species and the main difference between them is the lip size: 2 mm in *D. zebrina* and 3 mm in *D. edwallii*, and proportionally larger sepal tails and margins of lip minutely denticulate in *D. wuerstlei* margins (Luer 2005).

The difficulty of taxonomic delimitation between these taxa can be noted in Pabst & Dungs (1975), on plates 1088 of *D. edwallii* and 1096 of *D. zebrina* (pages 332 and 333, respectively) in which the morphology is very similar, and it is not possible to distinguish the species. Doubts about the taxonomy of these taxa are also common among orchidologists and collectors, especially between *D. zebrina* and *D. edwallii*, since *D. wuerstlei* is currently known only by type.

Besides, the nomenclatural types and corresponding protologues are not very informative, and the identifications are often dubious, sometimes with different determinations for the same specimen. Overlapping morphological variation is even more critical among herborized material due to the loss of qualitative data used in the original descriptions to delimit the species, such as the presence of purple spots forming bars on the dorsal sepal, which is considered a diagnostic characteristic of *D. zebrina* (Luer 2005).

Morphometry is an approach that can indicate potential phenotypic variation within species complexes (Pinheiro *et al.* 2018) through measures of morphological differences or similarities between the sampled units (Sokal & Michener 1958). These phenotypic evidences (variables), when submitted to multivariate analysis, facilitate the recognition of groups (Rohlf 1990), allowing the evaluation of the phenetic relationships between the taxa and the recognition of distinctive characters to delimit them (Tyteca & Dufrene 1994; Pinheiro & Barros 2007). Several plant groups were studied from the morphometric point of view to elucidating the taxonomy of complex species, e.g., *Anthurium augustinum* complex (Araceae), endemic to the Brazilian Atlantic (Cardozo *et al.* 2014) and herbaceous bamboos of the *Raddia brasiliensis* complex (Poaceae-Bambusoideae, Oliveira *et al.* (2008)). In Orchidaceae, different genera were studied involving species complexes or populations, focusing on taxonomy or conservation, such as *Acianthera* Scheidw. (Melo & Borba 2011), *Bulbophyllum* Thou. (Ribeiro *et al.* 2008), *Pleurothallis* R. Br. (Borba *et al.* 2002), *Brasiliorchis* R.B. Singer, S. Koehler & Carnevali (Pinheiro & Barros 2009); *Bonatea cassidea* and *Bonatea speciosa* complexes (Ponsie *et al.* 2007; 2009), *Pseudolaelia* Porto & Brade (Menini Neto *et al.* 2019) and *Habenaria* Willd. (Cruz-Luster *et al.* 2020) among others.

Following this context, we explored the morphological variation of flowers among populations of the *D. edwallii*, *D. wuerstlei* and *D. zebrina* over the entire geographic range from Brazil. We performed Principal Component Analysis (PCA) and hierarchical clustering (UPGMA) using the phenotypic variables measured to assess the importance of flower characters in recognising species boundaries. We aim to decide whether we should consider these specimens as a single, widespread species with high phenotypic plasticity, or as different taxa, with proper diagnostic characters to delimit them. We also present a treatment taxonomic for these species complexes as a proposal for consistent identification of the species.

Materials and methods

Sampled populations

We sampled 145 operational taxonomic units (OTUs), each one in a different phorophyte to avoid clones, from 23 populations of the *Dryadella zebrina*, *D. edwalli* and *D. wuerstlei* (Tab. 1). Four to ten OTUs were collected per population, considering that the *Dryadella* are not found in large abundances. These populations were collected from North-East to Southern Brazil to sample all morphological variation and cover the entire geographic distribution of the morphotypes (Figs. 1; 2). We searched for locations of historical collections in herbaria, which was successful for *D. edwalli* and *D. zebrina*, but not for *D. wuerstlei*, which occurrence was unknown. The first sample of *D. wuerstlei* was collected in São Francisco de Paula city (*Toscano de Brito* 2563, Tab. 1), South Brazil, and identified from the protologues, and from then on, we expanded our efforts to collect more populations. Samples outside Brazil, in Bolivia and Peru, were not collected due to logistical difficulties and for not obtaining collection licenses outside the country.

We used only flowers in the analyses because the taxa were initially separated by Porsch (1905), Cogniaux (1906), and especially Luer (2005) by qualitative and quantitative floral characteristics, for example, the size and shape of the sepals and tail, the size and shape of the lip, and the presence of dark spots that are often arranged into transverse bars on the dorsal sepal in *D. zebrina*. Furthermore, we observed high phenotypic plasticity in vegetative characters of OTUs in the field and after acclimating the specimens at the greenhouse. The flowers of all OTUs were conserved in 70% alcohol solution. Materials were collected randomly, and sample sizes varied according to the availability of flowers and the size of the populations in the different localities. One individual per population was preserved in spirit and deposited in the UPCB herbarium of the Universidade Federal do Paraná, Brazil.

Morphological characters and measurement procedures

We measured 465 flowers recently opened (1–2 days) and preserved them in 70% alcohol solution to avoid the effect of floral senescence. Initially, the morphotypes were identified based on the criteria used by Luer (2005), especially petal size, shape and distribution of vinaceous spots, length of sepal tails, shape, size and consistency of

leaves. We selected three flowers per OTU, dissected them, and photographed the floral pieces with an adapted camera in the Olympus SZX7 Stereomicroscope.

Table 1 – Identification (ID), Species, sample size, voucher, and collection sites of the 23 populations studied.

ID	Species	N.	Voucher	UF	Locality, municipality
ZB1	<i>D. zebrina</i>	9	Imig, 362	SC	Fazenda do Zinco, Benedito Novo
ZB2	<i>D. zebrina</i>	8	Imig, 373	PR	Mãe Catira, Morretes
ZB3	<i>D. zebrina</i>	6	Imig, 393	PR	Morro do Bruninho, Piraquara
ZB4	<i>D. zebrina</i>	6	Imig, 485	MG	Serra do Cipó, Santana do Riacho
ZB5	<i>D. zebrina</i>	5	Imig, 488	PR	Morro lateral do Bruninho, Piraquara
ZB6	<i>D. zebrina</i>	6	Imig, 489	BA	P. N. do Pau Brasil. Porto Seguro
ZB7	<i>D. zebrina</i>	9	Imig, 405	PR	Rio do Corvo, Piraquara
ZB8	<i>D. zebrina</i>	9	Imig, 453	BA	Os quatro, Camacan
ZB9	<i>D. zebrina</i>	5	Imig, 626	SC	Chácara Antônio Cziczeck, São Bento
ZB10	<i>D. zebrina</i>	5	Imig, 637	SC	Trilha dos Porcos, Praia Grande
ZB11	<i>D. zebrina</i>	5	Imig, 632	SC	Chácara Schlogel, São Bento
ZB12	<i>D. zebrina</i>	6	Imig, 378	PR	Trilha do Olimpo, P.E.Pico Marumbi, Morretes
ZB13	<i>D. zebrina</i>	6	Toscano, 3478	ES	Venda Nova do Imigrante
ED1	<i>D. edwallii</i>	7	Imig, 377	PR	Reserva Nhandara Guaricana, São José dos Pinhais
ED2	<i>D. edwallii</i>	5	Imig, 374	PR	P. E. do Marumbi, Piraquara
ED3	<i>D. edwallii</i>	8	Klingelfuss, 157	PR	Represa do Vossoroca, Tijucas do Sul
ED4	<i>D. edwallii</i>	6	Santos, 298	PR	Estação Marumbi, Piraquara
ED5	<i>D. edwallii</i>	6	Imig, 482	MG	Trilha da Pedra Redonda, Camanducaia
ED6	<i>D. edwallii</i>	6	Imig, 454	SP	Campos do Jordão
WU1	<i>D. wuerstlei</i>	5	Imig, 462	RS	Kaingang de Iraí, Iraí
WU2	<i>D. wuerstlei</i>	7	Toscano, 2646	RS	São Francisco de Paula
WU3	<i>D. wuerstlei</i>	4	Toscano, 2563	RS	São Francisco de Paula
WU4	<i>D. wuerstlei</i>	6	Imig, 408	SC	Rio do Sul

Total Sampled 145

Table 2 – Flower morphological characters measured.

Code	Character description
SD1	Dorsal sepal, perimeter (outline)
SD2	Dorsal sepal, area
SD3	Dorsal sepal, length without the tail
SD4	Dorsal sepal, width at the widest portion
SD5	Dorsal sepal, base width
SD6	Dorsal sepal, tail length
SD7	Dorsal sepal, tail width
SL8	Lateral sepal, apex angle
SL9	Lateral sepal, perimeter (outline)
SL10	Lateral sepal, total area
SL11	Lateral sepal, total length without the tail
SL12	Lateral sepal, total width at the widest portion
SL13	Lateral sepal, apex width before the tail
SL14	Lateral sepal, base width
SL15	Lateral sepal, tail length
SL16	Lateral sepal, tail width
SL17	Lateral sepal, apex angle
PT18	Petal, perimeter (outline)
PT19	Petal, area
PT20	Petal, length
PT21	Petal, apex width at the widest portion
PT22	Petal, width of the narrowest portion
PT23	Petal, base width
PT24	Petal, apex angle between the lobes
PT25	Petal, lower lobe apex internal angle
PT26	Petal, upper lobe apex internal angle
PT27	Petal, lower lobe apex external angle
PT28	Petal, upper lobe apex external angle
OV29	Ovary, perimeter (outline)
OV30	Ovary, area
OV31	Ovary, length
OV32	Ovary, width
LB33	Lip, perimeter (outline, including foot)
LB34	Lip, area including foot
LB35	Lip, length including foot
LB36	Lip, blade width median at the widest portion
LB37	Lip, base width

LB38	Lip, distance between the lobules at the base
LB39	Lip, apex angle
LB40	Lip, angle between the basal lobules apices

The images were obtained with ZEN lite software (Zeiss Oberkochen, Germany), with a scale of 1 mm. We established 40 variables, which included measures of dorsal sepal (8), lateral sepal (9), petal (11), ovary (4), and lip (8) (Fig. 1; Tab. 2). All measures were taken at the largest point using the ImageJ 1.46r software (Rasband 2004).

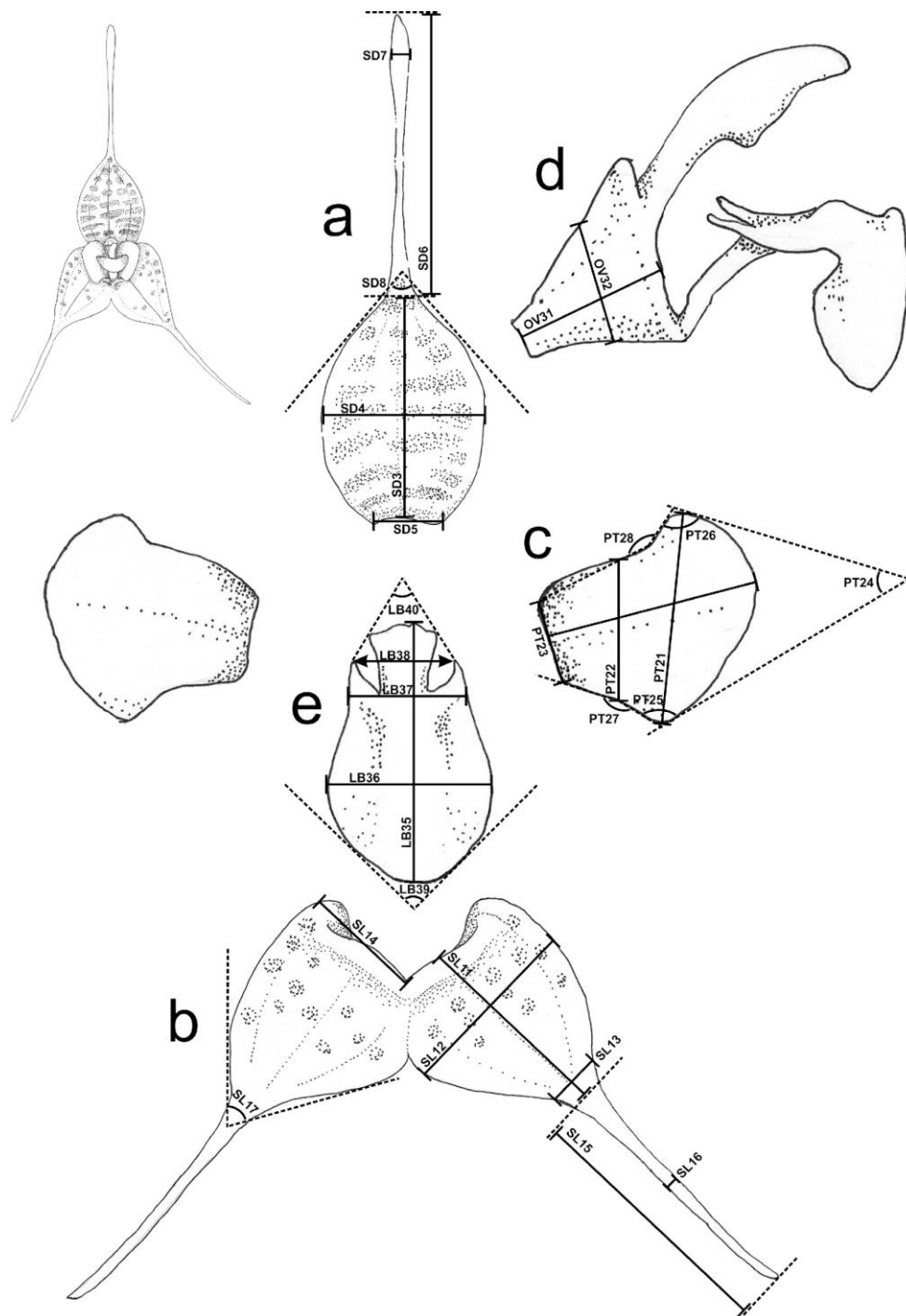


Figure 1 – a-e. Details of the flower morphological characters measured – a. Dorsal sepal; b. Lateral sepal; c. Petal; d. Ovary; e. Lip. (Character codes are listed in Tab. 2).

Statistical analyses

We used the mean values of each variable in statistical analyses for each OTU, and standardised the dataset. We calculated a distance matrix using the Gower method (Gower 1966), which was used in all subsequent analyses developed in the software R v. 4.1.1 (R Core Team 2018). The scripts and the dataset used are available in the supplementary material.

We performed a PCA to summarise all the morphological variation among OTUs based on the 40 quantitative variables surveyed and identify the characters that contributed most to a possible clustering pattern (Sneath & Sokal 1973; Henderson 2006). The principal components (PCs) were selected based on the broken-stick model, and the most important variables were visualised through the cosine² chart. To verify whether the morphological variation between the groups formed by the PCA is significant, we performed a permutational multivariate analysis of variance (PERMANOVA, Anderson 2001). To assess the phenetic relationships among OTUs, populations, and species, we generated a UPGMA (Sokal & Michener 1958) dendrogram. To validate the information generated by the dendrogram, we performed a correlation test between Gower's distance matrix and the cophenetic distance matrix (Gower 1966).

Results

Morphometric differentiation of populations of *Dryadella* species

According to the broken-stick model, only PC1 presented greater variation than expected by random (eigenvalue > broken-stick; Fig. 3). PC1 accounted for 27.3% of the total variance, and PC2 for 10.5%. Two groups were formed, one constituted by all OTUs of *D. wuerstlei* and the other with the remaining OTUs from the *D. edwalli* and *D. zebrina* together (Fig. 2a). The most important variables for the differentiation of these two groups were PT23, SD2, PT19, PT21, SL10, SD1, PT22, LB36, LB38, and SL15 (Fig. 2a,b; for character codes and definitions see Tab. 2). The PERMANOVA showed that both groups are significantly different ($R^2 = 0.31$, $F = 65.114$, $p = 0.0009$).

A similar pattern was observed in the dendrogram, in which two main groups were formed. The first is composed by all populations of *D. wuerstlei*, and the second by the interspersed populations of *D. edwallii* and *D. zebrina*. (Fig. 4).

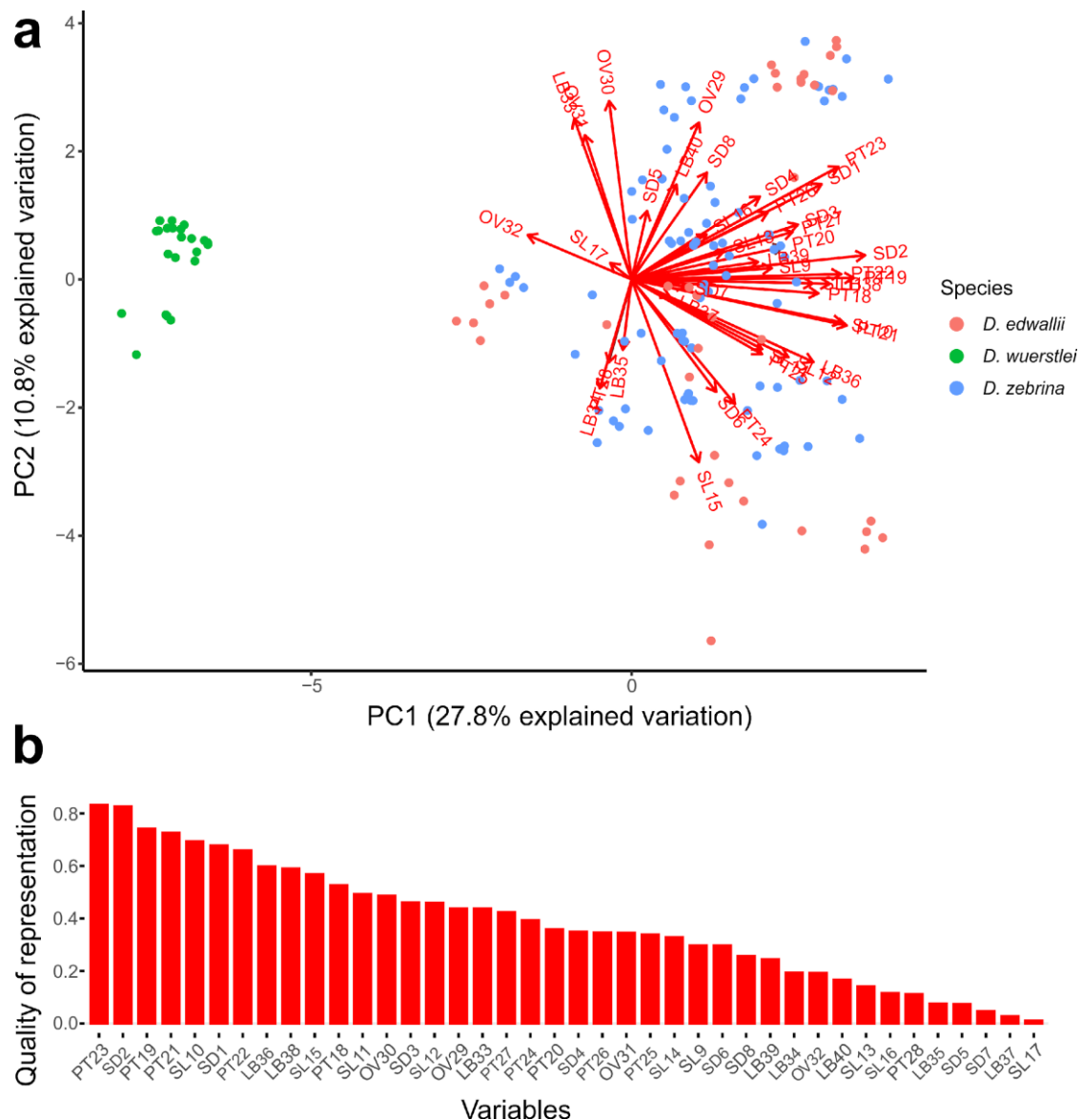


Figure 2 – a-b. Principal Component analysis (PCA) result – a. Ordination plot based on PC1 and PC2. Morphological characters are represented by their codes in red, and the size of the arrows is proportional to each character relative importance for the clustering structure. OTUs of *Dryadella wuerstlei* (green), *D. zebrina* (blue), and *D. edwallii* (pink) are represented by dots; b. Cosine² barplot with the contributions of each variable for the PCs.

Principal Components validation

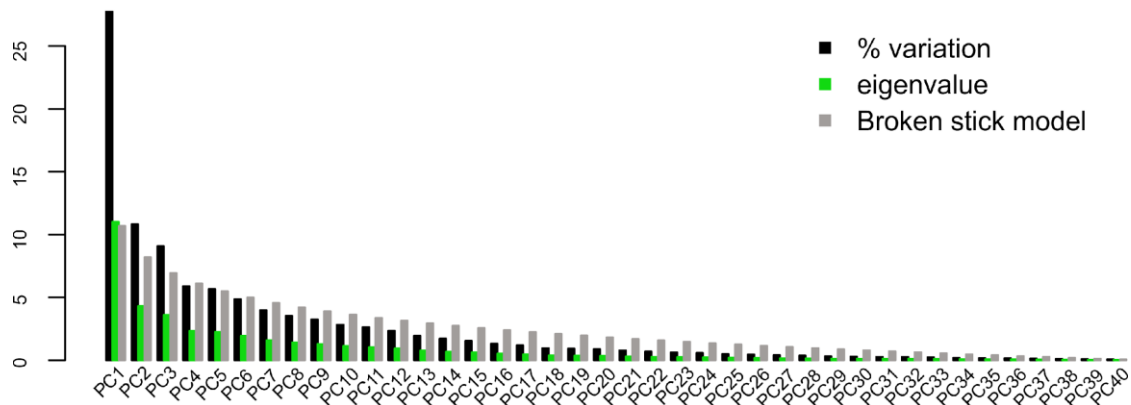


Figure 3 – Percentage of variation, eigenvalue and broken stick model for the first 40 Principal Components (PCs). Variation was considered significant when the eigenvalue is greater than the broken stick.

