Universidade Estadual de Campinas

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ESTUDOS FILOGENÉTICOS E ANATÔMICOS DA TRIBO MESECHITEAE MIERS (APOCYNACEAE, APOCYNOIDEAE).

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RESUMO GERAL

Mesechiteae Miers é uma das tribos da subfamília Apocynoideae (Apocynaceae *s.l.*), e segundo Endress & Bruyns (2000) é formada por nove gêneros (*Allomarkgrafia, Galactophora, Macrosiphonia, Mandevilla, Mesechites, Quiotania, Secondatia, Telosiphonia, Tintinnabularia*) e cerca de 150 espécies. O presente trabalho visa um amplo estudo da tribo baseado em dados filogenéticos e anatômicos.

De forma a testar o monofiletismo da tribo e determinar os limites genéricos, em especial no caso de *Mandevilla*, o maior gênero da tribo com cerca de 120 espécies, foram realizadas duas análises filogenéticas baseadas em dados moleculares de regiões do DNA de cloroplasto e morfologia. Os resultados obtidos demonstraram que Mesechiteae *sensu* Endress & Bruyns (2000) é parafilética. Apenas a exclusão dos gêneros *Secondatia* e *Galactophora* e a inclusão de *Forsteronia* tornam a tribo monofilética. Assim circunscrita, Mesechiteae é composta por sete gêneros distribuídos em três subclados: o subclado *Mesechites* (incluindo *Tintinnabularia, Allomarkgrafia* e *Mesechites*), o subclado *Forsteronia* (composto exclusivamente pelo gênero *Forsteronia*) e o subclado *Mandevilla* (composto por *Macrosiphonia, Mandevilla* e *Telosiphonia*). *Secondatia* forma com *Odontadenia*, um gênero neotropical da tribo Apocyneae, um clado fortemente sustentado. A posição do gênero *Galactophora* é incerta, porém este não está relacionado com nenhum táxon do clado Mesechiteae. *Allomarkgrafia* e *Mesechites* formam um grupo monofilético, e o primeiro deve ser incluído na sinonímia deste ultimo. *Quiotania colombiana*, a única espécie deste gênero, é idêntica a *Mandevilla ligustrifolia* e, portanto, *Quiotania* deve ser incluído em *Mandevilla*. *Macrosiphonia* e *Telosiphonia* não são congenéricos, mas sim formam clados distintos em *Mandevilla*.

Uma análise mais detalhada do subclado *Mandevilla* mostrou que *Mandevilla sensu* Woodson (1933) é parafiletico, porém *Mandevilla sensu* Pichon (1948) é monofilético, com a inclusão de *Macrosiphonia* e *Telosiphonia* em sua circunscrição. Assim definido, *Mandevilla* forma um clado fortemente sustentado dividido em dois subclados principais: Clado I, formado por *Macrosiphonia* e 14 espécies de *Mandevilla*, e Clado II, formado por *Telosiphonia* e as demais espécies amostradas de *Mandevilla*. O Clado II, por sua vez, também é divido em dois outros clados: Clado III, incluindo as espécies sul-americanas de

Mandevilla com 2 nectários mais *M. pycnantha*, e Clado IV, incluindo as espécies de *Mandevilla* com 5 nectários e *Telosiphonia*. O Clado IV ainda pode ser divido em dois clados menores, um incluindo espécies de *Mandevilla* que ocorrem predominantemente no norte da América do Sul (Clado V), e o outro incluindo *Telosiphonia* e as espécies de *Mandevilla* do México e América Central (Clado VI).

Ainda de forma a investigar a morfologia do ginostégio e coléteres calicinais em Mesechiteae, foram analisadas séries transversais e longitudinais das flores de sete espécies (*Macrosiphonia longiflora*, *Mandevilla pycnantha*, *M. scabra*, *M. tenuifolia*, *Mesechites mansoana*, *Secondatia densiflora* e *S. floribunda*). A estrutura dos coléteres calicinais mostrou-se variada, com quatro tipos estruturais observados: standard, bifurcado, laminar e séssil. O tipo standard já havia sido relatado em outras espécies de Apocynaceae, porém os outros três tipos encontrados são descritos aqui pela primeira vez. Estes novos tipos morfológicos possivelmente originaram-se a partir de modificações do tipo standard por três mecanismos distintos: separação, proliferação e alongamento celular. Os coléteres calicinais podem estar organizados de três formas diferentes, em relação às lacínias do cálice: alternos, opostos ou indefinidamente distribuídos. O padrão alterno não é homogêneo, com três subtipos observados: um com quatro, outro com cinco e o último com 10 grupos de glândulas dispostas na margem das lacínias do cálice. Não foram detectados padrões estruturais, numéricos ou de distribuição dos coléteres calicinais nas espécies analisadas para caracterizar a tribo e corroborar os resultados obtidos pela análise filogenética.

Em *Macrosiphonia*, *Mandevilla* e *Mesechites* a estrutura do ginostégio segue o mesmo padrão, com cinco projeções da cabeça do estilete formadas por uma proliferação de células parenquimáticas adnatas ao conectivo dos estames. Em *Secondatia*, no entanto, a estrutura do ginostégio é diferente, sem a proliferação de células parenquimáticas na cabeça do estilete. Neste gênero, um tênue contato entre os estames e a cabeça do estilete é promovido por tricomas unicelulares do conectivo que tocam levemente a epiderme da cabeça do estilete. Os resultados obtidos corroboram as análises filogenéticas, com *Macrosiphonia*, *Mandevilla* e *Mesechites* em sua circunscrição e exclusão de *Secondatia*.

Palavras-chave: Apocynaceae, glândulas calicinais, ginostégio, *Macrosiphonia*, *Mandevilla*, *Mesechites*, Mesechiteae, *Secondatia*, sistemática filogenética.

GENERAL ABSTRACT

Mesechiteae Miers is one of the tribes of subfamily Apocynoideae (Apocynaceae *s.l.*), and according to Endress & Bruyns (2000) it comprises nine genera (*Allomarkgrafia*, *Galactophora*, *Macrosiphonia*, *Mandevilla*, *Mesechites*, Quiotania, *Secondatia*, *Telosiphonia*, *Tintinnabularia*) and about 150 species. The aims of the present work were to make a broad study of the tribe based on phylogenetic and anatomical data.

To test the monophyly of the tribe and evaluate intergeneric relationships, especially for *Mandevilla*, by far the largest genus of the tribe with about 120 species, two phylogenetic analyses were conducted based on DNA plastid regions and morphology. The results showed that Mesechiteae, as circumscribed by Endress and Bruyns (2000), was found to be parayphyletic. Only removal of *Secondatia* and *Galactophora* and inclusion of *Forsteronia* rendered the tribe monophyletic. Thus defined, Mesechiteae forms a strongly supported clade including seven genera in three subclades: the *Mesechites* subclade (comprising *Tintinnabularia*, *Allomarkgrafia*, and *Mesechites*), the *Forsteronia* subclade (containing only *Forsteronia*) and the *Mandevilla* subclade (comprising *Macrosiphonia*, *Mandevilla*, and *Telosiphonia*). *Secondatia* forms a strongly supported clade with *Odontadenia*, a neotropical genus from the tribe Apocyneae. The position of *Galactophora* is uncertain, but it never grouped with taxa from Mesechiteae clade. *Allomarkgrafia* is nested in *Mesechites*, and should be included in its synonymy. *Quiotania colombiana* is cospecific with *Mandevilla ligustrifolia* and thus should be included in the synonymy of the latter. *Macrosiphonia* and *Telosiphonia* are not congeneric, but also form two distinct, well-supported clades nestes within *Mandevilla*.

Mandevilla, as circumscribed by Woodson (1933), was found to be paraphyletic, but the circumscription of Pichon (1948) proved to be monophyletic, with the inclusion of *Macrosiphonia* and *Telosiphonia* in its synonymy. Thus defined, *Mandevilla* forms a strongly supported clade in divided in two major clades: Clade I, comprising *Macrosiphonia* and 14 species of *Mandevilla*, and Clade II, comprising the remaining species of *Mandevilla*. The Clade II also included two strongly supported clades: Clade III, comprising the South American species of *Mandevilla* with 2 nectaries plus *M. pycnantha*, and Clade IV, comprising the remaining *Mandevilla* with five nectaries plus *Telosiphonia*.

The clade IV also includes two other clades: one comprising species of *Mandevilla* species mainly distributed in northern South America (Clade V) and another comprising *Telosiphonia* plus the Mexican and Mesoamerican species of *Mandevilla* (Clade VI).

In order to investigate the morphology of the gynostegium and calycine colleters in Mesechiteae, serial sections of flowers from seven species of Mesechiteae (*Macrosiphonia longiflora*, *Mandevilla pycnantha*, *M. scabra*, *M. tenuifolia*, *Mesechites mansoana*, *Secondatia densiflora* and *S. floribunda*) were performed and analysed. The structure of calycine colleters was more variable than expected, with four structural types observed: standard, bifurcate, laminar and sessile. The standard type has already been reported in Apocynaceae, but the other three types are here described for the first time in the family. These new types are possibly originated from deviations of the standard type by three distinct mechanisms: separation, proliferation and elongation of cells. The calycine colleters can be organized in three different ways in relation to the calyx lobes: alternate, opposite or indefinitely distributed. The alternate pattern is not homogeneous, and three subtypes were observed: one with four groups, another with five groups and the latter with 10 groups disposed on the margins of the calyx lobes. No specific patterns of calycine colleters characterize the tribe Mesechiteae in terms of structure, number and distribution, but a combination of these characters can be diagnostic for some groups within the tribe, as the infrageneric classification of *Mandevilla*.

The gynostegium structure of *Macrosiphonia*, *Mandevilla* and *Mesechites* follow the same pattern, with a proliferation of parenchyma cells in the style head forming five projecting ribs that are adnated to the expanded conective. The gynostegium of *Secondatia* has a different pattern, with no detectable proliferation of parenchyma cells in the style head. The contact between stamens and style head is promoted by unicellular trichomes of the connective that are close to the secretory epidermis of the style head, with no adnation between the parts. Our results support the new phylogeny of the tribe, with *Macrosiphonia*, *Mandevilla* and *Mesechites* in its circumscription and the segregation of *Secondatia*.

Key words: Apocynaceae, calycine glands, gynostegium, *Macrosiphonia*, *Mandevilla*, *Mesechites*, Mesechiteae, phylogenetic systematics, *Secondatia*

INTRODUÇÃO GERAL

Apocynaceae *sensu lato* é uma das maiores e mais representativas famílias de Angiospermas, contendo em seus limites atuais cerca de 335 gêneros e 3700 espécies (Judd. et al. 2002). A autoria da família foi alvo de recentes mudanças, com a restauração da autoria de Adanson (1768) através do Código de Nomenclatura Botânica (Greuter et al., 2000). A determinação dos limites da família também é controversa, especialmente em relação à tradicional família Asclepiadaceae. O reconhecimento de Apocynaceae *s. str.* (correspondendo à circunscrição tradicional de Apocynaceae) e Asclepiadaceae como famílias distintas é inconsistente do ponto de vista filogenético, pois torna a primeira parafilética. A afinidade entre estas já era reconhecida desde Jussieu (1789), que incluiu na circunscrição da família as atuais Apocynaceae *s. str.* bem como as Asclepiadaceae e alguns gêneros de Loganiaceae. Brown (1810) desmembrou as Apocinae em duas famílias, propondo "Asclepiadeae", que correspondem às Asclepiadaceae, e Apocynae, compreendendo as Apocynaceae s. str. Trabalhos posteriores, tanto específicos para a família (p.ex., Schumman, 1895 e Woodson, 1930) quanto gerais para Angiospermas (p.ex., Cronquist, 1981) mantiveram a divisão em duas famílias, embora reconhecendo sua proximidade.

A proposta da fusão das duas famílias é aceita em várias das propostas de classificação recentes (Stebbins 1974, Thorne 1992, Judd et al. 1999, Endress & Bruyns 2000), e tem por base um considerável número de estudos filogenéticos baseados tanto em caracteres morfológicos quanto macromoleculares (Judd et al. 1994, Endress et al. 1996, Sennblad & Bremer 1996, 2002, Struwe et al. 1994). Os resultados obtidos nestes trabalhos evidenciam a estreita relação filogenética entre as duas famílias, propondo a sua união em uma grande família, as Apocynaceae *s.l.* Apesar da grande aceitação desta proposta no meio científico, Apocynaceae *s. str.* e Asclepiadaceae continuam a ser reconhecidas como famílias distintas em trabalhos taxonômicos e florísticos. Alguns autores propõem mudanças alternativas, como Hutchinson (1976), que propôs a criação de uma ordem separada exclusiva para as duas famílias (Apocynales), e como Nicholas & Baijnath (1994) e Rosatti (1989), que mantêm as Asclepiadaceae na categoria de família distinta de Apocynaceae e propõem uma nova subordem, Apocyninae, para englobar as duas famílias.

As profundas mudanças na circunscrição da família, no entanto, não atingem a classificação infra-familiar de Apocynaceae *s. str.*, que pouco mudou nas últimas décadas. Apenas recentemente, Endress & Bruyns (2000) propuseram modificações substanciais na delimitação de subfamílias e tribos, envolvendo as Apocynaceae *s.l.*

Tradicionalmente, duas subfamílias de Apocynaceae *s. str.* são reconhecidas, Rauvolfioideae (=Plumerioideae) e Apocynoideae (=Echitoideae), e a delimitação destas pouco mudou desde o seu estabelecimento. A subfamília Rauvolfioideae constitui um grupo bastante heterogêneo, que agrupa os táxons considerados mais "primitivos" dentro da família. Com poucas exceções, os caracteres florais são pouco variáveis, e portanto pouco úteis na delimitação de tribos. Para tal fim, são utilizados tradicionalmente tanto caracteres vegetativos quanto caracteres de frutos e sementes.

A subfamília Apocynoideae, por sua vez, constitui um grupo bastante homogêneo, englobando os táxons ditos mais derivados dentro da família. Como características marcantes da subfamília temos a especialização das partes do androceu e do gineceu, com o desenvolvimento de um tecido de conexão entre as anteras e a cabeça do estilete, denominado retináculo por Pichon (1948a). Esta homogeneidade estrutural das Apocynoideae torna difícil a delimitação de tribos, dificuldade esta reconhecida por vários autores, como Fallen (1986) e Leeuwenberg (1994). Uma das mais relevantes contribuições ao conhecimento das Apocynoideae foi realizada por Pichon (1950), que, em amplo estudo taxonômico e morfológico desta subfamília, reconheceu quatro tribos: Parsonieae, Nerieae, Ecdysanthereae e Ichnocarpae. A delimitação das tribos baseou-se principalmente no modo de conexão entre as anteras e a cabeça do estilete. Apesar do inegável valor desta proposta, sua aplicação era complicada, devido à dificuldade em se observar os padrões de conexão para várias espécies, e por isto foi pouco adotada posteriomente por outros autores. Leeuwenberg (1994), mesmo reconhecendo a dificuldade de definir categorias infra-familiares, propôs três tribos para Apocynoideae: Echiteae, Wrighteae e Apocyneae. As duas primeiras correspondem respectivamente às tribos Parsonieae e Nerieae sensu Pichon (1950), e Apocyneae corresponde a um conjunto das tribos Ecdysanthereae e Ichnocarpae de Pichon.

A mais recente proposta de classificação para a família foi realizada por Endress & Bruyns (2000), na qual são estabelecidas tanto subfamílias quanto tribos, sendo

Apocynoideae composta por cinco tribos: Wrightieae, Malouetieae, Echiteae, Apocyneae e Mesechiteae. Estas categorias infra-familiares foram baseadas principalmente em caracteres morfológicos e alguns resultados preliminares de estudos moleculares. Conforme os próprios autores ressaltam, esta classificação ainda não é definitiva, e novos estudos são necessários para estabelecer os limites reais entre as tribos.

A tribo Mesechiteae sensu Endress & Bruyns (2000) engloba nove gêneros (*Allomarkgrafia, Galactophora, Macrosiphonia, Mandevilla, Mesechites, Quiotania, Secondatia, Telosiphonia* e *Tintinnabularia*) e cerca de 150 espécies. Dela fazem parte representantes das tribos Ichnocarpeae e Ecdysanthereae *sensu* Pichon (1950) e da tribo Apocyneae *sensu* Leeuwenberg (1994). A tribo encontra-se distribuída ao longo dos neotrópicos em praticamente todos os tipos de vegetação, e apresenta considerável variação morfológica tanto em caracteres vegetativos quanto reprodutivos. A maioria das espécies são lianas, mas arbustos e subarbustos são comuns em alguns gêneros, como *Mandevilla*, *Macrosiphonia* e *Telosiphonia*. A variação na estrutura floral é particularmente notável, especialmente em relação às suas cores e dimensões, sendo encontradas desde flores tubulosas, brancas e menores que 1 cm de comprimento em *Mandevilla ligustrifolia* até flores infundibuliformes, arroxeadas e maiores que 5 cm em *Mandevilla atroviolacea*; de especial interesse são as flores brancas e tubulosas dos gêneros *Macrosiphonia* e *Telosiphonia*, onde o tubo da corola alcança as maiores dimensões dentro da família (até 17 cm em *Macrosiphonia longiflora*).

Endress & Bruyns (2000) caracterizaram a tribo a partir de um conjunto de caracteres morfológicos relacionados à estrutura floral, em especial do ginostégio (órgão formado pela fusão em diferentes graus de partes do androceu e gineceu): a cabeça do estilete apresenta formato pentagonal em secção transversal devido à presença de cinco projeções longitudinais bem desenvolvidas, e as anteras estão fortemente adnatas à cabeça do estilete a partir de fusão celular. Esta uniformidade nos caracteres diagnósticos da tribo, no entanto, não é refletida na delimitação dos gêneros, que em muitos casos é pouco precisa e freqüentemente os caracteres se sobrepõem. *Quiotania*, por exemplo, foi descrito recentemente por Zarucchi (1991), e o seu relacionamento com outros gêneros de Mesechiteae ainda é incerto. Do ponto de vista morfológico, *Mandevilla* é o gênero mais próximo de *Quiotania*, sendo este último distinto pelo tubo da corola de dimensões

reduzidas. Algumas espécies de *Mandevilla*, no entanto, (p.ex., *M. ligustrifolia* e *M. syrinx*) possuem o tubo da corola bastante reduzido e com as mesmas dimensões relatadas para *Quiotania*, tornando questionável o reconhecimento deste gênero. *Telosiphonia*, considerado um subgênero de *Macrosiphonia* por Woodson (1933), foi recentemente elevado à categoria genérica por Henrickson (1996). Pichon (1950) incluiu *Macrosiphonia* na sinonímia de *Mandevilla*, assim como *Allomarkgrafia* na sinonímia de *Mesechites*, alegando que os caracteres diagnósticos utilizados por Woodson (1933) na delimitação destes gêneros eram inconsistentes.

Coléteres são estruturas glandulares presentes na maioria dos gêneros de Apocynaceae s.l., podendo ocorrer tanto em partes vegetativas quanto reprodutivas. Coléteres calicinais são particularmente comuns e alguns de seus aspectos, como morfologia externa e distribuição, são largamente utilizados na taxonomia da família, em especial em Apocynoideae. Apesar de sua relevância taxonômica, pouco se sabe a respeito dos coléteres calicinais em Mesechiteae, uma vez que estas estruturas foram estudadas do ponto de vista anatômico apenas em poucas espécies da tribo até o presente.

De posse destas informações e visando à realização de um estudo abrangente da tribo Mesechiteae, foram abordados dois tópicos; estudos de anatomia floral e sistemática filogenética.

Os estudos filogenéticos tiveram dois objetivos principais: determinar as relações de Mesechiteae com outras tribos de Apocynoideae e as relações entre os seus gêneros constituintes. Primeiramente, buscou-se testar a monofilia da tribo de acordo com a delimitação proposta por Endress & Bruyns (2000) e também comparar os resultados obtidos com as classificações de Pichon (1950) e Leeuwenberg (1994). Em um segundo momento, buscou-se determinar os limites intergenéricos e responder questões taxonômicas levantadas em estudos anteriores, como: a relação entre *Allomarkgrafia* e *Mesechites*; a distinção entre *Macrosiphonia* e *Telosiphonia*; o reconhecimento genérico de *Quiotania* e seu relacionamento com *Mandevilla*; a relação entre o complexo *Macrosiphonia/Telosiphonia* com *Mandevilla*. Para *Mandevilla*, buscou-se ainda testar as categorias infra-genéricas propostas por Woodson (1933) e Pichon (1948b).

Os estudos anatômicos tiveram como objetivo principal a caracterização estrutural do ginostégio (estrutura reprodutiva formada pela fusão dos estames com a cabeça do

estilete) e como objetivo secundário a caracterização estrutural dos coléteres presentes na base da face adaxial das lacínias do cálice, de forma a testar a validade dos caracteres diagnósticos para a tribo propostos por Endress & Bruyns (2000) e buscando possíveis padrões estruturais em diferentes níveis hierárquicos.

Os resultados obtidos são apresentados em cinco capítulos. Com exceção do primeiro, todos os capítulos foram escritos na forma de artigo e em inglês, visando à sua publicação em periódicos de circulação internacional.

O Capítulo I refere-se à caracterização taxonômica da tribo Mesechiteae e de seus gêneros. Para cada táxon foi escrita uma descrição taxonômica, baseada tanto em dados de literatura quanto em observações morfológicas realizadas a partir da análise de exsicatas, acrescida de comentários taxonômicos. A principal finalidade da inclusão deste capítulo na tese é auxiliar na compreensão dos capítulos subseqüentes.

Os Capítulos II e III correspondem aos estudos filogenéticos da tribo Mesechiteae. Ambos foram desenvolvidos no laboratório de Sistemática Molecular da Universidade de Zürich, Suíça, em colaboração com a Dra. Mary Endress e a Profa. Dra. Elena Conti, pesquisadoras da referida instituição.

O Capítulo II, "Tribal and intergeneric relationships of Mesechiteae (Apocynaceae, Apocynoideae): evidence from three noncoding plastid DNA regions and morphology" tem como objetivo uma análise filogenética geral de Mesechiteae, visando testar sua monofilia e estudar, ao menos em parte, as relações entre os seus gêneros constituintes. Este capítulo foi publicado no periódico American Journal of Botany (vol. 91(9): 1409-1418).

O Capítulo III, "Phylogenetic relationships in *Mandevilla* Lindl. and related genera (Apocynaceae, Apocynoideae) based on five plastid DNA regions and morphology" é uma extensão do Capitulo II, desta vez focando as relações intergenéricas e, em especial, a delimitação do gênero *Mandevilla* e suas categorias infragenéricas propostas por Woodson (1933) e Pichon (1948b). Este capítulo será submetido ao periódico "Annals of the Missouri Botanical Garden".

Os Capítulos IV e V correspondem aos resultados obtidos na análise da estrutura floral de sete espécies pertencentes a quatro gêneros da tribo: *Macrosiphonia*, *Mandevilla*, *Mesechites* e *Secondatia*. Ambos foram desenvolvidos no Laboratório de Anatomia Vegetal

do Departamento de Botânica da Unicamp, sob a co-orientação da Profa. Dra. Marilia de Moraes Castro.

O capítulo IV, "Calycine glands of seven species of Mesechiteae Miers (Apocynaceae, Apocynoideae)", tem como tema a análise dos coléteres calicinais em termos de sua estrutura, disposição e número. Este trabalho foi desenvolvido a partir da análise de secções transversais e longitudinais da porção basal de flores adultas e será submetido ao periódico "Annals of Botany".

O capítulo V, "Gynostegium structure of Mesechiteae Miers (Apocynaceae, Apocynoideae)", tem como tema a análise do ginostégio em termos de sua estrutura e função, com uma re-avaliação da terminologia utilizada para denominar suas partes componentes. Este trabalho foi desenvolvido a partir da análise de secções transversais e longitudinais da porção apical de flores adultas e será submetido ao periódico "International Journal of Plant Sciences".

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CAPÍTULO I – CARACTERIZAÇÃO TAXONÔMICA DA TRIBO MESECHITEAE

RESUMO: A família Apocynaceae tem sido alvo de um grande número de estudos recentes, que resultaram em uma profunda reavaliação de seus limites. Dentre as novas categorias propostas está a tribo Mesechiteae, composta de 9 gêneros e cerca de 150 espécies de distribuição exclusivamente neotropical. O desconhecimento das relações filogenéticas de Mesechiteae com outras tribos e a dificuldade no estabelecimento dos limites entre seus gêneros tornam necessários mais estudos taxonômicos sobre o grupo. Tendo em vista estes problemas, buscou-se fazer um levantamento bibliográfico, reunindo dados disponíveis na literatura sobre o grupo. Baseado nestes dados, foram elaboradas descrições para a tribo e os gêneros.

INTRODUÇÃO

A tribo Mesechiteae foi estabelecida por Miers em 1878, englobando 31 gêneros. A proposta de classificação de Miers (1878), no entanto, mostrou-se pouco prática e confusa, e por isto não foi utilizada pelos especialistas da família. Endress & Bruyns (2000) reestabeleceram a tribo, porém com uma nova circunscrição, englobando 9 gêneros e cerca de 150 espécies de distribuição exclusivamente neotropical (*Allomarkgrafia, Galactophora, Macrosiphonia, Mandevilla, Mesechites, Quiotania, Secondatia, Telosiphonia* e *Tintinnabularia*). A estreita relação entre alguns de seus gêneros componentes já havia sido apontada por vários autores. Woodson (1933), apesar de não propor categorias supragenéricas, reconheceu uma afinidade morfológica entre os gêneros *Allomarkgrafia*, *Mesechites*, *Mandevilla* e *Macrosiphonia* (incluindo *Telosiphonia*) baseada na combinação dos seguintes caracteres: presença de coléteres na base da lâmina foliar, anteras com base auriculada a truncada e cabeça do estilete com cinco projeções longitudinais. Pichon (1950) reconheceu a mesma afinidade e estabeleceu a subtribo Mandevillineae para incluir os gêneros *Mandevilla* (incluindo *Macrosiphonia* e *Telosiphonia* em sua sinonímia) e *Mesechites* (incluindo *Allomarkgrafia* em sua sinonímia). Zarucchi (1991), ao estabelecer o gênero *Quiotania*, considerou este morfologicamente relacionado a estes mesmos quatro gêneros, sem, contudo, especificar as possíveis relações de proximidade. Endress & Bruyns (2000) reconheceram que estes gêneros, em conjunto com *Galactophora*, *Secondatia* e *Tintinnabularia*, apresentavam o mesmo conjunto de características diagnósticas e, portanto, deveriam ser incluídos em uma tribo própria, Mesechiteae.

A família Apocynaceae, apesar do crescente número de trabalhos envolvendo seus representantes, ainda carece de estudos taxonômicos e filogenéticos, especialmente em

nível supra-genérico e genérico. O estudo taxonômico dos gêneros de Mesechiteae tem por objetivo estabelecer critérios para identificação e delimitação destes, auxiliando na compreensão da tribo Mesechiteae e das Apocynaceae como um todo, além de servir como referência para os estudos filogenéticos e anatômicos desenvolvidos durante as atividades de doutorado.

MATERIAL E MÉTODOS

Inicialmente, foi realizada uma pesquisa bibliográfica, de forma a obter a maior quantidade de dados disponíveis em literatura sobre os gêneros e espécies de Mesechiteae e determinar os caracteres diagnósticos mais consistentes na delimitação dos gêneros. As principais obras consultadas foram as de Woodson (1932, 1933, 1935, 1936), Barban (1985), Zarucchi (1991), Sales (1993), Henrickson (1996), Morales (1996, 1997, 1998) e Williams (1999).

As descrições basearam-se principalmente na análise de exsicatas e também em dados de literatura. As exsicatas eram provenientes em sua maioria dos herbários A-GH (Arnold Arboretum of Harvard University and Gray Herbarium of Harvard University, Cambridge, EUA) e UEC (Universidade Estadual de Campinas, Campinas, Brasil). Foram também analisadas exsicatas dos herbários ESAL (Escola Superior de Agricultura de Lavras, Lavras, MG, Brasil), BHCB (Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil) e Z (Universidade de Zurich, Zurich, Suíça). No total, foram analisadas exsicatas de 80 espécies pertencentes a todos os gêneros da tribo. Uma listagem completa das espécies é incluída ao final da descrição de cada gênero, e todas as espécies para as quais foram observadas exsicatas estão indicadas em negrito na listagem.

A análise das características vegetativas foi realizada por observação do material herborizado a olho nu e também com o auxílio de estereomicroscópio. As características florais foram analisadas a partir de flores previamente fixadas em álcool 70% ou FAA 50, quando disponíveis, ou a partir de flores herborizadas submetidas a processo de reidratação. Foi utilizado estereomicroscópio para a análise das estruturas florais e câmara clara acoplada para a ilustração de detalhes. Todas as medidas foram tomadas com o auxílio de paquímetro e régua, e quando necessário, com auxílio de escala no estereomicroscópio.

Os termos morfológicos utilizados foram baseados em Radford *et al.* (1974). A terminologia também foi baseada, quando necessária, em trabalhos específicos desenvolvidos para a família Apocynaceae *s. str.* Foram utilizadas abreviaturas nas medidas das descrições, aqui listadas: compr. para comprimento e larg. para largura.

RESULTADOS E DISCUSSÃO

Tribo *Mesechiteae* Miers, Apocyn. S. Amer. 10 (1878)

(Descrição baseada em Endress & Bruyns, 2000)

Figs. 1.1-4

Lianas, arbustos, subarbustos ou ervas, às vezes com xilopódio; látex geralmente branco. **Folhas** opostas, raramente verticiladas, geralmente com coléteres na base ou dispostos sobre a nervura primária na face adaxial da lâmina foliar; domácias geralmente ausentes, e quando presentes dispostas na axila das nervuras secundárias com a primária na face abaxial. **Cálice** 5-partido, com coléteres alternos ou contínuos, raramente solitários ou opostos às lacínias. **Corola** actinomorfa ou levemente zigomorfa, geralmente infundibuliforme, mais raramente tubular ou hipocrateriforme, tubo inferior cilíndrico a giboso, tubo superior dilatado e de formatos variados; corona geralmente ausente; pré-floração dextrorsa. **Estames** 5, totalmente inclusos, inseridos na base do tubo superior da corola; anteras adnatas à cabeça do estilete, esclerificadas dorsalmente, com as tecas confinadas à porção superior. **Ovário** súpero, hemi-sincárpico, circundado por 2 nectários livres ou por um disco nectarífero inteiro a 5-lobado, menor a ligeiramente maior que o ovário; óvulos muitos, placentação marginal. **Cabeça do estilete** sem anel basal ou superior, com 5 expansões laterais limitadas à base ou dispostas ao longo de todo o seu comprimento, e aonde os estames estão adnatos; região estigmática confinada na porção abaixo da cabeça do estilete ou na face inferior desta. **Fruto** composto por 2 folículos, totalmente livres a levemente ligados no ápice. **Sementes** comosas.

A tribo possui 9 gêneros e cerca de 150 espécies. (*Allomarkgrafia*, 9spp.; *Galactophora*, 6 spp.; *Macrosiphonia*, 6 spp.; *Mandevilla*, ca. 120 spp.; *Mesechites*, 10 spp.; *Quiotania*, 1 spp.; *Secondatia*, 6 spp.; *Telosiphonia*, 6 spp.; *Tintinnabularia*, 3 spp.).

Ocorre exclusivamente no Novo Mundo, com predominância nos Neotrópicos e alguns poucos representantes alcançando o Sudoeste dos Estados Unidos.

Observações: A similaridade entre os táxons componentes de Mesechiteae já era reconhecida por diferentes autores, observada pela ocorrência de coléteres na lâmina foliar e pela cabeça do estilete pentagonal, com cinco projeções laterais pouco ou bastante evidentes. Pichon (1948) reconheceu esta uniformidade estrutural entre alguns dos atuais táxons de Mesechiteae. Para agrupá-los, ampliou a circunscrição de *Mandevilla*, que passou a incluir *Macrosiphonia sensu* Woodson (o qual incluía o subgênero *Telosiphonia*, elevado a categoria genérica por Henrickson em 1996) em seus limites. Para abrigar este gênero, criou em 1950 uma nova subtribo, Mandevillinae. Williams (1999) reconhece Mandevillinae como uma subtribo válida, porém discorda dos limites genéricos propostos por Pichon (1948, 1950). *Allomarkgrafia, Macrosiphonia, Mesechites e Telosiphonia* são reconduzidos à categoria genérica. Além disso, propõe a inclusão de *Quiotania* e *Tintinnabularia* na subtribo, que passa então a contar com sete gêneros. A circunscrição de Mesechiteae *sensu* Endress & Bruyns (2000) abrange as *Mandevillinae* conforme revistas por Williams (1999), propondo ainda o acréscimo de mais dois gêneros em seus limites, *Galactophora* e *Secondatia.*

1) *Allomarkgrafia* Woodson, Ann. Missouri Bot. Gard. 19: 45. 1932.

(Descrição baseada em Woodson, 1932 e Morales, 1997)

Fig. 1.2B

Lianas, latescentes, ramos volúveis. **Folhas** opostas, com muitos coléteres na base da face adaxial da lâmina foliar. **Inflorescência** axilar, alterna, multiflora, di a tricotomicamente ramificada, os ramos bostricóide-racemosos. **Flores** actinomorfas. **Cálice** profundamente 5-partido, lacínias escariosas, com muitos coléteres dispostos adaxialmente na base. **Corola** vistosa, infundibuliforme; lobos 5, iguais, oblíquo-ovados, reniformes. **Anteras** com base obtusa, glabras; pólen granuloso. **Cabeça do estilete** oblonga, levemente angulosa, com as projeções longitudinais restritas à sua base e apêndice apical bífido. **Ovário** circundado por um disco nectarífero 5-lobado, os lobos mais ou menos livres no

ápice, do mesmo tamanho ou maiores que o ovário. **Folículos** livres, cilíndricos, longo-acuminados, mais ou menos falcados.

Espécies: *A. brenesiana* Woodson; ***A. campanulata*** (Markgr.) J.F. Morales; *A. ecuatoriana* J.F. Morales; *A. foreroi* A.H. Gentry; *A. insignis* J.F. Morales; *A. laxiflora* A. H. Gentry; *A. ovalis* (Markgr.) Woodson; ***A. plumeriifolia*** Woodson; *A. tubiflora* Woodson ex Dwyer.

Gênero neotropical com nove espécies, ocorre da Nicarágua ao Peru, com centro de diversidade na região do Choco (Colômbia). No geral, as espécies são pouco coletadas, sendo algumas conhecidas apenas pelo exemplar tipo ou por coleções reduzidas. Morales (1997) sugere que este seja um dos gêneros de Apocynaceae mais propensos a endemismo.

Observações: O gênero foi estabelecido por Woodson (1932), baseado em uma única espécie, *A. ovalis*. Em recente monografia do gênero, Morales (1997) ampliou a sua circunscrição, reconhecendo nove espécies. Pichon (1950) afirmou que *Allomarkgrafia* não se sustentava como gênero por se incluir na circunscrição de *Mesechites*, propondo assim sua inclusão na sinonímia deste último. Esta proposta, no entanto, não foi adotada em trabalhos posteriores (Morales 1997, Wiliams 1999), e *Allomarkgrafia* continuou a ser reconhecido como gênero.

Allomarkgrafia apresenta afinidades com vários gêneros de Mesechiteae. Difere de *Mandevilla* e *Mesechites* pelo tipo de inflorescência, que é racemosa no primeiro e do tipo cimeira corimbosa no segundo. As maiores afinidades, porém, são com *Tintinnabularia*, da qual difere pela presença de brácteas escariosas e pela ausência de domácias. Williams (1999) descreve uma nova espécie de *Tintinnabularia, T. murallensis*, cujas brácteas são escariosas. Desta forma, apenas a presença ou ausência de domácias distingue os dois gêneros, o que expõe a necessidade de um estudo mais aprofundado dos dois gêneros em questão.

2) *Galactophora* Woodson, Ann. Missouri Bot. Gard. 19: 49-50. 1932.

(Descrição baseada em Woodson 1933)

Subarbustos eretos, latescentes, com ramos providos de pilosidade de aparência glandular. **Folhas** opostas, sem coléteres na face adaxial, com tricomas de aparência glandular principalmente na face abaxial. **Inflorescência** terminal, bostricóide-racemosa,

pauciflora. **Cálice** profundamente 5-partido, sem coléteres adaxialmente na base, glabro ou piloso. **Corola** vistosa, infundibuliforme, de cores variadas, glabra a pilosa, tubo inferior cilíndrico, tubo superior campanulado; lobos 5, obovado-oblíquos. **Anteras** com base sagitada, glabras. **Cabeça do estilete** oblonga, com 5 projeções laterais restritas à sua base. **Folículos** livres, cilíndricos, longo-acuminados, mais ou menos falcados.

Espécies: ***G. calycina*** Woodson; *G. colleana* G. Morillo; ***G. crassifolia*** (Müll.Arg.) Woodson; *G. magnifica* Woodson; *G. petiolata* Markgr.; ***G. pulchella*** Woodson; ***G. pumila*** Monach.; ***G. schomburgkiana*** Woodson.

Gênero com 8 espécies de distribuição predominantemente amazônica, ocorre no Brasil, Venezuela e Guiana.

Observações: Galactophora é um gênero de fácil identificação, pela ausência de coléteres foliares e calicinais e pela presença de tricomas de aparência glandular nos ramos, folhas e, em algumas espécies, também no cálice e corola. A sua inclusão em Mesechiteae por Endress & Bruyns (2000) deve-se exclusivamente à estrutura da cabeça do estilete, que apresenta cinco projeções laterais bastante pronunciadas. Woodson (1932), em comentários sobre o gênero, afirma que este apresenta características bastante peculiares, não apresentando afinidades diretas com nenhum outro gênero de Apocynaceae.

3) *Macrosiphonia* Müll.Arg. in Mart., Fl. Bras. 6(1): 137. 1860.

(Descrição baseada em Woodson, 1933 e Barban, 1985)

Figs. 1.2H,L; 1.4C,E

Subarbustos eretos, latescentes, providos de xilopódio; ramos eretos ou ascendentes. **Folhas** opostas ou verticiladas, com coléteres diminutos na base da face adaxial da lâmina foliar. **Inflorescência** terminal, de aspecto racemoso, 3-flora até reduzida a flores solitárias. **Flores** actinomorfas, vistosas, nictantes ou vespertinas, pedicelo ausente. **Cálice** profundamente 5-partido, com vários coléteres contínuos dispostos adaxialmente na base. **Corola** hipocrateriforme, branca; tubo inferior cilíndrico e muito longo; tubo superior mais curto, geralmente campanulado; lobos 5, geralmente crispados. **Anteras** curtamente sagitadas a truncadas, glabras. **Cabeça** do estilete deltóide, com projeções laterais bem evidentes e ocupando toda a sua extensão longitudinal e apêndice apical bífido reduzido.

Ovário circundado por um disco nectarífero 5-lobado, com os lobos mais ou menos concrescentes na base. **Folículos** livres, cilíndricos a torulosos.

Espécies: ***M. longiflora*** (Desf.) Müll.Arg.; ***M. martii*** Müll.Arg.; *M. petrea* (A. St.-Hil.) K. schum.; *M. undulata* C. Ezcurra; ***M. velame*** (A. St.-Hil.) Müll.Arg.; ***M. virescens*** Müll.Arg.

Gênero com seis espécies exclusivas da América do Sul, em formações de Cerrado ou campestres.

Observações: Macrosiphonia foi estabelecido por Müller (1860), com base em 10 espécies. Como principal caráter diagnóstico do gênero, cita o tamanho excepcionalmente grande do tubo da corola, caráter este que ainda é utilizado para distinguí-lo de *Mandevilla*. A similaridade morfológica entre os dois gêneros, já apontada por Müller (1860), também foi observada por todos os autores que produziram trabalhos taxonômicos com o gênero.

Woodson (1933) mantém o gênero *Macrosiphonia* com a mesma circunscrição inicial, e propõe a criação de dois subgêneros: *Eumacrosiphonia* (=*Macrosiphonia*), com tubo da corola mais longo e distribuição restrita à América do Sul, e *Telosiphonia*, com tubo da corola mais curto e distribuição restrita ao México e sudoeste dos Estados Unidos.

Henrickson (1996) propõe mudanças em *Macrosiphonia sensu* Woodson (1993), elevando os dois subgêneros à categoria genérica. Desta forma, as espécies sul-americanas de *Macrosiphonia* permanecem neste gênero, enquanto as espécies mexicanas e norte-americanas passam a compor um novo gênero, *Telosiphonia*. No geral, a distinção entre *Macrosiphonia*, *Mandevilla* e *Telosiphonia* é muito tênue, baseando-se principalmente nas dimensões do tubo floral.

4) Mandevilla Lindl., Edwards's Bot. Reg. 26: pl. 7. 1840.

(Descrição baseada em Woodson, 1933 e Sales, 1993)

Figs. 1.1; 1.2F-G; 1.3

Lianas, arbustos ou subarbustos, às vezes com xilopódio bem desenvolvido; ramos volúveis, eretos ou escandentes, cilíndricos a angulosos, às vezes subcarnosos ou alados, com coléteres interpeciolares delgado-cônicos, diminutos ou bastante desenvolvidos. **Folhas** opostas ou verticiladas, pecioladas ou sésseis, com 1 a numerosos coléteres restritos à base da face adaxial da lâmina foliar ou dispostos ao longo da nervura primária.

Inflorescência de aspecto racemoso, axilar, às vezes terminal ou subterminal, pauci a multiflora, laxa a congesta; brácteas escariosas ou foliáceas. **Flores** actinomorfas a ligeiramente zigomorfas, pequenas a vistosas. **Cálice** profundamente 5-partido, lacínias escariosas ou mais raramente foliáceas, com um a vários coléteres alternos, opostos ou contínuos na base da face adaxial de cada lacínia. **Corola** tubular, hipocrateriforme, subipocrateriforme ou infundibuliforme, em diversas tonalidades de branco, branco-esverdeado, amarelo, violeta, rosa a púrpura ou magenta; tubo inferior cilíndrico a giboso; tubo superior de diferentes formatos; lobos cinco, eretos a patentes. **Anteras** com base sagitada, truncada ou auriculada, glabras. **Cabeça do estilete** triangular a deltóide, com projeções laterais bem evidentes e ocupando toda a sua extensão longitudinal e apêndice apical bífido inconspícuo a bastante desenvolvido. Ovário circundado por 2 nectários alternos a este ou por um disco nectarífero inteiro a 5-lobado. **Folículos** geminados, subparalelos ou divergentes, às vezes unidos no ápice, cilíndricos a torulosos.

Maior gênero de Mesechiteae, com cerca de 130 espécies distribuídas em todo o Neotrópico, alcançando também o México e sudoeste dos Estados Unidos.

Divisões infragenéricas (2 subgêneros, 5 seções):

A) Subgênero *Mandevilla* Woodson: Coléteres dispostos apenas na base da lâmina foliar adaxialmente ou ausentes. Tubo da corola cilíndrico e reto, não giboso ou arqueado. Coléteres do cálice numerosos, dispostos alternadamente ou continuamente (opostos em *M. funiformis*).

A1) Seção *Tubiflorae* Woodson (8 spp.): Lianas. Corola hipocrateriforme ou tubular-hipocrateriforme. Anteras auriculadas. Nectários 5, do mesmo tamanho ou maiores que o ovário. México e América Central.
Espécies: 01- *M. acutiloba* (A.DC.) Woodson; *M. Donnel-Smithii* Woodson; ***M. platydactyla*** Woodson; *M. rosana* (Donn. Sm.) Woodson; *M. sertuligera* Woodson; *M. scorpioidea* Woodson; ***M. syrinx*** Woodson; ***M. tubiflora*** (Mart. & Gal.) Woodson.

A2) Seção *Torosae* Woodson (5 spp.): Lianas. Corola hipocrateriforme. Anteras auriculadas. Nectários 5, menores que o ovário. México e Jamaica.
Espécies: *M. apocynifolia* (A. Gray.) Woodson; ***M. foliosa*** (Müll.Arg.); Woodson; ***M. karwinskii*** (Müll.Arg.) Woodson; *M. mexicana* (Müll.Arg.) Woodson; ***M. torosa*** (Jacq.) Woodson.

A3) Seção *Montanae* Woodson (17 spp.): Lianas, mais raramente subarbustos. Corola hipocrateriforme ou tubular-hipocrateriforme. Anteras

truncadas. Nectários 2-5, menores que o ovário, ou ausentes ("obsolete"). América do Sul.
Espécies: *M. achrestogyne* Woodson; *M. bogotensis* (Humb., Bonpl. & Kunth) Woodson; *M. brachyloba* (Müll.Arg.) K. Schum.; *M. callacatensis* Markgr.; ***M. cercophylla*** Woodson; *M. congesta* (Humb., Bonpl. & Kunth.) Woodson; ***M. emarginata*** (Vell.) Ezcurra; *M. fragilis* Woodson; ***M. jamesonii*** Woodson; *M. montana* (Humb., Bonpl. & Kunth) Markgr.; ***M. pentlandiana*** (A.DC.) Woodson; ***M. pycnantha*** (Steud.) Woodson; *M. riparia* (Humb., Bonpl. & Kunth) Woodson; *M. scutifolia* Woodson; *M. subpaniculata* Woodson; *M. subsessilis* (A.DC.) Woodson; ***Mandevilla tricolor*** Woodson.

A4) Seção *Tenuifoliae* Woodson (2 spp.): Subarbustos ou ervas. Corola hipocrateriforme. Anteras truncadas. Nectários 2. América do Sul.
Espécies: ***M. myriophyllum*** (Taub.) Woodson; ***M. tenuifolia*** (Mikan) Woodson

A5) Seção *Laxae* Woodson (50 spp.): Lianas, subarbustos ou arbustos. Corola infundibuliforme. Anteras auriculadas a truncadas. Nectários 2-5. México, América Central e América do Sul.
Espécies: 01- *M. albo-viridis* (Rubsy) Woodson; *M. andrieuxii* Müll.Arg.) Hemsl.; *M. angustifolia* (Malme) Woodson; ***M. atroviolacea*** (Stadelm.) Woodson; *M. bella* (Pittier) Woodson; *M. boliviensis* (Hook. f.) Woodson; *M. bridgesii* (Müll.Arg.) Woodson; ***M. callista*** Woodson; *M. cereola* Woodson; ***M. coccinea*** (Hook. & Arn.) Woodson; ***M. convolvulacea*** (A.DC.) Hemsl.; *M. crassinoda* (Gardn.) Woodson; *M. cuspidata* (Rubsy) Woodson; ***M. duartei*** Markgr.; *M. equatorialis* Woodson; *M. eximia* (Hemsl.) Woodson; ***M. fragrans*** (Stadelm.) Woodson; ***M. funiformis*** (Vell.) K. Schum.; *M. glabra* (Rubsy) Woodson; ***M. glandulosa*** (Ruiz & Pavan) Woodson; *M. grata* Woodson; *M. immaculata* Woodson; ***M. illustris*** (Vell.) Woodson; ***M. laxa*** (Ruiz & Pavan) Woodson; ***M. ligustrifolia*** Woodson; *M. lucida* Woodson; *M. luetzelburgii* (Ross. & Markgr.) Woodson; ***M. martiana*** (Stadelm.) Woodson; *M. minor* Woodson; ***M. moricandiana*** (A.DC.); Woodson; *M. mueleri* Woodson; ***M. novocapitalis*** Markgr.; ***M. oaxacana*** (A.DC.) Hensl.; *M. oblongifolia* Woodson; *M. pendula* (Ule) Woodson; ***M. permixta*** Woodson; ***M. pohliana*** (Stadelm.) A. Gentry; *M. rigidifolia* J.F. Morales; ***M. sancta*** (Stadelm.) Woodson; *M. sanderi* (Hensl.) Woodson; ***M. sellowii*** (Müll.Arg.) Woodson; ***M. spigeliiflora*** (Stadelm.) Woodson; ***M. splendens*** (Hook.) Woodson; *M. subcordata* Rubsy; *M. superba* Herzog; *M. surinamensis* (Pulle) Woodson; ***M. urophylla*** (Hook. f.) Woodson; ***M. veraguasensis*** (Seem.) Hemsl.; ***M. venulosa*** (Müll.Arg.) Woodson; ***M. widgrenii*** Ezcurra

B) Subgênero *Exothostemon* (44 spp.): Coléteres poucos a vários dispostos adaxialmente ao longo da nervura primária da lâmina foliar. Tubo da corola mais ou menos giboso ou arqueado. Coléteres do cálice 5, opostos às lacínias (freqüentemente fimbriados em algumas espécies).

Espécies: *M. aracamunensis* Morillo; ***M. anceps*** Woodson; ***M. annularifolia*** Woodson; *M. antennacea* (A.DC.) K. Schum.; ***M. benthamii*** (A.DC.) K. Schum.; *M. bracteata* (Humb., Bonpl. & Kunth) O. Ktze.; *M. bracteosa* (Rubsy) Woodson; *M. caurensis* Markgr.; ***M. dodsonii*** A.H. Gentry; ***M. duidae*** Woodson; *M. fenderi* (Müll.Arg.) Woodson; *M. filifolia* Monach.; ***M. hirsuta*** (A. Rich.) K. Schum.; *M. holstii* Morillo; *M. huberi* Morillo; *M. javitensis* (Humb., Bonpl. & Kunth) K. Schum.; ***M. krukowii*** Woodson; ***M. lancifolia*** Woodson; *M. lasiocarpa* (A.DC.) Malme; *M. lancibracteata* Woodson; ***M. leptophylla*** (A.DC.) K. Schum.; *M. mollis* Lundell; *M. molissima* (Humb., Bonpl. & Kunth) K. Schum.; *M. moritziana* (Müll.Arg.) Donn.; ***Mandevilla nerioides*** Woodson; *M. pachyphylla* Woodson; *M. pavonii* (A.DC.) Woodson; *M. pohlyantha* K. Schum. ex Woodson; ***M. rugellosa*** (Rich.) L. Allorge-Boiteau; ***M. rugosa*** (Benth.) Woodson; *M. rutila* Woodson; *M. sagittarii* Woodson; *M. scaberula* N.E. Br.; ***M. scabra*** (Roem. & Schult.) K. Schum.; *M. schilimi* (Müll.Arg.) Woodson; *M. spruceana* (Müll.Arg.) K. Schum.; *M. steyermarkii* Woodson; *M. subcarnosa* (Benth.) Woodson; ***M. subsagittata*** (Ruiz & Pavan) Woodson; *M. symphitocarpa* (G.F.W. Mey.) Woodson; *M. trianae* Woodson; ***M. turgida*** Woodson; *M. ulei* Markgr.; *M. vanheurckii* (Müll.Arg.) Markgr.; *M. villosa* (Miers) Woodson.

