Diversidade de glândulas florais em Pleurothallidinae (Epidendroideae – Orchidaceae) ocorrentes no Brasil

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Yẹmọnja àwa
Ààbò a yó
Yẹmọnja
Awa ààbò a yó

Ìyáàbà ó dé iré ṣé
Akíì ẹ Yẹmọnja
A kọkọ pè ilé gbè a ó yó
Odò ó fí a ṣà
Wẹ̀ rẹ̀ ó

A ṣà wẹ̀ lé ó
Odò fí ó
A ṣà wẹ̀ lé ó
A ṣà wẹ̀ lé

Ìyá kòròba
Kòròba ní sábà
Ìyá kòròba
Kòròba ní sábà

RESUMO

A subtribo Pleurothallidinae destaca-se como um dos maiores grupos em Orchidaceae polinizado por dípteros. Esta subtribo tem passado por uma notável reformulação taxonômica, em que todas as sinapomorfias morfológicas já relatadas remetem às características florais. Devido à evolução com seus polinizadores, as flores dos representantes desta subtribo apresentam glândulas de características únicas, cujas relações evolutivas são pouco conhecidas. Sendo assim, este estudo promoveu análises estruturais de glândulas florais de espécies brasileiras de Pleurothallidinae, enfatizando as alterações morfológicas apresentadas pelas diferentes glândulas dentro da flor. Microscopia eletrônica de varredura e microscopia de luz foram utilizadas para examinar a estrutura e a ocorrência destas glândulas no ovário, sépalas e labelo das flores de 28 representantes brasileiros de Pleurothallidinae. Coléteres foram identificados nas invaginações da parede externa do ovário e, fora das invaginações, ocorreram glândulas constituídas por elevações com poro e tricomas glandulares unicelulares. Três morfotipos de osmóforos, sticky-exudate glands, e nectários formados por tricomas unicelulares, foram identificados em diferentes locais na superfície adaxial das sépalas e labelo. A presença de coléteres nas invaginações do ovário pode ser uma sinapomorfia de Pleurothallidinae. Também foram observadas, pela primeira vez, em Pleurothallidinae, a ocorrência de outras estruturas secretoras além dos coléteres na parede externa do ovário. Os resultados referentes à estrutura das glândulas florais enfatizam a grande semelhança entre Pleurothallidinae e *Bulbophyllum*, devida à convergência evolutiva relacionada à polinização por moscas. Além disso, o padrão de glândulas observado no labelo, quando comparado com as filogenias disponíveis, mostrou uma transição entre nectários, “sticky-exudate glands” e osmóforos. Essa transferência de função provavelmente é um caso de homoheterotopia na subtribo Pleurothallidinae.

Palavras-chave: Anatomia, coléteres, glands, morfologia, nectários, osmóforos, heterotopia, *Bulbophyllum*.

ABSTRACT

The subtribe Pleurothallidinae is highlighted as one of the largest group of Orchidaceae pollinated by Diptera. The taxonomy of this subtribe has undergone a taxonomic reorganization in which all the recognized synapomorphies are related to floral features. Due to the evolution with their pollinators, Pleurothallidinae flowers exhibit unique glands whose evolution is poorly known. Thus, this investigation provides structural studies of floral glands from Brazilian Pleurothallidinae species, emphasizing the morphological changes shown by different glands inside the flower. Scanning electron and light microscopy were utilized to examine the structure and occurrence of glands in ovary, sepals and labellum of flowers of 28 Brazilian Pleurothallidinae representatives. Colleters were identified in the invaginations of the external ovary wall and out of the invaginations other glands formed by elevations with stomatal pore and glandular unicellular trichomes occurred. Three morphotypes of osmophores, sticky-exudate glands, and nectaries formed by unicellular trichomes were identified in different localities in the adaxial surface of the sepals and labellum. The presence of ovarian colleters can be a sinapomorphy of Pleurothallidinae. The occurrence of other glands besides colleters in the external ovary wall were observed for the first time in Pleurothallidinae. The results concerning the structure of the floral glands emphasize the great similarity between Pleurothallidinae and *Bulbophyllum*, due to the evolutionary convergence related to the pollination by flies. In addition, the pattern of gland occurrence observed in the lip, when compared to the available phylogenies, showed a transition between nectaries, "sticky-exudate glands" and osmophores. This transfer of function is probably a case of homoheterotopy in the subtribe Pleurothallidinae.

Key-words: Anatomy, colleters, morphology, nectaries, osmophores, glands, heterotopy, *Bulbophyllum*.

SUMARIO

1 INTRODUÇÃO GERAL

1.1 Família Orchidaceae Juss.

Orchidaceae pertence à ordem Asparagales (APG, 2006) e possui aproximadamente 27.800 espécies (TPL, 2017), sendo considerada a segunda maior família em Angiospermas pelos atuais sistemas de classificação. A maior diversidade desta família é encontrada nas regiões tropicais (Atwood, 1986). O Brasil possui cerca de 2550 espécies distribuídas em 238 gêneros (Forzza et al., 2015) encontrados em todas as formações vegetais (Hoehne, 1949, Pabst e Dungs, 1975). Particularmente em florestas úmidas como a Mata Atlântica com 1.257 espécies distribuídas em 176 gêneros, sendo 791 destas espécies endêmicas (Barros et al. 2009).

A família é reconhecida pela alta complexidade e diversidade de suas flores resultado de relações coevolutivas com distintos polinizadores (Judd et al., 2009). Suas inflorescências podem ser terminais quando surgem do ápice, e laterais quando surgem da base ou de algum nó do caule (ou cauloma), sendo essencialmente racemos ou panículas. As flores são geralmente zigomorfas, monoclinas, sésseis ou pediceladas. Além disso, também pode ocorrer a ressupinação, fenômeno que pode ser ocasionado pela torção em 180° do pedicelo e/ou ovário, por mudança do sentido da inflorescência, ou por uma inclinação do ovário e/ou pedicelo, isto durante o desenvolvimento floral, que consequentemente altera a posição do labelo de superior para inferior. O perianto possui três sépalas e três pétalas, em que a pétala oposta ao estame fértil é morfologicamente diversa e denominada labelo, possuindo alto valor taxonômico (Fig. 1).

O androceu é constituído geralmente por uma antera fértil (raramente duas ou três) e opercular, ou seja, composta por um “capuz” que normalmente cai com a retirada do pólen. O pólen, geralmente, esta aglutinado em polínias podendo conter apêndices, compondo o polinário. O filete e o estilete estão adnados em uma estrutura denominada de ginostêmio ou coluna, em que a base pode apresentar-se morfologicamente variada constituindo um pé, que pode estar adnado as sépalas laterais formando um mento e ainda articulado com o labelo. O estigma é trilobado e esta frequentemente na região ventral, sendo um dos lobos morfologicamente modificado, promovendo uma separação entre a antera e o estigma, denominado rostelo. O ovário é ínfero, tricarpelar, unilocular (geralmente), com placentação parietal.

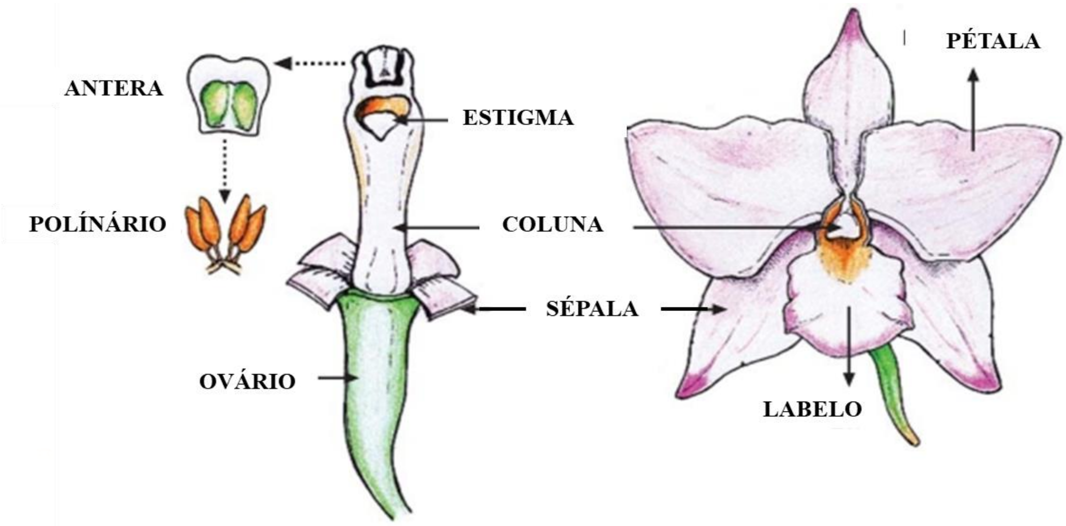

Fig.1: Diagrama de uma flor típica de orquídea (adaptado Aceto e Gaudio 2011).

Diversas classificações infra-familiares foram propostas para Orchidaceae (Dressler, 1993, Cameron et. al., 1999, Freudenstein e Ramussen, 1999, Cameron, 2004, Freudenstein et al., 2004). O mais recente sistema de classificação (Pridgeon et al., 2010) é baseado em caracteres morfológicos e moleculares e tende, gradativamente, a substituir os anteriores (Pridgeon et al., 1999, 2001, 2003, 2005). Neste sistema, a família Orchidaceae é dividida em cinco subfamílias: Apostasioideae,Cypripedioideae, Vanilloideae, Orchidoideae e Epidendroideae, em que Epidendroideae se destaca por possuir o maior número de espécies; entretanto, alguns grupos inseridos nesta subfamília apresentam problemas taxonômicos e não são monofiléticos.

1.2 Subtribo Pleurothallidinae Lindl.

A subtribo Pleurothallidinae é a maior da tribo Epidendreae (subfamília Epidendroideae), com cerca de 5000 espécies (Karremans, 2016), sendo conhecido como o maior grupo em Orchidaceae polinizado por dípteros (Pridgeon et al., 2001, 2010; Borba et al., 2011). A subtribo é restrita aos neotrópicos, ocorrendo principalmente no sul da Flórida, bacia do Caribe, México e Argentina. No Brasil, possui 627 espécies (Barros et. al. 2015) em que o gênero *Acianthera* se destaca com 104 espécies endêmicas (Barros et. al. 2015).

Esta subtribo tem passado por uma notável reformulação taxonômica, em que a filogenia atualmente aceita (Pridgeon et. al., 2001), baseada em caracteres moleculares, divide Pleurothallidinae em nove clados com gêneros poli e parafiléticos, e insere um pequeno clado composto por três gêneros ornitófilos e auto-compatíveis da América Central (*Dilomilis*, *Neocogniauxia* e *Tomzanonia*). Assim, a identificação de sinapomorfias morfológicas e anatômicas em Pleurothallidinae foi dificultada pela recorrente homoplasia observada nos caracteres vegetativos e florais (Pridgeon et. al., 2001).

No entanto, a concepção atualmente vigente (Borba et al., 2011) recomenda a divisão da subtribo em duas, uma considerando a antiga circunscrição (Luer, 1986) e a outra englobando o pequeno clado de espécies ornitófilas. Assim, a articulação entre o pedicelo e o ovário permaneceria como a principal sinapomorfia morfológica que unificava Pleurothallidinae (Luer, 1986), e são incluídas duas novas sinapomorfias biológicas, a miiofilia e a auto-incompatibilidade (Borba et al., 2011). Ainda, com base nos estudos recentes de Cardoso (2014), caso esta proposta seja aceita, duas novas sinapomorfias são reconhecidas: a redução do número de traços que vascularizam o ginostêmio e a presença de alcanos como constitutivos ubíquos dos voláteis florais (Cardoso-Gustavson et al., 2017). Estudos moleculares em desenvolvimento, envolvendo maior número de espécies (incluindo as brasileiras selecionadas para o presente estudo) têm disponibilizado novas filogenias (e.g., Pridgeon et al., 2010, Chiron et al., 2012) que consideram estas discussões e, assim, tendem a substituir as propostas anteriores.

Ressalta-se que todas as sinapomorfias já propostas para Pleurothallidinae envolvem características florais. De fato, as flores desta subtribo apresentam atrativos visuais, químicos e estruturais para guiar os seus polinizadores, em que as estruturas secretoras (ou glândulas) possuem um papel central ao sintetizar recompensas verdadeiras como o néctar, ou mimetizar voláteis e estruturas no engodo sexual ou alimentar (Dressler, 1993, Pemberton, 2010). Pleurothallidinae apresenta, ainda, outras glândulas florais que apresentam características únicas em seus representantes (veja detalhamento adiante), e cujas relações evolutivas ainda são completamente desconhecidas.