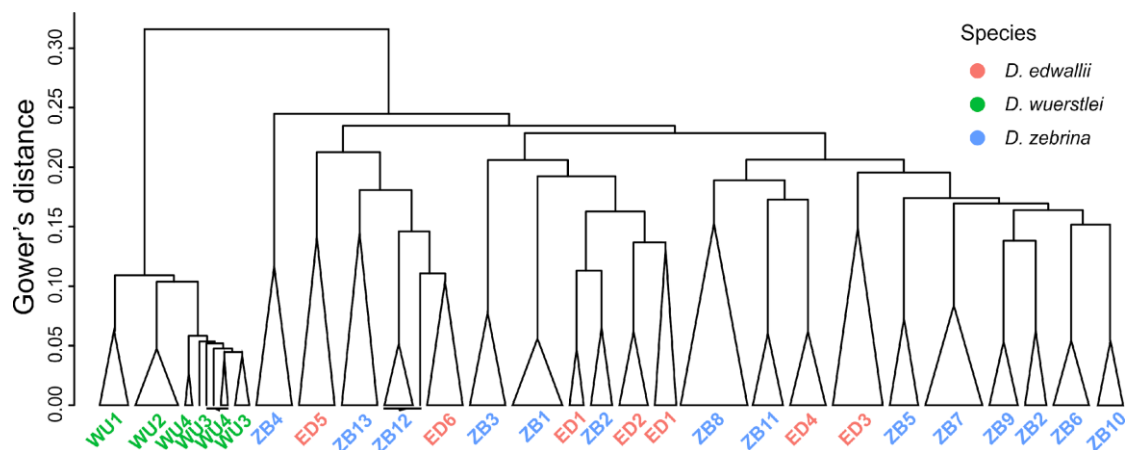


Figure 4 – UPGMA dendrogram showing phenetic relations among 23 populations of the *Dryadella zebrina* complex. ED (pink) = populations of *D. edwallii*, WU (green) = populations of *D. wuerstlei*, and ZB (blue) = populations of *D. zebrina*.

Discussion

Species delimitation

Previous studies in Orchidaceae have proposed that complex morphological patterns in populations can be driven by incipient divergence or hybridization (Ribeiro *et al.* 2008), and highlight the importance of using various sources of information before taking taxonomic decisions, being morphometry a valuable tool to assist taxonomists (Borba *et al.* 2002, 2007a,b; Ribeiro *et al.* 2008; Oliveira *et al.* 2008; Melo & Borba 2011; Adhikari & Wallace 2013). In addition, it is known that many plants in environments

with more aridity and altitudes have smaller vegetative and floral organs (Sapir *et al.* 2002, Kofidis *et al.* 2007). Morphological variations related to environmental and latitudinal gradients have already been observed in morphometric analyses of species from different plant families and different localities, such as Iridaceae (Sapir *et al.* 2002), Asteraceae (Fici & Lo Presti 2003), Orchidaceae (Goldman *et al.* 2004), Lamiaceae (Kofidis *et al.* 2007), Eriocaulaceae (Trovó *et al.* 2008), and Rosaceae (Shimono *et al.* 2009).

Morphometric methods used in studies with Orchidaceae showed that plants that are widely distributed can show strong morphological variation along with their distribution. This was observed, for example, in studies with *Epidendrum secundum* Jacq. (Pinheiro & Barros 2007); *Bulbophyllum exaltatum* Lindl. (Ribeiro *et al.* 2008); *Brasiliorchis picta* (Hook.) R.B.Singer, S.Koehler & Carnevali and *Brasiliorchis crysantha* (Barb. Rodr.) R.B.Singer, S.Koehler & Carnevali (Pinheiro & Barros 2009) and *Pseudolaelia vellozicola* (Menini Neto *et al.* 2019). Our results show this same pattern, especially the presence of interpopulation morphological variability in *D. zebrina*, a species that is widely distributed in the country, spreading to Bolivia and Peru and is frequently found in hills and mountains (e.g. voucher *Imig* 393, *Imig* 637 and *Imig* 378, collected on hill tops have proportionally smaller and darker colored flowers, and on the other hand, *Imig* 377 and *Klingelfuss* 157, collected in low places present larger flowers with and of lighter colors -see Tab. 01). This variation may possibly occur due to the isolation of populations since these inselbergs in which they live can act as barriers to the gene flow between populations (Barbará *et al.* 2007). *Dryadella wuerstley*, on the other hand, have a more restricted distribution than *D. zebrina* (only in South Brazil), and although the populations found are distant from each other and not very abundant, we observed less morphological variability among individuals when comparing with *D. zebrina*.

Although, determining the evolutionary causes of the morphological pattern found of these three morphotypes of *Dryadella* is beyond our scope, which will demand further population genetics studies and possibly cytologic and reproductive studies as well. Instead, we examined a great number of samples at the morphometric point of view, considering the particularities of the *Dryadella* (i.e. small populations that are hard to find in the field), in order to make better taxonomic decisions within the complex.

Our results showed the existence of two distinct morphological groups, therefore the acceptance of three autonomous taxa is not supported by our data. Instead, we propose the maintenance of *D. wuerstlei* and the synonymization of *D. edwallii* into *D. zebrina*. The great similarity between these two taxa was already pointed by Luer (2005), who differentiate them only by the lip size (3x3 mm in *D. edwallii* and 2x2 mm in *D. zebrina*) and the pattern of vinaceous spots in dorsal sepal (forming bars only in *D. zebrina*). However, based on our observations in acclimated individuals, this pattern of punctuations varies proportionally to sunlight exposure: the higher the exposure, the greater the number of vinaceous spots, which increases the likelihood that they will form bars. Besides, we sampled the same populations of *D. zebrina* and *D. edwallii* repeatedly between 2016–2021 and realised that the morphological variation is continuous within and among the populations. The analysis of the type of *D. zebrina* (published in 1905) and *D. edwallii* (published in 1906) also contributed to our decision to include them in a single taxon.

When describing *D. wuerstlei*, Luer (2005) highlights the smaller size of leaves, sepals, and petals in comparison to *D. zebrina*, as well as the fleshy leaves and lip with denticulate margins that differentiate these two species. Indeed, the ten variables that contributed most for the separation of the two morphological groups accounted for the size of dorsal sepals (SD1 and SD2), lateral sepals (SL10 and SL15), petals (PT19, PT21, PT22, and PT23), and lip (LB36 and LB38). With the addition of other qualitative characters, such as lip margin and leaf consistency, we can clearly circumscribe *D. wuerstlei* apart from *D. zebrina* and, hopefully, lessen the taxonomic confusion regarding these two species. For example, we can affirm that the illustration 1096 of *D. edwallii* (= *D. zebrina*) from Pabst and Dungs (1975, p.333), is actually a *D. wuerstlei*.

We provide a taxonomic note with the synonymisation of *D. edwallii* and taxonomic comments for *D. wuerstlei* and *D. zebrina*, as well as the identification key for the two species, photographs of specimens, and the updated distribution map. It is noteworthy that the type of *D. wuerstlei* have no defined locality (collection designated to Brazil), so the information about its current geographic range based on our extensive field work is of great taxonomic, ecological, and evolutionary importance. Complete taxonomic descriptions will be available in a broader taxonomic work, the flora of the *Dryadella* from Brazil (Imig *et al.*, in prep.).

Taxonomic conclusions

Key to taxa in the *Dryadella zebrina* complex

1. Plants with up to 3cm, semi-terete and caniculated leaves, flowers with strongly carinated sepals, pale green with diffuse dark purple punctuations and tail larger than blade size, lip with denticulated margins..***D. wuerstlei***
- 1'. Plants larger than 3cm, flat leaves, flowers with slightly carinated sepals, yellow green to dark yellow with diffuse dark purple punctuations and tail smaller than blade size, lip with entire margins.....***D. zebrina***

1. *Dryadella wuerstlei* Luer, Icones Pleurathallidinarum XXVII, 39, 2005.

Type: BRAZIL. Without collecting data purchased by B. Würstle from Ghillany, flowered in cultivation at Spielber, Germany, 23.V.1980, C. Luer 5269 (SEL!).

Taxonomic comments: Vegetatively *D. wuerstlei* is 2-3cm tall and densely caespitose, with leaves 15–25(30) × 2.5–3.3 mm, fleshy, elliptical, ridged, usually sub-erect, sessile. (Fig. 5a). The flowers of *D. wuerstlei* are proportionally large in relation to the vegetative and stand out from the leaves (Fig. 5a, b), sepals pale green, dorsal sepal 4.5–6.6 mm long, attenuating in a tail with 6–6.7mm long and narrow apex, the lateral sepals with 4.3–4.7(5) mm long, with attenuated apex, tailed with 5.2–6 (6.3)mm long and narrow apex, whose tail exceeds the size of the blade (Fig. 5a,c). The lip is obovate, light green in colour and dense with dark purple punctuations, margins minutely indented; blade 4.0–4.7 × 1.8–2.1 mm, claw 0.6–0.8 mm (Fig. 5d). Until the moment, the geographic distribution of *D. wuerstlei* was unknown, the type material comes from cultivation, and there was no specified origin. From our collections, we verified that it is probably endemic to the Brazilian Atlantic Forest, recorded here for the states of Santa Catarina and Rio Grande do Sul (Fig. 7).

Selected examined material: BRAZIL. RIO GRANDE DO SUL: Capão do Leão, 09 XI.1986, fl., J.A. Jarenkow 496 (FLOR). Iraí, 07.X.2016, fl., D.C. Imig 462 (UPCB); Pelotas, Distrito de Rincão da Cruz, Morro Quilongongo, 17.II.2008, fl., T. Perleberg 233 (ECT). São Francisco de Paula, 20.X.2009, fl., A.T. de Brito 2646 (UPCB); 17.XII.2008, fl., A.T. de Brito 2563 (UPCB). SANTA CATARINA: Calmon, Pinhelão, 08.III.2019, fl., A. Kassner-Filho et al. 5277 (FURB); Rio do Sul, 03.X.2015, fl., D.C. Imig, M. Bolson & J. Caetano 408 (UPCB). Idem, 23.XI.2016, fl., D.C. Imig 465 (UPCB); Vitor Meireles, Pratinha, 18.I.2012, fl., E. Caglioni et al. 132 (FURB).

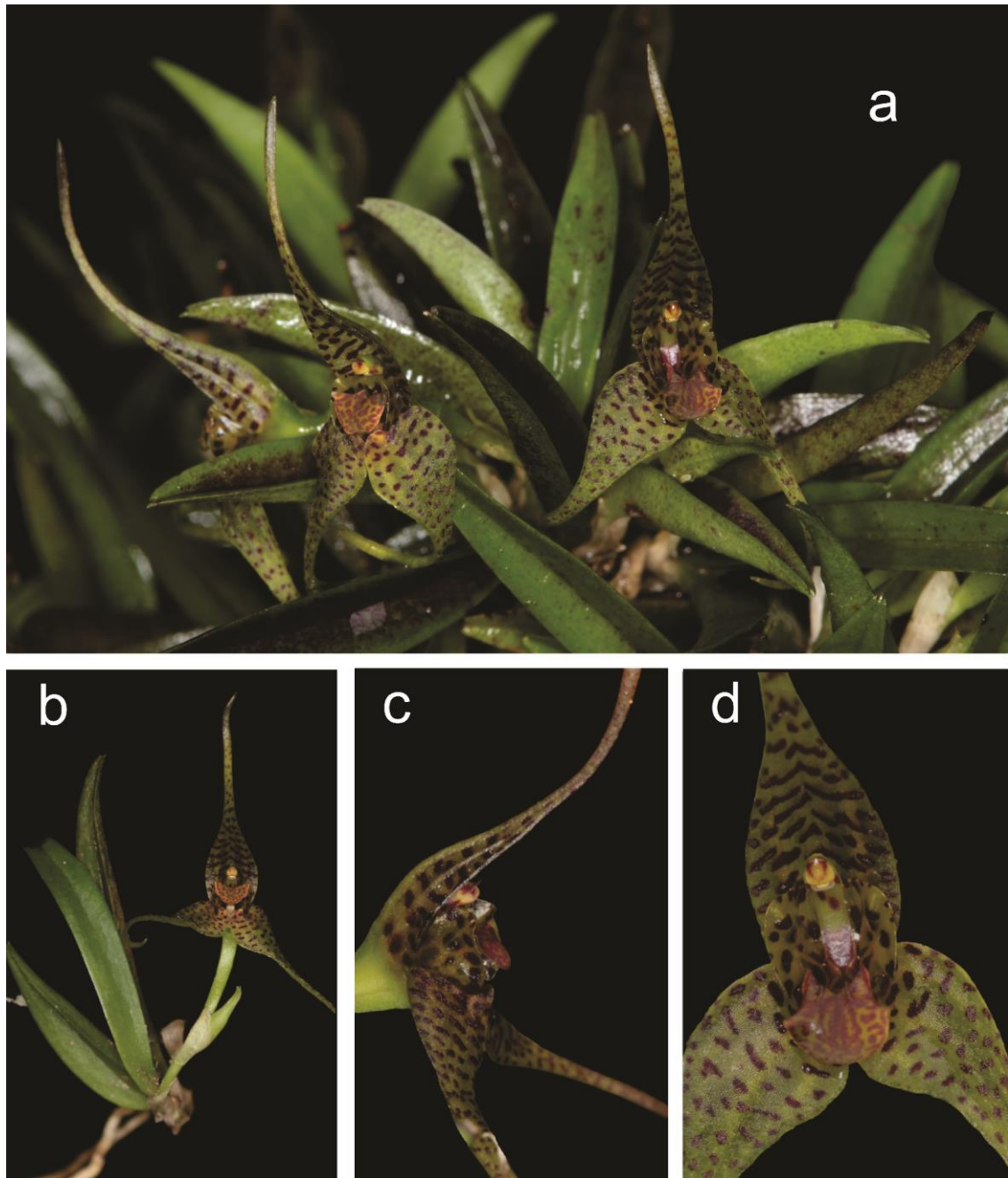


Figure 5 – a-d. *Dryadella wuerstlei* – a-b. habit; c. Side view of the flower; c. Front view of the flower. (a-d. D.C. Imig 462)

2. *Dryadella zebrina* (Porsch) Luer, Selbyana 2:209, 1978.

Type: BRAZIL. State unknown: Raiz da Serra, X.1901, *R. Wettstein & V.F. Schiffner* s.n. (destroyed at the B). **Neotype:** designated by Luer 2005: Rio de Janeiro, cultivated by orquidário Binot, 30. XI. 1989, *C. Luer 14500* (MO!).

Bas: *Masdevallia zebrina* Porsch, Oesterr. Bot. Z. 55:154, 1905.

Syn: *Masdevallia carinata* Cogn., Bull. Soc. Roy. Belgique 43:305, 1907.

Type: BRAZIL. Without locality, *A. Glaziov 17802* (BR997389!).

= *Dryadella edwallii* (Cogn.) Luer, Selbyana 2: 208, 1978. *syn nov.*

Type: BRAZIL. SÃO PAULO: Campos da Bocaina, (*Comm. Geogr. & Geol.* 2302), *G. Edwall 2302* (BR!, isotype K).

Taxonomic comments: *Dryadella zebrina* has high vegetative variability, even within the same population. Size can range from 40–85 (130) mm tall, with leaves ranging from dark green on both sides to fully purplish in the same population, leaves 40–65 (95) × 3.5–7 mm, elliptical to narrow-elliptical and flat and erect, petiolate. We noticed that in *D. zebrina*, the wide variation is repeated in the colour and characteristics of the flowers, ranging from translucent yellow to dark yellow, diffuse or densely spotted with dark purple, the spots more or less in transverse bars on the dorsal sepals, (4.5)6.5–8.5(9) mm long attenuating for a tail with (2.5) 5.5–7 (8.5) mm long and usually blunt apex, the lateral sepals with 6–7.5(8) mm long, attenuating for a (3)4.5–6.7(8) mm tail with a narrow apex, smaller in proportion to the blade (Fig. 6a–i). Lip yellow or densely mottled with dark purple, oblong to obovate, entire margins; blade 3–3.5 × 2.8–3.3 mm, claw 1.8–2.3 mm (Fig. 6d, e, f, j, k). *D. zebrina* has a wide geographic distribution, especially when compared to the other species of the genus. In Brazil it occurs in the Southern states (SC, RS and PR), Southeast (ES, SP, RJ and MG) and Bahia and is endemic to the Atlantic Forest in Brazil (Fig. 7). According to Luer (2005) it extends to La Paz, in Bolivia and Pomacochas, in Peru.

Selected examined material: BRAZIL. BAHIA: Camacã, 02.IX.2016, fl., *D.C. Imig 453* (UPCB); 18.IX.2016, fl., *D.C. Imig 455* (UPCB); 19.IX.2016, fl., *D.C. Imig 457* (UPCB). MINAS GERAIS: Camanducaia, Distrito de Monte Verde, Trilha da Pedra Redonda 28.VII.2017, fl., *D.C. Imig 484* (UPCB); 29.VII.2017, fl., *D.C. Imig 482* (UPCB). Santana do Riacho, Serra do Cipó, 18.X.2018, fl., *D.C. Imig 485* (UPCB). ESPÍRITO SANTO: Venda Nova do Imigrante, em cultivo no Orquidário Caliman, 21.XI.2015, fl., *A.T. de Brito 3478* (UPCB). PARANÁ: Guaratuba, Serra do Araçatuba,

Morro dos perdidos, 20.XI.1998, *E.P. Santos et al.* 641 (UPCB); 01.XII.1998, *G. Hatschbach et al.* 2639 (MBM); 27.XI.1998, fl., *E.P. Santos et al.* 661 (UPCB). Quatro Barras, Morro Sete, 17.XI.1994, fl., *O. Ribas et al.* 723 (MBM); Serra da Graciosa, 13.IV.2016, fl., *D.C. Imig* 406 (UPCB); 02.XII.2015, *D.C. Imig* 405 (UPCB); 22.XII.2016, fl., *D.C. Imig* 468 (UPCB); 28.XI.2014, fl., *A. T. de Brito et al.* 3311 (UPCB); 28.XI.2014, fl., *A. T. de Brito* 3453 (UPCB); 2.XII.2015, fl., *A. T. de Brito* 3312 (UPCB). Morretes, 19.X.2015, *D.C. Imig* 378 (UPCB); Cabeceiras do Arraial, 11.XI.1965, *G. Hatschbach* 13116 (V, US); Trilha da Estação Engenheiro, 19.X.2015, *D.C. Imig* 379 (UPCB); P.E. Marumbi, 11.X.1998, fl., *C. Giongo et al.* 64 (UPCB); Morro Facãozinho, 08.X.1998, fl., *C. Giongo* 75 (UPCB); Mãe Catira, 11.XI.2016, fl., *D.C. Imig* 463 (UPCB); 12.XII.1985, fl., *R. Kummrow* 2677 (MBM); XI.2010, fl., *E.C. Smidt* 1011 (UPCB); Serra da Prata, 23.XII.2008, fl., *C.T. Blum & J. Michelotti* 450 (UFC). Piraquara, Queimada, Morro Albino Souza, 27.XII.1947, fl., *G. Hatschbach* 819 (SP); Caiguava, 19.XI.1909, fl., *K. Dusén* 8987 (NY, V); Mananciais da Serra, XII.2005, fl., *M. Reginato* 636 (UPCB); X.2004, fl., *M. Reginato* 95 (UPCB); Morro do Canal, 30.X.2009, fl., *R. Kersten* 1387 (HUCP); P.E. Marumbi, 09.XI.2015, fl., *D.C. Imig* 389 (UPCB); Trilha do Olimpo, 09.XI.2015, fl., *D.C. Imig* 384 (UPCB); Trilha do Vigia, 03.XI.2017, fl., *T.F. Santos* 298 (UPCB); 06.XI.2017, fl., *T.F. Santos* 327 (UPCB); 27.XI.2003 fl., *J. Silva et al.* 3916 (MBM); Morro do Bruninho, 02.X.2017, fl., *D.C. Imig* 488 (UPCB); 10.XI. 2015, fl., *D.C. Imig* 393 (UPCB); Reservatório do Carvalho, 15.X.2015, fl., *D.C. Imig* 374 (UPCB); 15.X.2015, fl., *D.C. Imig* 376 (UPCB); Serra do Emboque; 03.XII.1970, fl., *G. Hatschbach* 25749 (MBM, K). São José dos Pinhais, Borda da Campo, 04.XII.2019, fl., *D.C. Imig, T. Imig & F. Imig* 654 (UPCB; CTBA); Morros Perdidos, 17.XI.2009, fl., *R. Kersten* 005 (HUCP); Nhandara Guaricana, 01.XI.2017, *D.C. Imig et al.* 493 (UPCB; CTBA); Nhandara Guaricana, 15.X.2015, fl., *D.C. Imig* 377 (UPCB); Rio Pequeno, 05.XI.1969, fl., *G. Hatschbach* 22850 (MBM). Tijucas do Sul, 15.IX.2015, fl., *D.C. Imig* 375 (UPCB); Represa Vossoroca, km160 09.XI.2017, fl., *M. klingelfuss* 157 (UPCB); 10.XI.2017, fl., *M. klingelfuss* 159 (UPCB); *M. klingelfuss* 160 (UPCB); *M. klingelfuss* 161 (UPCB); Serra de Papanduva, 15.XII.1997, fl., *O. Ribas et al.* 2121 (MBM). Telêmaco Borba, Monte Alegre, fl., 16.XI.1915, *P. Dusén* 13334 (K940348!). RIO GRANDE DO SUL: Canoas, 06.XI.2017, fl., *D.C. Imig* 483 (UPCB). Porto Alegre, Morro da Glória, 05.XI.1932 (SP50515). Torres, 07.IX.2020, fl., *D.C. Imig* 657 (UPCB); RIO DE

