

Filogenômica, morfologia e taxonomia na tribo  
Malmeeae (Malmeoideae, Annonaceae): implicações  
na evolução da androdioicia

Phylogenomics, morphology and taxonomy in tribe  
Malmeeae (Malmeoideae, Annonaceae): implications on  
the evolution of androdioecy

Jenifer de Carvalho Lopes

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Prof. Dr. Renato de Mello-Silva

Orientador

*Metafísica? Que metafísica têm aquelas árvores?*

*A de serem verdes e copadas e de terem ramos*

*E a de dar fruto na sua hora, o que não nos faz pensar,*

*A nós, que não sabemos dar por elas.*

*Mas que melhor metafísica que a delas,*

*Que é a de não saber para que vivem*

*Nem saber que o não sabem?*

Alberto Caeiro, heterônimo de Fernando Pessoa

Trecho de Guardador de Rebanhos, poema quinto

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**Resumo.** As flores possuem uma grande variedade de sistemas sexuais. O mais raro deles é a androdioicia, com poucos casos conhecidos, no qual as espécies apresentam indivíduos ou com flores masculinas ou com flores bissexuais. A maioria das espécies androdioicas evoluiu de ancestrais dioicos, sendo geralmente plantas herbáceas em populações com recorrente extinção local seguida de re-colonização. No entanto, há alguns exemplos de espécies androdioicas e lenhosas cujos ancestrais são hermafroditas. Este é o caso das Annonaceae, uma família pantropical de plantas lenhosas e predominantemente hermafrodita, no qual a androdioicia é frequente. Gêneros androdioicos surgiram várias vezes em diferentes linhagens de Annonaceae, tanto na subfamília Annonoideae, quanto na subfamília Malmeoideae. A maioria dos gêneros androdioicos pertence à subfamília Malmeoideae, um grupo predominantemente asiático. Nas Américas há cinco gêneros androdioicos, todos da tribo Malmeeae, que formam um grupo monofilético, com exceção de *Pseudoxandra* que, embora membro de Malmeeae, não está incluído no clado dos gêneros androdioicos. Assim, a tribo Malmeeae é um excelente modelo para o estudo da androdioicia em plantas lenhosas neotropicais. Para tanto a reconstrução da filogenia de 33 táxons da tribo Malmeeae foi realizada a partir de sequências de DNA de 66 marcadores moleculares do genoma do cloroplasto, obtidas por sequenciamento de nova geração. Foram realizadas análises de máxima verossimilhança, máxima parcimônia e inferência Bayesiana. A reconstrução dos estados ancestrais de caracteres relacionados ao sistema sexual e morfologia da flor foi realizada numa abordagem Bayesiana. Análises morfo-anatômicas das flores masculinas e bissexuais de *Pseudoxandra spiritus-sancti*, uma espécie androdioica, foram feitas com microscopia ótica e MEV. Por último, a filogenia morfológica e a revisão taxonômica de *Ephedranthus*, um gênero androdioico da tribo Malmeeae, são apresentadas.

**Abstract.** Flowers have a high diversity of sexual systems. The rarest among them is androdioecy, in which species present individuals with male flowers and others with bisexual flowers. The majority of androdioecious species, usually herbaceous plants with recurrent local extinction followed by re-colonization, has evolved from dioecious ancestors. Nevertheless, some woody and androdioecious plants have hermaphrodite ancestors. This is the case of Annonaceae, a pantropical family of woody and hermaphrodite plants, in which androdioecy is frequent. Androdioecious genera have arisen several times in different Annonaceae lineages, both in Annonoideae subfamily and in Malmeoideae subfamily. The majority of androdioecious genera belong to Malmeoideae, a mostly Asian group. In the Americas there are five androdioecious genera, all from tribe Malmeeae, where they compose a monophyletic group, with the exception of *Pseudoxandra*, which although being a member of Malmeeae, is not included in the androdioecious genera clade. Tribe Malmeeae is, thus, an excellent model to study androdioecy in Neotropical woody plants. The phylogenetic reconstruction of 33 taxa of this group was performed using DNA sequences of 66 molecular markers of the chloroplast genome, sequenced by next generation sequencing. Maximum likelihood, Bayesian inference and maximum parsimony were the methods used for the phylogenetic analyses. The reconstruction of ancestral states were performed to characters related to sexual system and floral morphology using a Bayesian approach. Morphological and anatomical analyses of male and bisexual flowers were done using LM and SEM. A morphological phylogeny and a taxonomic revision of *Ephedranthus*, an androdioecious genus of tribe Malmeeae, are also presented.

## Introdução

A flor é uma das estruturas que permitem reconhecer as angiospermas e também uma das principais novidades evolutivas deste grupo. As flores são as estruturas reprodutivas com maior variação nos seres vivos (Barrett 2002). A maioria das flores é bissexual, e apenas cerca de 10% são unissexuais (Barrett 2002). No entanto, essa pequena parcela das angiospermas que possuem flores de um único sexo exibe uma grande complexidade de sistemas sexuais. Os sistemas sexuais baseados na distribuição espacial dos órgãos reprodutivos masculinos e femininos podem ser classificados em sistemas monomórficos, com apenas uma categoria de indivíduos, e em sistemas dimórficos, no qual há duas classes de indivíduos. Sistemas sexuais monomórficos incluem o hermafroditismo, em que indivíduos têm flores bissexuais, a monoícia, na qual indivíduos têm flores masculinas e femininas, a andromonoícia, em que indivíduos têm flores masculinas e bissexuais, e a ginomonoícia, na qual indivíduos têm flores femininas e bissexuais. Já os sistemas sexualmente dimórficos incluem a dioícia, na qual indivíduos têm flores ou femininas ou masculinas, a ginodioícia, em que indivíduos têm flores ou femininas ou bissexuais, e a androdioícia, na qual indivíduos têm flores ou masculinas ou bissexuais (Bawa & Beach 1981).

A evolução dessa diversidade de sistemas sexuais se deve à competição reprodutiva controlada pela seleção sexual. O sucesso reprodutivo paterno é limitado pela dispersão do pólen para os estigmas das flores da mesma espécie, enquanto que o sucesso materno é dependente da quantidade de recursos nutricionais disponíveis para o desenvolvimento dos embriões, sementes e frutos (Charnov 1979). A dinâmica do sistema de polinização é um fator importante na determinação destes padrões sexuais, porque afeta a movimentação do pólen entre flores da mesma espécie (Bawa & Beach 1981). Dessa forma, cada sistema sexual é uma estratégia distinta para a alocação diferencial dos recursos paternos e maternos (Bawa & Beach 1981).

As mutações que causam esterilidade em um dos sexos teriam sido as responsáveis pela origem das flores unissexuais e, conseqüentemente, pela evolução da complexidade dos sistemas sexuais. Há duas vias evolutivas principais para a origem das plantas dioicas a partir de ancestrais hermafroditas (Barrett 2002). Estas vias incluem estágios intermediários com plantas ginodioicas, monoicas e andromonoicas (Ross 1982). O estágio final dessas vias é a androdioícia a partir de ancestrais dioicos

(Barrett 2002). A androdioicia, na qual há indivíduos com flores bissexuais e indivíduos com flores masculinas, é um sistema sexual muito raro, com poucos casos confirmados, seja em plantas, seja em animais (Pannell 2002). Espécies androdioicas são raras talvez porque os indivíduos masculinos devem ter a capacidade de deixar duas vezes mais descendentes do que os hermafroditas para conseguirem se perpetuar na população (Lloyd 1975, Charlesworth 1984). A maioria dos casos de plantas androdioicas evoluiu de ancestrais dioicos (Pannell 2002). As espécies androdioicas são geralmente de porte herbáceo e com populações que sofrem recorrente extinção local seguida de recolonização (Pannell 2001). No entanto, há algumas espécies androdioicas em que os ancestrais são hermafroditas, como em Oleaceae, um grupo de plantas lenhosas (Ishida & Hiura 1998, Lepart & Dommée 1992). Porém, a melhor compreensão destes casos requer mais estudos (Pannell 2002).

Outro grupo de plantas lenhosas, no qual a androdioicia é frequente, é Annonaceae. (Saunders 2010). Annonaceae, uma família pantropical, possui cerca de 2.440 espécies em 109 gêneros (Chatrou *et al.* 2012). Dentro da família, o gênero *Anaxagorea* é o grupo-irmão de todas as Annonaceae (Doyle & Le Thomas 1996, Doyle *et al.* 2000, Richardson *et al.* 2004) e o clado Ambavioide, com nove gêneros, emerge em sequência, como grupo-irmão das demais Annonaceae, exceto *Anaxagorea* (Doyle & Le Thomas 1996, Richardson *et al.* 2004). *Anaxagorea* está agora classificada na subfamília Anaxagoreoideae e o clado Ambavioide na subfamília Ambavioideae (Chatrou *et al.* 2012). Os gêneros restantes aparecem incluídos em dois grandes clados (Richardson *et al.* 2004). Um destes clados, correspondente ao grupo *Inaperturado* (Doyle & Le Thomas 1996, Doyle *et al.* 2000) e ao *Long Branch Clade* (Richardson *et al.* 2004), inclui a maioria das espécies de Annonaceae, cerca de 1.500, que possui pólen inaperturado. Este grupo corresponde, na atual classificação, à subfamília Annonoideae (Chatrou *et al.* 2012). O outro clado, denominado MPM (Malmeoides, Piptostigmoides e Miliusoides) por Doyle *et al.* (2000) ou *Short Branch Clade* por Richardson *et al.* (2004), inclui os grupos com pólen sulcado e o grupo Miliusoide, com pólen inaperturado (Doyle & Le Thomas 2012). Este grupo é tratado como a subfamília Malmeoideae (Chatrou *et al.* 2012). A maioria das Annonaceae possui flores hermafroditas, porém há vários gêneros androdioicos em diferentes linhagens (Chatrou *et al.* 2012, Saunders 2010). A androdioicia é encontrada nas duas subfamílias, Annonoideae e Malmeoideae. Em Annonoideae, há espécies androdioicas em *Anonidium* e *Annona* (van Heusden 1992, Westra 1995). Já na subfamília Malmeoideae

os gêneros androdioicos são *Greenwayodendron* e *Polyceratocarpus*, tribo Piptostigmateae (van Heusden 1992), *Pseuduvaria* e *Trivalvaria*, tribo Miliuseae (Su & Saunders 2006, van Heusden 1997) e *Ephedranthus*, *Klarobelia*, algumas espécies de *Oxandra*, *Pseudephedranthus* e *Pseudomalmea*, tribo Malmeeae (Chatrou 1998, Chatrou *et al.* 2012, Fries 1931, Oliveira & Sales 1999, van Heusden 1992).