1.3 Glândulas em Pleurothallidinae Lindl.

O néctar é o principal recurso floral em Orchidaceae (van der Pijl e Dodson 1966) oferecido em nectários florais estruturalmente diversos (Stpiczyńska, 2003, Davies et al., 2005, Stpiczyńska et al., 2005, Melo et al., 2010). Alguns grupos de Diptera são adaptados para obter seu alimento a partir das flores, mas outros são atraídos para elas por engano (van der Pijl e Dodson, 1966). As flores miiófilas apresentam nectários superficiais, produzem néctar em abundância e emitem odor adocicado, enquanto as sapromiiófilas produzem odores putrescentes e frequentemente não produzem néctar (van der Pijl, 1966, Faegri e van der Pijl, 1979). Estas síndromes estão associadas às famílias Mycetophilidae, Sciaridae, Drosophilidae e Syrphidae (van der Pijl e Dodson 1966), esta última considerada a mais importante em relação aos polinizadores de Orchidaceae (Endress 1994, Proctor et al., 1996), principalmente na subtribo Pleurothallidinae. No entanto, são poucos os estudos que analisam o papel efetivo destas moscas como polinizadores, principalmente nas regiões tropicais (Arruda e Sazima, 1996). Em Pleurothallidinae, os nectários são referidos como tricomas unicelulares que produzem pequenas quantidades de néctar e são encontrados no labelo (Borba e Semir, 2001, Barbosa et al., 2009, Melo et al., 2010, Cardoso, 2014). Este recurso floral não é comumente encontrado em espécies sapromiiófilas, entretanto parece ser um importante elemento no mecanismo de polinização por engano de espécies de *Acianthera* (Borba e Semir, 2001).

Osmóforos (ou glândulas de odor) são ubíquos nesta subtribo (Pridgeon et. al., 2010), produzindo e liberando compostos voláteis associados ou não à atração de polinizadores (Dudareva e Pichersky, 2006, Wiemer et al., 2009). A ocorrência de alcanos constituindo majoritariamente o bouquet floral foi proposta como uma nova sinapomorfia em Pleurothalldiinae (Cardoso, 2014). Estas glândulas são descritas como células epidérmicas simples, poros ou tricomas localizados na superfície do labelo, pétalas, sépalas e ovário (Swanson et al., 1980, Endress, 1994, Teixeira et al., 2004, Ascensão et al., 2005, Wiemer et al., 2009, Melo et al., 2010, Cardoso, 2014).

Em Pleurothalldiinae, os coléteres caracterizam-se como tricomas glandulares presentes nas brácteas e nas invaginações da parede externa do ovário (Cardoso-Gustavson et al., 2014). Estas glândulas produzem secreção mucilaginosa e/ou lipofílica (Esau 1977, Fahn, 1979, Cardoso-Gustavson et al., 2014) que protege os órgãos vegetativos e reprodutivos em diferenciação contra a dessecação (Kronestedt-Robards e Robards, 1991, Paula e Oliveira, 2007), enquanto a porção lipofílica pode atuar como dissuasivo contra microorganismos (Demarco, 2005).

Glândulas com características únicas foram identificadas e denominadas “sticky-exudate glands” por Cardoso (2014). Estas glândulas apresentam-se como elevações no ápice da superfície adaxial das sépalas laterais e no labelo de *Echinosepala aspasicensis*, e produzem um exsudato heterogêneo constituído por lipídios e polissacarídeos com propriedades adesivas, provavelmente relacionado à polinização por engodo alimentar/sexual (Cardoso, 2014).

2 OBJETIVOS

2.1 Objetivos gerais

Os objetivos propostos neste estudo pretenderam responder às seguintes questões:

(1) Quais são os tipos e a localização das glândulas presentes nos órgãos florais das espécies-alvo?

(2) Como é a estrutura geral destas glândulas e como elas são caracterizadas morfologicamente?

(3) Há alguma transição evolutiva quando as variações morfológicas de cada morfotipo glandular das espécies-alvo são comparadas às filogenias disponíveis?

2.2 Objetivo específico

Este trabalho propõe-se a: descrever e inventariar a micromorfologia e anatomia das glândulas de flores em antese sob microscopia eletrônica de varredura e óptica.

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DIVERSITY OF THE FLORAL GLANDS IN THE BRAZILIAN PLEUROTHALLIDINAE SPECIES (EPIDENDROIDEAE – ORCHIDACEAE)*

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4 INTRODUCTION

The subtribe Pleurothallidinae with about 5000 species (Karremans, 2016) has been through remarkable taxonomic revisions. The currently accepted phylogeny (Pridgeon et al., 2001) based on molecular characters, splits the subtribe in nine poly and paraphyletic clades and still includes a small clade composed of three ornithophylous and self-compatible genera of central America (*Dilomilis*, *Neocogniauxia* and *Tomzanonia*). This makes more difficult the identification of morphologic and anatomic synapomorphies in Pleurothallidinae due to the greater homoplasy of their characters (Pridgeon et al., 2001). However, the current proposal (Borba et al., 2011) recommends that this subtribe has to be divided in two, one considering the previous circumscription (e.g. Luer, 1986) and the other comprising the small ornithophylous genera. Thus, all the synaphomorphies are preserved and it is noteworthy that they are all related to the floral characters that are closely involved with the fly-pollination syndromes.

The pantropical genus *Bulbophyllum* and the American subtribe Pleurothallidinae are the two largest fly-pollinated groups within Orchidaceae (van der Pijl and Dodson, 1966), being the most interesting case of morphological convergence due to the analogous pollination syndrome (Dressler, 1981, 1993). Fly-pollination is the second biggest pollination syndrome in orchids (Borba and Semir, 2001) being related to about 20 families of Diptera (Christensen, 1994), and probably evolved in unrelated groups several times along the evolution of the family. Some Diptera groups are adapted to get their food from the flowers, but others are attracted by deception (van der Pijl and Dodson, 1966). The myophilous flowers normally have exposed nectaries, produce plenty of nectar and emit sweetish odors, while the sapromyophilous produce putrescent odors and usually do not produce nectar (van der Pijl, 1966, Faegri and van der Pijl, 1979, Cardoso-Gustavson et al. 2017). These syndromes are related to the Mycetophilidae, Sciaridae, Drosophilidae and Syrphidae families (van der Pijl and Dodson, 1966); the last seems to be the most important in relation to their pollinators in Orchidaceae (Endress, 1994, Proctor et al., 1996), mainly in Pleurothallidinae (Melo et. al. 2010).

Pleurothallidinae flowers show visual, chemical, and morphological attractives to guide their pollinators, in which the glands have a fundamental role. The nectaries in Pleurothallidinae have been described as labellar unicellular trichomes that release small amounts of nectar (Borba and Semir 2001, Barbosa et al., 2009, Melo et al., 2010, Cardoso, 2014). This reward is not commonly observed in sapromyophilus flowers, but seems to be important in the pollination syndrome in *Acianthera* (Borba and Semir 2001, Melo et al., 2010). The osmophores (or scent glands) are ubiquitous in this subtribe (Pridgeon et al., 2010), producing and releasing volatile compounds involved in long distance pollinators attraction (Melo et al. 2010). These glands had been described as pores or trichomes located at the labellum surface and sepals (Melo et al., 2010, Cardoso, 2014). The sticky-exudate glands have unique features and were identified as elevations at the apex of the adaxial surface of the lateral sepals and labellum of *Echinosepala aspasicensis*. These glands release a heterogenous exudate constituted of lipids and polysaccharides with adhesive properties (Cardoso, 2014). In addition to that, even though not related to the pollination, glands were also identified in the bracts and invaginations of the external ovary wall, being characterized as colleters (Cardoso-Gustavson et al., 2014).

Previous investigations of the flower ontogeny, focused on the floral glands of the Pleurothallidinae (Cardoso-Gustavson et al., in prep), showed important variations in the morphology of floral osmophores and nectaries, and the occurrence of unique glands within the subtribe. These variations could be associated to heterotopic changes of these glands along the different clades of the subtribe – considering the currently accepted phylogenies (Pridgeon et al., 2001, 2010; Pridgeon and Chase, 2001, Chiron et al., 2012). Here, we performed an inventory of floral glands in 28 species of Brazilian Pleurothallidinae representatives, describing their morphological and topological variations, looking for insights and propose some evolutionary hypothesis over the alterations of these glands in this subtribe.

5 MATERIAL AND METHODS

5.1 Plant material

Inflorescences of 28 Brazillian Pleurothallidinae species (Table 1) were collected from plants cultivated in the living orchid collection of the Instituto de Botânica (São Paulo, Brazil). Vouchers were deposited at SP Herbarium situated in the same institution.

5.2 Anatomical studies

The material was fixed in 2.5% glutaraldehyde solution buffered at pH 7.2 with 50 mM sodium cacodylate buffer and kept in this solution at 4℃ until further procedures.

For scanning electron microscopy (SEM), floral organs were isolated, dehydrated in an ethanol series, and dried in a Leica EM CPD300 critical point dryer. Samples were mounted on stubs, coated with gold in a Leica ACE200 sputtering system and viewed with a SEM FEI Quanta 250 at 10kV. Digital images were edited using Adobe Photoshop version 7.0.

For light microscopy, the material was embedded using standard methods for Technovit (Kulzer Technik) historesin, transversal and longitudinally sectioned at 2 μm thickness. Sections were stained with toluidine blue/p-phenylenediamine (1% aqueous/1% isopropanol: methanol, 1:1) for metachromasy and lipids identification, respectively (Feder and O'Brien, 1968; Kivimäenpää et al., 2004), and mounted in water. Observations and digital images were acquired with an Olympus BX53 compound microscope equipped with an Olympus I-Color 5 digital camera and Image Pro Express 6.3 software.

5.3 Gland’s identification

The identification was made based on the histochemical and ontogenetic study of seven Pleurothallidinae representatives (Cardoso 2014).

Table 1. List of living collection and voucher data of Pleurothallidinae species investigated. Species distributed according to the current phylogeny (Pridgeon et. al., 2001, 2010; Chiron et al., 2012).

Species / Clade	Collection site (Brazil)	Collection number	Voucher
Octomeria-Brachionidium clade			
Octomeria grandiflora Lindl.	Bertioga, SP; Ilha do Cardoso, Cananéia, SP; Nova Friburgo, RJ; Reserva Biológica de Paranapiacaba, Santo André, SP	16060; 10373, 12844, 12696; 5938; 11929	SP401779; SP401864, SP401846; SP401848; SP401793
O. juncifolia Barb.Rodr.	Parati, RJ; Parque Estância Turística do Parque do Ribeira, Iporanga, SP; São Sebastião, SP	3212, 3213; 17880; 13637	SP401794
O. gracilis Lodd. ex Lindl.	Morro do Diabo, Teodoro Sampaio, SP	14300	SP491492
Echinosepala-Barbosella clade			
Myoxanthus lonchophyllus (Barb.Rodr.) Luer	Camanducaia, MG; Porangaba, SP	1039; 10563, 10566, 10569, 10670	SP401885; SP401790, SP401862, SP401863
M. seidelii (Pabst) Luer	BA	13246	SP491485
Pleurothallopsis nemorosa (Barb.Rodr.) Porto & Brade	Parque Estadual da Serra do Mar, Caraguatatuba, SP; AM	17354; 14688	SP401832
Barbosella gardneri (Lindl.) Schltr.	South region of Bahia, BA	13533	SP491490
Acianthera clade			
Acianthera recurva (Lindl.) Pridgeon & M.W.Chase	Brotas, SP; Nova Friburgo, RJ; São Carlos, SP	3687, 3668; 6061; 392	SP401875; SP401870
A. pubescens (Lindl.) Pridgeon & M.W.Chase	Guaira, PR; Itaverava, Sítio da Cachoeira, MG; Tremembé, SP	P5856; 13266; 16739	SP401837; SP401811
A. saurocephala (Lodd.) Pridgeon & M.W.Chase	Guarulhos, SP; Ilha do Cardoso, Cananéia, SP; Poços de Caldas, MG; Serra da Bocaina, Bananal, SP; Serra do Ouro Branco, Ouro Branco, MG	P6730; 12924; 12269, 12270; 16659; P6801, P6802, P6803, P6811	SP401843; SP401851, SP401852
Sansonia bradei Chiron	Chapada dos Guimarães, Cuiabá, MT	15809	SP491495
Zootrophion-Trichosalpinx clade			
Trichosalpinx memor (Reichb.f.) Luer	Serra da Neblina, Pacaraima, RR	16304	SP491491
Anathallis modesta	*	*	SP491496