Observações: Woodson (1933), em extenso trabalho de revisão, definiu os limites atuais de gênero, compreendendo um total de 108 espécies. Desde então, várias espécies do gênero foram publicadas, e o número total de espécies gira em torno de 130. Além de ampliar a circunscrição do gênero, Woodson (1933) propôs um sistema de classificação infragenérico, dividido *Mandevilla* em dois subgêneros, *Exothostemon* e *Mandevilla*, sendo este último dividido em cinco seções. A delimitação entre os dois subgêneros dá-se a partir de caracteres vegetativos e florais, sendo o mais consistente a disposição dos coléteres foliares. Em *Exothostemon*, os coléteres dispõem-se nas axilas da nervura primária, enquanto no subgênero *Mandevilla* estão limitados à base da lâmina foliar. A distribuição dos coléteres sobre a nervura é uma característica exclusiva de *Exothostemon*, não sendo conhecida para nenhum outro grupo de Apocynaceae. Desta forma, constitui uma sinapomorfia para o grupo e permite uma fácil distinção com o subgênero *Mandevilla*. Caracteres florais de cálice e corola também foram utilizados por Woodson (1933) na separação entre os subgêneros. Em *Exothostemon* o tubo inferior da corola é curvado em maior ou menor grau, enquanto no subgênero *Mandevilla* o tubo inferior é sempre reto. Os coléteres do cálice em *Exothostemon* são sempre opostos, em número de 1 por lacínia, enquanto no subgênero *Mandevilla* apresentam-se variáveis tanto na disposição quanto no número. Apesar da distinção baseada nestes caracteres florais ser adequada para a maioria

das espécies, há algumas exceções. O caso mais notável é o de *M. funiformis*, que apesar de estar no subgênero *Mandevilla* encaixa-se melhor na circunscrição do subgênero *Exothostemon*, pois apresenta 5 coléteres opostos e o tubo inferior da corola curvado. A justificativa para mantê-la no subgênero *Mandevilla* é o fato de possuir coléteres foliares restritos à base da lâmina foliar. Woodson (1933) já havia observado a ocorrência de algumas espécies com características intermediárias entre os dois subgêneros, e sugeriu que os limites destes devessem ser melhor testados e reavaliados futuramente.

O subgênero *Mandevilla* é o maior dos dois, com 77 espécies. Woodson (1933) dividiu-o em cinco seções, baseado em caracteres florais da corola (forma), das anteras (forma da base) e dos nectários (número e tamanho). São estas as seções *Tubiflorae* (8 spp.), *Torosae* (5 spp.), *Montanae* (16 spp.), *Tenuifoliae* (2 spp.) e *Laxae* (46 spp.) Esta divisão revelou-se pouco prática, criando grupos pouco consistentes e de difícil delimitação. Trabalhos recentes (Sales 1993, Morales 1998) não adotaram as seções propostas por Woodson (1933), mantendo apenas a divisão em subgêneros.

Pichon (1948) ampliou a circunscrição do gênero, o qual passou a incluir *Macrosiphonia sensu* Woodson (1933). Para agrupar este gênero ampliado, propôs a criação de uma subtribo exclusiva, Mandevillinae. A uniformidade estrutural e o íntimo relacionamento destes gêneros já eram reconhecidos e continuou o sendo posteriormente, porém a circunscrição mais ampla de *Mandevilla* não foi aceita e trabalhos recentes reconhecem o status genérico de *Allomarkgrafia, Mesechites* e *Macrosiphonia* (Sales 1993, Morales 1997, 1998, Williams 1999).

5) *Mesechites* Müll.Arg. in Mart., Fl. bras. 6(1): 150. 1860.

(Descrição baseada em Woodson, 1933)

Figs. 1.2C,J; 1.4B

Lianas, ramos volúveis. **Folhas** opostas, com 1-vários coléteres na base da face adaxial da lâmina foliar, diminutos a deltóides, formando ou não um denso aglomerado. **Inflorescência** axilar, bostricóide-racemosa, di ou tricotomicamente ramificada, multiflora ou reduzida a 1-2 flores. **Flores** actinomorfas. **Cálice** 5-partido, com coléteres contínuos dispostos adaxialmente na base. **Corola** hipocrateriforme; tubo inferior cilíndrico; tubo

superior dilatado a levemente urceolado. **Anteras** com base curtamente sagitada, glabras. **Cabeça do estilete** oblonga, levemente angulosa, com as projeções longitudinais restritas à sua base e apêndice apical bífido. **Ovário** circundado por um disco nectarífero 5-lobado, os lobos concrescentes ou parcialmente livres entre si, com o mesmo tamanho que o ovário. **Folículos** livres, cilíndricos.

Gênero com 10 espécies de distribuição neotropical, na América Central, Caribe e América do Sul.

Divisões infragenéricas (2 subgêneros):

A) Subgênero *Mesechites* Woodson: Coléteres da lâmina foliar 1-4, laminados ou irregularmente pectinados, agrupados de forma concêntrica; corola branco-esverdeada salpicada de vermelho ou púrpura, ou amareladas; lianas da América do Sul e Central, incluindo Trinidad e Tobago
Espécies: *M. acuminata* (Ruiz & Pavan) Müll.Arg.; *M. bicorniculata* (Rubsy) Woodson; *M. citrifolia* Woodson; ***M. mansoana*** (A. DC.) Woodson; *M. pilossisima* Woodson; *M. sanctae-crucis* Woodson; ***M. trifida*** (Jacq.) Müll.Arg.

B) Subgênero *Dydimnadenia* Woodson: Coléteres da lâmina foliar 2, fusiformes, agrupados de forma radial; corola creme ou rósea; lianas de Cuba e Hispaniola
Espécies: *M. angustifolia* (Poir.) Miers; ***M. minima*** Woodson; *M. repens* (Jacq.) Miers; ***M. rosea*** Miers.

Observações: O gênero foi descrito por Müller (1860), baseado em *Echites mansoana*. Woodson (1933) estabeleceu os limites atuais do gênero, criando dois subgêneros: Me*sechites* (=*Mesechites*), com 3 espécies da América Central e do Sul, e *Didymnadenia*, com 3 espécies de Cuba e Hispaniola.

Mesechites apresenta claras afinidades com *Mandevilla* e *Macrosiphonia*, diferindo pelo tipo de inflorescência e pela estrutura da cabeça do estilete. O padrão "umbraculiforme" de cabeça do estilete também ocorre em *Mesechites*, porém o corpo principal apresenta formato oblongo e as projeções laterais, apesar de proeminentes, estão limitadas à sua base, padrão este também observado em *Allomarkgrafia* e *Tintinnabularia*.

6) *Quiotania* Zarucchi, Novon 1: 33. 1991.

(Descrição baseada em Zarucchi, 1991)

Liana, ramos volúveis. **Folhas** opostas, com coléteres pouco evidentes dispostos na base da face adaxial. **Inflorescência** axilar, de aspecto racemoso, multiflora, congesta. **Flores** actinomorfas. **Cálice** profundamente 5-partido, com coléteres contínuos dispostos adaxialmente na base. **Corola** amarelada, infundibuliforme, com tubo bastante rudizido. **Anteras** com base truncada, glabras. **Cabeça do estilete** deltóide, com projeções laterais bem evidentes e ocupando toda a sua extensão longitudinal e apêndice apical bífido reduzido. **Ovário** circundado por um disco nectarífero 5-lobado. **Folículos** livres, cilíndricos.

Observações: Gênero monotípico, baseado em *Q. colombiana*. Zarucchi (1991) enquadra o gênero dentro da circunscrição de Mesechiteae, mas afirma não ter evidências claras para determinar quais são os gêneros a ele mais relacionados. Apesar disto, observa haver uma grande similaridade deste com *Mandevilla*, diferindo unicamente pela ausência de um tubo da corola diferenciado (a corola é reduzida, sem distinção aparente entre tubo superior e inferior), não havendo além deste outros caracteres confiáveis que possam separar os dois gêneros. Williams (1999), apesar de não opinar sobre o reconhecimento do gênero, afirma que uma espécie de *Mandevilla, M. syrinx*, também possui o tubo da corola reduzido e sem diferenciação aparente, semelhante ao descrito por Zarucchi (1991) para *Quiotania*. O reconhecimento do status genérico de *Quiotania*, portanto, é bastante questionável, uma vez que não há caracteres que o distingam de *Mandevilla*.

7) *Secondatia* A.DC. in DC., Prodr. 8: 445. 1844.

(Descrição baseada em Woodson, 1935)

Figs. 1.2A,I; 1.4D

Lianas; ramos volúveis. **Folhas** opostas, sem coléteres na lâmina foliar. **Inflorescência** terminal, às vezes também axilar, tirsiforme, multiflora. **Cálice** profundamente 5-partido, com coléteres alternos dispostos adaxialmente na base. **Corola** hipocrateriforme, tubo cilíndrico. **Estames** 5, inclusos, inseridos na base do tubo superior da corola; anteras adnatas à cabeça do estilete, base sagitada. **Anteras** pilosas dorsalmente, com a base sagitada. **Cabeça do estilete** oblongo-fusiforme, sem projeções laterais, com

apêndice apical bífido, séssil. **Ovário** circundado por um disco nectarífero 5-lobado, os lobos parcial ou totalmente concrescentes entre si. **Folículos** livres, fusiformes.

Espécies: ***S. densiflora*** A. DC.; *S. duckei* Markgr.; ***S. floribunda*** A. DC.; *S. macnabii* (Urb.) Woodson; *S. peruviana* Poepigg; *S. schlimiana* Müll.Arg.

Gênero neotropical, com 6 espécies. Ocorre predominantemente na América do Sul, sendo que uma espécie, *S. macnabii*, ocorre na Jamaica.

Observações: A inclusão de *Secondatia* em Mesechiteae por Endress & Bruyns (2000) é bastante discutível. O gênero não apresenta o conjunto de caracteres que definem a tribo: os coléteres foliares estão ausentes e a estrutura da cabeça do estilete é similar à que caracteriza a Tribo Apocyneae, sem projeções laterais.

8) *Telosiphonia* (Woodson) Henrickson, Aliso 14(3): 179. 1995.

(Descrição baseada em Henrickson, 1996)

Fig. 1.4F

Arbustos ou subarbustos eretos, providos de xilopódio. **Folhas** opostas, com coléteres diminutos na base da face adaxial da lâmina foliar. **Inflorescências** paucifloras, formando monocásios ou dicásios, às vezes reduzidas a flores solitárias. **Flores** actinomorfas, vistosas, vespertinas, pediceladas. **Cálice** profundamente 5-partido, com vários coléteres dispostos de forma contínua adaxialmente na base das lacínias. **Corola** branca, hipocrateriforme; tubo inferior longo, cilíndrico; tubo superior largamente cilíndrico a campanulado; lobos 5, patentes. **Anteras** com base truncada, às vezes subcordada, glabras; pólen tricolpado, esferoidal. **Ovário** circundado por um disco nectarífero 5-lobado, os lobos retangulares e unidos em sua quase totalidade. **Cabeça** do estilete deltóide, com projeções laterais bem evidentes e ocupando toda a sua extensão longitudinal e apêndice apical bífido reduzido. **Folículos** livres, cilíndricos a torulosos.

Espécies: ***T. brachysiphon*** (Torr.) Henrickson; ***T. hesperia*** (I.M. Johnston) Henrickson; ***T. hypoleuca*** (Benth.) Henrickson; ***T. lanuginosa*** (Mart. & Gal.) Henrickson; ***T. macrosyphon*** (Torr.) Henrickson; ***T. nacalpulensis*** Felger & Henrickson;

O gênero possui 6 espécies, distribuídas no México e sudoeste dos Estados Unidos, em formações abertas.

Observações: Telosiphonia foi estabelecido por Henrickson (1996), englobando as cinco espécies de *Macrosiphonia* subgênero *Telosiphonia sensu* Woodson (1933) e uma nova espécie por ele descrita, *T. nacapulensis*. Woodson (1933) estabeleceu os dois subgêneros por achar que, apesar de apresentarem diferenças significativas entre si (especialmente quanto à distribuição geográfica e detalhes na estrutura floral), estas não eram suficientes para justificar a separação em dois gêneros distintos. No entanto, reconhece que esta posição pode estar equivocada, e os dois grupos possam compor na verdade gêneros distintos. Para ele, cada grupo teria uma história evolutiva particular, e a similaridade estrutural que apresentam não indicaria uma relação filogenética, mas sim uma evolução convergente por ocuparem ambientes semelhantes e sofrerem o mesmo tipo de pressão seletiva.

Henrickson (1996) propõe a elevação de *Telosiphonia* à categoria genérica, afirmando haver um conjunto de caracteres florais (aspectos da inflorescência, da estrutura da cabeça do estilete e do pólen) suficientemente distinto nos dois grupos para justificar sua separação em nível genérico. Esta argumentação retoma as idéias de Woodson (1933), aceitando a hipótese da evolução paralela de duas linhagens isoladas geograficamente. A proposta de Henrickson (1996) nos pareceu razoável, por apresentar um conjunto consistente de caracteres distintivos em relação às espécies de *Macrosiphonia* sul-americanas. No entanto, a delimitação entre os dois gêneros e destes com *Mandevilla* ainda é questionável, conforme atestam outros autores (Zarucchi 1991, Williams 1999), e, portanto, uma análise profunda envolvendo todos estes gêneros faz-se necessária.

9) *Tintinnabularia* Woodson, Ann. Missouri Bot. Gard. 23: 387-388. 1936.

(Descrição baseada em Woodson, 1936)

Fig. 1.2D; 1.4G

Lianas, latescentes; ramos volúveis. **Folhas** opostas, com poucos e inconspícuos coléteres na base da face adaxial da lâmina foliar; domácias elípticas, pouco conspícuas presentes na superfície abaxial junto às axilas da nervura principal. **Inflorescência** axilar, alterna, corimbosa-tricasial, multiflora ou reduzida a 2-3 flores; brácteas foliáceas. **Cálice** profundamente 5-partido, lacínias foliáceas, com coléteres opostos dispostos adaxialmente

na base. **Corola** infundibuliforme, tubo inferior cilíndrico, tubo superior dilatado. **Anteras** com base obtusa, glabras. **Cabeça do estilete** oblonga, levemente angulosa, com as projeções longitudinais restritas à sua base e apêndice apical bífido. **Ovário** circundando por um disco nectarífero 5-lobado, os lobos separados ou mais ou menos concrescidos, do mesmo tamanho que o ovário. **Frutos** desconhecidos, provavelmente folículos livres, cilíndricos.

Espécies: ***T. gratissima*** J.F. Morales; ***T. mortonii*** Woodson; *T. murallensis* J.K. Willians.

Gênero pequeno, com três espécies restritas às florestas pluviais de altitude do México, Guatemala e Honduras.

Observações: O gênero, estabelecido por Woodson (1932), foi baseado em uma única espécie, *T. mortonii*. Recentemente teve sua circunscrição ampliada para três espécies, com a descrição de *T. gratissima* por Morales (1996) e *T. murallensis* por Willians (1999).

Woodson (1932) comenta sobre possíveis afinidades deste com representantes de Mesechiteae, observando haver uma grande similaridade com *Allomarkgrafia*. Entretanto, o autor afirma que *Tintinnabularia* apresenta uma maior proximidade com *Beaumontia*, por ambos possuírem corola infundibuliforme, cálice foliáceo, estames com filetes longos e pela presença de domácias nas folhas. Esta possível proximidade entre os dois gêneros foi aceita por Pichon (1950), que não incluiu *Tintinnabularia* na subtribo Mandevillinae, e sim na subtribo Forsteroniinae, possivelmente pela presença de domácias. Leeuwenberg (1994) concorda com as idéias de Woodson (1932) e posiciona *Tintinnabularia* na subtribo Wrightiinae, onde também está incluído *Beaumontia*. Williams (1999) não concorda com Leeuwenberg e posiciona *Tintinnabularia* na subtribo Mandevillinae, por apresentar três caracteres que também ocorrem nos outros representantes desta subtribo: coléteres na base da lâmina foliar, cabeça do estilete com 5 projeções laterais e distribuição restrita ao Novo Mundo. Dos caracteres compartilhados com *Beaumontia*, apenas a presença de filetes longos sustenta uma hipotética afinidade entre os dois. Domácias ocorrem em gêneros comprovadamente não relacionados filogeneticamente, inclusive pertencentes a diferentes subfamílias, e devem ter tido origens paralelas durante a história evolutiva de Apocynaceae. Corolas infundibuliformes ocorrem em diversos gêneros da família, e demonstram mais uma especialização à polinização do que refletem uma verdadeira relação filogenética.

Desta forma, os argumentos que sustentam a inclusão de *Tintinnabularia* em Mesechiteae são mais fundamentados do que os que propõe a manutenção deste gênero no grupo de *Beaumontia*.

Dentre os seis caracteres definidos por Woodson (1932) para caracterizar o gênero, apenas dois continuam válidos, em face da recente descoberta de duas novas espécies. Desta forma, há uma necessidade da reavaliação do status genérico de Tintinnabularia e de sua relação com outros gêneros de Apocynaceae. Os dois caracteres que permanecem distintivos do gênero são a presença de domácias e as inflorescências do tipo dicasial ou sub-umbelada, enquanto os quatro que não mais desempenham este papel são a presença de corola infundibuliforme (tubular-campanulada em *T. murallensis*), lacínias do cálice foliáceas (escariosas em *T. murallensis*), filamentos da antera 3-5 vezes maiores que as anteras (curtos em *T. gratissima*) e anteras com apêndices apicais (ausentes em *T. gratissima*).

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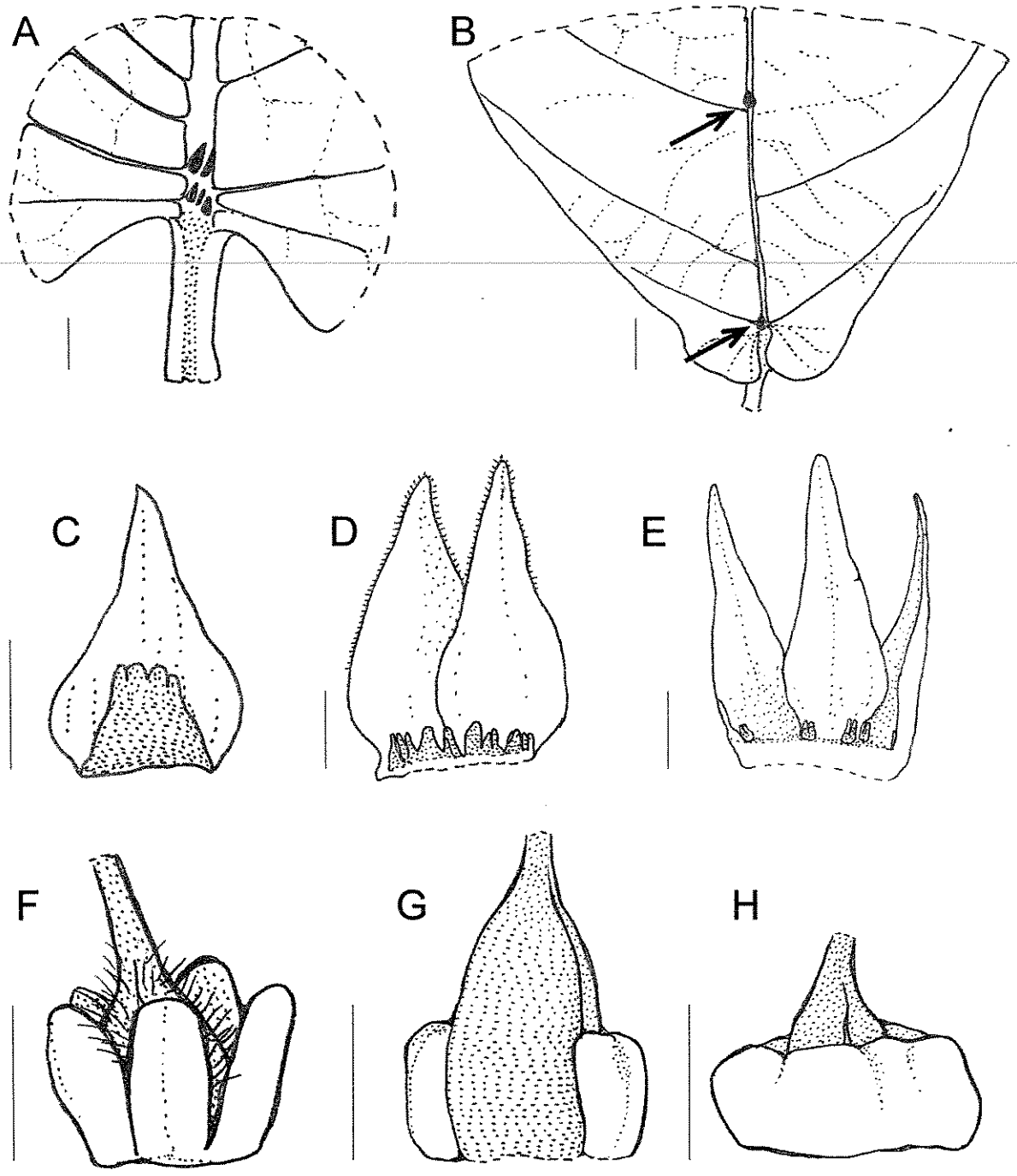

Figura 1.1. Tipos de coléteres e nectários em Mesechiteae. A-B: Coléteres foliares. C-E: Coléteres calicinais. F-H: Nectários. A. *Mandevilla oaxacana*, coléters na base da lâmina foliar. B. *M. hirsuta*, detalhe de dois coléteres (setas) dispostos ao longo da lâmina foliar. C. *M. subsagittata*, coléter oposto à lacínia do cálice; D. *M. syrinx*, coléteres formando um anel contínuo; E. *M. atroviolacea*, coléteres alternos. F. *M. convolvulacea*, 5 nectários do mesmo tamanho que o ovário. G. *M. atroviolacea*, 5 nectários. H. *M. subsagittata*, 5 nectários menores que o ovário. Escala = 1mm.

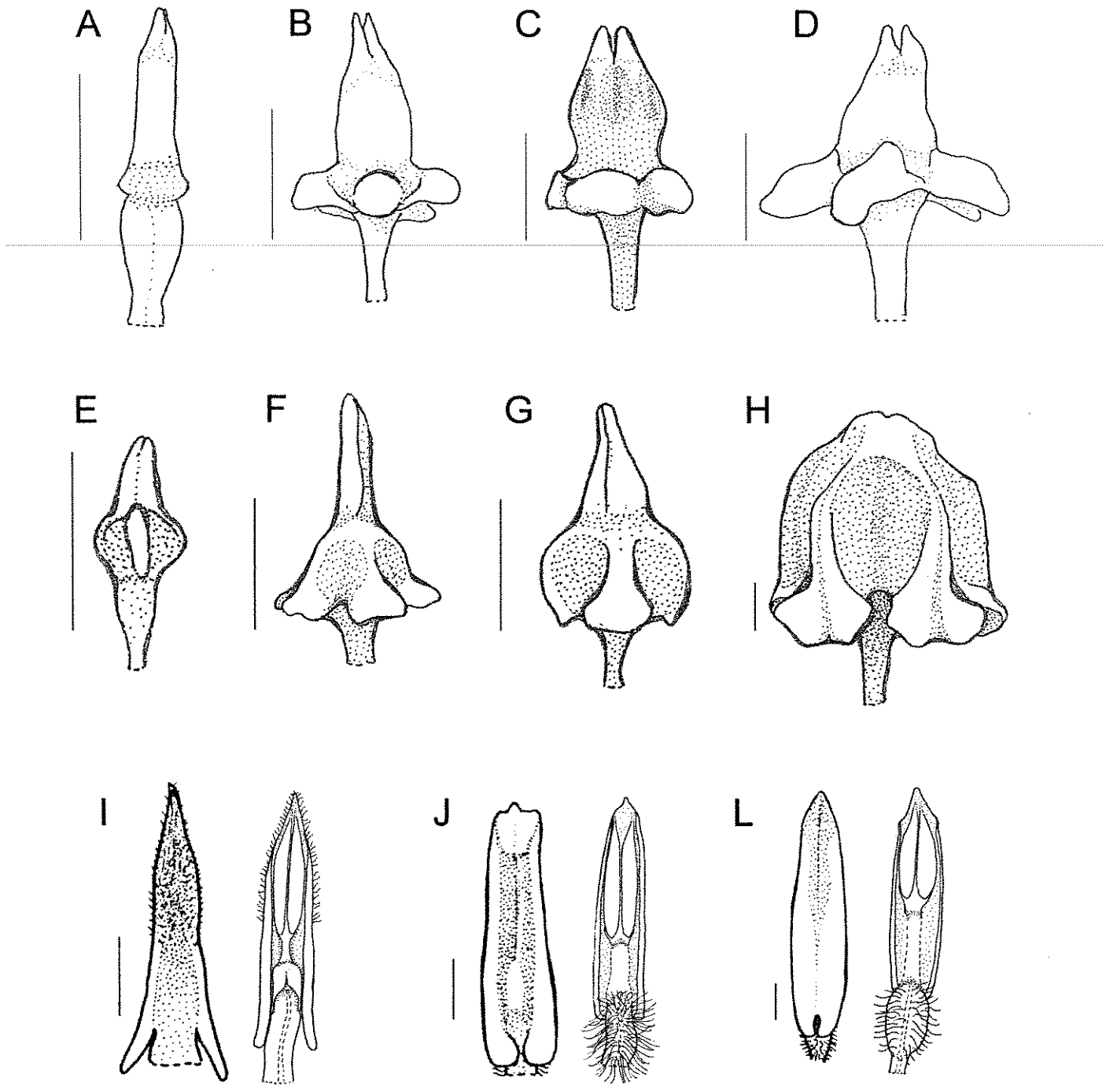

Figura 1.2. Estrutura da cabeça do estilete e estames em espécies de Mesechiteae e Apocyneae (*Forsteronia*). A-H: Cabeça do estilete. I-L: Estames. A,I. *Secondatia densiflora*; B. *Allomarkgrafia brenesiana*; C,J. *Mesechites mansoana*; D. *Tintinnabularia mortonii*; E. *Forsteronia velloziana*; F. *Mandevilla syrinx*; G. *M. mexicana*; H,L. *Macrosiphonia longiflora.* Escala = 1mm.

Figura 1.3: Repesentantes do gênero *Mandevilla*. A: *M. atroviolacea* (Stadelm.) Woodson, ramo florido. B: *M. coccinea* (Hook. & Arn) Woodson, ramo florido. C: *M. illustris* (Vell.) Woodson, ramo florido. D-E: *M. hirsuta* (A. Rich,) K. Schum - d) flor, vista frontal; e) flor, vista lateral. F: *M. pycnantha* (Steud.) Woodson, inflorescência. G: *M.* cf. *rugosa* (Benth.) Woodson, ramo florido. H: *M. pentlandiana* (A. DC.) Woodson, inflorescência. I: *M. myriophyllum* (Taub.) Woodson, indivíduo.

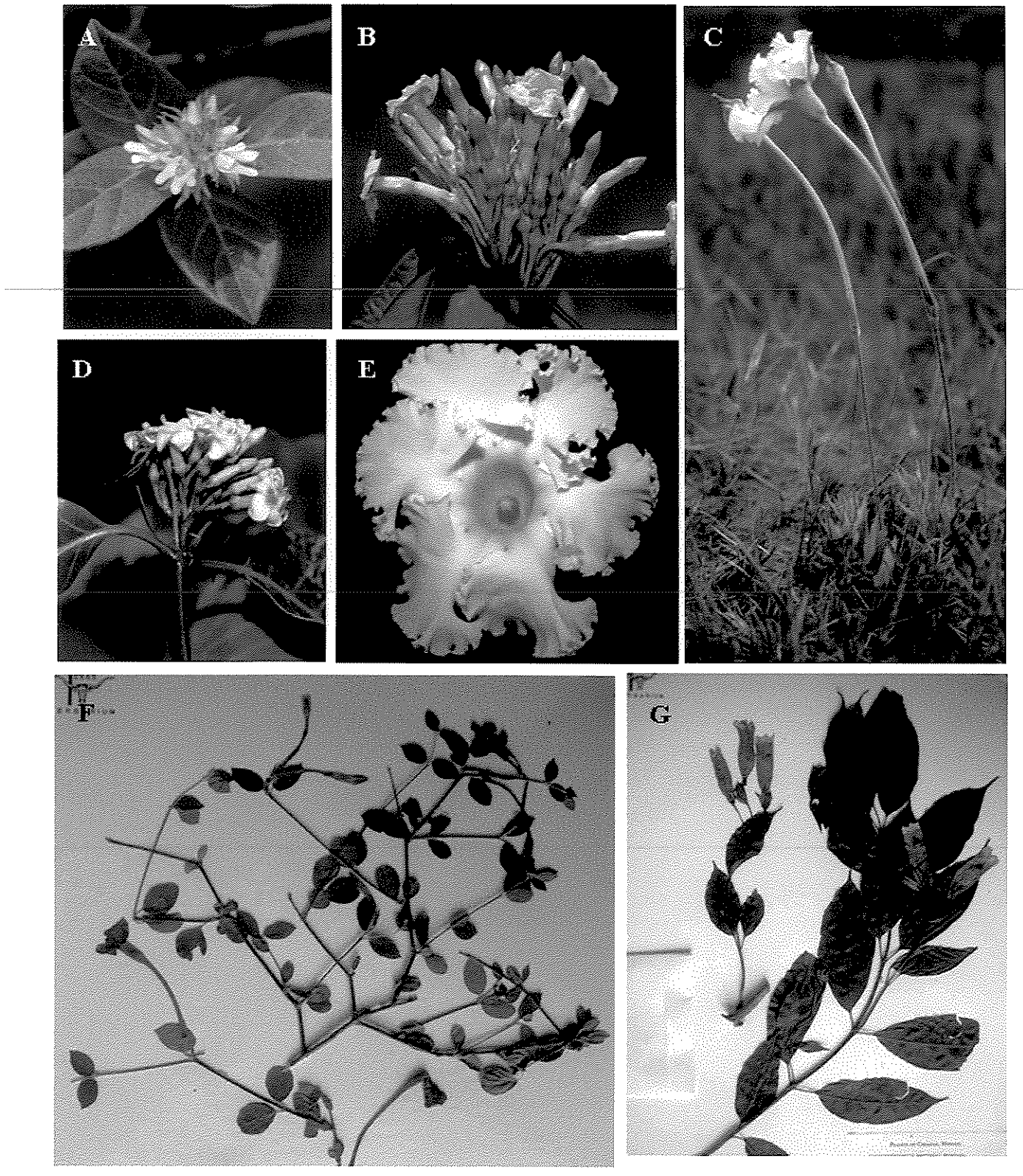

Figura 1.4: Repesentantes de Mesechiteae. A: *Forsteronia velloziana* Müll.Arg., inflorescência. B: *Mesechites mansoana (*A. DC.) Woodson, inflorescência. C,E: *Macrosiphonia longiflora* (Desf.) Müll.Arg. c) ramo florido; e) flor, vista frontal. D: *Secondatia densiflora* A. DC., inflorescência. F) *Telosiphonia lanuginosa* (Mart. & Gal.) Henrickson, ramo florido. G) *Tintinnabularia mortonii* Woodson, ramo florido.

CAPÍTULO II

TRIBAL AND INTERGENERIC RELATIONSHIPS OF MESECHITEAE (APOCYNOIDEAE, APOCYNACEAE): EVIDENCE FROM THREE NONCODING PLASTID DNA REGIONS AND MORPHOLOGY[1]

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Abstract - The Neotropical tribe Mesechiteae (Apocynaceae) is currently considered to include nine genera: *Allomarkgrafia*, *Galactophora*, *Macrosiphonia*, *Mandevilla*, *Mesechites*, *Quiotania*, *Secondatia*, *Telosiphonia*, and *Tintinnabularia*. Tribal and intergeneric relationships, however, are in dispute. To test the monophyly of the tribe and evaluate intratribal relationships, a maximum parsimony analysis was conducted based on DNA sequences from the plastid *rpl16* intron, *rps16* intron, and *trnS-G* intergenic spacer region as well as morphological data for 23 taxa of Mesechiteae and 11 taxa from other tribes of Apocynoideae. Mesechiteae, as currently circumscribed, was found to be paraphyletic. Only removal of *Secondatia* and *Galactophora* and inclusion of *Forsteronia* rendered the tribe monophyletic. Thus defined, Mesechiteae forms a strongly supported clade including seven genera in three subclades: the *Mesechites* subclade (comprising *Tintinnabularia*, *Allomarkgrafia*, and *Mesechites*), the *Forsteronia* subclade (containing only *Forsteronia*) and the *Mandevilla* subclade (comprising *Macrosiphonia*, *Mandevilla*, and *Telosiphonia*). *Allomarkgrafia* is nested in *Mesechites*. *Macrosiphonia* and *Telosiphonia* form two distinct monophyletic clades. Both, however, are nested in *Mandevilla*. Results suggest upholding the following genera in Mesechiteae: *Allomarkgrafia*, *Forsteronia*, *Mandevilla*, *Mesechites*, and *Tintinnabularia*. The status of *Quiotania* could not be evaluated.

Key words: Apocynaceae; Apocynoideae; Mesechiteae; morphology; phylogenetic systematics; *rpl16*; *rps16*; *trnS-G*

INTRODUCTION

Mesechiteae is one of the five tribes comprising the subfamily Apocynoideae (Endress and Bruyns, 2000) and includes nine genera and about 150 species. It is restricted to the Neotropics, where it has a broad distribution, ranging from the southwestern United States throughout Mesoamerica and Caribbean to southern South America in rainforests, montane forest, savanna, and desert habitats. The tribe is extremely variable in habit and includes vines, erect subshrubs, and small trees. Floral structure is also remarkably diverse, especially the corolla, which ranges from small, inconspicuous, whitish, and tubular to large, variously colored, and infundibuliform.

Although several recent phylogenetic studies have addressed the circumscription of the Apocynaceae and their relationships with the Asclepiadaceae (Judd et al., 1994; Sennblad and Bremer, 1996, 2002; Potgieter and Albert, 2001), resulting in the amalgamation of these two families into Apocynaceae sensu lato (Endress and Bruyns, 2000), many other aspects of classification within the family remain controversial. One main controversy has been the delimitation and composition of tribes (Table 1). This is exemplified by Mesechiteae, for which relationships with other tribes are unknown and the generic circumscription is confusing and shows little consistency (Zarucchi, 1991; Henrickson, 1996; Williams, 1999).

Pichon (1950) recognized four tribes in Apocynoideae: Parsonsieae, Nerieae, Ecdysanthereae, and Ichnocarpeae. His tribal delimitations were based mainly on the form of the "retinacle," a term he coined for the specialized region of the anther that unites it with the style head (Pichon, 1948a). Except for *Galactophora* and *Secondatia*, most of the genera included in the ingroup in our study were placed in his tribe Ichnocarpeae, which was defined by the presence of a glabrous, concave retinacle. Within Ichnocarpeae, five subtribes were recognized, only two of which are pertinent to our study: Forsteroniinae and Mandevillinae.

A new classification for the Apocynaceae sensu stricto was published by Leeuwenberg (1994). Although his work was clearly influenced by Pichon's ideas, only three tribes were recognized in Apocynoideae: Echiteae, Wrightieae, and Apocyneae. This was the first classification to take into account the priority rule, necessitating changing the

name of Pichon's (1950) tribe Parsonsieae to Echiteae and Nerieae to Wrightieae. Leeuwenberg's third tribe, Apocyneae, was more or less equivalent to Pichon's tribes Ecdysanthereae and Ichnocarpeae combined. Leeuwenberg's tribes, however, are difficult to distinguish, and Sennblad et al. (1998) showed that they are artificial. Leeuwenberg (1994) arranged the genera he included in his Echiteae into two subtribes, Echitinae and Parsonsiinae, without any explanation of the criteria he used to delimit them. In his classification, most of the genera that comprise the ingroup in this study were placed in Echitinae. The only exception is *Tintinnabularia,* which was placed near *Beaumontia* in Wrightieae. Although Leeuwenberg (1994) gave no reasons for this placement of *Tintinnabularia*, presumably he was influenced by Woodson's (1936) comment that *Tintinnabularia* and *Beaumontia* share three character states: (1) domatia on the lower surface of the leaves, (2) long anther filaments, and (3) foliaceous sepals. The first two are relatively rare in the family.

The most recent classification of the family is that by Endress and Bruyns (2000). Theirs was a unified classification taking into account both Apocynaceae and Asclepiadaceae, and the first one to incorporate phylogenetic considerations. Two of the five subfamilies they recognized, Rauvolfioideae and Apocynoideae, correspond to the traditional Apocynaceae s. str., the other three to the Asclepiadaceae as traditionally circumscribed. Their classification differed most dramatically from those of previous authors with regard to the circumscription of Rauvolfioideae and Apocynoideae. Although the subfamilies and tribes proposed by Endress and Bruyns (2000) were based mainly on morphological characters, this was the first classification to incorporate evidence from molecular studies as well (e.g., Endress et al., 1996; Sennblad and Bremer, 1996; Sennblad et al., 1998; K. Potgieter and V. Albert, University of Illinois, personal communication.).

Endress and Bruyns (2000) recognized five tribes in Apocynoideae: Wrightieae, Malouetieae, Echiteae, Mesechiteae, and Apocyneae. Two of the most important morphological characters they used to define these tribes are the structure of the style head and the retinacle, both of which are also important characters in Pichon's (1948a, 1950) classification, but were not considered reliable by A. Leeuwenberg (Wageningen Agricultural University, personal communication). Endress and Bruyns (2000) significantly changed tribal circumscription and composition from those in the classification systems of

Pichon (1950) and Leeuwenberg (1994). Most taxa included in a more narrowly circumscribed Mesechiteae by Endress and Bruyns (2000) were part of the heterogeneous Ichnocarpeae of Pichon (1950) and the Echiteae of Leeuwenberg (1994).

Another controversy concerns whether or not smaller satellite genera should be recognized as distinct from their closely related, larger genus. Within Mesechiteae, one such case is whether *Allomarkgrafia* should be included in the synonymy of *Mesechites* (as proposed by Pichon, 1950 and followed by Leeuwenberg, 1994) or recognized as a distinct genus (as in the classification of Endress and Bruyns, 2000). Another problematic taxonomic question concerns the large genus *Mandevilla* and its smaller satellite genera *Macrosiphonia*, *Telosiphonia* and, more recently, *Quiotania*. In this group of taxa, one to four genera have been recognized by specialists in the family (Zarucchi, 1991; Leeuwenberg, 1994; Henrickson, 1996; Williams, 1999).

The classification of Apocynaceae s. l. proposed by Endress and Bruyns (2000) represents a considerable advance in the systematics of the family and is a logical starting point for studies that test the phylogenetic relationships of the groupings proposed therein. This type of analysis was done by Potgieter and Albert (2001), based on the *trnL-F* intergenic spacer and morphology in a broad phylogenetic study of the family. They found well-defined clades in Rauvolfioideae, several of which are reflected in the current classification. For Apocynoideae, however, no clearly defined groups were retrieved using that plastid region.

The aims of the present article are to test the monophyly of Mesechiteae sensu Endress and Bruyns (2000) and the relationships among its constituent genera using both morphology and molecular sequence data from the plastid *rpl16* intron, *rps16* intron and *trnS-G* intergenic spacer. The resulting hypothesis of phylogenetic relationships within Mesechiteae is compared with the current classification, morphological features that characterize clades are discussed and a modified circumscription of Mesechiteae is proposed.

MATERIALS AND METHODS

Taxon sampling—Twenty-three taxa, representing eight of the nine genera currently recognized in Mesechiteae by Endress and Bruyns (2000) were defined as the ingroup and included in this study (*Allomarkgrafia*, *Galactophora*, *Macrosiphonia*, *Mandevilla*, *Mesechites*, *Secondatia*, *Telosiphonia*, and *Tintinnabularia*). *Mandevilla tenuifolia* exhibhits a high degree of polymorphism in its habit and vegetative parts, one morphotype (*Mandevilla tenuifolia*2) of which is very similar to *M. myriophyllum*. Therefore, in order to determine the relationship of these two species, we included two samples of the most extreme forms of *M. tenuifolia* and one from *M. myriophyllum*. *Quiotania* could not be included, as none of the type material could be located, suggesting that it was never distributed. Outgroup taxa were chosen from all but the basalmost tribe of the subfamily (Wrightieae), based largely on previous studies, which suggest that the closest relative of Mesechiteae is either Apocyneae or Echiteae (Sennblad et al., 1998; Sennblad and Bremer, 2002). Two genera from Echiteae (*Prestonia* and *Rhodocalyx*) and five genera from Apocyneae (*Beaumontia*, *Chonemorpha*, *Forsteronia*, *Odontadenia*, and *Trachelospermum*) were included. Three representatives of Malouetieae, (two species of *Pachypodium* and one species of *Mascarenhasia*) were used to root the cladograms. Taxon names, voucher information, and GenBank accession numbers are given in Appendix 1 (see Supplemental Data accompanying online version of this article).

DNA extraction, amplification and sequencing—Total genomic DNA was extracted from silica-dried leaf material or from herbarium specimens using DNeasy Plant mini kits (Qiagen, Valencia, California, USA) following the manufacturer's protocol. Three noncoding plastid regions, the rpl16 intron, rps16 intron and trnS-G intergenic spacer, were amplified for all taxa. Double-stranded DNA was amplified by polymerase chain reaction (PCR) on a Biometra Tgradient machine (Biometra, Göttingen, Germany), applying a thermal cycling program consisting of 34 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 1 min and extension at 72°C for 90 sec. Reactions were terminated with a final extension of 4 min at 72°C. All PCR reactions were performed in a total volume of 25 μL

reactions, using 2.5 mM MgCl, 10% PCR* Buffer (Amersham Biosciences, Otelfingen, Switzerland), 0.25 mM dNTP, 0.5 units Taq DNA polymerase (Amersham Biosciences, Otelfingen, Switzerland), 1–4 μl BSA (bovine serum albumin, Sigma, Steinheim, Germany) and 0.1 mM of each primer. Primer information is presented in Appendix 2 (see Supplemental Data accompanying online version of this article). For a few taxa, internal primers were also used to amplify the rpl16 intron and trnS-G intergenic spacer, with the following changes in the thermal cycling program: 40 instead of 34 cycles and extension time shortened to 1 min. Successfully amplified PCR products were then purified using GFX PCR DNA and a gel band purification kit (Amersham Biosciences, Otelfingen, Switzerland).

The same primers used for PCR amplification were also used for the cycle-sequencing reactions, carried out with the ABI Prism Big Dye Terminator Cycle Sequencing Ready Extraction Kit (Perkin Elmer, Applied Biosystems, Applera Europe BV, Rotkreuz, Switzerland). Sequence products were purified on MicroSpin G-50 columns (Amersham Pharmacia Biotech Europe, Dübendorf, Switzerland) and loaded on an ABI Prism 377 DNA sequencer (Perkin Elmer). Complementary strands were edited and assembled with Sequencher 3.1.1 (Gene Codes, Ann Arbor, Michigan, USA).

Data matrix composition and parsimony analysis—Nucleotide sequences of the rpl16 intron, rps16 intron, and trnS-G intergenic spacer were aligned using Clustal W, version 1.8 (Thompson et al., 1994) and adjusted by eye. Regions of ambiguous alignment were excluded from the analysis. Individual gap positions were treated as missing data, unequivocally aligned gaps being coded as presence/absence of characters with the software Gapcoder (Young and Healy, 2003) and then added to the sequence matrix.

Twenty-nine morphological characters were scored using herbarium and fresh specimens, pickled flowers, and when available, flower sections provided by the second author. For some taxa, the literature was also consulted (e.g., Woodson, 1933; Pichon, 1950; Leeuwenberg, 1997; Morales, 1998, 2002). The morphological matrix, a list of the characters, character states, and explanatory notes on characters are given in Appendices 3 and 4. Exemplars were used as terminal taxa, because morphological variation is

considerable in the two larger genera, Mandevilla and Forsteronia, which would lead to difficulties in coding character states.

The following data sets were subjected to phylogenetic analysis: (1) rpl16 intron, (2) rps16 intron, (3) trnS-G intergenic spacer, and (4) morphology. As the results of individual analyses did not show any major topological conflict, data partitions were combined in the following ways: all molecular data sets combined together (molecular combined), and all molecular and morphological data sets combined (total evidence).

Maximum parsimony analyses were performed using PAUP* 4.0b (Swofford, 2000). All characters were unordered and equally weighted. Polymorphisms in the data matrix were treated as such, rather than as uncertainties. A heuristic search for most-parsimonious trees (MPT) included an initial round of tree searches with 1000 random addition sequence replicates (RASR), holding 10 trees at each step, tree bisection-reconnection (TBR) branch swapping with MULTREES and steepest descent in effect, saving a maximum of 100 trees at each replicate. All shortest trees retained in memory were then included in a second round of searches involving exhaustive TBR branch swapping. Relative support for each node was estimated using the bootstrap resampling procedure (Felsenstein, 1985) as implemented in PAUP employing a full heuristic search with 1000 replicates, 250 RASR, three trees held at each step, TBR branch swapping with steepest descent and MULTREES in effect, saving 10 trees at each RASR.

Morphological characters were optimized onto the strict consensus tree of the total evidence analysis using Winclada, version 1.00.08 (Nixon, 2002) in order to identify the synapomorphies that were congruent with each of the major clades of the ingroup retrieved in our analyses. The proportion of nodes in the individual molecular data partitions that were congruent with the topology of the total evidence tree was assessed in WinClada by optimizing the unambiguous character changes of each data set separately onto the topology of the strict consensus tree obtained from the total evidence matrix. Subsequently, the ratio of nodes supported by such character changes divided by the total number of nodes was calculated. This method is analogous to the Partition Bremer Support (Baker and DeSalle, 1997), although it has the disadvantage that, unlike Partition Bremer Support, it cannot identify whether or not a data partition contradicts a particular node.

RESULTS

Size and structure of individual and combined data sets—Detailed information for both individual and combined data sets is given in Table 2. Multiple sequence alignment was straightforward for the rps*16* intron, but proved to be more difficult for the rpl*16* intron and the *trnS-G* intergenic spacer due to the large number of gaps and AT-rich regions. A total of 382 characters, including nucleotides and gaps, were excluded from this latter data set because of ambiguous alignment.

Parsimony analyses —To test hypotheses of the tribal delimitation proposed by Endress and Bruyns (2000) (see Table 1), *Galactophora* was also included in the initial round of taxon sampling. Results from a heuristic search showed that *Galactophora* did not group with the Mesechiteae, but rather came out as sister to the rest of the ingroup species, as illustrated in Fig. 2.1. Based on its position in the unrooted tree and its long branch compared to other taxa in our sampling, *Galactophora* might actually be less closely related to the ingroup than the taxa used for rooting (*Pachypodium geayi*, *P. lamerei* and *Mascarenhasia lisianthiflora*). Due to this uncertainty, *Galactophora* was excluded from further analysis.

Tree length, consistency index (CI), and retention index (RI) for the cladograms that resulted from the analyses of the individual and combined data sets are summarized in Table 2. Individual analyses of the three plastid regions showed similar results, and visual inspection of the strict consensus trees of the individual molecular data sets showed no topological conflict. Assuming that simultaneous analysis of combined data is the best approach to phylogenetic inference (e.g., Brower, 1996; Nixon and Carpenter, 1996), and because none of the nodes involved in topological discrepancies in our analysis were supported by high bootstrap values (Fig. 2.2), the individual molecular data sets were combined. The best resolved cladograms were provided by the *rpl*16 and *rps16* intron data sets, with most of the internal nodes receiving bootstrap support (BS) higher than 50%. Of the cladograms generated by the separate molecular data sets, only the *rps16* intron tree defined a clade, corresponding to what will later be defined as the Mesechiteae clade, with

BS higher than 50%, identical to that of the molecular combined and total evidence trees. The least resolved cladogram was that based on the *trnS-G* intergenic spacer data set.

Analysis of the morphological data set resulted in a poorly resolved cladogram, with only a few groups supported by a bootstrap value higher than 50% (Fig. 2.3). Except for a weakly supported clade composed of the species of *Macrosiphonia* and *Telosiphonia* (BS = 54%), which is not found in any of the molecular trees, no incongruence was detected when comparing the morphological tree with either the strict consensus of the individual or combined molecular trees. Therefore, the morphological and combined molecular data sets were combined into a total evidence data set. All further discussion will be based on the total evidence tree (Fig. 2.4).

A clade including representatives of *Allomarkgrafia*, *Forsteronia*, *Mesechites*, *Mandevilla*, *Macrosiphonia*, *Telosiphonia*, and *Tintinnabularia* is strongly supported (BS = 97%), and will be referred to hereafter as the Mesechiteae clade. Within this clade, three other subclades are defined: (1) a strongly supported (BS = 100%) subclade comprised of *Allomarkgrafia*, *Mesechites*, and *Tintinnabularia*, and hereafter referred to as the *Mesechites* subclade; 2) a strongly supported (BS = 99%) subclade comprising the two *Forsteronia* species (the *Forsteronia* subclade) and 3) a larger, moderately supported (BS = 77%) subclade composed of taxa of *Mandevilla*, *Macrosiphonia*, and *Telosiphonia*, hereafter referred to as the *Mandevilla* subclade. *Mandevilla tenuifolia* is paraphyletic to *M. myriophyllum*. One morphotype (*Mandevilla tenuifolia*1) forms a strongly supported clade (BS = 94%) with *M. myriophyllum* rather than with the other morphotype of *M. tenuifolia* (*Mandevilla tenuifolia*2). The tree topology resulting from MP analyses of the total evidence data set showed a clade formed by *Odontadenia* and *Secondatia* to be sister to the Mesechiteae clade, although this relationship received a BS of less than 50%.