A maioria dos gêneros androdioicos pertence à subfamília Malmeoideae. Neste grupo, há um exemplo de evolução da androdioicia a partir de ancestrais dioicos em algumas espécies de *Pseuduvaria*, tribo Miliuseae (Su *et al.* 2008). No entanto, este não deve ser o caso dos demais táxons de Annonaceae, uma vez que o hermafroditismo é plesiomórfico na família (Saunders 2010). Na tribo Malmeeae estão incluídos todos os gêneros androdioicos da região neotropical, exceto por algumas espécies de *Annona* (Paulino-Neto & Teixeira 2006). Além dos cinco gêneros androdioicos mencionados acima, *Pseudoxandra spiritus-sancti* Maas, outra espécie da tribo Malmeeae, revelou-se androdioica, no primeiro registro de androdioicia no gênero (Lopes & Mello-Silva 2014). Por contar com praticamente todas as espécies androdioicas da região neotropical, Malmeeae é um excelente modelo para o estudo deste caráter em plantas lenhosas neotropicais.

Malmeeae é um grupo monofilético e inclui todos os gêneros neotropicais da subfamília Malmeoideae, que é predominantemente asiática. *Ephedranthus*, *Klarobelia*, *Oxandra*, *Pseudephedranthus* e *Pseudomalmea*, os gêneros androdioicos de Malmeeae, pertencem a um clado dentro da tribo (Pirie *et al.* 2006). No entanto, *Oxandra* não é um gênero monofilético (Pirie *et al.* 2006) e apenas algumas de suas espécies são androdioicas, como *Oxandra martiana* (Schltdl.) R.E.Fr. (Fries 1931). Por sua vez, *Pseudoxandra* não está incluído neste clado de gêneros androdioicos e seu monofiletismo apresenta baixo suporte (Pirie *et al.* 2006).

Nesta tese apresentamos a filogenia da tribo Malmeeae obtida por meio de 64 genes codificadores de proteínas do cloroplasto e dois espaçadores intergênicos também do cloroplasto. Foram amostrados 33 táxons da tribo Malmeeae e seis espécies como grupo externo. As sequências foram obtidas por sequenciamento de nova geração e a reconstrução filogenética foi feita por inferência Bayesiana, máxima verossimilhança e parcimônia. Uma análise de reconstrução de estados ancestrais é apresentada, na qual discutimos a evolução da androdioicia e de outros caracteres morfológicos (Capítulo 1).

Análises morfo-anatômicas das flores bissexuais e masculinas de *Pseudoxandra spiritus-sancti* foram realizadas por meio de microscopia ótica e microscopia eletrônica

de varredura. Pranchas com cortes anatômicos e eletromicrografias das flores bissexuais e masculinas são apresentadas (Capítulo 2).

Por último, a revisão taxonômica do gênero *Ephedranthus*, um gênero androdioico da tribo Malmeeae, é apresentada. Este gênero foi revisado há 17 anos por Oliveira & Sales (1999), e desde então duas novas espécies foram descritas, totalizando sete, e novas informações sobre biologia, distribuição geográfica e morfologia das espécies foram acumuladas. A revisão conta com reconstrução filogenética das espécies de *Ephedranthus* baseada em dados morfológicos, descrições das espécies, chaves de identificação, mapas de distribuição e ilustrações das espécies (Capítulo 3).

## Referências bibliográficas

- Barrett, S.C.H. 2002. The evolution of plant sexual diversity. *Nature Reviews Genetics* 3: 274–284.
- Bawa, K.S. & Beach, J.H. 1981. Evolution of sexual systems in flowering plants. *Annals of the Missouri Botanical Garden* 68: 254–274.
- Charlesworth, D. 1984. Androdioecy and the evolution of dioecy. *Botanical Journal of the Linnean Society* 22: 333–48.
- Charnov, E.L. 1979. Simultaneous hermaphroditism and sexual selection. *Proceedings of the National Academy of Sciences of the United States of America* 76: 2480–82.
- Chatrou, L.W. 1998. Changing genera. Systematic studies in Neotropical and West African Annonaceae. Ph.D. Thesis, Utrecht University, Utrecht.
- Chatrou, L.W., Pirie, M.D., Erkens, R.H.J., Couvreur, T.L.P., Neubig, K.M.J., Abbott, R., Mols, J.B., Maas, J.W., Saunders, R.M.K. & Chase, M.W. 2012. A new subfamilial and tribal classification of the pantropical flowering plant family Annonaceae informed by molecular phylogenetics. *Botanical Journal of the Linnean Society* 169(1): 5–40.
- Doyle, J.A. & Le Thomas, A. 1996. Phylogenetic analysis and character evolution in Annonaceae. *Bulletin du Muséum National d'Histoire Naturelle, Section B, Adansonia* 18: 279–334.
- Doyle, J.A. & Le Thomas, A. 2012. Evolution and phylogenetic significance of pollen in Annonaceae. *Botanical Journal of the Linnean Society* 169(1): 190–221.

- Doyle, J.A., Bygrave, P. & Le Thomas, A. 2000. Implications of molecular data for pollen evolution in Annonaceae. Pp. 259–284. In: Harley, M.M.; Morton, C.M. & Blackmore, S. (eds), *Pollen and Spores: morphology and biology*. Royal Botanic Gardens, Kew.
- Fries, R.E. 1931. Revision der Arten einiger Annonaceen-Gattungen II. *Acta Horti Bergiani* 10(2): 129–341.
- Ishida, K. & Hiura, T. 1998. Pollen fertility and flowering phenology in an androdioecious tree, *Fraxinus lanuginosa* (Oleaceae), in Hokkaido, Japan. *International Journal of Plant Sciences* 159: 941–47.
- Lepart, J. & Dommée, B. 1992. Is *Phillyrea angustifolia* L. (Oleaceae) an androdioecious species? *Botanical Journal of the Linnean Society* 108: 375–87.
- Lloyd, D.G. 1975. The maintenance of gynodioecy and androdioecy in angiosperms. *Genetica* 45: 325–339.
- Lopes, J.C. & Mello-Silva, R. 2014. Annonaceae da Reserva Natural Vale, Linhares Espírito Santo. *Rodriguésia* 65(3): 599–635
- Oliveira, J. & Sales, M.F. 1999. Estudos taxonômicos dos gêneros *Ephedranthus* e *Pseudephedranthus* Aristeg.: Annonaceae. *Boletim do museu paraense “Emílio Goeldi”*. Nova série, botânica 15(2): 117–166.
- Pannell, J.R. 2001. A hypothesis for the evolution of androdioecy: the joint influence of reproductive assurance and local mate competition in a metapopulation. *Evolutionary Ecology* 14: 195–211.
- Pannell, J.R. 2002. The evolution and maintenance of androdioecy. *Annual Review of Ecology and Systematics* 33: 397–425.
- Paulino-Neto, H.F. & Teixeira, R.C. 2006. Florivory and sex ratio in *Annona dioica* St. Hil. (Annonaceae) in the Pantanal at Nhecolândia, southwestern Brazil. *Acta Botanica Brasilica* 20: 405–409.
- Pirie, M.D., Chatrou, L.W. Mols, J. B., Erkens, R.H. J. & Oosterhof, J. 2006. ‘Andean-centred’ genera in the short-branch clade of Annonaceae: Testing biogeographical hypotheses using phylogeny reconstruction and molecular dating. *Journal of Biogeography* 33: 31–46.
- Richardson, J.E., Chatrou, L.W., Mols, J.B., Erkens, R.H.J. & Pirie, M.D. 2004. Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and Rhamnaceae. *Philosophical Transactions of the Royal Society B* 359: 1495–1508.

- Ross, M.D. 1982. Five evolutionary pathways to subdioecy. *The American Naturalist* 119: 297–318.
- Saunders, R.M.K. 2010. Floral evolution in the Annonaceae: hypotheses of homeotic mutations and functional convergence. *Biological Reviews* 85: 571–591.
- Su, Y.C.F. & Saunders, R.M.K. 2006. Monograph of *Pseuduvaria* (Annonaceae). *Systematic Botany Monographs* 79: 1–204, pl. 1–3.
- Su, Y.C.F., Smith, G.J.D. & Saunders, R.M.K. 2008. Phylogeny of the basal angiosperm genus *Pseuduvaria* (Annonaceae) inferred from five chloroplast DNA regions, with interpretation of morphological character evolution. *Molecular Phylogenetics and Evolution* 48: 188–206.
- van Heusden, E.C.H. 1992. Flowers of Annonaceae: morphology, classification, and evolution. *Blumea* 7 (Supplement): 1–218.
- van Heusden, E.C.H. 1997. Revision of the Southeast Asian genus *Trivalvaria* (Annonaceae). *Nordic Journal of Botany* 17(2): 169–180.
- Westra, L.Y.T. 1995. Studies in Annonaceae. XXIV. A taxonomic revision of *Raimondia* Safford. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 117: 273–297.