(Barb.Rodr.) Pridgeon & M.W.Chase			
A. aristulata (Lindl.) Luer	Serra do Ouro Branco, Ouro Branco, MG	P6666, P6691, P6693	SP491494
A. rubens (Lindl.) Pridgeon & M.W.Chase	Juquitiba, SP	13350	SP491487
A. sclerophylla (Lindl.) Pridgeon & M.W.Chase	Carangola, MG; Ilha do Cardoso, Cananéia, SP	14997D, 14997; 12880, 15737; 11795	SP401827; SP401861
Pabstiella-Pleurothallis-Stelis clade			
Pabstiella punctatifolia (Barb.Rodr.) Luer	*	13480	SP491488
P. ramphastorhyncha (Barb.Rodr.) L.Kollmann	Ji-Paraná, RO	15223	SP491493
P. sp. nov.	Fazenda Salto do Indaia, Chapadão do Sul, MG	P5337, P5338, P5339	*
Pleurothallis loranthophylla Rchb.f.	*	*	SP491497
P. ruscifolia (Jacq.) R.Br.	Maranguape, CE	13402	SP401836
Stelis aprica Lindl.	Reserva Biológica do Alto da Serra de Paranapiacaba, Santo André, SP	13015	SP401841
S. deregularis Barb.Rodr.	Caraguatatuba, SP; Ilha do Cardoso, Cananéia, SP; Ourilândia, São Felix do Xingú, PA; Porangaba, SP; Reserva Biológica de Paranapiacaba, Paranapiacaba, SP	15764; 12434, 12441, 12442, 12694, 13136, 15940,17936; 14907; 10567; 11931; 12355	SP401822; SP401830
S. aff. *papaquerensis* Rchb.f.	Reserva Florestal do Rio Grande, SP	652; P6198	SP491486
Scaphosepalum-Andinia clade			
Dryadella lilliputana (Cogn.) Luer	Bananal, Serra da Bocaina, SP; São José do Barreiro, SP	P5712, P5713; 12733	SP401847
Specklinia grobyi (Batem. ex Lindl.) F.Barros	Curitibanos, SC; Serra da Bocaina, Bananal, SP	4718; 16659; A856	SP401873; SP401887
S. spiculifera (Lindl.) Pridgeon & M.W.Chase	Reserva Biológica de Una, Una, BA	P4808	SP491489
Trisetella-Dracula clade			
Masdevallia infracta Lindl.	Parque Nacional de Sete Quedas, Guaira, PR; Presidente Figueiredo, AM; Serra da Neblina, Pacaraima, RR; Serra da Bocaina, Bananal, SP	6522; 13497; 16307; 17123	SP401835

*number/information pending or material under preparation.

6 RESULTS

6.1 Flower morphology

The pivotal morphological features of each species evaluated here (Table 1) are described below. All flowers are resupinate and have the labellum articulated with the column foot.

In *Octomeria grandiflora* (Fig. 1A), *O. juncifolia* (Fig. 1B) and *O. gracilis* (Fig. 1C), the inflorescence is fasciculate, sepals and petals are free from each other (and petals smaller than sepals), yellowish, membranaceous. The labellum is trilobed and auriculated, with a purple color in the central region in *O. grandiflora*.

In *Myoxanthus seidelii* (Fig. 1D) and *M. lonchophyllus* (Fig. 1E) the inflorescence is fasciculate 1-flowered, the flowers are yellowish and produced, successively, the sepals are linear-lanceolate, lateral ones connate at the base. The petals are linear and the labellum is trilobed, sub-deltoid with the margin fimbriate, and in *M. seidelii* the labellum is reddish.

In *Pleurothallopsis nemorosa* (Fig. 1F) the inflorescence is 1-flowered with yellowish flowers, the dorsal sepal lanceolate and the lateral ones connate in a lanceolate synsepal, the petals are lanceolate and the labellum is yellowish and lingulate.

In *Barbosella gardneri* (Fig. 1G) the inflorescence is 1-flowered with greenish-yellow flowers, the dorsal sepal is lanceolate, the lateral ones connate in an obovate synsepal, the petals are lanceolate and the labellum is yellowish, fleshy and trilobed.

In *Acianthera recurva* (Fig. 1H), *A. pubescens* (Fig. 1I), *A. saurocephala* (Fig. 1J), the inflorescence is a raceme, the flowers are purple with the outer surface pubescent. The dorsal sepal is linear-laceolate with yellowish acute apex in *A. recurva*, while in *A. pubescens* is lanceolate and in *A. saurocephala* is obovate. All the species have the lateral sepals connate in an obovate synsepal, the petals is obovate to oblong and the labellum trilobed in *A. recurva*. The petals have margin serrate and labellum lingulate in *A. pubescens* and in *A. saurocephala* the petals have apex acuminate and labellum panduriform, bilobed at the base.

In *Sansonia bradei* (Fig. 1K) the inflorescence is 1-flowered with purple flowers, the sepals are linear-lanceolate, the dorsal one connate with the lanceolate lateral ones in a synsepal, the petals are lanceolate, the labellum is lingulate with apex fimbriate.

In *Tricosalpinx memor* (Fig. 1L) the inflorescence is a raceme with brownish flowers, the dorsal sepal is oblong, with apex cream-colored and yellowish at the center, the lateral sepals are connate in a obovate synsepal the petals are oblong and yellowish, and the labellum is lingulate.

In *Anathallis modesta* (Fig. 1M) and *A. aristulata* (Fig 1N) the inflorescence is 1-flowered, the flowers in *A. modesta* are purplish and greenish-yellow in *A. aristulata*, the sepals in both are free, lanceolate, the petals are lanceolate and the labellum in *A. modesta* is trilobed and auriculate at the base, while in *A. aristulata* is lingulate. In *A. rubens* (Fig. 1O) and *A. sclerophylla* (Fig. 1P) the inflorescence is a raceme. *A. rubens* have greenish flowers, the sepals are free, linear-lanceolate, the petals are obovate, and the labellum is trilobed and panduriform, while in *A. sclerophylla* flowers are yellow, dorsal sepal are free and linear-lanceolate, the lateral ones connated only at base, the petals are oblong, and the labellum is trilobed and sub-deltoid with a purple spot.

In *Pabstiella punctatifolia* (Fig 1Q) and *Pabstiella ramphastorhyncha* (Fig. 1R) the inflorescence is a raceme, in *Pabstiella* sp. nov. (Fig. 1S) the inflorescence is a few-flowered raceme. The flowers in *P. punctatifolia* are purple, linear-lanceolate, the dorsal sepal is free and the lateral connate in a lanceolate synsepal, the petals are linear to linear-lanceolate and the labellum is trilobed and panduriform. In *P. ramphastorhyncha* the flowers are yellow with longitudinal purple stripes, while in *Pabstiella* sp. nov. are only yellow, both have linear-lanceolate, and free dorsal sepals, the lateral ones in *P. ramphastorhyncha* connate in a obovate synsepal, the petals are obtrullate and the labellum trilobed and sagittate. In *Pabstiella* sp. nov., the lateral sepals are connate at the base, the petals are linear and the labellum is panduriform.

In *Pleurothallis loranthophylla* (Fig. 1T) the inflorescence is a 1-flowered successive raceme with purple flowers, the dorsal sepal are free, and lanceolate and the lateral ones connate in an obovate synsepal, the petals are linear-lanceolate and the labellum are panduriform. In *Pleurothallis ruscifolia* (Fig. 1U) the inflorescence is fasciculate with greenish-yellow flowers, with dorsal sepal lanceolate, the lateral ones connate in a lanceolate synsepal, the petals are linear and the labellum is trilobed and sub-deltoid.

In *Stelis aprica* (Fig. 1V) and *S. deregularis* (Fig. 1W) the flowers are membranaceous and greenish-yellow, while in *S.* aff. *papaquerensis* the flowers are purple (Fig. 1X) and in all the species the inflorescence is a raceme with the sepals ovate and connate at the base in a pateliform synsepal. The petals are concave and the labellum is trilobed with mucronate apex in *S. aprica*. Petals are ovate and the labellum is fleshy and trilobed in *S deregularis* and *S.* aff. *papaquerensis*.

In *Dryadella liliputana* (Fig. 1Y) the inflorescence is 1-flowered, the flowers have greenish-cream color and purple spots, the sepals are ovate, the dorsal one free, the lateral ones connate at the base and obovate, the petals are obtrullate, and the labellum is lingulate.

In *Specklinia grobyi* (Fig. 1Z) the inflorescence is a raceme and the flowers have outer surface yellow with longitudinal purple stripes, the sepals are lanceolate, the dorsal one free, the lateral ones connate in a wide-lanceolate to obovate synsepal, the petals are elliptical and the labellum is reddish and lingulate. While in *Specklinia spiculifera* (Fig. 1AA) the inflorescence is a 1-flowered with purple flowers, the sepals are purple, lanceolate, free, the petals are lanceolate and the labellum is lingulate.

In *Masdevallia infracta* (Fig. 1AB) the inflorescence is 1-flowered, with purplish flowers, the sepals are fleshy, concave, connate at the base in a campanulate synsepal with greenish-yellow aristate apex, the petals are greenish-yellow, reduced with rounded apex, and the labellum is greenish-yellow and lingulate.

The ovary, sepals and the labellum of the selected Brazilian Pleurothallidinae species exhibited different types of glands at varied locations that are described in the anatomical chapter below.

6.2 Micromorphology and Anatomy

External ovary wall (Figs. 2–3): colleters and other glands

In all species examined, the external ovary wall had six invaginations where the colleters were located (Fig. 2A–B, 3A–B). These glands were formed by uniseriate and bicellular trichomes with a clear distinction between the head and stalk cells (Fig. 3C), and release a profuse exudate (Fig. 3A–C).

Most of the species showed a glabrous epidermis outside of the invagination (Fig. 2C, 3A), except *Acianthera recurva*, *A. pubescens* and *A. saurocephala*, which had non-glandular trichomes all over the epidermis (Fig. 2D, 3B). *Myoxanthus lonchophyllus* and *M. seidelii* exhibited two glandular structures: unicellular trichomes (Fig. 2E, 3D–E), with a dense protoplast and lipid droplets (Fig. 3E), and rounded elevated structures with a stomatal pore (Fig. 2E, 3D, F). The latter also showed glandular unicellular trichomes flanking it and together with the adjacent glandular parenchyma have lipid droplets (Fig. 3F). These elevations with stomatal pore were also identified in *Octomeria gracilis* (Fig. 2F, 3G–H), however, the cells flanking the pore were ordinary epidermal cells without lipid droplets (Fig. 3H).

Osmophores (Fig. 4–5)

Three morphotypes of osmophores (Table 2) described below were identified in the adaxial surface of the sepals (Fig. 4A–H) and labellum (Fig. 5A–N):

(1) Elevations of the epidermis (Fig. 4A–C), observed at the apex of *Masdevallia infracta* sepals (Fig. 4A), and in the central region of this organ in *M. lonchophyllus and M.*

seidelii (Fig. 4B). Non-glandular trichomes were observed all over the edge and abaxial surface of sepals (Fig. 4B). These glands were formed by the elevations of the palisade epidermis and the adjacent glandular parenchyma. The palisade epidermis had a dense protoplast with many lipid droplets and a large vacuole (Fig. 4C).

(2) Elevations with stomatal pore (Fig. 4D–E), located at the apex of sepals in *M. lonchophyllus*, *M. seidellii*, *P. nemorosa* and *A. pubescens* (Fig. 4D). Unicellular glandular trichomes flanked the stomatal pore. The abaxial surface of *A. pubescens* and *A. recurva* exhibited non-glandular trichomes. The glandular cells that constitute the elevations formed by the proliferation of parenchyma tissue had dense protoplast with lipid droplets and a large vacuole (Fig. 4E).

(3) Unicellular glandular trichomes (Fig. 4F–H, 5A–N), observed from the base up to the center of the fused sepals in *Pabstiella ramphastorhyncha* and *Stelis aprica* (Fig. 4F, H), and from the center to the apex in all sepals of *Anathallis modesta* (Fig. 4G). These glands appeared only at the apex in *Octomeria grandiflora*, *O. juncifolia*, *O. gracilis*, *Barbosella gardneri*, *Acianthera recurva*, *Sansonia bradei*, *Trichosalpinx memor*, *A. aristulata*, *A. rubens*, *A. sclerophylla* (co-occurring with non-glandular trichomes), *Pabstiella punctatifolia*, *P. ramphastorhyncha*, *P.* sp. nov., *Pleurothallis loranthophylla*, *P. ruscifolia*, *Stelis aprica*, *S. deregularis*, *S.* aff. *papaquerensis*, *Dryadella lilliputiana*, *Specklinia grobyi*, and *S. spiculifera*. These glands at this position are restricted to the dorsal sepal in *Pabstiella punctatifolia* and *Dryadella lilliputiana*, but occurred in both sepals' surface in *Octomeria* species. The unicellular trichomes exhibited smooth cuticle, dense protoplast with many lipid droplets and a large vacuole (Fig. 4H).