The taxa belonging to tribe Apocyneae sensu Endress and Bruyns (2000) are paraphyletic, with the six representatives included in this study dispersed among three different parts of the cladogram. The Neotropical genus *Forsteronia* is nested in the Mesechiteae clade, whereas *Odontadenia*, a large genus widely distributed in the Neotropics, forms a strongly supported clade (BS = 98%) with *Secondatia*, a Neotropical genus included in tribe Mesechiteae by Endress and Bruyns (2000). *Beaumontia*, *Chonemorpha*, and *Trachelospermum*, all genera of tropical and subtropical regions of

Asia, form a strongly supported clade (BS = 96%) somewhat closer to the base of the tree. A clade composed of *Prestonia* and *Rhodocalyx*, all Neotropical members of Echiteae sensu Endress and Bruyns (2000), is strongly supported (BS = 100%).

Analysis of the percentage of nodes supported by unambiguously optimized characters for each individual partition onto the strict consensus tree of the total evidence analysis showed that the individual data sets had varying degrees of resolving power (Table 3). The highest power of resolution consistent with the total evidence tree was found for nucleotides in the *rpl16* intron, with more than 80% of the nodes in the strict consensus tree of the combined analysis supported by at least one unambiguously optimized nucleotide substitution of the *rpl16* intron. Slightly lower percentages were found for the *rps16* intron and *trnS-G* intergenic spacer, with the lowest percentage provided by the gaps in the *rps16* intron. A relatively high percentage (45%) of the nodes in the strict consensus of the total evidence tree was supported by at least one unambiguously optimized morphological character.

DISCUSSION

Molecular characteristics of noncoding plastid DNA—The three plastid regions used in our analysis showed a set of characteristics similar to that reported by Kelchner (2000) for noncoding plastid regions, such as the occurrence of strings of mononucleotide repeats and small tandem repeat units. Considerable length variation among the individual sequences required the insertion of a large number of gaps in the alignments (Table 2), especially for the *trnS-G* intergenic spacer, which is consistent with the results reported by Perret et al. (2003) for the same region. Gaps provided a considerable amount of information for our analysis, representing one-quarter of the total number of informative characters. The nucleotide substitutions were not uniform across sequences of the same DNA region, but rather alternated between more conserved and more variable regions. This heterogeneity was highest in the *trnS-G* intergenic spacer region. These more variable regions, which could provide potential sources of phylogenetic information at lower taxonomic levels, were excluded from our analysis because of ambiguous alignment.

Phylogenetic utility of individual data partitions—All three plastid regions were characterized by similar percentages of parsimony informative characters, ranging from 8.6% in the *trnS-G* intergenic spacer to 10.6% in the *rps16* intron, consistent with the results reported by Schönenberger and Conti (2003). In contrast to this uniformity in the percentage of parsimony informative characters provided by each data set, marked differences were observed in the resolving power of the separate data partitions. The highest resolving power was found in the *rpl16* intron, which yielded the highest percentage of nodes supported by at least one unambiguously optimized character onto the strict consensus of the total evidence tree (Table 3). All individual data partitions, however, including morphology, contributed partially to the resolution in the total evidence tree.

Although the *rpl16* intron, *rps16* intron, and *trnS-G* intergenic spacer have been used in several recent studies, either individually or combined with other plastid and/or nuclear regions (e.g., Oxelman et al., 1997; Downie et al., 2000; Asmussen and Chase, 2001; Perret et al., 2003), the combination of all three regions in the same data matrix has been applied only recently (Schönenberger and Conti, 2003). This study shows their phylogenetic utility to resolve relationships at the tribal and intergeneric level, especially when combined with morphology.

Comparison between phylogenetic hypothesis and current classification—The total evidence tree does not support the monophyly of Mesechiteae as circumscribed by Endress and Bruyns (2000) with its nine constituent genera: *Allomarkgrafia*, *Galactophora*, *Quiotania*, *Macrosiphonia*, *Mandevilla*, *Mesechites*, *Secondatia*, *Telosiphonia*, and *Tintinnabularia*. Of the eight genera included in this study, six (*Allomarkgrafia*, *Macrosiphonia*, *Mandevilla*, *Mesechites*, *Telosiphonia*, and *Tintinnabularia*) form a strongly supported clade together with *Forsteronia*, a genus placed in Apocyneae by Endress and Bruyns (2000). *Secondatia*, on the other hand, groups with *Odontadenia*, the Neotropical representative of Apocyneae included among the outgroup taxa in our study. *Galactophora* clearly does not belong in Mesechiteae, but its relationships are uncertain at present (see Results and Fig. 2.1). The status of *Quiotania* could not be evaluated, because collection of leaf tissue was not possible (see Materials and Methods).

Addition of morphological characters to the combined molecular data set increased bootstrap support for the Mesechiteae clade from 81 to 97%. Four morphological synapomorphies were identified, which are congruent with the Mesechiteae clade, as defined in the total evidence tree: (1) leaf blade with one to many colleters on the adaxial surface, (2) anthers with a blunt-cordate to truncate base, (3) retinacle strongly united with the style head by cellular fusion, and (4) style head with five strongly protruding longitudinal ribs (Fig. 2.4). The structure of the style head and the manner in which it is united with the anthers (retinacle type) are both key characters in the specialized flowers of the Apocynaceae. The style head is a product of post-genital fusion of the two carpel apices, which develop into an enlarged, secretory structure. The retinacle is the region of the anther that becomes post-genitally united with the style head, thus forming a gynostegium (Fallen, 1986; Sennblad et al., 1998). All members of the Mesechiteae clade have a style head that is often referred to in the earlier literature as "umbraculiform" (umbrella shaped). This type of style head is characterized by having a starlike shape in cross section, from the five strongly projecting vertical ribs, which may extend along its entire length or be more or less restricted to the base. The anthers are post-genitally united with these ribs by the retinacle, which in Mesechiteae consists of a strip of specialized cells that are unusually short and strongly attached to the style head by cellular fusion. This type of retinacle sets the Mesechiteae clade apart from other Neotropical Apocynoideae, in which the retinacle is composed of longer hairs, characterized by a weaker attachment to the style head, without cellular fusion.

In contrast to these "good" characters, many of the morphological characters (e.g., habit, presence of domatia, absence vs. presence or arrangement of calycine colleters, corolla shape, length of staminal filaments, and nectary height), which have previously been used to define taxonomic groups (Woodson, 1933; Zarucchi, 1991; Morales, 1997, 1998; Williams, 1999, 2002), appear to be phylogenetically unreliable. For example, Woodson (1936) and Leeuwenberg (1994) considered *Tintinnabularia* to be related to *Beaumontia* (a genus of the outgroup tribe Apocyneae in this study), based on their shared possession of domatia and long staminal filaments.

Relationships between genera—Next we briefly discuss relationships among the three subclades of Mesechiteae, focusing on the morphological synapomorphies that are consistent with these clades .

Mesechites subclade – The *Mesechites* subclade is defined by one morphological synapomorphy: having the ribs of the style head restricted to the base (Fig. 2.4). This distinguishes the *Mesechites* subclade from the other two subclades of Mesechiteae, both of which are characterized by having ribs along the entire length of the style head. In the literature (e.g., Woodson, 1933; Williams, 1999), the inflorescences of *Allomarkgrafia*, *Mesechites*, and *Tintinnabularia* are often said to be cymose (to distinguish these genera from *Mandevilla*, which is considered to have racemose inflorescences). However, inflorescence type is a character that is difficult to interpret, especially as the inflorescence is often reduced (e.g., in *Tintinnabularia mortonii*, *Mesechites minima*, and *M. rosea*).

In the *Mesechites* subclade, *Tintinnabularia* is sister to a strongly supported clade composed of *Allomarkgrafia* and *Mesechites*. *Tintinnabularia*, described by Woodson (1936) and comprising three species, is restricted to Mexico, Guatemala, and Honduras. It is one of the most rarely collected and thus poorly known genera in the Apocynoideae. It has been considered to be allied to other members of the Mesechiteae by most Neotropical Apocynaceae specialists (e.g., Zarucchi, 1991; Henrickson, 1996; Williams, 1999), but except for Pichon (1950), who considered *Tintinnabularia* to be most closely related *Forsteronia*, no other specialist in the family has made an attempt to elucidate it closest relatives within the tribe.

Mesechites currently comprises 10 species, divided between the two subgenera, *Eumesechites* and *Didymadenia*, described by Woodson (1933). Of the three species of *Mesechites* included in our study, the two Cuban species, *M. minima* and *M. rosea*, representing subgenus *Didymadenia*, are strongly supported as sister taxa (BS = 94%). The South American representative from subgenus *Eumesechites, M. mansoana*, however, groups with *Allomarkgrafia* rather than with the other *Mesechites* species. This suggests that *Mesechites* may not be a monophyletic genus as currently circumscribed. The two genera have long been considered to be closely related. Pichon (1948b, 1950) considered *Allomarkgrafia* to be a synonym of *Mesechites*, arguing that the style head form was the

same for the two genera and that no diagnostic character states supported the generic distinction of *Allomarkgrafia*. Our current results do not contradict this taxonomic interpretation; no morphological synapomorphy that could distinguish the two genera was found. More species need to be analyzed, however, before firm conclusions as to generic circumscription can be reached.

Forsteronia – The inclusion of *Forsteronia* in the Mesechiteae clade is somewhat unexpected; this relationship has never been proposed. *Forsteronia* is a relatively large Neotropical genus with some 46 recognized species, characterized by having small, subrotate flowers in thyrsiform, often dense inflorescences. Although easily distinguished from other Neotropical genera, the placement of *Forsteronia* within the Apocynoideae has proved difficult. Pichon (1950) placed *Forsteronia* in his tribe Ichnocarpeae based on its glabrous, concave retinacle, and created the subtribe Forsteroniinae to accommodate this genus together with *Tintinnabularia*. This subtribe was mainly defined by the presence of domatia on the abaxial surface of the leaves. Leeuwenberg (1994) agreed for the most part with Pichon and placed *Forsteronia* in his tribe Apocyneae, together with many of the genera originally placed in Pichon's Ichnocarpeae. He did not recognize subtribe Forsteroniinae, however, and he placed *Tintinnabularia* in a different tribe, Wrightieae. Endress and Bruyns (2000) included *Forsteronia* in Apocyneae, assuming that it shares the diagnostic characters states of the tribe: style head fusiform and retinacle formed by a horseshoe-shaped rim and a narrow longitudinal strip of hairs.

One of the main difficulties concerning the placement of *Forsteronia* is due to the morphological variation within the genus. In order to estimate this variation and the consistency of our phylogenetic results, the flower structure of an additional five species of *Forsteronia, F. australis* Müll.-Arg., *F. portoricensis* Woodson, *F. rufa* Müll.-Arg., *F. spicata* (Jacq.) G. Mey. and *F. velloziana* (A. DC.) Woodson was examined, based on herbarium vouchers, pickled flowers and, when available, sections of flowers provided by the second author. All species were found to share two of the key characters states that define the Mesechiteae clade: the presence of colleters at the leaf base and anthers with a bluntly cordate to truncate base. Other characteristics, however, are more variable and are more or less intermediate between Mesechiteae and Apocyneae. One such character is the

structure of the retinacle. As in other Mesechiteae, the retinacle of *Forsteronia* has a glabrous, concave region; but it also has a small to well-developed row of hairlike cells beneath this. These hairlike cells form a weak union between the anthers and the base of the style head, generally only by agglutination but sometimes with accompanying cellular fusion (e.g., *F. spicata*), as in other Mesechiteae. Furthermore, the longitudinal ribs of the style head are not always well developed. In some species they are quite conspicuous, with the characteristic "Mesechiteae" starlike shape in cross section. In other species, however, the ribs are scarcely developed (e.g., *F. acouci*), so that the style head is more or less pentagonal in cross section, which is more characteristic of the Apocyneae. Despite this variation in some of its morphological features, the results of our analyses of morphological variation indicate that the inclusion of *Forsteronia* in the Mesechiteae clade is warranted. In order to test the monophyly of *Forsteronia*, however, a broader taxon sampling of species chosen to adequately represent the range of variation within the genus is required.

The Mandevilla subclade – The three genera of this subclade (*Macrosiphonia*, *Mandevilla*, and *Telosiphonia*) have always been considered to be closely related and sometimes even synonymous. They all have racemose inflorescences, sometimes reduced to a single flower, and strongly protruding ribs extending along the entire length of the style head.

Mandevilla is the largest Neotropical genus in the Apocynoideae. It is extremely variable, with about 120 species distributed throughout the Neotropics, from Mexico to Argentina, and includes vines, erect shrubs, and even epiphytes. The flower size and structure also spans a broad range, from inconspicuous whitish, tubular flowers less than 1 cm long to brightly colored, showy infundibuliform flowers up to 5 cm long. *Macrosiphonia* and *Telosiphonia*, in contrast, contain only five and six species, respectively, which share a number of morphological characteristics. Both are erect shrubs or subshrubs with leaves covered by a dense, wooly indument on the abaxial side, and occur in savannas or arid habitats. The flowers are white with a long slender tube and are presumably adapted to hawkmoth pollination. *Telosiphonia* was originally described as a subgenus of *Macrosiphonia* by Woodson (1933). Henrickson (1996), however, elevated subgenus *Telosiphonia* to generic rank based on characters such as inflorescence type, style

head structure and pollen size. He suggested that the many similarities between *Macrosiphonia* and *Telosiphonia* are the result of adaptation to a similar habitat and pollination syndrome. The distribution of the two genera roughly coincides with the extreme northern and southern distribution of *Mandevilla*. *Telosiphonia* is restricted to the arid zones of Mexico and the southwestern United States, whereas *Macrosiphonia* is found in the savannas of central Brazil to Argentina. The separation of *Macrosiphonia* and *Telosiphonia* into two distinct clades strongly supports Henrickson's (1996) ideas that the two taxa are not congeneric. Nevertheless, their recognition as distinct genera probably cannot be upheld in light of the present data because both clades are nested in *Mandevilla*. The morphological characteristics that have been used to distinguish *Mandevilla* from *Macrosiphonia* and *Telosiphonia* are rather minor, being based only on leaf indument and superficial aspects of flower structure. Woodson (1933, p. 778) maintained them as distinct genera, but stated that "The existing distinctions between *Macrosiphonia* and *Mandevilla* are extremely tenuous." Pichon (1948b) proposed the inclusion of *Macrosiphonia* in the synonymy of *Mandevilla*, arguing that the distinguishing characters used by Woodson (1933) to differentiate between the two genera were inconsistent and arbitrary, making impossible an unambiguous distinction between them. Our morphological analyses identified no apomorphies exclusive to either genus, reinforcing the difficulty of upholding their current generic rank.

In his taxonomic revision of *Mandevilla*, Woodson (1933) recognized two subgenera: *Eumandevilla* and *Exothostemon*. Within subgenus *Eumandevilla*, he recognized five sections: *Laxae*, *Montanae*, *Tenuifoliae*, *Torosae*, and *Tubiflorae*. The monophyly of subgenus *Exothostemon*, represented in our study by *M. rugosa*, *M. rugellosa* and *M. scabra*, is strongly supported (BS = 100%). However, these results must be interpreted as preliminary due to the small number of *Mandevilla* species sampled for a genus of this size. Similarly, no conclusions can be made at this time about relationships among the sections of *Mandevilla* sensu Woodson (1933), also due to the insufficient taxon sampling. Our finding of paraphylly in *M. tenuifolia* with regard to *M. myriophyllum* suggests that the latter could be merely an extreme morphotype of the former. However, a significantly broader taxon sampling in *M. tenuifolia* would need to be undertaken in order

to determine this with more certainty. Further studies based on more intensive taxon sampling in *Mandevilla*, needed to address these questions, are underway.

Galactophora and Secondatia—The exclusion of *Secondatia* and *Galactophora* from the Mesechiteae, suggested by our phylogenetic analyses, is congruent with morphology. In both genera, leaf colleters are absent, the anther base is strongly sagittate, and the retinacle is protuberant with no detecs concave region—all character states that are at odds with the synapomorphies that support the Mesechiteae clade. In *Secondatia*, the style head is almost circular in cross section, with no longitudinal ribs, similar to that found in *Odontadenia*. The retinacle structure is also of the same type as that in *Odontadenia*. *Secondatia* and *Odontadenia* comprised Pichon's (1950) subtribe Secondatinae of Ecdysanthereae, and our results also support a close relationship between these two genera. In *Galactophora*, in contrast, the style head has five well-developed projecting ribs at the base. The presence of these ribs was the main reason for the inclusion of *Galactophora* in Mesechiteae by Endress and Bruyns (2000). Our results, however, suggest that the ribs on the style head are independently derived in *Galactophora* and the Mesechiteae clade. Preliminary analysis of the style-head structure of *G. calycina* reinforces this hypotheses. In this species, the ribs are not continuous with the main body of the style head, as in taxa of the Mesechiteae clade, but rather are formed by soft tissue that is distinct from that of the rest of the style head. The union between the style head and the anthers in *Galactophora* is also quite weak; the anthers are easily detached from the style head. This is in sharp contrast to the situation in taxa of the Mesechiteae clade in which the anthers are so strongly united with the style head that they can usually only be removed by ripping off an adjacent piece of the style head.

Apocyneae—Our results, especially in light of the position of *Forsteronia* in Mesechiteae, strongly suggest that Apocyneae sensu Endress and Bruyns (2000) is not monophyletic. This is not unexpected, confirming their prediction (Endress and Bruyns, 2000, p. 8) that, "The Apocyneae, especially, will probably need to be divided in some way, and some rearrangement of taxa will no doubt be necessary as more data accumulate."

No further conclusions can be drawn about relationships within the Apocyneae, however, due to the small number of taxa from this tribe sampled in our analysis.

Conclusions—The phylogenetic analysis presented here provides the first broad study of the Mesechiteae including representatives of all but one of its constituent genera, using both morphological and molecular characters. This represents the first step towards resolving long-standing disputes over generic delimitation and intergeneric relationships within the tribe. The newly defined Mesechiteae comprise taxa previously ascribed to eight genera: *Allomarkgrafia*, *Forsteronia*, *Macrosiphonia*, *Mandevilla*, *Mesechites*, *Quiotania*, *Telosiphonia*, and *Tintinnabularia*.

Topics to be addressed in a future study include testing the monophyly and determining the systematic position of *Forsteronia* in the Mesechiteae; defining the generic circumscription of *Allomarkgrafia*, *Macrosiphonia*, and *Telosiphonia*; testing the monophyly of the currently recognized sections within *Mandevilla*; and elucidating character evolution and the biogeographic history of the group.

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Table 1. Comparative tribal placement of genera that have been included in Mesechiteae.

Genus	Pichon (1950)	Leeuwenberg (1994)	Endress and Bruyns (2000)	This project
Allomarkgrafia	-[a]	-	Mesechiteae	Mesechiteae
Forsteronia	Ichnocarpeae	Apocyneae	Apocyneae	Mesechiteae
Galactophora	Parsonsieae	Echiteae	Mesechiteae	?[a]
Macrosiphonia	-	Echiteae	Mesechiteae	Mesechiteae
Mandevilla	Ichnocarpeae	Echiteae	Mesechiteae	Mesechiteae
Mesechites	Ichnocarpeae	Echiteae	Mesechiteae	Mesechiteae
Quiotania	-	-	Mesechiteae	?
Secondatia	Ecdysanthereae	Echiteae	Mesechiteae	Apocyneae
Telosiphonia	-	-	Mesechiteae	Mesechiteae
Tintinnabularia	Ichnocarpeae	Wrightieae	Mesechiteae	Mesechiteae

[a] - = genus not recognized, ? = uncertain placement.

Table 2. Summary of sequence lenght, variability and parsimony-tree parameters for individual and combined data sets. Tree length, consistency index (CI) and retention index (RI) were calculated based on parsimony-informative characters only.

	rpl16 intron	*rps16* intron	*trnS-G* intergenic spacer	Molecular combined	Morpholog y	Total evidence
Aligned length	1436	902	1365	3708	29	3737
Range of sequence length	900-1092	790-815	445-801	-	-	-
No. of coded gaps (no. of parsimony-informative gaps)	179 (36)	71 (19)	164 (27)	420 (82)	-	420 (82)
No. of characters excluded (nucleotides + gaps)	195	56	382	633	-	633
Total no. of parsimony-informative characters (percentage of total number of characters)	143 (10%)	98 (10.7%)	99(8.6%)	346 (9.9%)	29 (100%)	375 (10.7%)
Tree length	241	141	131	525	117	659
CI	0.718	0.762	0.671	0.723	0.350	0.680
RI	0.818	0.854	0.811	0.826	0.550	0.801

Table 3. Percentage of nodes supported by at least one unambiguously optimized character for each individual partition on the strict consensus tree of total evidence analysis.

	rpl16 intron	*rps16* intron	*trnS-G* intergenic spacer	Morphology
Nucleotides	83.8%	67.7%	64.5%	-
Gaps	51.6%	25.8%	35.4%	-
Morphological characters	-	-	-	45.1%

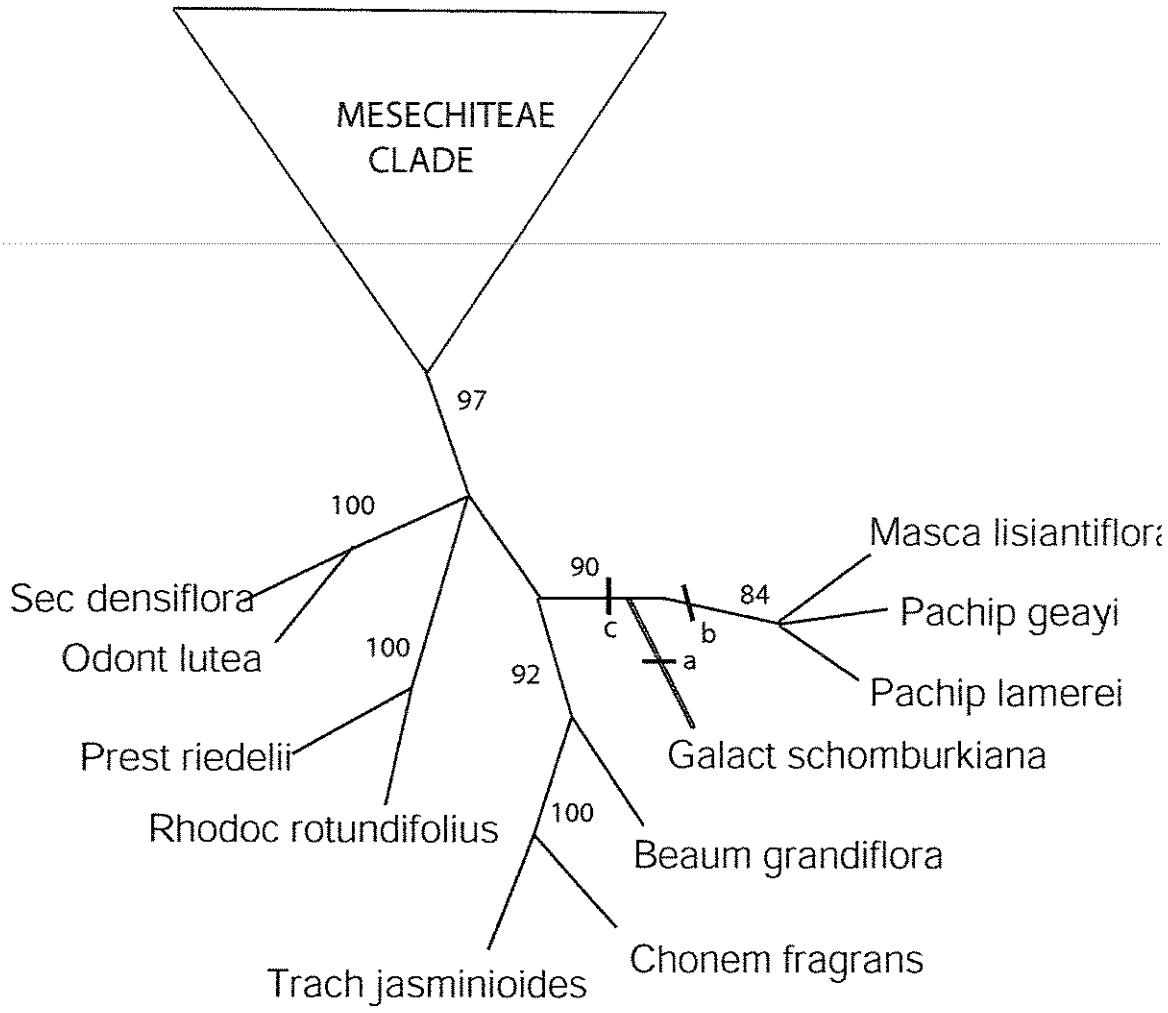
FIGURE LEGENDS

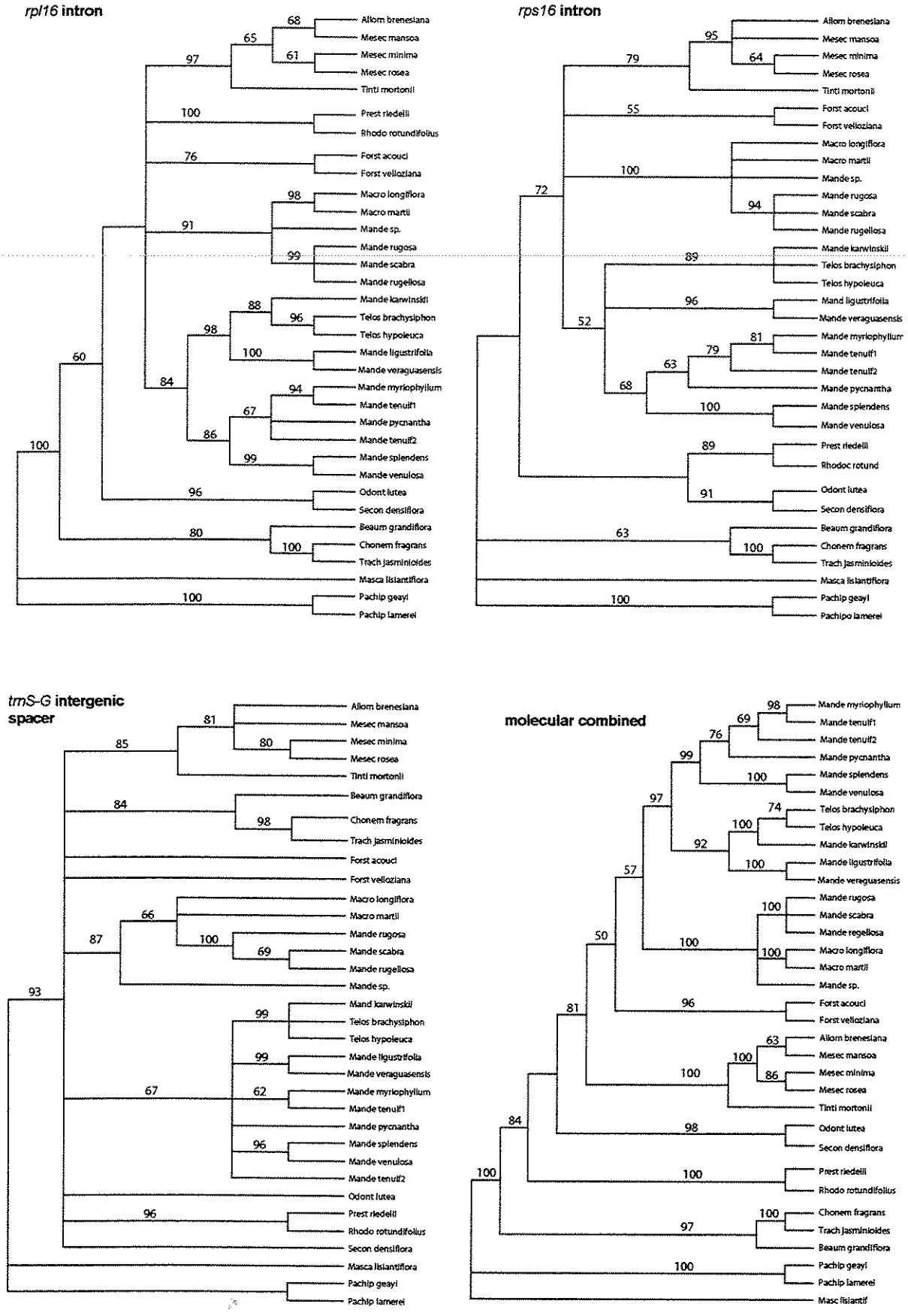
Fig. 2.1. Unrooted cladogram based on the total evidence data set, showing the relationship of *Galactophora* to the other taxa used in this analysis. Different root positions (a, b, c) show that *Galactophora* never groups with the Mesechiteae clade. Full taxon names are given in Appendix 3 (see Supplemental Data accompanying online version of this article).

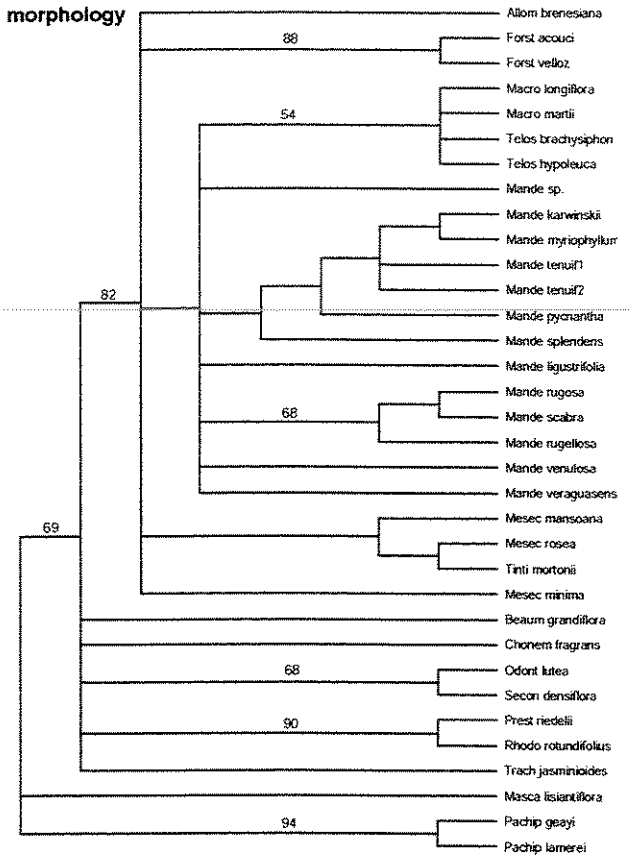
Fig. 2.2. Strict consensus of the most parsimonious trees generated by the three individual molecular data sets and the combined molecular data set. Bootstrap values >50% are indicated above the branches. Full taxon names are given in Appendix 3 (see Supplemental Data accompanying online version of this article).

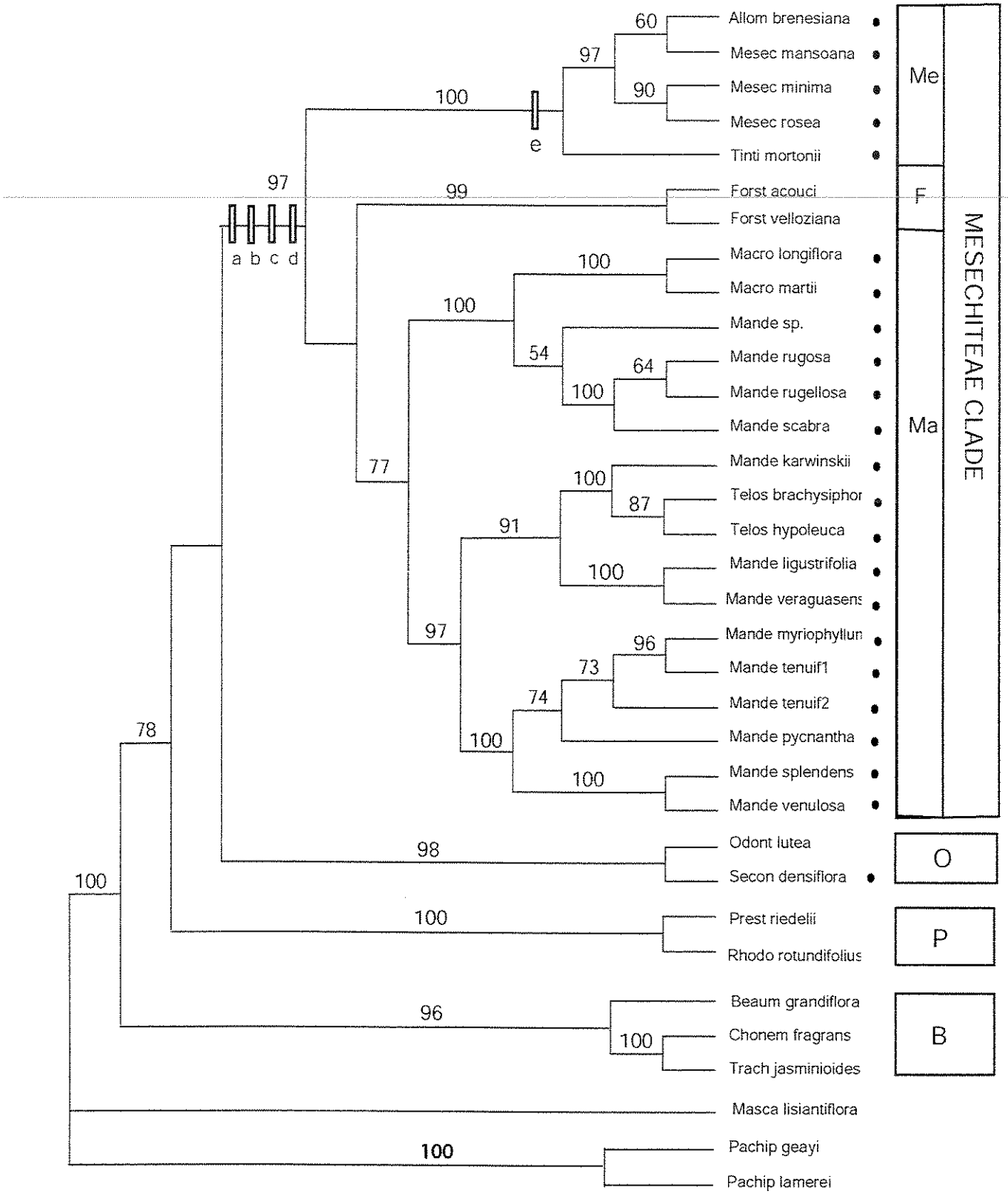
Fig. 2.3. Strict consensus of the most parsimonious trees generated by the morphological data set. Bootstrap values >50% are indicated above the branches. Full taxon names are given in Appendix 3 (see Supplemental Data accompanying online version of this article).

Fig. 2.4. Strict consensus of the most parsimonious trees generated by the total evidence data set. Bootstrap values >50% are indicated above the branches. Clade names are indicated as following: Me = *Mesechites* clade; F = *Forsteronia* clade; Ma = *Mandevilla* clade; O = *Odontadenia* clade; P = *Prestonia* clade: B = *Beaumontia* clade. The letters a, b, c, d, e denote key morphological synapomorphies diagnostic for the Mesechiteae and Mesechites clades and are taken from the morphological data set (see Appendices 3 and 4 on Supplemental Data accompanying online version of this article); a: leaf colleters on the adaxial surface of the leaf; b: anther base bluntly cordate to truncate; c: anthers attached to the style head by cellular fusion; d: style head with five strongly protruding ribs in cross section; e: ribs of the style head restricted to the base. Circles indicate species previously placed in Mesechiteae by Endress and Bruyns (2000). Full taxon names are given in Appendix 3 (see Supplemental Data accompanying online version of this article).


MESECHITEAE
CLADE
97
100
Sec densiflora
Odont lutea
100
Prest riedelii
Rhodoc rotundifolius
90
c
b
a
84
Masca lisiantiflora
Pachip geayi
Pachip lamerei
92
Galact schomburkiana
100
Beaum grandiflora
Chonem fragrans
Trach jasminioides


rpl16 intron
Allom breneslana
Mesec mansoa
Mesec minima
Mesec rosea
Tinti mortonii
Prest riedelii
Rhodo rotundifolius
Forst acouci
Forst velloziana
Macro longiflora
Macro martii
Mande sp.
Mande rugosa
Mande scabra
Mande rugellosa
Mande karwinskii
Telos brachysiphon
Telos hypoleuca
Mande ligustrifolia
Mande veraguasensis
Mande myriophyllum
Mande tenuif1
Mande pycnantha
Mande tenuif2
Mande splendens
Mande venulosa
Odont lutea
Secon densiflora
Beaum grandiflora
Chonem fragrans
Trach jasminioides
Masca lisiantiflora
Pachip geayi
Pachip lamerei
rps16 intron
Allom breneslana
Mesec mansoa
Mesec minima
Mesec rosea
Tinti mortonii
Forst acouci
Forst velloziana
Macro longiflora
Macro martii
Mande sp.
Mande rugosa
Mande scabra
Mande rugellosa
Mande karwinskii
Telos brachysiphon
Telos hypoleuca
Mand ligustrifolia
Mande veraguasensis
Mande myriophyllum
Mande tenuif1
Mande tenuif2
Mande pycnantha
Mande splendens
Mande venulosa
Prest riedelii
Rhodoc rotund
Odont lutea
Secon densiflora
Beaum grandiflora
Chonem fragrans
Trach jasminioides
Masca lisiantiflora
Pachip geayi
Pachipo lamerei
trnS-G intergenic spacer
Allom breneslana
Mesec mansoa
Mesec minima
Mesec rosea
Tinti mortonii
Beaum grandiflora
Chonem fragrans
Trach jasminioides
Forst acouci
Forst velloziana
Macro longiflora
Macro martii
Mande rugosa
Mande scabra
Mande rugellosa
Mande sp.
Mand karwinskii
Telos brachysiphon
Telos hypoleuca
Mande ligustrifolia
Mande veraguasensis
Mande myriophyllum
Mande tenuif1
Mande pycnantha
Mande splendens
Mande venulosa
Mande tenuif2
Odont lutea
Prest riedelii
Rhodo rotundifolius
Secon densiflora
Masca lisiantiflora
Pachip geayi
Pachip lamerei
molecular combined
Mande myriophyllum
Mande tenuif1
Mande tenuif2
Mande pycnantha
Mande splendens
Mande venulosa
Telos brachysiphon
Telos hypoleuca
Mande karwinskii
Mande ligustrifolia
Mande veraguasensis
Mande rugosa
Mande scabra
Mande regellosa
Macro longiflora
Macro martii
Mande sp.
Forst acouci
Forst velloziana
Allom breneslana
Mesec mansoa
Mesec minima
Mesec rosea
Tinti mortonii
Odont lutea
Secon densiflora
Prest riedelii
Rhodo rotundifolius
Chonem fragrans
Trach jasminioides
Beaum grandiflora
Pachip geayi
Pachip lamerei
Masc lisiantif




Allom brenesiana
Mesec mansoana
Mesec minima
Mesec rosea
Tinti mortonii
Forst acouci
Forst velloziana
Macro longiflora
Macro martii
Mande sp.
Mande rugosa
Mande rugellosa
Mande scabra
Mande karwinskii
Telos brachysiphor
Telos hypoleuca
Mande ligustrifolia
Mande veraguasens
Mande myriophyllun
Mande tenuif1
Mande tenuif2
Mande pycnantha
Mande splendens
Mande venulosa
Odont lutea
Secon densiflora
Prest riedelii
Rhodo rotundifolius
Beaum grandiflora
Chonem fragrans
Trach jasminioides
Masca lisiantiflora
Pachip geayi
Pachip lamerei
60
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98
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Me
F
Ma
MESECHITEAE CLADE
O
P
B

Appendix 1. Taxa names, voucher information and Genbank acession numbers for all *rpl16* intron, *rps16* intron and *trnS-G* intergenic spacer sequences.

SPECIES	Voucher/Citation	*rpl16* intron	*rps16* intron	*trnS-G* intergenic spacer
Allomarkgrafia brenesiana Woodson	Costa Rica, M. Endress 97-06 (Z)	AY597546	AY597580	AY597614
Beaumontia grandiflora Wall.	Cult., Bot. Gard. Munich, Gerlach s.n. unvouchered *India, coll. Native collector, s.n. (Z)	AY597547	AY597581	AY597615
Chonemorpha fragrans (Moon) Alston	Cult., Queensland, Australia, Forster 2009 * India, Ridsale 109 (Z)	AY597548	AY597582	AY597616
Forsteronia acouci (Aubl.) A. DC.	French Guiana, Prévost 3720 (CAY) * Peru, Revilla 291 (Z); Venezuela, Breteler 5029 (Z)	AY597549	AY597583	AY597617
Forsteronia velloziana (A. DC.) Woodson	Brazil, Simões 343 (UEC)	AY597550	AY597584	AY597618
Macrosiphonia longiflora (Desf.) Müll.Arg.	Brazil, Flores s.n. (UEC)	AY597551	AY597585	AY597619
Macrosiphonia martii Müll.Arg.	Brazil, Simões 1245 (UEC)	AY597552	AY597586	AY597620
Mandevilla sp.	Brazil, Simões 1303 (UEC)	AY597559	AY597593	AY597627
Mandevilla karwinskii (Müll.Arg.) Hemsl.	Mexico, Fishbein 2966 (ARIZ)	AY597553	AY597587	AY597621
Mandevilla ligustrifolia Woodson	Ecuador, Matezki 340 (Z)	AY597554	AY597588	AY597622
Mandevilla myriophyllum	Brazil, Pansarin 878 (UEC)	AY597555	AY597589	AY597623

(Taub.) Woodson				
Mandevilla pycnantha (Steud. ex A. DC.) Woodson	Brazil, Yamamoto s.n. (UEC)	AY597556	AY597590	AY597624
Mandevilla rugosa (Benth.) Woodson	Brazil, Simões 1204 (UEC)	AY597557	AY597591	AY597625
Mandevilla rugellosa (Vahl) Markgr.	French Guiana, Prévost 3721 (CAY) * Suriname, Lindeman 1976 (Z)	AY597561	AY597595	AY597629
Mandevilla splendens (Hook.) Woodson	Brazil, Simões 1268 (UEC)	AY597560	AY597594	AY597628
Mandevilla scabra (Hoffmans. Ex Roem. & Schult.) K. Schum.	Brazil, Simões 1126 (UEC)	AY597558	AY597592	AY597626
Mandevilla tenuifolia1 (J.C. Mikan) Woodson	Brazil, Simões 1171 (UEC)	AY597562	AY597596	AY597630
Mandevilla tenuifolia2 (J.C. Mikan) Woodson	Brazil, Kinoshita s.n. (UEC)	AY597563	AY597597	AY597631
Mandevilla venulosa (Müll.Arg.) Woodson	Brazil, Simões 1107 (UEC)	AY597564	AY597598	AY597632
Mandevilla veraguasensis (Seem.) Hemsl.	Costa Rica, Endress 97-76 (Z)	AY597565	AY597599	AY597633
Mascarenhasia lisianthiflora A. DC.	Madagascar, Schönenberger A147 (UPS) * Madagascar, Schlieben 8242 (Z)	AY597566	AY597600	AY597634
Mesechites	Brazil, Simões	AY597567	AY597601	AY597635

mansoana (A. DC.) Woodson	1087 (UEC)			
Mesechites minima (Britton & P. Wilson) Woodson	Cuba, Nilsson s.n. (Z)	AY597568	AY597602	AY597636
Mesechites rosea (A. DC.) Miers	Cuba, Nilsson s.n. (Z)	AY597569	AY597603	AY597637
Odontadenia lutea (Vell.) Markgr.	Brazil, Kinoshita 2002/56 (UEC)	AY597570	AY597604	AY597638
Pachypodium geayi Costantin & Bois	cultivated, Bot. Gard. Chèvreloup, Lieberherr s.n., unvouchered	AY597571	AY597605	AY597640
Pachypodium lamerei Drake	Cultivated, Zürich Bot. Gart., Endress s.n., unvouchered	AY597572	AY597606	AY597639
Prestonia riedelii (Müll.Arg.) Markgr.	Brazil, Simões 1274 (UEC)	AY597573	AY597607	AY597641
Rhodocalyx rotundifolius Müll.Arg.	Brazil, Kinoshita 2000/66 (UEC)	AY597574	AY597608	AY597642
Secondatia densiflora A. DC.	Brazil, Simões 1218 (UEC)	AY597575	AY597609	AY597643
Telosiphonia brachysiphon (Torr.) Henr.	USA, Jenkins 00-185 (TUC) * USA, Worthington 25068 (TEX)	AY597576	AY597610	AY597644
Telosiphonia hypoleuca (Benth.) Henr.	Mexico, Reinag 2000-362 (Z) * Mexico, Richardson 1526 (TEX)	AY597579	AY597611	AY597645
Tintinnabularia mortonii Woodson	Mexico, Breedlove 34900 (TEX)	AY597578	AY597612	AY597646
Trachelospermum jasminioides (Lindl.) Lem.	Cultivated, Zürich Bot. Gard., Endress s.n.,	AY597577	AY597613	AY597647

unvouchered

Appendix 2. Primer sequences of the three plastid regions used in this study

cpDNA region		Primers	Primer source
Rpl16 intron	F71	5'-GCTATGCTTAGTGTGTGACTCGTTG–3'	Baum et al., 1998
	R1516	5'- CCCTTCATTCTTCCTCTATGTTG –3'	Baum et al., 1998
	513F	5'– GGGAACGATGGAAGCTGTGAATGC – 3'	This project
	542R	5' – CGCGGGCGAATATTTACTCTTC – 3'	This project
Rps16 intron	rpsF	5'- TGGTAGAAAGCAACGTGCGACTT –3'	Oxelman et al., 1997
	rpsR2	5'- TCGGGATCGAACATCAATTGCAAG –3'	Oxelman et al., 1997
	387F	5' – CACCGAAGTAATGCCTAAACC – 3'	This project
	497R	5' – GGATTCTKAAGTCTGGCCCAG – 3'	This project
trnS-G intergenic spacer	trnS	5'- GCCGCTTTAGTCCACTCAGC –3'	Hamilton, 1999
	trnG	5'- GAACGAATCACACTTTTACCAC –3'	Hamilton, 1999
	309F	5' – GATGATTTTTCATTTATMTGA – 3'	This project
	527R	5'- GTGCTWAAATATTTCYYATTMAC – 3'	This project

Appendix 3. Morphological matrix of 29 selected characters from the 34 species of Apocynaceae used in this study. All characters are unordered and equally weighted.

Taxon	Character states
Allomarkgrafia brenesiana	1001100001?10001101032100000[a]
Beaumontia grandiflora	10110–0011010000102021000010 0
Chonemorpha fragrans	10210–0001010000101021000000 0
Forsteronia acouci	001100001230-111011{2/3}22000111
Forsteronia velloziana	1001100001030-11010{2/3}22000101
Macrosiphonia longiflora	2001101101010001101032200001 0
Macrosiphonia martii	2001101101010001101032200001 0
Mandevilla karwinskii	2001100101300001101032200001 0
Mandevilla ligustrifolia	1001100101020001101032201001 1
Mandevilla myriophyllum	2001100101300001101032202001 0
Mandevilla pycnantha	2021100101300001101032200000 0
Mandevilla rugosa	1001110101110101101132201001 0
Mandevilla rugellosa	1001110111110101101032201001 0
Mandevilla scabra	1001110101110101101132201001 0
Mandevilla splendens	1021100101310001101032202000 0
Mandevilla sp.	2001100101110101101132200000 0
Mandevilla tenuifolia1	2021100101000001101032202001 0
Mandevilla tenuifolia2	2021100101000001101032202001 0
Mandevilla venulosa	2001100101010001101032202000 0
Mandevilla veraguasensis	1001100101010001101032201000 0
Mascarenhasia lisianthiflora	0011000001010000100-000010100
Mesechites mansoana	1001100001000001101032101100 0
Mesechites minima	1001100?01?0000110103210?00?0
Mesechites rosea	1001100?01100001101032101100 0
Odontadenia lutea	10210–0001210000111021000000 0
Pachypodium geayi	01-00–0000–00010000-000000000
Pachypodium lamerei	00-00–0000–00000000-000000000
Prestonia riedelii	11110–0111101000111010010001 0
Rhodocalyx rotundifolius	21010–0011101000101010011100 0
Secondatia densiflora	10210–0001200000111021000000 0
Telosiphonia brachysiphon	2001101101010001101032200000 0
Telosiphonia hypoleuca	2001101101010001101032200001 0
Tintinnabularia mortonii	1001100?01110001102032101101
Trachelospermum jasminioides	10210–0001000000101021001000 0

[a] ? = missing data

Appendix 4. Characters, character states, and explanatory notes on characters used in the cladistic analyses of the morphological matrix.

1 **Habit**: 0 – trees; 1 – lianas or vines; 2 – erect shrubs or subshrubs, these often with a xylopod.

2 **Latex**: 0 – white; 1 – translucent. Latex in Apocynaceae is most commonly white; however, translucent latex is characteristic for many genera of Echiteae and is also present in some Wrightieae and Malouetieae.

3 **Nodal colleters**: 0 – interpetiolar; 1 – intrapetiolar; 2 – continuous. Colleters are multicellular glands at the nodes and on the inner surface of the calyx in families of the Gentianales (Wagenitz, 1992). In Apocynaceae, the position of nodal colleters can be helpful for distinguishing taxa (Simões and Kinoshita, 2002).

4 **Phyllotaxis**: 0 – alternate; 1 – opposite. In the Apocynoideae, the phyllotaxis is usually opposite. However, some genera of Wrightieae and Malouetieae, the two basalmost tribes in the subfamily, have alternate leaves.