# Capítulo 1

## **Phylogenomics and evolution of androdioecy in tribe Malmeeae (Annonaceae), a Neotropical woody group**

**Abstract.** Flowers have a high diversity of sexual systems. The rarest among them is androdioecy, in which species present individuals with male flowers and others with bisexual flowers. The majority of androdioecious species, usually herbaceous plants with recurrent local extinction followed by re-colonization, has evolved from dioecious ancestors. Nevertheless, some woody and androdioecious plants have hermaphrodite ancestors. This is the case of Annonaceae, a pantropical family of woody and hermaphrodite plants, in which androdioecy is frequent. Androdioecious genera have arisen several times in different Annonaceae lineages. The majority of androdioecious genera belong to the Neotropical tribe Malmeeae. Malmeeae is, thus, an excellent model to study androdioecy in Neotropical woody plants. The phylogenetic reconstruction of 33 taxa of this group was performed using DNA sequences of 66 molecular markers of the chloroplast genome, sequenced by next generation sequencing. Maximum likelihood, Bayesian inference and maximum parsimony were the methods used for the phylogenetic analyses. The reconstruction of ancestral states was performed to characters related to sexual system and floral morphology using a Bayesian approach. The phylogenetic analyses reconstructed three main groups in Malmeeae, ((*Malmea* (*Crematosperma*, *Pseudoxandra*)) sister to the rest of the tribe, and ((*Unonopsis* (*Bocageopsis*, *Onychopetalum*)) sister to (*Mosanonna*, *Ephedranthus*, *Klarobelia*, *Oxandra*, *Pseudephedranthus fragrans*, *Pseudomalmea*, *Ruizodendron ovale*). Hermaphroditism is plesiomorphic in the tribe, with four independent evolutions to androdioecy, which is a synapomorphy of two groups, one that includes three genera and 14 species, and other with one genus of seven species.

**Key words:** ancestral state analysis, next generation sequencing.

## Introduction

Androdioecious plants are those that possess male and bisexual flowers in different individuals (Bawa & Beach 1981). Confirmed cases of androdioecious taxa are few (Pannell 2002), because for males to persist in the population they must have the ability to produce twice more descendants than the hermaphrodites (Charlesworth 1984, Lloyd 1975). Moreover, the evolutionary pathway that originates androdioecious species is very complex (Barrett 2002, Ross 1982). Most confirmed cases of androdioecy originated from dioecious ancestors (Pannell 2002). These species are usually herbaceous and form populations that suffer recurrent local extinction followed by re-colonization (Pannell 2001). Nevertheless, some androdioecious species have hermaphrodite ancestors and are woody plants, such as Oleaceae and Tapisciaceae (Ishida & Hiura 1998, Lepart & Dommée 1992, Wallander 2008, Zhou et al 2016).

Annonaceae is also a group of woody plants with hermaphrodite ancestors, in which androdioecy is frequent (Saunders 2010). It is a pantropical family with 2,440 species in 109 genera (Chatrou et al. 2012), and androdioecious taxa have been reported in its two main subfamilies, Annonoideae and Malmeoideae. In Annonoideae, *Anonidium* and *Annona*, tribe Annoneae, have androdioecious species (van Heusden 1992, Westra 1995). In Malmeoideae, *Greenwayodendron* and *Polyceratocarpus*, from tribe Piptostigmatae (van Heusden 1992), *Pseuduvaria* and *Trivalvaria*, from tribe Miliuseae (Su & Saunders 2006, van Heusden 1997), and *Ephedranthus*, *Klarobelia*, *Oxandra*, *Pseudephedranthus*, and *Pseudomalmea*, from tribe Malmeeae (Chatrou 1998, Fries 1931, Oliveira & Sales 1999, van Heusden 1992), also have androdioecious taxa.

The majority of androdioecious genera belong to Malmeoideae subfamily. In tribe Miliuseae, some androdioecious species of *Pseuduvaria* are originated from dioecious ancestors (Su et al. 2008). However, this may not be the case in the remaining Annonaceae, once hermaphroditism is plesiomorphic in the family (Saunders 2010). All androdioecious genera from the Neotropical region are included in tribe Malmeeae, except for some species of *Annona* (Paulino-Neto & Teixeira 2006, Westra 1995). Apart from the five androdioecious genera already reported, another species from tribe Malmeeae, *Pseudoxandra spiritus-sancti*, has been shown to be androdioecious, accounting for the first record of androdioecy in *Pseudoxandra* (Lopes & Mello-Silva 2014, Lopes et al. *in prep*).

Malmeeae is monophyletic and includes all Neotropical genera from the subfamily Malmeoideae, whose majority of genera are from Asia. *Ephedranthus*, *Klarobelia*, *Oxandra*, *Pseudephedranthus* and *Pseudomalmea*, the androdioecious genera of Malmeeae, belong to a single clade in the tribe (Chatrou et al. 2012, Pirie et al. 2006). However, *Oxandra* is not monophyletic (Pirie et al. 2006). This genus includes at least one androdioecious species, *Oxandra martiana* R.E.Fr. (Fries 1931) and it has not yet been sampled in any phylogenetic analysis. Besides, *Pseudoxandra* is not included in that clade of androdioecious genera, and its monophyly presents low support (Pirie et al. 2006). The aim of this paper is to reconstruct the phylogeny of tribe Malmeeae using DNA sequences from 66 molecular markers from the chloroplast genome, 64 protein-coding genes and two intergenic spacers, and analyze the evolution of androdioecy in the tribe. Malmeeae is a model for understanding the evolution of androdioecy from hermaphrodite woody plants, once hermaphroditism is plesiomorphic in Annonaceae.

## Material & Methods

### *Taxon sampling*

A total of 33 species from all genera of tribe Malmeeae were sampled. The number of species sampled by genus/the total of species in each genus are *Bocageopsis* (2/4), *Crematosperma* (2/35), *Ephedranthus* (2/7), *Klarobelia* (2/12), *Malmea* (2/6), *Mosanonna* (2/14), *Onychopetalum* (1/2), *Oxandra* (10/30), *Pseudephedranthus* (1/1), one of *Pseudomalmea* (1/3), *Pseudoxandra* (5/24), *Ruizodendron* (1/1), and *Unonopsis* (2/48). Six taxa, including one Magnoliaceae, one Anaxagoreoideae, one Ambavioideae, two Annonoideae and one Malmeoideae, represent the outgroup species (Table 1, plate 1).

### *DNA extraction and sequencing.*

Total genomic DNA was extracted from 20 mg of leaves dried in silica gel or from herbarium material using cetyl trimethyl ammonium bromide (CTAB) method (Doyle & Doyle 1987). The genomic DNA was purified using the Wizard<sup>®</sup> DNA Clean-Up System following the manufacturer's instructions (Promega, The Netherlands).

**TABLE 1.** Species of Malmeeae and outgroups sampled for the phylogenetic analyses.

<b>Taxon</b>	<b>Origin</b>	<b>Voucher</b>
<b>Outgroup</b>		
<i>Magnolia kobus</i> DC.	Japan	Chatrou 93 (U)
<i>Anaxagorea phaeocarpa</i> Mart.	Ecuador – Napo	Maas 8592 (U)
<i>Tetrameranthus duckei</i> R.E.Fr.	Brazil – Amazonas	Stevenson 1002 (U)
<i>Annona muricata</i> L.	Neotropics	Maas 8759 (U)
<i>Monanthes buehneri</i> (Engl.) Verdc.	Tanzania	Bidgood 2706 (WAG)
<i>Desmopsis bibracteata</i> (B.L.Rob) Saff.	Costa Rica	Chatrou 728 (WAG)
<b>Tribe Malmeeae</b>		
<i>Bocageopsis pleiosperma</i> Maas	Brazil – Amazonas	Miralha 300 (U)
<i>Crematosperma cauliflorum</i> R.E.Fr.	Peru – Loreto	Chatrou 224 (U)
<i>Crematosperma leiophyllum</i> R.E. Fr.	Bolivia - Santa Cruz	Pirie 2 (U)
<i>Ephedranthus guianensis</i> R.E.Fr.	Guyana	Scharf 64
<i>Ephedranthus amazonicus</i> R.E.Fr.	Peru – Loreto	Chatrou 173 (U)
<i>Klarobelia cauliflora</i> Chatrou	Peru – Loreto	Chatrou 161 (U)
<i>Klarobelia inundata</i> Chatrou	Peru – Loreto	Chatrou 205 (U)
<i>Malmea dielsiana</i> R.E. Fr.	Peru - Madre de Dios	Chatrou 122 (U)
<i>Malmea dimera</i> Chatrou	Panama – Panama	Croat 34626 (U)
<i>Mosannonna costaricensis</i> (R.E. Fr.) Chatrou	Costa Rica – Limón	Chatrou 90 (U)
<i>Mosannonna discolor</i> (R.E. Fr.) Chatrou	Guyana	Jansen-Jacobs 6000 (U)
<i>Onychopetalum periquino</i> (Rusby) D.M.Johnson & N.A.Murray	Bolivia – Beni	Chatrou 425 (U)
<i>Oxandra asbeckii</i> (Pulle) R.E. Fr.	Guyana	University of Guyana, course Neotropical Botany UG-NB-55 (U)
<i>Oxandra espiantana</i> (Spruce ex Benth.) Baill.	Peru - Madre de Dios	Chatrou 133 (U)
<i>Oxandra euneura</i> Diels	Peru – Loreto	Chatrou 235 (U)
<i>Oxandra guianensis</i> R.E.Fr.	Guyana	Scharf 67 (U)
<i>Oxandra laurifolia</i> (Sw.) A.Rich	Dominican Republic	Maas 8375 (U)
<i>Oxandra longipetala</i> R.E. Fr.	Costa Rica – Osa	Chatrou 114 (U)
<i>Oxandra macrophylla</i> R.E. Fr.	Peru – Loreto	Chatrou 204 (U)
<i>Oxandra martiana</i> (Schltdl.) R.E.Fr.	Brazil - Espírito Santo	Lopes 363 (SPF)
<i>Oxandra polyantha</i> R.E.Fr.	Peru – Loreto	Chatrou 215 (U)