Non-glandular trichomes are observed all over the labellum edge in *Myoxanthus lonchophyllus* (Fig. 5A) and in *Anathallis modesta* (Fig. 5B), while glandular trichomes were visualized at the center of labellum in the following species: *Octomeria gracilis* (Fig. 5C), *Octomeria juncifolia*, *Acianthera recurva*, *A. pubescens*, *Sansonia bradei*, *Trichosalpinx* *memor* (with an acute apex; Fig. 5D), *Anathallis aristulata*, *Pabstiella punctatifolia*, *P. ramphastorhyncha* (Fig. 5E), *Stelis aprica* (Fig. 5F), *Stelis deregularis*, *Dryadella liliputana*, *Specklinia grobyi*, and *Masdevallia infracta* (Fig. 5G). These glands occurred from the center up to the apical region only in *Stelis* aff. *papaquerensis* (Fig. 5H). Glandular trichomes were also observed in saliences at the region of the labellum (Fig. 5I). These cells showed cuticle ornamentation in *Pleurothallis ruscifolia* (Fig. 5J) Glandular trichomes over saliences of parenchyma tissue were observed in *Octomeria juncifolia* (Fig. 5K). The glandular unicellular trichomes and the adjacent parenchyma (proliferated or not) had a dense protoplast with many lipid droplets and a large vacuole (Fig. 5L–N).

Sticky-exudate glands (Fig. 6)

These glands were located from the center to the apex of the sepals in *Acianthera saurocephala* (Fig. 6A), and were constituted by smooth rounded elevations of palisade epidermis and proliferated parenchyma, a stomatal pore were also observed (Fig. 6B).These glands released a profuse exudate (Fig. 6C). Both glandular epidermis and parenchyma showed a dense protoplast and large vacuole.

These glands were also identified in the adaxial surface of the labellum of some species, showing similarities with the sepal afore mentioned ones. However, the glands in the labellum did not show stomatal pores. Structurally, they were also formed by elevations of the palisade epidermis and the proliferation of the adjacent glandular parenchyma. In *A. saurocephala* (Fig. 6D–E), the glandular epidermis had many small starch grains (Fig. 6E). The labellum had a grooved aspect in *M. seidelii* (Fig. 6F–G), in which the cell wall of the epidermis exhibit a strong metachromasy, while the protoplast was dense and had many lipids droplets and a large vacuole (Fig. 6H). In *A. sclerophylla* these elevations reflected edges along the labellum (Fig. 6I). Interestingly, a large aerenchyma is observed in the mesophyll (Fig. 6J). The same structure was observed in *Pleurothallis loranthophylla* (Fig. 6K–M). However, the palisade epidermis is not continuous, i.e, there is an absence of bound between the anticlinal walls and the epidermis appeared as groups of cells, giving a fimbriate aspect to this tissue (Fig. 6K, L). The cells that had anticlinal bounds exhibited many plasmodesmata (Fig. 6M). The vacuolar content and the cell wall of these cells possessed a strong metachromasy (Fig. 6M).

Nectaries (Fig. 7)

These glands were identified as glandular unicellular trichomes in the labellum of *Barbosella gardneri*, *Octomeria grandiflora*, *Pleurothallopsis nemorosa* and *Anathallis rubens*. The trichomes occurred since the base up to the center, or were restricted to the central region of the adaxial surface of the labellum (Fig. 7A–B, D–E), exhibiting a dense protoplast with several nucleoli and a large vacuole (Fig. 7C, F)

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Fig. 1. Inflorescence and flowers of Brazilian Pleurothallidinae species. (A) *Octomeria grandiflora*. (B) *O. juncifolia*. (C) *O. gracilis*. (D) *Myoxanthus lonchophyllus*. (E) *M. seidelii*. (F) *Pleurothallopsis nemorosa*. (G) *Barbosella gardneri*. (H) *Acianthera recurva*. (I) *A. pubescens*. (J) *A. saurocephala*. (K) *Sansonia bradei*. (L) *Trichosalpinx memor*. (M) *Anathallis modesta*. (N) *A. aristulata*. (O) *A. rubens*. (P) *A. sclerophylla*. (Q) *Pabstiella punctatifolia*. (R) *P. ramphastorhyncha*. (S) *Pabstiella.* sp. nov. (T) *Pleurothallis loranthophylla*. (U) *P. ruscifolia*. (V) *Stelis aprica*. (W) *S. deregularis*. (X) *S. aff. papaquerensis*. (Y) *Dryadella liliputana*. (Z) *Specklinia grobyi*. (AA) *S. spiculifera*. (AB) *Masdevallia infracta*. Scale bars = 1 cm (A-E, G-J, L- P, W, X, Z, AB); 0,5 cm (F, K, N, Q-V, Y, AA).

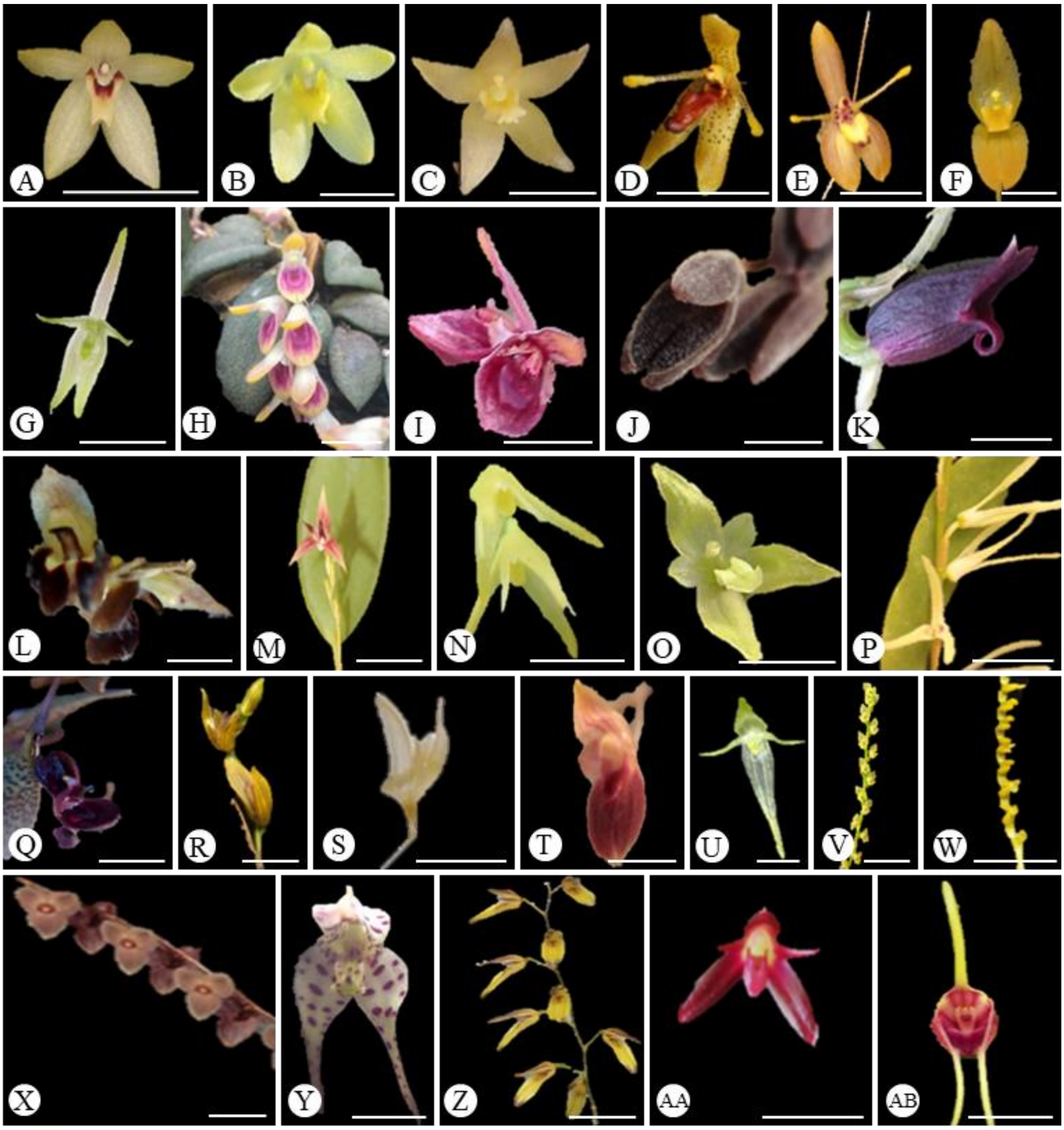
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Fig. 2. Scanning electron micrographs of the glands located at the external ovary wall of selected Brazilian Pleurothallidinae species. Transverse sections (A–B) and frontal view (C–F) of the external ovary walls. (A) *Octomeria grandiflora*, note the invaginations (asterisks) where colleters are found. (B) Colleter (glandular trichomes, arrowhead) in *Sansonia bradei*. (C) Glabrous epidermis of *Stelis aprica*. (D) Non-glandular trichomes in *Acianthera saurocephala*. (E) Glandular trichome (dashed square) and rounded elevations with a stomatal pore (red arrows) in *Myoxanthus lonchophyllus*. (F) Stomatal pores (yellow arrows) in *Octomeria gracilis*. Scale bars = 500 μm (C); 250 μm (E); 200 μm (A, D, F); 150 μm (B).

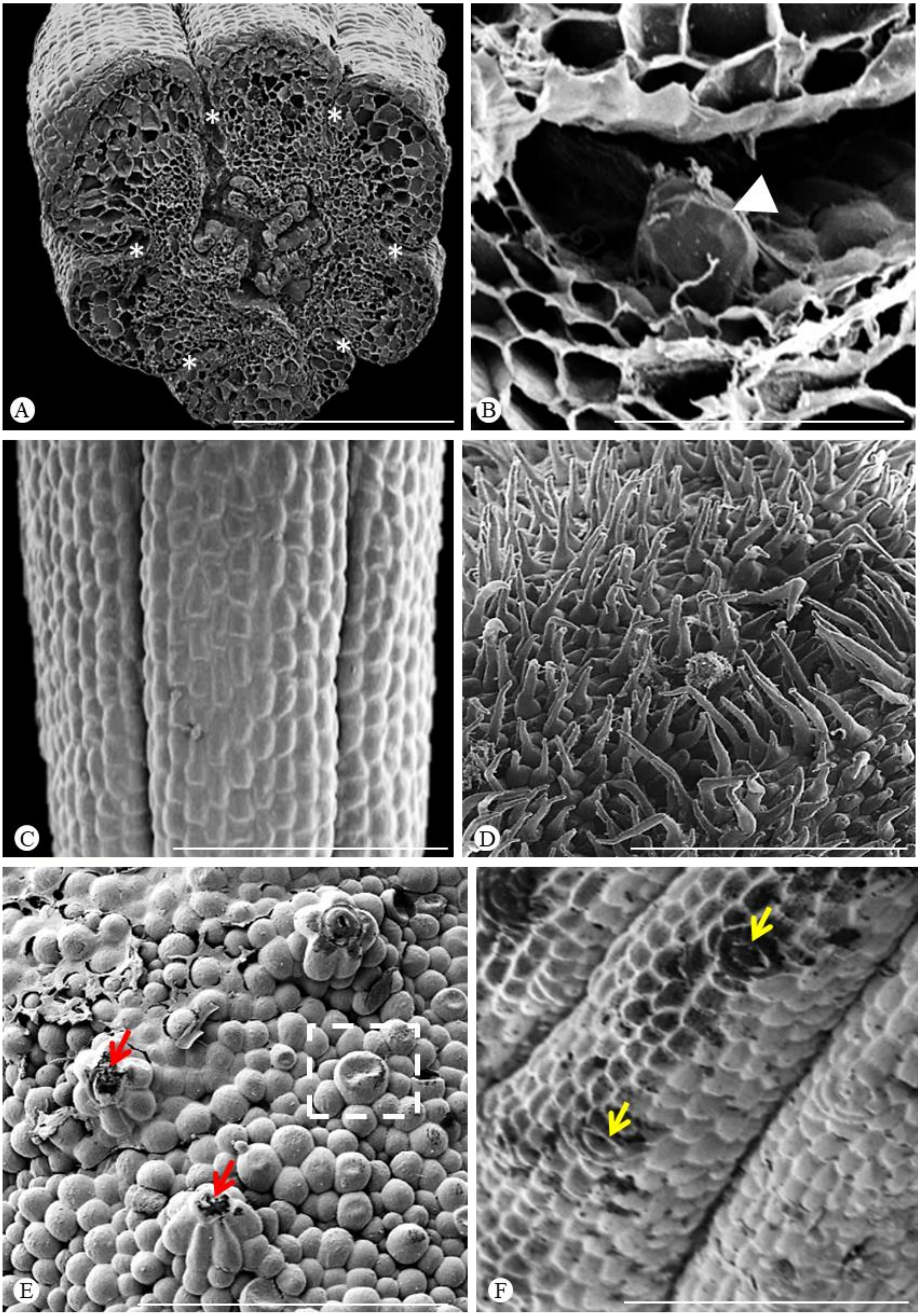
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Fig. 3. Transverse sections of the ovary of selected Brazilian Pleurothallidinae species. (A) Epidermis without ornamentation in *Tricosalpinx memor*. (B–C) *Acianthera saurocephala*, note the epidermis with non-glandular trichomes and the profuse exudate from colleters in the invagination of external ovary wall (yellow asterisks in B). Detail of a colleter (C). (D–F) *Myoxanthus lonchophyllus*. (D–E) Glandular unicellular trichomes (black arrow in D) consisting the glandular epidermis, some of the trichomes have a dense protoplast with lipid droplets (black arrowheads in E). (D, F) Elevated rounded structures with stomatal pores (red arrow in D). Stomatal pore (red arrowhead in F) and the air chamber (black asterisk) below the cells; note the glandular unicellular trichomes (blue arrows) flanking the pore and together with the adjacent glandular parenchyma have lipid droplets (black arrowheads). (G, H) Elevation with pores in *Octomeria gracilis* (green arrowhead in G); note the detail of the stoma and he glandular parenchyma with lipid droplets (black arrowheads in H). (A–H) Toluidine blue + p-phenylenidiamine (TBO+phe). Scale bars = 25 µm.