JANEIRO: Alto Macahé, 05.XI.1888, fl., A. *Glaziou* 17802 (K; BR; P493105!). Proximo a Petrópolis, 30.XI.1989, fl., C. *Luer* 14500 (Neotypus MO!). Angra Dos Reis, arredores do P. N. Serra da Bocaina, 02.IX.2016, fl., D.C. *Imig* 454 (UPCB). SANTA CATARINA: Angelina, 28.X.2009, fl., A. *Cadorin et al.*, 316 (FURB). Antônio Carlos, 09.XI.2009, fl., A. *Cadorin et al.*, 482 (FURB). Benedito Novo, Trilha da Cruz de Pedra, 13.IX. 2018, fl., L.A. *Funez* 7742 (FURB); Fragmento florestal ao lado da cachoeira Campo do Zinco, 22.VII.2012 fl., (FURB); Bom Retiro, 17.X.1973 fl., A. *Bresolin* 877 (FLOR); Campo Alegre, Rio dos Bugres, 26.X.2019, fl., P. *Schwirkowski & C.R. Hantschel* 3555 (FURB; FPS); Serra do Quiriri, 27.XII.2004, fl., O. *Ribas et al.* 6580 (MBM); Rio Manso, 02.II.2010, fl., T. *Cadorin et al.* 1182 (FURB); Corupá, 07.IX.2020, fl., D.C. *Imig* 659 (UPCB); Palmeiras II, 24.VIII.2010, fl., A. *Korte & A. Kniess* 4197 (FURB). Doutor Pedrinho, 25.V.2010, fl., T. *Cadorin et al.* 2423 (FURB). Florianópolis, Residencial dos Araças, 04.I.2010, Siqueira 210 (FLOR); 28.IX.1967, fl., R.M. *Klein & A. Bresolin* 7587 (FLOR); Morro do Ribeirão, 24.X.1967, fl., R.M. *Klein & A. Bresolin* 7609 (FLOR). Garuva, Serra do Quiriri, 24.XI.2010, fl., A. *Korte* 5101 (FURB). Governador Celso Ramos, 11.VIII.1971, R.M. *Klein* 9649 (FLOR); Vargem do Macário, 20.III.1972, A. *Bresolin* 527 (FLOR). Ilhota, Morro do Baú, 16.IX.2016, fl., K. *Gaglioli & N. Junckes* 501 (UPCB). Imaruí, Morro D'una, 18.X.1973, fl., A. *Bresolin* 900 (FLOR). Joinville, Cubatão, 06.VII.2010, fl., T. *Cadorin et al.*, 2879 (FURB); Serra Dona Francisca, 06.VI.2010, fl., T. *Cadorin et al.* 1975 (FURB); 10.XII.2009, fl., T. *Cadorin et al.*, 870 (FURB); Serra do Piraí, 18.X.2010, fl., A. *Korte & A. Kniess* 4090 (FURB); Serra Queimada, 03.IX.2010, fl., W. *Mancinelli & A. Mancinelli* 1301 (JOI); Morro do Tromba, 20.III.2006, fl., W. *Mancinelli* 557 (JOI). Lages, Encruzilhada, 30.X.1962, fl., R.M. *R. Reitz & R.M Klein* 13924 (MBM, US). Monte Castelo, Serra da Garganta, 17.IX.2010, fl., A. *Korte & A. Kniess* 4381 (FURB). Nova Trento, 9.IX.2017, fl., A. *Kassner-Filho et al.* 1031 (FURB); Monte Barão, RPPN Prima Luna, 16.VI.2006, T. *Cadorin et al.*, 2573 (FURB). Palhoça, Morro Cambirela, 20.VIII.1971, A. *Bresolin* 349 (FLOR); 20.VIII.1971, fl., A. *Bresolin* 342 (FLOR). Praia Grande, Morro em frente ao Canyon Itaimbezinho, Thilha dos porcos, 24.XII.2018, fl., D.C. *Imig & G.A. Mantovani* 637 (UPCB); 26.XII.2018, fl., D.C. *Imig* 644 (UPCB); Serra do Faxinal, 13. V.2010, fl., J.L. *Schmitt et al.* 2216 (FURB). São Bento do Sul, 08.X.2018, fl., D.C. *Imig* 632 (UPCB); fl., D.C. *Imig* 630 (UPCB); fl., D.C. *Imig* 632 (UPCB); fl., D.C. *Imig* 629 (UPCB). Ponte Alta, Morro do Funil, 06.X.2009, fl., J.L.

Schmitt et al. 160 (FURB). Rancho Queimado, BR 282, 10.X.1993, fl., *F.F. Neves* 82 (FLOR). Rio dos Cedros, 27.VII.2017, fl., *A. Kassner-Filho et al.* 604 (FURB). Rodeio, Eremitério Frei Egídio, 24.IX.2016, *L.A. Funez et al.* 5552 (FURB). São Martinho, 26.I.2010, fl., *J.L. Schmitt et al.* 995 (FURB). Vargem do Cedro/P. E. da Serra do Tabuleiro, 15.VI.2010, fl., *A. Verdi et al.* 5023 (FURB). São José, Serra da Boa Vista, 14.X.1962, fl., *R. Reitz & R.M. Klein* 10217 (FLOR, US); 24.X.1957, fl., *R. Reitz & R.M. Klein* 5397 (US). Santo Amaro da Imperatriz, 13.X.2006, *J.Z. Matos* 24 (FLOR). Taió, Passo Manso, 09.X.2009, fl., *T. Cadorin et al.* 201 (FURB); Gramado, 15.IX.2010, fl., *A. Korte & A. Kniess* 4347 (FURB). Treviso, Brasília, 24.XI.2009, *J.L. Schmitt et al.* 594 (FURB). Vidal Ramos, Águas Frias, 21.X.2009, *A. Korte & A. Kniess* 686 (FURB). SÃO PAULO: Bananal, 10.IX.2020, FL., *M. Klingelfuss* 309 (UPCB). Capão Bonito, 15.IX.2020, fl., *D.C. Imig* 658 (UPCB). São José do Barreiro, Campos da Bocaina, Invernada Pinhal, 26.III.1894, fl., *A. Loefgren & G. Edwall* 2302 (SP 29265!, K, BR657020! Typus *D. edwallii*). Serra do Mar Monte Alegre, fl., XI.1914, fl., *P. Dusén* 15842 (MO-1109578).

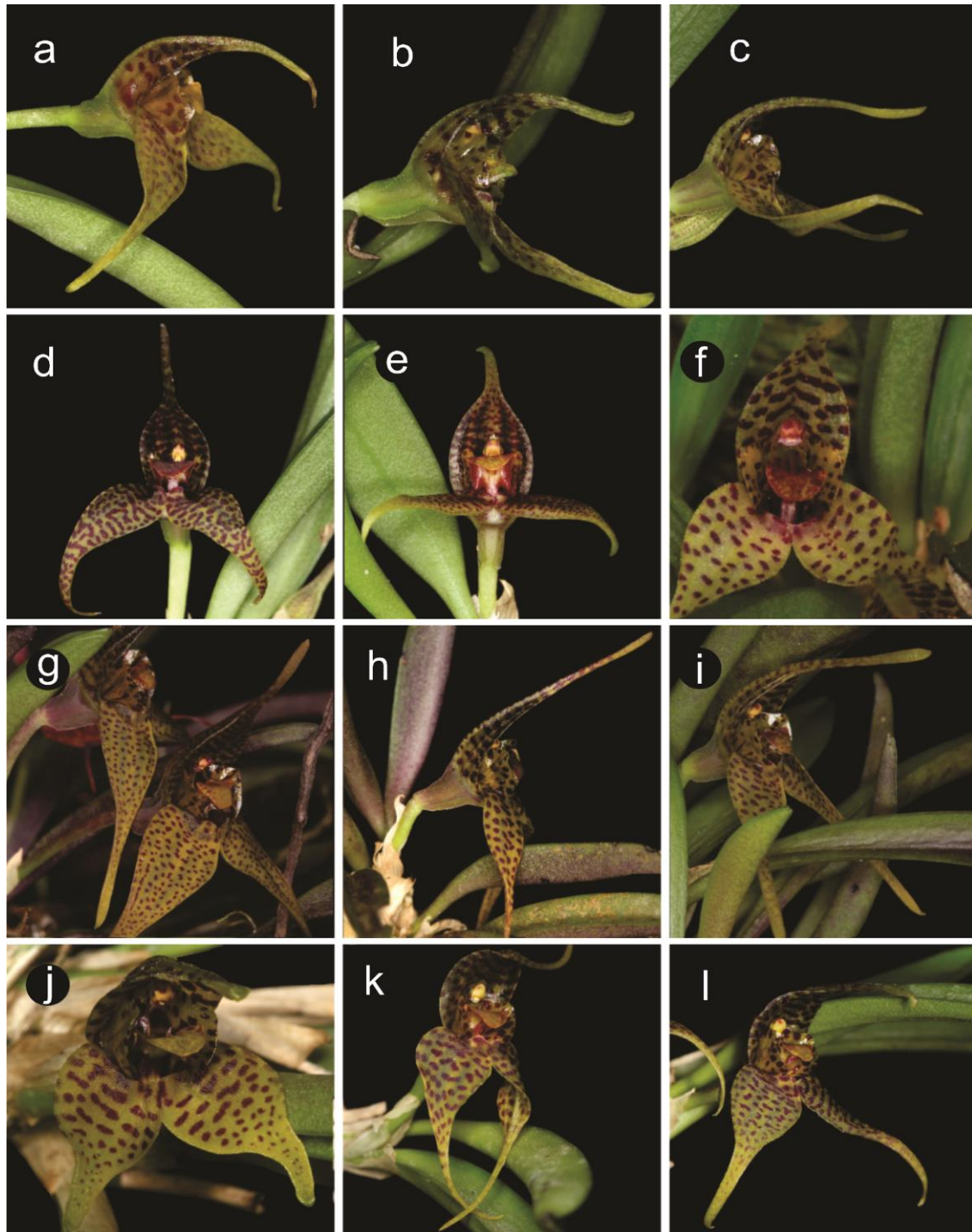


Figure 6 – a-l. *Dryadella zebrina*: vegetative and reproductive morphological intrapopulation variations. (a-c. *D.C. Imig* 362; d. *D.C. Imig* 374; e-f. *D.C. Imig* 393; g-i. *D.C. Imig* 632; j-l. *D.C. Imig* 405).

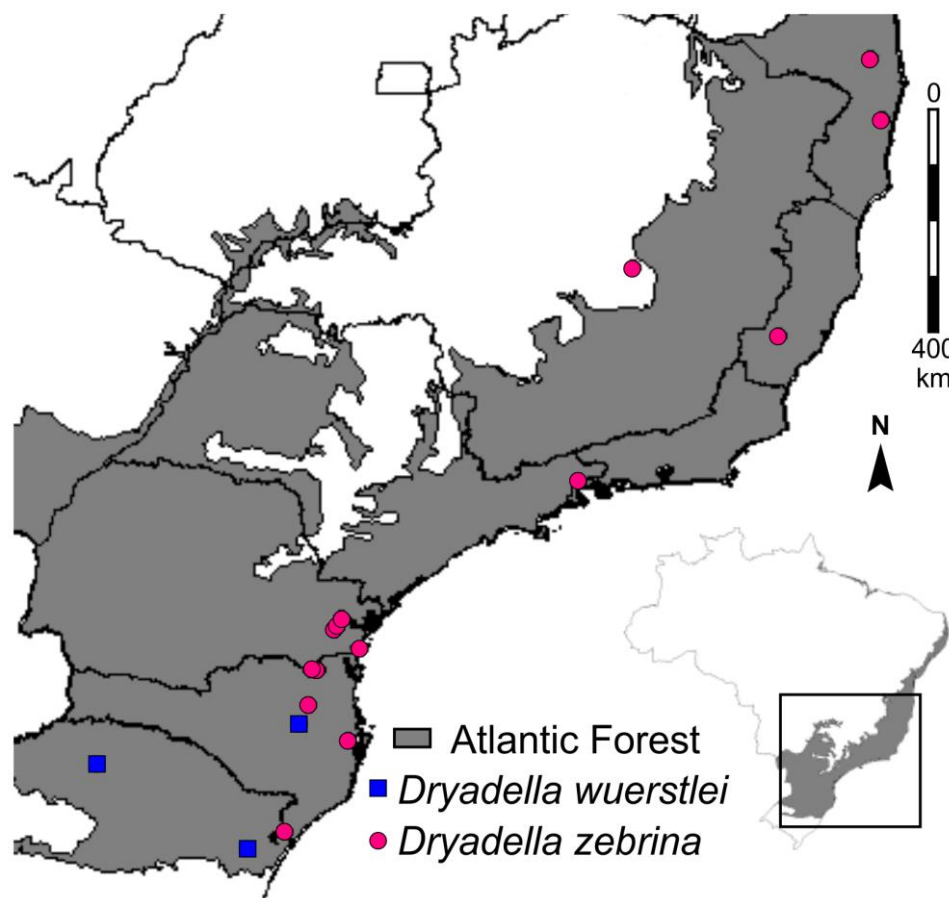


Figure 7 – Map of distribution of *D. zebrina* (red circle) and *D. wuestlei* (blue square) in the Brazilian Atlantic Rainforest (in grey).

Acknowledgements

We would like to thank Marcos Klingelfuss of Curitiba, who cultivated with a lot of expertise and passion our *Dryadella* samples. ECS thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research productivity award grant (Bolsa de Produtividade em Pesquisa - Nível 2, Proc. 308460/2017-0). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) with scholarships granted to DCI (168348/2017-9).

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Supplementary material

Taxonomic update in *Dryadella* (Orchidaceae: Pleurothallidinae) based on morphometric analyses of three species endemic to the Atlantic Rainforest

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Loading the packages:

```
library(vegan)
```

```
library(FactoMineR)
```

```
library(factoextra)
```

```
library(ggplot2)
```

```
library(devtools)
```

```
library(ggbiplot)
```

Loading the data:

```
morfo <- read.table("dadosmorfo_spp.txt", header = T)
```

```
morfo1 <- morfo[, -c(1,2)]
```

```
morfo.std <- decostand(morfo1, method = "standardize")
```

```
morfo.std
```

PCA Analysis

```
pca.morfo <- princomp(morfo.std)
```

```
summary(pca.morfo)
```

```
#Eigenvalues
```

```
ev <- pca.morfo$sdev^2
```

```
#% explanation
```

```
pov <- pca.morfo$sdev^2 / sum(pca.morfo$sdev^2)
```

```
evplot = function(ev) {
```

```
  # Broken stick model (MacArthur 1957)
```

```
  n = length(ev)
```

```
  bsm = data.frame(j = seq(1:n), p = 0)
```

```
  bsm$p[1] = 1/n
```

```
  for (i in 2:n) {
```

```

    bsm$p[i] = bsm$p[i-1] + (1/(n + 1 - i))
  }
  bsm$p = 100*bsm$p/n
  # Plot eigenvalues and % of variation for each axis
  barplot(t(cbind(100*pov, ev, bsm$p[n:1])), beside = T,
    main = "Principal Components validation",
    col = c("blue", "green", "red"), las = 3)
  legend("topright", c("% variation", "eigenvalue", "Broken stick model"),
    pch = 15, col = c("blue", "green", "red"), bty = "n")
}
evplot(ev) #Barplot with pov, ev, and broken stick for each principal component
#cos2 barplot, indicating the relative importance of each variable
cos2.plot <- fviz_cos2(pca.morfo, choice = "var", axes = 1:2)
#PCA biplot
pca.plot <- ggbiplot(pca.morfo, choices = c(1, 2), groups = morfo[,1],
  obs.scale = 1, var.scale = 1) +
  theme_classic()
#Agrupando os pontos por especie (groups = morfo[,1])
Dendrogram
#Calculating the distance matrix
morfo.dist <- vegdist(morfo.std, method = "gower")
morfo.dist
#Dendrogram
cl.morfo <- hclust(morfo.dist, method = "average")
plot(cl.morfo)
plot(cl.morfo, labels = morfo[,2], hang = -1)
#To validate the information generated by the dendrogram
cofresult <- cophenetic(cl.morfo)
cor(cofresult, morfo.dist)
PERMANOVA
#Among populations
permanova_pop <- adonis2(morfo.std ~ morfo[,2],
  method = "gower", permutations = 1000)

```

```
permanova_pop
#Among species
permanova_spp <- adonis2(morfo.std ~ morfo[,1],
                        method = "gower", permutations = 1000)
permanova_spp
#Among clusters
clusters <- c(rep("A", times = 123), (rep("B", times = 22)))
clusters
permanova_clusters <- adonis2(morfo.std ~ clusters,
                             method = "gower", permutations = 1000)
permanova_clusters
```

CAPÍTULO IV / CHAPTER IV***A new *Dryadella* Luer (Orchidaceae: Pleurothallidinae) from the South Atlantic Rainforest***

(Artigo publicado em 2021 na revista Phytotaxa 508 (2): 206–212 – *Phytotaxa* ISSN - 1179-3155 (versão impressa) / ISSN - 1179-3163 (versão eletrônica)). DOI: <https://doi.org/10.11646/phytotaxa.508.2.9>

A new *Dryadella* Luer (Orchidaceae: Pleurothallidinae) from the South Atlantic Rainforest

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DCI and WSM collected samples; DCI, ECS and WSM wrote the manuscript.

Abstract: *Dryadella catharinensis*, a new orchid species found in the north-northeast part of the Santa Catarina state, in southern Brazil, is described here. The tiny new species was found in the dense ombrophilous submontane forest (Floresta Ombrófila Densa Submontana) in the Atlantic Rainforest. It is similar to *Dryadella susanae*, which is endemic of the Espírito Santo state and differs in the larger vegetative size, the narrow and suberect leaf, yellow flowers, the sepals with slightly denticulate margins, the petals with an obtuse apex and the cuneate lip with denticulated margins and a annular callus in the median portion of the claw. It is the fifteenth *Dryadella* species restricted to the Atlantic Rainforest (~25% of the genus), evidencing the importance of the conservation of this biome — of which only 11% remains — for the genus. Due to the rarity and low density of this species in the field, allied to the degradation of the habitat, we suggest a conservation status of Endangered. Further fieldwork is needed to locate additional populations and study the biological aspects for their conservation.

Key-words: Conservation; *Dryadella catharinensis*; IUCN; Santa Catarina; Taxonomy

Introduction

The 60 or so species of the Neotropical genus *Dryadella* Luer (1978: 207) occur from Mexico to southern Brazil, with centers of diversity in the Andes and in the Atlantic Rainforest (Luer 2005; POWO 2020). *Dryadella* species are recognised among the Pleurothallidinae by having a thickened callus close to the base of the lateral sepals, multi-angled petals, and a long unguiculate lip with a blade provided with two basal, usually retrorsed, lobes (Luer 1978; Luer 2005; Pridgeon 2005). The taxonomy of the genus is complex as species are remarkably similar. Luer (2005) provided a determination key based on the length and width of the leaf. He stated that the morphology of the petals and lip is variable in many species, and the sepals often become the most reliable feature for identification.

In Brazil, ca. 16 species are currently known to occur; 12 are endemic to the country, and 14 species are restricted to the Atlantic Rainforest, mainly in the Southern Atlantic Forest/Paraná Forests and in the Northern Atlantic Forest super-bioregions (sensu Reginato & Michelangeli 2020). The Amazon (*D. osmariniana* (Braga) Garay & Dunst. (1979: 172) and the Cerrado (*D. ana-paulae* V.P.Castro, B.P.Faria & A.D.Santana (2004: 198) biomes register only one species each (BFG 2015; 2018; Imig *et al.* 2020). Three species have been reported for the Santa Catarina state in southern Brazil: *D. lilliputiana* (Cogn.) Luer (1978: 208), *D. zebrina* (Porsch) Luer (1978: 209) and *D. edwallii* (Cogn.) Luer (1978: 208) (Pabst & Dungs 1975; Luer 2005; BFG 2015; 2018; Imig *et al.* 2020).