<i>Oxandra venezuelana</i> R.E.Fr.	Costa Rica – Osa	Chatrou 120
<i>Pseudophedranthus fragrans</i> (R.E. Fr.) Aristeg.	Venezuela – Amazonas	Maas 6878 (U)
<i>Pseudomalmea diclina</i> (R.E. Fr.) Chatrou	Peru - Madre de Dios	Chatrou 136 (U)
<i>Pseudoxandra angustifolia</i> Maas	Peru – San Martín	Pirie 139 (U)
<i>Pseudoxandra bahiensis</i> Maas	Brazil – Bahia	Lopes 414 (SPF)
<i>Pseudoxandra lucida</i> R.E. Fr.	Peru – Loreto	Chatrou 212 (U)
<i>Pseudoxandra obscurinervis</i> Maas 1	Brazil – Amazonas	Martins 15 (INPA)
<i>Pseudoxandra obscurinervis</i> Maas 2	Brazil – Amazonas	Webber s.n. (U)
<i>Pseudoxandra spiritus-sancti</i> Maas	Brazil - Espírito Santo	Lopes 436 (SPF)
<i>Ruizodendron ovale</i> (Ruiz & Pav.) R.E. Fr.	Ecuador – Napo	Maas 8600 (U)
<i>Unonopsis pittieri</i> Saff.	Costa Rica - Braulio Carillo	Chatrou 68 (U)
<i>Unonopsis rufescens</i> (Baill.) R.E. Fr.	French Guiana	Orava 9 (U)

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The samples were sent to Beijing Genomics Institute, Hong Kong, China to be sequenced via Illumina platform in two lanes. The same company also performed the library preparation. After verification of the concentration, sample integrity and purification, 1.5 µg DNA was fragmented in 500 bp by Covaris, then the Gel-Electrophoretometric was used to test the fragmented DNA. The fragmented DNA was combined with End Repair Mix, incubated at 20 °C for 30 min. The end-repaired DNA was purified with QIAquick PCR Purification Kit (Qiagen). A-Tailing Mix was then added and incubated at 37 °C for 30 min. The purified Adenylate 3'Ends DNA, Adapter and Ligation Mix were combined and incubated at 20 °C for 15 min. Adapter-ligated DNA was selected by running a 2% agarose gel to recover the target fragments. The gel was purified with QIAquick Gel Extraction kit (Qiagen). Several rounds of PCR amplification with PCR Primer Cocktail and PCR Master Mix were performed to enrich the Adapter-ligated DNA fragments. Then the PCR products were selected by running another 2% agarose gel to recover the target fragments. The gel was purified again with QIAquick Gel Extraction kit (Qiagen).

The final library was quantified by determining the average molecule length using the Agilent 2100 bioanalyzer instrument (Agilent DNA 1000 Reagents), and by quantifying the library by real-time quantitative PCR (QPCR) (TaqMan Probe).



**Plate 1.** A. *Crematosperma cauliflorum*. B. *Ephedranthus amazonicus*. C. *Klarobelia cauliflora*. D. *Malmea dielsiana*. E. *Oxandra longipetala*. F. *Pseudomalmea dielina*. Photos: Lars Chatrou.

The qualified libraries were amplified on cBot to generate the cluster on the flowcell (HiSeq PE Cluster Kit v4 cBot, Illumina). The amplified flowcell were sequenced paired end on the HiSeq 2500 System (HiSeq SBS Kit V4, Illumina).

The above library preparation protocol described refers to the normal library preparation. For the sample with low amount of DNA, less than 1 µg of DNA, low input library preparation and WGA treatment were used (Table 2). The low input library preparation follows the same protocol mentioned above with some changes in the selection of fragmented DNA, PCR enzyme and an increase in the PCR cycles number. The WGA treatment uses the REPLI-g® Midi Kit (Qiagen) as a first step before the normal library preparation.

**TABLE 2.** Details of library preparation.

<b>Taxon</b>	<b>Library preparation</b>	<b>Taxon</b>	<b>Library preparation</b>
<i>Anaxagorea sylvatica</i>	WGA treatment	<i>Oxandra longipetala</i>	normal library
<i>Annona muricata</i>	normal library	<i>Oxandra macrophylla</i>	low input library
<i>Crematosperma cauliflorum</i>	normal library	<i>Oxandra martiana</i>	normal library
<i>Crematosperma leiophyllum</i>	low input library	<i>Oxandra nitida</i>	WGA treatment
<i>Ephedranthus amazonicus</i>	low input library	<i>Oxandra polyantha</i>	normal library
<i>Ephedranthus guianensis</i>	WGA treatment	<i>Pseudomalmea diclina</i>	normal library
<i>Klarobelia inundata</i>	normal library	<i>Pseudoxandra angustifolia</i>	low input library
<i>Magnolia Kobus</i>	normal library	<i>Pseudoxandra bahiensis</i>	low input library
<i>Malmea dielsiana</i>	low input library	<i>Pseudoxandra bahiensis</i>	WGA treatment
<i>Malmea dímera</i>	normal library	<i>Pseudoxandra lucida</i>	low input library
<i>Mosanonna costaricensis</i>	low input library	<i>Pseudoxandra obscurinervis</i>	low input library
<i>Mosanonna discolor</i>	normal library	<i>Pseudoxandra obscurinervis</i>	WGA treatment
<i>Onychopetalum periquino</i>	low input library	<i>Pseudoxandra polyphleba</i>	WGA treatment
<i>Oxandra asbeckii</i>	low input library	<i>Pseudoxandra spiritus-sancti</i>	WGA treatment
<i>Oxandra espintana</i>	low input library	<i>Ruizodendron ovale</i>	low input library
<i>Oxandra euneura</i>	WGA treatment	<i>Unonopsis pittieri</i>	normal library
<i>Oxandra guianensis</i>	low input library	<i>Unonopsis rufescens</i>	WGA treatment
<i>Oxandra laurifolia</i>	normal library		

### *Chloroplast genome assembly and molecular sampling*

The chloroplast genome assembly was performed using the Interactive Organelle Genome Assembly (IOGA) pipeline (Bakker et al. 2016). The raw sequence data, forward and reverse, for each sample was used as input and genomes of different taxa, including *Liriodendron tulipifera* L. (Magnoliaceae), were the references. The best assemblies, those ones with the best Assembly Likelihood Estimation (Clark et al. 2013), were used as the final assembly. The best assemblies for each sample were input in Geneious R8 (Kearse et al. 2012) and map and gene annotated to *Liriodendron tulipifera* (GenBank accession DQ899947). Gene annotations were extracted in

Geneious R8. Gene sequences for samples that could not be retrieved in the way cited above were accessed by BLAST (Altschul et al. 1990).

#### *Sexual system and morphological characters*

The sexual system in Malmeeae is analysed in the ancestral state and parsimony analyses. The taxa analysed are either hermaphrodite or androdioecious (Character 1, table 3). The remaining morphological characters were chosen because of their importance in classifications and delimitation of the genera involved (Table 3, Doyle & Le Thomas 1996, Koek-Noorman et al. 1990), allowing investigation of their role in a phylogenetic context rather than performing a total evidence analysis. A broad analysis of evolution of morphological characters in Annonaceae is in Doyle & Le Thomas (1996).

The relative position of primary vein on the adaxial surface of the leaf (Doyle & Le Thomas 1996) is an important feature to recognizing genera (Maas et al. 2007). Midrib are or concave to flat, also called impressed, or convex, other term to raised primary vein (Character 2).

Flowers are either always solitary or rarely in pairs, or born in determinate inflorescences that varies in the same species from one to many, normally with many flowers (Doyle & Le Thomas 1996; character 3). The term Rhipidium is often used to describe this inflorescence (Maas et al. 2007).

Petals aestivation in Annonaceae are valvate, reduplicate-valvate, imbricate, transversely folded or apert (van Heusden 1992). Valvate and imbricate petals are the most common and the only states found in the group analysed (Character 4). This character has been important in previous classifications (Koek-Noorman et al. 1990).

The number of ovules per carpel ranges from one to numerous. In most genera, there are less than 10 ovules (van Heusden 1992). In *Anaxagorea* there are generally two ovules per carpel (Maas et al. 1984), and in Malmeeae one ovule is found in the majority of the genera with few genera showing two to five ovules per carpel (Maas et al. 2007, van Heusden 1992; character 5).

Stigma shape is either lobed, capitate, or cylindrical (Doyle & Le Thomas 1996). Capitate stigma is present in the majority of tribe Malmeeae, with few exceptions (Maas et al. 2007; Pirie 2005; character 6)

The stamens of Annonaceae are narrowly oblong to oblanceoloid, with a very short filament, two linear thecae and a connective with shield-like prolongation (van

Heusden 1992). Stamen shape is a phylogenetically informative character and the division into three states, laminar, narrow with a tongue-like connective, and peltate-truncate follows Doyle & Le Thomas (1996; character 7). Laminar stamens are found in *Anaxagorea* and the other two states are present in Malmeeae.

Staminodes are observed in few genera in Annonaceae, such as *Anaxagorea* and *Xylopia* (van Heusden 1992). In tribe Malmeeae, staminodes are found in *Pseudoxandra* only (Lopes et al. *in prep.*; character 8).

Rumination in seeds, a synapomorphy of Annonaceae, is due to the infolding of dark inner layer of the seed coat into the lighter coloured endosperm (van Setten & Koek-Noorman 1992, character 9).

**TABLE 3.** Characters analysis and coding. D&LT = Doyle & Le Thomas (1996), vH = van Heusden (1992) and, vS&K = van Setten & Koek-Noorman (1992).