5 **Leaf colleters**: 0 – absent; 1 – present. In addition to the colleters at the nodes and in the calyx, some genera in Apocynaceae typically have colleters on the adaxial surface of the leaf blade. Among the Neotropical taxa of the Apocynoideae, the presence of colleters at the base of the leaf blade is characteristic only for members of Mesechiteae. This is one of key characters of Mesechiteae, and a character that is easily seen with the naked eye.

6 **Leaf colleter position**: 0 – clustered at the base of the leaf blade adaxially; 1 – spread along the midrib of the leaf blade adaxially. Not only the presence of leaf colleters, but also their distribution is an important character for distinguishing genera within Mesechiteae.

7 **Abaxial leaf surface**: 0 – thick indument of white, wooly trichomes absent; 1 – thick indument of white, wooly trichomes present.

8 **Inflorescence type**: 0 – branched (cymose); 1 – unbranched (racemose). An unbranched inflorescence was used by Woodson (1933) to distinguish *Mandevilla* from its closest relatives in the Neotropical Apocynoideae.

9 **Bracts**: 0 – scarious; 1 – petaloid.

10 **Calycine colleters**: 0 – absent; 1 – present. The absence or presence of calycine colleters is often as an aid to distinguish genera in the Apocynaceae (Woodson, 1933; Rosatti, 1989; Ezcurra et al., 1992).

11 **Calycine colleter arrangement**: 0 – continuous; 1 – opposite; 2 – alternate, solitary; 3 – alternate, in groups. Most Apocynoideae have calycine colleters and their arrangement with relation to the sepals is an important character distinguishing genera or subgenera (Simões and Kinoshita, 2002).

12 **Corolla shape**: 0 – infundibuliform or campanulate to tubular-campanulate; 1 – salverform; 2 – tubular; 3 – rotate.

13 **Annular corona**: 0 – absent; 1 – present. The presence or absence of an annulus in the mouth of the corolla tube is often used to distinguish *Prestonia* and some closely related taxa in the New World Apocynoideae (Woodson, 1933; Williams, 2002).

14 **Lower corolla tube**: 0 – straight; 1 – curved. Here we distinguish between an upper corolla tube (the postgenitally fused part above the insertion of the stamens) and a lower corolla tube (the congenitally fused part below the insertion of the stamens)

(Boke, 1948; Nishino, 1982). A curved lower corolla tube is one of the key characteristics used by Woodson (1933) to delimit *Mandevilla* subgenus *Exothostemen* from *Mandevilla* subgenus *Mandevilla* (with a straight lower tube).

15 **Stamens**: 0 – completely included; 1- tips of the anthers exserted or stamens ± completely exserted. *Forsteronia* is variable for this character. The two species used in this analysis have the anthers partially or almost completely exserted, but had a different species been used, coding could have been different.

16 **Anther base**: 0 – strongly sagittate; 1 – bluntly cordate to truncate. In most Apocynoideae, the base of the anthers is sagittate with slender, tapering appendages. In most taxa of Mesechiteae, in contrast, the anther base is nearly truncate. Truncate anther bases was one of the key characters used by Woodson (1933) to delimit genera of the Mesechiteae.

17 **Anther guide-rails**: 0 – composed mainly of endothecial thickenings; 1 –composed mainly of sclerenchyma. In almost all taxa of Apocynoideae, the anthers are fertile only in the upper part; the lower part is enlarged and sterile and involved in the postgenital fusion with the style head. Guide-rails are a specialization of the lateral parts of the anther that function to guide the proboscis of the pollinator into one of the five pollination chambers of the flower (Fallen, 1986).

18 **Dorsal side of anthers**: 0 – completely glabrous; 1 – with trichomes.

19 **Filament length**: 0 – anthers ± sessile; 1 – less than 1 cm long; 2 – greater than 3 cm long.

20 **Junction of filament and anther connection**: 0 – flat; 1 – with a globose swelling.

21 **Anther/style-head union (retinacle):** 0 – anthers attached by a circular patch of trichome-like cells; 1 – anthers attached by a horseshoe-shaped rim of hairs; 2 – anthers attached by a horseshoe-shaped rim of hairs and a narrow longitudinal strip; 3 – anthers attached by cellular fusion. In Apocynoideae, the androecium and gynoecium are postgenitally united to form a gynostegium. The different manner of union and its significance was first noted by Pichon (1948a). Although it is not an easy character to discern, it provides a wealth of information, especially at the tribal level (Sennblad et al., 1998), and has been employed in the most recent classification of the family by Endress and Bruyns (2000).

22 **Style-head shape in cross section**: 0 – circular or subcircular; 1 – pentagonal; 2 – with five strongly projecting ribs. In Apocynaceae, the uppermost part of the gynoecium develops into an enlarged organ with secretory regions called the style-head. Style-head shape and differentiation is quite variable and very important for distinguishing taxa, particularly at the tribal level (Endress et al., 1996; Sennblad et al., 1998; Endress and Bruyns, 2000). To avoid the difficulties of coding the many different and complex shapes of the style head, here we use the shape in cross-section, which, for the taxa used in this study, is sufficient.

23 **Style-head ribs**: 0 – absent; 1 - restricted to the base; 2 – along the entire length of the body of the style head. A strongly five-ribbed style head is characteristic for Mesechiteae. However, the ribs do not run the entire length of the style head in all taxa. In some genera, they are restricted to the base only; thus, this character is useful within the tribe for distinguishing genera.

24 **Collar or wreath at base of style-head**: 0 – absent; 1 – present. Within the Apocynoideae, a collar or wreath is characteristic for the tribes Wrightieae as well as Echiteae, and thus is a useful character at the tribal level.

25 **Nectaries**: 0 – five lobes, more or less free; 1 – two free lobes; 2 – fused halfway or more into an annulus.

26 **Nectary height**: 0 – shorter than the ovary; 1 – equal to or taller than the ovary.

27 **Ovary indument**: 0 – absent; 1 – present.

28 **Follicles**: 0 – cylindrical; 1 – torulose; 2 – moniliform. In most genera of Neotropical Apocynoideae, the follicles are cylindrical, but are moniliform or torulose in *Tintinnabularia*, as well as in some species of *Forsteronia* and *Mandevilla*.

29 **Domatia**: 0 – absent; 1 – present. Domatia are small cavities in the axils of the mid-vein with the secondary veins on the abaxial leaf surface. They are rare in Apocynaceae, but are diagnostic for a few genera (Woodson, 1936; Williams, 1999).

CAPÍTULO III

PHYLOGENETIC RELATIONSHIPS IN *MANDEVILLA* LINDL. (APOCYNACEAE, APOCYNOIDEAE) AND RELATED GENERA BASED ON FIVE PLASTID DNA REGIONS AND MORPHOLOGY[1]

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Abstract - *Mandevilla* Lind., the largest neotropical genus in the subfamily Apocynoideae, is included in tribe Mesechiteae and comprises about 150 species. To test the monophyly of the genus and its relationship with other genera in Mesechiteae, a maximum parsimony analysis was conducted based on DNA sequences from the plastid *rpl16* intron, *rps16* intron, *trn*K intron, *trnS-G* intergenic spacer and *mat*K exon as well as morphological data for 65 taxa of Mesechiteae (48 of which belonging to *Mandevilla*) and 9 taxa from other tribes of Apocynoideae. *Allomarkgrafia* is nested in *Mesechites*, with *Tintinnabularia* sister to this group. *Mandevilla*, as circumscribed by Woodson (1933), was found to be paraphyletic, but the circumscription of Pichon (1948) proved to be monophyletic. Only inclusion of *Quiotania*, *Macrosiphonia* and *Telosiphonia* rendered the genus monophyletic. Thus defined, *Mandevilla* forms a strongly supported clade including three genera in two clades: the clade I, comprising *Macrosiphonia* and 11 species of *Mandevilla* subgenus *Exothostemon* plus three species from subgenus *Mandevilla, M. callista*, *M. funiformis* and *Mandevilla sp.*; and the clade II, comprising the remaining *Mandevilla* and *Telosiphonia*. The clade II also included two strongly supported clades: the clade III, comprising the South American species of *Mandevilla* with 2 nectaries plus *M. pycnantha*, and the clade IV, comprising the remaining *Mandevilla* with five nectaries plus *Telosiphonia*. The clade IV includes two other clades: the clade V, comprising *Telosiphonia* and species of *Mandevilla* distributed in Mexico and Mesoamerica; and the clade VI, comprising a heterogeneous group of *Mandevilla* species mainly distributed in northern South America.

Key words: Apocynaceae; Mesechiteae; *Mandevilla*; phylogenetic systematics; *matK*; *rpl16*; *rps16*; *trnS-G; trnK;*

INTRODUCTION

Mandevilla is the largest Neotropical genus in the subfamily Apocynoideae, with about 150 species distributed throughout the Neotropics, from Mexico to Argentina in a wide variety of different habitats such as deserts, savannas, tepuis, open grasslands and forests. Morphological variation is remarkable in the genus, not only in vegetative but also in reproductive parts. Most species are vines, but erect shrubs are also common, and unbranched subshrubs and even epiphytes can be found. Flower size and structure also spans a broad range, from inconspicuous whitish, tubular flowers less than 1 cm long to brightly colored, showy infundibuliform flowers up to 5 cm long.

A combination of high morphological diversity and broad geographic distribution makes *Mandevilla* one of the most challenging and complex genera of neotropical Apocynaceae, a fact that is reflected in its taxonomic history. The currently accepted circumscription of *Mandevilla* was defined by Woodson in 1933. In a broad taxonomic study of the Neotropical species of subfamily Apocynoideae, he make significant improvements in the circumscription of *Mandevilla*, including in its synonymy other genera like *Exothostemon* G. Don, *Dipladenia* A.DC., *Laseguea* A.DC, *Amblyanthera* Müll.Arg., *Heterotryx* Müll.Arg. and part of *Echites* P. Browne. He maintained *Macrosiphonia* Müll.Arg., a small group of shrubby species with long, white tubular flowers with a disjunct distribution in the arid zones of the southwestern U.S.A. and Mexico, and the savannas of southern South America as a separate genus, even though he recognized that the existing distinctions between *Macrosiphonia* and *Mandevilla*, based on plant habit, flowering time and style-head structure, were extremely tenuous. He recognized two subgenera in *Macrosiphonia*, corresponding to the two geographically distinct groups: *Telosiphonia*, comprising all species in the Northern Hemisphere, and *Eumacrosiphonia* (=*Macrosiphonia*), comprising all species from the Southern Hemisphere.

In addition to broadening the limits of *Mandevilla*, Woodson also proposed an infrageneric classification of the genus, dividing it into two major subgenera: subgenus *Exothostemon* and subgenus *Eumandevilla* (=*Mandevilla*). The two subgenera were differentiated based on the following set of morphological characters: species of subgenus *Exothostemon* have leaf colleters distributed along the entire length of the midrib, calycine colleters with an opposite arrangement and curved corolla tubes, whereas species of

subgenus *Mandevilla* have leaf colleters restricted to the base of the midrib, calycine colleters with an alternate or continuous arrangement and straight corolla tubes.

Within subgenus *Mandevilla*, Woodson (1933) also proposed a new subdivision recognizing five sections: *Laxae*, *Montanae*, *Tenuifoliae*, *Torosae* and *Tubiflorae*. These sections were differentiated based mainly on flower characters concerning corolla shape, anther base shape and number and size of nectaries. The largest section, *Laxae*, has 46 species distributed throughout South America and was characterized by having infundibuliform corollas. Section *Montanae* comprises 16 species also distributed in South America, and was defined by having flowers with salverform to tubular-salverform corolla tubes, anthers with a truncate base and nectaries varying in number from two to five or even obsolete in some species. The small section *Tenuifoliae*, comprising only two South American species, *M. myriophyllum* and *M. tenuifolia*, was distinguished from section *Montanae* by having anthers with auriculate bases and two nectaries. The two remaining sections, *Torosae* and *Tubiflorae*, have five and eight species, respectively, and are distributed in Mexico and Mesoamerica. Both sections were characterized by flowers having salverform to tubular-salverform corollas, anthers with auriculate bases and five nectaries surrounding the ovary, and were differentiated by the size of the nectaries, which were said to be equal to or taller than the ovary in *Tubiflorae* and shorter than the ovary in *Torosae*.

A new classification of *Mandevilla* was published by Pichon in 1948. He expanded Woodson's (1933) circumscription of *Mandevilla* by including *Macrosiphonia* in its synonymy. Pichon justified this by arguing that the distinguishing characters used by Woodson to differentiate between the two genera were inconsistent and arbitrary, making impossible an unambiguous distinction between them. He did not view Woodson's subgenera *Macrosiphonia* and *Telosiphonia* especially closely related, however, and differentiated them in two distinct sections, based on the absence of a pedicel, longer filaments and larger pollen grains of the former. Within *Mandevilla*, Pichon recognized Woodson's subgenera *Mandevilla* and *Exothostemon* as valid groups, but not his sections within subgenus *Mandevilla*. His arguments were that the characters supporting the two subgenera were reliable, but the characters supporting the sections were highly inconsistent, with no real diagnostic states to define them. Pichon (1948) proposed a new infrageneric

classification within *Mandevilla*, recognizing four sections: 1) section Orthocaulon, corresponding to Woodson's subgenus *Mandevilla*; 2) section Exothostemon, corresponding to Woodson's subgenus *Exothostemon*; 3) section Megasiphon, corresponding to Woodson's *Macrosiphonia* subgenus *Macrosiphonia*; and 4) section Telosiphonia, corresponding to Woodson's *Macrosiphonia* subgenus *Telosiphonia*. A summarized comparison between the infrageneric classification of Woodson (1933) and Pichon (1948) is provided in Table 1.

Since Pichon's work little was undertaken in the taxonomy of the *Mandevilla* and related genera in Mesechiteae until relatively recently. In 1991 Zarucchi published the description of *Quiotania*, a monotypic genus very closely related to *Mandevilla*, Woodson's subgenus *Telosiphonia* was elevated to generic status by Henrickson (1996), and *Salpinctes* was included of in the synonymy of *Mandevilla* by Morales (1998a). Another relevant work was a synopsis of the Mexican and Central American species of *Mandevilla* by Morales (1997), with new taxonomic combinations in the species from Woodson's sections *Tubiflorae* and *Torosae*, many of them reduced to synonymy. In addition, a large number of new species of *Mandevilla* have been described in the last decades, increasing the number of published species from the 108 recognized by Woodson (1933) to about 150 at the present. Even though new information has been accumulated for the genus, no overall concept for taxonomic classification within *Mandevilla* as a whole was proposed after Pichon (1948). Taxonomic difficulties in generic and infrageneric concepts, which have persisted for the past seven decades still remain, and as pointed out by Zarucchi (1991, p. 35), "The last word concerning generic limits of the *Mandevilla-Mesechites-Macrosiphonia* complex and near relatives has obviously not yet been written."

The use of phylogenetic methods has been successfully applied in Apocynaceae to solve controversial aspects of classification within the family. Most of these studies have addressed the circumscription of the Apocynaceae s. str. and their relationships with the former Asclepiadaceae (e.g., Judd et al., 1994; Sennblad and Bremer, 1996, 2002; Potgieter and Albert, 2001), but a significantly increasing number of works are focusing on relationships within the traditional Apocynaceae. Two examples are Sennblad et al. (1998) for tribe Wrightieae and, more recently, Endress et al. (submitted) for Alyxieae), and a

larger-scale study of subfamily Apocynoideae, which is currently being conducted at Harvard University (D. Middleton, pers. comm.).

Simões et al. (2004) provided the first phylogenetic study of the tribe Mesechiteae, with suggestions for taxonomic improvements in tribal and intergeneric delimitations. Preliminary results were obtained for *Mandevilla* and related genera, but no conclusive statements could be made due to the limited taxon sampling within *Mandevilla*. Using a broader taxon sampling, our present study represents a logical second step in interpreting the phylogeny of Mesechiteae, by focusing on the inter- and infrageneric relationships of its largest genus, *Mandevilla*.

The aims of the present article are to test the monophyly of *Mandevilla* and to determine its relationships to the closely related genera, *Macrosiphonia*, *Telosiphonia* and *Quiotania*, using both morphology and molecular sequence data from five different regions: *rpl16* intron, *rps16* intron, *trnK* intron, *trnS-G* intergenic spacer and *matK* exon. The resulting hypotheses of monophyly and intergeneric relationships of *Mandevilla* are compared with the classification of Woodson (1933) and Pichon (1948). Morphological features that characterize clades and/or are used to define taxonomic ranks are discussed and a list of recombinations in the studied genera is provided (Appendix 4).

MATERIALS AND METHODS

TAXON SAMPLING

Sixty-five taxa of Mesechiteae including representatives from all genera of the tribe recognized by Simões et al., 2004) (*Allomarkgrafia, Forsteronia, Macrosiphonia, Mandevilla, Mesechites, Telosiphonia* and *Tintinnabularia*) were defined as the ingroup and included in this study (Appendix 1). In order to test the infrageneric classifications of *Mandevilla* proposed by Woodson (1933) and Pichon (1948) (Table 1), 48 specimens (from 47 species) of *Mandevilla* representing all subgenera and sections proposed by these authors were sampled (Table 2). Nine outgroup taxa were chosen from all but the basalmost tribe (Wrightieae) of the subfamily Apocynoideae based largely on previous studies, which suggest that the closest relative of Mesechiteae is either Apocyneae or Echiteae (Sennblad et al., 1998; Sennblad & Bremer, 2002; Simões et al, 2004). Two genera from Echiteae

(*Prestonia* and *Rhodocalyx*) and five genera from Apocyneae (*Beaumontia, Chonemorpha, Odontadenia, Secondatia* and *Trachelospermum*) were included. Two species of *Pachypodium* (Malouetieae) were used to root the cladograms.

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Total genomic DNA was extracted from silica dried leaf material or from herbarium specimens using DNeasy Plant Mini Kits (Qiagen, Valencia, California, USA) following the manufacturer's protocol. Four noncoding plastid regions, rpl16 intron, rps16 intron, trnK intron and trnS-G intergenic spacer, and one coding regions, matK exon, were amplified. Double-stranded DNA was amplified by polymerase chain reaction (PCR) on a Biometra Tgradient machine (Biometra, Göttingen, Germany), applying a thermal cycling program consisting of 34 cycles of denaturation at 95°C for 30 sec, annealing at 52°C for 1 min and extension at 72°C for 90 sec. For trnK intron and matK exon, the thermal cycling program was modified in the following steps: denaturation at 94°C for 30sec and annealing at 54°C for 1 min. Reactions were terminated with a final extension of 4 min at 72°C for rpl16 intron, rps16 intron and trnS-G spacer, and of 7 min for trnK intron and matK exon. All PCR reactions were performed in a total volume of 25 microliter reactions, using 2.5 mM MgCl, 10x PCR* Buffer (Amersham Biosciences), 0.25 mM of dNTP's, 0,5 units of Taq DNA Polymerase (Amersham Biosciences, lot 17544), 1 to 4 µl of BSA (Bovine Albumine, Sigma, Steinheim, Germany) and 0,1 mM of each primer. Primer information is presented in Table 3. For some taxa, internal primers were also used to amplify the rpl16 intron and trnS-G intergenic spacer, with the following changes in the thermal cycling program: 40 instead of 34 cycles and extension time shortened to 1 min. For most of the taxa, amplification of the whole trnK intron – matK exon region was made in one single step; for some taxa, however, this region could only be amplified when broken into two halves by using a combination of one external and one internal primer. Successfully amplified PCR products were then purified using GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences).

For some taxa with DNA extracted from herbarium vouchers, no amplified products were obtained with our initial PCR protocols and primer sets. Amplification was only

successful when the following two-step amplification method was used: 1) a first round of PCR amplification using the total genomic DNA as a template; and 2) a second round of amplification using 1 external and 1 internal primer and using the 10% diluted product of the first amplification as a template. For *rpl*16 intron, *rps*16 intron and *trn*S-G intergenic spacer the entire region was amplified in the first PCR by using only external primers. The *trn*K intron/*mat*K exon region was broken into two parts after the first amplification round by using a combination of external and internal primers. Each amplified part was then broken into two smaller parts during the second amplification round by using a new set of internal primers. Since this method is very sensitive and prone to contamination, a series of additional steps was applied to avoid any possible source of contamination and double-check the results obtained: 1) Additional amplifications and sequences were generated for all species in which this method was used; 2) A new set of external and internal primers was designed for *rpl16* and *rps*16 introns (see Table 3) the total genomic DNA of two species (*Mandevilla anceps* and *M. krukowii*) was re-extracted.

The same primers used for PCR amplification were also used for the cycle-sequencing reactions, carried out with the ABI Prism Big Dye Terminator Cycle Sequencing Ready Extraction Kit (Perkin Elmer, Applied Biosystems, Applera Europe BV, Rotkreuz, Switzerland). Sequence products were purified on MicroSpin G-50 columns (Amersham Pharmacia Biotech Europe, Dübendorf, Switzerland) and loaded on an ABI Prism 377 DNA sequencer (Perkin Elmer). Complementary strands were edited and assembled with Sequencher 3.1.1 (Gene Codes, Ann Arbor, Michigan, USA).

For two species, *Mandevilla lancifolia* and *M. nerioides,* amplification of *rpl*16 intron, *rps*16 intron and *trn*S-G spacer was only possible with the two-step amplification method. A new set of external and internal primers was designed for amplification of *rpl*16 intron and *rps*16 intron, since amplification with the original primer was not successful. The two-step amplification procedure was also used to obtain products of *trn*K intron and *mat*K exon for six species (*Mandevilla anceps*, *M. krukowii*, *M. lancifolia*, *M. leptophylla*, *M. nerioides, M. tricolor* and *Tintinnabularia mortonii*). With this method, we amplified the complete sequence for *M. lancifolia* and only partial sequences for *M. anceps*, *M. leptophylla* and *T. mortonii*. No amplified products were obtained for *Mandevilla krukowii*,

M. nerioides and *M. tricolor*, and for this reason they were excluded from further individual analyses of *trn*K and *mat*K data sets.

DATA MATRIX COMPOSITION AND PARSIMONY ANALYSIS

Nucleotide sequences of the *rpl16*, *rps16* and *trn*K introns, *trnS-G* intergenic spacer and *mat*K exon were aligned using Clustal W version 1.8 (Thompson et al., 1994) and adjusted by eye. Regions of ambiguous alignment were excluded from the analysis. Individual gap positions were treated as missing data, unequivocally aligned gaps being coded as presence/absence characters with the software Gapcoder (Young and Healy, 2003) and then added to the sequence matrix. For *trn*K intron and *mat*K exon, gaps were coded manually as additional, binary characters applying the coding method described by Simmons and Ochoterena (2000), due to their reduced number when compared to the other studied plastid regions.

Thirty-two morphological characters were scored using herbarium and fresh specimens, pickled flowers and, when available, flower sections provided by the second author. For some taxa, the literature was also consulted (e.g., Woodson, 1933; Pichon, 1950; Leeuwenberg, 1997; Morales, 1996, 1998b, 2002). The morphological matrix, a list of the characters, character states and explanatory notes on characters are given in Appendix 2 and 3.

The following data sets were subjected to phylogenetic analysis: 1) rpl16 intron, 2) rps16 intron, 3) trnS-G intergenic spacer, 4) trnK intron 5) matK exon and 6) morphology. As the results of individual analyses did not show any significant topological conflict, data partitions were combined as follows: all molecular data sets combined together (molecular combined), and all molecular and morphological data sets combined (total evidence).

Maximum parsimony analyses were performed using PAUP* 4.0b (Swofford, 2000). All characters were unordered and equally weighted. Polymorphisms in the data matrix were treated as such, rather than as uncertainties. A heuristic search for most-parsimonious trees (MPT) included an initial round of tree searches with 1000 random addition sequence replicates (RASR), holding ten trees at each step, tree bisection-reconnection (TBR) branch swapping with MULTREES and steepest descent in effect,

saving a maximum of 50 trees at each replicate. All shortest trees retained in memory were then included in a second round of searches involving exhaustive TBR branch swapping. Relative support for each node was estimated using the bootstrap resampling procedure (Felsenstein, 1985) as implemented in PAUP employing a full heuristic search with 500 replicates, 250 RASR, three trees held at each step, TBR branch swapping with steepest descent and MULTREES in effect, saving ten trees at each RASR.

Morphological characters were optimized onto the strict consensus tree of the total evidence analysis using Winclada ver. 1.00.08 (Nixon, 2002) in order to identify the synapomorphies that were congruent with each of the major clades of the ingroup retrieved in our analyses.

RESULTS

Detailed information for both individual and combined data sets is given in Table 4. Multiple sequence alignment was straightforward in the *mat*K exon, with a small number of gaps and no detectable mononucleotide repeats. For the four noncoding regions used in this study, alignment was also straightforward in the *trn*K intron and *rps*16 intron, but proved to be more difficult for the *rpl*16 intron and *trn*S-G spacer due to the larger number of gaps and mononucleotide repeats. A total of 165 characters, including nucleotides and gaps, were excluded from the noncoding regions, mainly from the *rpl*16 intron.

PARSIMONY ANALYSES

Tree length, consistency index (CI) and retention index (RI) for the cladograms that resulted from the analyses of individual and combined data sets are summarized in Table 4. Assuming that simultaneous analysis of combined data is the best approach to phylogenetic inference (Nixon and Carpenter, 1996), we tested the combinability of all molecular information by searching for incongruence between individual data sets. For this, we compared the results on a node-to-node basis of all individual data sets with respect to levels of resolution and bootstrap support, as applied by many authors (e.g., Wiens, 1998; Sheahan and Chase, 2000, Whitten et al., 2000; Reeves et al., 2001). No strongly supported

(>85%) incongruent clades were found, thus the individual molecular data sets were combined (Fig. 3.4). From the individual molecular data sets, the best resolved cladogram was provided by the *matK* exon, with most of the internal nodes receiving bootstrap support (BS) higher than 50%. (Fig. 3.2) The highest proportion of parsimony informative characters and CI and RI values were also provided by this data set. Of the other data sets, *rpl16* intron, *rps16* intron and *trnK* intron had similar levels of resolution; the least resolved cladogram was provided by *trnS-G* intergenic spacer (Figs. 3.3). Of the cladograms generated by the separate molecular data sets, only the *mat*K exon and *trnS-G* intergenic spacer trees defined a clade, corresponding to what will later be defined as the *Mandevilla* clade, with BS higher than 50%, identical to that of the molecular combined and total evidence trees.

Analysis of the morphological data set resulted in a poorly resolved cladogram with only a few groups supported by a BS value higher than 50% (Fig. 3.3). No incongruence was detected when comparing the morphological tree with either the strict consensus of the individual or combined molecular trees. Therefore, the morphological and combined molecular data sets were combined into a total evidence data set. All further discussion will be based on the total evidence tree (Fig. 3.5).

A clade including representatives of *Allomarkgrafia*, *Forsteronia*, *Macrosiphonia*, *Mandevilla*, *Mesechites* and *Tintinnabularia* is strongly supported (BS = 100%). The same results were obtained by Simões et al (2004) in a phylogenetic analyses of the tribe Mesechiteae, with a clade composed by representatives of the same genera, designated as the Mesechiteae clade, also strongly supported (BS = 97%). Within this clade, the same three strongly supported subclades as observed in our previous study are defined: 1) the *Mesechites* subclade (BS = 100%), comprised of *Allomarkgrafia*, *Mesechites* and *Tintinnabularia*; 2) the *Forsteronia* subclade (BS = 100%), comprising three species of this genus; and 3) the *Mandevilla* subclade (BS = 94%), comprising species of *Macrosiphonia*, *Mandevilla* and *Telosiphonia*.

Within the *Mandevilla* clade, two major, strongly supported (BS = 100%) clades are defined: 1) one comprised of *Macrosiphonia*, 11 species of *Mandevilla* subgenus *Exothostemon* Woodson (1933) and three species of subgenus *Mandevilla* Woodson (1933), *M. callista*, M. *funiformis* and *Mandevilla sp*, hereafter referred to as clade I; and 2)

one comprised of *Telosiphonia* and the remaining species of *Mandevilla*, hereafter referred as the clade II.

Within the clade II, the most diverse in *Mandevilla* and extensively sampled in our study, two strongly supported clades are defined: 1) a subclade comprised of mostly species of *Mandevilla* from central to southern South America, hereafter referred as clade III, and 2) a clade comprised of *Telosiphonia* and a wide range of species of *Mandevilla* distributed from Mexico to southern South America, hereafter referred as the clade IV. The clade IV is subdivided in two major clades: 1) clade V, a more heterogeneous clade composed of the South American species of *Mandevilla* with truncate anther bases; and 2) clade VI, comprised by *Telosiphonia* and all Mexican species of *Mandevilla*. All 6 clades within *Mandevilla* clade are not named and defined at the present, thus maintaining flexibility for further taxonomic revisions.

DISCUSSION

PHYLOGENETIC HYPOTHESIS AND CURRENT CLASSIFICATION

In our study, a clade composed of species of *Mandevilla*, *Macrosiphonia* and *Telosiphonia* (*Mandevilla* clade) largely corresponds to the circumscription of *Mandevilla* proposed by Pichon (1948), but only partially to Woodson's (1933) circumscription the genus. The main difference between the two classifications concerns the status of *Macrosiphonia/Telosiphonia*. Woodson (1933) recognized *Macrosiphonia* as a distinct genus with two subgenera, subgenus *Macrosiphonia* and subgenus *Telosiphonia*. Pichon (1948), on the other hand, included *Macrosiphonia* in the synonymy of *Mandevilla*, arguing that the distinguishing characters used by Woodson (1933) to differentiate the two genera were inconsistent and arbitrary, being based only on leaf indument and superficial aspects of flower structure. He cited a set of morphological characters to differentiate between Woodson's subgenus *Macrosiphonia* and subgenus *Telosiphonia* (presence versus absence of a pedicel, structure of staminal filaments and pollen size) and recognized them as two different sections of *Mandevilla*, section *Megasiphon* and section *Telosiphonia* respectively. Our results are largely consistent and complementary to the preliminary results from our previous study (Simões et al., 2004).

Most of the infrageneric ranks of *Mandevilla* proposed by Woodson (1933) are not supported in our analyses. The two subgenera he proposed, *Exothostemon* and *Mandevilla*, although not monophyletic, correspond for the most part to the two major clades within *Mandevilla* identified in our analyses, clades I and II, respectively (Fig. 3.5). To render Woodson's subgenera monophyletic: 1) *Macrosiphonia* must be included in subgenus *Exothostemon*, 2) *Telosiphonia* must be included in subgenus *Mandevilla*, and 3) *Mandevilla callista*, *M. funiformis* and *Mandevilla sp.* must be transferred from subgenus *Mandevilla* to subgenus *Exothostemon*. Of the five sections of subgenus *Mandevilla* proposed by Woodson (1933), only the smallest, section Tenuifolia, containing two species, constitute a monophyletic group (BS = 100%) in our study. All of Woodson's (1933) other sections are paraphyletic, with their constituent taxa scattered throughout *Mandevilla* clade (Fig. 3.5). The most extreme case of paraphyly is his section *Laxae*, the largest section of the subgenus *Mandevilla*, which he characterized by having infudibuliform corollas. In our study, the 24 species sampled from this section are scattered among all larger subclades of the *Mandevilla* clade (Table 2).

Compared to the infrageneric ranks proposed by Pichon (1948), our results support the monophyly of two of his sections, section Megasiphon and section Telosiphonia. His other sections, section Orthocaulon and section Exothostemon, correspond to Woodson's subgenus *Mandevilla* and subgenus *Exothostemon*, respectively, and consequently do not constitute monophyletic groups. Despite their strongly supported monophyly, recognition of sections *Megasiphon* and *Telosiphonia* is untenable both taxonomically as well as a morphologically, due to the considerable number of additional sections without morphological synapomorphies that would need to be recognized in *Mandevilla*. The same justification can be applied for not recognizing Woodson's section *Tenuifoliae*, despite its monophyly.

CLADE I

The clade I contains taxa from three different taxonomic ranks of Woodson's (1933) classification. From its 17 constituent taxa, the majority (11) belong to Woodson's subgenus *Exothostemon*. All sampled species of *Exothostemon* in our study are within this clade. From the six remaining taxa, three (*Macrosiphonia longiflora*, *M. martii* and *M.*

velame) belong to Woodson's genus *Macrosiphonia*, and the other three (*Mandevilla callista, M. funiformis* and *M. sp.*) are under the circumscription of his subgenus *Mandevilla*.

The subgenus *Exothostemon* forms a morphologically easily distinguished group within *Mandevilla*. Flower structure is quite homogeneous, with the presence of two character states noted by both Woodson (1933) and Pichon (1948) as diagnostic for the subgenus and section, respectively: 1) leaf surface with of many colleters distributed along the midrib on the adaxial surface, and 2) calycine colleters in an opposite distribution pattern (see Appendix 3 for details). Variation in vegetative characters and geographic distribution, however, is remarkable in the subgenus, and morphological groups within clade I can be characterized. The first group, represented in our study by seven species (*M. dodsonii, M. hirsuta, M. krukowii, M. leptophylla, M. rugosa, M. rugellosa, M. scabra* and *M. subsagittata*), is composed by taxa that show the most common morphological pattern in the subgenus: vines with terete stems and yellow-flowers, often with a red center (white flowers in *M. rugosa*) that occur mainly in forest and in its bordering zones throughout the Neotropics. The second group, represented in our study by three species (*M. anceps, M. lancifolia* and *M. nerioides*), is composed by taxa that have a unique set of characters within the subgenus: they are shrubs or woody lianas with ridged to winged stems (tetragonal in cross section), and flowers of various colours that are found mainly in the open habitats of white sand savannas and tepuis of northern South America. None of these two groups are monophyletic, though: all species from the first group are nested together in a strongly supported clade, together with one species from the second group, *M. lancifolia*. The two remaining species of the second group, *Mandevilla anceps* and *M. nerioides*, form a strongly supported clade (BS = 100%), but its relationship to the other remaining species of clade I is not resolved in our analysis. Based on these results, no further conclusions on relationships and patterns of evolution within *Exothostemon* can be made. A broader taxon sampling, especially including representatives from the poorly collected species of the tetragonal-stem group, is needed to address these questions.

The inclusion of three species from subgenus *Mandevilla* (*M. callista, M. funiformis* and *Mandevilla sp.*) in clade I is key to understanding the evolution of characters within *Mandevilla*, since they possess characteristics from both subgenera of Woodson (1933).

Both *M. funiformis* and *Mandevilla sp.*, have five calycine colleters arranged in an opposite distribution pattern, as in subgenus *Exothostemon*, but at the same time have leaf colleters restricted to the base of the midrib, a key character of subgenus *Mandevilla*. *M. callista*, in contrast, has leaf colleters spread along the midrib, as is characteristic for taxa in subgenus *Exothostemon*, but according to Woodson (1933), it has calycine colleters in a continuous ring. Woodson (1933) recognized this "intermediate" status of *M. callista* and *M. funiformis*, but justified their inclusion in subgenus *Mandevilla* by the presence of continuous calycine colleters in the former and leaf colleters restricted to the base in the latter. We examined vouchers from both *M. funiformis* and *M. callista* in order to estimate this morphological variation as compared with the taxonomic descriptions provided by Woodson (1933). We found that *M. funiformis* and *Mandevilla sp.* both have leaf colleters restricted to the base of the midrib and calycine colleters in an opposite distribution pattern . Our observations in *M. callista*, however, do not agree completely with Woodson's (1933) original description of the species. We agree with him that leaf colleters are spread along the entire midrib, but in the specimens we studied, calycine colleters had the opposite distribution pattern typical for most taxa in the clade I.

The inclusion of *Macrosiphonia* in the clade I is somewhat unexpected from a morphological point of view, but this relationship was previously observed by Simões et al. (2004). In *Macrosiphonia,* leaf colleters are restricted to the base of the midrib and calycine colleters form a continuous ring, whereas in the other species in this clade, leaf colleters are spread along the midrib and calycine colleters are arranged in an opposite pattern. Thus, no morphological synapomorphies were found to define clade I. Since subclades in the clade I are only weakly to moderately resolved, no further conclusions on relationships can be made at this time. Increased taxon sampling and additional studies focused on aspects never or scarcely addressed in *Mandevilla* and *Macrosiphonia* (e.g., palynology, floral ontogeny and anatomy) could provide useful information to help clarify relationships within this clade.

CLADE II

This clade comprises *Telosiphonia* and the bulk of all *Mandevilla* species, and corresponds to Pichon's (1948) sections Orthocaulon and Telosiphonia, and in largely with

Woodson's (1933) subgenus *Mandevilla* plus genus *Telosiphonia*. Almost the entire spectrum of morphological variation, in both vegetative and reproductive parts of the whole *Mandevilla* and related genera can be found within the clade II, from the subshrubs with large, showy, lilac to pink infundibuliform flowers of *M. sancta*, for example, to the vines with small, inconspicuous, white, tubular flowers of *M. ligustrifolia*. This clade is also represented throughout the entire geographic area of *Mandevilla* in the Neotropics, from southwestern U SA and Mexico to the subtropical regions of Argentina. All species from this clade share one morphological synapomorphy: leaf colleters are restricted to the base of midrib. The same character state, however, is also found in some taxa of the clade I (e.g., all *Macrosiphonia* species, as well as *Mandevilla funiformis* and *Mandevilla sp.*); thus no morphological synapomorphy exclusively for the clade II could be detected. Within the clade II, two main clades can be distinghished: clades III and IV.

CLADE III

This clade is composed for the most part of species of *Mandevilla* occurring in the forests, savannas and "campo rupestre" formations of northeastern to southern Brazil, and also reaching Paraguay and Argentina. Most of these species belong to Woodson's (1933) section *Laxae*, but two of them (*Mandevilla myriophyllum* and *M. tenuifolia*) belong to his section *Tenuifoliae* and one, *M. pycnantha*, to his section *Montanae*. With the exception of *M. pycnantha*, all species from this clade share one morphological character state: the presence of only two nectaries, in a position alternate to the carpels. Other morphological characters, however, are more variable within this subclade, in both vegetative and reproductive parts. Some species, like *Mandevilla pendula* and *M. urophylla*, are vines from the southwestern Brazilian forests, but others, like *Mandevilla illustris*, *M. pohliana* and *M. spigeliiflora*, are small, unbranched subshrubs of savannas and "campo rupestre" formations. Branched, woody shrubs are also common, with some species, like *Mandevilla duartei* and *M. venulosa*, endemic to specific mountain formations of southwestern Brazil. Flowers are showy and variously coloured, and, in most cases, have an infundibuliform corolla. Woodson (1933) used corolla shape as a diagnostic character, and defined one entire section, *Laxae*, based on this single character. Even though species with infundibuliform corolla form a strongly supported clade (BS = 100%) within the clade III,

this character state is also dispersed among species from different clades of *Mandevilla*, undermining its utility as a phylogenetic marker.

The sister position of *M. pycnantha* to a clade composed of *M. myriophyllum* and *M. tenuifolia* is consistent from a morphological point of view, as these species have small, purplish flowers with salverform corollas. The presence of five nectaries surrounding the ovary in the former makes impossible to characterize the clade III based solely on morphological characters, but in counterpart all species from this clade occur in the same geographic area. *Mandevilla pycnantha* is endemic to the "campo rupestre" formations of southern Brazil, where it occurs simpatrically with other species from the clade III such as *Mandevilla martiana*, *M. pohliana* and *M. tenuifolia*, among others.

CLADE IV

This clade comprises a heterogeneous group of 15 species of *Mandevilla* from sections *Laxae*, Montaneae, *Torosae* and *Tubiflorae* plus the three sampled species of *Telosiphonia*. In contrast to clade III, all species have five nectaries surrounding the ovary. Another character state found in most species of this clade is the presence of elongate apical appendages of style-head that are the same size or taller than the main body of the style-head. Two clades within the clade IV lack elongate style-head apical appendages, however; one is represented by the *Telosiphonia* species, and the other by *Mandevilla emarginata*, *M. laxa* and *M. pentlandiana*. The apical appendages of the style-head are usually short in *Mandevilla* and related genera, ranging from inconspicuous to about half the length of the main body of style-head (which corresponds to the region with the protruding ribs, see Appendix 3 for details). Long style-head appendages in *Mandevilla* are found exclusively in the clade IV and therefore constitute an important synapomorphy for the group, but their role in an evolutionary context remains uncertain. Within the clade IV, two main clades can be distinguished: clades V and VI.

CLADE V

This clade is mainly composed of species from Woodson's (1933) *Mandevilla* section *Montanae*, but three species (*M. glandulosa*, *M. laxa* and *M. veraguasensis*) belong to section *Laxae*. Most of the species are vines, with the exception of *M. emarginata*, an

unbranched subshrub and *M. pentlandiana*, which has both vine and shrub forms. Flowers are in general salverform or tubular, white to greenish, but *M. veraguasensis*, *M. glandulosa* and *M. laxa* have showy, infundibuliform to campanulate corollas. One morphological synapomorphy defines this clade: the anther base is truncate, with no discernible auricles or protruding extensions. In *M. emarginata*, *M. laxa* and *M. pentlandiana* auriculate anther bases can sometimes be found in some individuals, but in most cases the base is truncate. The presence of truncate anthers was used by Woodson (1933) to distinguish his section *Montanae*. Indeed this holds for most of the species from this section, although *M. pycnantha* has conspicuously auriculate anthers. In addition, the three species from Woodson's (1933) section *Laxae*, which come out in this subclade (*M. glandulosa*, *M. laxa*, and *M. veraguasensis*) also have truncate anthers. This character has some phylogenetic relevance of within *Mandevilla*, but not in the way it was used by Woodson (1933) to define an entire section.

An interesting aspect of the clade V is its geographical distribution. In contrast to the clade VI, which is restricted to one geographical region, two geographically disjunct groups can be distinguished in the clade V. The majority of its species are found in the forests of Central America and northern South America. Three species, however (*M. emarginata*, *M. laxa* and *M. pentlandiana*) occur mainly in Argentina and southern Brazil, with *M. emarginata* also reaching the savannas of central Brazil. One possible explanation of this geographic pattern is a putative corridor provided by the Andes, which could have played a role as a connection between northern and southwestern South America. This is a potential hot topic for future studies in biogeography of Apocynaceae.

CLADE VI

This clade is composed of species from Woodson's *Mandevilla* sections *Laxae* (*Mandevilla convolvulacea*, *M. oaxacana*), *Torosae* (*M. foliosa*, *M. karwinskii*) and *Tubiflorae* (*M. syrinx*, *M. tubiflora*), plus the genus *Telosiphonia*, all of which occur in the deserts and forests of Mexico and southwestern United States. Despite this relatively tight geographic distribution, morphological traits are extremely variable, especially those related to flower structure. *Mandevilla syrinx* and *M. tubiflora* have many-flowered inflorescences bearing small, tubular, white flowers, whereas *Mandevilla convolvulacea*

and *M. oaxacana* have few-flowered inflorescences bearing showy yellow, infundibuliform flowers. The most extreme member of this subclade, however, is *Telosiphonia*, in which the long, narrowly tubular white flowers are in much-reduced, few-flowered inflorescences, sometimes reduced to a single flower.

Although species of Woodson's (1933) sections *Torosae* and *Tubiflorae* are restricted to this subclade, they do not form distinct, monophyletic groups and therefore do not deserve recognition as valid sections. Distinction between these two sections is based on the nectary height: in section *Torosae*, the nectaries are shorter than the ovary, and in section *Tubiflorae* the nectaries are the same size or taller than the ovary. We observed that species of section *Torosae* always have nectaries taller than the ovary, but the same condition occurs in three other species from this subclade, which all belong to different sections sensu Woodson (1933): *M. foliosa* (section *Torosae*), *M. convolvulacea* and *M. oaxacana* (both in section *Laxae*). Shorter nectaries are found in all *Telosiphonia* species and *M. karwinskii*, which form a strongly supported clade (BS = 100%).

The sister relationship of M. *karwinskii* and *Telosiphonia*, which has never been proposed previously, is congruent with their geographic distribution and habitat. Both species are branched, rhizomatous shrubs occurring sympatrically in the deserts of Mexico and the southwestern USA. Apart from the short nectaries, other morphological traits are quite different between *M. karwinskii* and *Telosiphonia*, especially leaf indument, flower size and style-head structure. This exemplifies the remarkable morphological plasticity of *Mandevilla*, even for species occupying the same habitat.

MACROSIPHONIA AND TELOSIPHONIA

Macrosiphonia and *Telosiphonia* are traditionally recognized as two genera of Mesechiteae with five and six species, respectively, that share a number of morphological characteristics. Both are erect shrubs or subshrubs, sometimes rhizomatous, with a well-developed underground system, leaves covered by a dense, wooly indument on the abaxial side and white flowers with a long slender tube. Despite morphological affinities, the two genera occur in disjunct geographical areas, which roughly coincide with the extreme northern and southern distribution of *Mandevilla*. *Macrosiphonia* is found in the savannas

of Central Brazil and Argentina, and *Telosiphonia* is restricted to the arid zones of Mexico and southwestern United States.

Simões *et al.* (2004) investigated the phylogenetic relationships within Mesechiteae and found that *Macrosiphonia* and *Telosiphonia* are not congeneric, forming two distinct, strongly supported clades nested in *Mandevilla*. Our study, based on a broader taxon sampling of *Mandevilla*, support these results, with *Macrosiphonia* and *Telosiphonia* forming two distinct, monophyletic groups within *Mandevilla*. The disjunct pattern of species of *Macrosiphonia* and *Telosiphonia* is of great interest in Apocynaceae, by constituting a case in which a convergence of morphological characters is proved to occur in two lineages from the same genus.

Convergence in vegetative characters of *Macrosiphonia* and *Telosiphonia* could be explained as an adaptation to similar environmental conditions. A shrubby, erect habit, together with the presence of a dense indument covering both vegetative and reproductive organs are common traits of plants that inhabit open, moderately-dry to arid habitats. Convergence in flower structure, however, is of special interest, since the recognition of *Macrosiphonia* and *Telosiphonia* as congeneric by Woodson (1933) was mainly based on floral characters. Flowers in the two genera present a set of characters (white flowers with a long corolla tube, vespertine opening and production of scent) that, when considered together, is unique in the family and are possibly associated to hawkmoth pollination. This correlation between flower morphology and pollination is highly suggested but not confirmed, due to the lack of studies on pollination biology of *Macrosiphonia* and *Telosiphonia*. Some evidence, however, was provided by L. Galleto (pers. comm.), who observed hawkmoth visits on flowers of *Macrosiphonia petrea*. Another evidence of hawkmoth pollination in *Macrosiphonia* was obtained from observations of *M. longiflora* and *M. velame* in their natural habitat made by the first author. Flowers of these species produce a considerable quantity of nectar and seem to have a vespertine activity, producing a strong scent and fully opening the corolla lobes in the first hours of night.

THE STATUS OF *QUIOTANIA*

Quiotania is a monotypic genus described by Zarucchi (1991) based on only two collections from Antioquia, Colombia. He stated that it is clearly a member of Mesechiteae,

and that it would key out to *Mandevilla* using Woodson's 1938 key in North American Flora. The only distinguishing character for *Quiotania* given by Zarucchi (1991) was its lack of a pronounced corolla tube, which can be only 2--3mm long. However, in some species of *Mandevilla* (e.g., *M. syrinx* and *M. torosa*) the length of the corolla tube is quite variable, and in some specimens is only 4 mm long (Morales, 1998).

During preparation of the Apocynaceae treatment for the Flora of Ecuador, two specimens of an undetermined species were received by the second author, which after detailed examination were identified as *Mandevilla ligustrifolia*, a species known only from the holotype. In Simões et al. (2004), *M. ligustrifolia* was clearly nested within *Mandevilla*, with a strong bootstrap support, and with the increased taxon sampling in the current paper this relationship is also strongly supported. At the same time, however, a strong similarity to *Quiotania* was noticed, which was further supported by the excellent illustration that accompanied the original description of the genus. Comparing our specimens with photocopies of the type collection of *Quiotania*, we determined that they were cospecific. The nomenclatural consequences will be undertaken in a second paper.

THE *MESECHITES* CLADE

In Simões et al. (2004), a clade composed of *Allomarkgrafia*, *Mesechites* and *Tintinnabularia* (Mesechites clade) was identified, in which one of the three species of *Mesechites* included, *M. mansoana*, grouped with *Allomarkgrafia* rather than with the other species of *Mesechites*, suggesting that *Mesechites* may not be a monophyletic genus. In order to test monophyly of *Mesechites*, we added one additional species each of *Allomarkgrafia* (*A. plumeriifolia*) and *Mesechites* (*M. trifida*), and the results were consistent with those obtained by Simões *et al.* (2004). Based on the tree topology alone, there are two logical interpretations. One could recognize either the Caribbean species of *Mesechites* (*M. minima* and *M. rosea*), which form a strongly supported clade, as a distinct genus from the continental species of *Mesechites* and *Allomarkgrafia*, or *Mesechites* could be circumscribed so as to include both taxa. Considering morphology as well, however, we concur with Pichon's (1950) circumscription of *Mesechites*, including *Allomarkgrafia* in its synonymy.

Tintinnabularia was supported as a good genus in Simões et al. (2004), as sister to a clade including *Mesechites* and *Allomarkgrafia*. The type species of the genus, *T. mortonii*, which was included in that paper, is distinguished from all other genera in Mesechiteae by its long staminal filaments and conspicuous hairy anther apical appendages. In 1996 a new species, *T. gratissima*, was described by Morales, which lacked both key characters. Especially because it deviated from the original generic circumscription, we decided to include *T. gratissima* in our taxon sampling. Despite their morphological differences, they form a strongly supported clade (BS = 100%) in this study and therefore *Tintinnabularia* should be maintained as a valid genus. Within the Mesechites clade, two morphological synapomorphies define *Tintinnabularia*: presence of domatia on abaxial leaf surface and lower corolla tubes which are more than three times shorter than the upper tube (for additional information on corolla tube divisions, see Simões and Kinoshita 2002).