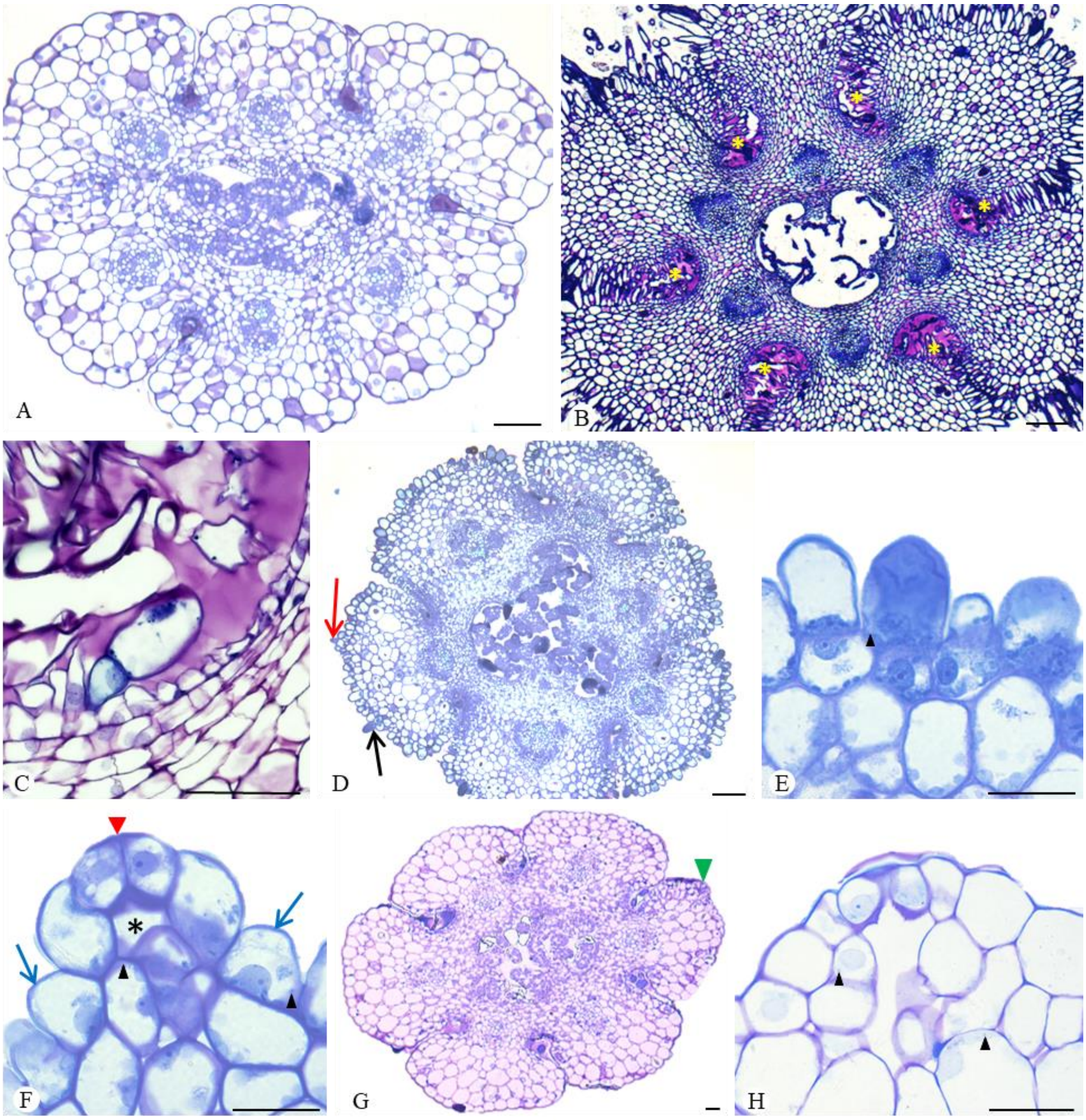
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Fig. 4. Osmophores in the adaxial surface of the sepals of selected Brazilian Pleurothallidinae species. (A–C) Saliences formed by the elevations of the palisade epidermis and the adjacent secretory parenchyma. (D–E) Elevated rounded structures with a stomatal pore at the apex. (F–H) Unicellular glandular trichomes. (A) *Masdevallia infracta*; saliences all over the apex (yellow arrowhead). (B–C) *Myoxanthus seidelii*; non-glandular trichomes (red arrowhead in B) and elevations of the epidermis (red arrow in B) formed by cells with dense protoplast and lipid droplets (black arrowheads in C). (D–E) *Pleurothallopsis nemorosa*; stomatal pore (yellow arrow in D), note the stoma and the air chamber (black asterisk in E) below the cells, and the unicellular trichomes (blue arrows in E) flanking the stoma. (F) *Pabstiella ramphastorhynca*; the glandular trichomes (*inset*) from the base up to the center of the fused sepals (red asterisk). (G) *Anathallis modesta*. (H) *Tricosalpinx memor*; longitudinal section: glandular trichomes exhibited dense protoplast with lipid droplets (black arrowheads) and a large vacuole. (A–B, D, F–G) SEM. (C,E, H) TBO+phe. Scale bars = 1mm (F), 500 µm (B, C, H), 200 µm (A, D, G), 25 µm (C, E, H), 10 µm (*inset* in F).

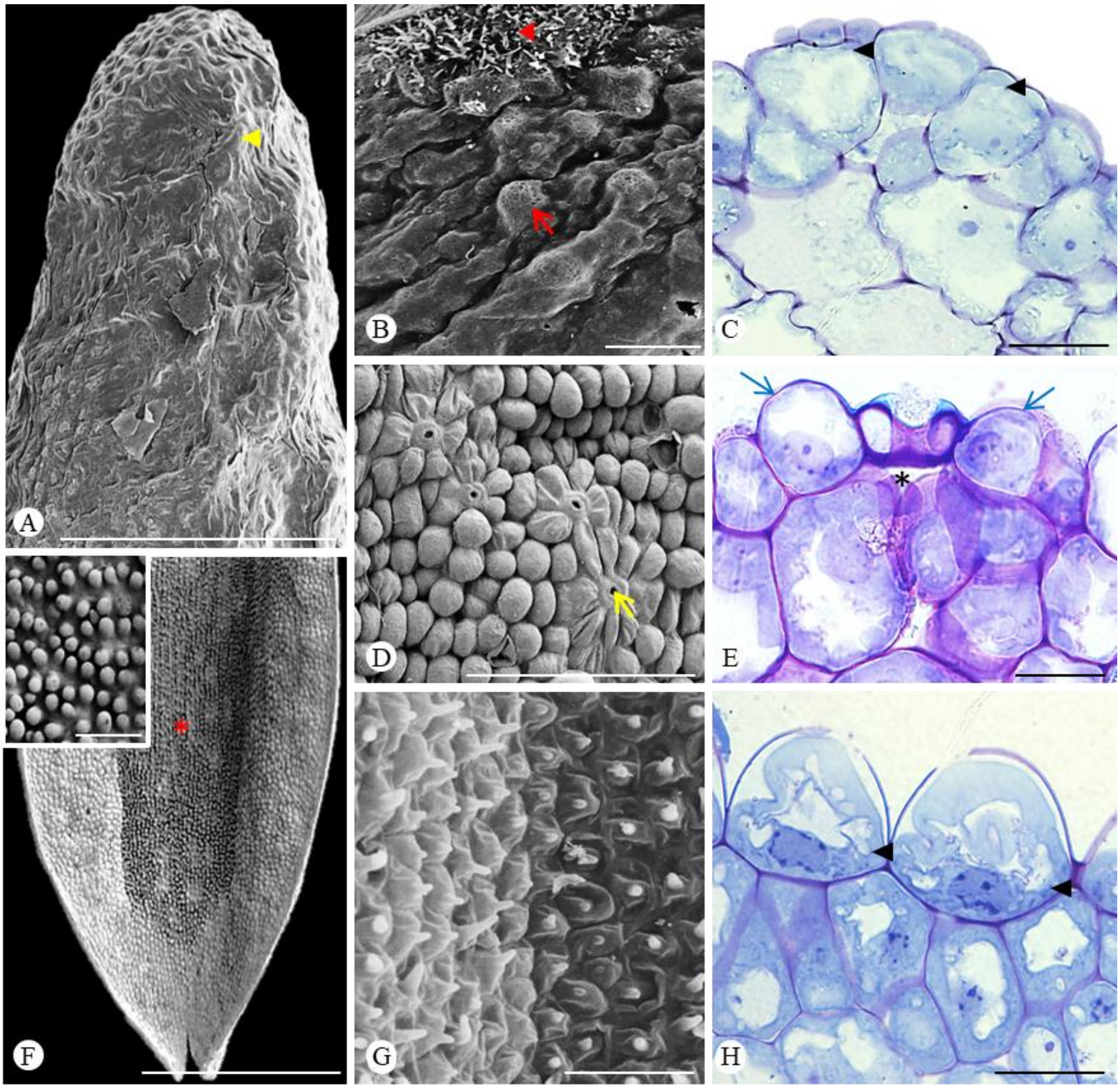
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Fig. 5. Osmophores (unicellular glandular trichomes) in the labellum of selected Brazilian Pleurothallidinae species. (A) *Myoxanthus lonchophyllus*. (B) *Anathallis modesta*. (A–B) Glands occur in the central region (red asterisk in A) and non-glandular trichomes all over the edge of the labellum (red arrowhead in A and B). (C) *Octomeria gracilis*. (D) *Tricosalpinx memor*. (E) *Pabstiella ramphastorhyncha*. (F) *Stelis aprica*. (G) *Masdevallia infracta*. (H) *Stelis* aff. *papaquerensis*; these glands occur from the center up to the apical region of the labellum (yellow asterisk). (I–J) *Pleurothallis ruscifolia*, note some glandular trichomes in salience (I) and their cuticle ornamentation (J). (K–L) *Octomeria juncifolia*, the glandular trichomes in salience (K) have a dense protoplast and a large vacuole (black arrows in L). (M) *Acianthera recurva*. (N) *Sansonia bradei*. (M–N) The glandular trichomes and the adjacent parenchyma have a dense protoplast with lipid droplets (black arrowheads) and a large vacuole. (A–K) SEM. (L–N) TBO+phe. Scale bars = 1 mm (A), 200 µm (B, E), 100 µm (C, G, H, K), 50 µm (D, J), 25 µm (F, I, L–N).