Materials previously identified as *Dryadella aviceps* (Rchb.f.) Luer (1978: 208) (Mancinelli & Esemann-Quadros 2016) were carefully reexamined during the preparation of a monograph on Brazilian members of the genus. The analysis of these field collected, and herbarium specimens led to the discovery of an undescribed *Dryadella* species. We provide a formal description of the new species here with taxonomic and ecological comments, an assessment of the conservation status, photographic plates, and an ink drawing.

Materials and methods

The plant material was collected and herborised according to Fidalgo & Bononi (1989) and deposited at UPCB and MBM (acronym according to Thiers, 2020, continuously updated). The morphological terminology follows Stearn (2004), and the descriptions

follow Luer (1978, 2005). The figures and illustrations were edited using CorelDRAW X8 (<https://www.coreldraw.com/>). The conservation status was assessed using IUCN (2019) categories and criteria. The GeoCAT Tool (Bachman *et al.* 2011) was used to calculate the area of occupancy (AOO) with a default cell width of 2 km² and the extent of occurrence (EOO).

Taxonomy

Dryadella catharinensis* Imig, Mancinelli & E.C.Smidt *sp nov. (Figs. 1–2)

Type:—BRAZIL. Santa Catarina: Joinville, Morro do Tromba, 03 September 2009, [fl.], W.S. Mancinelli 1007 (holotype JOI).

Diagnosis:— The new species is morphologically similar to *Dryadella susanae* (Pabst 1976: 68) Luer (1978: 371) but can be distinguished by the larger vegetative size, the narrow and suberect leaf, the yellow flower, the sepals with slightly denticulate margins, the petals with an obtuse apex and cuneate lip, with denticulated margins and the presence of ring callus in the median portion of the claw.

Description:—Epiphytic **herb**, caespitose, minute, 16–23 mm tall. **Roots** thick, 2–3 at each rhizome node, 0.5–0.7 mm diameter. **Rhizome** 1–1.5 mm between the internodes. **Ramicaul** 1.5–2 mm long, suberect, covered by two paleaceous, deltoid sheaths, truncated at the base, obtuse, mucronate, entire, 0.8–1.2 × 0.6–0.8 mm. **Leaf** dark green on the adaxial side and vinaceous on the abaxial side, coriaceous, narrowly elliptic, 13–19.5 × 2.1–3.3 mm; petiole 1–1.5 mm long; blade suberect, retuse, entire. **Inflorescence** a successive, single-flowered, congested raceme; bearing two pale, imbricate, mucronate, entire basal bracts; peduncle 5.5–8.5 mm long; pedicel plus ovary light green with purple dots, slightly triolate, 0.8–1.3 mm long. **Flower** resupinate; dorsal sepal pale yellow, densely dark-purple spotted, ovate, inflexed, three-veined, carinate, 4.5–5.2 × 2–2.5 mm, base truncate, connate 0.5 mm with the lateral sepals forming a short sepaline cup, obtuse, entire; lateral sepals light pale yellow, with sparse light purple spots at base, ovate, three-veined, asymmetrical, 3.3–3.7 × 1.8–2.3 mm, connate at the truncate base, short caudate, margin slightly denticulate, subacute, transversal callus purple; petals dark-yellow, red-spotted, rhomboid, two-veined, asymmetrical, 1.0–1.4 × 1.3–1.5 mm, base truncate, obtuse, superior margin erect, lobe acute, slightly projected forward; inferior margin excavated, lobe acute; lip dark yellow, unguiculate;

claw 0.7–0.8 mm, bilobed at base, caniculate with a transversal annular callus in the median portion; blade cuneate, three-veined, $1.8\text{--}2.1 \times 1.5\text{--}1.6$ mm, base with two lateral, acute retrorse lobes, a pair of lamellar callus at the base; apex reflexed, obtuse, denticulated; column dark-yellow, red in the ventral margin, 1.5–1.7 mm long, curved, winged on the distal half, acute-toothed at winged base; apex minutely denticulate, foot equally long; anther red, apiculate; pollinarium not seen. **Fruit** not seen.

Additional material examined (paratypes): — BRAZIL. Santa Catarina: Corupá, Bairro Izabel, 01 September 2020, [fl.], *D.C. Imig* 656 (UPCB). Joinville, Serra Dona Francisca, next to Estrada Piraí, 29 August 2020, fl., *W.M. Mancinelli & D.C. Imig* 1591 (MBM).

Discussion:—The tiny *Dryadella catharinensis* is similar to *D. susanae*, they share the ovate lateral sepals with short caudate apex. The new species may be distinguished vegetatively in the larger size of the plant 16–23 mm (vs. 10–13.5 mm) and in the larger $13\text{--}19.5 \times 2.1\text{--}3.3$ mm (vs. $3\text{--}5 \times 1.5\text{--}3$ mm), narrowly elliptical, suberect and petiolate leaves (vs. leaves broadly elliptical, prostrate and sessile). Florally, *D. catharinensis* is distinguished by the yellow (vs. dark red) flower, the dorsal sepal 4.5–5.2 mm long (vs. 3.8–4.0 mm), ovate, obtuse and thin (vs. triangular-ovate, acute, thickened), the lateral sepals with the margin slightly denticulate (vs. margin entire), the 1.0–1.4 mm long petals (vs. petals 1.5 mm) and the larger lip blade $1.8\text{--}2.1 \times 1.5\text{--}1.6$ mm (vs. lip blade 1.5×1.4 mm), cuneate, with margins denticulated, and a ring callus in the median portion of the claw (vs. obovate, margins entire, without callosities in the claw).

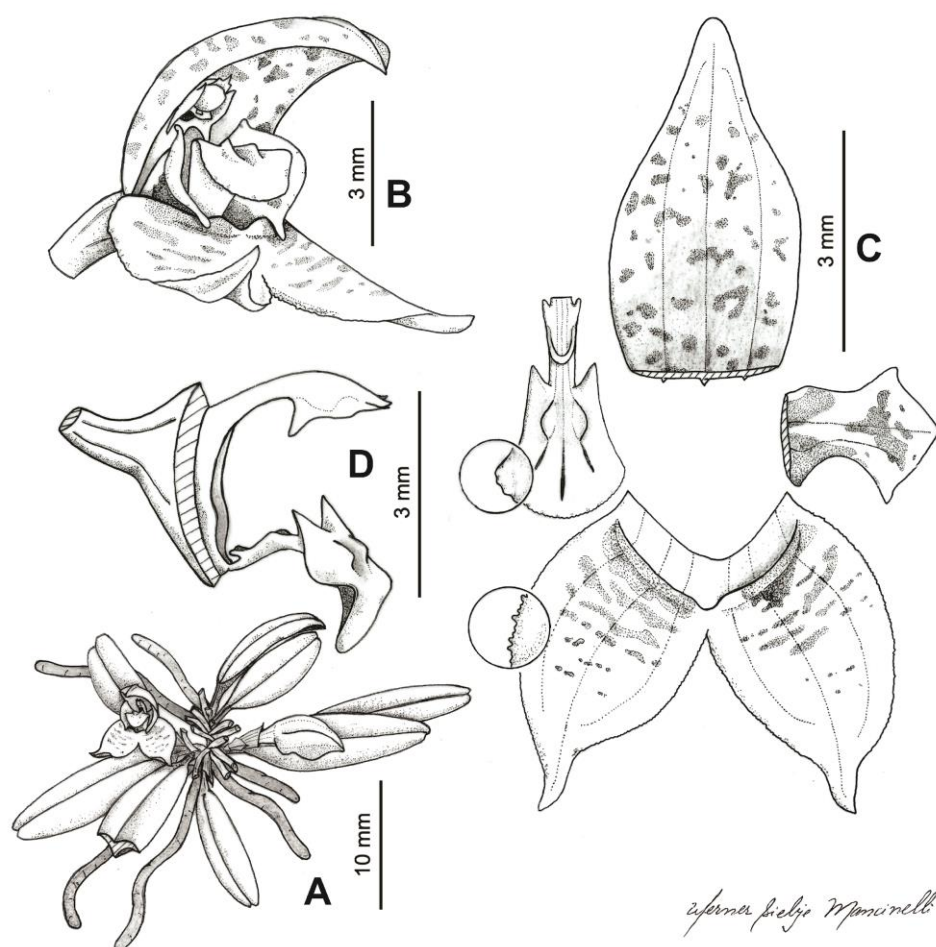


FIGURE 1.— *Dryadella catharinensis* Imig, Mancinelli & Smidt. — A. Habit. B. Flower. C, D. Detail of the flowers. Drawn by W. Mancinelli from W.S. Mancinelli 1007 (holotype JOI).



FIGURE 2.— *Dryadella catharinensis* Imig, Mancinelli & Smidt. — A. Habit. B. Detail of the flower in front view. C. Detail of the lip and column. D. Detail of the leaves and sheaths. Photos by E.C. Smidt from *D.C. Imig 656* (paratype UPCB).

In terms of geographic distribution, *D. susanae* is currently restricted to the state of Espírito Santo, Northern Atlantic Forest super-bioregion (Reginato & Michelangeli 2020) in the Serra do Mar Area of Endemism (Peres *et al.* 2020), where it is found growing at elevations between 800–1000 m alt. *Dryadella catharinensis* is restricted to the Santa Catarina state, Southern Atlantic Forest/Paraná Forest super-bioregion (Reginato & Michelangeli 2020), in the Paraná / Araucária Area of Endemism (Peres *et al.* 2020) at 200–300 m alt. (see Fig. 3).

Dryadella catharinensis was misidentified as *D. aviceps* in Mancinelli & Esemann-Quadros (2016) despite not being morphologically similar. *Dryadella aviceps* has its southern distribution limit west of the state of Paraná (Foz do Iguaçu and Guaíra) and extends to the region of the Ybycuí National Park, in Minas Kue, Paraguay. There is no record of *D. aviceps* in the Santa Catarina state (Luer 2005; CRIA 2016, Imig *et al.* 2020). In southern Brazil, three other species of *Dryadella* have been registered. *Dryadella lilliputiana* can be easily recognized by the terete, rather than flat, leaves. *Dryadella catharinensis* differs from *D. edwallii* and *D. zebrina* in the smaller plants (< 3 cm); *D. edwallii* has narrowly elliptic, green leaves with purplish nuances and a dorsal sepal with a clavate tail and *D. zebrina* has elliptical, green leaves and a dorsal sepal with a slender tail.

Distribution, habitat and phenology:—*Dryadella catharinensis* was found in Joinville and Corupá, north-northeast of the Santa Catarina state, at an elevation between 200–300 m in the Dense Ombrophilous submontane rainforest. In Joinville, it occurs in the Morro da Tromba (type locality) and Morro Pelado, both within the Serra Dona Francisca Environmental Protection Area (APA) with an extension of 401.77 km². Few individuals were found at both sites, growing at 2–3 m from the ground next to bifurcations of the central stem of the phorophyte. Flowering was registered from August to October.

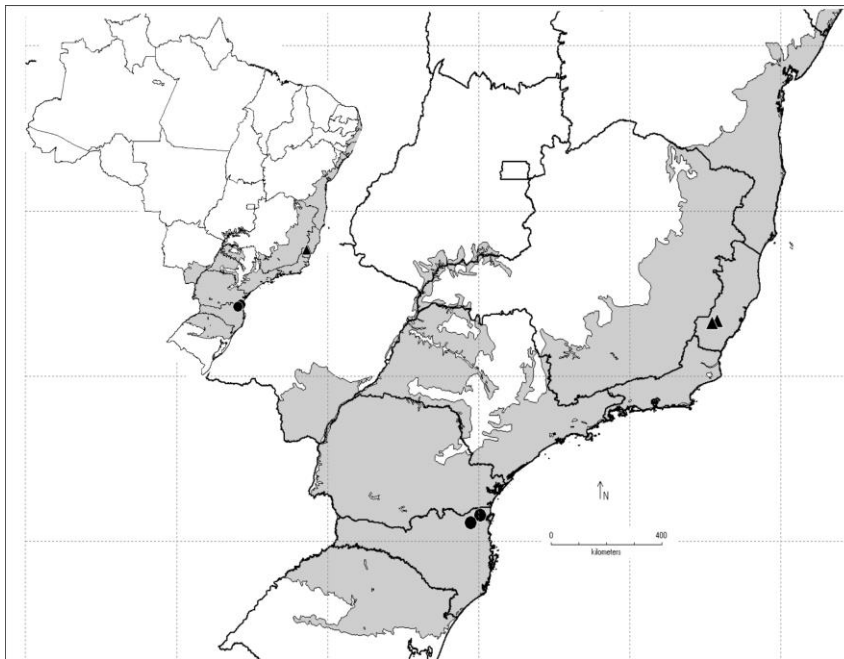


FIGURE 3.— Distribution map of *Dryadella catharinensis* (circles) and *D. susanae* (triangles).

Etymology:—Named after Santa Catarina State, where the new species was collected.

Conservation status:—According to the Categories and Criteria of IUCN Red List (2019), *Dryadella catharinensis* should be assigned a conservation status of “Endangered” [EN B1ab(i,iii)+2ab(ii,iii)]. The geographic range where the new species was found is in a fragmented area of the Atlantic Rainforest, north-northeast of the Santa Catarina state. The two localities (three collection points) are in the same habitat, at a distance of only ca. 40 km. The Extent of Occurrence (EOO) is estimated to be 66,7 km², whereas its Area of Occupancy (AOO) is 8 km². In several excursions to the type locality and surrounding areas, very few specimens (>10) were located in a large area, indicating a low density of the known populations. Recently, the epiphytic flora of Santa Catarina State was inventoried (Vibrans *et al.* 2013), and this species was not sampled, which evidences its rarity. *Dryadella catharinensis* is under numerous threats, including deforestation, overgrazing, habitat fragmentation, random cutting, human interference, and climate change. The northern region of the State of Santa Catarina is under anthropic pressure, with the replacement of native vegetation by invasive species of *Pinus* spp. and *Eucalyptus* spp., which degrades the quality of the adjacent forest. Recent problems with fires arising from human activities have increased, including in

the Morro da Tromba and the Morro Pelado, in Joinville, causing severe environmental damage to the type population habitat.

Further fieldwork is needed to locate additional populations and increase the extent of occurrence and occupation area. The study of floral biology, the phorophyte preference, and mycorrhizal associations are also needed to conserve this tiny, rare orchid.

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CAPÍTULO V / CHAPTER V**Anatomia vegetativa e sua importância sistemática na *Dryadella* Luer
(Orchidaceae: Pleurothallidinae)**

(Artigo publicado em 2020 na revista Feddes Repertorium, 131, 175–187- Feddes Repertorium 2020, ISSN on-line: 1522-239X). DOI:10.1002/fedr.202000010

Vegetative anatomy and its systematic significance in the *Dryadella* Luer (Orchidaceae: Pleurothallidinae)

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Running title: Anatomical investigations in *Dryadella*

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Abstract

Dryadella comprises 54 neotropical species with disjunct distribution in the Andean region and the Atlantic Rainforest. This work investigated the vegetative anatomy of 13 species of *Dryadella*, and *Masdevallia infracta* selected as an outgroup, to search for distinctive characteristics of the genus, characters to differentiate disjunct Atlantic Rainforest and the Andean groups, and the traditional infrageneric classification, based mainly in vegetative characters. Twenty-seven anatomical vegetative characters were analysed in light microscope-LM and scanning electron microscopy-SEM, and evaluated by Cluster and Principal Coordinates Analysis - PCoA. Results from statistical and Cluster analysis exhibited morpho-anatomical similarity among *Dryadella* species. PCoA indicated separation between *Dryadella ataleiensis* and *D. pusiola* from other species with average support by silhouette analysis. The two most relevant characteristics to distinct clusters were the shape of the ramicaul in cross section, and the ornamentation of leaf cuticle in adaxial surface. Statistical analyses indicated that endemic groups of the Atlantic Rainforest and the Andean region do not present morpho-anatomical differences, and infrageneric sections are not supported by the characters investigated. This similarity may be related to the recent origin and rapid diversification of species, as shown in previous studies on Pleurothallidinae. Therefore, the morpho-anatomical characters observed here are stable and may be useful for taxonomy and genus characterisation.

Keywords: Andes; Atlantic Rainforest; Andes; Cluster; disjunct distribution; Neotropical orchids; PCoA.

Introduction

Epiphytic orchids have morphological and functional adaptations to prevent desiccation, such as multiseriate epidermis in the roots (velamen; Carlswald *et al.* 2006; Dressler 1981). In addition to absorbing water and mineral salts, the velamen also reduces transpiration and provides mechanical and UV light protection (Dressler 1981; Pridgeon 1982; Benzing 1982; Carlswald *et al.* 2006; Chomicki *et al.* 2014). Therefore, from a comparative approach, studies of vegetative anatomy in Orchidaceae may highlight ecological, and habitat adaptation issues as well as assist in identifying difficult-to-delimitate taxa (Pridgeon 1982; Neyland *et al.* 1995; Carlswald and Stern 2009; Smidt *et al.* 2013; Piazza *et al.* 2015; Kowsalya *et al.* 2017; Almeida *et al.* 2019).

The subtribe Pleurothallidinae (Epidendroideae, Epidendreae) represents approximately 20% of species richness of Orchidaceae, comprising 44 genera and ca. 5,100 species of exclusively Neotropical distribution (Pridgeon *et al.* 2005; Higgins and Williams 2009; Karremans 2016). General anatomical studies for the subtribe were focused on roots and leaves because they have characteristics that may be determinant for genera identification, contributing to the knowledge of phylogenetic relationships in the subtribe (Pridgeon 1982; Neyland *et al.* 1995; Almeida *et al.* 2019).

Pleurothallidinae is a monophyletic group, probably composed by nine clades (Pridgeon *et al.* 2001; Karremans 2016). The *Specklinia* clade (Sp) encompasses the genera *Acostaea* Schltr., *Dryadella* Luer, *Platystele* Schltr., *Pleurothallis* R. Br., and *Scaphosepalum* Pfitzer. (Karremans 2016), being *Dryadella* the early divergente lineage and focus of this study. The genus is characterised by the presence of a callus near the base of the lateral sepals and the unguiculated lip with retroverted basal lobules. These were the main characteristics used by Luer (1978) to propose the genus from some species that belonged to the genus *Masdevallia* Ruiz & Pav.. Currently, *Dryadella* comprises 58 accepted names, whose species inhabit tropical and subtropical forests from Mexico to southern Brazil (Pridgeon 2005; POWO 2020).

In South America, the distribution of *Dryadella* species is disjunct between the Andean region and the Atlantic Rainforest, except for *D. ana-paulae* V.P. Castro, B.P. Faria & A. D. Santana, found in gallery forests of the South American Dry Diagonal, and *D. osmariniana* (Braga) Garay & Dunst., which occurs in the Amazon Rainforest (Pridgeon 2005; Imig *et al.* 2020 *in construction*). The Atlantic Rainforest hosts 14 species of the genus, of which only two are not endemic to the biome: *D. zebrina* (Porsch) Luer, and *D. lilliputiana* (Cogn.) Luer, that also occur in the Andes, where

other South American species are restricted (Pridgeon 2005; Luer 1978, 2005; Chase *et al.* 2015; Imig *et al.* 2019).

Despite the wide geographical distribution of *Dryadella*, species of the genus are morphologically very similar. Luer (2005) reported subtle differences in size and shape of petals, sepals, and lip, as well in size of the leaves in his taxonomic review of *Dryadella*. Based on leaf morphology, the author suggested a possible classification of *Dryadella* species in three informal sections: “The Needle Leaves”, “The Tiny Mites” and “All The Rest” (Luer 2005). In “Needle Leaves” group are the species with narrow linear leaves less than 3 mm wide; in “The Tiny Mites” group are the small species with leaves less than 25 mm long; and in the “All The Rest” group are all species that have leaves more than 3 mm wide and 25 mm long (Luer 2005) (Fig. 01).