MORPHOLOGY AND EVOLUTION OF CHARACTERS

Addition of morphological characters to the molecular combined data set caused no increase in bootstrap support for any of the observed clades. Some of the terminal clades, on the contrary, showed a decrease in bootstrap values when morphological characters were considered, in some cases with minor changes in tree topology (e.g., the relationship between *Allomarkgrafia* and *Mesechites* in the *Mesechites* clade). Comparison of parsimony-tree parameters between the molecular combined and total evidence data sets showed a decrease in CI and RI values and a significant increase in tree length with the addition of morphological characters (Table 4). This illustrates the extreme variation in morphology within *Mandevilla* and related genera and the difficulties in selecting and coding characters, which explains at least in part the problems faced by taxonomists to provide a reliable classification for this group.

From the 32 morphological characters used in our analyses, some proved to be phylogenetically reliable and constitute interesting topics for discussion of character evolution in Mesechiteae. Simões *et al.* (2004) discussed in detail some of these characters, especially the ones defining the tribe Mesechiteae and the *Mesechites* clade, and therefore they will not be discussed here. Next we discuss in details six of the "useful" morphological characters within *Mandevilla* found in our study. Further we also discuss characters

previously used to define taxonomic groups that are discordant with our phylogenetic hypotheses. Some selected characters were traced in the *Mandevilla* clade, in order to illustrate possible trends in character evolution in *Mandevilla*, and some examples of erroneous statements provided by the current classification (Figs. 3.6-9).

The arrangement of calycine colleters in relation to the calyx lobes was one of the key characters that Woodson (1933) used to distinguish subgenera *Mandevilla* and *Exothostemon* (a detailed explanation of colleter structure and arrangement is provided in Simões and Kinoshita, 2002). We agree in great part with this classification, since opposite colleters are present only in the clade I, mainly composed by species of *Exothostemon* and alternate or continuous colleters occur in all remaining *Mandevilla*, *Macrosiphonia* and *Telosiphonia* (Fig. 3.6). When coding characters, we decided to consider alternate and continuous arrangement as one single state, since the distinction between these two organizational models was uncertain for some species, like *Mandevilla martiana* and *M. syrinx*. Opposite colleters, on the other hand, are straightforward to identify, with no intermediate states. Colleters with opposite distribution also have the same structural characteristics, with five, largely deltoid colleters sometimes fimbriate at the apex occupying the whole circumference of calyx base. The combination of large and deltoid colleters opposite to the calyx lobes is exclusive from this group of species within *Mandevilla*, attesting the phylogenetic value of this set of characters.

Colleters are also present at the leaf blade, and their arrangement is another important character used to distinguish the two subgenera. Colleters distributed along the entire extension of the midrib were observed only in clade I, except for the species of *Macrosiphonia*, *Mandevilla funiformis* and *Mandevilla sp* (Fig. 3.6). This character state is unique and has never been reported for any other group within Apocynaceae.

Two characters related to the flower nectaries (number and size) were informative and congruent with our phylogenetic results. The number of nectaries (two or five) is a fixed, easily-defined character, with no intermediate states. Tracing these characters in the tree topology (Fig. 3.9) shows that the presence of two nectaries is restricted to one clade, reinforcing the idea that this character carries a strong phylogenetic signal. The sister position of *M. pycnantha*, with five nectaries, to a clade composed of *M. myriophyllum* and *M. tenuifolia*, with two nectaries each, in clade III leads to two equally-parsimonious

hypotheses to explain the evolution of two nectaries in the clade:1) The change from five to two nectaries occurred only once at the base of the clade III, with a reversal to the five nectaries condition in *M. pycnantha* or 2) The change from five to two nectaries occurred twice, once in the clade composed by *M. myriophyllum* and *M. tenuifolia* and again in the clade composed by the remaining species of the clade III. The size of nectaries, a character used by Woodson (1933) to distinguish sections *Tubiflorae* and *Torosae*, is also informative in *Mandevilla*. Even tough none of these two sections proved to be monophyletic in our study, species with nectaries the same size or taller than ovary are only found in one of the subclades of *Mandevilla*, the clade IV, which encompasses all Mexican species of the genus (Fig. 3.9).

The proportion between apical appendages and the main body of style head, a character never used for taxonomic purposes in Apocynaceae, is easily coded and proved to be informative in *Mandevilla*. Appendages with the same size or longer than the main body are restricted to species of one clade of *Mandevilla* (clade IV) with the exception of two subclades within, one formed by *M. karwinskii* and *Telosiphonia*, and the other formed by *M. emarginata*, *M. laxa* and *M. pentlandiana* (Fig. 3.8). At first we believed in a possible correlation between the sizes of flower and style head appendages, with long appendages occurring only in species with small flowers, shorter than 2 cm. Our hypothesis proved wrong, however, since four species with showy flowers larger than 3 cm (*M. convolvulacea*, *M. oaxacana*, *M. glandulosa* and *M. veraguasensis*) also showed long style head appendages. The evolutionary role of elongated style head appendages is unknown, and no further statements can be done at the present.

The form of the anther base also constitutes a useful character in *Mandevilla*. Woodson (1933) used this character as diagnostic for some of his sections, defining section *Montanae* by the truncate anther base, opposed to the emarginate or auriculate anther base (margins with different degrees of expansion, but never straight or sagittate, see Fig. 3.8) of the other sections of subgenus *Mandevilla*. We observed that species with truncate anthers occur only in clade V, which largely corresponds to Woodson's section *Montanae*. However, this character is not exclusive of this section, as explained before (see "Clade II" topic in discussion), and also is not easy to define and code. Three species of the clade V (*M. emarginata*, *M. laxa* and *M. pentlandiana*) also have individuals with slightly

emarginate anther bases. Moreover, the gradation between emarginate and auriculate margins is not clear, and that was one of the main reasons pointed out by Pichon (1948) for not recognizing Woodson's sections of *Mandevilla*. In conclusion, we consider truncate anther base as a reliable character to distinguish species from the clade V, but do not recognize defined states between emarginate to auriculate bases.

In contrast to these "good" characters, some of the morphological characters that have been previously used to define taxonomic groups appear to be phylogenetically unreliable. The most obvious is the corolla shape, a character used by Woodson (1933) to distinguish his section *Laxae* (infundibuliform corollas) from the other sections of subgenus *Mandevilla* (tubular or salverform corollas). Our results show that infundibuliform corollas appear in many different clades within *Mandevilla*, making its use virtually impossible in an infrageneric classification of the genus (Fig. 3.7). Other characters of the flower, like size and colour, are possibly subject to convergent evolution and therefore may not reflect phylogenetic relationships. Habit is also a possible candidate for convergent evolution, since it seems to have a strong correlation with habitat requirements. In *Mandevilla*, life form can be remarkably variable even within species, with some taxa (e.g., *M. martiana*, *M. sancta*) having both twining and erect individuals.

The presence of a curved lower corolla tube was considered by Woodson (1933) as one of the main diagnostic characters of subgenus *Exothostemon*. Indeed we observed that, in our sampled species of *Mandevilla*, curved corolla tubes occur exclusively in the clade I, but this character state cannot be considered diagnostic for the entire subgenus. Three species of subgenus *Exothostemon* (*Mandevilla anceps*, *M. lancifolia* and *M. nerioides*) have straight lower corolla tubes, and three species of subgenus *Mandevilla* (*M. callista*, *M. funiformis* and *M. sp.*) have curved tubes. Other species of *Exothostemon* not sampled in our study, (e.g., *Mandevilla duidae* and *M. turgida*) also have straight corolla tubes, showing that the status of this character must be reviewed in *Mandevilla*. Besides, the observation of a curvature in the lower corolla tube is not straightfoward and dubious for most species, especially when dried flowers from vouchers are the only available source of information.

All species with curved tubes sampled in our study share a set of characters in flower structure: the corolla tube is yellow (white in *M. callista* and *M. rugosa*) turning to

orangish or reddish in the base of the lobes and inner surface of the upper tube, and the staminal filaments are laterally enlarged. The biological significance of this model of flower architecture is uncertain, but a possible relationship with hummingbird pollination can be hypothesized. A curvature in the lower corolla tube force the flower to remain in a horizontal position in the inflorescence, with its reddish center exposed. The enlarged staminal filaments could play a role in preventing any physical damage to the ginostegium structure caused by the pollinator, and could also constrain the access to the nectar at the base of the flower. Reports on hummingbird pollination in flowers of *M. hirsuta* (Linhart & Feinsinger, 1980) reinforce our hypotheses, but the question remains open for future studies in pollination biology of this *Mandevilla* group.

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Table 1. Comparison between Pichon's (1948) sections of *Mandevilla* and its corresponding ranks in Woodson's (1933) classification.

Woodson (1933)	Pichon (1948)
Mandevilla subgenus *Exothostemon*	*Mandevilla* section Exothostemon
Mandevilla subgenus *Mandevilla*	*Mandevilla* section Orthocaulon
genus *Macrosiphonia*	*Mandevilla* section Megasiphon
genus *Telosiphonia*	*Mandevilla* section Telosiphonia

Table 2. List of the sampled taxa of *Macrosiphonia*, *Mandevilla* and *Telosiphonia*, comparing the classification of Woodson (1933) and the clades observed in our study.

TAXON NAME AND CURRENT CLASSIFICATION	THIS STUDY
Genus *Macrosiphonia*	
Macrosiphonia longiflora (Desf.) Müll. Arg.	Clade I
Macrosiphonia martii Müll.Arg.	Clade I
Macrosiphonia velame (A. St.-Hil.) Müll.Arg.	Clade I
Genus *Mandevilla*	
Subgenus *Mandevilla*	
Section Laxae	
Mandevilla atroviolacea (Stadelm.) Woodson	Clade III (in Clade II)
Mandevilla callista Woodson	Clade I
Mandevilla coccinea (Hook. & Arn.) Woodson	Clade III (in Clade II)
Mandevilla convolvulacea (A. DC.) Hemsl.	Clade IV (in Clade II)
Mandevilla duartei Markgr.	Clade III (in Clade II)
Mandevilla fragrans (Stadelm.) Woodson	Clade III (in Clade II)
Mandevilla funiformis (Vell.) K. Schum.	Clade I
Mandevilla glandulosa (Ruiz & Pav.) Woodson	Clade IV (in Clade II)
Mandevilla illustris (Vell.) Woodson	Clade III (in Clade II)
Mandevilla laxa (Ruiz & Pav.) Woodson	Clade IV (in Clade II)
Mandevilla ligustrifolia Woodson	Clade IV (in Clade II)
Mandevilla martiana (Stadelm.) Woodson	Clade III (in Clade II)
Mandevilla moricandiana (A. DC.) Woodson	Clade III (in Clade II)
Mandevilla oaxacana (A. DC.) Hemsl.	Clade IV (in Clade II)
Mandevilla pendula (Ule) Woodson	Clade III (in Clade II)
Mandevilla pohliana (Stadelm.) A.H. Gentry	Clade III (in Clade II)
Mandevilla sancta (Stadelm.) Woodson	Clade III (in Clade II)

Mandevilla sellowii (Müll.Arg.) Woodson	Clade III (in Clade II)
Mandevilla sp.	Clade I
Mandevilla spigeliiflora (Stadelm.) Woodson	Clade III (in Clade II)
Mandevilla splendens (Hook.) Woodson	Clade III (in Clade II)
Mandevilla urophylla (Hook.) Woodson	Clade III (in Clade II)
Mandevilla venulosa (Müll.Arg.) Woodson	Clade III (in Clade II)
Mandevilla veraguasensis (Seem.) Hemsl.	
Section Montanae	
Mandevilla cercophylla Woodson	Clade IV (in Clade II)
Mandevilla emarginata (Vell.) C. Ezcurra	Clade IV (in Clade II)
Mandevilla jamesonii Woodson	Clade IV (in Clade II)
Mandevilla pentlandiana (A. DC.) Woodson	Clade IV (in Clade II)
Mandevilla pycnantha (Steud. ex. A. DC.)	Clade III (in Clade II)
Mandevilla tricolor Woodson	Clade IV (in Clade II)
Section Tenuifoliae	
Mandevilla myriophyllum (Taub.) Woodson	Clade III (in Clade II)
Mandevilla tenuifolia (J.C. Mikan) Woodson	Clade III (in Clade II)
Section Torosae	
Mandevilla foliosa (Müll.Arg.) Hemsl.	Clade IV (in Clade II)
Mandevilla karwinskii (Müll.Arg.) Hemsl.	Clade IV (in Clade II)
Section Tubiflorae	
Mandevilla syrinx Woodson	Clade IV (in Clade II)
Mandevilla tubiflora (M. Martens & Galeotti) Woodson	Clade IV (in Clade II)
Subgenus *Exothostemon*	
Mandevilla anceps Woodson	Clade I
Mandevilla dodsonii A.H. Gentry	Clade I
Mandevilla hirsuta (Rich.) K. Schum.	Clade I
Mandevilla krukowii Woodson	Clade I
Mandevilla lancifolia Woodson	Clade I
Mandevilla leptophylla (A. DC.) K. Schum.	Clade I
Mandevilla nerioides Woodson	Clade I

Mandevilla rugellosa (Vahl) Markgr.	Clade I
Mandevilla rugosa (Benth.) Woodson	Clade I
Mandevilla scabra (Hoffmans. Ex Roem. & Schult.) K. Schum.	Clade I
Mandevilla subsagittata (Ruiz & Pav.) Woodson	Clade I
Genus *Telosiphonia*	
Telosiphonia brachysiphon (Torr.) Henr.	Clade IV (in Clade II)
Telosiphonia hypoleuca (Benth.) Henr.	Clade IV (in Clade II)
Telosiphonia nacalpulensis Felger & Henr.	Clade IV (in Clade II)

Table 3. Primer sequences of the five plastid regions used in this study. Primers especially designed for the two-step amplification are indicated with an asterisk (*)

cpDNA region		Primers	Primer source
rpl16 intron	F71	5'-GCTATGCTTAGTGTGTGACTCGTTG–3'	Baum et al., 1998
	R1516	5'- CCCTTCATTCTTCCTCTATGTTG –3'	Baum et al., 1998
	513F	5'– GGGAACGATGGAAGCTGTGAATGC –3'	Simões et al. (in press)
	542R	5' – CGCGGGCGAATATTTACTCTTC – 3'	Simões et al. (in press)
	*F73	5' – CYCATTACTTCGCATTATCTC – 3'	This study
	*IR582	5' – CGACCAGTGAATCATTAAGAT – 3'	This study
	*IF479	5' – ACAAATTTCATTATGAGCTCC – 3'	This study
	*R1060	5' – GCGAATAAAAGAATTMAAA – 3'	This study
rps16 intron	rpsF	5'- TGGTAGAAAGCAACGTGCGACTT –3'	Oxelman et al., 1997
	rpsR2	5'- TCGGGATCGAACATCAATTGCAAG –3'	Oxelman et al., 1997
	387F	5' – CACCGAAGTAATGCCTAAACC – 3'	Simões et al. (in press)
	497R	5' – GGATTCTKAAGTCTGGCCCAG – 3'	Simões et al. (in press)
	*IF486	5' – WAACTGGGCCAGACTTMAGAA – 3'	This study
	*R768	5' – CGAATAAATTACATAAAAGG – 3'	This study
	*R782	5' – ATGGAATTCGAATAAATTACA – 3'	This study
trnS-G intergenic spacer	trnS	5'- GCCGCTTTAGTCCACTCAGC –3'	Hamilton, 1999
	trnG	5'- GAACGAATCACACTTTTACCAC –3'	Hamilton, 1999
	309F	5' – GATGATTTTTCATTTATMTGA – 3'	Simões et al. (in press)
	527R	5'- GTGCTWAAATATTTCYYATTMAC – 3'	Simões et al. (in press)
*trn*K intron + *mat*K exon	*trn*K 3914F	5' – GGGGTTGCTAACTCAACGG – 3'	Civeyrel and Rowe, 2001
	*mat*K -8F	5' – AATTTCAAATGGAAGAAATC – 3'	Civeyrel and Rowe, 2001
	*mat*K 174R	5' – CGAKTAATTAAMCGTTTCAC – 3'	Civeyrel and Rowe, 2001
	*mat*K 8F	5' – AATTTCAAATGGAAGAAATC – 3'	Civeyrel and Rowe, 2001
	*mat*K 503R	5' – GCATCTTTTACCCAATAGCG – 3'	Civeyrel and Rowe, 2001
	*mat*K 503F	5' – TCGCTATTGGGTAAAAGATGC – 3'	Civeyrel and Rowe, 2001
	*mat*K 681F	5' – GTGAATACGAATCYATTTTC – 3'	Civeyrel and Rowe, 2001
	*mat*K 900F	5' – TGGAAATTTTACCTTGTCAA – 3'	Civeyrel and Rowe, 2001
	*mat*K 1628R	5' – CATGCTACATCAACATTTCAG – 3'	Civeyrel and Rowe, 2001
	*mat*K 1309F	5' – GACTTTCTTGTGCTAGAACT – 3'	Civeyrel and Rowe, 2001
	*trn*K -2R	5' – AACTAGTCGGATGGAGTA – 3'	Civeyrel and Rowe, 2001

Table 4. Summary of sequence lenght, variability and parsimony-tree parameters for individual and combined data sets. Tree length, consistency index (CI) and retention index (RI) were calculated based on parsimony-informative characters only.

	rpl16 intron	*rps16* intron	*trnS-G* intergenic spacer	trnK intron	matK exon	Molecular combined	Morpholog y	Total evidence
Aligned length	1554	931	1275	1110	1554	6344	32	6376
Range of sequence length	908-1092	789-830	437-801	903-1005	1497-1554	-	-	-
No. of coded gaps	212	131	177	20	6	546	-	546
No. of characters excluded (nucleotides + gaps)	62	21	63	19	-	165	-	165
Total no. of parsimony-informative characters (percentage of total number of characters)	242 (13,7%)	127 (12%)	127 (8,8%)	149 (13,3%)	216 (13,9%)	858 (12,5%)	32(100%)	893 (12,9%)
Tree length	460	283	305	273	430	1653	117	1796
CI	0.521	0.551	0.475	0,667	0,644	0,619	0	0.594
RI	0.777	0.825	0.756	0,862	0,865	0.848	0	0.836

FIGURE LEGENDS

Fig. 3.1. Strict consensus of the most parsimonious trees generated by the rpl16 intron and rps16 intron data sets. Bootstrap values >50% are indicated above the branches. Full taxon names are given in Appendix 1.

Fig. 3.2. Strict consensus of the most parsimonious trees generated by the trnK intron and matK exon data sets. Bootstrap values >50% are indicated above the branches. Full taxon names are given in Appendix 1.

Fig. 3.3. Strict consensus of the most parsimonious trees generated by the trnS-G intergenic spacer and morphological data sets. Bootstrap values >50% are indicated above the branches. Full taxon names are given in Appendix 1.

Fig. 3.4. Strict consensus of the most parsimonious trees generated by the molecular combined data set. Bootstrap values >50% are indicated above the branches. Full taxon names are given in Appendix 1.

Fig. 3.5. Strict consensus of the most parsimonious trees generated by the total evidence data set. Bootstrap values >50% are indicated above the branches. The six subclades within the Mandevilla clade are indicated as I, II, III, IV, V, and VI. Full taxon names are given in Appendix 1.

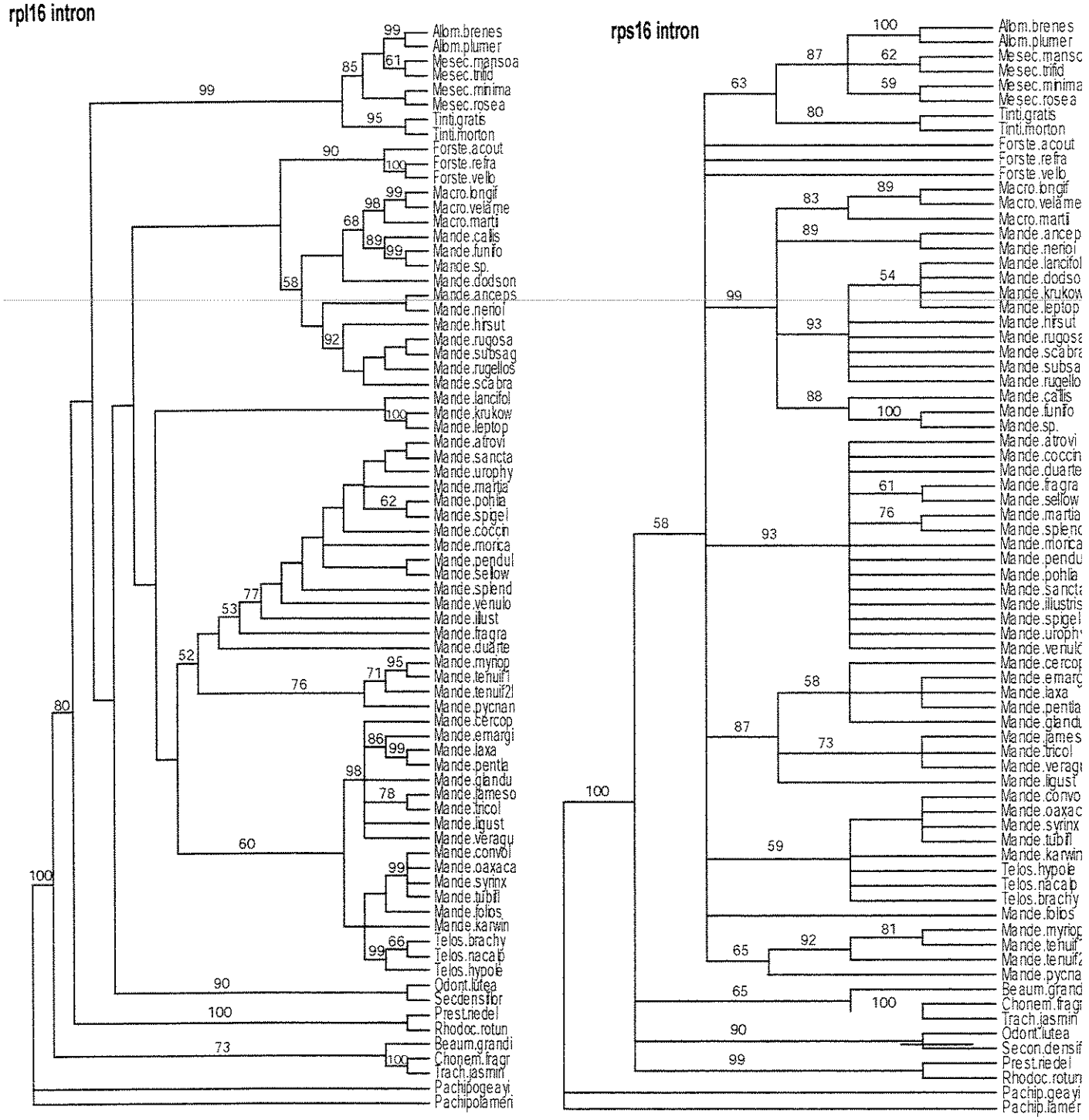
Fig. 3.6. One of the most parsimonious trees generated by the total evidence data set tracing the distribution of two morphological characters into the tree topology: leaf colleter position (left) and the arrangement of calycine colleters (right). Only the *Mandevilla* clade is illustrated. Full taxon names are given in Appendix 1.

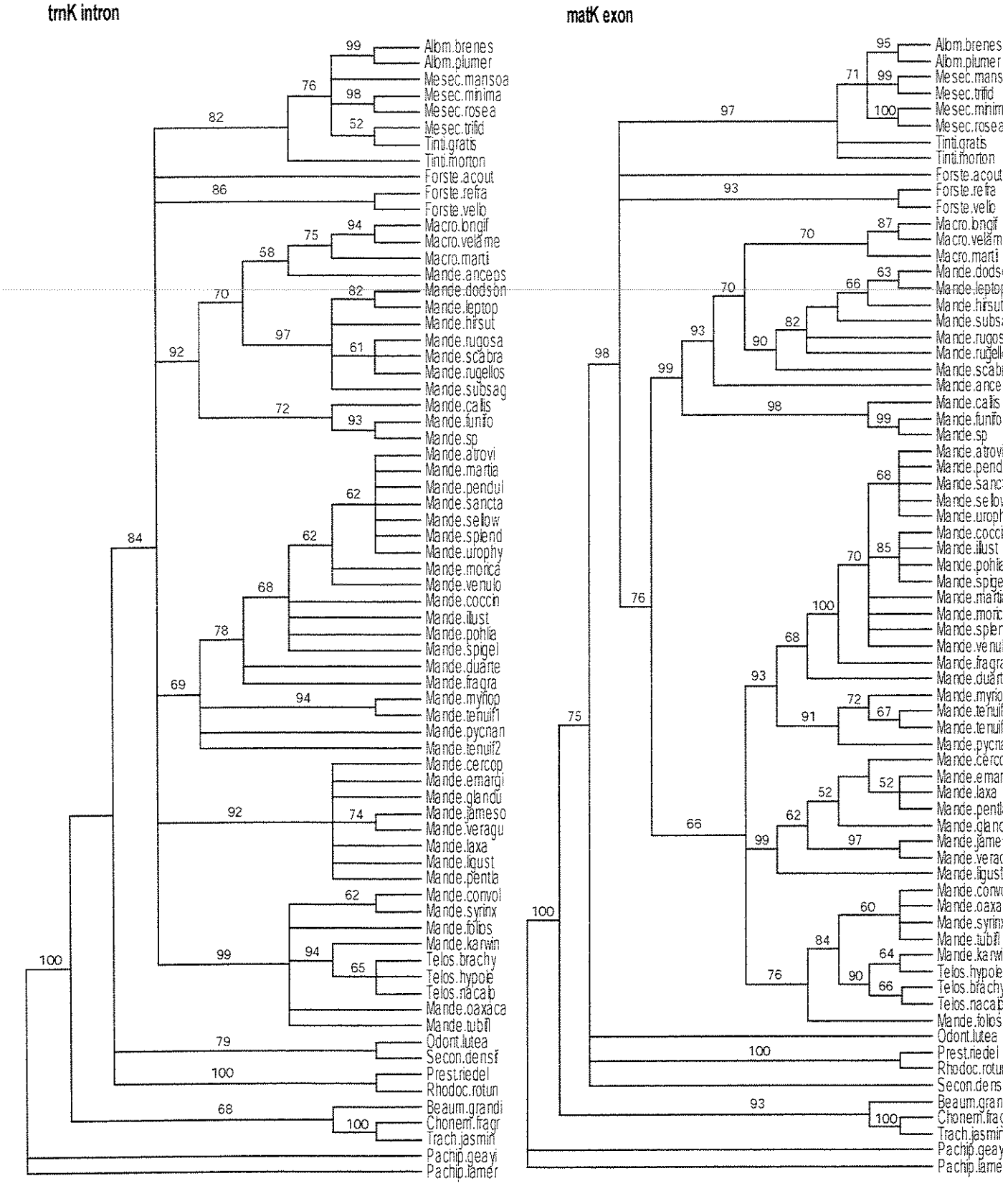
Fig. 3.7. One of the most parsimonious trees generated by the total evidence data set tracing the distribution of two morphological characters into the tree topology: corolla shape (left)

and the form of the lower corolla tube (right). Only the *Mandevilla* clade is illustrated. Full taxon names are given in Appendix 1.

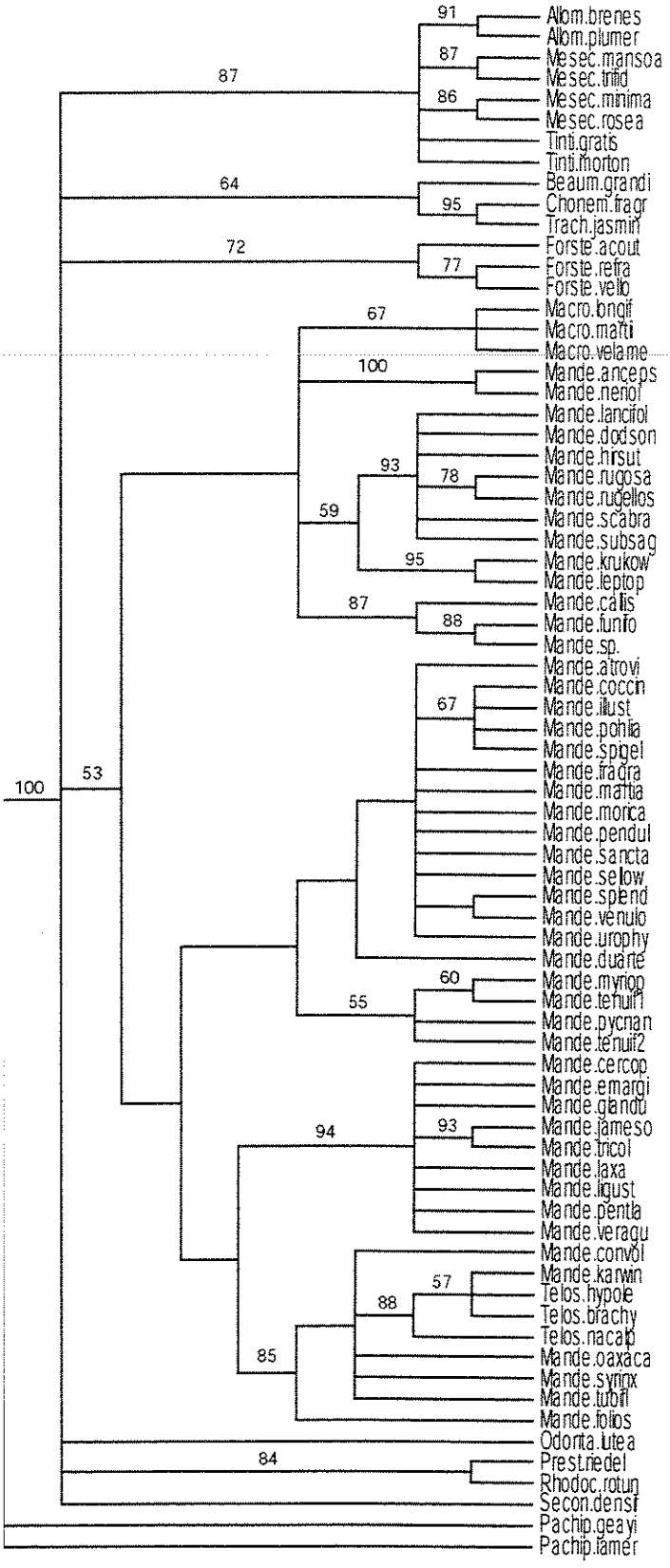
Fig. 3.8. One of the most parsimonious trees generated by the total evidence data set tracing the distribution of two morphological characters into the tree topology: form of the anter base (left) and the proportion between the apical appendages and main body of the style head (right). Only the *Mandevilla* clade is illustrated. Full taxon names are given in Appendix 1.

Fig. 3.9. One of the most parsimonious trees generated by the total evidence data set tracing the distribution of two morphological characters into the tree topology: nectaries number (left) and nectaries height (right). Only the *Mandevilla* clade is illustrated. Full taxon names are given in Appendix 1.

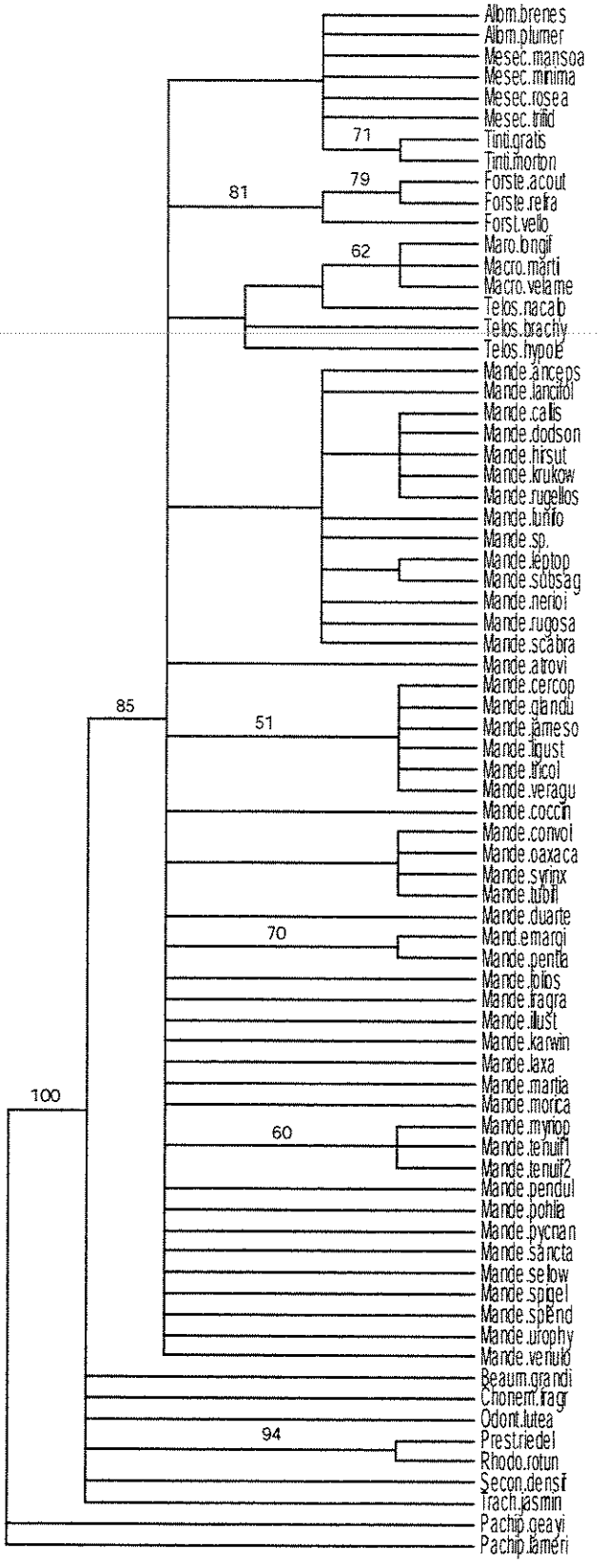

rpl16 intron
Alom.brenes
Alom.plumer
Mesec.mansoa
Mesec.trifid
Mesec.minima
Mesec.rosea
Tinti.gratis
Tinti.morton
Forste.acout
Forste.refra
Forste.vello
Macro.longif
Macro.velame
Macro.marti
Mande.callis
Mande.fumifo
Mande.sp.
Mande.dodson
Mande.anceps
Mande.neriol
Mande.hirsut
Mande.rugosa
Mande.subsaq
Mande.rugellos
Mande.scabra
Mande.lancifol
Mande.krukow
Mande.leptop
Mande.atrovi
Mande.sancta
Mande.urophy
Mande.martia
Mande.pohlia
Mande.spigel
Mande.coccin
Mande.monica
Mande.pendul
Mande.sellow
Mande.splend
Mande.venulo
Mande.illust
Mande.fragra
Mande.duarte
Mande.myriop
Mande.tenuif1
Mande.tenuif2l
Mande.pycnan
Mande.cercop
Mande.emargi
Mande.laxa
Mande.pentia
Mande.glandu
Mande.jameso
Mande.tricol
Mande.ligust
Mande.veraqu
Mande.convol
Mande.oaxaca
Mande.synnx
Mande.tubifl
Mande.folios
Mande.karwin
Telos.brachy
Telos.nacalp
Telos.hypole
Odont.lutea
Secdensflor
Prest.riedel
Rhodoc.rotun
Beaum.grandi
Chonem.fragr
Trach.jasmin
Pachipogeayi
Pachipolameri
rps16 intron
Alom.brenes
Alom.plumer
Mesec.mansc
Mesec.trifid
Mesec.minima
Mesec.rosea
Tinti.gratis
Tinti.morton
Forste.acout
Forste.refra
Forste.vello
Macro.longif
Macro.velame
Macro.marti
Mande.ancep
Mande.neriol
Mande.lancifol
Mande.dodso
Mande.krukow
Mande.leptop
Mande.hirsut
Mande.rugosa
Mande.scabra
Mande.subsa
Mande.rugello
Mande.callis
Mande.fumifo
Mande.sp.
Mande.atrovi
Mande.coccin
Mande.duarte
Mande.fragra
Mande.sellow
Mande.martia
Mande.splend
Mande.monca
Mande.pendu
Mande.pohlia
Mande.sancta
Mande.illustris
Mande.spigel
Mande.urophy
Mande.venulo
Mande.cercop
Mande.emargi
Mande.laxa
Mande.pentia
Mande.glandu
Mande.jamesb
Mande.tricol
Mande.veragu
Mande.ligust
Mande.convo
Mande.oaxac
Mande.syrinx
Mande.tubifl
Mande.karwin
Telos.hypole
Telos.nacalp
Telos.brachy
Mande.folios
Mande.myriop
Mande.tenuif1
Mande.tenuif2
Mande.pycna
Beaum.grand
Chonem.fragr
Trach.jasmin
Odont.lutea
Secon.densif
Prest.riedel
Rhodoc.rotun
Pachip.geayi
Pachip.lameri


tmK intron
Albm.brenes
Albm.plumer
Mesec.mansoa
Mesec.minima
Mesec.rosea
Mesec.trifid
Tinti.gratis
Tinti.morton
Forste.acout
Forste.refra
Forste.vello
Macro.longif
Macro.velame
Macro.marti
Mande.anceps
Mande.dodson
Mande.leptop
Mande.hirsut
Mande.rugosa
Mande.scabra
Mande.rugellos
Mande.subsag
Mande.callis
Mande.funifo
Mande.sp
Mande.atrovi
Mande.martia
Mande.pendul
Mande.sancta
Mande.sellow
Mande.splend
Mande.urophy
Mande.monca
Mande.venulo
Mande.coccin
Mande.illust
Mande.pohlia
Mande.spigel
Mande.duarte
Mande.fragra
Mande.myriop
Mande.tenuif1
Mande.pycnan
Mande.tenuif2
Mande.cercop
Mande.emargi
Mande.glandu
Mande.jameso
Mande.veragu
Mande.laxa
Mande.ligust
Mande.pentla
Mande.convol
Mande.syrinx
Mande.folios
Mande.kanwin
Telos.brachy
Telos.hypole
Telos.nacalp
Mande.oaxaca
Mande.tubifl
Odont.lutea
Secon.densif
Prest.riedel
Rhodoc.rotun
Beaum.grandi
Chonem.fragr
Trach.jasmin
Pachip.geayi
Pachip.lamer
matK exon
Albm.brenes
Albm.plumer
Mesec.mansoa
Mesec.trifid
Mesec.minima
Mesec.rosea
Tinti.gratis
Tinti.morton
Forste.acout
Forste.refra
Forste.vello
Macro.longif
Macro.velame
Macro.marti
Mande.dodson
Mande.leptop
Mande.hirsut
Mande.subsa
Mande.rugosa
Mande.rugell
Mande.scabra
Mande.ancep
Mande.callis
Mande.funifo
Mande.sp
Mande.atrovi
Mande.pendul
Mande.sancta
Mande.sellow
Mande.urophy
Mande.cocci
Mande.illust
Mande.pohlia
Mande.spigel
Mande.martia
Mande.monca
Mande.splend
Mande.venulo
Mande.fragra
Mande.duarte
Mande.myriop
Mande.tenuif
Mande.tenuif
Mande.pycna
Mande.cercop
Mande.emarg
Mande.laxa
Mande.pentla
Mande.glandu
Mande.jameso
Mande.veragu
Mande.ligust
Mande.convol
Mande.oaxaca
Mande.syrinx
Mande.tubifl
Mande.kanwin
Telos.hypole
Telos.brachy
Telos.nacalp
Mande.folios
Odont.lutea
Prest.riedel
Rhodoc.rotun
Secon.densif
Beaum.grandi
Chonem.fragr
Trach.jasmin
Pachip.geayi
Pachip.lamer

trnS-G intergenic spacer



Morphology



Molecular combined

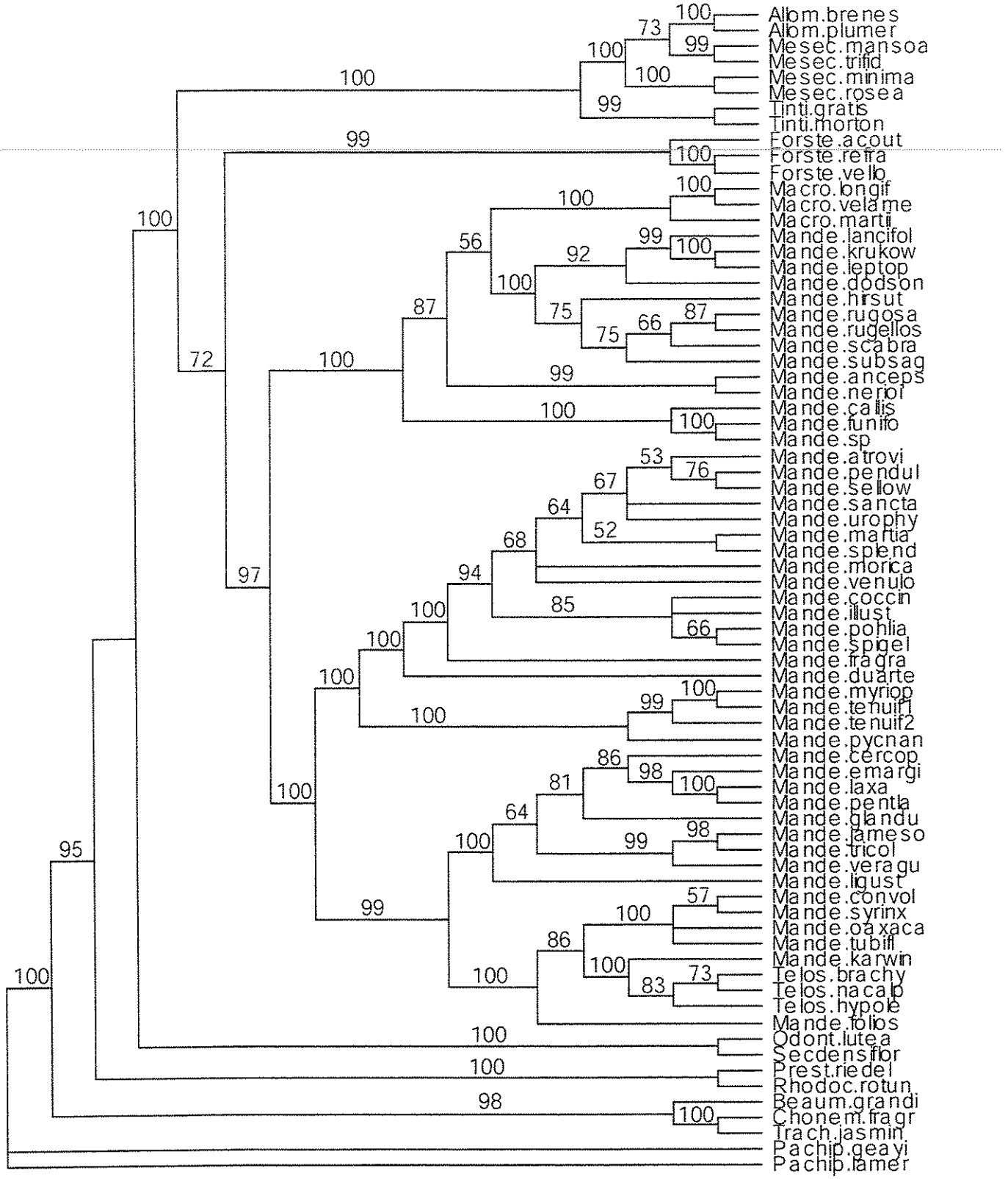


Figure 3.5 Simões et al.