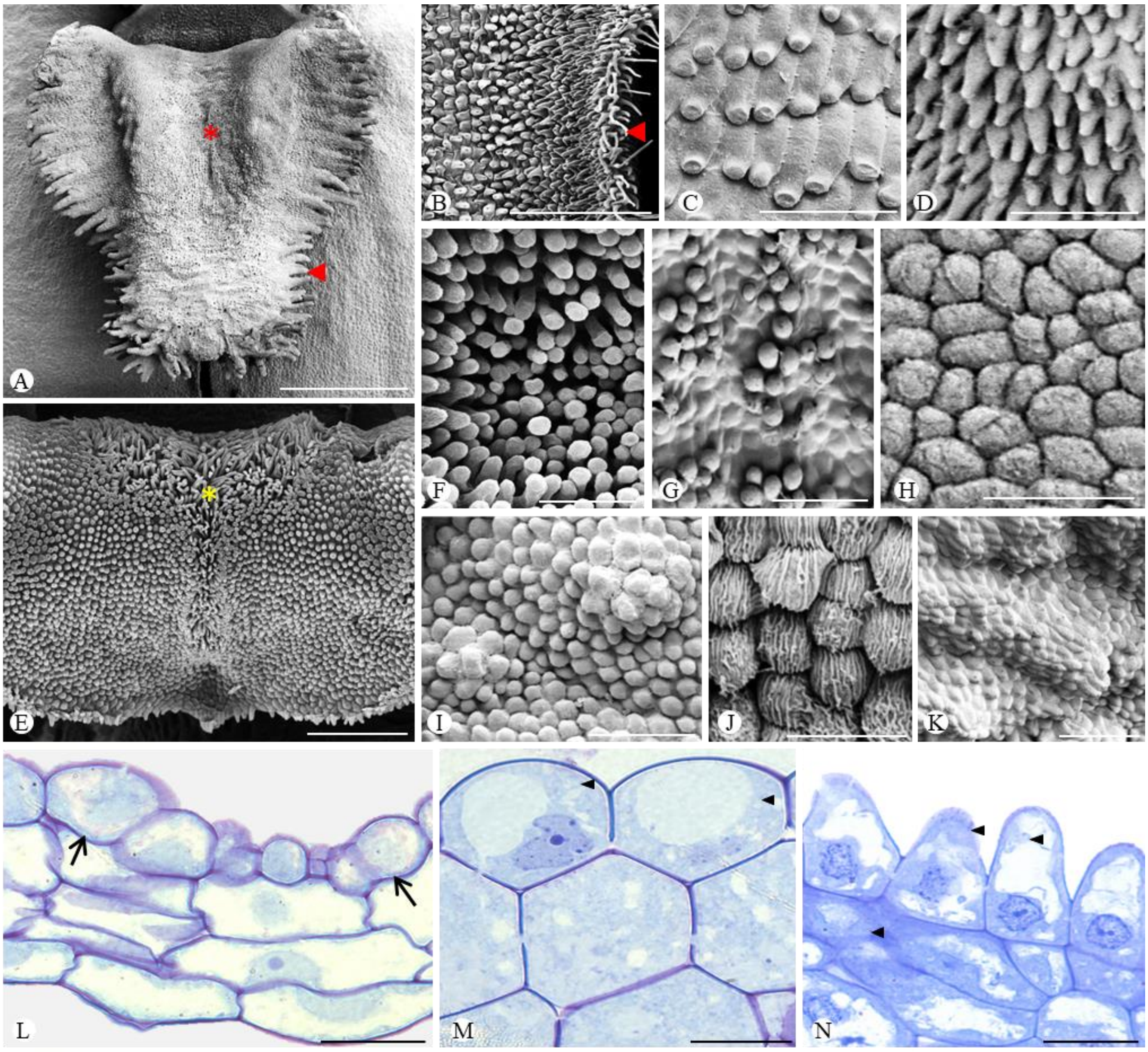
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Fig. 6. Sticky-exudate glands of the selected Brazilian Pleurothallidinae species. Saliences formed by the elevations of the glandular palisade epidermis and the adjacent glandular parenchyma occur from the central to the apex of the sepals and in the central region of the labellum. (A–E) *Acianthera saurocephala*; rounded saliences with stoma pore occurred in the adaxial surface of the sepals (A–B, green arrowhead in C). The glandular epidermis and adjacent parenchyma exhibits dense protoplast and a large vacuole, note the profuse exudate (*) released by the glands (C). The glandular epidermis occurred in the central region of the labellum (yellow asterisk in D), and, together with the adjacent parenchyma, shows many small starch grains (black arrowheads in E). (F–H) *Myoxanthus seidelii*; note the grooved aspect of the labellum (G). The glandular epidermis shows a strong metachromasy of the cell wall, many plasmodesmata (green arrowheads in H) and dense protoplast with many lipid droplets (red arrowheads in H). (I, J) *Anathallis sclerophylla*. The glandular epidermis constitutes edges along the labellum (I). The glandular epidermis and adjacent parenchyma exhibits dense protoplast and a large vacuole; note the occurrence of a large aerenchyma (black asterisks in J). (K–M) *Pleurothallis loranthophylla*: note the elevations on the entire surface (K, L); these glands are formed by palisade cells, but some of them do not show anticlinal bounds (M). These cells have a dense protoplast and the vacuolar content shows a strong metachromasy. Note the occurrence of many plasmodesmata in the anticlinal walls (green arrowheads in M). (A–B, D, F–G, I, K–L) SEM. (C, E, H, J, M) TBO+phe. Scale bars = 2 mm (F), 1 mm (A, D, K), 300 µm (L), 250 µm (G), 100 µm (B, I), 25 µm (C, E, H, J, M).

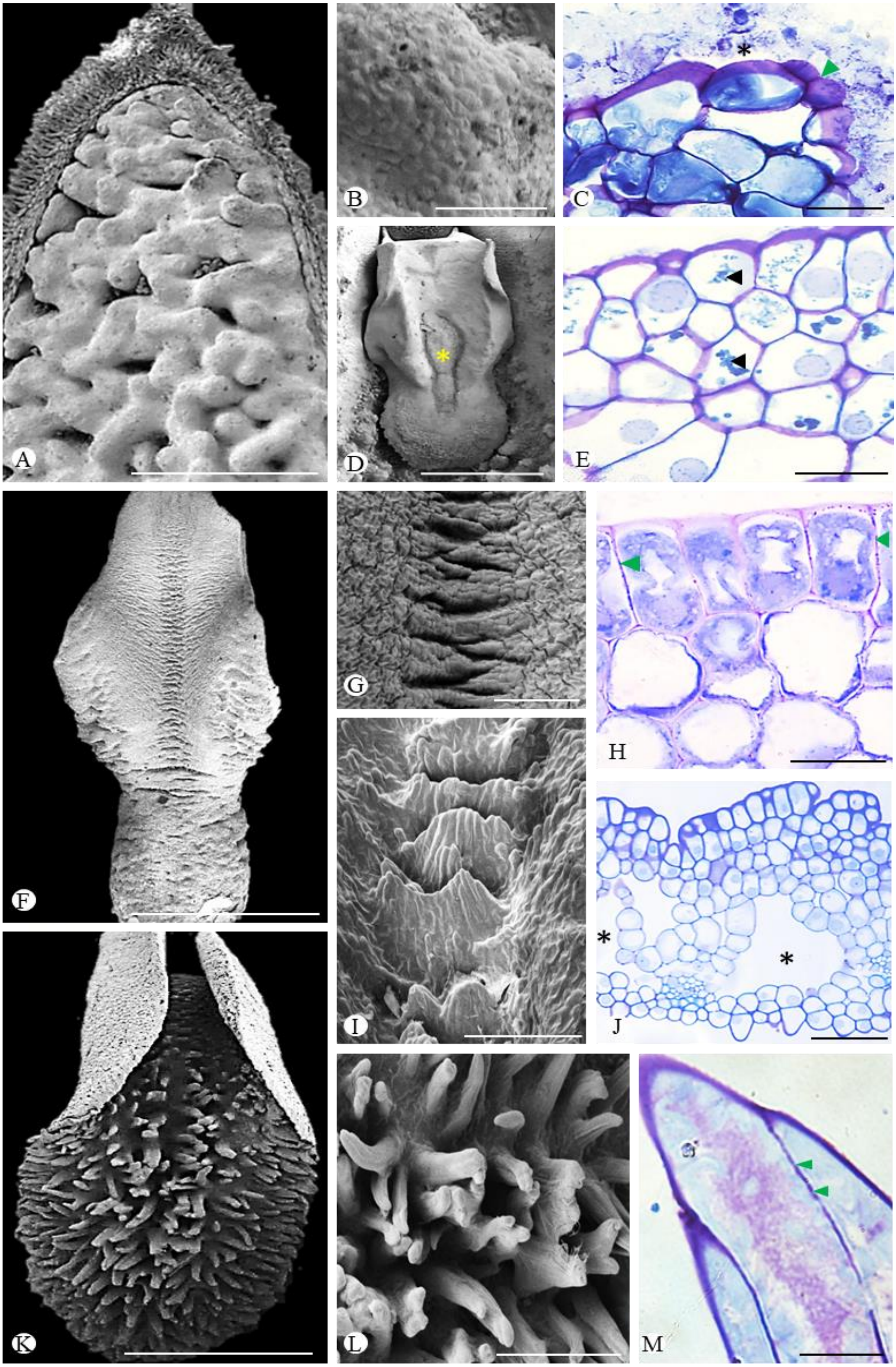
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Fig. 7. Nectaries in the labellum of selected Brazilian Pleurothallidinae species. (A-C) Glandular unicellular trichomes of *Barbosella gardneri* from the base up to the center of the labellum (red asterisks in A); these glands are restricted to the central region (B) and the glandular trichomes exhibit dense protoplast and several nucleoli (red arrowheads in C). (D-F) Glandular unicellular trichomes occur in the central region of the labellum of *Anathallis rubens* (D, dashed square); these glands present smooth cuticle (E) and the secretory epidermis exhibit dense protoplast and a large vacuole (black arrowhead in F). (A–B, D–E) SEM. (C, F) TBO+phe. Scale bars = 2 mm (D), 1 mm (A), 400 µm (E), 300 µm (B), 25 µm (C, F).

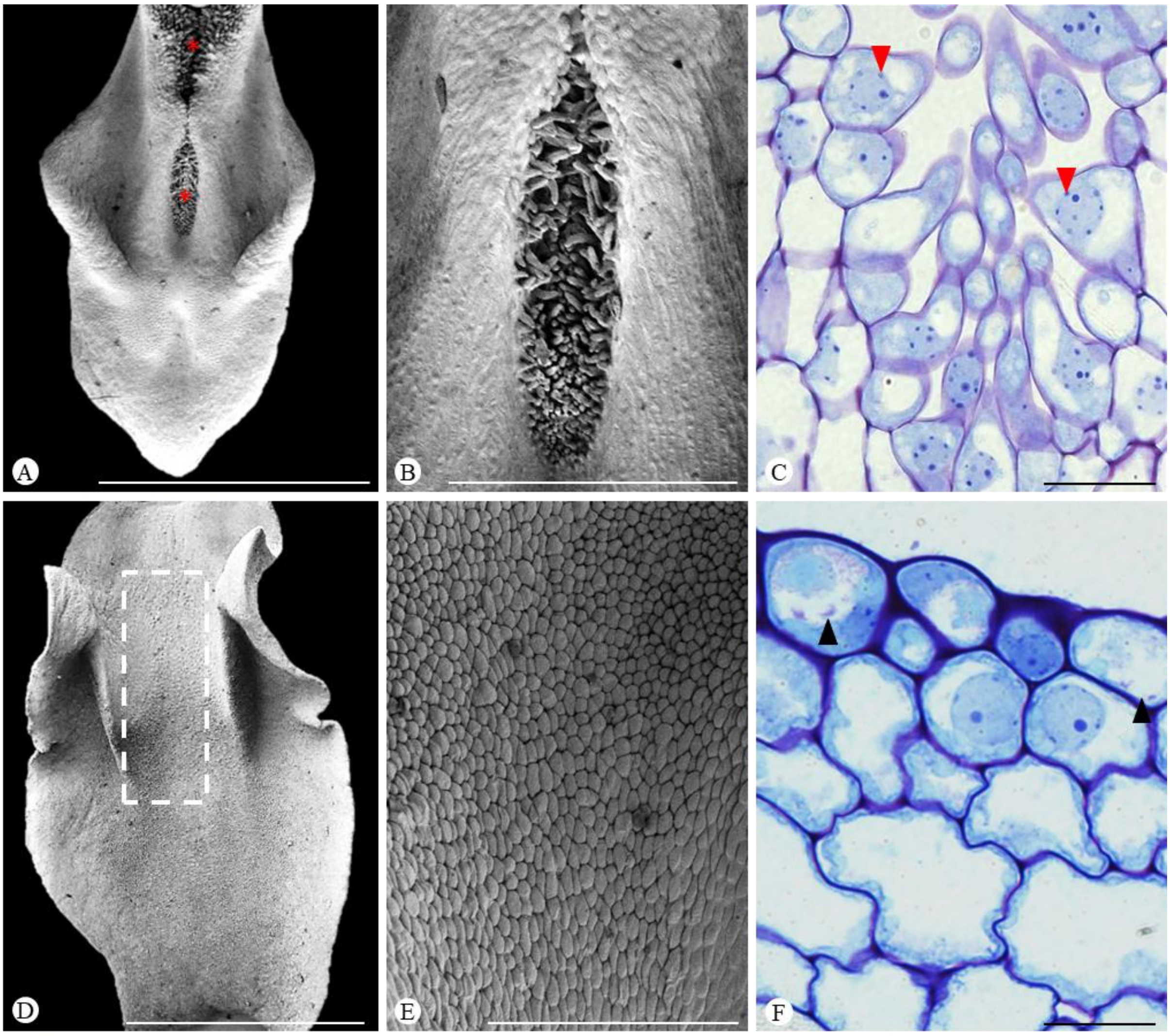
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Table 2. List of the glands identified in the selected Brazillian Pleurothallidinae species.