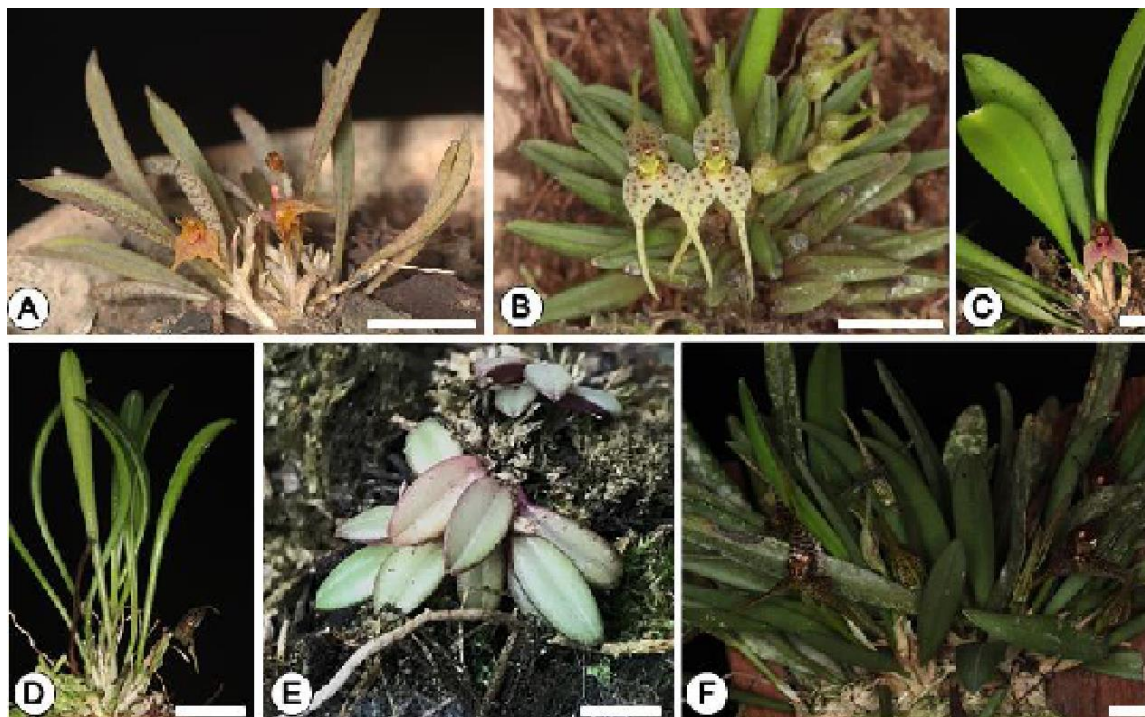


Figure 1. Main vegetative morphology types of the species. (A). *Dryadella ataleiensis* (The Needle Leaves). (B). *D. lilliputiana* (The Tiny Mites). (C). *D. litoralis* (All the Rest). (D). *D. summersii* (All the Rest). (E). *D. Susanae* (The Tiny Mites). (F). *D. zebrina* (All the Rest). Scale bars = 1 cm.

Pridgeon (1982) in the study on Pleurothallidinae, sampled species of *Dryadella* and declared that this genus has some anatomical differences in relation to *Masdevallia*, such as epidermal cells with thickened walls and loss of leaf idiosblasts in some species.

Furthermore, the velamen of *Dryadella* roots is uniseriate, while in *Masdevallia* it is biseriate or multiseriate.

In the absence of a comparative anatomical approach, specify to the genus with wide sampling along the distribution of *Dryadella*, that is, the main objective of this study will be to carry out a descriptive comparative morpho-anatomical investigation in *Dryadella* species, focusing on the Andean and Atlantic Forest groups and verifying as vegetative anatomy it is congruent with Luer (2005) infrageneric classification of the genus.

The following hypotheses were tested: 1) there are morphoanatomical differences between the Andean and Atlantic Rainforest species, given the disjunct distribution of the genus in South America. It is expected that different adaptations to these two distinct environments have led to some degree of anatomical differentiation between the endemic species of each region. 2) The anatomical characters investigated in this study may support the sections proposed by Luer, which are based on vegetative morphological characteristics. To test these hypotheses, data were submitted to cluster analysis and principal coordinate analysis (PCoA), as well as permutational multivariate analysis of variance (PERMANOVA), expecting enough variation on characters of the vegetative organs to discriminate groups in the genus.

2. Materials and Methods

2.1. Taxon sampling

Thirteen species of *Dryadella* were sampled in this work, of which four are endemic representatives of the Atlantic Rainforest (*D. vitorinoi*, *D. aviceps*, *D. susanae*, *D. litoralis*, *D. ataleiensis*), six are endemic of Andes region (*D. cristata*, *D. pachyrhiza*, *D. pusiola*, *D. simula*, *D. summersii*, *D. hirtzi*), and two occur in both biomes: *D. lilliputiana* and *D. zebrina*. For anatomical comparisons and descriptions, we used *Masdevallia infracta* as an outgroup, because it represents the genus from which *Dryadella* was dismembered. According to infrageneric sections proposed by Luer (2005), *D. ataleiensis* Campacci, *D. pachyrhiza* Luer & Hirtz and *D. pusiola* (Rchb.f.) Luer represent the section “The Needle Leaves” (*D. ataleiensis* was added to this group by the researchers based on morphology), *D. susanae* (Pabst) Luer and *D. lilliputiana* belong to “The Tiny Mites”, and the remaining eight species are from the “All the

Rest”. One to three individuals were selected for each species, whose information of voucher and collection locality is available in Table 1, for each image used in the figures the respective voucher is mentioned in the caption between parentheses.

Table 1. List of species examined. UPCB: Herbário do Departamento de Botânica da Universidade Federal do Paraná. AF: Atlantic Rainforest, AR: Andean Region.

Táxon	Voucher (Herbário)	Locality
<i>Dryadella ataleiensis</i> Campacci	D.C.Imig 471 (UPCB)	Brasil, Espírito Santo, Venda Nova do Imigrante (AF)
<i>D. aviceps</i> (Rchb.f.) Luer	D.C.Imig 475 (UPCB)	Brasil, São Paulo, Campos do Jordão (AF)
<i>D. cristata</i> Luer & R.Escobar	D.C.Imig 399 (UPCB)	Colômbia, Vale del Cauca-Cali (AR)
<i>D. cristata</i>	D.C.Imig 640 (UPCB)	Ecuagenera Cia. Ltda., Equador (AR)
<i>D. hirtzii</i> Luer	D.C.Imig 505 (UPCB)	Ecuagenera Cia. Ltda., Equador (AR)
<i>D. lilliputiana</i> (Cogn.) Luer	D.C.Imig 467 (UPCB)	Brasil, Paraná, Piraquara (AF and AR)
<i>D. lilliputiana</i>	D.C.Imig 395 (UPCB)	Brasil, Bahia, Camacã (AF)
<i>D. lilliputiana</i>	M. Bolson 559 (UPCB)	Brasil, Rio de Janeiro (AF)
<i>D. litoralis</i> Campacci	D.C.Imig 354 (UPCB)	Brasil, São Paulo, Caraguatatuba (AF)
<i>D. litoralis</i>	D.C.Imig 476 (UPCB)	Brasil, Espírito Santo (AF)
<i>D. pachyrhiza</i> Luer & Hirtz	D.C.Imig 507 (UPCB)	Ecuagenera Cia. Ltda., Equador (AR)
<i>D. pusiola</i> (Rchb.f.) Luer	D.C.Imig 360 (UPCB)	Ecuagenera Cia. Ltda., Northwestern South America (AR)
<i>D. simula</i> (Rchb.f.) Luer	D.C.Imig 383 (UPCB)	Ecuagenera Cia. Ltda., Northwestern South America (AR)
<i>D. simula</i>	D.C.Imig 618 (UPCB)	Ecuagenera Cia. Ltda., Northwestern South America (AR)
<i>D. simula</i>	D.C.Imig 501 (UPCB)	Equador, Guayaquil (AR)
<i>D. summersii</i> (L.O.Williams) Luer	D.C.Imig 481 (UPCB)	Ecuagenera Cia. Ltda., Equador (AR)
<i>D. susanae</i> (Pabst) Luer	D.C.Imig 491 (UPCB)	Brasil, Espírito Santo (AF)
<i>D. vitorinoi</i> Luer & Toscano	D.C.Imig 506 (UPCB)	Brasil, Minas Gerais, Boa Esperança (AF)
<i>D. zebrina</i> (Porsch) Luer	D.C.Imig 405 (UPCB)	Brasil, Paraná, Quatro Barras (AF)
<i>D. zebrina</i>	D.C.Imig 408 (UPCB)	Brasil, Santa Catarina, Rio do Sul (AF, AR)
<i>D. zebrina</i>	D.C.Imig 456 (UPCB)	Brasil, Bahia, Camacã (AF)
<i>Masdevallia infracta</i> Lindl.	D.C.Imig 387 (UPCB)	Brasil, Minas Gerais, Serra do Cipó (AF)
<i>M. infracta</i>	D.C.Imig 496 (UPCB)	Brasil, Espírito Santo, Alfredo Chaves (AF)

2.2. Light microscopy-LM and scanning electron microscopy-SEM

Samples were obtained from 2 to 3 cm from the root apex, the middle third of the ramicaul and the middle portion of the fully developed leaf blade (third youngest leaf), and were fixed in FAA 50 (Johansen 1940). The samples were sectioned by hand using a razor blade, stained with Astra blue and safranin (Bukatsch 1972) and mounted in glycerol gelatin (Kaiser 1880). Histochemical tests were performed for identification of starch using Lugol solution and potassium iodide (Johansen 1940), lignin with acidic phloroglucinol (Foster 1949) and lipids using Sudan III (Sass 1951). The dissociation of the leaf epidermis was performed using a 1:1 solution of glacial acetic acid and hydrogen peroxide at 60°C for 12 hours (Franklin 1945) and stained with 50% alcoholic safranin (Berlyn and Miksche 1976) for observation of the stomata type, stomata distribution and epidermis cells. The autofluorescence of wall thickening of tracheoidal idioblasts was recorded by fluorescence microscope Olympus BX51 (Liu *et al.* 1994) using RFP or Texas red filters. Roots and leaves fixed in FAA 50 were dehydrated in ethyl alcohol series, dried to the critical point and coated with gold (Robards 1978). Electron micrographs were produced using a TESCAN VEGA3 LMU scanning electron microscope.

2.3. Terminology

The description of the epicuticular wax deposition patterns followed Barthlott *et al.* (1998), and cell wall thickness classification followed IAWA Committee (1989). The tilosomes were classified using the terminology used by Pridgeon *et al.* (1982).

2.4. Statistical analysis

The data obtained with Light microscopy-LM and scanning electron microscopy- SEM observations were categorised and coded to build a morpho-anatomical matrix. Fourteen from 23 macro and micro-morphological characters analysed are binary, and nine multistate (Table 2). Paired distance between species was calculated using the Gower coefficient (1971), and the resulting distance matrix was used in all subsequent statistical analyses developed in the software R v.3.6.0 (R Core Team 2019). The scripts are available in the supplementary material.

A dendrogram was generated by Unweighted Pair Group Method with Arithmetic Mean (UPGMA; - Sokal and Michener 1958) to access the robustness of the infrageneric

sections and the disjunct groups in relation to the anatomical characters, whose clusters were validated by the silhouette method (Rousseeuw 1987). Principal Coordinates Analysis (PCoA) was also performed to identify relevant characters in the clusters formation. Species were represented by icons and connected according to their clusters using the ordcluster function (vegan v.2.4-2), with colours being the same for UPGMA and PCoA for easy viewing. The cluster analysis graph was edited using the factoextra v.1.0.7 package, and minor visual edits in the dendrogram and PCoA were made in CorelDraw v.18.1.0.661 (<https://www.coreldraw.com/>). Original graphics are available in the supplementary material.

The morpho-anatomical difference between the groups referring to the infrageneric sections ("The Needle Leaves" x "The Tiny Mites" x "All The Rest") and the geographic regions (Andes x Atlantic Rainforest) was tested by permutational multivariate analysis of variance (PERMANOVA;- Anderson, 2001). For the geographic region test, *Dryadella* species occurring in both biomes were disregarded.

Table 2. Categorical macro and micro-morphological descriptors used in the statistical analyses.

Character number	Character code	Character: character states
1	C2R	Root, cortex, cell layers (excluding exodermis), number: (0) 5, (1) 6-7, (2) 8-9, (3) 10.
2	C3R	Root, endodermis, passage cells, number: (0) 2, (1) 3, (2) 4.
3	C4R	Root, protoxylem, poles, number: (0) 3-5, (1) 6-11, (2) 12-13.
4	C5R	Root, calcium oxalate crystals (rafids): (0) absent, (1) present.
5	C1RM	Ramical, shape in cross-section: (0) round, (1) sulcate.
6	C2RM	Ramical, epidermis, cells, shape: (0) elliptic, (1) circular.
7	C3RM	Ramical, cortex, cell layers, number: (0) 4-5, (1) 6-8, (2) 10-13.
8	C4RM	Ramical, vascular bundles, number: (0) 3, (1) 6-9, (2) 10-13, (3) 14-16.
9	C5RM	Ramical, vascular bundles, structure: (0) bundles have same caliber, (1) bundles have different calibers.
10	C7RM	Ramical, length (mm): (0) 3-7, (1) 8-15, (2) 30.
11	C1L	Leaf, shape in cross-section: (0) flat or semi-flat, (1) circular-sulcate.
12	C2L	Leaf, cuticle, wax deposition pattern: (0) granular, (1) in platelets.
13	C3L	Leaf, adaxial surface, cuticle, ornamentation: (0) verrucous, (1) smooth.
14	C4L	Leaf, abaxial surface, cuticle, ornamentation: (0) verrucous, (1) smooth.

15	C5L	Leaf, mesophyll, cell layers, number: (0) 9–11, (1) 10–13.
16	C6L	Leaf: aquiferous parenchyma, cell organization: (0) 2 layers in adaxial surface, (1) 3 layers in adaxial surface, (2) dispersed on mesophyll.
17	C7L	Leaf, mesophyll, starch grains: (0) absent, (1) present.
18	C8L	Leaf, mesophyll, tracheoidal idioblasts with helicoidal thickening: (0) absent, (1) present.
19	C9L	Leaf, mesophyll, crystal rafids: (0) absent, (1) present.
20	C10L	Leaf, mesophyll, vascular bundles, location: (0) close to abaxial surface, (1) central.
21	C11L	Leaf, trichome scars: (0) absent, (1) present.
22	C12L	Leaf, length (mm): (0) 5, (1) 24–50, (2) 60–90, (3) 95–110.
23	C13L	Leaf, width (mm): (0) 2–3, (1) 4–6, (2) 13, (3) 16.

3. Results

3.1. Anatomical characters

3.1.1. Root

Dryadella species presented a bi-layered velamen (Fig. 2A–C), epi-velamen with U-shaped wall thickening (Fig 2B–C), endovelamen with elongated cells radially (Fig 2B) or tangentially (Fig 2C). *Masdevallia infracta* presented velamen with four or five cell layers, epi-velamen with perforated cells (Fig 2D). Tilosomes are spongy type in *Dryadella* (Fig 2F–H) and lamellar in *Masdevalia* (Fig 2I). All species analysed have exodermis cells lignified with U-shaped wall thickening (Fig 2C, E), the cortex composed by five to ten cell layers of parenchyma (Fig 2A, J, K), and with starch grains (Fig 2K). *D. pachyrhiza*, *D. simula*, *D. vitorinoi* (Fig 2L) and *M. infracta* present raphides in the cortex. Tracheoidal idioblasts were not observed (Fig. 2M). The endodermis is lignified and presents O-shaped wall thickening with thin or thin-to-thick walls (Fig 2N–P). Passage cells in the endodermis appear in groups of two to four (Fig 2N). The pith present lignified cells with very thick walls (Fig 2M–P). The number of protoxylem poles varies from three to five in *D. hirtzii*, *D. susanae*, *D. pachyrhiza*, *D. cristata* and *D. zebrina* (Fig. 2F, Q), 11 to 13 in *D. vitorinoii*, *D. simula* (Fig. 2P) and *M. infracta* (Fig. 2R).

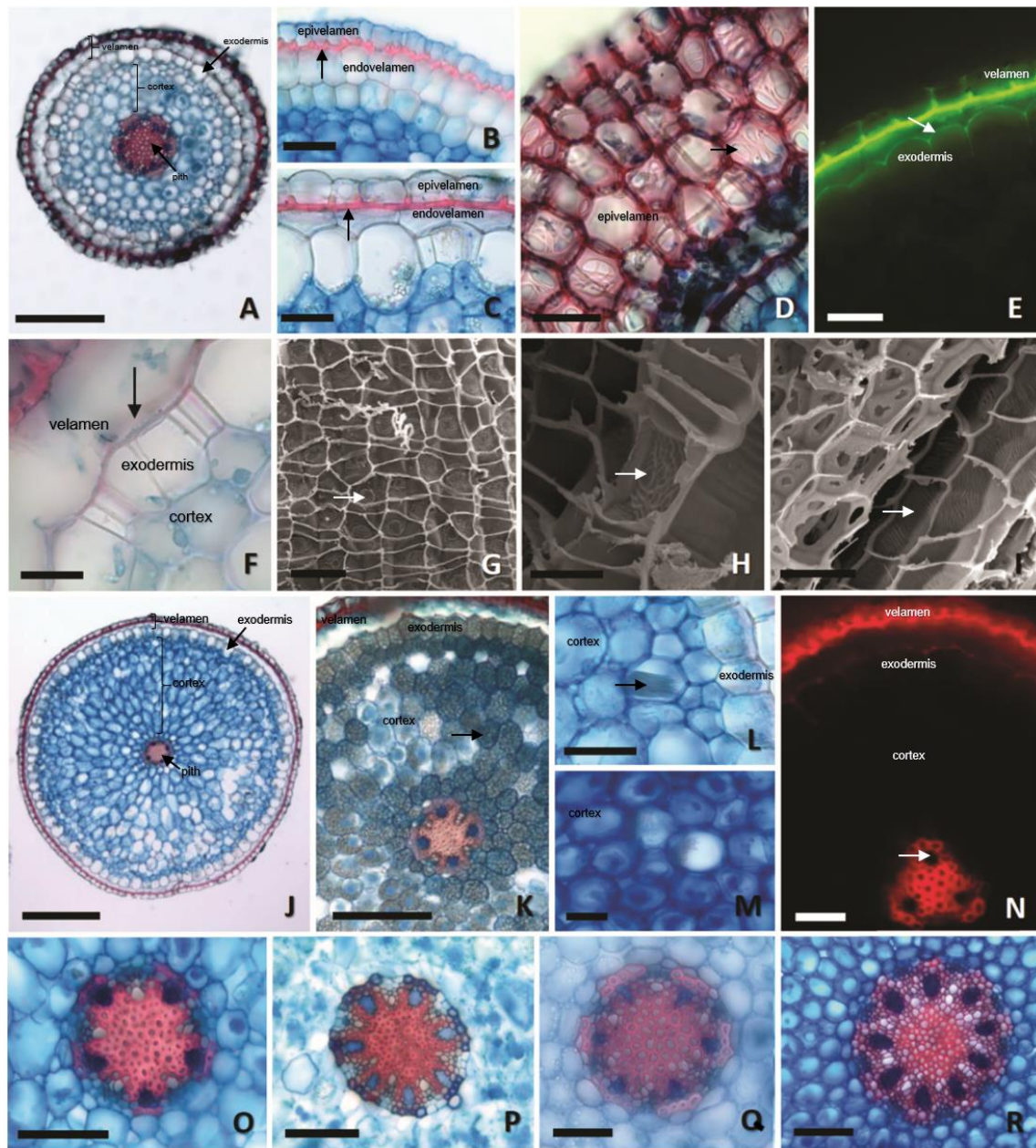


Figure 2. Root anatomy of *Dryadella* species and *Masdevallia infracta*. (A) *D. zebrina* (Imig 405) root in cross-section in LM. (B) *D. litoralis* (Imig 476) and (C) *D. pachyrrhiza* (Imig 507) roots in longitudinal section in LM, evidencing the U-shaped cell-wall thickening of epivelamen (arrow). (D) *M. infracta* (Imig 496) root in cross-section in LM, highlighting the multilayered velamen, with perforated cells in epivelamen (arrow). (E) *D. pachyrrhiza* (Imig 507) root in cross-section in fluorescence microscopy-FM with lignin in green, showing the exodermis U-shaped cell-wall thickening (arrow). (F) *D. zebrina* (Imig 408) root in cross-section in LM, showing a spongy-type tilosome (arrow) adjacent to passage cells of exodermis. (G) *D. summersii* (Imig 481), (H) *D. pachyrrhiza* (Imig 507), and (I) *M. infracta* (Imig 496) exodermis in

longitudinal section in SEM, evidencing spongy-type tilosomes (arrow). (J) *D. pachyrhiza* (Imig 507), root in cross-section in LM. (K) *D. pusiola* (Imig 360) root in cross-section in LM, with starch grains (arrow) visible in the cortex. (L) *D. vitorinoi* (Imig 506) cortex in cross-section in LM with raphides (arrow). (M) *M. infracta* (Imig 387) cortex in cross-section in LM. (N) *D. susanae* (Imig 491) root in cross-section in FEM, showing lignin in red and O-shaped cell-wall thickening (arrow) in endodermis. (O) *D. hirtzii* (Imig 505), (P) *D. simula* (Imig 383), (Q) *D. zebrina* (Imig 408) and (R) *M. infracta* (Imig 496) roots in cross-section in LM, showing the pith with lignin. Scale bar: A, J-K = 200 μ m; B-E, G, I, L-P = 50 μ m; F, H = 20 μ m; Q-R = 100 μ m. In parentheses are the vouchers of the specimen mentioned in the figure.