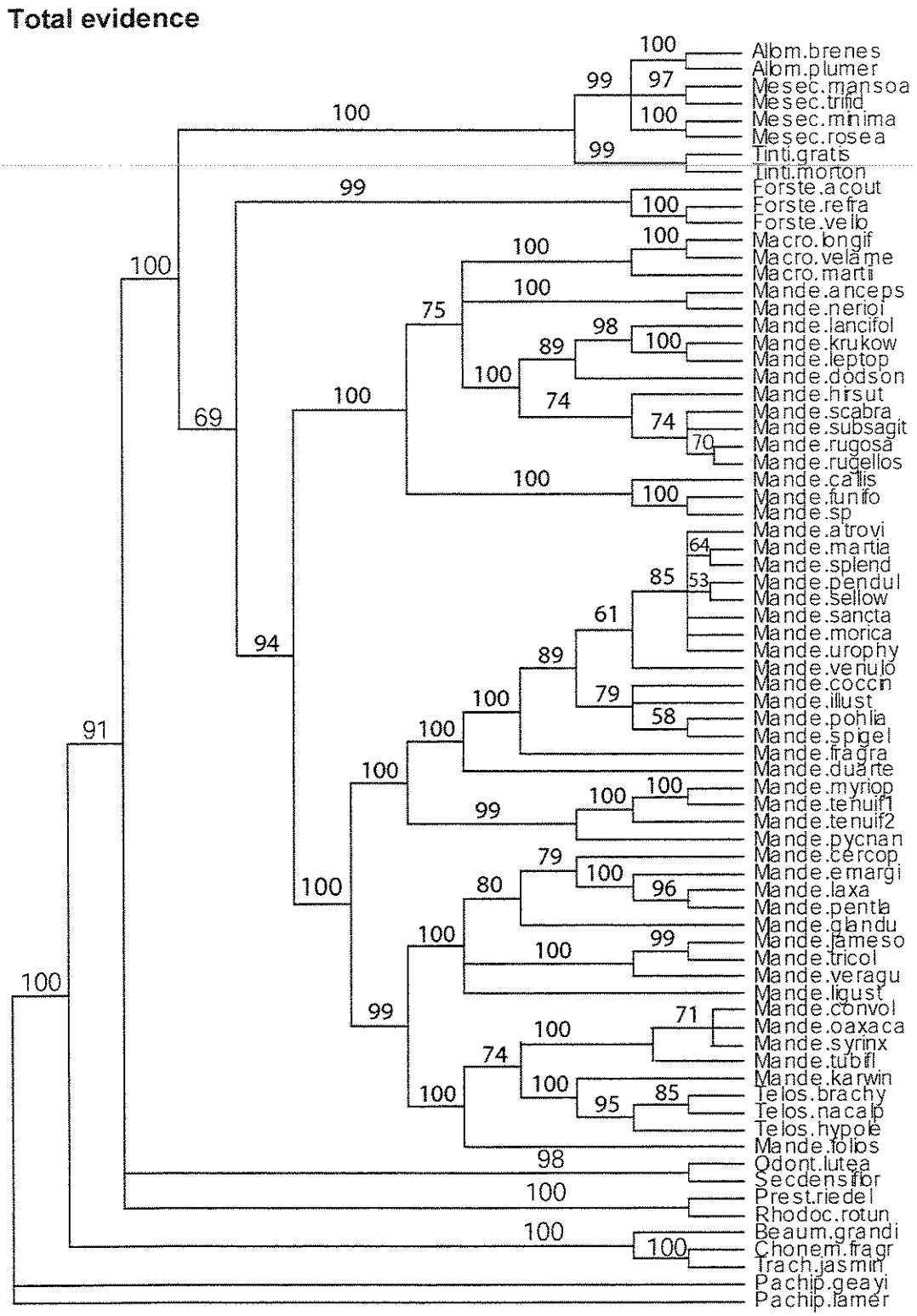


Figure 3.6 Simões et al. - Phylogenetic relationships of *Mandevilla*

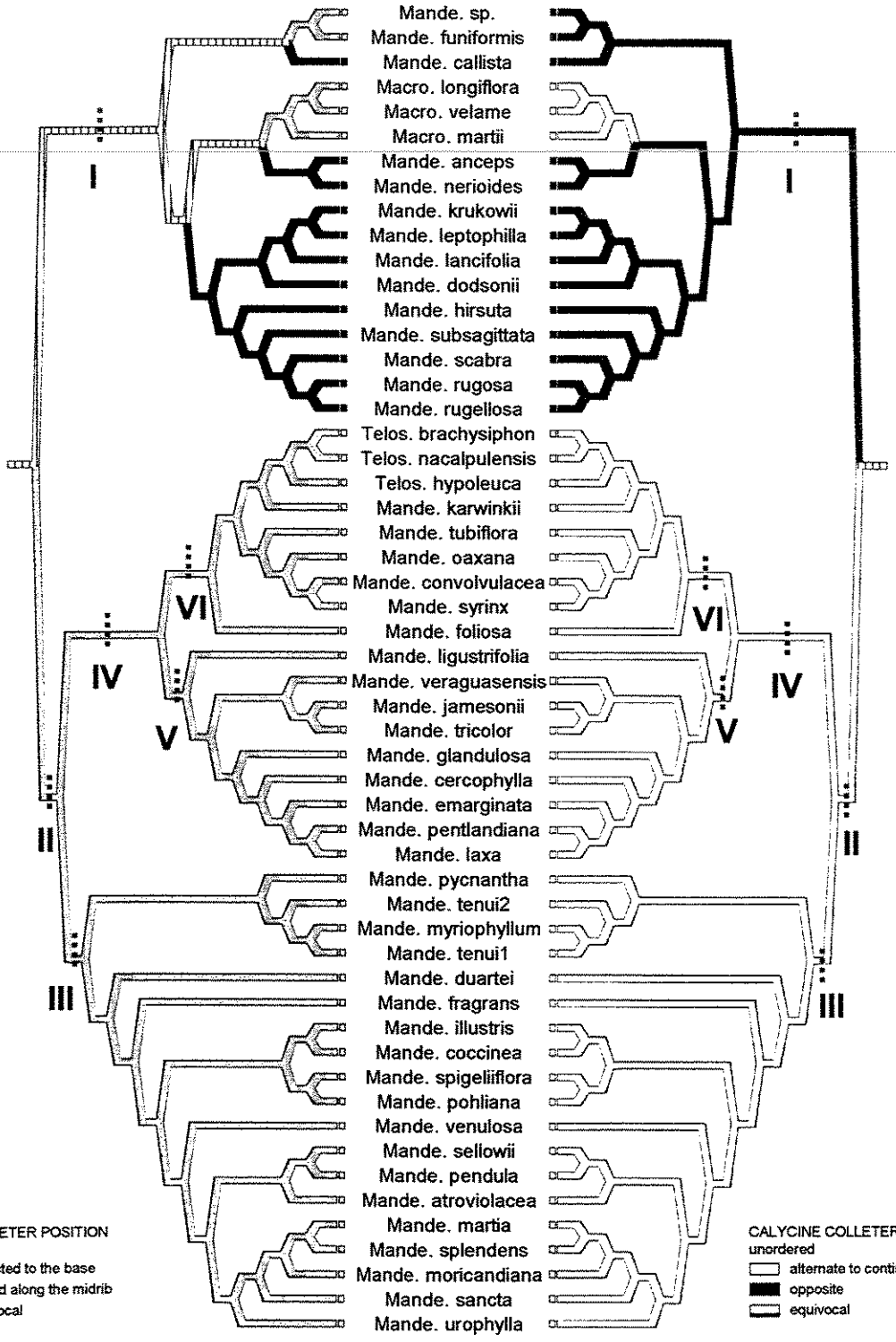


Figure 3.7 Simões et al. - Phylogenetic relationships of *Mandevilla*

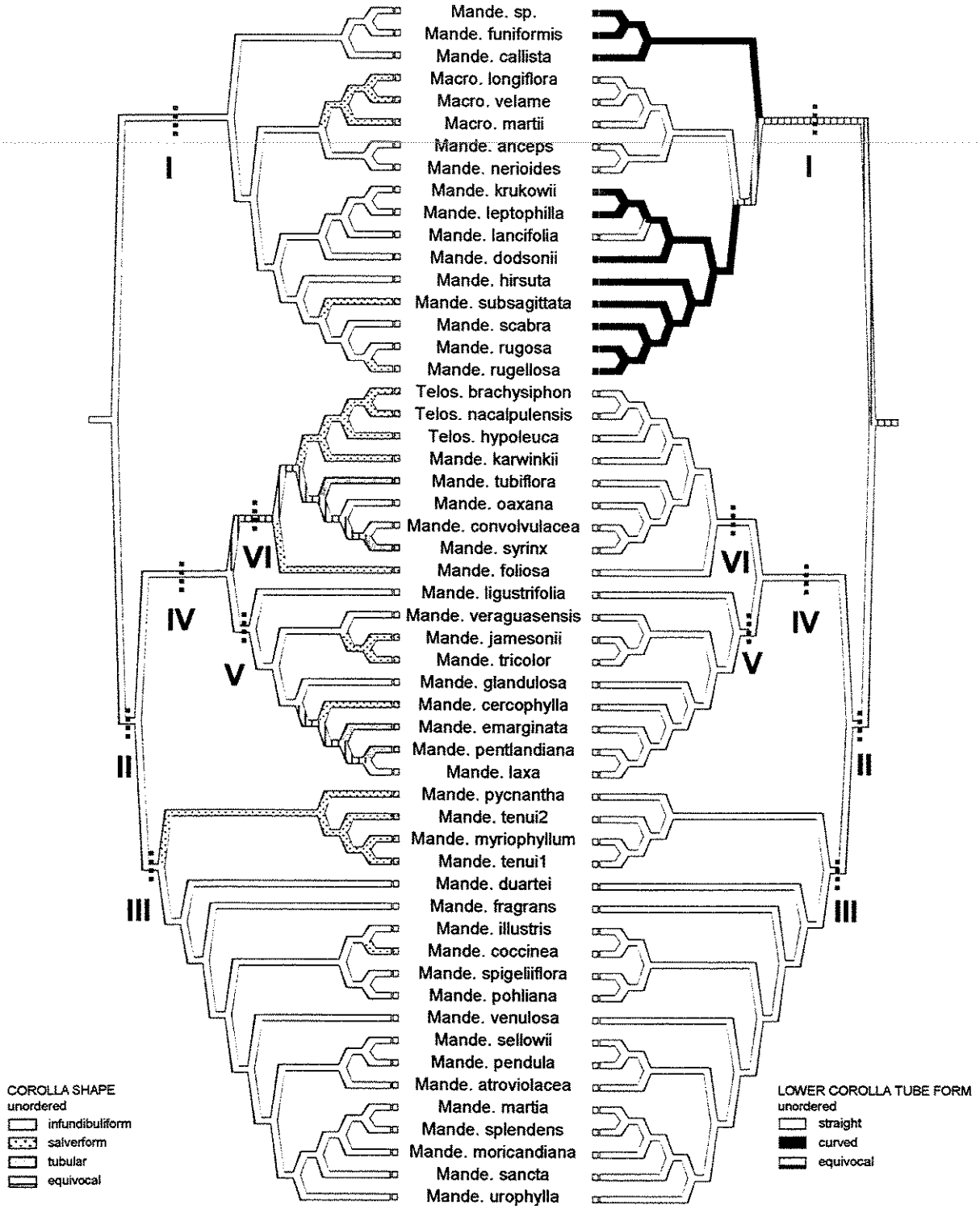


Figure 3.8 Simões et al. - Phylogenetic relationships of *Mandevilla*

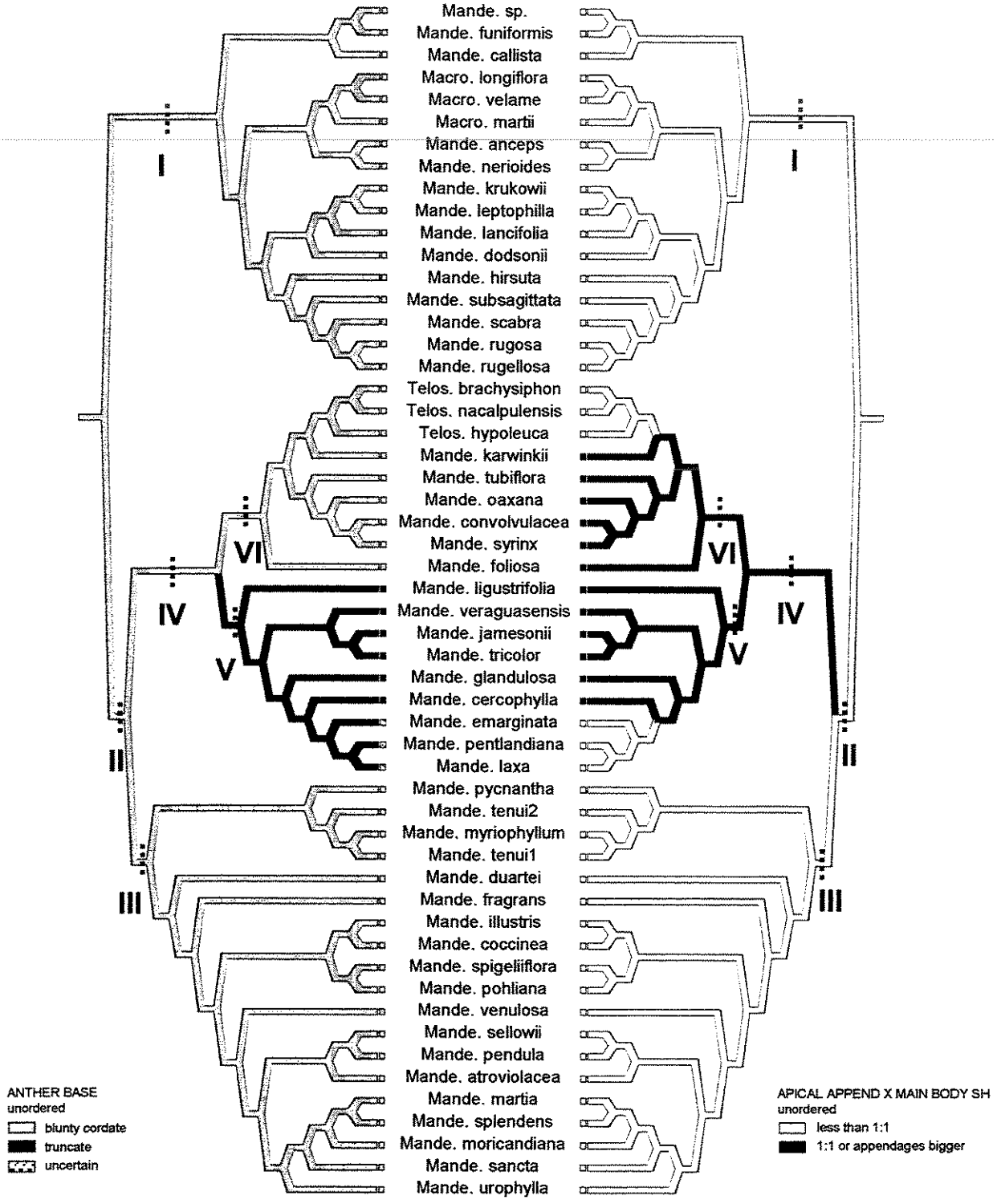
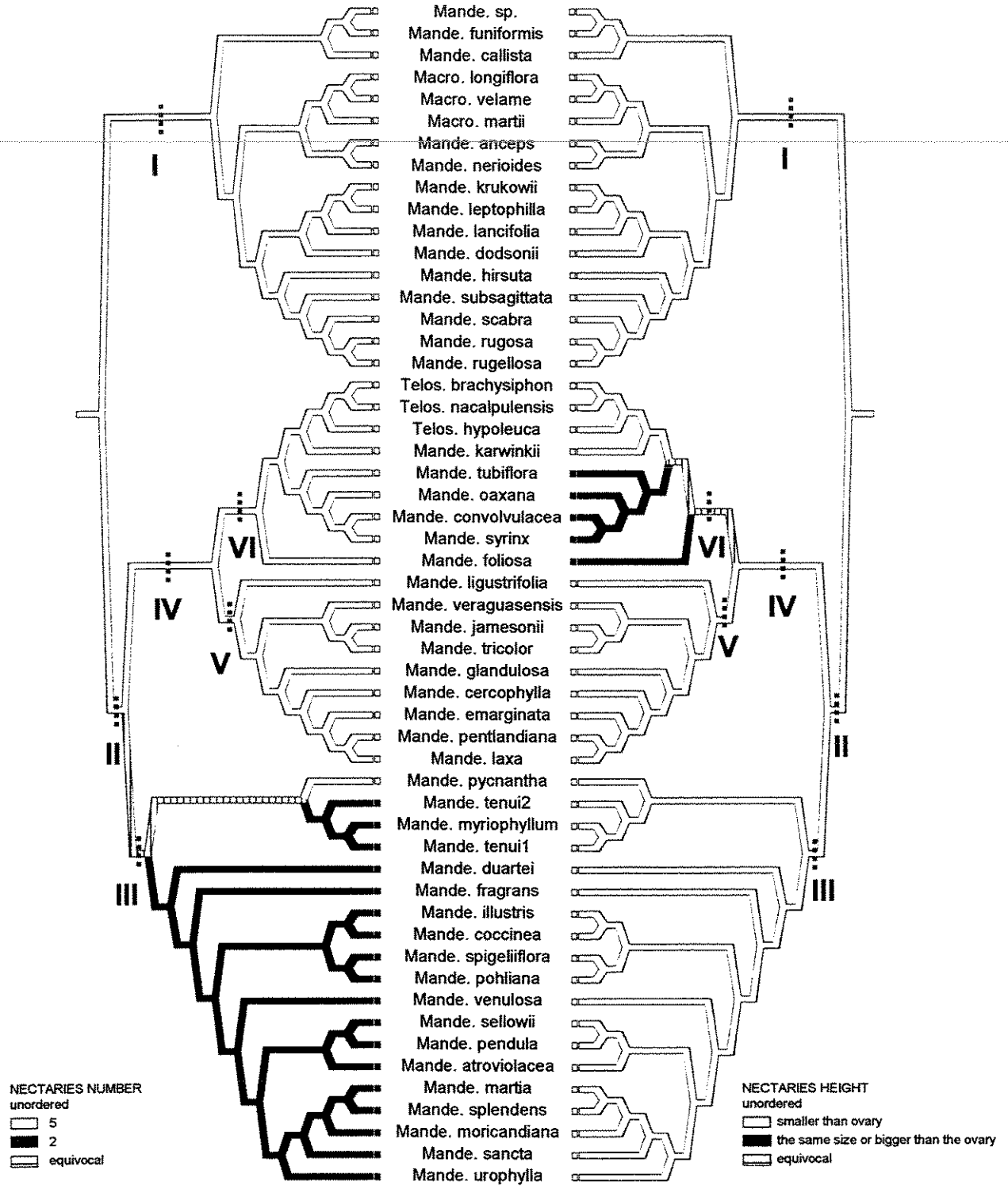


Figure 3.9 Simões et al. - Phylogenetic relationships of *Mandevilla*



Appendix 1. Voucher information and Genbank acession numbers for the taxa used in this paper.

SPECIES	Voucher/Citation	*rpl16* intron	*rps16* intron	*trnS-G* intergenic spacer	*trn*K intron	*mat*K exon
Allomarkgrafia brenesiana Woodson	Costa Rica, M. Endress 97-06 (Z)	AY597546	AY597580	AY597614		
Allomarkgrafia plumeriifolia Woodson	Costa Rica, Morales 9338 (INB)					
Beaumontia grandiflora Wall.	Cult., Bot. Gard. Munich, Gerlach s.n. unvouchered *India, coll. Native collector, s.n. (Z)	AY597547	AY597581	AY597615		
Chonemorpha fragrans (Moon) Alston	Cult., Queensland, Australia, Forster 2009 * India, Ridsale 109 (Z)	AY597548	AY597582	AY597616		
Forsteronia acouci (Aubl.) A. DC.	French Guiana, Prévost 3720 (CAY) * Peru, Revilla 291 (Z); Venezuela, Breteler 5029 (Z)	AY597549	AY597583	AY597617		

Forsteronia refracta Müll. Arg.	Brazil, Kinoshita s.n. (UEC)			
Forsteronia velloziana (A. DC.) Woodson	Brazil, Simões 343 (UEC)	AY597550	AY597584	AY597618
Macrosiphonia longiflora (Desf.) Müll.Arg.	Brazil, Flores s.n. (UEC)	AY597551	AY597585	AY597619
Macrosiphonia martii Müll.Arg.	Brazil, Simões 1245 (UEC)	AY597552	AY597586	AY597620
Macrosiphonia velame (A. St.-Hil.) Müll. Arg.	Brazil, Simões et al. 1199 (UEC)			
Mandevilla anceps Woodson	Venezuela, Hubber & Medina 5793 (Z)			
Mandevilla atroviolacea (Stadelm.) Woodson	Brazil, Meireles 1290 (UEC)			
Mandevilla callista Woodson	Equador, Webster & Castro 31319 (Z)			
Mandevilla cercophylla Woodson	Ecuador, Matezki 420 (Z)			
Mandevilla coccinea (Hook.	Brazil, Flores 452 (UEC)			

& Arn.) Woodson	
Mandevilla convolvulacea (A. DC.) Hemsl.	Mexico, Alvarado 162 (MEXU)
Mandevilla dodsonii A.H. Gentry	Equador, Fallen 875 (Z)
Mandevilla duartei Markgr.	Brazil, Simões 1281 (UEC)
Mandevilla emarginata (Vell.) C. Ezcurra	Brazil, Quast 1 (UEC)
Mandevilla foliosa (Müll.Arg.) Hemsl.	Mexico, Reina 2000-447 (Z)
Mandevilla fragrans (Stadelm.) Woodson	Brazil, Pansarin & Micheliunas 1022 (UEC)
Mandevilla funiformis (Vell.) K. Schum.	Brazil, Simões 1105 (UEC)
Mandevilla glandulosa (Riuz & Pav.) Woodson	Ecuador, Matezki 427 (Z)
Mandevilla hirsuta (Rich.)	Brazil, Simões 1215 (UEC)

K. Schum.				
Mandevilla illustris (Vell.) Woodson	Brazil, Kinoshita sn. (UEC)			
Mandevilla jamesonii Woodson	Ecuador, Jorgensen 1467 (Z)			
Mandevilla karwinskii (Müll.Arg.) Hemsl.	Mexico, Fishbein 2966 (ARIZ)	AY597553	AY596587	AY597621
Mandevilla krukowii Woodson	Brazil, Kirkbride & Lleras 2907 (Z)			
Mandevilla lancifolia Woodson	Venezuela, Davidse & Huber 14887 (Z)			
Mandevilla laxa (Ruiz & Pav.) Woodson	Argentina, Galetto 809 (CORD)			
Mandevilla leptophylla (A. DC.) K. Schum.	Venezuela, Steyermark 119835 (Z)			
Mandevilla ligustrifolia Woodson	Ecuador, Matezki 340 (Z)	AY597554	AY596588	AY597622
Mandevilla martiana (Stadelm.) Woodson	Brazil, Simões & Pansarin 1100 (UEC)			
Mandevilla	Brazil, Simões			

moricandiana (A. DC.) Woodson	et al. 1130 (UEC)			
Mandevilla myriophyllum (Taub.) Woodson	Brazil, Pansarin 878 (UEC)	AY597555	AY596589	AY597623
Mandevilla nerioides Woodson	Colombia, Franco 618 (Z)			
Mandevilla oaxacana (A. DC.) Hemsl.	Mexico, Alvarado 190 (MEXU)			
Mandevilla pendula (Ule) Woodson	Brazil, Ribeiro 2520 (UEC)			
Mandevilla pentlandiana (A. DC.) Woodson	Brazil, Simões 1272 (UEC)			
Mandevilla pohliana (Stadelm.) A.H. Gentry	Brazil, Kinoshita sn. (UEC)			
Mandevilla pycnantha (Steud. ex A. DC.) Woodson	Brazil, Yamamoto s.n. (UEC)	AY597556	AY596580	AY597625
Mandevilla rugellosa (Vahl) Markgr.	French Guiana, Prévot 3720 (CAY) * Suriname,	AY597561	AY597595	AY597629

	Lindeman 1976 (Z)			
Mandevilla rugosa (Benth.) Woodson	Brazil, Simões 1204 (UEC)	AY597557	AY597591	AY597625
Mandevilla sancta (Stadelm.) Woodson	Brazil, Simões 1060 (UEC)			
Mandevilla scabra (Hoffmans. Ex Roem. & Schult.) K. Schum.	Brazil, Simões 1126 (UEC)	AY597558	AY597592	AY597626
Mandevilla sellowii (Müll.Arg.) Woodson	Brazil, Ribeiro sn. (UEC)			
Mandevilla sp.	Brazil, Simões 1303 (UEC)	AY597559	AY597593	AY597627
Mandevilla spigeliiflora (Stadelm.) Woodson	Brazil, Gomes 513 (UEC)			
Mandevilla splendens (Hook.) Woodson	Brazil, Simões 1268 (UEC)	AY597560	AY597594	AY597628
Mandevilla subsagittata (Riuz & Pav.)	Mexico, Alvarado 288 (MEXU)			

Woodson				
Mandevilla syrinx Woodson	Mexico, Calzada 21305 (MEXU)			
Mandevilla tenuifolia1 (J.C. Mikan) Woodson	Brazil, Simões 1171 (UEC)	AY597562	AY597596	AY597630
Mandevilla tenuifolia2 (J.C. Mikan) Woodson	Brazil, Kinoshita s.n. (UEC)	AY597563	AY597597	AY597631
Mandevilla tricolor Woodson	Equador, Jorgensen 1484 (Z)			
Mandevilla tubiflora (M. Martens & Galeotti) Woodson	Mexico, Alvarado 106 (MEXU)			
Mandevilla urophylla (Hook.) Woodson	Brazil, M.P. Quast 6 (UEC)			
Mandevilla venulosa (Müll.Arg.) Woodson	Brazil, Simões 1107 (UEC)	AY597564	AY597598	AY597632
Mandevilla veraguasensis (Seem.) Hemsl.	Costa Rica, Endress 97-76 (Z)	AY597565	AY597599	AY597633
Mascarenhasia	Madagascar,	AY597566	AY597600	AY597634

lisianthiflora A. DC.	Schönenberger A147 (UPS) * Madagascar, Schlieben 8242 (Z)			
Mesechites mansoana (A. DC.) Woodson	Brazil, Simões 1087 (UEC)	AY597567	AY597601	AY597635
Mesechites minima (Britton & P. Wilson) Woodson	Cuba, Nilsson s.n. (Z)	AY597568	AY597602	AY597636
Mesechites rosea (A. DC.) Miers	Cuba, Nilsson s.n. (Z)	AY597569	AY597603	AY597637
Mesechites trifida (Jacq.) Müll.Arg.	Equador, Liede & Meve 3471 (?)			
Odontadenia lutea (Vell.) Markgr.	Brazil, Kinoshita 2002/56 (UEC)	AY597570	AY597604	AY597638
Pachypodium geayi Costantin & Bois	cultivated, Bot. Gard. Chèvreloup, Lieberherr s.n., unvouchered	AY597571	AY597605	AY597640
Pachypodium lamerei Drake	Cultivated, Zürich Bot. Gart., unvouchered	AY597572	AY597606	AY597639
Prestonia riedelii	Brazil, Simões 1274 (UEC)	AY597573	AY597607	AY597641

(Müll.Arg.) Markgr.				
Rhodocalyx rotundifolius Müll.Arg.	Brazil, Kinoshita 2000/66 (UEC)	AY597574	AY597608	AY597642
Secondatia densiflora A. DC.	Brazil, Simões 1218 (UEC)	AY597575	AY597609	AY597643
Telosiphonia brachysiphon (Torr.) Henr.	USA, Jenkins 00-185 (TUC) * USA, Worthington 25068 (TEX)	AY597576	AY597610	AY597644
Telosiphonia hypoleuca (Benth.) Henr.	**Mexico, Reinag 2000-362 (Z)** * Mexico, Richardson 1526 (TEX)	AY597579	AY597611	AY597645
Telosiphonia nacalpulensis Felger & Henr.	USA, Arizona			
Tintinnabularia gratissima J.F. Morales	Mexico, Ventura 107 (Herbario de la Escuela de Ciencias Biologicas, Inst. Politecnico Nacional, Mexico)			
Tintinnabularia	Mexico,	AY597578	AY597612	AY597646

mortonii Woodson	Breedlove 34900 (TEX)			
Trachelospermum jasminioides (Lindl.) Lem.	Cultivated, Zürich Bot. Gard., unvouchered	AY597577	AY597613	AY597647

Appendix 2. Morphological matrix. ? = missing data.

Taxon	Character states
Allomarkgrafia brenesiana	10000111000001000001101032100010
Allomarkgrafia plumeriifolia	10000111000001000001101032100010
Beaumontia grandiflora	10010100000101000000102021000001
Chonemorpha fragrans	10020100000001000000101021000000
Forsteronia acouci	10000111010001030011101{23}2200001
Forsteronia refracta	10000111010001030011101{23}2200001
Forsteronia velloziana	10000111000001030011101{23}2200001
Macrosiphonia longiflora	20000111101011010001101032200000
Macrisophonia martii	20000111101011010001101032200000
Macrosiphonia velame	20000111101011010001101032200000
Mandevilla anceps	20100112001001100001101132200000
Mandevilla atroviolacea	10000111001001000001101032200100
Mandevilla callista	10000112001101100101101132200000
Mandevilla cercophylla	10000111011001010002101032201000
Mandevilla coccínea	20000111001001010001101032200100
Mandevilla convolvulacea	10000111001001000001101032201011
Mandevilla dodsonii	10000112001101100101101132200000
Mandevilla duartei	20000111001001000001101032200100
Mandevilla emarginata	20000111001101020000{12}101032200000
Mandevilla foliosa	20000111001001010001101032201010
Mandevilla fragrans	10000111001001000001101032200100
Mandevilla funiformis	10000111001001100101101132200000
Mandevilla glandulosa	10000111001001000002101032201000
Mandevilla hirsuta	100?011200110110010110113220000l
Mandevilla illustris	20020111001001000001101032200100
Mandevilla jamesonii	10000111001001010002101032201000
Mandevilla karwinskii	20000111001001010001101032201000
Mandevilla krukowii	10000112001101100101101132200000
Mandevilla lancifolia	{12}0100{12}120010011000011010322000000
Mandevilla laxa	10021111001001000000{12}101032200000
Mandevilla leptophylla	10021112001001100101101132200000
Mandevilla ligustrifolia	10000111011001000002101032201000
Mandevilla martiana	{12}00211110010010000011010322001000
Mandevilla moricandiana	10021111001001000001101032200100
Mandevilla myriophyllum	20020111001001010001101032200100
Mandevilla nerioides	20100112001001100001101?32200000
Mandevilla oaxacana	10000111001001000001101032201011
Mandevilla pendula	10000111001001000001101032200100
Mandevilla pentlandiana	{12}0000111001101020000{12}101032200000
Mandevilla pohliana	20020111001001000001101032200100
Mandevilla pycnantha	20000111001001010001101032200000
Mandevilla rugosa	10000112001001100101101132200000

Mandevilla rugellosa	10000112001101110101101132200000
Mandevilla sancta	{12}00211110010010000011010322001 00
Mandevilla scabra	10000112001001100101101132200000
Mandevilla sellowii	10021111001001000001101032200100
Mandevilla sp.	20000111001001100101101132200000
Mandevilla spigeliiflora	20000111001001000001101032200100
Mandevilla splendens	10021111001001000001101132200101
Mandevilla subsagittata	10020112001001110101101?32200000
Mandevilla syrinx	10000111001001020001101032201011
Mandevilla tenuifolia 1	20020111001001010001101032200100
Mandevilla tenuifolia 2	20020111001001010001101032200100
Mandevilla tricolor	10000111001001010002101032201000
Mandevilla tubiflora	10000111001001020001101032201010
Mandevilla urophylla	10021111001001000001101032200100
Mandevilla venulosa	20000111001001000001101032200100
Mandevilla veraguasensis	10000111001001000002102032201000
Mesechites mansoana	10000111000001010001101032100010
Mesechites mínima	1000011100?001010001101032100010
Mesechites rosea	1000011100?001010001101032100010
Mesechites trifida	10000111000001010001101032100010
Odontadenia lútea	10020100000001000000111021000000
Pachipodium geayi	000-0000000000-00010000-00000000
Pachipodium lamerei	000-0000000000-00000000-00000000
Prestonia riedelii	11010100001101111000111010010000
Rhodocalyx rotundifolius	21000100000101111000101010010010
Secondatia densiflora	10020100000001010000111021000000
Telosiphonia brachysiphon	20000111101001010001101032200000
Telosiphonia hypoleuca	20000111101001010001101032200000
Telosiphonia nacalpulensis	20000111101001010001101032200000
Tintinnabularia gratissima	1000011101?001000001101032100010
Tintinnabularia mortonii	1000011101?101000001102032100010
Trachelospermum jasminioides	10020100000001010000101021000000

Appendix 3- Characters and states for the morphological matrix used in the cladistic analyses

1 **Habit**: 0 – trees; 1 – lianas or vines; 2 – erect shrubs or subshrubs, these often with a xylopod.

2 **Latex**: 0 – white; 1 – translucent.

3 **Stem in cross section:** 0 – circular; 1 – pentagonal.

4 **Nodal colleters**: 0 – interpetiolar; 1 –intrapetiolar; 2 – continuous.

5 **Spiny ring of nodal colleters:** 0 – absent; 1 –present. In some species of *Mandevilla*, the nodal colleters are greatly expanded and form a somewhast spiny "crown" around the nodes.

6 **Phyllotaxis**: 0 – alternate; 1 – opposite.

7 **Leaf colleters**: 0 – absent; 1 – present.

8 **Leaf colleter position**: 0 – clustered at the base of the leaf blade adaxially; 1 – spread along the midrib of the leaf blade adaxially.

9 **Abaxial leaf surface**: 0 – thick indument of white wooly trichomes absent; 1 – thick indument of white wooly trichomes present

10 **Domatia**: 0 – absent; 1 – present.

11 **Inflorescence type**: 0 – branched (cymose). 1 – Unbranched (racemose).

12 **Bracts**: 0 – scarious; 1 – petaloid

13 **Pecidel**: 0 – present; 1 – abscent

14 **Calycine colleters**: 0 – absent; 1 – present.

15 **Calycine colleter arrangement**: 0 – alternate to continuous; 1 – opposite.

16 **Corolla shape**: 0 – infundibuliform or campanulate to tubular-campanulate; 1 – salverform; 2 – tubular; 3 – rotate

17 **Annular corona**: 0 – absent; 1 – present.

18 **Form of the lower corolla tube**: 0 – straight; 1 – curved.

19 **Stamens**: 0 – completely included; 1- tips of the anthers exserted; stamens ± completely exserted.

20 **Anther base**: 0 – strongly sagittate; 1 – cordate; 2- truncate.

21 **Anther guide-rails**: 0 – composed mainly of endothecial thickenings; 1 –composed mainly of sclerenchyma.

22 **Dorsal side of anthers:** 0 – completely glabrous; 1 – with trichomes

23 **Filament length**: 0 – anthers ± sessile; 1 – less than 1 cm long; 2 – greater than 3 cm long.

24 **Junction of filament and anther connection**: 0 – flat; 1 – with a globose swelling

25 **Anther/style-head union:** 0 – anthers attached by a circular patch of trichome-like cells; 1 – anthers attached by a horseshoe-shaped rim of hairs; 2 – anthers attached by a horseshoe-shaped rim of hairs and a narrow longitudinal strip; 3 – anthers attached by cellular fusion.

26 **Style-head shape in cross section**: 0 – circular or subcircular; 1 – pentagonal; 2 – with five strongly projecting ribs.

27 **Style-head ribs**: 0 – absent; 1 - restricted to the base; 2 – along the entire length of the body of the style-head.

28 **Collar or wreath at base of style-head**: 0 – absent; 1 – present.

29 **Proportion between the apical appendages and main body of the style-head:** 0 – less than 1:1; 1 – 1:1 or appendages bigger than the main body. The style head is divided in two portions: two apical appendages and a massive main body. The appendages are variable in size within different species of *Mandevilla*, and their size in proportion to the main body constitute a character that has never been used before in the genus.

30 **Nectaries number**: 0 – five; 1 – two.

31 **Nectaries height**: 0 – smaller than the ovary; 1 – equal or greater than the ovary.

32 **Ovary indument**: 0 – absent; 1 – present

Appendix 4- List of taxonomic recombinations in Mesechiteae. All new combinations are indicated in asterisks (*).

1) *Mandevilla* Lindl., Edwards's Bot. Reg. 26: pl. 7. 1840.

Macrosiphonia Müll.Arg. in Mart., Fl. Bras. 6(1): 137. 1860.

Macrosiphonia subgenus *Eumacrosiphonia* Woodson, Ann. Missouri Bot. Gard. 20: 784. 1933.

Macrosiphonia subgenus *Telosiphonia* Woodson, Ann. Missouri Bot. Gard. 20: 778. 1933.

Quiotania Zarucchi, Novon 1: 33. 1991.

Telosiphonia Henrickson, Aliso 14(3): 179. 1995.

1.1. *Mandevilla brachysiphon* (Torr.) Pichon, Bull. Mus. Nat. Hist., ser. 2: 106. 1948.

Macrosiphonia brachysiphon (Torr.) A. Gray, Syn. Fl. N. Amer. 2(1): 83. 1878.

Telosiphonia brachysiphon (Torr.) Henr., Aliso 14(3): 187. 1995.

*1.2. *Mandevilla hesperia* A.O. Simões, M. Endress & L.S. Kinoshita, comb. nov.

Macrosiphonia hesperia I.M. Johnst., Proc. Calif. Acad. Sci., ser. 4, 12: 1125. 1924.

Telosiphonia hesperia (I.M. Johnst.) Henr., Aliso 14(3): 191. 1995.

1.3. *Mandevilla hypoleuca* (Benth.) Pichon, Bull. Mus. Nat. Hist., ser. 2: 106. 1948.

Macrosiphonia hypoleuca (Benth.) Müll.Arg., Linnaea 30: 452. 1860.

Telosiphonia hypoleuca (Benth.) Henr., Aliso 14(3): 185. 1995.

1.4. *Mandevilla lanuginosa* (M. Martens & Galleoti) Pichon, Bull. Mus. Nat. Hist., ser. 2: 106. 1948.

Macrosiphonia lanuginosa (M. Martens & Galleoti) Hemsl., Biol. Cent.-Amer., Bot. 2(10): 316. 1881.

Telosiphonia lanuginosa (M. Martens & Galleoti) Henr., Aliso 14(3): 189. 1995.

1.5. *Mandevilla ligustrifolia* Woodson, Ann. Missouri Bot. Gard. 37(3): 404-405. 1950.

Quiotania colombiana Zarucchi, Novon 1(1): 33-36. f. 1. 1991.

1.6. *Mandevilla longiflora* (Desf.) Pichon, Bull. Mus. Nat. Hist., ser. 2: 107. 1948.

Macrosiphonia longiflora (Desf.) Müll.Arg. in Mart., Fl. Bras. 6(1): 140. 1860.

1.7. *Mandevilla macrosiphon* (Torr.) Pichon, Bull. Mus. Nat. Hist., ser. 2: 106. 1948.

Telosiphonia macrosyphon (Torr.) Henr., Aliso 14(3): 187. 1995.

1.8. *Mandevilla martii* (Müll.Arg.) Pichon, Bull. Mus. Nat. Hist., ser. 2: 107. 1948.

Macrosiphonia martii Müll.Arg. in Mart., Fl. Bras. 6(1): 130. 1860.

1.9. *Mandevilla petrea* (A. St.-Hil.) Pichon, Bull. Mus. Nat. Hist., ser. 2: 107. 1948.

Macrosiphonia petrea (A. ST.-Hil.) K. Schum, Nat. Pflanzenfam. 4(2): 168. 1895.

*1.10. *Mandevilla nacapulensis* (Felger & Henr.) A.O. Simões, M. Endress & L.S. Kinoshita, comb. nov.

Telosiphonia nacapulensis Felger & Henr., Aliso 14(3): 194-195, f. 4E-J, 5. 1995.

* 1.11. *Mandevilla undulata* (Ezcurra) A.O. Simões, M. Endress & L.S. Kinoshita, comb. nov.

Macrosiphonia undulata Ezcurra, Hickenia 1(45): 243-245, f. 2. 1981.

1.12. *Mandevilla velame* (A. St.-Hil.) Pichon, Bull. Mus. Nat. Hist., ser. 2: 107. 1948.

Macrosiphonia velame (A. St.-Hil.) Müll.Arg. in Mart. Fl. Bras. 6(1): 138. 1860.

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CAPÍTULO IV

CALYCINE COLLETERS OF SEVEN SPECIES OF MESECHITEAE MIERS (APOCYNACEAE, APOCYNOIDEAE)

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Abstract - In order to investigate the structure of calycine colleters in Apocynaceae, seven species belonging to four genera of Apocynoideae (*Macrosiphonia longiflora*, *Mandevilla pycnantha*, *M. scabra*, *M. tenuifolia*, *Mesechites mansoana*, *Secondatia densiflora* and *S. floribunda*) were studied. Calycine colleters were found in all species. All colleters had the same histological compositon, with a central core of parenchyma cells surrounded by a secretory layer of palisade epidermal cells, but differences in morphological traits were observed in four species. Most colleters were of standard type, and possible deviations from this pattern resulted in three morphological types, here described for the first time in the family: bifurcated, laminar and sessile. The structure is more variable than expected and mechanisms of cell separation, proliferation and elongation may explain the origin of these three new observed types. As a consequence of the observed morphological changes in these types, the secretory surface is expanded when compared to colleters of standard type. Most colleters were in a postsecretory phase, but a hidrophyllic secretion was observed in *Mandevilla pycnantha* and *Mesechites mansoana*. A larger secretory surface, together with the extension of the secretory phase in adult flowers, is major shifts in the calycine colleters of Apocynaceae, possibly correlated to the functional and ecological aspects of these organs. The distinct patterns of distribution of calycine colleters were observed: alternate, opposite or indefinitely distributed. The alternate pattern is not homogeneous and three subtypes were observed, with four, five or 10 groups of colleters attached to the sepals. The number of calycine colleters is variable among taxa and even within the same species, except in *S. floribunda* (four), and *S. densiflora* and *Mandevilla scabra* (five each). No patterns of morphology, distribution and number of calycine colleters were found to characterize Mesechiteae and support the exclusion of *Secondatia* from its circumscription.

Key words: anatomy, Apocynaceae, calycine colleters, Mesechiteae, *Macrosiphonia, Mandevilla, Mesechites, Secondatia.*

INTRODUCTION

Colleters are glandular structures that produce a sticky secretion constituted by a mixture of mucilage and terpenes, with the main function of protecting and lubricanting buds at initial stages of development (Fahn, 1979). These glands are found on the margin or in axilar position to both vegetative and reproductive organs such as nodes, leaves, bracts and flowers in about 59 Angiosperm families (Thomas 1991). Despite their great representation in both basal and more advanced groups of Angiosperms, the external morphology of the colleteres is homogeneous, being constituted by a long, conical to deltoid and undivided main body (named "head" by Thomas, 1991) on the top of a short stalk. Colleters of such type, originally described as "glandular shaggy hairs" by Solereder (1908), were termed "standard" by Lersten (1974a,b) in Rubiaceae, a name that has been used by many authors to describe similar colleters in other Angiosperm families since then. Variation in the standard type was observed only in species of Rubiaceae, in which six additional morphological types were described: filiform, winged, reduced standard, intermediate, dendroid and brush-like, as summarized in Thomas (1991).

In Apocynaceae s.l., one of the largest Angiosperm families with 355 genera and about 3700 species (Judd et al., 2002), colleters have been reported in 67 genera (Woodson and Moore, 1938; Rao and Ganguli, 1963; Ramayya and Bahadur, 1968; Fjell, 1983; Fallen, 1986; Dave et al., 1987; Thomas et al., 1989; Thomas and Dave 1989a,b,c, 1990; Thomas, 1991; Galetto, 1997; Appezzato-da-Glória and Estelita, 2000; Rio et al., 2002; Simões and Kinoshita, 2002). These structures have a great taxonomic importance in the family, and their aspect, distribution and number have been traditionally used as taxonomic characters by many authors (e.g., Woodson, 1933, 1935, 1936; Ezcurra, 1981; Ezcurra et al., 1992; Simões & Kinoshita, 2002). According to Thomas (1991), only colleters of standard type have been described in Apocynaceae, but this homogeneity in form is questionable. Descriptions of colleters or colleter-like structures of Apocynaceae as "pectinate", "lacerate", "laciniate" or "fimbriate" are largely found in the literature (e.g., Woodson 1933, 1936; Ezcurra et al., 1992; Simões & Kinoshita, 2002; Rio & Kinoshita, submitted to Hoehnea), suggesting that variations in the standard type can occur in the family.

Calycine colleters are found attached to the inner surface of the sepals in about 55 genera of Apocynaceae (Thomas, 1991), a number underestimated due to the presence of these structures in other genera of the family that were not considered by him. Woodson (1933) applied for the first time aspects of calycine colleters in taxonomy, and since then their number and distribution are used as key diagnostic characters in the family, especially at generic and specific levels (e.g., Woodson, 1933, 1935; Pichon, 1950; Ezcurra, 1981; Ezcurra et al., 1992; Simões and Kinoshita, 2002). In contrast to their frequency and taxonomic relevance, anatomical aspects of calycine colleters are poorly known and were studied only by few authors (e.g., Woodson and Moore, 1938; Fallen, 1986; Thomas et al., 1989; Galetto, 1997). Most of these studies were focused mainly in the morphology and distribution of calycine colleters, with little attention to their ontogeny or histochemical aspects.

Mesechiteae sensu Endress and Bruyns (2000) encompasses nine genera and about 150 species distributed in the neotropics. Simões et al. (2004), based on phylogenetic results, proposed significant changes in the tribe, with the exclusion of *Galactophora* Woodson and *Secondatia* A. DC. and inclusion of *Forsteronia* G. Mey. in its circumscription. Calycine colleters were reported for all genera of Mesechiteae (e.g., Woodson, 1933; Pichon, 1950; Ezcurra, 1981; Ezcurra et al., 1992; Morales, 1997, 1998; Simões and Kinoshita, 2002), but few studies on the structure of these glands have been made. The most relevant was conducted by Woodson and Moore (1938), who studied the calycine colleters of eight species (*Forsteronia viridescens* S.F. Blake, *Mandevilla bridgesii* (Müll.Arg.) Woodson, *M. hirsuta* (A. Rich.) K. Schum., *M. illustris* (Vell.) Woodson, *M. laxa* (Ruiz & Pav.) Woodson, *M. subsagittata* (Ruiz & Pav.) Woodson, *Mesechites trifida* (Jacq.) Müll.Arg. and *Telosiphonia brachysiphon* (Torr.) Henr.) Other works are the ones of Fallen (1986) for *Mandevilla laxa* and Galetto (1997) for *Macrosiphonia petraea* (A. St.-Hil.) K. Schum., *Mandevilla laxa* and *M. pentlandiana* (A. DC.) Woodson. Evidence of morphological variation of these organs in the tribe is provided in the literature for species of *Mesechites* A. DC. and *Mandevilla* subgenus *Exothostemon* Woodson (Woodson 1933; Simões & Kinoshita, 2002).

The aims of the present work are: 1) characterize the structure of calycine colleters in seven species of Apocynaceae in terms of morphology, number and distribution; and 2)

verify the occurrence of patterns in the analysed aspects of calycine colleters to support the exclusion of *Secondatia* from Mesechiteae.

MATERIAL AND METHODS

Seven species from four genera of Apocynaceae included in Mesechiteae by Endress & Bruyns (2000) were selected for this study: one of *Macrosiphonia* Müll.Arg. (*M. longiflora* (Desf.) Müll.Arg.), three of *Mandevilla* Lind. (*M. pycnantha* (Steud) Woodson, *M. tenuifolia* (Mikan) Woodson and *M. scabra* (Roem. & Schult.) K. Schum., the first two sampled from subgenus *Mandevilla* Woodson and the latter from subgenus *Exothostemon*), one of *Mesechites* (*M. mansoana* (A. DC.) Woodson) and two of *Secondatia* (*S. densiflora* A. DC. and *S. floribunda* A. DC.). From the studied genera, three (*Macrosiphonia, Mandevilla* and *Mesechites*) belong to Mesechiteae and the other, *Secondatia*, was included in the tribe by Endress & Bruyns (2000) and later transferred to Echiteae by Morales (2003) and to Apocyneae by Simões et al. (2004).

Only adult, anthetic flowers were selected. Samples were collected in their natural habitat on southeastern Brazil and later identified by the first author. All vouchers were deposited in the herbarium UEC. Flowers of *M. tenuifolia* were obtained from cultivated specimens maintained at the greenhouse of the Department of Botany at the University of Campinas, Brazil.

Herbarium specimens. *Macrosiphonia longiflora*: Brazil, Minas Gerais, Carrancas, A. Flores et al., X/2001 (UEC); *Mandevilla pycnantha*: Brazil, Minas Gerais, Grão-Mogol, A.O. Simões et al. 1176, 24/I/2002 (UEC); *Mandevilla scabra*: Brazil, Bahia, Andaraí, A.O. Simões et al. 1127, 19/I/2002 (UEC); *Mandevilla tenuifolia*: Brazil, São Paulo, Atibaia, A.O. Simões et al. 1048, X/2000 (UEC).; *Mesechites mansoana*: Brazil, Minas Gerais, Grão-Mogol, A.O. Simões & E.R. Pansarin 1087, 10/II/2001 (UEC); *Secondatia densiflora*: Brazil, Minas Gerais, Serra do Cipó, L.S. Kinoshita et al., 24/IX/2002 (UEC); *Secondatia floribunda*: Brazil, Minas Gerais, Pedra Azul, A.O. Simões & R.B. Singer 1303, IV/2002 (UEC).

Flowers were collected and fixed in FAA (Johanson, 1940) for at least 24 h at room temperature and placed under a low vacuum to aid the penetration of the fixative. The

flowers were then stored in ethanol 70%. For anatomical sections, only the basal part of the flower with 8-15 mm long was used. All materials were dehydrated using the tertiary butyl alcohol series, embebbed in paraplast and then sectioned (Johanson, 1940). Longitudinal and transverse serial sections were cut 10-12 μm thick with a rotary microtome. Deparaffinized sections were stained with Safranin O and Astra Blue (Gerlach, 1969). Light microscope observations were carried out on an Olympus BX51 microscope. Photomicrographs were captured with Kodak Pro Image (100 ASA) film and electronically processed using the software Adobe Photoshop 7.0.

RESULTS

A. Structure

In all species, the calyx is gamossepalous and 5-parted. The aestivation is quincuncial: of the five lobes, two are exterior, two interior and one intermediate with one margin overlapping the flank of one interior lobe and the other covered by one of the exterior lobes (Fig. 4.3A). Calycine colleters were found on the adaxial surface of the calyx in all species, attached to the margins or axils of the sepals.

In all colleters, the main body is composed of a central core of parenchyma cells elongated in the direction of the stalk and surrounded by a secretory layer of palisade epidermal cells covered by a thin cuticle. The stalk, when present, is composed of parenchyma cells covered by a non-secretory epidermis. No vascularization was observed in the colleters of any species. In *Mesechites mansoana*, the secretory epidermis covers the whole gland, forming a dense palisade. The epidermal cells of the main body have thin cell walls, a relatively large nucleus and dense cytoplasm, a set of characteristics common to secretory cells. No secretory compounds were found inside the calycine colleters or in their vicinities, exception for *Mandevilla pycnantha* and *Mesechites mansoana*. In these two species, a hydrophylic secretion stained in pink was released to the outside in the space between the sepals and the corolla tube, also accumulating within the epidermal cells (Fig. 4.2D,E). In the other five species, the epidermal cells showed a retraction of the protoplast, an indicator that the colleters were in a postsecretory phase. No cuticle rupture was

observed in any species, even in glands with the protoplast retracted. In *S. densiflora* and *S. floribunda* the secretory epidermis of the main body stained in deep red.

The morphology of the calycine colleters is greatly variable, with four observed morphological types:

Standard type: This type was found in *Macrosiphonia longiflora*, *Mandevilla pycnantha*, *M. tenuifolia*, *Secondatia densiflora* and *S. floribunda* (Table 1). In these species, colleters are differentiated into a conical to deltoid, undivided main body on the top of a short-thinned, non-secretory stalk. (Fig. 4.1A-B).

Bifurcated type: This type was found in two species of *Mandevilla*, *M. pycnantha* and *M. tenuifolia*. Standard and bifurcated colleters are almost identical in form, differing only by the division of the upper half ot the main body in the latter. These two divided parts are completely free from each other on the top of the gland and surrounded by the secretory epidermis (Figs. 4.1C-E, 4.2A-C). In *M. pycnantha* and *M. tenuifolia* both standard and bifurcated colleters were observed in the same flower (Table 1), but standard ones were always more frequent.

Laminar type: This type was found only in *Mandevilla scabra*, being distinct from the standard type by the shape of the main body and the structure of the stalk. In standard colleters, the main body is circular to ellipsoid in cross section; laminar colleters, on the other hand, are dorsiventral in cross section and much more wide than thick (Figs. 4.1F, 4.4A-C). The stalk of laminar colleters are greatly enlarged on the abaxial side opposite to the sepals due to a proliferation of parenchyma cells.

Sessile type: This type was seen only in *Mesechites mansoana* (Table 1). Sessile colleters are composed by two parts with about the same size: the main body, free from the calyx, and a large, compressed base fused to the calyx. No discernible stalk was observed. The main body is massive and dorsiventral, like in laminar colleters, but the enlarged and flat base formed by a single layer of palisade secretory cells was not observed in any other gland type. The epidermal cells are longitudinally elongated and form a very dense palisade, covering the whole gland (Figs. 4.2E-G, 4.4D-E).

B. Number

The number of calycine colleters was greatly variable, ranging from four to many. Constant and easily quantified numbers were found in three species (five in *Mandevilla scabra* and *S. densiflora* (Fig. 4.3A), and four in *S. floribunda*). In the remaining species, however, the colleters were many, always more than five, and this number varied even in the same plant. The number of calycine colleters in *Mandevilla pycnantha* and *M. tenuifolia* could not be unambiguously determined due to the occurrence of both standard and bifurcated colleters in the same flower.

C. Distribution

Calycine colleters are distributed in three different patterns in relation to the sepals: **alternate**, **opposite** or **indefinitely distributed** (Table 1).

Alternate colleters are attached to the margins of the sepals and were observed in four species (*Mandevilla pycnantha*, *M. tenuifolia*, *S. densiflora* and *S. floribunda*). Three subtypes of alternate colleters were recognized: 4, 5 and 10-grouped. In *S. densiflora*, the colleters are disposed in five groups, each one composed by one gland positioned at the sinus between two adjacent sepals. Four of these groups are attached to the margins of the inner sepals, and the other to the internal margin of the intermediate sepal (Fig. 4.3A-B). In *S. floribunda*, the colleters are disposed in four groups attached to the inner sepals, with no gland or group of glands attached to the internal margin of the intermediate sepal. In *M. pycnantha* and *M. tenuifolia*, 10 groups of one to three glands each are disposed in the petal sinuses, with two groups attached to the margins of each sepal, in the area of contact between two adjacent sepals.

Opposite colleters occur only in *Mandevilla scabra* (Table 1). In this species, the calycine colleters are in number of five, each one attached to the axils of a sepal. They are considerably large and occupy the entire width of the sepals (Fig. 4.4A-C).

Indefinitely distributed colleters were found in *Macrosiphonia longiflora* and *Mesechites mansoana*. In these species, the calycine colleters are many and form a continuous ring at the base of the sepals. In *M. mansoana* most of the calycine colleters were attached to the margins of the sepals, but colleters attached to the axils of the sepals

were also found (Fig. 4.4D-E). In *Macrosiphonia longiflora*, the colleters are attached to both margins and axils of the sepals.

DISCUSSION

The terminology traditionally used to characterize and describe calycine colleters in Apocynaceae is somewhat confusing and adjustments are necessary to avoid misenterpretations. **Standard** colleters have been traditionally described as "finger-like" (Lersten, 1974b) or "finger-shaped" (Thomas, 1991). The term "head", proposed by Thomas (1991) to name the main body of colleters, has ben used in Apocynaceae for that purpose. A finger-like shape implies a constant diameter along the length of an organ, and the term head is more properly used to designate spherical structures, like the glandular trichomes of Lamiaceae (Ascensão *et al.*, 1999). Colleters with conical shape have been described in Apocynaceae: *Allamanda* and *Thevetia* (Fjell (1983); *Allamanda* (Thomas and Dave (1989a); *Prestonia* Rio et al. (2002), and all colleters observed in our study are conical to deltoid or laminar, with a progressive decrease in diameter towards the apex. Based on these assumptions, a description of the calycine colleters as conical to deltoid or laminar instead of finger-shaped and the use of the term main body instead of head are more appropriate to reflect the architecture of these organs in Apocynaceae.