Species	Ovary[a]	Labellum	Sepals
	External ovary wall (outside the invaginations)		
Octomeria grandiflora	*	nectaries	osmophores
O. juncifolia	*	osmophores	osmophores
O. gracilis	elevation with pores	osmophores	osmophores
Myoxanthus lonchophyllus	glandular unicellular trichomes and elevated rounded structures with pores	osmophores	osmophores
M. seidelii	glandular unicellular trichomes and elevated rounded structures with pores	sticky-exudate glands	osmophores
Pleurothallopsis nemorosa	*	nectaries	osmophores
Barbosella gardneri	*	nectaries	osmophores
Acianthera recurva	*	osmophores	osmophores
A. pubescens	*	osmophores	osmophores
A. saurocephala	*	sticky-exudate glands	sticky-exudate glands
Sansonia bradei	*	osmophores	osmophores
Trichosalpinx memor	*	osmophores	osmophores
Anathallis modesta	*	osmophores	osmophores
A. aristulata	*	osmophores	osmophores
A. rubens	*	nectaries	osmophores
A. sclerophylla	*	sticky-exudate glands	osmophores
Pabstiella punctatifolia	*	osmophores	osmophores
P. ramphastorhyncha	*	osmophores	osmophores
P. sp. nov.	*	osmophores	osmophores
Pleurothallis loranthophylla	*	sticky-exudate glands	osmophores
P. ruscifolia.	*	osmophores	osmophores
Stelis aprica	*	osmophores	osmophores
S. deregularis	*	osmophores	osmophores
S. aff. *papaquerensis*	*	osmophores	osmophores
Dryadella lilliputana	*	osmophores	osmophores
Specklinia grobyi	*	osmophores	osmophores
S. spiculifera	*	osmophores	osmophores
Masdevallia infracta	*	osmophores	osmophores

[a] all species examined present colleters in the invagination of the external ovary wall; *glabrous epidermis

7 DISCUSSION

7.1 Morphology, glands and pollination

The nectaries here identified occur in flowers with myophilous features, since their colors are between yellow and greenish-yellow, as also occurred in the flowers of a previous investigation (Cardoso, 2014). However, in some Pleurothallidinae nectaries also occur in flowers with sapromyophilous features (Melo et al., 2010), where sticky-exudate glands apparently occur with purplish flowers as in *Echinosepala aspasicensis* (Cardoso, 2014). An exception is *Anathallis sclerophylla*, that has yellow flowers and only a purple spot in the labellum but seems to be a myophilous flower (Barbosa et al., 2009). Also in *Bulbophyllum*, nectaries and glands with similar characteristics to sticky-exudate glands, occur in flowers with sapromyophilous features, bearing colors between brownish and purplish (Teixeira et al., 2004; Davies and Stpiczyńska, 2014).

The osmophores are related to fly attraction, changing the floral odors composition to pleasant odor with nectar reward in myophylous orchids (Barbosa et. al., 2009; Borba et al., 2011; Cardoso-Gustavson et al., 2017) or unpleasant odors such as of a decomposing organism, fecal-like odors, and mimicking odors emitted by mushrooms (Garner et al., 2007; Urru et al., 2011; Siddique et al., 2012, Woodcok et al., 2014). Thus, the sticky-exudate glands may be involved in the food/brood deception pollination (Cardoso, 2014).

7.2 Colleters and glands at the external ovary wall

Colleters are glands that occur in vegetative and/or reproductive organs, release an exudate composed mostly of mucilage, lipophilic compounds, or both and to a lesser extend terpenes, proteins and phenolic compounds (Fahn, 1979). Structurally, can be multicellular trichomes or emergences (Fahn, 1979). Among their possible functions, it has been mentioned the protection and lubrication of developing organs (Thomas, 1991), and their action as a chemical barrier against insects and microorganisms (Miguel et al., 2006; Muravnik and Kostina, 2011; Ribeiro et al., 2017).

There are few reports of colleters in monocots, firstly described as "axillary squamules" in the axis of vegetative leaves (Dahlgren and Clifford, 1982). In Orchidaceae the first report was in bracts of *Rodriguezia venusta* (Leitão and Cortelazzo, 2008), and later in leaf primordium of protocorms, apical and axillary buds, and at the base of bracts and sepals of *Oncidium flexuosum* (Mayer et al., 2011). Glandular trichomes at the invaginations of the external ovary wall of seven Pleurothallidinae representatives were identified as colleters by Cardoso-Gustavson et al. (2014). According to these authors, the occurrence of colleters contrasts with the occurrence of nectaries at this position in other Epidendroideae, being possibly a case of homeosis. Here, 28 Pleurothallidinae species were analyzed, all of them bear colleters. Comparing to other Epidendroideae subtribes, it is possible to hypothesize the ovarian colleters as another synapomorphy of Pleurothallidinae. The occurrence of colleters is probably an apomorphy in other groups of monocots, as in Rapateaceae (Poaceae) (Oriani and Scatena, 2012; Ferrari and Oriani, 2017), thus proving to be taxonomically important. This highlights the importance of these glands in future investigations about group circumscriptions in monocots.

Non-glandular trichomes were observed all over the external ovary wall of the studied species of *Acianthera*, and also reported in *Echinosepala aspasicensis* (Cardoso-Gustavson et al., 2014). There are few investigations about the possible functions of these structures in angiosperm families (Corsi and Bottega, 1999; Bottega and Corsi, 2000; Machado et al., 2006). These non-glandular trichomes create a mechanical layer isolating the flower from the rest of the plant, protecting the floral organs from herbivory, and also against the excess of transpiration and solar radiation as had been previously reported (Corsi and Bottega, 1999; Bottega and Corsi, 2000; Machado et al., 2006). This flower isolation might also reduce the access of robbers to floral resources – e.g. nectar by ants in species that do offer a reward, such as some *Acianthera* spp. (Melo et al., 2010; Almeida, 2016).

Due to the shortage of floral biology studies concerning Pleurothallidinae species, it is only possible to make inferences about the glands at the external ovary wall (besides colleters) comparing with other similar structures observed in other regions of the flower. Therefore, glands at this position may be identified as osmophores or extrafloral nectaries, and may assume functions in pollination syndromes that are beyond the scope of this study.

Glandular trichomes at the external ovary wall that release lipophilic compounds have been reported in other angiosperm families, such as Bignoniaceae (*Zeyheria montana*; Machado et. al., 2006) and Asteraceae (*Helichrysum stoechas*; Ascensão et al., 2000). In Orchidaceae, the only available description is in the ovary of *Cypripedium reginae* (Cypripedioideae, Orchidaceae; Swanson et al., 1980). In Pleurothallidinae, these glands were observed in *Myoxanthus*, in which the occurrence of lipid droplets in the protoplast was another evidence of the chemical nature of these glands.

Elevations with stomatal pore were also identified here as the osmophores in sepals of *Acianthera* species (Melo et al., 2010; Cardoso-Gustavson, 2014; Pansarin et al., 2016). However, glands with similar morphology proved to be nectar-producing in the pedicel of *Coleogyne* (Subedi et al., 2011) and in bracts of *Epidendrum tridactylum* (Pansarin and Pansarin, 2014). Here, these structures were identified in *Octomeria gracilis*, co-occurring with glandular trichomes in the ovary of *Myoxanthus* spp., but with no evidence of these glands acting as nectaries (e.g., the occurrence of nectar droplets). However, the observation of lipid droplets in the adjacent glandular parenchyma was noteworthy and enabled us to consider them as osmophores. In addition, the occurrence of nectaries in other *Octomeria* species was also related to the occurrence of nectar droplets and the glandular trichome morphology (Melo et al., 2011; Cardoso, 2014).

7.3 Osmophores

Osmophores (scent-producing glands) are ubiquitous in the Pleurothallidinae (Pridgeon et al., 2010), and the presence of alkanes in the floral bouquet of its representatives was recently proposed as another synapomorphy of the subtribe (Cardoso-Gustavson et al., 2017). In this subtribe, the osmophores assume different morphology, location (sepals or labellum) and position (apex, center, base) within the bearing organs (Vogel, 1962; Pridgeon and Stern, 1983; Pridgeon and Stern, 1985; Melo et. al., 2010; Cardoso, 2014; Millner and Baldwin, 2016; Almeida, 2016; Pansarin et. al., 2016). This large variation may probably be related to the volatiles released and the behavior of the pollinator within the flower. Indeed, the volatiles act as guides conducting the pollinator to the floral resources and sexual structures to perform the pollination (Woodcock et al., 2014). The presence of osmophores is strongly associated with sapromiophylous orchids (Teixeira et al., 2004, Melo et al., 2010), in which the pollinators are deceit by the flower mimicking flesh and exhaling fetid odors, but these glands are also found in myophylous flowers, acting as nectar guides (Melo et. al., 2010; Woodcok et al., 2014)

Glandular trichomes, elevations, and elevations with stomatal pore were the osmophores' morphology observed in the sepals, while only glandular trichomes were identified in the labellum. The location of these different morphologies varied according to the species; e.g., the elevations were observed in the sepals of *Masdevallia infracta* and *Myoxanthus* spp., however they were previously described at the base of the labellum of *Phloeophila nummularia* (Cardoso, 2014).

Elevations with stomatal pore at the adaxial surface of the sepals seems to be common in *Acianthera* species (Melo et al. 2010; Cardoso, 2014; Almeida 2016; Pansarin et al., 2016), but were also identified here in *Pleurothallopsis nemorosa* and *Myoxanthus* spp., indicating that some morphology are not restricted to certain genera. Regarding the morphology of osmophores, it seems a common feature that elevations with pore in the sepals co-occurs with unicellular trichomes, as in the labellum of *Myoxanthus* spp. On the other hand, the same elevations in the sepals, but without pores, appeared with unicellular trichomes in the labellum of *Masdevallia infracta*, being the unicellular trichomes the widespread morphology in in the sepals and labellum Pleurothallidinae representatives (fig. 8).

The cuticle ornamentation, as well as nectar and volatile guides, are linked with the behavior of the pollinator inside the flower (Woodcock et al., 2014), being noteworthy in sexual deceptive species in which the cuticle ornamentation mimic the texture of the female body (Singer et al., 2004).

The vacuole was large in all osmophores here observed, and this may be related to the occurrence of phenolic compounds inside (e.g., anthocyanin in sapromyophilous species) (Poustka et al., 2007). In addition, all the osmophores here identified have lipid droplets in the protoplast, which may be precisely involved with the low polarity of these volatile organic compounds (VOC). These volatiles are commonly monoterpenes, isoprenoids, benzenoids, phenylpropanoids, all with low polarity, low molecular weight, and high vapor pressure, properties that promote volatility (Piechulla and Pott, 2003). This is the case of the compounds in the floral bouquet of Plerothallidinae representatives, as pointed out by Cardoso-Gustavson et al. (2017).