3.1.2. Ramicaul

In all species analysed, the ramicaul is round in cross-section (Fig 3A, B), except in *D. ataleiensis* and *D. pusiola*, which is round-sulcate (Fig 3C). The epidermis is uniseriate with dome-shaped cells with thin cuticle (Fig 3D). Cortex is parenchymatous and has four to five cell layers in *D. ataleiensis*, *D. litoralis* and *D. pusiola* (Fig. 3E), 10 to 13 cell layers in *M. infracta* (Fig 3F), and six to eight in other species (Fig 3A). Starch grains were observed in all species (Fig. 3D). The vascular system is formed by collateral bundles organized in concentric circles (Fig 3A-C, E-F). Round-sulcate ramicauls have three vascular bundles (Fig. 3B), while round ramicauls varies from 10 to 13 in *D. litoralis*, *D. vitorinoi* and *M. infracta*, 14 to 16 in *D. aviceps* and six to nine in other species, being that the larger bundles are interspersed with the smaller bundles (Fig 3A, C, E, F). The interfascicular fundamental tissue consists of sclerified cells only in *M. infracta* (Fig 3F, G).

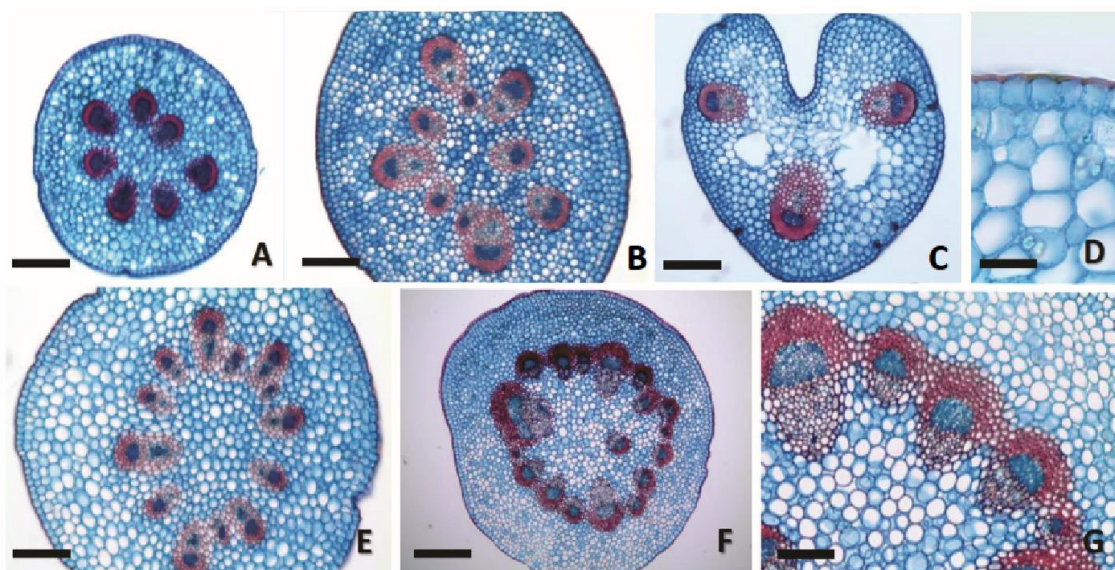


Fig. 3. Ramicaul anatomy of *Dryadella*. (A) *D. hirtzii* (Imig 505). (B) *D. litoralis* (Imig 476). (C) *D. pusiola* (Imig 360). (D) *M. infracta* (Imig 496). (E) *D. litoralis* (Imig 476). (F) *M. infracta* (Imig 387). (G) *M. infracta* (Imig 387). Scale bar: A, E, G = 200um, B-C = 100 um; D = 50 um; F = 500um.

3.1.3. Leaf

In cross-section, the leaves of *D. ataleiensis*, *D. hirtzii* and *D. lilliputiana*, are round-sulcate (Fig 4A) and in other species analysed are flat or semi-flat (Fig 4B). The leaf surface in frontal view is smooth in most species of *Dryadella* and in *M. infracta* (Fig 4C), and verrucous in *D. ataleiensis* and *D. pusiola* (Fig 4D). Wax deposition pattern is granular in *D. pusiola*, *D. simula* and *D. ataleiensis* (Fig 4E), and in platelets in the other species. All species presented hypostomatic leaves (Fig 4C, D, F, H). The stomata consist of two guard cells surrounded by four to five subsidiary cells (Fig 4F). The epidermal cells in surface view have straight (Fig 4G) or curved walls (Fig 4H). Trichome scars are present in the epidermis of both surfaces (Fig 4I), except in *D. lilliputiana*, *D. pusiola* and *D. summersii*. In cross-section, the epidermal cells are dome-like (Fig 4J) or polygonal (Fig 4K). All species have a single-layered abaxial hypodermis, conspicuous (Fig 4L) or discontinuous (Fig 4M). The mesophyll presents nine to 15 cell layers, with aquiferous parenchyma predominant in adaxial face (Fig 4A,B, L–M) or dispersed throughout the mesophyll (Fig 4N). All species analysed present an homogeneous chlorenchyma (Fig 4L–N), except for *D. hirtzii* and *D.*

summersii (Fig 4O). In *M. infracta* the chlorenchyma has brachiform cells with intercellular spaces (Fig 4P). The tracheoidal idioblasts with helicoidal lignification are cylindrical (Fig 4Q), globose (Fig 4R), or both (Fig. 4S).

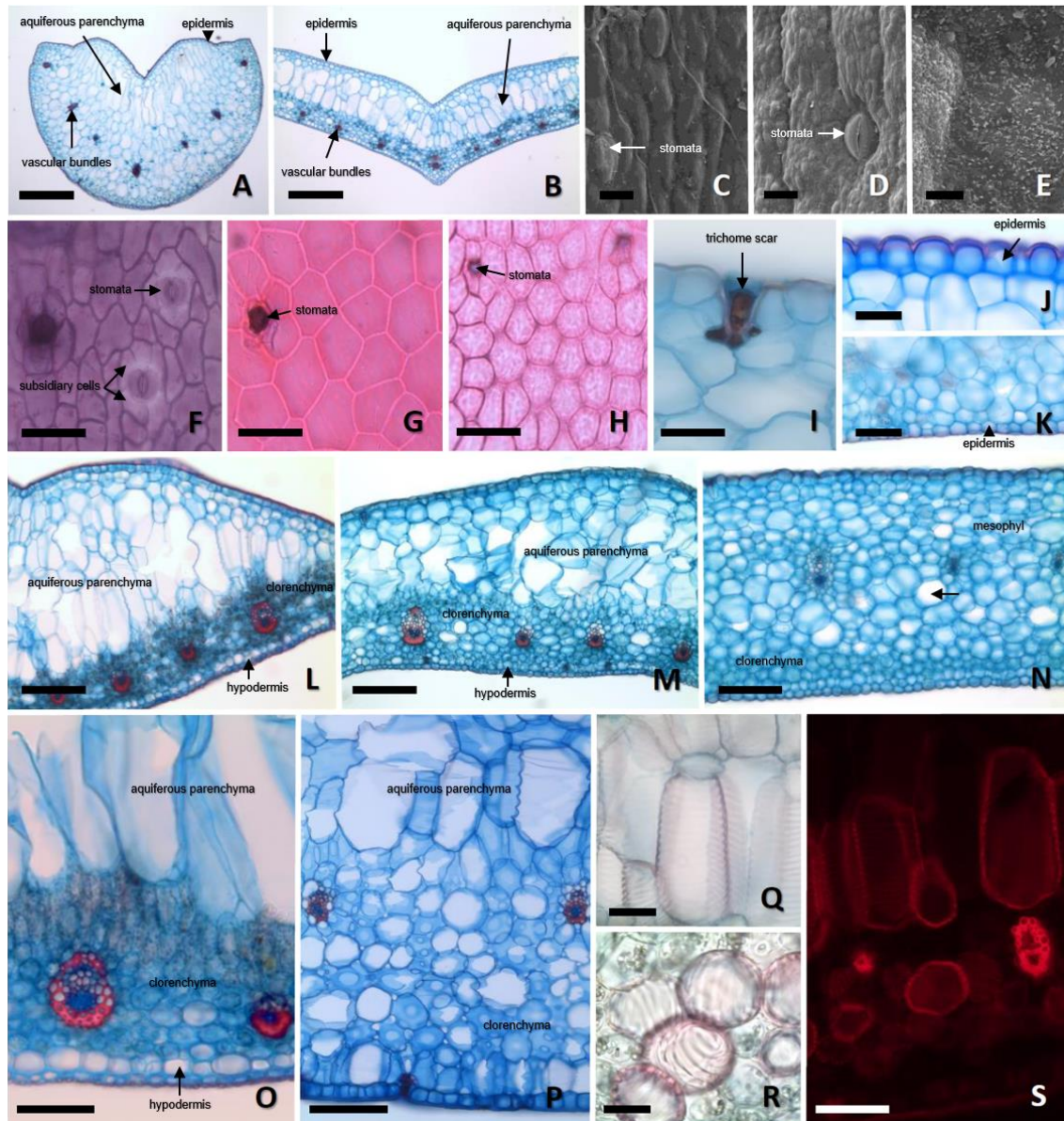


Fig. 4. Leaf anatomy of *Dryadella*. A-B, F-R. Transversal sections in light microscopy. C-E. SEM. (A) *D. lilliputiana* (Imig 395) ou *D. atalaiensis* (Imig 471). (B) *D. simula* (Imig 618). (C) *D. zebrina* (Imig 405); (D) *D. pusiola* (Imig 360) abaxial; (E) *D. pusiola* (Imig 360); (F) *D. simula* (Imig 501); (G) *M. infracta* (Imig 387), (H) *D. lilliputiana* ab (Imig 467). (I) *D. zebrina* (Imig 408) TC ad; (J) *D. aviceps* (Imig 475) ad; (K) *D. litoralis* (Imig 476) ab, (L) *D. pachyrhiza* (Imig 507), (M) *D. simula* (Imig 383), (N) *D. vitorinoi* (Imig 506), (O) *D. hirtzii* (Imig 505), (P) *M. infracta* (Imig 387), (Q-R) *D. zebrina* (Imig 405) (S) *D. zebrina* (Imig 456). Scale bar: A-B = 500 μ m; C-D = 20 μ m;

E = 5 μ m; F-I = 50 μ m; K = 100 μ m, L-N = 200 μ m; O = 100 μ m; P = 200 μ m, Q-R = 50 μ m; S = 100 μ m.

The cylindrical tracheoidal idioblasts are located in the adaxial surface and the globose ones in the abaxial surface in most species. Collateral vascular bundles are surrounded by perivascular fibers (Fig 4L-O), and located near the abaxial surface in all species (Fig 4A-B, L-M), except in *D. cristata*, *D. litoralis* and *D. vitorinoi*, which is in the middle region of the mesophyll (Fig 4N).

3.2. Statistics

3.2.1. Cluster analysis

Silhouette analysis results (Table 3) indicated that the best number of clusters (k) is three, with average silhouette width= 0.34, separating *M. infracta* (cluster 1) from the clade formed by *D. ataleiensis* and *D. pusiola* (cluster 2), and from the other *Dryadella* species (cluster 3; Fig. 5A).

3.2.2. PCoA

The first two PCoA axes together represent 64.48% of the total data variation, with PCo1 accounting for 43.19% of the variance and PCo2 accounting for 21.29%. Both axes presented variance more significant than expected by random, given by the eigenvalues greater than the broken-stick (Table 4). Nine, of the twenty-three variable characters that support the formation of clusters 1 and 2 were identified (Fig 5B).

Table 3: Results of silhouette analysis for cluster validation.

	Test 1	Test 2	Test 3
Cluster number (k)	3	4	5
Average silhouette width	0.34	0.27	0.21

Tabela 04: Summary of Principal Coordinates Analysis for *Dryadella* dataset.

Principal coordinate	% of variance	Eigenvalues	Broken-sticks
PCo1	54.97	0.4193	0.2586

PCo2	18.48	0.1409	0.1752
PCo3	15.82	0.1206	0.1336
PCo4	7.01	0.0535	0.1058
PCo5	5.34	0.0407	0.0850
PCo6	4.44	0.0339	0.0683
PCo7	2.01	0.0153	0.0544
PCo8	0.94	0.0071	0.0425
PCo9	0.00	0.0000	0.0321
PCo10	-0.17	-0.0013	0.0228
PCo11	-0.73	-0.0055	0.0145
PCo12	-1.81	-0.0138	0.0069
PCo13	-2.38	-0.0181	0.0000
PCo14	-3.93	-0.0299	0.0000

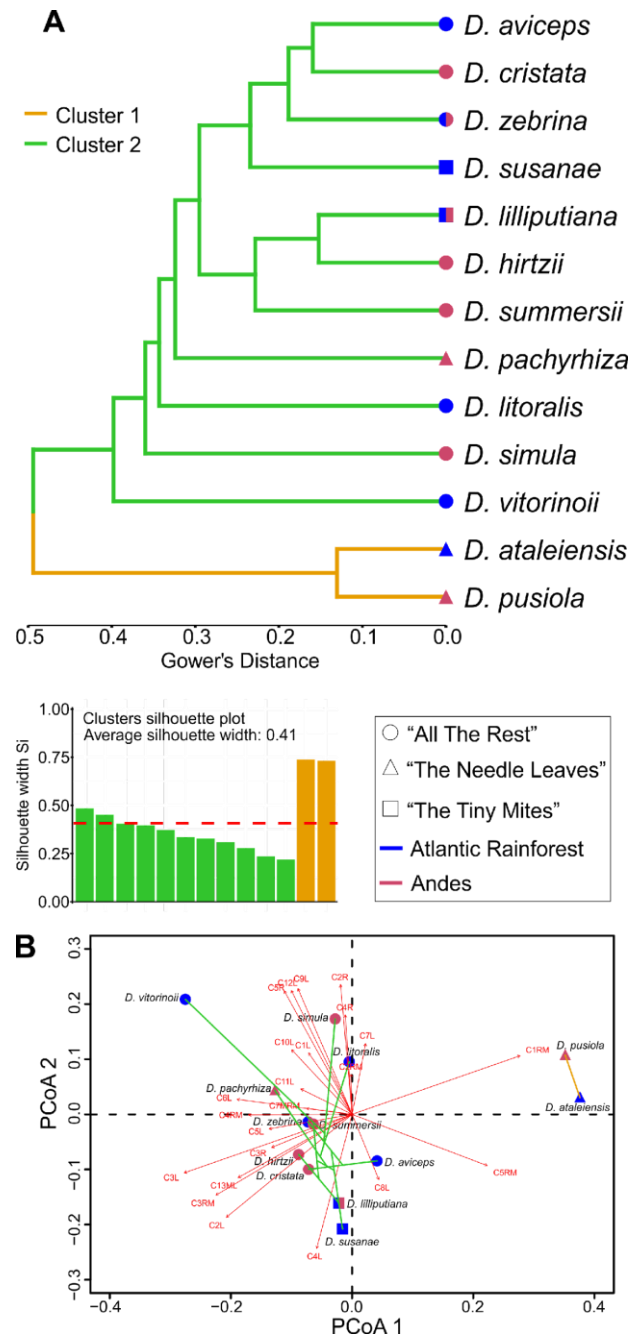


Fig. 5. Result of cluster analysis with the mean silhouette width value for each number of clusters (k), and the members of each cluster highlighted in the UPGMA dendrogram. Below is the PCoA chart with the morphological characters represented by their codes in red. The relevance of each character to the variance in the data is proportional to the size and inclination of the arrow. Species, represented by icons, were connected according to their clusters using the `ordicluster` function (`vegan` v.2.4-2).

3.2.3. Permutational multivariate analysis of variance

The PERMANOVA results indicated that there are no significant differences between the endemic species of the Andes and Atlantic Forest in relation to the anatomical variables analysed ($p = 0.8342$, $F = 0.3632$). Also, there was no difference between the infrageneric sections ($p = 0.07992$, $F = 1.8018$), although the p -value was less than 0.1 in this case.

4. Discussion

Based on anatomical data from previous studies for Pleurothallidinae including *Dryadella*, there are three new findings for the genus. 1) Oxalate crystal raphides were found in the roots and leaves of *D. simula*, and *D. vitorinoii* as well as in the roots of *D. pachyrhiza*. 2) No trichomes or scars of these were observed on the leaf surface of *D. liliputiana*, *D. pusiola*, and *D. summersii*, in opposition to Pridgeon (1981) statement, which claimed that glandular or submerged trichomes occur on leaf surfaces in all genera with two pollinia, which includes *Dryadella* and *Masdevallia*. 3) *D. ataleiensis*, *D. litoralis*, and *D. pusiola* have a verrucous cuticular surface, a character not previously described for Pleurothallidinae, whose pattern is smooth or minutely papillous (Pridgeon 1982).

When comparing *Dryadella* species with *M. infracta* it is possible to identify some distinctive anatomical characteristics for *Dryadella*. Concerning the roots, the velamen is bi-layered (four-layered in *M. infracta*), the epi-velamen cells have U-shape wall thickening (perforated in *M. infracta*), and the tilosomes are spongy-type (lamellar-type in *M. infracta*). The ramicaul has 4–8 cell layers in the cortex (10–13 cell layers in *M. infracta*), and the interfascicular fundamental tissue is composed by non-sclerified cells (sclerified cells in *M. infracta*). In the leaves, the chlorenchyma is homogeneous, yet it can be heterogeneous in some species, but without intercellular spaces. In *M. infracta*, the chlorenchyma has brachiform cells with intercellular spaces.

The number of cell layers in the cortex of ramicaul and the presence of sclerified cells in the fundamental tissue are features responsible for resistance and support to the thin vegetative organs (Oliveira and Sajo 1999). The fact that these traits are present in *M. infracta* and absent in *Dryadella* is probably directly related to plant size: *M. infracta*

ramicaul measures 1– 3 cm, whereas *Dryadella* ramicaul is no longer than 1.5 cm in length.

Therefore, we found that anatomical root traits were the most informative in distinguishing the species studied at the genus level. Pridgeon (1982) had already pointed out differences between *Dryadella* and *Masdevallia* based on root characteristics, indicating that *Dryadella* has uniform cell wall thickening in the epidermis and uni-stratified velamen, while *Masdevallia* has no uniform cell wall thickening in epidermis and bi or multi-stratified velamen.

The presence of tilosomes is almost unique to epiphyte taxa and is related to water intake and possibly reservoir, since these structures are responsible for condensation and absorption of steam from the atmosphere, preventing tissue desiccation (Pridgeon 1982; Benzing *et al.* 1982). Spongy-type tilosomes are predominant in Pleurothallidinae (Pridgeon *et al.* 2005). However, among the phylogenetically related taxa of *Dryadella*, only *Pleurothallis*, of the *Pleurothallis* affinity, also has this spongy tilosomes (Pridgeon *et al.* 2005; Karremans 2016). The other genera of *Specklinia* affinity do not follow the subtribe pattern: *Platystele* has no tilosomes, and in *Scaphosepalum* the tilosomes are lamellar (Pridgeon *et al.* 2005; Karremans 2016).

The number of ramicaul vascular bundles varied among species, from four in *D. pusiola*, 6– 13 in other species (including *M. infracta*) and 14– 16 in *D. aviceps*.

The number of vascular bundles is directly related to the diameter of the ramicaul (Stern 2014). However, *D. aviceps* presented the highest number of vascular bundles and is considered a small species within the genus. Studies focused on the ecology of *D. aviceps* may be fundamental to understand this lack of relationship between the diameter of the ramicaul and the number of vascular bundles in this species.

Other characteristics that varied among the species are related to the presence and morphology of tracheoidal idioblasts in the mesophyll. *Dryadella hirtzii*, *D. pachyrhiza*, *D. pusiola*, and *D. summersii*, exclusively Andean, and *D. vitorinoii*, endemic from the Atlantic Rainforest, are the only species that do not have idioblasts. In the other species it was observed globose idioblasts on both faces in flat or semi-flat leaves, and in cylindrical leaves, they were restricted to the adaxial face. This variation in the morphology of tracheoidal idioblasts between species of the same genus and in relation to the mesophyll face is apparently common in Pleurothallidinae (Pridgeon 1982).

Little morpho-anatomical variation was observed within the genus *Dryadella*. Regarding the sections proposed by Luer (2005), the grooved ramicaul and the verrucous adaxial cuticular surface are unique characteristics of *D. ataleiensis* and *D. pusiola*, both from “The Needle Leaves”. However, *D. pachyrhiza* also belongs to this section and has round ramicaul in cross-section and smooth cuticle on the adaxial surface, as well as the other species of the genus. There were also no unique characteristics in relation to the biomes, just a tendency for Andean species to have fewer protoxylem poles in root, of 3–7, compared to Atlantic Rainforest species, where the number ranged from 6–11. However, the number of protoxylem poles may vary between individuals of the same species (Rosso 1966; Signhg 1986; Silva 2010). The morpho-anatomical similarity between *Dryadella* species is evident from the results of the statistical analyzes.