Characters	States			
1 - Sexual system	hermaphrodite: 0	androdioecious: 1		
2 - Midrib (D&LT)	concave or flat: 0	convex: 1		
3 - Flower number (D&LT)	always 1(-2): 0	1-many: 1		
4 - Petals aestivation (vH)	imbricate: 0	valvate: 1		
5 - Ovule (D&LT)	more than 6: 0	two to five: 1	always two : 2	one : 3
6 - Stigma (D&LT)	lobed: 0	capitate: 1	cylindrical: 2	
7 - Stamen morphology (D&LT)	laminar: 0	narrower with tongue-like extension connective: 1	peltate-truncate: 2	
8 - Staminodes (vH)	absent: 0	present: 1		
9 - Endosperm (vS&K)	ribbon shaped (0)	peg-shaped (1)	spiniform (2)	lamellate (3)

Character states follows Maas & Westra (1984, 1985, *Anaxagorea*), Pontes et al. (2004, *Annona*), Maas et al. (2007, *Bocageopsis*, *Onychopetalum*, and *Unonopsis*), Pirie (2005, *Crematosperma*) Safford (1916 *Desmopsis*) Oliveira & Sales (1999, *Ephedranthus*), Lopes & Mello-Silva (unpublished data, *Ephedranthus*), Chatrou (1998, *Klarobelia*, *Malmea*, *Mosanonna*, and *Pseudomalmea*), Chen & Nootboom (1993; *Magnolia*), Paul Hoekstra (unpublished data, *Monanthotaxis*), Paul Maas (unpublished data, *Oxandra*, *Pseudephedranthus*, and *Ruizodendron*), Maas & Westra (2003, 2005, *Pseudoxandra*), and Westra & Maas (2012, *Tetrameranthus*) (Table 4).

**TABLE 4.** Morphological matrix.

Taxa / Characters	1	2	3	4	5	6	7	8	9
<i>Magnolia kubos</i>	0	0	0	0	1	?	?	0	-
<i>Anaxagorea phaeocarpa</i>	0	0	1	1	2	0	0	1	0
<i>Tetrameranthus duckei</i>	0	0	0	0	2	0	1	0	-
<i>Monanthotaxis buchananii</i>	0	0	1	1	0	2	2	1	3
<i>Ammona muricata</i>	0	0	0	1	3	1	2	0	-
<i>Desmopsis bibracteata</i>	0	?	0	1	1	1	2	0	3
<i>Bocageopsis pleiosperma</i>	0	1	1	1	1	1	1	0	2
<i>Unonopsis pittieri</i>	0	1	1	1	3	2	1	0	2
<i>Unonopsis rufescens</i>	0	1	1	1	3	2	2	0	2
<i>Onychopetalum perequino</i>	0	1	1	1	1	2	1	0	1
<i>Crematosperma cauliflorum</i>	0	0	0	0	3	0	2	0	2
<i>Crematosperma leiophyllum</i>	0	0	0	0	3	0	2	0	2
<i>Ephedranthus guianensis</i>	1	0	0	0	3	1	2	0	3
<i>Ephedranthus amazonicus</i>	1	0	0	0	3	1	2	0	3
<i>Klarobelia cauliflora</i>	1	0	0	0	3	1	2	0	3
<i>Klarobelia inundata</i>	1	0	0	0	3	1	2	0	3
<i>Malmea dielsiana</i>	0	0	0	0	3	1	2	0	2
<i>Malmea dimera</i>	0	0	0	0	3	1	2	0	2
<i>Mosanonna costaricensis</i>	0	1	?	0	3	1	2	0	3
<i>Mosanonna discolor</i>	0	1	?	0	3	1	2	0	3
<i>Oxandra asbeckii</i>	0	1	0	0	3	1	1	0	1
<i>Oxandra espintana</i>	0	0	0	0	3	1	1	0	1
<i>Oxandra euneura</i>	0	1	0	0	3	1	1	0	1
<i>Oxandra guianensis</i>	0	0	0	0	3	1	1	0	2
<i>Oxandra laurifolia</i>	0	0	1	0	3	1	1	0	2
<i>Oxandra longipetala</i>	0	1	0	0	3	1	1	0	-
<i>Oxandra macrophylla</i>	0	0	0	0	3	1	1	0	2
<i>Oxandra martiana</i>	1	0	0	0	3	1	1	0	2
<i>Oxandra polyantha</i>	0	1	1	0	3	1	1	0	2
<i>Oxandra venezuelana</i>	0	0	1	0	3	1	1	0	-
<i>Pseudephedranthus fragrans</i>	1	1	?	0	3	1	1	?	3
<i>Pseudomalmea dielina</i>	1	0	0	0	3	1	2	0	3
<i>Pseudoxandra angustifolia</i>	0	0	0	0	3	1	1	0	2
<i>Pseudoxandra bahiensis</i>	0	0	1	0	3	1	1	0	2
<i>Pseudoxandra lucida</i>	0	0	0	0	3	1	1	0	2
<i>Pseudoxandra obscurinervis</i>	0	0	1	0	3	1	1	0	2
<i>Pseudoxandra obscurinervis</i>	0	0	1	0	3	1	1	0	2
<i>Pseudoxandra spiritus-sancti</i>	1	1	0	0	3	1	1	1	2
<i>Ruizodendron ovale</i>	0	?	0	0	3	1	1	0	3

*Ancestral state analysis*

The evolution of sexual system and morphological characters in Malmeeae was inferred by estimating the marginal posterior probability of ancestral states using a

Bayesian framework. This approach allows incorporation of phylogenetic uncertainty during the inference, because instead of optimizing states changes in a consensus tree, it takes a pool of different trees generated during the Bayesian phylogenetic analyses for estimating the ancestral states in each node (Pagel et al. 2004).

Ancestral states were estimated using BayesTraits V2 (Pagel & Meade 2014). The analyses were performed for each character separately using the module MultiState as implemented in BayesTraits and the sample of 1,000 post-burn-in trees. Initially, a maximum likelihood analysis was run to derive an empirical prior. In addition, hypotheses about the rate of transformation between states were tested. For characters with two states, two hypotheses were tested. The first hypothesis considers that the rate of transformation from state 0 to state 1 is equal to the rate from 1 to 0. In the second hypothesis, the rates of transformation from state 0 to state 1 are different from 1 to 0. A third model was tested for characters with more than two states, with symmetrical rates of transformation. The rate of transformation from state 0 to state 1 is equal to the rate from 0 to 2 and from 1 to 2, the remaining rates, from state 1 to state 0, from 2 to 0, and from 2 to 1, are different (Table 5). For testing which of these models best fit the data, comparisons among them were done using the Akaike information criterion corrected for small data (Sugiura 1978) implemented on R (R Development Core Team. 2008). Character 5 (number of ovules), with four states, was analysed with the reversible jump strategy due to over parameterisation. Bayesian analyses were run with a Markov chain Monte Carlo approach, an exponential hyper prior of 0–20, 20 million generations, sampling every 1,000th generation, discarding the first 5 million generations (25%) as burn-in. Ancestral states were estimated for all nodes, except for character 5 (petal aestivation) in which the analysis was done just for nodes of interest. For nodes with posterior probability less than 1.0, the MRCA (most recent common ancestral) command was applied (Pagel et al. 2004). The results of ancestral state evolution are showed in the Bayesian consensus tree. Phylograms and posterior probabilities graphs were elaborated on R (R Development Core Team. 2008).

**TABLE 5.** Hypotheses for state transformation for each character.

Character	Parameters	Hypotheses (LnL)			Hypothesis selected
		Equal rates (ER)	Symmetric (SYM)	All rates different (ARD)	
Sexual system	2	-18,32699	–	-16,03995	ARD
Midrib	2	-16,38611	–	-16,03995	ER
Flower number	2	-21,43273	–	-20,97848	ER
Petal aestivation	2	-9,312,956		-8,857,237	ARD
Ovule number	4	-15,47725	-14,23541	-12,6095	Reversible jump
Stigma	3	-16,43255	-16,21346	-15,04159	ARD
Stamen morphology	3	-28,02906	-22,66235	-21,28504	ARD
Staminodes	2	-8,138146	–	-7,82789	ER

### *Phylogenetic analyses*

One challenge that emerges with the analysis of a huge data matrix generated by next generation sequencing is to choose the best way to partition and analyse the data. The junction of matrix with incomplete character sampled and phylogenetic analysis with low quality heuristic search could create false results, with high resolution and support values for clades that are in fact unsupported (Simmons 2014). One way to prevent it in Bayesian and maximum likelihood analyses is choosing an adequate partition scheme (Kainer & Lanfear 2015). For this reason, the best partition scheme was algorithmically chosen with a priori partition into codon position for protein coding genes (Lanfear et al. 2014). For parsimony analysis, the RATCHET, an algorithm that reduce the risk of the analysis getting stuck in a suboptimal tree island, was used to improve the analysis search (Nixon 1999a).

### *Data partition and selection of substitution model*

The selection of the best partitioning scheme and substitution model for each partition was performed in PartitionFinder v1.1.1 (Lanfear et al. 2012). This software compares numerous partitioning schemes and selects the optimal one. The alignment was divided into codon positions for the 64 protein coding genes, 192 data blocks were created, plus the two intergenic region (*psbA-trnH* and *trnL-trnF*) in a total of 194 data blocks (Table 6, molecular markers). The data blocks were then submitted to a heuristic algorithm, relaxed clustering algorithm (search = rcluster), that analyses a percentage of all possible partition schemes and the respective substitution model. The substitution models analysed were GTR+G and GTR+I+G, due to the selected algorithm. The

percentage chosen was 30% because optimize results and computational time (Lanfear et al. 2014). This approach was chosen due to the huge amount of possibilities to be analysed, 38,175 data subsets, which were generated with the 194 data blocks. The best partition scheme was identified using the Bayesian information criterion (BIC).