The subtribe Pleurothallidinae together with the genus *Bulbophyllum* are the two largest unrelated myophilous groups within Orchidaceae, thus, sharing similarities in floral morphology and adaptations (van der Pijl and Dodson, 1966). Indeed, they share convergent adaptations as the emission of pheromones in their pollination system (Tan et al., 2002; Nishida et al., 2004; Karremans et. al., 2015). This convergence can be exemplified here by the occurrence of glandular trichomes in the labellum of *Octomeria juncifolia* and *Pleurothallis ruscifolia* and also in this same location in *Bulbophyllum levanae* (Wiśniewska

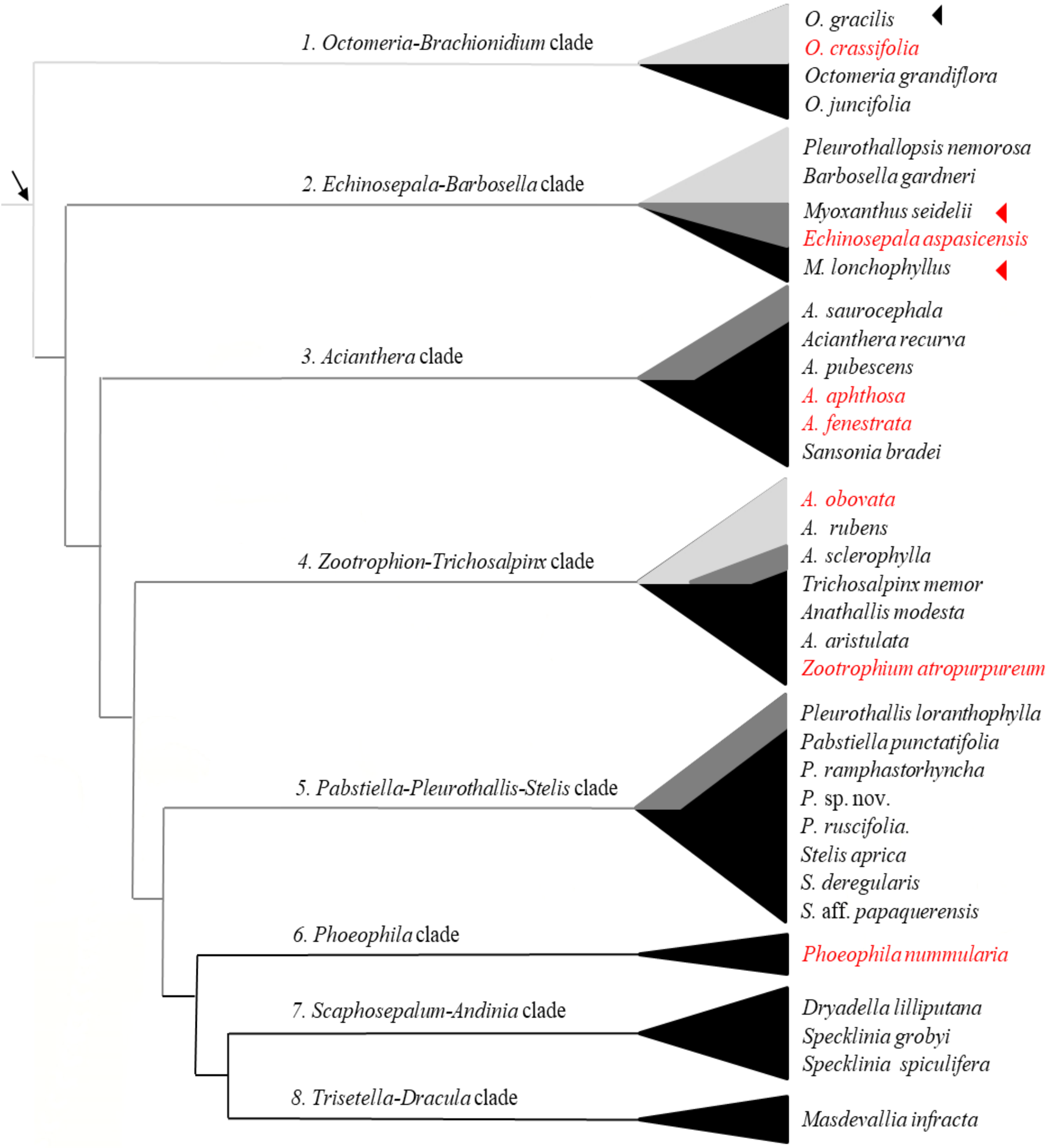

Fig. 8. Simplified phylogenetic tree of the subtribe Pleurothallidinae based on Pridgeon et al. (2001), Chiron et al. (2012) and Karremans (2016) showing the main clades currently recognized and the respective glands identified in the median region of the labellum. Light grey represents species that have nectaries, dark grey,species with sticky-exudate glands and black, species bearing only osmophores (however all the species possessed these glands, except *A. saurocephala*). Black arrow indicates the presence of colleters, also ubiquitous in the subtribe. Regarding the glands at the external ovary wall, black arrowheads indicate the presence of elevations with stomata pore, and red arrowheads the presence of elevations with stomata pore and glandular trichomes. Names written in red belong to a previous investigation in subtribe Pleurothallidinae (Cardoso-Gustavson et al., *in prep.*).

et al., 2017). However, while osmophores are usually reported in the labellum of *Bulbophyllum* species (Teixeira et. al., 2004; Nunes et al., 2014, 2015), in Pleurothallidinae they are usually located at the sepals, co-occurring with other glands in the labellum (Vogel, 1962; Pridgeon and Stern, 1983, 1985; Cardoso-Gustavson, 2014; Millner and Baldwin, 2016).

7.4 Sticky-exudate glands

Sticky-exudate glands are glands with unique characteristics that release a heterogenous exudate, composed of lipids and polysaccharides (Cardoso, 2014). These glands appeared in the sepals and labellum of *Acianthera saurocephala*, and were restricted to the labellum in *Myoxanthus seidelli*, *Anathallis sclerophylla* and *Pleurothallis loranthophylla*. The anatomical features are similar to the first report, described in *Echinosepala aspacisensis* by Cardoso (2014). The release of the heterogenous exudate apparently could be similar to the previous description, because of the accumulation of the exudate in the subcuticular space, cuticle detachment and further rupture. However, the exudate seems to be released via stomatal pore in *A. saurocephala*, this species also lacks osmophores as reported in other Pleurothallidinae species evaluated here. Interestingly, the pollination probably occurs as in some *Bulbophyllum* species in which the pollination is made exclusively by female flies attracted to flowers by their oviposition instinct (Borba and Semir, 1998).

Glands with similar characteristics had been also reported in *Bulbophyllum* (Davies and Stpiczyńska, 2014; Stpiczyńska et al., 2015; Kowalkowska et al., 2017; Stpiczyńska and Davies, 2016). The ultrastructural investigation carried out by Davies and Stpiczyńska (2014), showed that glands occurring in the labellum of *Bulbophyllum* spp. release an exudate composed of protein-rich mucilage, in which the mucilage precursor is firstly produced in sub-epidermal cells and pass through plasmodesmata until reaching the epidermis, where this mucilage is completely synthesized. The exudate is released similarly, by cuticle rupture as observed in sticky-exudate glands of Pleurothallidinae (Cardoso, 2014).

Regarding *Bulbophyllum*, the presence of aerenchyma in the labellum had been reported (Stpiczyńska et al., 2015), although in subtribe Pleurothallidinae, it is apparently the first report here. The presence of a spongy parenchyma in the labellum limb seems to be related to wind-assisted pollination, in which any moving promoted by the wind may cause the contact of the flies to the column (Borba and Semir; 1998; Teixeira et al. 2004). In this context, the occurrence of aerenchyma in the labellum of *Anathallis sclerophylla* may also contribute to this mechanism.

7.5 Nectaries

The structure of the nectaries identified is the same as described in previous studies (Melo et al., 2010; Cardoso, 2014; Almeida, 2016). They occurred as glandular unicellular trichomes in the labellum of *Barbosella gardneri*, *Octomeria grandiflora*, *Pleurothallopsis nemorosa*, and *Anathallis rubens*. In contrast, in a recent study, Karremans et al. (2015) observed nectaries formed by stomata in the sepals in *Specklinia*, although few reports of these nectaries morphology are available in orchids (Davies et al., 2005; Hobbhahn et al., 2013). The cuticle of the nectaries here observed may be permeable facilitating the nectar reabsorption as identified in other Pleurothallidinae species by Cardoso (2014). The unicellular trichomes may increase the secretion surface as have been previously described (Teixeira et al., 2004; Stpiczynska et al., 2005, Melo et al., 2010). There is only one report of several nucleoli in the cells of the nectary parenchyma in *Echinacea purpurea* (Asteraceae) (Wist and Davis, 2006), but the role of this alteration in these glands remains unknown.

Noteworthy, the presence of exposed nectaries seems to be common in myophilous orchids as Pleurothallidinae and *Bulbophyllum* (Borba and Semir 1998, 2001; Melo et al., 2010). Their fundamental role in sapromyophilous orchids seems to be to increase the time of the insect's stay inside the flower until the pollination effectively occur, even in a case of attraction by oviposition instinct (Borba and Semir, 1998; Melo et al. 2010).

7.6 Evolutionary considerations

The simplified phylogenetic tree presented here is based on the available data for subtribe Pleurothallidinae (Pridgeon et al., 2001, Chiron et al., 2012, Karremans et al., 2015). However, a character mapping was not made, because this analysis would request an entire correlation with the species examined and those utilized to construct the phylogenetic tree. As some taxa are very rare this correlation would be difficult. We have only correlated the species studied here with the accepted phylogenetic tree. Finally, the pattern of the glands was examined and it was possible to identify some evolutionary transitions in the subtribe (Fig. 8).

The studied species bear in the same region of the labellum: a) nectaries in 1 to 2 clades, b) sticky-exudate glands in 3 to 5 clades, and c) osmophores in 6 to 8 clades. In this context, all the glands identified have the machinery to produce carbohydrates and lipophilic compounds. Glands that release essentially monosaccharides (e.g., glucose, fructose, and galactose) probably become able to produce polysaccharides (carbohydrate of high molecular weight) even mixed with lipophilic compounds. Subsequently the ability of sugar production was lost, remaining only the production of increasingly complex lipophilic compounds until reaching the volatile organic compounds. Indeed, the cell machinery is the same, and the release of these compounds is essentially done by repeated cycles of contraction and expansion of the protoplast (Paiva, 2016).

Other characteristic that reinforces this hypothesis is that sticky-exudate glands in the clade 3 are formed by rounded saliences (e.g. *Echinosepala aspasicencis*; Cardoso, 2014 and *Acianthera saurocephala* Fig. 6A–C, E) or edges in the clade 4 (e.g. *Anathallis sclerophylla*

Fig. 6I–J) and, in the clade 5, the epidermis appeared as a group of cells, forming a fimbriate tissue (e.g. *Pleurothallis loranthophylla* Fig. 6K–L). Tissues in growth and differentiation tend to isolate groups or individual cells by loss of functional plasmodesmata, thus forming different sympatric domains: the cells within the domain and the cells of de border (Burch-Smith et al. 2011). Therefore, this loss of functional plasmodesmata probably triggered an absence of anticlinal bounds. This transition to bifurcate glands in the phylogeny had been reported in glands that release also heterogenous exudate (Thomas, 1991; Simões et al., 2006). Even the isolation may increase the specificity of the cells until becoming osmophores in clades of 6 to 8. Thus, it was proposed that this transference of function may be also a special case of homeoheterotopy named as homeosis (Baum and Donogue, 2002).

Overall, these findings reinforce the hypothesis of Borba et al. (2011) that myophilly is a plesiomorphic condition being a transition of the melittophily to deceptive sapromyophily within the subtribe Pleurothallidinae. Finally, this investigation shows that the presence of ovarian colleters can be a sinapomorphy of Pleurothallidinae, and also, for the first time, the occurrence of other secretory glands besides colleters in the external ovary wall. More investigations have to be performed for understanding the relevance of these structures in floral biology and pollination syndromes. The results concerning the structure of the floral glands emphasize the great similarity between Pleurothallidinae and *Bulbophyllum,* due to the evolutionary convergence related to the pollination by flies. Some important evolutionary transitions about the floral glands in the subtribe are discussed; although future investigations concerning this matter have to be considered. This data may also be useful in further phylogenetic studies within this subtribe.

8 LITERATURE CITED

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9 CONSIDERAÇÕES FINAIS

Os resultados obtidos durante o desenvolvimento da pesquisa permitiram responder satisfatoriamente às questões inicialmente levantadas; as perguntas e suas respectivas respostas são detalhadas adiante:

(1) Quais são os tipos e a localização das glândulas presentes nos órgãos florais das espécies-alvo?

Na parede externa do ovário, foram identificados coléteres nas invaginações e, descritas pela primeira vez na subtribo, glândulas na região fora das invaginações. Osmóforos foram identificados na face adaxial e somente em *Octomeria* spp. na face abaxial das sépalas, enquanto no labelo somente na face adaxial, ocorrendo da região apical a basal dependendo da espécie. "Sticky-exudate glands" foram observadas na face adaxial, sendo no labelo na região central e nas sépalas de *Acianthera saurocephala* da região central a apical. Por fim, nectários ocorreram apenas na região central do labelo.

(2) Como é a estrutura geral destas glândulas e como elas são caracterizadas morfologicamente?

Coléters ocorreram como tricomas unicelulares ou bicelulares, as glândulas presentes na região fora das invaginações: são elevações com poros e tricomas unicelulares. Osmóforos apresentaram três morfotipos distintos: elevações da epiderme, elevações da epiderme com poros e tricomas unicelulares. "Sticky-exudate glands" foram observadas como saliências formadas pela elevação da epiderme glandular em paliçada e do parênquima glandular adjacente, apresentando poros nas sépalas de *Acianthera saurocephala*. Nectários ocorreram apenas como tricomas unicelulares.

(3) Há alguma transição evolutiva quando as variações morfológicas de cada morfotipo glandular das espécies-alvo são comparadas às filogenias disponíveis?

Sim, principalmente no labelo em que foram identificados na mesma região nectários, "sticky-exudate glands" e osmóforos, estes últimos se mostraram como uma transição evolutiva na subtribo, ocorrendo assim, provavelmente, um caso específico de transferência de função denominada homeose.