Variation in morphology and series of transformation

Thomas (1991) stated that colleters of Apocynaceae (including calycine colleters) are finger-shaped structures differentiated into a long "head" (main body) on the top of a short stalk, a set of characteristics associated to the **standard** type. Our results support his idea only in terms of histological composition. All observed colleters were formed by a core of longitudinally elongated cells surrounded by a layer of epidermal palisade cells, but variations in morphological traits were seen in four species. The three observed additional types (**bifurcated, laminar** and **sessile**), described here for the first time, demonstrate that the structure of calycine colleters in Apocynaceae is not always homogeneous, as previously assumed, but instead more varied than expected. This variation may not be exclusive of species of *Mandevilla* and *Mesechites*, though, but instead occur in other

genera of Apocynaceae. Calycine colleters of neotropical genera from tribe Echiteae, such as *Peltastes, Prestonia* and *Temnadenia* have been described as "laciniate" or "fimbriate" (e.g., Woodson, 1935; Ezcurra, 1981; Simões and Kinoshita, 2002), with a large and flattened main body divided at the apex. Apparently, these colleters have characteristics of both laminar and bifurcated type, but no further conclusions can be made based only in the information found in the literature. Anatomical studies of calycine colleters of neotropical species of Apocynaceae, especially from Echiteae, are greatly desired to address this question.

The standard pattern is by far the most frequent morphological type of colleters found in Angiosperms, with additional types reported only in Rubiaceae before our study. As standard colleters are so frequent and occur in basal families of Angiosperms, such as Magnoliaceae and Ranunculaceae, it is reasonable to assume that the standard type is the plesiomorphic condition and all other types are derived from deviations of this original pattern. Lersten (1974b) proposed a series of transformation to explain the origin of 4 morphological types of colleters in Rubiaceae. He argued two deviations from the standard type originated new morphological types in that family: 1) reduction in size of epidermal cells and; 2) elongation and separation of epidermal cells, sometimes coupled with either a reduction of the axis in size or number of cells. The same approach can be made with our results, and a hypothetical series of transformation from the standard to the three additional morphological types is shown (Fig. 5). Three mechanisms are assumed to provide morphological variation in the colleters of Apocynaceae: separation, proliferation and elongation of cells. Bifurcated colleters are an example of the first mechanism, with the cells of the main body being segregated in two distinct parts, each one free from the other at the top and covered by a secretory epidermis. Laminar and sessile colleters, on the other hand, are examples of the second mechanism. The proliferation of cells, especially from the parenchyma of the main body, results in the enlargement of the gland. A more extreme stage in this typological sequence is represented by the sessile type, in which a vertical expansion of the epidermis downwards, giving origin to a well-developed secretory basis, occurs together with a lateral enlargement of the gland. In addition to that changes, the epidermal cells become more elongated and form a dense palisade. From a morphological point of view, it is more logical to assume that the laminar type is an intermediate state

between the standard and sessile ones. However, the phylogenetic results provided by Simões et al. (2004) show that, in the newly circumscribed Mesechiteae, *Mesechites* is more basal than *Mandevilla*, which makes more parsimonious to assume an origin of laminar colleters from the reduction in size of sessile colleters. An independent origin of both sessile and laminar colleters is equally parsimonious, though, resulting in three putative evolutionary trends. Structural studies of calycine colleters of other taxa from Mesechiteae could give more evidence to better address this question.

Even though bifurcated, laminar and sessile types have significant differences in shape and size, they both share one characteristic: deviations from the standard pattern resulted in an increase on the area of the secretory epidermis. The observed morphological variation of calycine colleters illustrates how plastic these structures are and suggests a functional utility of these characters by increasing the secretory phase in Apocynaceae. This variation, however, is strongly constrained by the narrow space between the calyx and corolla where these glands are located, which greatly reduces the number of possible forms that they can assume. Lateral expansion and separation of cells are two alternatives to promote structural changes in calycine colleters and, at the same time, optimize their function by increasing the secretory surface.

Secretion

The absence of secretion in most of the studied species is in agreement with other results reported for Apocynaceae, in which the calycine and/or vegetative colleters are active at the initial stages of development and, consequently, in a post-secretory stage in adult flowers (Thomas et al., 1999; Appezzato-da-Glória and Estelita, 2000; Rio et al., 2002). The presence of secretory compounds in the calycine colleters of *Mesechites mansoana* and *Mandevilla pycnantha*, however, shows that these structures can be still active even in fully developed flowers. The epidermal cells strongly stained in red in the calycine colleters of *S. densiflora* and *S. floribunda*. It is possibly caused by the presence of phenolic compounds, suggesting that these cells are senescent or can still be in a secretory phase.

Patterns of distribution

The three chief categories of colleters with respect to their relationships with the sepals recognized by Woodson & Moore (1938), **alternate, opposite** and **indefinitely distributed,** were found in our study. **Alternate** colleters occur in many genera of Apocynaceae (Woodson 1933, 1935, 1936; Ezcurra, 1981; Simões and Kinoshita, 2002) and are particularly frequent in subfamily Apocynoideae. The alternate pattern, however, is heterogeneous and may not be homologous in the whole Apocynaceae, as suggested by the observation of three subtypes (four, five and 10-grouped glands) in our study. The four-grouped subtype is very similar to the 5-grouped one and may represent, in fact, a variation of the latter by the senescence and subsequent loss of one gland or group of glands in adult flowers. The 10-grouped subtype is very distinct from the other two subtypes by having two groups of glands, instead of one, attached to the margin of a sepal. Our initial hypothesis was that the duplicated number of groups of glands was originated from the expansion and/or division of the five original groups, but our results showed that each adjacent group is attached to the margin of a different sepal. Calycine colleters disposed in 10 alternate groups have been observed in other species of *Mandevilla*, suggesting that this pattern is frequent within the genus and has a potential taxonomic relevance at infrageneric level. Woodson and Moore (1938) illustrated this pattern in *M. bridgesii*, and observations on the external morphology of other species of *Mandevilla* by Simões and Kinoshita (2002) showed the same pattern in *M. atroviolacea, M. coccinea, M. illustris, M. spigeliiflora* and *M. widgrenii*.

Opposite colleters are frequent in most genera belonging to tribe Echiteae (Woodson, 1935, 1936; Endress and Bruyns, 2000; Simões and Kinoshita, 2002). In Mesechiteae, opposite colleters are known only in species of *Mandevilla* subgenus *Exothostemon*, a group of about 45 species that include *M. scabra*. The position of calycine colleters was defined by Woodson (1933) as one of the key characters to characterize the subgenera of *Mandevilla*. Opposite colleters were observed and illustrated by Woodson and Moore (1938) in another species of subgenus *Exothostemon, M. subsagittata*, reinforcing the taxonomic value of this character within *Mandevilla*. Woodson and Moore (1938) suggested that opposite calycine colleters were derived by the fusion of two marginal glands in a position adaxial to the sepal which they subtend. This hypothesis was based on

their observations in the calycine colleters of *M. subsagittata*, in which the base of the opposite gland is bipartite and attached to the margins of the sepal. The same pattern of development was observed by us in *M. scabra*, but we disagree with their hypothesis. In *M. scabra*, the very base of the colleters are attached to the middle part of the sepals and become bipartite only in a more superior position.

Indefinitely distributed colleters are known for many taxa in Apocynaceae and are highly variable in number. Distinction between alternate and indefinitely distributed colleters is not always straightfoward, though. This is clearly exemplified by *Mandevilla*, in which a morphological analysis made by Simões (pers. obs.) showed that the calycine colleters of *M. martiana* and *M. moricandiana* have a somewhat alternate placement, with 10 more or less recognizable groups of glands close to the margins of the sepals forming a continous ring at the calyx base. These results suggest that the indefinitely distributed pattern may be originated by the lateral expansion of groups of alternate colleters in these two species of *Mandevilla*, supporting in part the hypothesis of Woodson and Moore (1938), who proposed the origin of colleters with indefinite distribution by laceration of either alternate or opposite colleters.

Applications on systematics

No patterns of morphology, distribution and number of calycine colleters were found to characterize Mesechiteae *sensu* Simões et al. (2004). The combination of these parameters, however, may be useful in lower taxonomic levels of some groups within the tribe, as the infrageneric categories of *Mandevilla*. In that genus, the largest one of the tribe with about 130 species, the subgenus *Exothostemon* is characterized by having five, opposite calycine colleters in contrast to subgenus *Mandevilla*, in which the colleters are variable in number and distribution but never opposite to the sepals (Woodson, 1933). The same pattern was observed in our study, and the description of laminar colleters in *Mandevilla scabra*, a species from subgenus *Exothostemon*, suggests that this morphological type of colleters can occur in other species of the subgenus and, consequently, be of great utility in the systematics of the genus. Some of these parameters may also be useful in other taxonomic levels of Apocynaceae, as the three observed subtypes of alternate colleters.

Conclusions - In general terms, the main conclusions of our study are: 1) The structure of calycine colleters in Apocynaceae is more variable than expected, and three new morphological types are here described for the first time: **laminar**, **sessile** and **bifurcated**. Deviations from the standard pattern by mechanisms of cell separation, proliferation and elongation are assumed to explain the origin to the three observed new types; 2) Observation of secretory compounds on calycine colleters of *Mandevilla pycnantha* and *Mesechites mansoana* shows that these organs can be still active in adult flowers in some species. 3) The number of calycine colleters can be variable among different taxa and even within the same species. As a consequence, the application of this character in taxonomy must be considered only after a careful analysis of the structure of these organs on a given taxa; 4) the increase on the secretory surface on the three new morphological types and the production of secretory compounds in two species in adult flowers are major shifts on the calycine colleters of Apocynaceae, and are possibly associated to functional and ecological aspects of these organs. 5) Three main patterns of distribution of calycine colleters in relation to the calyx were observed: **alternate**, **opposite** and **indefinitely distributed**. The alternate pattern is not homogeneous, and three subtypes were seen: one with four, another with five and the latter with 10 groups of glands disposed on the margins of the sepals; 6) No specific patterns of calycine colleters support the exclusion of Secondatia from Mesechitea in terms of structure, number or distribution. Some patterns, however, could be potentially useful to characterize groups in other taxonomic levels, like the five laminar colleters with opposite distribution in the subgenus *Exothostemon* of *Mandevilla* and the distinction between the three subtypes of alternate colleters.

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Table 1. Structural types and distribution of calycine colleters in seven species of Mesechiteae. S= standard; B= bifurcate; L= laminar; Se= sessile; A4= 4-grouped alternate; A5= 5-grouped alternate; A10= 10-grouped alternate; In= indefinitely distributed; O=opposite

Species	Structural type	Distribution
Macrosiphonia longiflora	S	In
Mandevilla pycnantha	S, B	A10
Mandevilla scabra	L	O
Mandevilla tenuifolia	S, B	A10
Mesechites mansoana	Se	In
Secondatia densiflora	S	A5
Secondatia floribunda	S	A4

FIGURE LEGENDS

Figure 1. Types of calycine colleters of *Macrosiphonia* and *Mandevilla*. A-B. *Macrosiphonia longiflora*: standard type. C-E. *Mandevilla tenuifolia*: bifurcated type; C-D: sequence of bifurcation. F. *Mandevilla scabra*: laminar type. A,E,F: longisections; B,C,D: transections. ca: calyx; cg: calycine colleters; co: corolla.

Figure 2. Types of calycine colleters of *Mandevilla* and *Mesechites*. A-D. *Mandevilla pycnantha*: bifurcated type; B-C: sequence of bifurcation; D: detail of a gland showing secretion inside the cells (arrow). E-G. *Mesechites mansoana*: sessile type; E. detail of a gland showing secretion in the space between the calyx and the corolla tube (arrow). A,D,E,G: longisections; B,C,F: transections. ca: calyx; cg: calycine gland; co: corolla.

Figure 3. Distribution of calycine colleters in relation to the sepals: alternate pattern. A-B. *Secondatia densiflora*: five-grouped alternate colleters; A. groups of calycine colleters indicated in arrows; B. detail of one gland attached to the margin of a sepal. C-D. *Mandevilla tenuifolia* and E-F. *Mandevilla pycnantha*: 10-grouped alternate colleters. C,E: four groups indicated in arrows; D,F: detail of colleters attached to the margin of a sepal. A-F: transections. ca: calyx; cg: calycine colleters; co: corolla.

Figure 4. Distribution of calycine colleters in relation to the sepals: opposite and indefinitely distributed patterns. A-C. *Mandevilla scabra*: topological sequence of one opposite gland; A: lower part of the gland, attached to the sepal in an axilar position; B: median part, attached to the sepal in two points; C: upper part of the gland, completely free from the sepal. D-E. *Mesechites mansoana*: continuous colleters; D: gland with alternate origin; E: colleters with opposite origin . A-E: transections. ca: calyx; cg: calycine gland; co: corolla.

Figure 5. Hypothetical series of transformation from standard to bifurcated, laminar and sessile colleters in the studied species. All colleters are shown in longitudinal and transversal view. Dashed lines indicate two alternative trends for the evolution of sessile and laminar colleters.

Figure 4.1 – Calycine glands of Mesechiteae

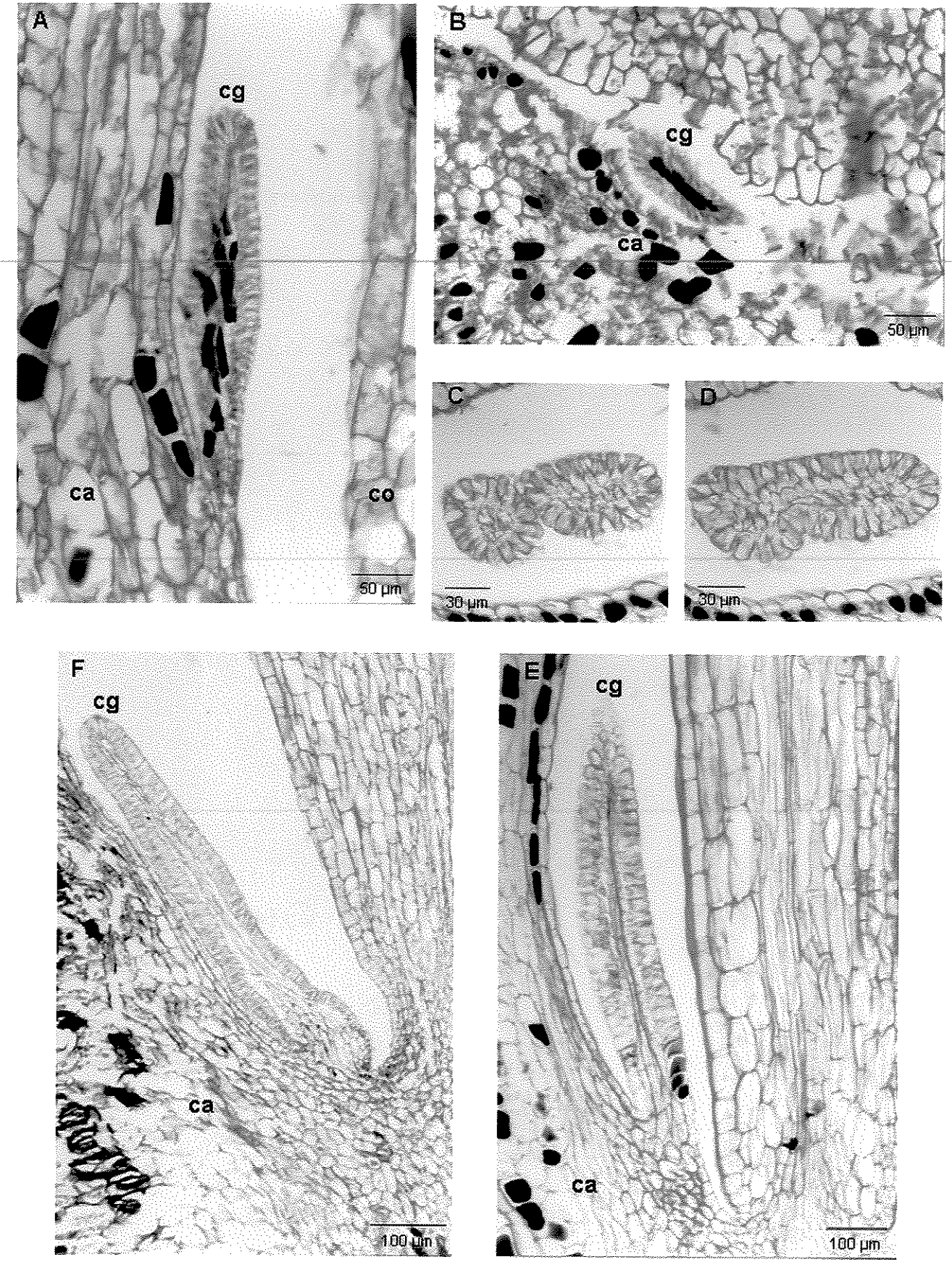


Figure 4.2 – Calycine glands of Mesechiteae

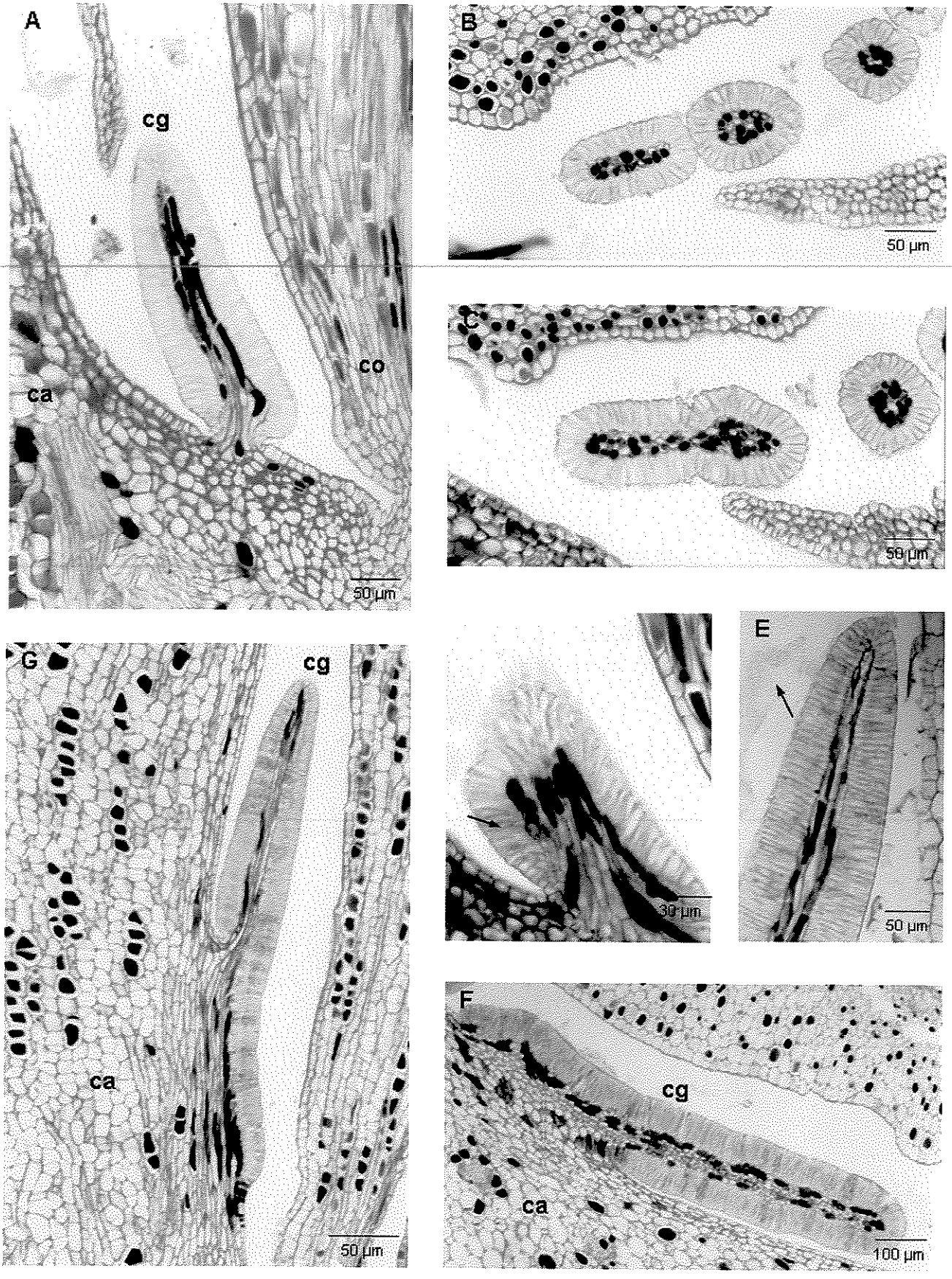


Figure 4.3 – Calycine glands of Mesechiteae

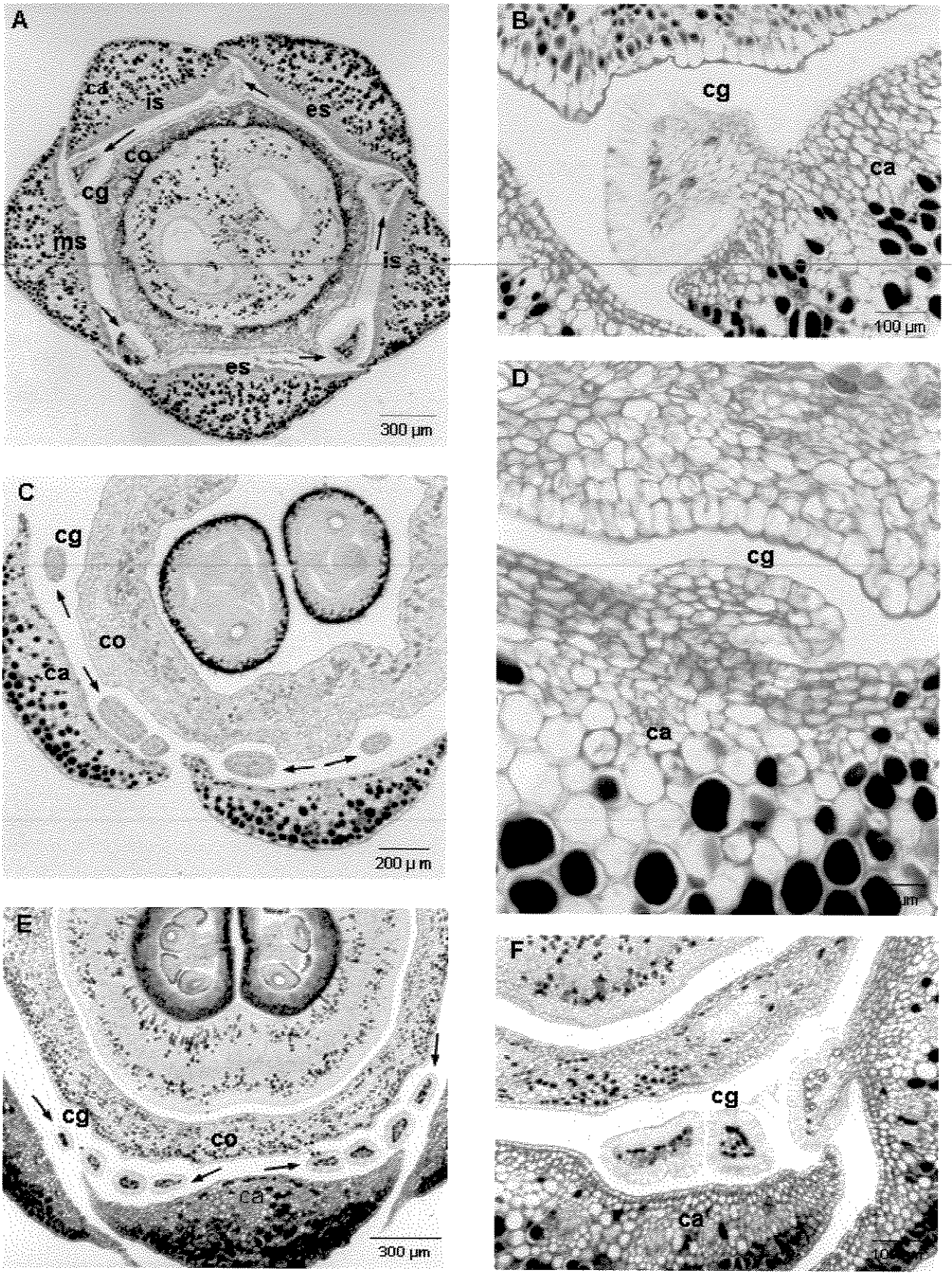


Figure 4.4 – Calycine glands of Mesechiteae

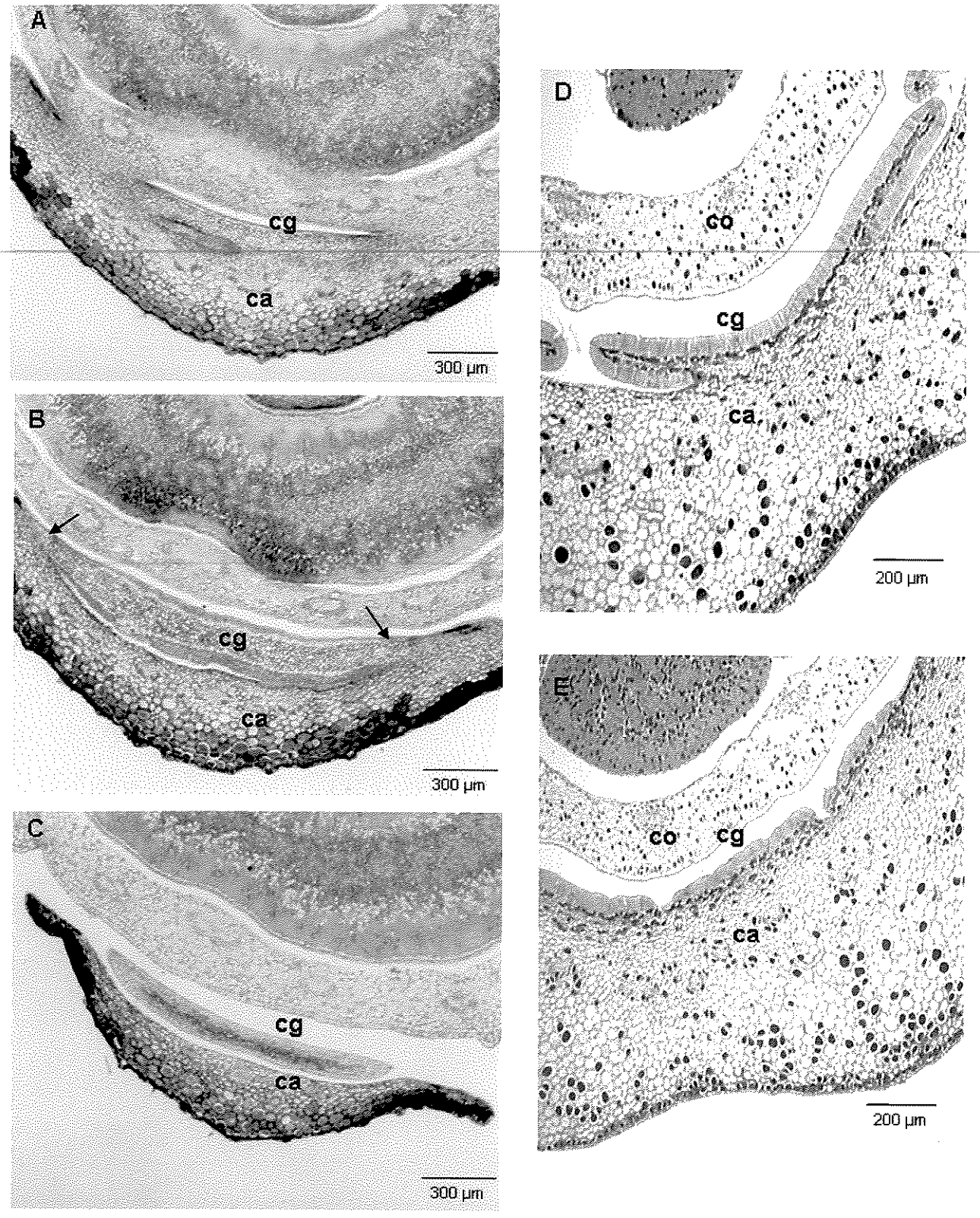
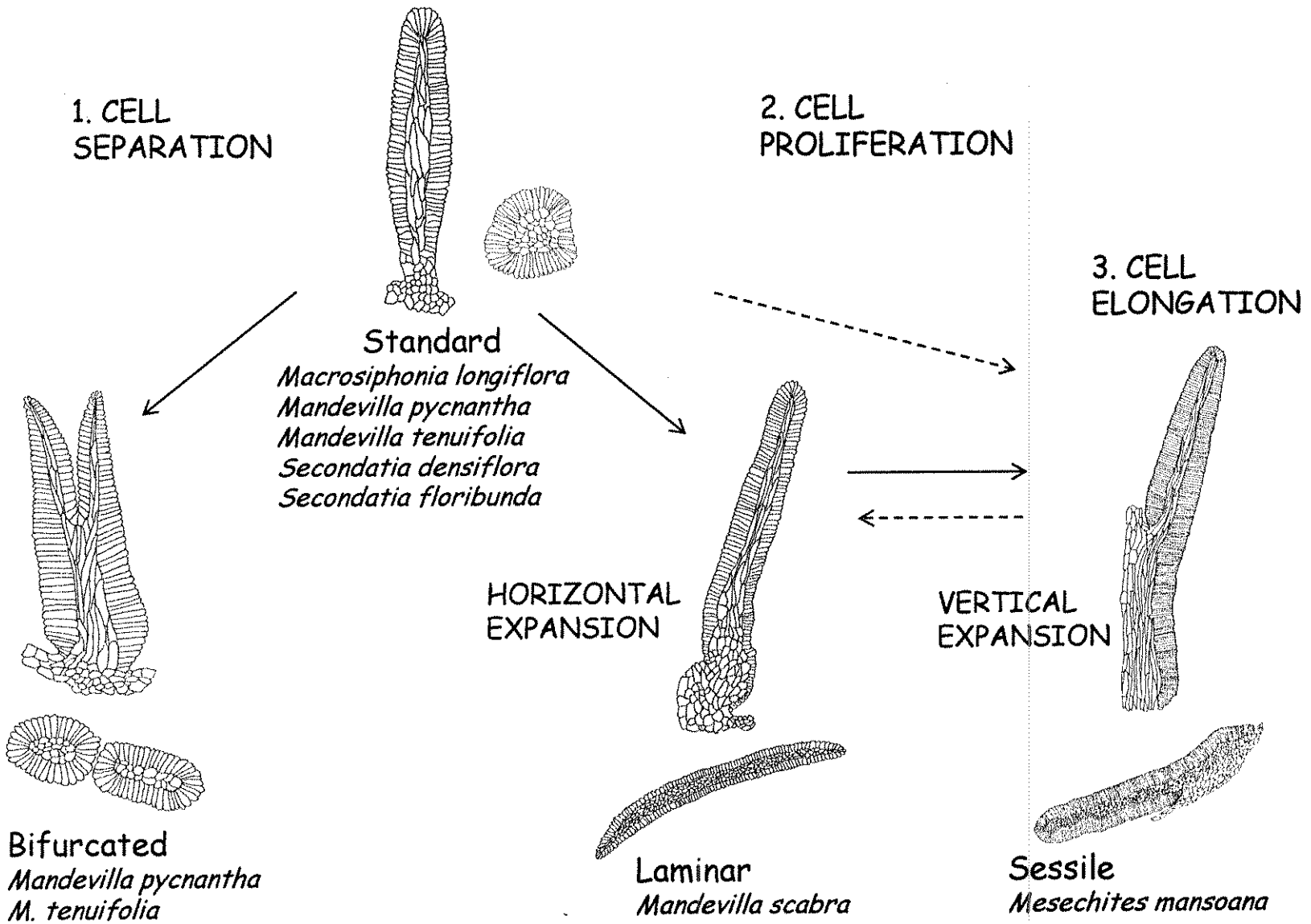


Figure 4.5

Calycine glands of Mesechiteae



CAPÍTULO V

GYNOSTEGIUM MORPHOLOGY OF MESECHITEAE MIERS (APOCYNACEAE, APOCYNOIDEAE)

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Abstract - In order to investigate morphological patterns in Mesechiteae and test the new circumscription of the tribe, the gynostegium structure of seven species belonging to four genera (*Macrosiphonia longiflora*, *Mandevilla pycnantha, M. scabra, M. tenuifolia*, *Mesechites mansoana, Secondatia densiflora* and *S. floribunda*) was studied. In all species the style-head is composed by two portions, apical appendages and main body, and is totally or partially covered by a secretory epidermis. Our results corroborate the new circumscription of Mesechiteae, with the inclusion of *Macrosiphonia*, *Mandevilla* and *Mesechites* on its limits and segregation of *Secondatia.* The gynostegium of *Macrosiphonia*, *Mandevilla* and *Mesechites* have the same pattern, with a proliferation of parenchyma cells in the style-head forming five projecting ribs that are adnated to the expanded portion of the staminal connective. The adnation occurs in one single zone, restricted to the base or extending along the main body of the style-head. The gynostegium of *Secondatia* has a distinct pattern, which has been already reported in species of Apocyneae: the style-head is terete and smooth, with no projecting ribs. Unicellular trichomes of the expanded connective grow towards the center of the flower and touch the secretory epidermis of the style-head, with no adnation between these parts. In all species, the gynostegium is divided in three main portions: one **stigmatic chamber**, five **secretory zones** and one **pollen-depository chamber**. In *Macrosiphonia*, *Mandevilla* and *Mesechites* the secretory zones are not homogeneous but instead formed by two structural and functional portions, one involved in the adnation process and the other in the production and storage of secretion. This distinction is less evident in *Mesechites*, in which the projecting ribs are restricted to the base of the style-head and the main body above is 5-angled. The expanded portion of the connective just below the thecae, traditionally called "retinacle" in the taxonomic literature, is the only portion of the androecium adnated or close to the style-head and directly involved in the formation of the gynostegium. Evidence for the location of the stigmatic region was provided by *Mandevilla scabra*, in which pollen tube growth was observed at the transition zone between the style and the style-head.

Key words: gynostegium, style-head, retinacle, anatomy, Apocynaceae, Mesechiteae, *Macrosiphonia*, *Mandevilla*, *Mesechites*, *Secondatia*,.

INTRODUCTION

Apocynaceae *sensu lato* is one of the largest Angiosperm families, with about 335 genera and 3700 species (Judd et al., 2002). The family is characterized by having a complex flower structure, with a progressive morphological and functional specialization of its parts (Woodson, 1933; Ezcurra, 1981; Fallen, 1986; Rosatti, 1989; Nicholas and Baijnath, 1994; Endress and Bruyns, 2000; Simões and Kinoshita, 2002). A major evolutionary trend in Apocynaceae is the progressive synorganization of gynoecium and androecium that are postgenitally fused in different degrees to form a single functional unit, the gynostegium (Endress and Bruyns, 2000). This term was originally used only in the former Asclepiadaceae (corresponding to subfamilies Secamonoideae and Asclepiadoideae sensu Endress and Bruyns, 2000), but Fallen (1986) expanded the use of this term to the subfamily Apocynoideae. She argued that, even tough postgenital fusion in Apocynoideae is less complete than in the former Asclepiadaceae, the basic design and function of the reproductive parts is the same in both groups. We agree with Fallen (1986), and the term gynostegium will be used in this work hereafter.

The structure of the gynostegium has been traditionally used as key characters in the taxonomy of Apocynaceae, especially in Apocynoideae (Woodson 1933, 1935, 1936; Pichon, 1950; Ezcurra, 1981; Fallen, 1986; Endress and Bruyns, 2000; Simões and Kinoshita, 2002). In contrast to its great relevance in systematics, few studies focused on anatomical aspects of the gynostegium were made (Woodson and Moore, 1938; Rao and Ganguli, 1963; Allorge, 1976; Schick 1980, 1982; Fallen, 1986; Galetto, 1997).

Mesechiteae, as circumscribed by Endress and Bruyns (2000), is one of the tribes of Apocynoideae and comprises nine genera and about 150 species. Its characterization was mainly based on the gynostegium structure, with two diagnostic characters: the style-head had five strongly projecting ribs and was attached to the anthers by "hairs" or by "cellular fusion". Simões et al. (2004), in a broad phylogenetic study of the tribe, proposed rearrangements in its composition, with the maintenance of *Allomarkgrafia* Woodson, *Macrosiphonia* Müll. Arg., *Mandevilla* Lindl., *Mesechites* A. DC., *Telosiphonia* (Woodson) Henr. and *Tintinnabularia* Woodson, inclusion of *Forsteronia* Müll.Arg., and exclusion of *Galactophora* Woodson and *Secondatia* A. DC from its circumscription. A comparison between the gynostegium morphology of *Secondatia* to other genera from

Mesechiteae can provide useful information to corroborate the new classification of the tribe.

Description of the morphology of a complex organ as the gynostegium is difficult and requires a well-defined set of terms. However, a variable and confuse terminology has been used by many authors to describe and characterize the gynostegium of Apocynaceae. One of the clearest examples of that are the terms used to describe the stamens of Apocynoideae. Pichon (1948) adopted the term "retinacle" to name the portion of the stamens that is adjacent or adnated to the style-head to form the gynostegium. The word retinacle, however, has been traditionally used to designate the adhesive disc or corpusculum of a pollinarium that clasps or attaches to the pollinator in the subfamilies Secamonoideae and Asclepiadoideae of Apocynaceae (corresponding to the former Asclepiadaceae) and Orchidaceae. The highly specialized anthers are formed by a combination of sterile and fertile portions, but the term "thecae" has been adopted by Sennblad et al. (1998) to designate the unit formed by anther plus connective. The terminology used to characterize the cells of the "retinacle" and their relation to the style-head is also confusing. Terms like "hairs", "trichomes", "adnation", "agglutination", "cellular fusion" and "contact", even though poorly defined, have been extensively used in the taxonomic literature.

The aims of the present study are: 1) characterize the gynostegium structure of seven species belonging to four genera previously included in Mesechiteae by Endress and Bruyns (2000); 2) verify the existence of structural patterns of the gynostegium in Mesechiteae *sens*u Simões et al. (2004); 3) compare the classifications of Endress and Bruyns (2000) and Simões et al. (2004) with the obtained results, especially in terms of the placement of *Secondatia*; 4) revise the terminology required to describe the gynostegium in the studied species.

MATERIAL AND METHODS

Seven species from four genera of Apocynoideae included in Mesechiteae by Endress & Bruyns (2000) were selected for this study: one of *Macrosiphonia* (*M. longiflora* (Desf.) Müll.Arg.), three of *Mandevilla* (*M. pycnantha* (Steud.) Woodson, *M. tenuifolia*

(J.C. Mikan) Woodson and *M. scabra* (R. & S.) K. Schum., the first two sampled from subgenus *Mandevilla* Woodson and the latter from subgenus *Exothostemon* Woodson), one of *Mesechites* (*M. mansoana* A. DC.) and two of *Secondatia* (*S. densiflora* A. DC. and *S. floribunda* A. DC.).

Only adult, anthetic flowers were selected. Samples were collected in their natural habitat on southeastern Brazil and later identified by the first author, and vouchers were deposited in the herbarium UEC. Flowers of *Mandevilla. tenuifolia* were obtained from cultivated specimens maintained at the greenhouse of the Department of Botany at the University of Campinas, Brazil.

Herbarium specimens. *Macrosiphonia longiflora*: Brazil, Minas Gerais, Carrancas, R.S. Rodrigues *et al.* 1227, X/2001 (UEC); *Mandevilla pycnantha*: Brazil, Minas Gerais, Grão-Mogol, A.O. Simões *et al.* 1176, 24/I/2002 (UEC); *Mandevilla scabra*: Brazil, Bahia, Andaraí, A.O. Simões *et al.* 1127, 19/I/2002 (UEC); *Mandevilla tenuifolia*: Brazil, São Paulo, Atibaia, A.O. Simões *et al.* 1048, X/2000 (UEC).; *Mesechites mansoana*: Brazil, Minas Gerais, Grão-Mogol, A.O. Simões & E.R. Pansarin 1087, 10/II/2001 (UEC); *Secondatia densiflora*: Brazil, Minas Gerais, Serra do Cipó, L.S. Kinoshita *et al.*, 24/IX/2002 (UEC); *Secondatia floribunda*: Brazil, Minas Gerais, Pedra Azul, A.O. Simões & R.B. Singer 1288, IV/2002 (UEC).

Flowers were collected and fixed in FAA (Johanson, 1940) for at least 24 h at room temperature and placed under a low vacuum to aid the penetration of the fixative. The flowers were then stored in ethanol 70%. For anatomical sections only the portion of the corolla surrounding the gynostegium, at the transition zone between the lower and upper corolla tube was used. In *Macrosiphonia longiflora* and *Mandevilla scabra*, the whole upper corolla tube was removed in order to expose the gynostegium. All materials were dehydrated using the tertiary butyl alcohol series, embebbed in paraplast and then sectioned (Johanson, 1940). Longitudinal and transverse serial sections were cut 10-12 μm thick with a rotary microtome. Deparaffinized sections were stained with Safranin O and Astra Blue (Gerlach, 1969). Light microscope observations were carried out on an Olympus BX51 microscope. Photomicrographs were captured with Kodak Pro Image (100 ASA) film and electronically processed using the software Adobe Photoshop 7.0.

RESULTS

A comparison between the gynostegium structures of all studied species is summarized in Table 1. The styles are in number of two and located between the ovary and style-head. They are fused to form a slender tube, becoming enlarged in their upper portion at the base of the style-head. The styles are long and cylindrical in *Macrosiphonia*, *Mandevilla* and *Mesechites* but reduced in *Secondatia*, in which only the enlarged portion is present. In all studied species, the style-head is composed by two portions: the main body, a conical to terete structure located above the styles, and the apical appendages, two expansions of the style-head free from each other and positioned on the top of the main body.

In all species the style-head is covered by a epidermis formed by a single layer of palisade cells, sometimes very elongated. The epidermal cells are longitudinally elongated, have thin cell walls, a relatively large nucleus and dense cytoplasm, a set of characteristics common to secretory cells. This secretory epidermis is absent only at the top of the apical appendages. In the two species of *Secondatia*, the whole surface of the style-head is covered by that epidermis, even in its lower portion below the basal ring and close to the ovary. In all studied species, secretion was observed inside these cells and also recovering the main body of the style-head and adjacent portions of the stamens. On the upper half of the main body close to the base of the anther thecae, these cells reach their maximum length and in some species (e.g., *Mandevilla pycnantha*, *Mesechites mansoana*) a considerable amount of secretion is accumulated outside (Figs. 5.2C; 5.3B,C; 5.4B; 5.6A,C,E).

The apical appendages show great variation in size, from very small, almost inconspicuous in *Macrosiphonia longiflora* and *Mandevilla scabra* (Fig. 5.1B) to well-developed, with almost the same size as the main body of the style-head in *Mandevilla pycnantha* (Fig. 5.1A) and *Mesechites mansoana* (Fig. 5.3A). They are composed in its most part by parenchyma cells covered by a single epidermal layer, secretory in *Secondatia* and non-secretory in the other genera. In three species (*Mandevilla pycnantha*, *M. tenuifolia* and *Mesechites mansoana*) cells from the parenchyma core were intensely stained in red or purple, a condition particularly remarkable in *M. pycnantha*, in which the whole appendages are deeply stained in red (Fig. 5.1A, 5.2A).

In the species of *Macrosiphonia*, *Mandevilla* and *Mesechites*, the parenchyma cells of the main body of the style-head proliferate in five portions and grow towards the androecium, forming five strongly projecting ribs. In *Macrosiphonia* and *Mandevilla*, the ribs are well-developed and extend along the entire length of the main body (Figs. 5.1, 5.2A, 5.7C), but in *Mesechites* the ribs are much shorter and restricted to the base of the main body (Fig. 5.3A,E, 5.7B). The space confined between two adjacent ribs and the surrounding stamens form a chamber closed at the bottom by the extended base of the style-head ribs and opened at the top, partially to completely filled with secretion, in a total of five per flower (secretory chambers of the gynostegium, see details in Discussion). In *Mesechites* these chambers are well-defined only at the base of the style-head, where the ribs are located, being slightly delimited by the 5-angled shape of the main body above (Fig. 5.7D). In *Mandevilla tenuifolia*, *M. pycnantha* and *Mesechites mansoana*, the secretion can surpass the limits of the chambers and fills the space between the apical appendages of the style-head and the anther thecae, as observed.

The style-head of the two studied species of *Secondatia* follows a distinct morphological pattern (Fig. 5.4). The main body is long, cylindrical and smooth, with a small enlarged portion in the form of a ring in its lower portion. No evidence of ribs was found in the main body. In cross sections, the style-head has a circular shape (Fig. 5.4B-D, 5.7C), in contrast to *Macrosiphonia*, *Mandevilla* and *Mesechites*, in which the style-head is strongly pentagonal, at least at base (Fig. 5.1C-E, 5.7E).

The stamens are inserted in the corolla at the level corresponding to the base of the upper tube, forming a cone around the style-head. They are divided in three main portions: anthers, connective and filaments. The anthers are complex structures, formed by a combination of sterile and fertile tissues. The dorsal part is mainly composed by sclerenchyma, extending below the thecae to form two side wings, sagitate in *Secondatia* and more or less cordate in *Macrosiphonia*, *Mandevilla* and *Mesechites*; the thecae are reduced and restricted to the upper half of the ventral part of the anther. The connective is large and divided into two portions: a regular one, between the two thecae, and an expanded one, distributed from the base of the thecae to the region in which the anther side wings are originated (Fig. 8). The expanded part of the connective is composed by unicelular trichomes (*Secondatia*, Figs. 5.4C,E, 5.6F) or parenchyma cells (*Macrosiphonia*,

Mandevilla and *Mesechites*, Figs. 5.5, 5.6B,D), being adjacent or contacting the style-head. In *Mandevilla pycnantha*, *M. tenuifolia* and *Mesechites mansoana*, the expanded connective is flat and formed by a reduced number of parenchyma cells, not clearly distinct from the sterile tissue of the anther (Fig. 5.5A-B, 5.6B). In *Macrosiphonia longiflora* and *Mandevilla scabra* the expanded connective is rounded (*M. scabra*, Fig. 5.5C-D) or triangular (*M. longiflora*, Fig. 5.5E-F), formed by multiple layers of parenchyma cells and clearly distinct from the anther. The filaments are located just below the connective and are densely covered by long unicellular trichomes directed to the base of the style-head. No secretory activity was detected in the stamens.

In *Macrosiphonia*, *Mandevilla* and *Mesechites* the contact between each stamen and the style-head is strong and restricted to one single region (adnation zone). In that zone, the parenchyma cells of the style-head ribs grow towards the androecium and the epidermal cell walls of the style-head are cemented to the epidermal cell walls of the expanded connective (Figs. 5.5, 5.6,B,D). In *Mandevilla tenuifolia* and *Mesechites mansoana*, the adnation is restricted to the base of the style-head, where the projecting ribs reach their maximum size (Table 1; Figs. 5.1C; 5.3A; 5.7B). In *Macrosiphonia longiflora, Mandevilla pycnantha* and *M. scabra* the adnation zone is larger, occurring along the whole extension of the ribs (Table 1; Figs. 5.2A; 5.7C).

In *Secondatia* each stamens touch the style-head in one single zone, but no adnation was observed between these organs. Long, unicellular trichomes of the connective grow directed to the center of the flower (Figs.5.4A,C,E; 5.6F). The tip of the trichomes touches the epidermal cells of the style-head only in a very superficial way, and the stamens can be easily detached from the style-head when flowers are dissected. No proliferation of parenchyma cells was observed in the main body of the style-head or in the connective.

In all species, another region in which stamens and style-head are very close to each other was detected. In that region, the upper part of the style-head next to the apical appendages is adjacent to the base of the anther thecae, but no adnation between the parts was observed (Fig. 5.6A,C,E). The secretory epidermal cells of the style-head reach their maximum size in that region and their cytoplasm are intensely stained in pink or blue.

Evidence from the location of the stigmatic region was observed only in *M. scabra.* In this species, pollen tube growth was detected at the transition zone between the style-

head and the style, on the concave surface just below the projecting ribs. No pollen tube growth was detected in any other species.

DISCUSSION

1. Gynostegium structure and the current classification of Mesechiteae

The structure and organization of the gynostegium are key characters in the taxonomy of Apocynaceae and were used by Endress and Bruyns (2000) and Simões et al. (2004) to characterize Mesechiteae. Our results reinforce the diagnostic value of these characters and make possible to compare the circumscription of the tribe as defined by Endress and Bruyns (2000) with the most recent one proposed by Simões et al. (2004). Some comments are also made on the classification of Pichon (1950).

Mesechiteae sensu Endress and Bruyns (2000) include nine genera that, according to the authors, have a similar structure of the gynostegium: the style-head is typically **pentagonal** in cross section, with five projecting ribs, and strongly attached to the stamens. This set of characters was observed in all studied species of *Macrosiphonia*, *Mandevilla* and *Mesechites* (Figs. 1-3), but not in *Secondatia*. In *S. densiflora* and *S. floribunda*, that represent half of the species of the genus as circumscribed by Morales (2003), the style-head is **circular** in cross section, with no discernible projecting ribs and is very close, but never adnated, to the stamens by trichomes. The distinct gynostegium structure of *Secondatia* makes inconsistent Mesechiteae as circumscribed by Endress and Bruyns (2000), since no one of the morphological characters diagnostic for the tribe were observed in that genus, and corroborates the new circumscription of Mesechiteae proposed by Simões et al. (2004).