### Matrix

For phylogenetic analyses, 66 molecular markers were used. From this amount, 33 samples have nearly complete dataset and just six have only three markers, *psbA-trnH*, *rbcL* and *trnL-trnF* (Table 6). Six samples with incomplete data were selected to improve the taxonomic sampling. They correspond to species sampled by Pirie et al. (2006; table 7). Alignments were performed at MAFFT (Kato et al. 2002) in Geneious R8 package (Kearse et al. 2012) and were checked in Mesquite (Maddison & Maddison 2015). Data concatenation was done with SequenceMatrix, version 1.78 (Vaidya et al. 2011). Matrices used for maximum likelihood and Bayesian inference analyses have both 65,002 base pairs

**TABLE 6.** Molecular markers sampled for the phylogenetic analyses.

<i>atpA</i>	<i>atpB</i>	<i>atpE</i>	<i>atpH</i>	<i>atpI</i>
<i>ccsA</i>	<i>cemA</i>	<i>infA</i>	<i>matK</i>	<i>ndhA</i>
<i>ndhB</i>	<i>ndhC</i>	<i>ndhD</i>	<i>ndhE</i>	<i>ndhG</i>
<i>ndhH</i>	<i>ndhI</i>	<i>ndhJ</i>	<i>ndhK</i>	<i>petA</i>
<i>petB</i>	<i>petD</i>	<i>petG</i>	<i>petL</i>	<i>petN</i>
<i>psaA</i>	<i>psaB</i>	<i>psaC</i>	<i>psaJ</i>	<i>psbA-trnH</i>
<i>psbA</i>	<i>psbB</i>	<i>psbC</i>	<i>psbD</i>	<i>psbH</i>
<i>PsbI</i>	<i>psbK</i>	<i>psbM</i>	<i>psbN</i>	<i>psbT</i>
<i>PsbZ</i>	<i>rbcL</i>	<i>rpl2</i>	<i>rpl14</i>	<i>rpl16</i>
<i>rpl23</i>	<i>rpl32</i>	<i>rpl36</i>	<i>rpoA</i>	<i>rpoB</i>
<i>rpoC1</i>	<i>rpoC2</i>	<i>rps2</i>	<i>rps3</i>	<i>rps4</i>
<i>rps7</i>	<i>rps8</i>	<i>rps11</i>	<i>rps14</i>	<i>rps15</i>
<i>rps16</i>	<i>rps19</i>	<i>trnL-trnF</i>	<i>ycf2</i>	<i>ycf4</i>
<i>ycf15</i>				

**TABLE 7.** Samples with incomplete dataset and GenBank accession for molecular markers.

Taxon	<i>rbcL</i>	<i>trnL-trnF</i>	<i>psbA-trnH</i>
<i>Bocageopsis pleiosperma</i>	AY841601 <sup>b</sup>	AY841679 <sup>b</sup>	AY841446
<i>Klarobelia cauliflora</i>	AY841627 <sup>b</sup>	AY841705 <sup>b</sup>	AY841468
<i>Oxandra venezuelana</i>	AY841645 <sup>b</sup>	AY841723 <sup>b</sup>	AY841495
<i>Pseudephedranthus fragrans</i>	AY841651 <sup>b</sup>	AY841729 <sup>b</sup>	AY841504
<i>Tetrameranthus duckei</i>	AY841658 <sup>b</sup>	AY841736 <sup>b</sup>	AY841439
<i>Unonopsis rufescens</i>	AY743455 <sup>a</sup>	AY743474 <sup>a</sup>	AY841518

### *Maximum Likelihood (ML) analysis*

For maximum likelihood (ML) analysis was used the RAxML (Stamatakis 2014) web-server program available at the CIPRES portal in San Diego, CA, USA (Miller et al. 2010, <http://www.phylo.org/portal2>). A rapid Bootstrap analysis (Stamatakis et al. 2008) and search for the best-scoring ML tree were conducted in one single program run (-f a). The option “let RaxML halt bootstrapping automatically” was select and the bootstrapping criterion was the extended majority rule consensus tree (-N autoMRE). The substitution model selected was GTRGAMMA under 13 partitions as recovered by PartitionFinder (Lanfear et al. 2012).

### *Bayesian Inference (BI)*

Bayesian analysis was conducted in MrBayes 3.2.6 (Ronquist et al. 2012) via CIPRES platform (Miller et al. 2010). Two independent analyses were run (nruns = 2) with three heated chains and one cold chain (nchain = 4). The first 25% of the cold chain were discarded as burn-in. The number of generations of the Markov chain Monte Carlo was set to 100 million, but the analyses were stopped after 71,204,000 generations, 168 CPUs hours, because the average standard deviation of split frequencies had reached 0.009, an indication that the analyses converged. Both the print and sample frequencies were set to 1,000. The data were partitioned following the results obtained by PartitionFinder (Lanfear et al. 2012). The substitution model GTR (nst = 6) was set for each partition, with gamma shape distribution frequencies and invariants sites for some of the partitions, as described in the partition scheme results.

### *Maximum Parsimony (MP) analysis*

Matrix for parsimony analysis was derived from the 65,002 bp matrix used for ML and BI analyses, from which 61,669 uninformative characters were excluded, and 9 morphological characters were included (Table 3 and 4), resulting in a matrix with 3,342 characters. The exclusion of uninformative characters had the goal to speed up the processing time of the analysis. Data concatenation and edition were performed on Winclada (Nixon 1999b).

Parsimony analyses were conducted on TNT (Goloboff et al. 2003). First, a tree bisection reconnection (TBR) analysis was performed. To improve the search for the most parsimonious trees, the RATCHET was implemented with 100 iterations and five seeds and default settings (Nixon 1999a). Standard bootstrap analysis was performed

with 1,000 replications. Ambiguous transformations are indicated, and shown as ACTRAN in the cladogram.

## Results

### *Data partition and selection of substitution model*

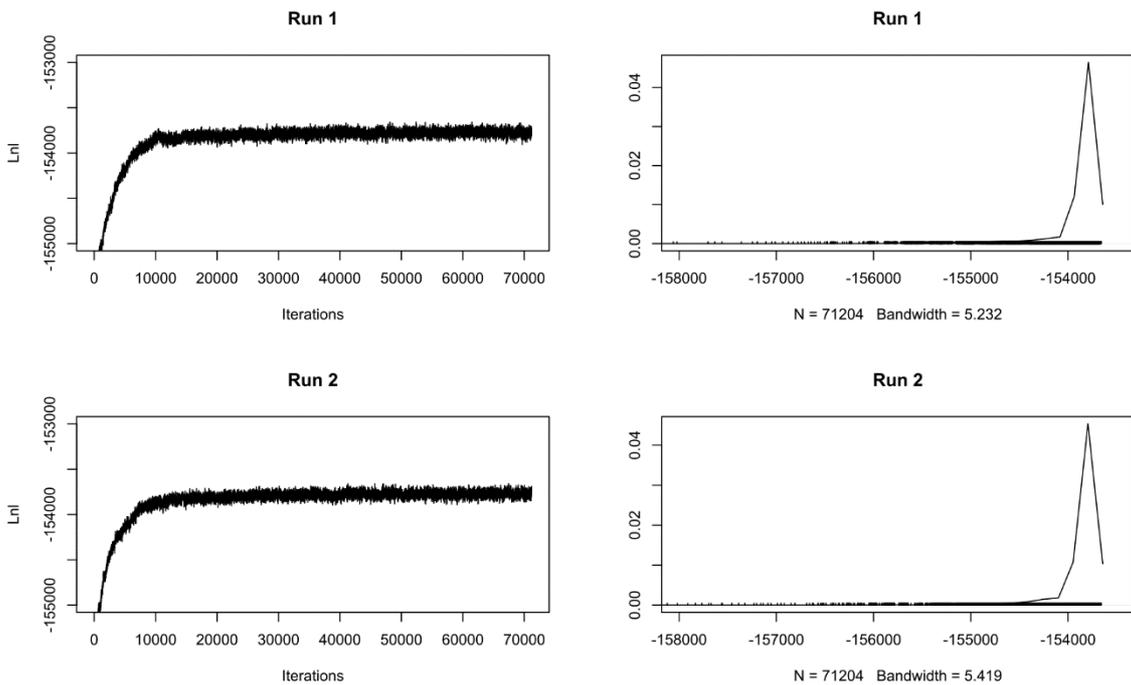
The best partition scheme has 13 subsets. Table 8 presents the partition subsets and substitution models for each subset.