Cluster analysis and PCoA indicated the separation *D. ataleiensis* + *D. pusiola* clade (cluster 1) from the other species of the genus (cluster 2), with average support by the silhouette analysis (Fig. 5 A,B). Grooved ramicaul in cross section (C1RM), and leaf with verrucous cuticle in adaxial surface (C3L) were the main characters distinguishing cluster 1 from cluster 2. Other seven anatomical features contributed to clusters distinction, that are: the number of vascular bundles in the ramicaul (C4RM), the number of cell layers in mesophyll (C5L), the number of passage cells in root endodermis (C3R), leaf width (C13ML), the number of cortical cell layers (C3RM), wax deposition pattern on leaf cuticle (C2L), and verrucous cuticle on leaf abaxial surface (C4L).

However, none of the two clusters corresponded to the expected groups of species. In addition, the characteristics explored in this study do not show differences between the Atlantic Rainforest and Andes groups or between the sections, as verified with PERMANOVA. This refutes our predictive assumptions that there would be differences between Andean and Atlantic Rainforest taxa, and that anatomical characters would support Luer infrageneric classification.

Most Andean orchids originated in the last 15 to 20 million years (Ma) from migrating lineages derived from the Amazon plain (Pérez-Escobar *et al.* 2017). It is suggested that Pleurothallidinae, however, originated in Central America or in the Antilles at the beginning of the Miocene period, and then migrated to the northern Andes around 16 Ma, at a time when the mountain range had already reached average elevations of 1500 m (Pérez-Escobar *et al.* 2017).

Therefore, the elevation of the Andes did not act as a geographical barrier to Pleurothallidinae lineages, and even facilitated the dispersal of species to South America. This fact is related to the high dispersal capacity of orchid seeds, which are small and easily wind-dispersed over long distances and in different elevation zones (Perez-Escobar *et al.* 2017). The same did not happen to other plant groups, whose seeds do not have the same characteristics, such as Annonaceae and Rubiaceae (Pirie *et al.* 2006, Antonelli *et al.* 2009). In South America some lineages have diversified explosively and occupied montane habitats and hazy forest environments of an average altitude of 1200–1300 m (Perez-Escobar *et al.* 2017; Hoorn *et al.* 2010; Givnish *et al.* 2015).

Subsequently, Neotropic forests were fragmented by the advance of open vegetation in response to the drier climate, giving rise to the current South American Dry Diagonal (Werneck 2011). Currently, seasonally dry tropical forests or Caatinga (northeastern Brazil) and Cerrado savanna (central Brazil) separate the Atlantic Rainforest from the Amazon Rainforest, while the Pampas (northeastern Argentina, western Paraguay and southeastern Bolivia) divided the Atlantic Rainforest of the Andean Rainforest (Werneck 2011, Turchetto-Zolet *et al.* 2012). Such distribution of biomes is also recent and was finally shaped during the Pleistocene period (Ab'Saber 1977).

Considering the recent origin of the subtribe and the rapid diversification of lineages in tropical forests, which occurred when they were still undergoing fragmentation and shrinkage, it can be concluded that time was not sufficient for *Dryadella* species to differentiate. This is reflected in the anatomical similarity between species, as seen here, as well as macro-morphological, noted by Luer (2005). These data should be compared to genetic and morphological data in a phylogenetic context to understand the ecology and evolution of *Dryadella* species.

5. Conclusion

Some distinctive anatomical characters were found for *Dryadella* in relation to *Masdevallia infracta*, as well as anatomical novelties for the genus, which may help to establish future synapomorphies for possible infrageneric groups and also in the general context of Pleurothallidinae.

The classification of species in "The Needle Leaves", "The Tiny Mites" and "All The Rest" was not supported by the data collected. Statistical analyses of anatomical and

morphological variables also showed that the Andean and Atlantic Rainforest species do not have distinct characteristics, despite their disjunct distribution. This fact may be related to the time of origin and diversification of the species since time was not long enough for the accumulation of genetic diversity to express differences in anatomy and morphology. Therefore, the characters investigated do not provide sufficient information to establish a fine infrageneric classification of *Dryadella* species, however, it have seen that such characters are stable since they have not differed in relation to habitat and among individuals, and can be useful for systematic studies in the genus.

Conflicts of interest

The authors declare no conflicts of interest.

We thank the Instituto Chico Mendes de Conservação da Biodiversidade (SISBIO/ICMBio), and Instituto Ambiental do Paraná (IAP) for the collecting permits. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) with scholarships granted to DCI (168348/2017-9). ECS thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research productivity award grant (Bolsa de Produtividade em Pesquisa - Nível 2, Proc. 308460/2017-0).

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Supplementary Material

Vegetative anatomy and its systematic importance in the *Dryadella* Luer (Orchidaceae: Pleurothallidinae)

Data frame

In the data frame species names are with abbreviated genus epithet *D.* for *Dryadella*. The first 23 columns are the characters of vegetative anatomy, followed by the regional groups (Andes and Atlantic Forest), and by the taxonomic sections, respectively.

```
anatomy <- read.table("data_dryadella.txt", header = TRUE)
```

```
anatomy
```

```
##           C2R C3R C4R C5R C1RM C2RM C3RM C4RM C5RM C7MRM C1L
C2L C3L C4L
```

```
## D. cristata    1 3 0 0 0 0 1 1 0 1 1 1 1 1
## D. hirtzii     1 4 1 0 0 0 1 1 0 0 0 1 1 1
## D. pachyrhiza  3 4 0 1 0 0 1 1 0 1 1 1 1 1
## D. pusiola     2 3 1 0 1 0 0 0 1 0 1 0 0 0
## D. simula      2 2 1 1 0 0 1 1 0 1 1 0 1 0
## D. summersii   2 4 1 0 0 0 1 1 0 0 1 1 1 0
## D. ataleiensis 2 3 1 0 1 0 0 0 1 0 0 0 0 0
## D. aviceps     2 3 1 0 0 0 1 3 1 1 1 1 1 1
## D. litoralis   2 4 1 0 0 1 0 2 0 0 1 1 1 0
## D. susanae     1 4 0 0 0 0 1 1 1 0 1 1 1 1
## D. vitorinoii  3 4 2 1 0 0 1 2 0 0 1 1 1 0
## D. lilliputiana 1 3 1 0 0 0 1 1 0 0 0 1 1 1
## D. zebrina     1 4 1 0 0 0 1 1 0 1 1 1 1 0
```

```
##           C5L C6L C7L C8L C9L C10L C11L C12L C13ML  distribution
## D. cristata    0 0 1 1 0 1 1 1 1      Andes
## D. hirtzii     1 0 1 0 0 0 1 2 1      Andes
## D. pachyrhiza  0 1 1 0 0 0 1 2 0      Andes
## D. pusiola     0 0 1 0 0 0 0 1 0      Andes
## D. simula      0 0 1 1 1 0 1 2 1      Andes
## D. summersii   1 1 0 0 0 0 0 1 1      Andes
## D. ataleiensis 0 0 1 1 0 0 1 1 0 Atlantic_Rainforest
## D. aviceps     0 0 1 1 0 0 1 1 1 Atlantic_Rainforest
## D. litoralis   0 0 1 1 0 1 1 2 1 Atlantic_Rainforest
## D. susanae     0 1 0 1 0 0 1 0 1 Atlantic_Rainforest
## D. vitorinoii  1 2 1 0 1 1 1 2 1 Atlantic_Rainforest
## D. lilliputiana 1 1 1 1 0 0 0 1 1      both
```

```

## D. zebrina      0 1 1 1 0 0 1 2 1      both
##
## sections
## D. cristata     All_The_Rest
## D. hirtzii      All_The_Rest
## D. pachyrhiza   The_Needle_Leaves
## D. pusiola      The_Needle_Leaves
## D. simula       All_The_Rest
## D. summersii    All_The_Rest
## D. ataleiensis  The_Needle_Leaves
## D. aviceps      All_The_Rest
## D. litoralis    All_The_Rest
## D. susanae      The_Tiny_Mites
## D. vitorinoii   All_The_Rest
## D. lilliputiana The_Tiny_Mites
## D. zebrina      All_The_Rest
anatomyData <- anatomy[,-c(24,25)]
anatomyData
##          C2R C3R C4R C5R C1RM C2RM C3RM C4RM C5RM C7MRM C1L
C2L C3L C4L
## D. cristata  1 3 0 0 0 0 1 1 0 1 1 1 1 1
## D. hirtzii   1 4 1 0 0 0 1 1 0 0 0 1 1 1
## D. pachyrhiza 3 4 0 1 0 0 1 1 0 1 1 1 1 1
## D. pusiola   2 3 1 0 1 0 0 0 1 0 1 0 0 0
## D. simula    2 2 1 1 0 0 1 1 0 1 1 0 1 0
## D. summersii 2 4 1 0 0 0 1 1 0 0 1 1 1 0
## D. ataleiensis 2 3 1 0 1 0 0 0 1 0 0 0 0 0
## D. aviceps   2 3 1 0 0 0 1 3 1 1 1 1 1 1
## D. litoralis 2 4 1 0 0 1 0 2 0 0 1 1 1 0
## D. susanae   1 4 0 0 0 0 1 1 1 0 1 1 1 1
## D. vitorinoii 3 4 2 1 0 0 1 2 0 0 1 1 1 0
## D. lilliputiana 1 3 1 0 0 0 1 1 0 0 0 1 1 1
## D. zebrina   1 4 1 0 0 0 1 1 0 1 1 1 1 0
##          C5L C6L C7L C8L C9L C10L C11L C12L C13ML
## D. cristata  0 0 1 1 0 1 1 1 1

```

```
## D. hirtzii      1 0 1 0 0 0 1 2 1
## D. pachyrhiza  0 1 1 0 0 0 1 2 0
## D. pusiola     0 0 1 0 0 0 0 1 0
## D. simula      0 0 1 1 1 0 1 2 1
## D. summersii   1 1 0 0 0 0 0 1 1
## D. ataleiensis 0 0 1 1 0 0 1 1 0
## D. aviceps     0 0 1 1 0 0 1 1 1
## D. litoralis   0 0 1 1 0 1 1 2 1
## D. susanae     0 1 0 1 0 0 1 0 1
## D. vitorinoii  1 2 1 0 1 1 1 2 1
## D. lilliputiana 1 1 1 1 0 0 0 1 1
## D. zebrina     0 1 1 1 0 0 1 2 1
```

Distance matrix

We calculated a distance matrix from the morphological data for further analyses of clustering and ordination. We chose Gower's metric, since the variables are both categorical and quantitative.

```
library(cluster)
```

```
gdMorphology <- cluster::daisy(anatomyData, metric = "gower")
```

Cluster Analysis

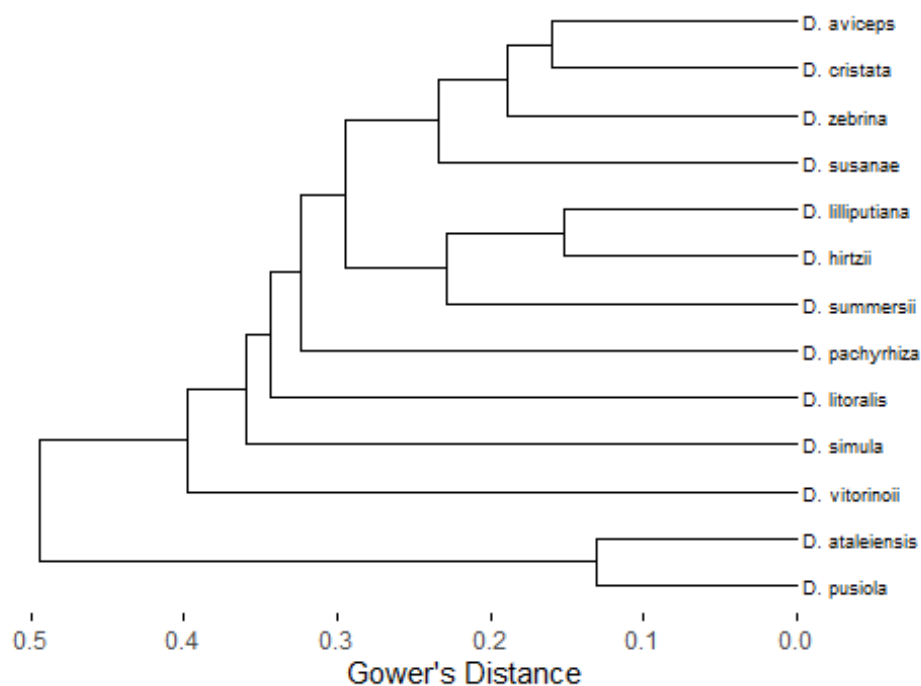
We built an UPGMA dendrogram with the distance matrix above to access how the sampled species are related according to our variables, as well as possible clusters. We also tested if the UPGMA method suited well for our dataset through correlation test between the resulted dendrogram and the cophenetic distances among samples.

```
dendMorphology <- hclust(gdMorphology, method = "average")
```

```
library("factoextra")
```

```
factoextra::fviz_dend(dendMorphology, cex = 0.5, main = "UPGMA Dendrogram",
  ylab = "Gower's Distance", horiz = TRUE)
```

UPGMA Dendrogram



```
res.coph <- cophenetic(dendMorphology)
```

```
cor.test(gdMorphology, res.coph)
```

```
##
```

```
## Pearson's product-moment correlation
```

```
##
```

```
## data: gdMorphology and res.coph
```

```
## t = 14.39, df = 76, p-value < 2.2e-16
```

```
## alternative hypothesis: true correlation is not equal to 0
```

```
## 95 percent confidence interval:
```

```
## 0.7814935 0.9054784
```

```
## sample estimates:
```

```
## cor
```

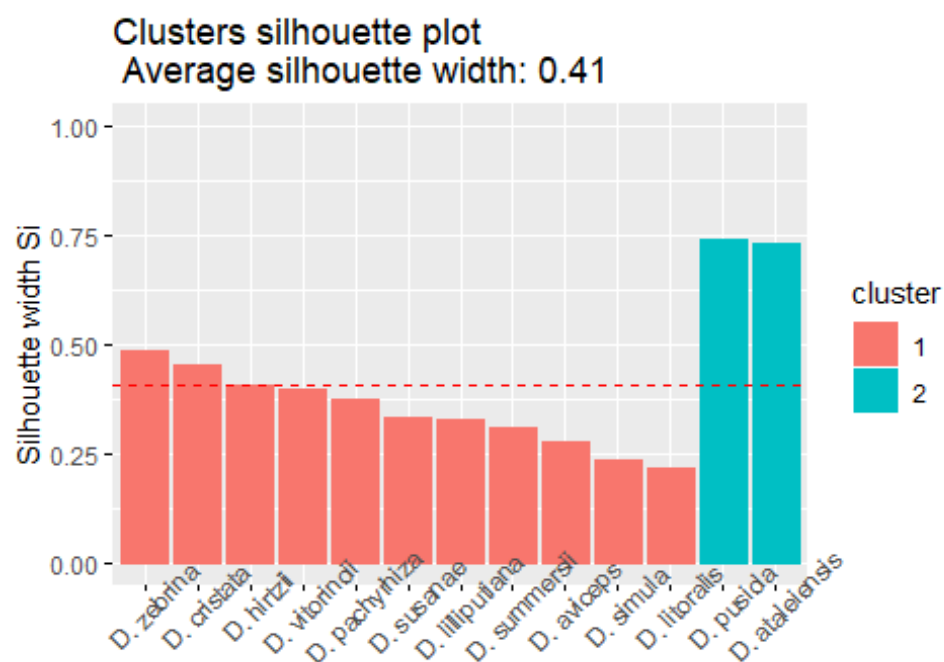
```
## 0.8552848
```

Since the correlation is strong (> 0.84), we are confident that the UPGMA is a good clustering method for our dataset. Next, we performed silhouette analysis in order to validate the clusters formed in the dendrogram. We tested for two to five clusters number (k).

```

sil_2 <- silhouette(cutree(dendMorphology, k = 2), gdMorphology)
row.names(sil_2) <- row.names(anatomyData)
fviz_silhouette(sil_2, gdMorphology)
## cluster size ave.sil.width
## 1    1  11    0.35
## 2    2   2    0.74

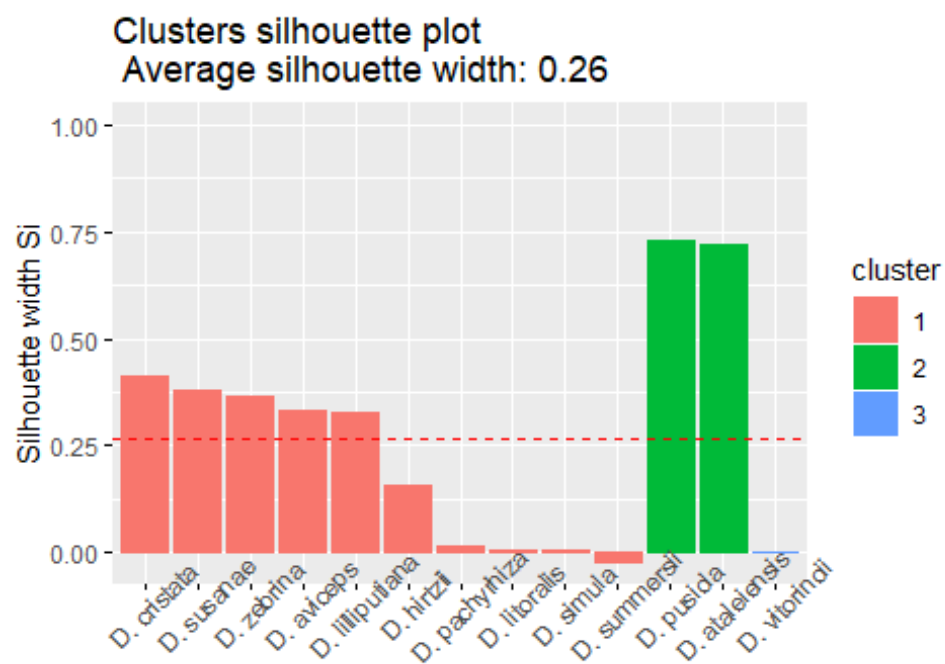
```



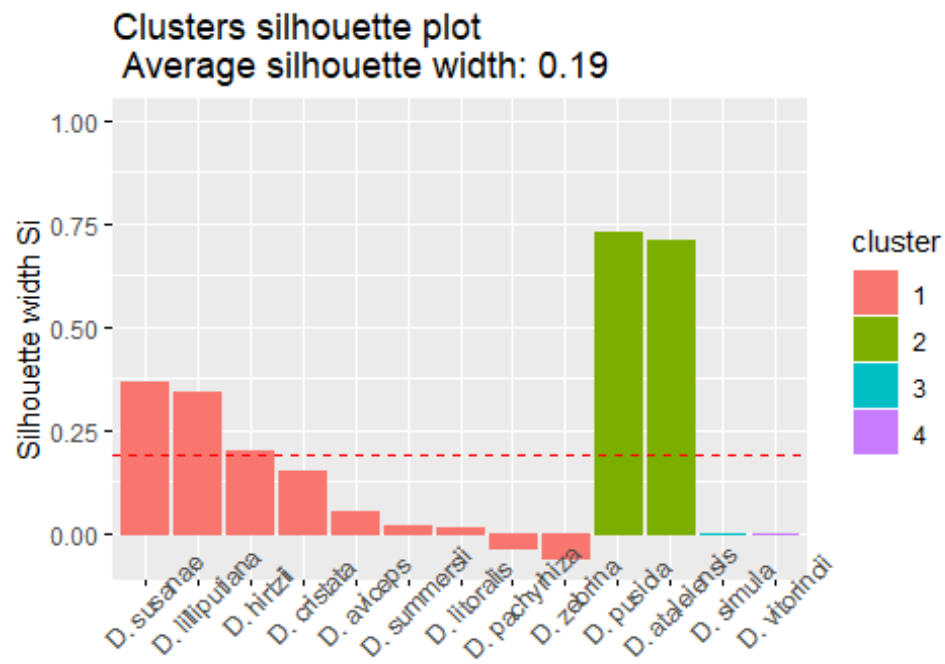
```

sil_3 <- silhouette(cutree(dendMorphology, k = 3), gdMorphology)
row.names(sil_3) <- row.names(anatomyData)
fviz_silhouette(sil_3, gdMorphology)
## cluster size ave.sil.width
## 1    1  10    0.20
## 2    2   2    0.73
## 3    3   1    0.00

```



```
sil_4 <- silhouette(cutree(dendMorphology, k = 4), gdMorphology)
row.names(sil_4) <- row.names(anatomyData)
fviz_silhouette(sil_4, gdMorphology)
## cluster size ave.sil.width
## 1 1 9 0.12
## 2 2 2 0.72
## 3 3 1 0.00
## 4 4 1 0.00
```



```
sil_5 <- silhouette(cutree(dendMorphology, k = 5), gdMorphology)
```

```
row.names(sil_5) <- row.names(anatomyData)
```

```
fviz_silhouette(sil_5, gdMorphology)
```

```
## cluster size ave.sil.width
```