The distinction between *Secondatia* and the group formed by *Macrosiphonia*, *Mandevilla* and *Mesechites* was previously noticed by Pichon (1950). In his classification, which was based on the structure of the expanded connective (named by him as"retinacle"), *Mandevilla* (with *Macrosiphonia* in its synonymy) and *Mesechites* were placed in subtribe Mandevilineae of tribe Ichnocarpeae and *Secondatia* in tribe Ecdysanthereae. Ichnocarpeae was defined by the presence of a reduced and concave expanded connective, and Ecdysanthereae for an expanded connective composed by a horseshoe-shaped fringe of

hairs with a narrow longitudinal strip of hairs above. The same differences in the "retinacle" structure were observed in our study, corroborating Pichon's ideas and attesting the taxonomic value of this structure. Pichon (1950) also recognized the affinities of *Secondatia* and *Odontadenia* Benth., a neotropical genus of tribe Apocyneae, and placed both genera in the subtribe Odontadenieae. Pichon's ideas were confirmed later by Simões et al. (2004), who showed that the two genera form a strongly supported clade and that *Secondatia* should be transferred to Apocyneae. The gynostegium structure of one species of *O. hoffmannseggiana* (Steud.) Woodson was described and illustrated by Allorge (1976), showing that the same pattern observed by us in *Secondatia* occurs in *Odontadenia*. Morales (2003) observed that, in *Secondatia*, the style-head do not have longitudinal or basal ribs, and should not be included in Mesechiteae. He then transferred the genus to Echiteae, justifying his position by assuming that the gynostegium of *Secondatia* has a set of characters diagnostic of that tribe, as the anthers strongly attached at two points to the style-head. We do not agree with his proposal however, as our results show that the stamens contact the style-head in one single point, never in two, in *Secondatia*.

The size of the style-head ribs was a character used by Simões et al. (2004) to support the distinction of *Mesechites* from *Macrosiphonia* and *Mandevilla*. Our results confirm that the ribs of *Mesechites* are smaller and restricted to the base of the style-head when compared to *Macrosiphonia* and *Mandevilla*, confirming the taxonomic utility of this character. Even though no significant changes were observed in structure of the gynostegium of the three genera, the larger ribs of *Macrosiphonia* and *Mandevilla* carry some potential evolutive advantages. The extension of the ribs through the main body of the style-head up to the apical appendages increase the contact area between style-head and stamens and, consequently, the surface involved in the adnation process. A stronger adnation leads to a more complex organization of the gynostegium, and, by consequence, to a specialization of the pollination mechanism. This hypothesis, however, is preliminary and should be taken into consideration with caution. This is illustrated by *Mandevilla tenuifolia*, in which the ribs are long but the adnation is restricted to the base of the style-head. It is possible that the extended portion of the style-head ribs is not only involved in the adnation process, but also have other functions like acting as a wall to delimit the areas of production and storage of secretion or also serving as a support to the stamens in the gynostegium

architecture. Observations on the gynoecium of other species from *Macrosiphonia* and *Mandevilla* are necessary to testify this hypothesis.

2. Organization of the gynostegium

The complex organization of the gynostegium in Apocynaceae and its correlation to pollination has been proposed by some authors (Shick, 1980, 1982; Fallen, 1986; Galetto, 1997). Schick (1982) divided the gynostegium of Apocynaceae in three main portions, according to their organizational and functional aspects: 1) the "**stigmatic chamber**", a space delimited by the inner surface of the staminal filaments and the portion of the corolla tube just above the stamens, both densely covered by trichomes; 2) the "**glue zone**", which is composed by five cavities delimited by two contiguous stamens and the main body of the style-head; and 3) the "**pollen depository**", a platform of the style-head just above the glue zone. Functionally, each portion is involved in one aspect of the pollination mechanism. The anthers dehisceise introrsely before flower anthesis and the released pollen accumulates in the pollen depository, with no contact to the stigmatic region. A sticky secretion is produced by the glandular epidermis of the style-head of the glue zone and fulfills its cavities. During the pollination process, secretion of the glue zone touches the pollinator and stick to a mass of pollen in the pollen depository, with the whole unit being carried by the pollinator to another flower. The foreign pollen carried by the pollinator is captured by the staminal and corolline trichomes at the stigmatic chamber and then transferred to the receptive stigmatic surface.

The same organizational pattern was seen in all species, with the three portions of the gynoecium clearly differentiated. We observed, however, that in *Macrosiphonia*, *Mandevilla* and *Mesechites* the **glue zone** of the gynostegium is not homogeneous, but instead formed by two structural and functional portions: the **adnation zone** and the **secretory chambers**. No subdivision of the glue zone was observed in *Secondatia*, again supporting its exclusion from Mesechiteae.

The adnation zone is formed by the area in which the five projecting ribs of the style-head extend towards the androecium and are cemented to the expanded connective. The extension of this zone is variable, from small and restricted to the base of the style-head in *Mesechites mansoana* and *Mandevilla tenuifolia* to large and disposed along the

main body of the style-head in *Macrosiphonia longiflora, Mandevilla pycnantha* and *M. scabra*. The degree of adnation is correlated to the size of the style-head ribs, occurring along their whole longitudinal surface. The only exception is *M. tenuifolia*, in which the ribs are disposed along the main body of the style-head, as usual in *Macrosiphonia* and *Mandevilla*, but only the basal portion is adnated to the stamens, as in *Mesechites*. Even though differences in the extension of the adnation zone were observed, one aspect is constant for all species: the most enlarged portion of the style-head is always adnate to the stamens, corresponding to the whole extension of the style-head ribs in *Macrosiphonia* and *Mandevilla* or to their basal portion in *Mesechites*.

The secretory chambers are in number of five and correspond to the concave space delimited by two contiguous style-head ribs and the surrounding stamens. In *Macrosiphonia longiflora*, *Mandevilla pycnantha* and *M. scabra*, the separation between the chambers is totally made by the adnation zone, which involves both stamens and style-head. In *Mandevilla tenuifolia*, on the other hand, the separation is made by the upper part of the ribs not involved in the adnation process, and in *Mesechites mansoana* it is made by the walls of the style-head. The chambers act as storage areas to the secretion produced in the epidermis of the main body of the style-head. The epidermal cells of the style-head reach their maximum size in that region, especially in the upper part of the chambers where the style-head is adjacent to the base of the thecae, and are less developed or even absent in the adnation zone. Based on the size and distribution of that secretory epidermis, it is possible to speculate that the secretion is produced only in specific areas at the main body of the style-head, not in its whole surface. The secretory activity is concentrated on the epidermis that covers the inner walls of the secretory chambers close to the pollen depository, with the epidermis at the adnation zone having a reduced or even absent activity. No further conclusions can be made with our results, though, and future studies focused on histochemical aspects are necessary to elucidate these questions.

3. Stamen structure and terminology

In Apocynoideae, the stamens are adjacent or adnated to the style-head by an expanded portion of the connective. Pichon (1948) named this region as "retinacle", assuming that it was formed by specializations of the ventral surface of the connective. Our

results are in agreement with Pichon (1948) showing that the "retinacle" is indeed formed by a portion of the connective located below the fertile portion of the anther that extends downwards to the top of the staminal filaments (Fig. 8). We consider, however, that the term "retinacle" is improper and must be avoided for two reasons: 1) it has been used to describe a portion of the pollinarium of subfamilies Asclepiadoideae and Secamonoideae that has a different structure, composition and origin; and 2) this term carries no information about its composition or from which portion of the stamen it is originated. We therefore propose the term "expanded connective", since this name reflects its histological composition and is not used in Asclepiadoideae and Secamonoideae.

Another controversial aspect is to determine the exact extension of the contact between stamens and the style-head. Fallen (1986) described the flower structure of *Mandevilla laxa* (Ruiz & Pav.) Woodson and reported that the lower half of the stamens below the thecae was specialized for adnation with the style-head. Galetto (1997) described the flower structure of *Macrosiphonia petraea* (A. St.-Hil.) K. Schum., *Mandevilla pentlandiana* (A. DC.) Woodson and *M. laxa*, but made no comments about the relation between stamens and style-head. Some statements, however, can be made based exclusively on his schemes of the gynostegium structure of *Macrosiphonia petraea*. In his drawings, the style-head is adnated to the stamens in two zones, one between the base of the style-head and the sterile portion of the stamens and the other between the upper portion of the style-head with the fertile portion of anthers. Our results are in agreement with Fallen (1986), with only the expanded connective involved in the adnation process in one single zone. We believe that the results obtained by Galetto (1997) are questionable and could be explained in two ways: the difficulties of interpreting the complex, tri-dimensional structure of the gynostegium of *Macrosiphonia*; and the close proximity between the thecae and the upper portion of the style-head at the zone of the pollen depository, which can be easily misinterpreted as adnation in longitudinal sections.

Conclusions - In general terms, the main conclusions of our study are: 1) The gynostegium structure of *Macrosiphonia*, *Mandevilla* and *Mesechites* follows the same pattern: the style-head have five projecting longitudinal ribs formed by a ploriferation of parenchyma cells that grow towards the stamens. The epidermal cells are cemented to the

epidermal cells of the expanded connective in one single zone. In *Secondatia*, however, the style-head has no projecting ribs and unicellular trichomes of the anther are adjacent to the epidermal cells of the style-head, but never cemented to them, a pattern already described for species of Apocyneae; 2) The distinction between the gynostegium morphology of *Secondatia* to *Macrosiphonia*, *Mandevilla* and *Mesechites*, supports the new circumscription of Mesechiteae of Simões et al. (2004), who proposed the replacement of *Secondatia* to another tribe, Apocyneae; 3) The division of the gynostegium in three organizational and functional portions (stigmatic chamber, glue zone and pollen depository) proposed by Shick (1982) was observed in all studied species. In *Macrosiphonia*, *Mandevilla* and *Mesechites*, however, the glue zone is not homogeneous and can be divided in another two structural and functional portions: the adnation zone and the secretory chambers; 4) The expanded portion of the connective just below the thecae is the part of the stamens just below the thecae adjacent or adnated to the style-head that form the gynostegium.

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Table 1. Summary of the gynostegium structure in the seven studied species of Mesechiteae. + = present; - = absent.

Species	Style	Style head in transection	Projecting ribs	Secretory epidermis	"Glue zone" of the style head	Expanded connective	Adnation
Macrosiphonia longiflora	Entire	pentagonal	Present, along the whole main body	Partially covering the style head	Heterogeneous, divided in adnation zone and secretory chambers	Parenchyma cells, triangular	+, one zone along the whole main body
Mandevilla pycnantha	Entire	Pentagonal	Present, along the whole main body	Partially covering the style head	Heterogeneous, divided in adnation zone and secretory chambers	Parenchyma cells, flat	+, one zone along the whole main body
Mandevilla scabra	Entire	Pentagonal	Present, along the whole main body	Partially covering the style head	Heterogeneous, divided in adnation zone and secretory chambers	Parenchyma cells, circular	+, one zone along the whole main body
Mandevilla tenuifolia	Entire	Pentagonal	Present, along the whole main body	Partially covering the style head	Heterogeneous, divided in adnation zone and secretory chambers	Parenchyma cells, flat	+, one zone at the base of the main body
Mesechites mansoana	Entire	Pentagonal	Present, at the base of the main body	Partially covering the style head	Heterogeneous, divided in adnation zone and secretory chambers	Parenchyma cells, flat	+, one zone at the base of the main body
Secondatia densiflora	Reduced to the enlarged apex	Circular	Absent	Totally covering the style head	Homogeneous	Layer of unicellular trichomes	-
Secondatia floribunda	Reduced to the enlarged apex	Circular	Absent	Totally covering the style head	Homogeneous	Layer of unicellular trichomes	-

FIGURE LEGENDS

Figure 1. Longitudinal sections of the gynostegium of *Macrosiphonia* and *Mandevilla*. A, *Mandevilla pycnantha*. B, *Mandevilla scabra*. C, *Mandevilla tenuifolia*. D, *Macrosiphonia longiflora*. a, anther; c, connective; f, filament; sh, style-head. The transition between connective and filament is indicated by a traced line.

Figure 2. Gynostegium structure of *Mandevilla pycnantha*. A, general view; numbers 1,2,3 and 4 correspond to the level of the transections illustrated in figures 4B,C,D and E, respectively. B, upper portion of the gynostegium, with the anther thecae adjacent to the style-head epidermis. C, adnation zone at the top of the style-head ribs. D, middle portion of the adnation zone. E, base of style-head, in which the style-head ribs reach their maximum size and adnation is stronger. A, longitudinal sections. B,C,D,E, cross sections. a, anther; c, connective; f, filament; sh, style-head. The transition between connective and filament is indicated by a traced line.

Figure 3. Gynostegium structure of *Mesechites mansoana*. A, general view. B,C, the anther thecae are adjacent to the style-head. D,E, adnation zone. A,B,E, longitudinal sections. C,D, cross sections. a, anther; c, connective; f, filament; sh, style-head. Secreted products in B and C are indicated by arrows. The transition between connective and filament is indicated by a traced line.

Figure 4. Gynostegium structure of *Secondatia*. A-C, *S. densiflora*. D-E, *S. floribunda*. A, general view. B, upper portion of the gynostegium. C, lower portion of the gynostegium, showing the proximity between staminal trichomes and the epidermal cells of the style-head. D, base of the style-head. E, staminal trichomes, adjacent to the epidermis of the style-head. A,E, longitudinal sections. B,C,D, crosss sections. a, anther; c, connective; f, filament; sh, style-head.

Figure 5. Cross sections of the gynostegium of *Mandevilla* and *Macrosiphonia*, showing the adnation zone in detail. A,B, *Mandevilla pycnantha*, connective flat. C,D, *Mandevilla*

scabra, connective enlarged. E,F, *Macrosiphonia longiflora*, connective enlarged. a, anther; c, connective; sh, style-head.

Figure 6. Cross sections of the zone in which the anther thecae are adjacent to the epidermis of the style-head (A,C,E,) and the zone in which the expanded connective contact (B,D) or is adjacent (F) to the epidermis of the style-head. A,B, *Mandevilla tenuifolia.* C,D, *Mesechites mansoana.* E,F, *Secondatia densiflora.* a, anther; c, connective; s, secretion; sh, style-head.

Figure 7. Style-head structure of *Secondatia*, *Mesechites* and *Macrosiphonia.* A,D, *Secondatia densiflora.* B,E, *Mesechites mansoana.* C,F. *Macrosiphonia longiflora.* D-F, cross sections of A,B and C, respectively. The dashed lines indicate the level in which the cross sections were made. The adnation zone is painted in gray.

Figure 8. Stamens of *Secondatia*, *Mesechites* and *Macrosiphonia.* A, *Secondatia densiflora.* B, *Mesechites mansoana.* C, *Macrosiphonia longiflora.* The expanded connective is painted in gray.

Figure 5.1 – Gynostegium of Mesechiteae

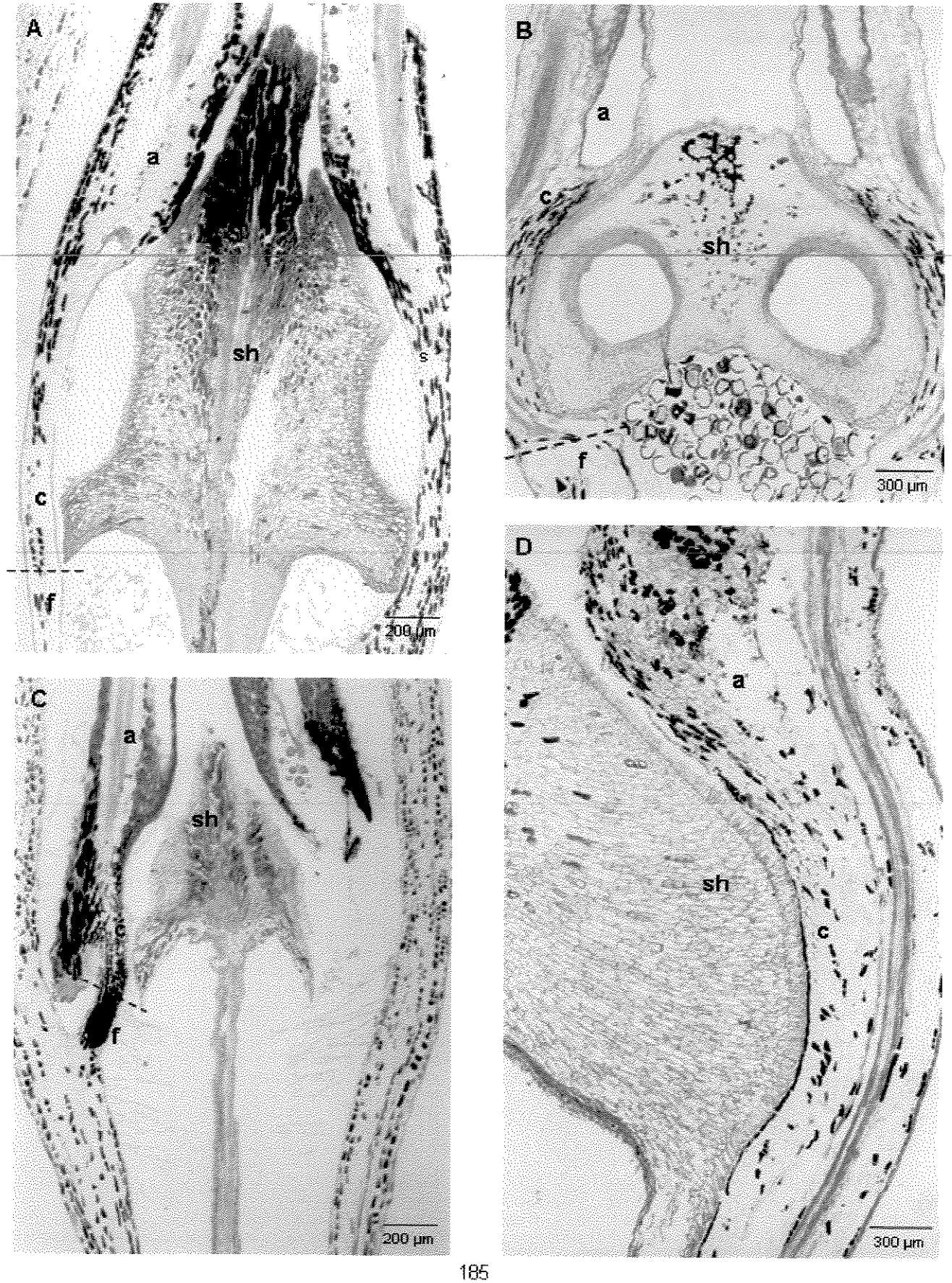


Figure 5.3 – Gynostegium of Mesechiteae

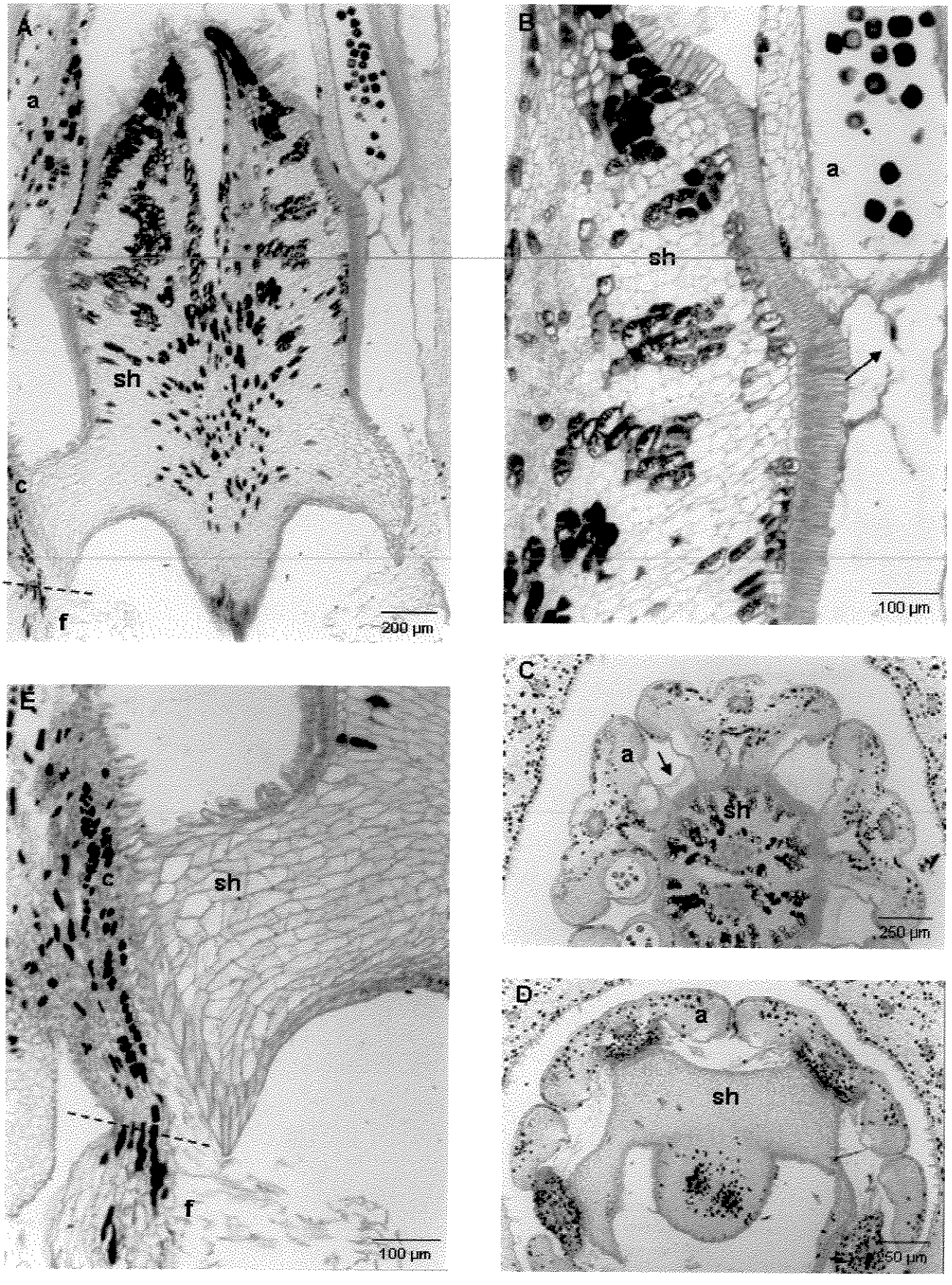


Figure 5.4 – Gynostegium of Mesechiteae

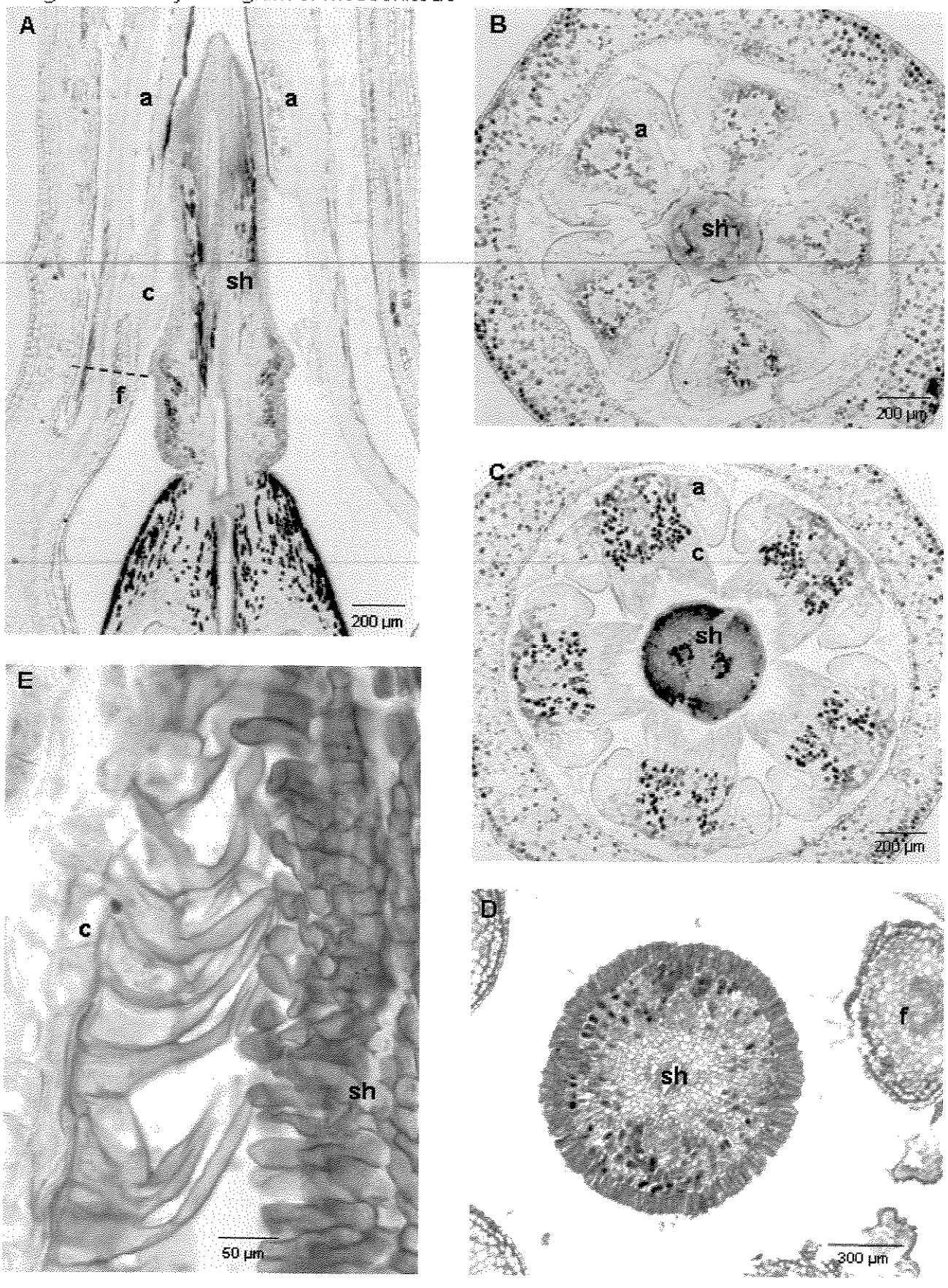


Figure 5.5 Gynostegium of Mesechiteae

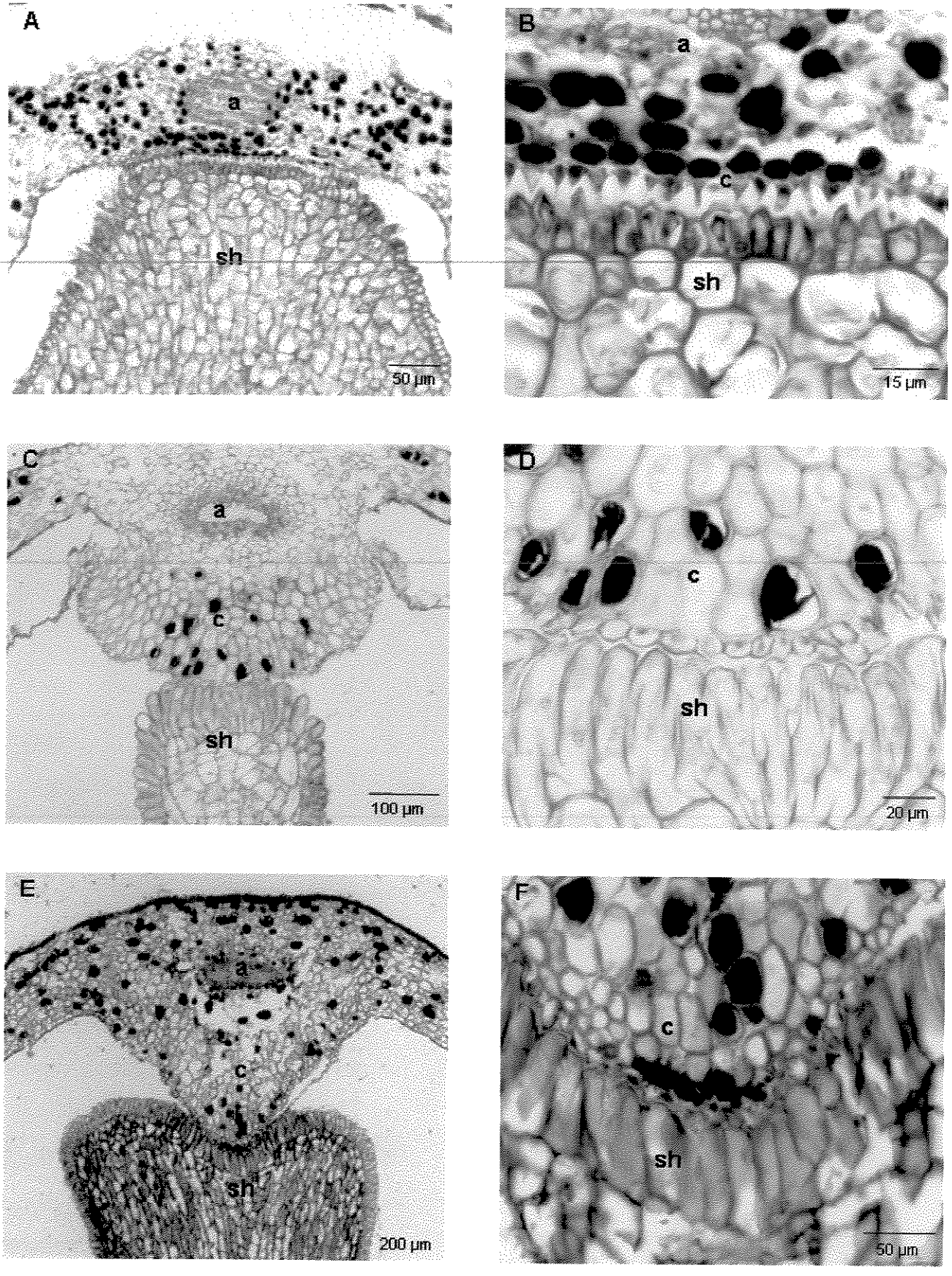


Figure 5.6 – Gynostegium of Mesechiteae

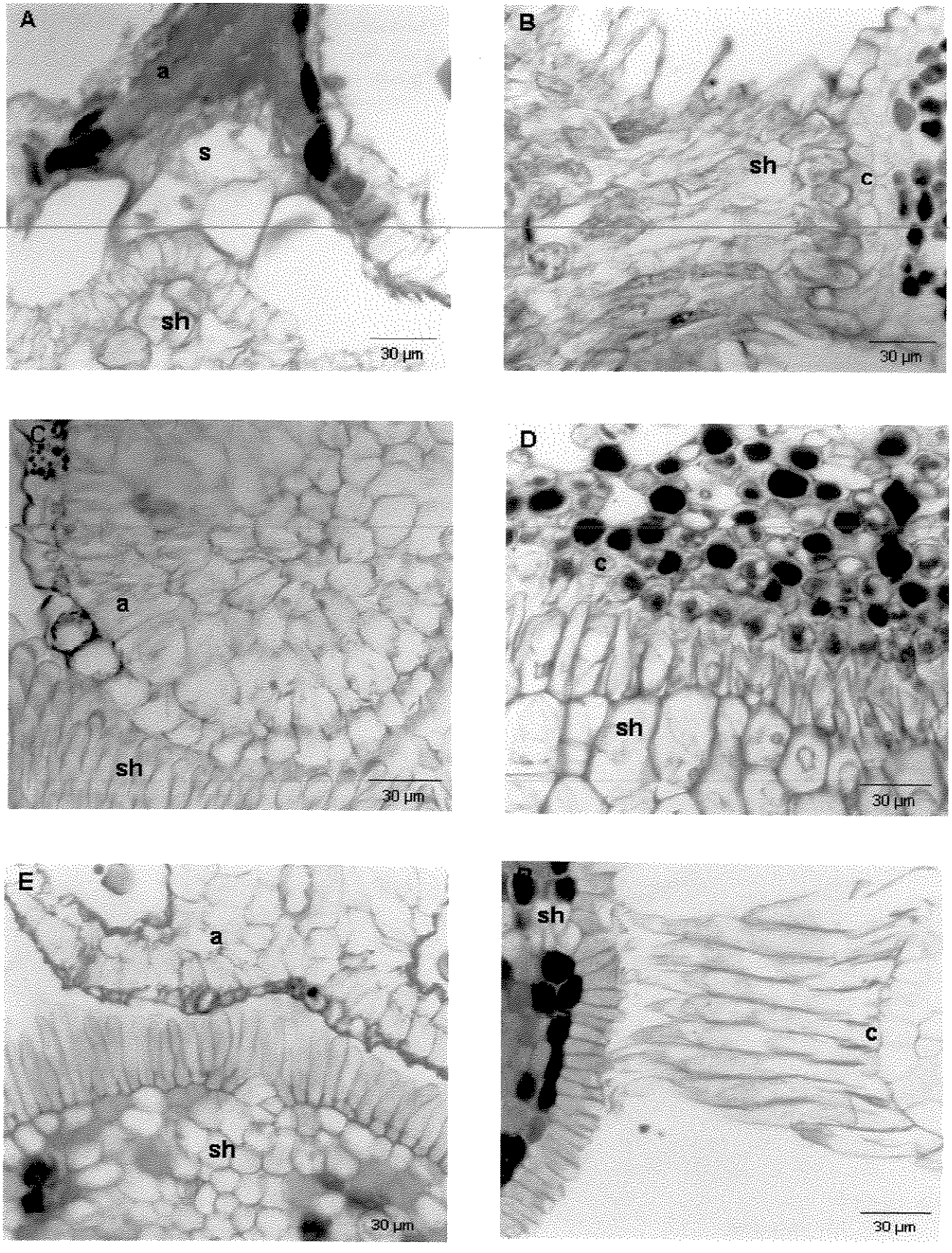


Figure 5.7 **Gynostegium of Mesechiteae**

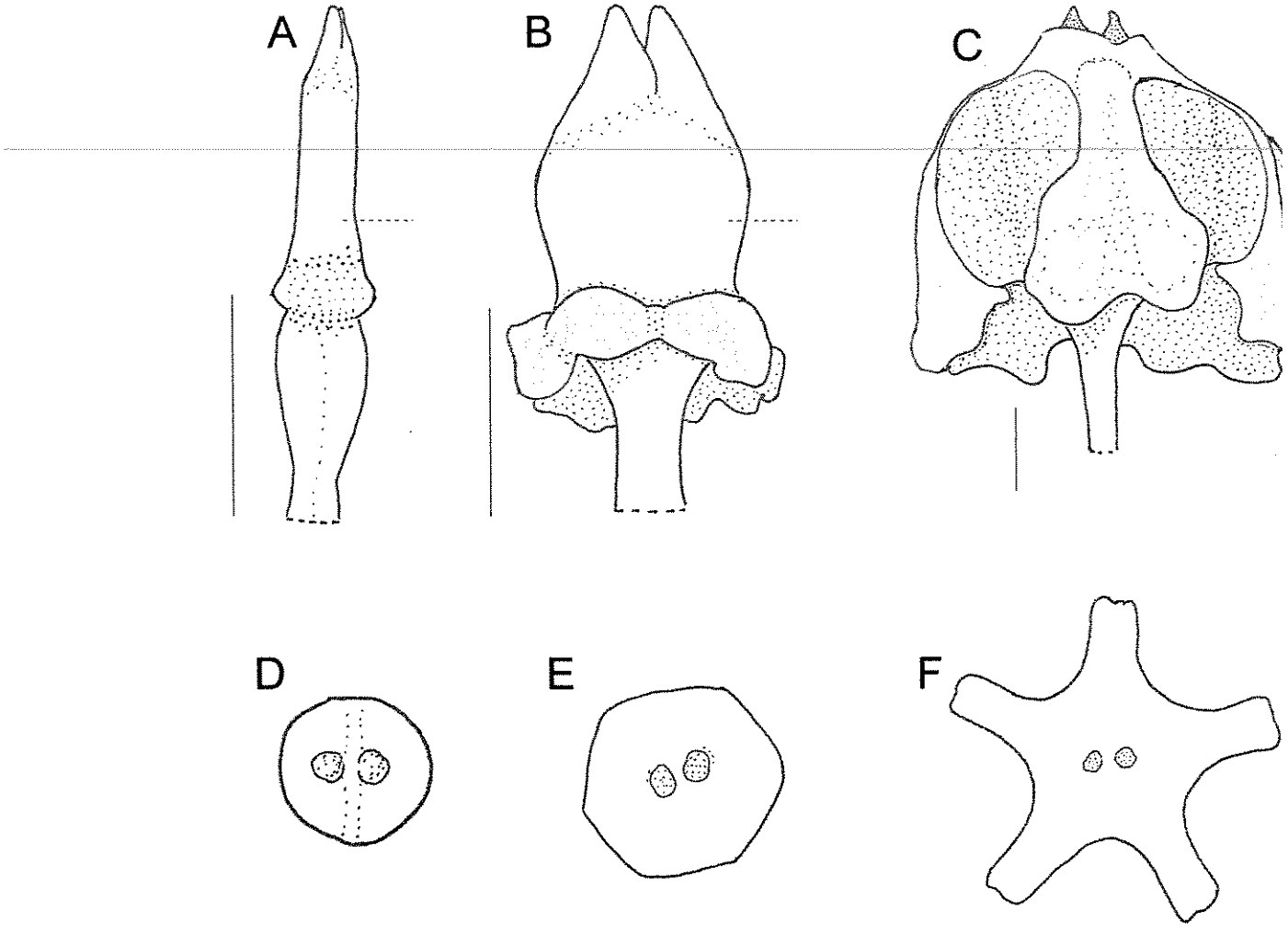
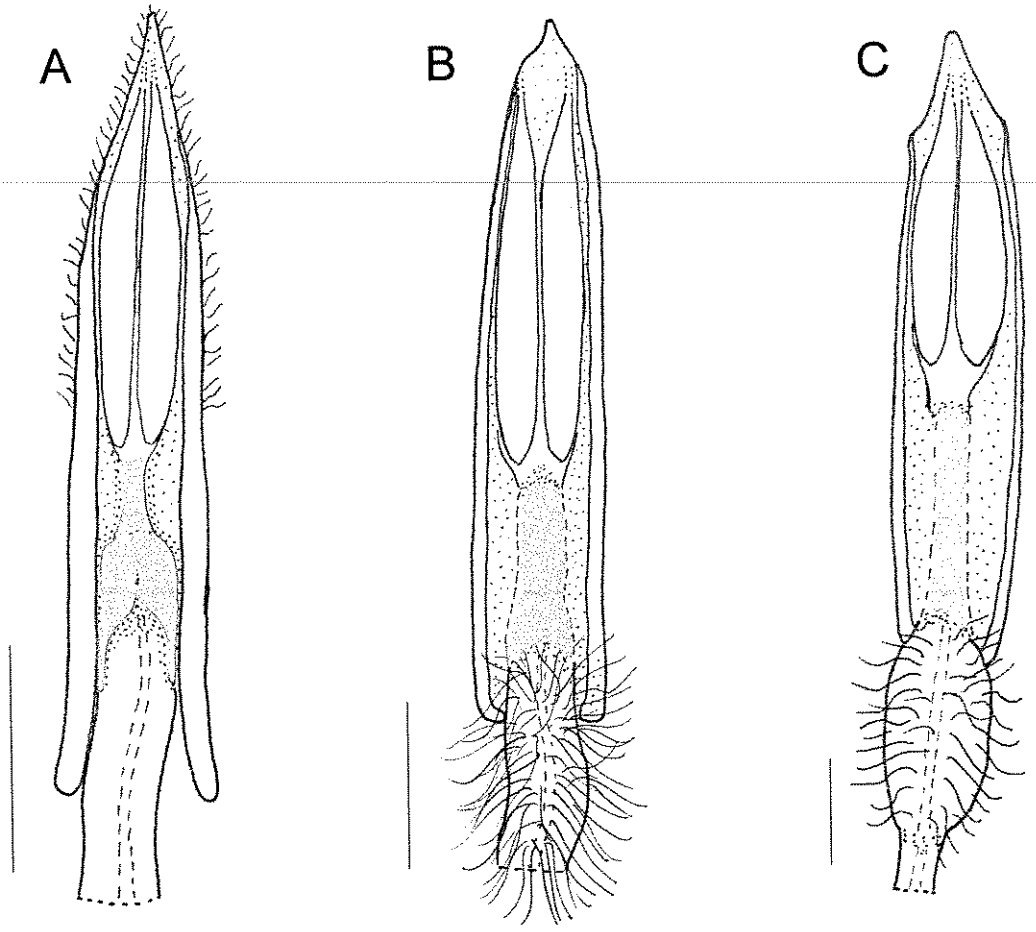


Figure 5.8 **Gynostegium of Mesechiteae**



CONCLUSÕES GERAIS

1) A tribo Mesechiteae *sensu* Endress & Bruyns (2000) não é monofilética. Para torná-la monofilética, são propostas a exclusão dos gêneros *Galactophora* e *Secondatia* e a inclusão de *Forsteronia*. A tribo assim circunscrita é sustentada por quatro caracteres morfológicos: presença de coléteres na base da lâmina foliar; anteras com base auriculada a truncada; cabeça do estilete com cinco projeções longitudinais ao menos em sua base; e adnação entre estames e cabeça do estilete envolvendo fusão celular. A análise da estrutura do ginostégio corroborou estes resultados, com o mesmo padrão morfológico encontrado em espécies de *Macrosiphonia*, *Mandevilla* e *Mesechites*: a cabeça do estilete apresenta cinco projeções laterais formadas pela proliferação de células parenquimáticas do corpo principal da cabeça do estilete, estando estas projeções adnatas, ao menos em parte, com o conectivo expandido dos estames localizado logo abaixo da porção fértil da antera.

2) A exclusão de *Secondatia* da tribo Mesechiteae e seu reposicionamento na tribo Apocyneae também é sustentada nos estudos anatômicos realizados com *S. densiflora* e *S. floribunda*. Ambas as espécies possuem um padrão estrutural do ginostégio diferente do observado em *Macrosiphonia*, *Mandevilla* e *Mesechites*: a cabeça do estilete é lisa, sem projeções laterais, e o contato entre os estames e a cabeça do estilete é tênue, sem adnação entre as partes. Tricomas unicelulares originados no conectivo expandido dos estames crescem em direção à cabeça do estilete e entram em contato com as células epidérmicas do mesmo. A organização alterna dos coléteres calicinais em *Secondatia* também segue um padrão diferente do observado em *Mandevilla*, com apenas cinco séries de um coléter ao invés de 10 séries de 1-3 coléteress cada, mas os resultados são pouco conclusivos para sustentar o posicionamento do gênero.

3) A posição taxonômica do gênero *Galactophora* em Apocynaceae é incerta; no entanto, e possível concluir com base em nossos estudos de filogenia que *Galactophora* não está relacionado com os demais gêneros do clado Mesechiteae. Morfologicamente, as projeções na cabeça do estilete presentes neste gênero não são homólogas às projeções observadas nos gêneros de Mesechiteae.

4) *Forsteronia* é um gênero que apresenta considerável variação morfológica na estrutura floral, mas sua inclusão em Mesechiteae é sustentada por apresentar todos os caracteres morfológicos que definem este clado.

5) A estreita relação entre os gêneros *Allomarkgrafia*, *Mesechites* e *Tintinnabularia* é comprovada, com estes formando um clado fortemente sustentado e apresentando uma importante sinapomorfia morfológica: as projeções da cabeça do estilete estão restritas à base da cabeça do estilete. *Tintinnabularia* e *Mesechites* são gêneros reconhecidos, porém não *Allomarkgrafia*, que deve ser incluído na sinonímia de *Mesechites*.

6) Vários dos caracteres morfológicos tradicionalmente utilizados na taxonomia de Mesechiteae, como a presença de domácias, presença e organização de coléteres calicinais, forma da corola, tamanho dos filetes das anteras e tamanho dos nectários, são homoplásicos e, portanto, possuem baixo valor filogenético.

7) *Macrosiphonia* e *Telosiphonia* não são congenéricos, mas sim formam clados distintos dentro de *Mandevilla*; desta forma, não constituem gêneros reconhecidos e devem ser incluídos na sinonímia deste último. A similaridade morfológica entre os dois gêneros constitui um notável exemplo de convergência adaptativa a um mesmo tipo de habitat e possivelmente ao mesmo tipo de polinizador (esfingofilia).

8) *Quiotania* também deve ser incluído na sinonímia de *Mandevilla*. A única espécie conhecida para o gênero, *Q. colombiana*, foi reconhecida como sinônimo de *Mandevilla ligustrifolia* após comparação com os fototipos e descrições das mesmas. Baseado na posição de *M. ligustrifolia* no grande clado *Mandevilla* observada na análise filogenética, o reconhecimento do status genérico de *Quiotania* implicaria em profundas modificações na taxonomia de *Mandevilla*, *Macrosiphonia* e *Telosiphonia*, razão pela qual decidiu-se por incluí-lo na circunscrição de *Mandevilla*.

9) *Mandevilla sensu* Woodson (1933) não é um gênero monofilético, tal como é *Mandevilla sensu* Pichon (1948). A diferença entre as duas circunscrições está no fato de que Pichon (1948) incluiu o complexo *Macrosiphonia/Telosiphonia* na sinonímia de *Mandevilla*, enquanto Woodson elevou este complexo à categoria genérica em *Macrosiphonia*.

10) Com exceção da seção *Tenuifoliae*, as categorias infra-genéricas de *Mandevilla* propostas por Woodson (1933) não são monofiléticas. Das quatro seções propostas por Pichon (1948), duas (*Megasiphon* e *Telosiphonia*) são monofiléticas, mas as duas restantes (*Orthocaulon* e *Exothostemon*, que correspondem aos subgêneros *Mandevilla* e *Exothostemon* sensu Woodson (1933), respectivamente), não são monofiléticas.

11) Em *Mandevilla*, todas as espécies do subgênero *Exothostemon* apresentam um conjunto de caracteres morfológicos distintos, e estão incluídas em um clado (clado I). A presença de três espécies do subgênero *Mandevilla* neste clado (*M. callista*, *M. funiformis* e *Mandevilla sp.*) é sustentada do ponto de vista morfológico, uma vez que estas apresentam características intermediárias entre as que definem os dois subgêneros. Por outro lado, a inclusão de *Macrosiphonia* neste mesmo clado é de certa forma surpreendente, pela ausência de sinapomorfias morfológicas.

12) Os subclados de *Mandevilla* são bastante heterogêneos do ponto de vista morfológico, mas alguns destes apresentam uma notável uniformidade do ponto de vista geográfico (clado III, espécies distribuídas no centro e sul da América do Sul; clado IV, predominantemente espécies do México, América Central e norte da América do Sul; clado VI, espécies distribuídas no México e sudoeste dos Estados Unidos; clado V, espécies predominantemente distribuídas no norte da América do Sul).

13) Dos caracteres morfológicos tradicionalmente usados na delimitação de categorias infra-genéricas em *Mandevilla*, muitos se mostraram incongruentes com a filogenia obtida (p.ex., hábito, forma, cor e dimensões da corola, curvatura do tubo inferior da corola), enquanto outros (disposição dos coléteres foliares e calicinais, forma da base das anteras,

proporção entre os apêndices apicais e o corpo principal da cabeça do estilete, número e tamanho dos nectários) mostraram-se filogeneticamente informativos.

14) Quatro tipos estruturais de coléteres calicinais foram observados: a. tipo "standard", com um corpo principal secretor, grande e inteiriço sustentado por um pedúnculo curto, não secretor; b. tipo bifurcado, semelhante ao "standard" exceto pela bifurcação do corpo principal em sua metade superior; c. laminar, também semelhante ao "standard", porém diferindo por apresentar o pedúnculo engrossado e o corpo principal laminar; d. séssil, formado por um corpo principal grande e inteiriço e uma base bastante desenvolvida, não pedunculada e também secretora. Os novos tipos descritos podem ter se originado do tipo standard a partir de três mecanismos distintos: separação, proliferação e alongamento celular.

15) Não foi observado um padrão estrutural e/ou organizacional dos coléteres calicinais característico para Mesechiteae. A combinação destes aspectos, no entanto, revela-se potencialmente úteis para a identificação de grupos menores dentro da tribo, como a presença de 5 coléteres laminares e opostos em espécies de *Mandevilla* subgênero *Exothostemon* e coléteres do tipo séssil em *Mesechites*..

16) O número de coléteres calicinais pode ser bastante variável, servindo como um bom caracter taxonômico apenas quando combinado com aspectos estruturais e de distribuição destas glândulas, como no caso dos cinco coléteres laminares de distribuição oposta em *Mandevilla scabra*.

17) Três padrões organizacionais de coléteres calicinais foram observados: alterno, oposto ou contínuo. Quando alternos, podem estar dispostos de três formas diferentes. Na primeira, observado em *Secondatia densiflora*, os coléteres estão agrupados em cinco séries de uma glândula cada, na margem das duas sépalas internas e na margem interna da sépala intermediária. Na segunda, observada em *S. floribunda*, estão agrupados em quatro séries de uma glândula cada, com um coléter ausente na margem interna da sépala intermediária.

Na terceira, observada em *Mandevilla pycnantha* e *M. tenuifolia*, 10 séries de uma a três glândulas ocorrem na margem de todas as sépalas, duas por sépala.

18) A divisão do ginostégio em três unidades funcionais (câmara estigmática, zona de adesão e zona acumuladora de pólen), proposta por Shick (1982) foi observada nas espécies estudadas. Para os gêneros *Macrosiphonia*, *Mandevilla* e *Mesechites*, no entanto, a zona de adesão pode ser subdividida em duas porções, uma envolvida com o processo de adnação, e a outra envolvida na produção e acúmulo de secreção.

19) A estrutura dos estames envolvida na formação do ginostégio e que está adjacente ou adnata à cabeça do estilete corresponde a uma expansão do conectivo sobre a porção estéril e esclerificada da antera. Esta estrutura foi denominada de retináculo por Pichon (1948), porém este nome é inadequado para descrevê-la e deve ser substituído pelo termo conectivo expandido.