**TABLE 8.** Best partition scheme found by PartitionFinder and the substitution model for each partition.

Subset	Model	Subset Partitions
1	<b>GTR+I+G</b>	atpA_1, atpB_1, atpH_1, atpI_1, ndhC_1, ndhH_1, petA_1, petD_1, petN_1, psaA_1, psaB_1, psaC_1, psbB_1, psbC_1, psbH_1, rpl2_1, rpl2_3, rpoB_1, rps14_1, rps7_1, ycf4_1
2	<b>GTR+I+G</b>	atpA_2, atpB_2, atpI_2, cemA_2, ndhB_1, ndhB_2, ndhB_3, ndhD_2, ndhG_2, ndhJ_2, petN_2, psaA_2, psbB_2, psbC_2, psbD_1, psbD_2, psbH_2, psbK_2, psbN_2, psbZ_2, rpl14_2, rpoB_2, rps15_2, rps8_3
3	<b>GTR+G</b>	atpA_3, atpE_3, ccsA_3, matK_3, ndhA_3, ndhC_3, ndhE_3, ndhG_3, ndhH_3, ndhI_3, petA_3, petD_3, petL_3, petN_3, psbH_3, psbT_3, rpl16_3, rpoB_3, rpoC1_2, rpoC2_3, rps15_3, rps16_1, rps2_3, rps3_3, rps4_3, rps8_3
4	<b>GTR+G</b>	atpB_3, infA_1, infA_2, matK_1, matK_2, ndhA_1, ndhA_2, ndhC_2, ndhD_3, ndhE_1, ndhE_2, ndhF_1, ndhI_1, petB_1, petB_2, petL_1, psaA_3, psbC_3, psbD_3, psbI_1, psbI_2, psbI_3, psbM_1, psbN_3, psbT_2, psbZ_1, psbZ_3, rpl14_3, rpl32_1, rpl32_2, rpl36_1, rpl36_2, rpoC1_3, rpoC2_1, rpoC2_2, rps11_1, rps11_2, rps14_2, rps16_2, rps16_3, rps19_1, rps2_1, rps3_1, rps3_2, rps4_1, rps8_1, trnL_trnF, ycf15_3, ycf2_1, ycf2_2, ycf2_3
5	<b>GTR+I+G</b>	atpE_1, rbcL_1, rpl14_
6	<b>GTR+G</b>	atpE_2, atpH_2, ccsA_1, ccsA_2, cemA_1, cemA_3, ndhD_1, ndhG_1, ndhH_2, ndhI_2, ndhK_1, ndhK_2, petA_2, petB_3, petD_2, petG_2, psaC_2, psbN_1, psbT_1, rpl23_1, rpl23_2, rpl23_3, rpl2_2, rpl36_3, rpoC1_1, rps15_1, rps2_2, rps4_2, rps7_2, rps7_3, ycf15_1, ycf15_2, ycf4_2
7	<b>GTR+I+G</b>	atpH_3, atpI_3, ndhF_2, ndhJ_3, ndhK_3, psaB_3, psaC_3, psbA_3, psbB_3, psbK_1, psbK_3, rbcL_3, rps14_3, rps19_3, ycf4_3
8	<b>GTR+G</b>	infA_3, ndhF_3, psaJ_3, psbM_3, rpl32_3, rpoA_1, rpoA_2, rpoA_3, rps11_3
9	<b>GTR+I+G</b>	ndhJ_1, psaJ_1, psaJ_2, psbA_1, psbA_2, rbcL_2, rpl16_2
10	<b>GTR+I+G</b>	petG_1, petG_3, psbM_2, rpl16_1, rps19_2
11	<b>GTR+I+G</b>	petL_2
12	<b>GTR+I+G</b>	psaB_2
13	<b>GTR+I+G</b>	psbA_trnH

### *Phylogenetic relationship*

The two runs of the Bayesian inference (BI) analysis converged (Figure 1). The consensus tree is presented on figure 2. The majority rule consensus tree generated from maximum likelihood (ML) analysis is showed on figure 3. Three most parsimonious trees were found with 5565 steps each. Strict consensus tree is showed on figure 4.

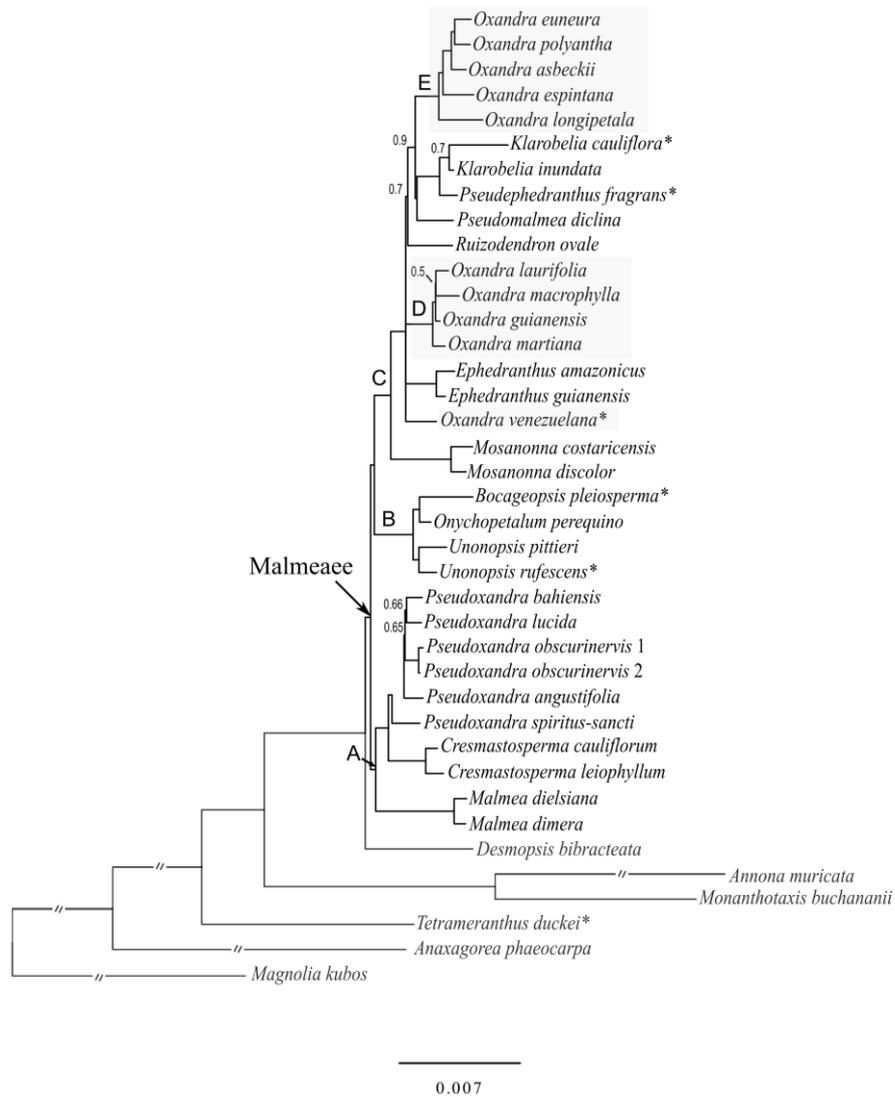


**Figure 1.** Likelihood distribution per generations of the MCMC in the two independent runs, showing the runs convergence.

The three analyses have recovered three highly supported groups in tribe Malmeeae (0.95 of posterior probability (PP) in BI analysis and more than 95% Bootstrap (BS) in ML and maximum parsimony (MP) analyses; figures 2, 3 and 4). Tribe Malmeeae is monophyletic (1.0 BI, 86% ML, 87% MP) and supported by imbricate petals (character 4: state 0, ambiguously homoplastic), and spiniform rumination (9: 2, homoplastic), with a change to valvate petals in *Unonopsis*, *Bocageopsis*, *Onychopetalum* group, and two changes each to lamellate and peg-shaped rumination (Figure 4). *Malmea* sister to *Crematosperma* and *Pseudoxandra* (1.0 BI, 98% ML, 88% MP) are sister to the remaining genera. *Pseudoxandra* emerged as monophyletic, supported by narrow stamen with tongue-like extension connective (7: 1, homoplastic), with *P. spiritus-sancti* sister to the rest of the species (1.0 BI, 94% ML, 88% MP). ((*Unonopsis* (*Bocageopsis*, *Onychopetalum*)) (*Mosanonna* (*Ephedranthus*, *Klarobelia cauliflora* (oxandra laurifolia group (((*Pseudephedranthus*, *Klarobelia inundata*) *Pseudomalmea*) oxandra eunera group) (*Ruizodendron*, *Oxandra venezuelana*)))))) is supported by convex midrib (2:1, ambiguously homoplastic) (1.0 BI, 94% ML, 90% MP). *Bocageopsis* and *Onychopetalum* sister to *Unonopsis* is supported by many flowers (3:1, homoplastic), valvate petals (4:1, ambiguously

homoplastic), cylindrical stigma (6:2 homoplastic), and narrow stamen with tongue-like connective extension (7:1, homoplastic, change in *Unonopsis rufescens*) (1.0 BI, 100% ML, 98% MP; node B, figures 2, 3 and 4). *Mosanonna* is monophyletic and supported by lamellate rumination (9:3, homoplastic) (1.0 BI, 100% ML, 100% MP). *Mosanonna* sister to (*Ephedranthus*, *Klarobelia cauliflora* (oxandra laurifolia group (((*Pseudephedranthus*, *Klarobelia inundata*) *Pseudomalmea*) oxandra euneura group) (*Ruizodendron*, *Oxandra venezuelana*)))) is supported by concave to flat midrib (2:0, ambiguously homoplastic, reversion in *Pseudephedranthus* and in oxandra euneura group) (node C, figures 2, 3 and 4). In this polytomy, there are two highly supported groups of *Oxandra*, (((*O. laurifolia*, *O. macrophylla*) *O. guianensis*) *O. martiana*), the oxandra laurifolia group (1.0 BI, 100% ML, 75% MP; node D, figures 2, 3 and 4) and (((*O. euneura*, *O. polyantha*) *O. asbeckii*) *O. espintana*) *O. longipetala*), the oxandra euneura group supported by convex midrib (2:1, ambiguously homoplastic) (1.0 BI, 100% ML, 99% MP; node E, figures 2, 3 and 4). ((*Ruizodendron ovale*, *Oxandra venezuelana*) ((*Pseudomalmea diclina* (*Pseudephedranthus fragrans*, *Klarobelia inundata*)) oxandra euneura group)) is supported by lamellate rumination (9:3, homoplastic) with a change to peg-shaped rumination in oxandra euneura group (0.7 BI, 53% ML, 2% MP). The group (*Pseudomalmea diclina* (*Pseudephedranthus fragrans*, *Klarobelia inundata*)) is supported by androdioecy (1:1, homoplastic) (0.95 BI, 48% ML, 11% MP), which also supports *Ephedranthus* (1.0 BI, 100% ML, 100% MP).