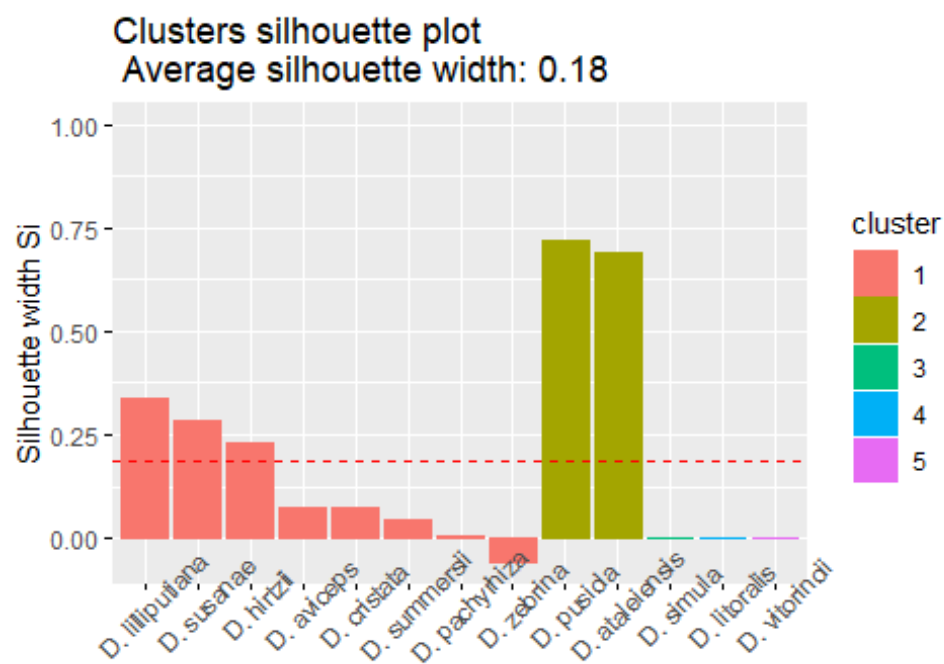
```
## 1 1 8 0.12
```

```
## 2 2 2 0.70
```

```
## 3 3 1 0.00
```

```
## 4 4 1 0.00
```

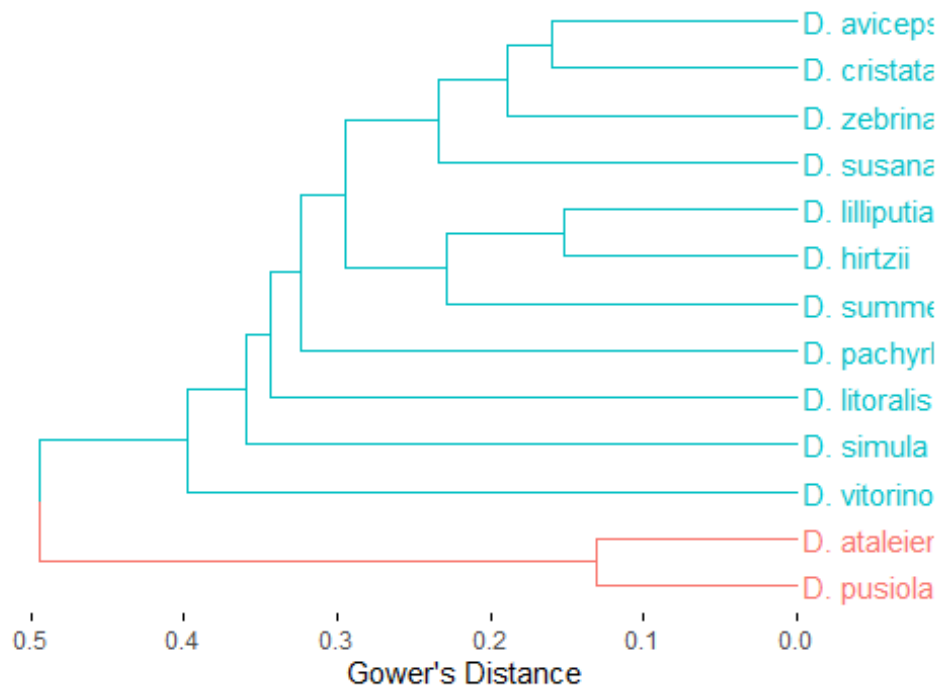
```
## 5 5 1 0.00
```



The results showed that $k = 2$ is the best clustering due to the highest silhouette average width of all clusters. Also, the *D. ataleiensis* + *D. pusida* group seems to be a good cluster.

```
hc.cut <- hcut(gdMorphology, k = 2, hc_method = "average")
fviz_dend(hc.cut, show_labels = TRUE, rect = FALSE, horiz = TRUE,
          ylab = "Gower's Distance")
```

Cluster Dendrogram



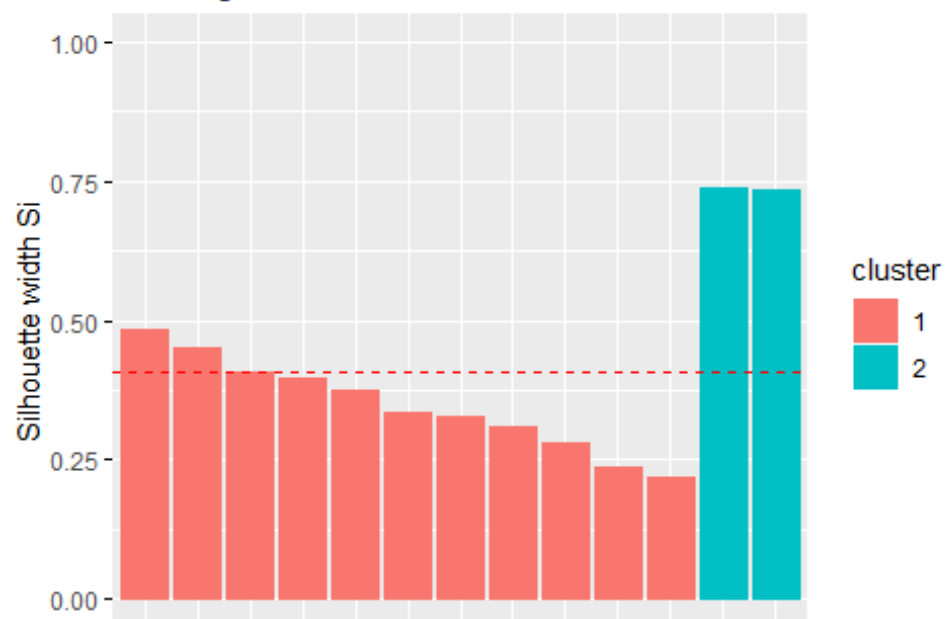
```
fviz_silhouette(hc.cut)
```

```
## cluster size ave.sil.width
```

```
## 1 11 0.35
```

```
## 2 2 0.74
```

Clusters silhouette plot Average silhouette width: 0.41



Principal Coordinate Analysis (PCoA)

The PCoA was performed to show which characters were responsible for clusters formation.

```
library(ape)
```

```
library(vegan)
```

```
distribution <- anatomy$distribution
```

```
sections <- anatomy$sections
```

```
pcoa_dry <- ape::pcoa(gdMorphology)
```

```
efit <- envfit(pcoa_dry$vectors, anatomyData)
```

```
plot(pcoa_dry$vectors, col = c("red", "blue", "purple")[distribution],
```

```
  pch = c(19,17,15)[sections],
```

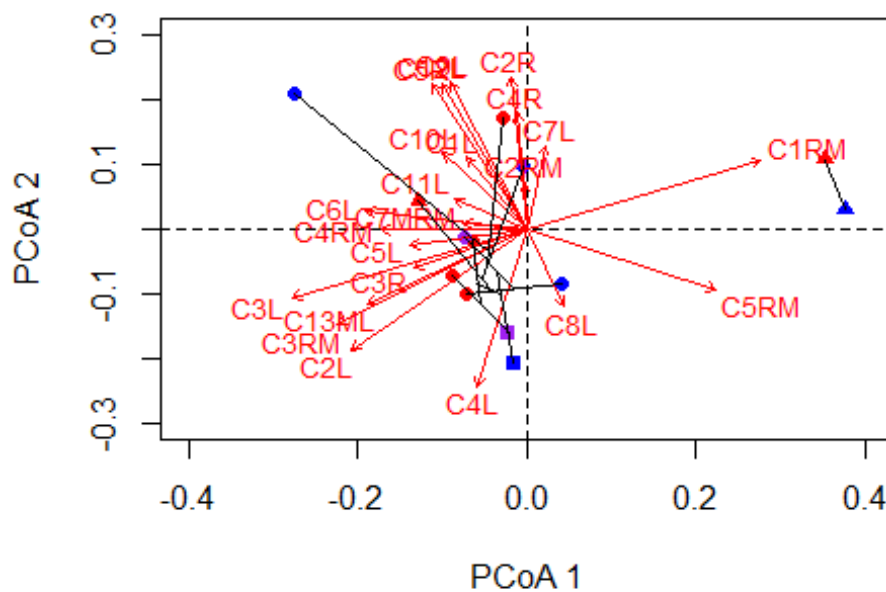
```
  xlim = c(-.4,0.4), ylim=c(-.3, .3),
```

```
  xlab = "PCoA 1", ylab = "PCoA 2")
```

```
abline(h = 0, v = 0, lty = 2)
```

```
plot(efit, col = "red", cex = 0.9) -> pcoa_plot
```

```
ordicluster(pcoa_dry$vectors, dendMorphology, prune = 1, col = "black")
```



Permutational Multivariate Analysis of Variance (PERMANOVA)

We chose PERMANOVA to compare the morphological attributes between the species of the taxonomic sections, and between the geographical areas of the Andes and the Atlantic Rainforest. We removed all *Dryadella* species that are not endemic to one geographical region from the data frame, as well as the columns of species distribution and taxonomy, since they are the variables to be tested.

```
endemic <- anatomy[-c(12:13),]
```

```
endemic
```

```
##          C2R C3R C4R C5R C1RM C2RM C3RM C4RM C5RM C7MRM C1L
C2L C3L C4L
```

```
## D. cristata    1 3 0 0 0 0 1 1 0 1 1 1 1 1
## D. hirtzii     1 4 1 0 0 0 1 1 0 0 0 1 1 1
## D. pachyrhiza  3 4 0 1 0 0 1 1 0 1 1 1 1 1
## D. pusiola     2 3 1 0 1 0 0 0 1 0 1 0 0 0
## D. simula      2 2 1 1 0 0 1 1 0 1 1 0 1 0
## D. summersii   2 4 1 0 0 0 1 1 0 0 1 1 1 0
## D. ataleiensis 2 3 1 0 1 0 0 0 1 0 0 0 0 0
## D. aviceps     2 3 1 0 0 0 1 3 1 1 1 1 1 1
## D. litoralis   2 4 1 0 0 1 0 2 0 0 1 1 1 0
```

```

## D. susanae      1 4 0 0 0 0 1 1 1 0 1 1 1 1
## D. vitorinoii  3 4 2 1 0 0 1 2 0 0 1 1 1 0
##              C5L C6L C7L C8L C9L C10L C11L C12L C13ML  distribution
## D. cristata    0 0 1 1 0 1 1 1 1      Andes
## D. hirtzii     1 0 1 0 0 0 1 2 1      Andes
## D. pachyrhiza  0 1 1 0 0 0 1 2 0      Andes
## D. pusiola     0 0 1 0 0 0 0 1 0      Andes
## D. simula      0 0 1 1 1 0 1 2 1      Andes
## D. summersii   1 1 0 0 0 0 0 1 1      Andes
## D. ataleiensis 0 0 1 1 0 0 1 1 0 Atlantic_Rainforest
## D. aviceps     0 0 1 1 0 0 1 1 1 Atlantic_Rainforest
## D. litoralis   0 0 1 1 0 1 1 2 1 Atlantic_Rainforest
## D. susanae     0 1 0 1 0 0 1 0 1 Atlantic_Rainforest
## D. vitorinoii  1 2 1 0 1 1 1 2 1 Atlantic_Rainforest
##              sections
## D. cristata     All_The_Rest
## D. hirtzii      All_The_Rest
## D. pachyrhiza   The_Needle_Leaves
## D. pusiola      The_Needle_Leaves
## D. simula       All_The_Rest
## D. summersii    All_The_Rest
## D. ataleiensis The_Needle_Leaves
## D. aviceps      All_The_Rest
## D. litoralis    All_The_Rest
## D. susanae      The_Tiny_Mites
## D. vitorinoii   All_The_Rest
data_endemic <- endemic[,-c(24:25)]
data_endemic
##              C2R C3R C4R C5R C1RM C2RM C3RM C4RM C5RM C7MRM C1L
C2L C3L C4L
## D. cristata    1 3 0 0 0 0 1 1 0 1 1 1 1 1
## D. hirtzii     1 4 1 0 0 0 1 1 0 0 0 1 1 1
## D. pachyrhiza  3 4 0 1 0 0 1 1 0 1 1 1 1 1
## D. pusiola     2 3 1 0 1 0 0 0 1 0 1 0 0 0

```

```

## D. simula      2 2 1 1 0 0 1 1 0 1 1 0 1 0
## D. summersii   2 4 1 0 0 0 1 1 0 0 1 1 1 0
## D. ataleiensis 2 3 1 0 1 0 0 0 1 0 0 0 0 0
## D. aviceps     2 3 1 0 0 0 1 3 1 1 1 1 1 1
## D. litoralis   2 4 1 0 0 1 0 2 0 0 1 1 1 0
## D. susanae     1 4 0 0 0 0 1 1 1 0 1 1 1 1
## D. vitorinoii  3 4 2 1 0 0 1 2 0 0 1 1 1 0
##              C5L C6L C7L C8L C9L C10L C11L C12L C13ML
## D. cristata    0 0 1 1 0 1 1 1 1
## D. hirtzii     1 0 1 0 0 0 1 2 1
## D. pachyrhiza  0 1 1 0 0 0 1 2 0
## D. pusiola     0 0 1 0 0 0 0 1 0
## D. simula      0 0 1 1 1 0 1 2 1
## D. summersii   1 1 0 0 0 0 0 1 1
## D. ataleiensis 0 0 1 1 0 0 1 1 0
## D. aviceps     0 0 1 1 0 0 1 1 1
## D. litoralis   0 0 1 1 0 1 1 2 1
## D. susanae     0 1 0 1 0 0 1 0 1
## D. vitorinoii  1 2 1 0 1 1 1 2 1

```

Then we performed the PERMANOVA for species distribution (Andes and Atlantic Rainfores).

```
distr <- endemic$distribution
```

```
permanova_distr <- adonis2(data_endemic ~ distr, endemic,
```

```
  method = "gower", permutations = 1000)
```

```
permanova_distr
```

```
## Permutation test for adonis under reduced model
```

```
## Terms added sequentially (first to last)
```

```
## Permutation: free
```

```
## Number of permutations: 1000
```

```
##
```

```
## adonis2(formula = data_endemic ~ distr, data = endemic, permutations = 1000,
```

```
method = "gower")
```

```
##      Df SumOfSqs    R2    F Pr(>F)
```

```
## distr    1  0.03171 0.03879 0.3632 0.8472
```

```
## Residual 9  0.78579 0.96121
```

```
## Total   10  0.81749 1.00000
```

And finally for the taxonomic sections proposed by Luer (The Needle Leaves, The Tiny Mites, All The Rest).

```
sect <- endemic$sections
```

```
permanova_sect <- adonis2(data_endemic ~ sect, endemic,
  method = "gower", permutations = 1000)
```

```
permanova_sect
```

```
## Permutation test for adonis under reduced model
```

```
## Terms added sequentially (first to last)
```

```
## Permutation: free
```

```
## Number of permutations: 1000
```

```
##
```

```
## adonis2(formula = data_endemic ~ sect, data = endemic, permutations = 1000,
  method = "gower")
```

```
##      Df SumOfSqs    R2    F Pr(>F)
```

```
## sect    2  0.25388 0.31056 1.8018 0.08891 .
```

```
## Residual 8  0.56362 0.68944
```

```
## Total   10  0.81749 1.00000
```

```
## ---
```

```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

CONCLUSÃO GERAL

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

O Capítulo I aborda a filogenia e a biogeografia do gênero onde, a partir de 30 espécies de *Dryadella* em conjunto com outros gêneros relacionados, análises filogenéticas foram realizadas usando dados moleculares nrITS e *matk*, a fim de testar o monofiletismo do gênero, dada a sua distribuição disjunta, a relação infra-genérica e hipóteses da origem e diversificação das espécies. Os resultados obtidos demonstram que *Dryadella* é um grupo monofilético, altamente suportado, e que as relações dos cladogramas internos não com as propostas de seções baseadas em morfologia vegetativa. A partir da datação molecular e as análises de biogeografia, os resultados demonstraram que a provável origem de *Dryadella* ocorreu no Mioceno Médio-Superior, por volta de 7 Ma, e hoje são disjuntas por uma imensa área seca, com uma única espécie para o Cerrado, em matas de galerias. A diversificação inicial do gênero ocorreu por um evento de dispersão e, posteriormente, por múltiplas dispersões em ambas as áreas. Os resultados deste capítulo contribuem para o nosso melhor entendimento da evolução de *Dryadella*, bem como evidências sobre a conexão histórica das florestas costeiras da América do Sul, pelos Andes e Floresta Atlântica.

O Capítulo II trata da revisão taxonômica das espécies brasileiras de *Dryadella*, resultante de esforços de coleta em campo, do estudo dos materiais históricos e protólogos e ilustrações, bem como da análise de exsicatas provenientes de vários herbários do mundo. A identificação das espécies de *Dryadella* não é uma tarefa fácil, já que são espécies diminutas e muito semelhantes morfologicamente, poucos caracteres diferenciam uma espécie da outra, além disso, exibe plasticidade fenotípica, vegetativa e reprodutiva. Anteriormente eram citadas 18 espécies para o Brasil, mas no presente trabalho atualizamos a listagem para 14 espécies reconhecidas no país: *Dryadella anapaulae*, *D. auriculigera*, *D. aviceps*, *D. crenulata*, *D. kautskyi*, *D. krenakiana*, *D. lilliputiana*, *D. gnoma*, *D. susanae*, *D. toscanoi*, *D. vitorinoi*, *D. wuerstlei* e *D. zebrina*. Através deste tratamento taxonômico atualizamos as descrições morfológicas, realizadas por Luer em 2005; apresentamos ilustrações mais detalhadas, juntamente

com fotografias; elaboramos uma chave de identificação e novos registros de ocorrência para alguns estados brasileiros e oito lectótipos foram designados.

O Capítulo III Aborda morfometricamente três espécies ocorrentes na Floresta Atlântica: *D. edwallii*, *D. wuerstlei* e *D. zebrina*. Os resultados obtidos a partir das análises multivariadas de 40 variáveis levantadas, demonstraram que *D. edwallii* e *D. zebrina* devem ser tratadas como sinônimos. Apresentamos a nova circunscrição para *D. zebrina* (= *D. edwallii*) e para *D. wuerstlei*, com fotos e comentários taxonômicos.

O Capítulo IV trata de uma nova espécie descrita para o Brasil, endêmica do Sul da Floresta Atlântica, em Santa Catarina. *Dryadella catharinensis* que anteriormente foi citada como *D. aviceps* durante estudos da flora de orquídeas de Joinville-SC. O Capítulo V descreve e compara a anatomia de raiz, rizoma, ramicaule e folhas espécies de *Dryadella*, a fim de investigar a morfologia interna dos órgãos vegetativos, entre espécies de distribuição disjunta. Os resultados demonstraram caracteres anatômicos inéditos para o gênero em relação à subtribo, mas não encontramos diferenças entre os grupos de Floresta Atlântica e dos Andes, tão poucos entre as seções propostas para o gênero. Dada a quantidade de caracteres anatômicos vegetativos sinapomórficos dentro gênero, a partir deste estudo foi possível compreender mais a fundo as relações evolutivas em *Dryadella*.

Vale ressaltar que, apesar dos esforços recentes em Pleurothallidinae, muito ainda tem a ser feito para compreender a relação entre os gêneros da subtribo. Os resultados aqui apresentados para o gênero *Dryadella*, embora de grande importância e filogeneticamente bem suportados, representam apenas uma parcela no conhecimento geral deste enorme grupo. Esta tese também reforça que estudos filogenéticos e biogeográficos têm auxiliado na compreensão de eventos que influenciaram a diversificação de Orchidaceae no espaço e no tempo. Outros estudos sobre biologia reprodutiva e polinização são fortemente sugeridos e podem contribuir ainda mais para as hipóteses filogenéticas e compreensão do gênero e da subtribo. Os trabalhos taxonômicos e nomenclaturais também são essenciais para a compreensão da diversidade em geral, pois, sem esses trabalhos, é impossível estimar a diversidade real. As orquídeas são alvo de interesse de muitos orquidófilos e colecionadores, e neste sentido, é importante que taxonomistas e entusiastas considerem que o trabalho deve ser conjunto, especialmente na área da taxonomia e que tenham domínios dos critérios para descrição de novos táxons bem como da taxonomia e morfologia geral dos grupos, pois a taxonomia é muito importante e quando realizada de forma errônea, afeta o andamento

de outras áreas, com a conservação e regulamentação para a comercialização, por exemplo, bem como a estimativa do número correto da biodiversidade.