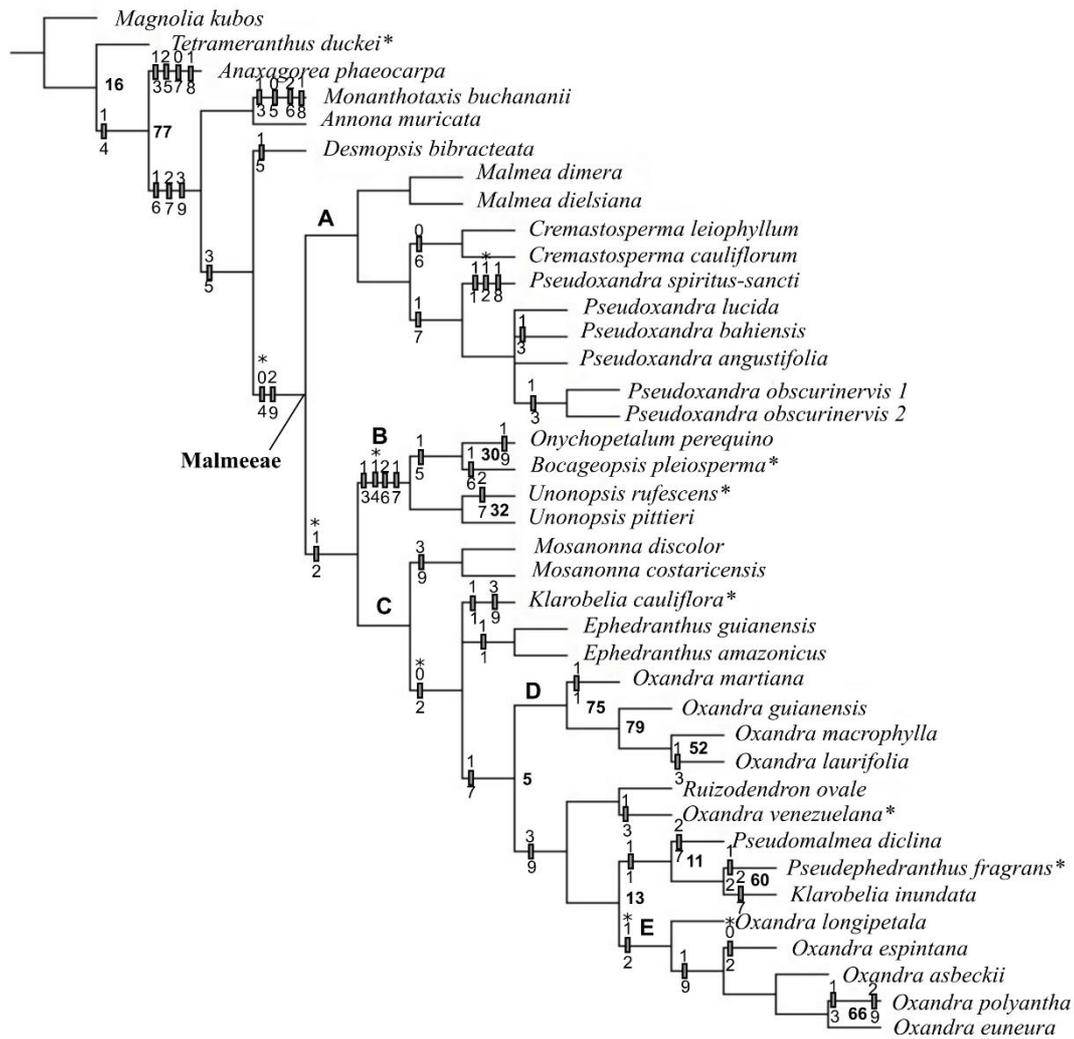
*Klarobelia cauliflora* is not placed together with *K. inundata* in the ML and MP phylogenies, but in the main polytomy that includes *Ephedranthus*, both groups of *Oxandra*, *Pseudephedranthus fragrans*, *Pseudomalmea*, and *Ruizodendron ovale* (Figures 3 and 4). Nevertheless, *Klarobelia cauliflora* emerges as sister to *K. inundata* in the Bayesian analysis (0.7 BI; figure 2). *Oxandra venezuelana* has a different position in each analysis. In a polytomy together with *Ephedranthus*, *Klarobelia*, *Oxandra*, *Pseudephedranthus fragrans*, *Pseudomalmea*, and *Ruizodendron ovale* in the Bayesian analysis (Figure 2), sister to *Ephedranthus* in maximum likelihood phylogeny (Figure 3), and sister to *Ruizodendron ovale* in parsimony analysis (Figure 4). *Ruizodendron ovale* is sister to (oxandra euneura group ((*Klarobelia*, *Pseudephedranthus fragrans*) *Pseudomalmea diclina*)) in BI and ML analyses (0.7 BI, 53% ML; figures 2 and 3).



**Figure 2.** Consensus tree from Bayesian Inference analysis. Nodes without indication were recovered with 0.95 to 1.0 of posterior probability. Asterisks (\*) denote species with incomplete data. Letters in the nodes are cited in the text.



**Figure 3.** Consensus tree generated from Maximum Likelihood analysis. Nodes without indication were recovered with 85 to 100% of Bootstrap percentage. Asterisks (\*) denote species with incomplete data. Letters in the nodes are cited in the text.



**Figure 4.** Strict consensus tree from Maximum Parsimony analysis. Nodes without indication were recovered with 85 to 100% of Bootstrap percentage. Asterisks after species names (\*) denote species with incomplete data. Letters in the nodes are cited in the text. Bars are states changes, numbers below are character and above states, asterisks (\*) above states indicate states with ambiguous optimization.

#### Ancestral state analysis

The reconstruction of the sexual system suggests that the ancestral state in Malmeaceae was hermaphroditism with PP 0.97 (Figure 5A, node 6). The analysis indicates that hermaphroditism remained ancestral up to node 11 in the ancestral that leads to the lineage formed by *Klarobelia*, *Pseudephedranthus*, *Pseudomalmea*, *Ruizodendron* and *Oxandra espintana* group with moderate posterior probability of 0.72 PP (Figure 5BB). Besides, the analysis suggests that androdioecy has evolved three

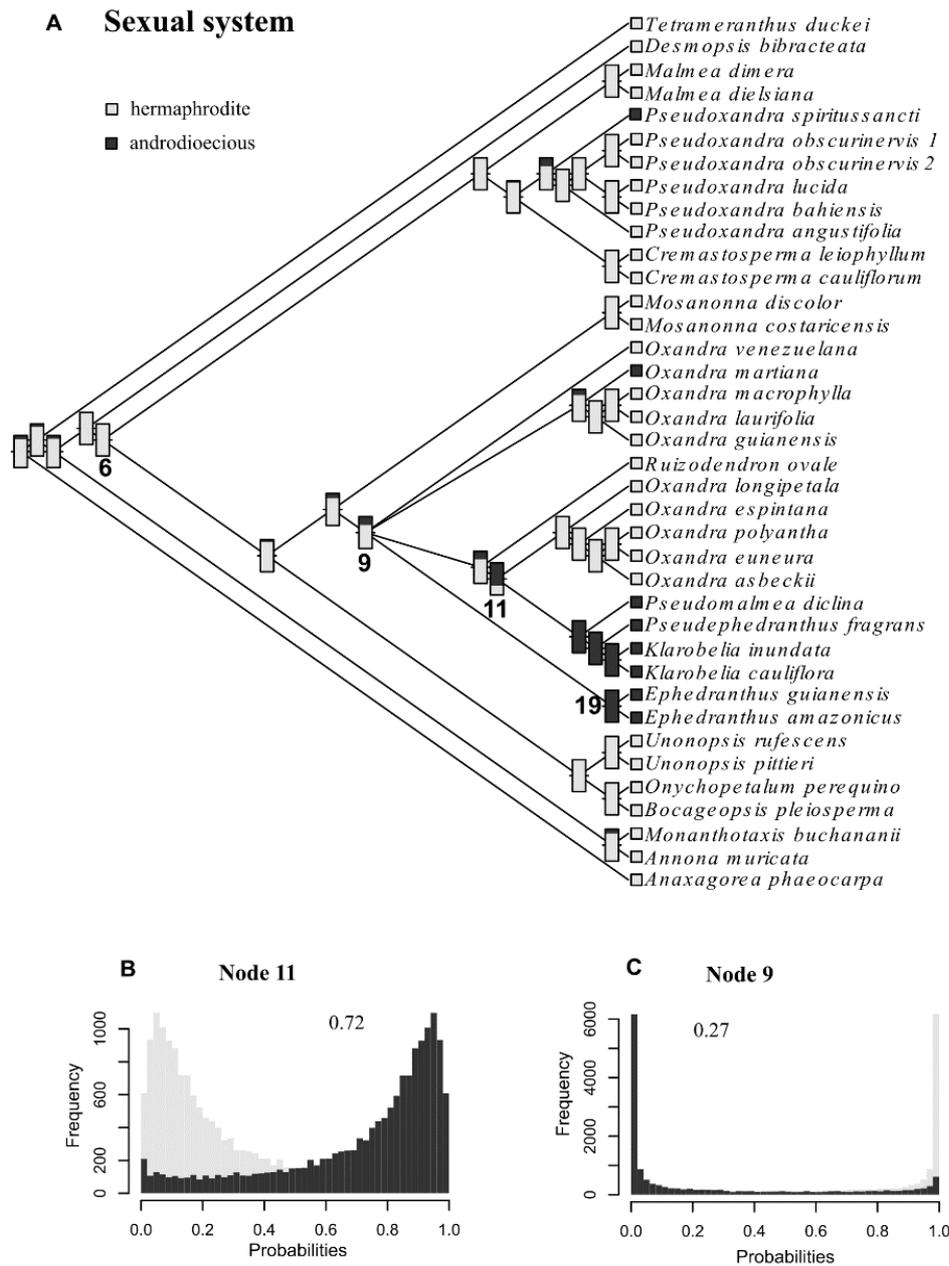
times more, in *Ephedranthus* (node 19), *Oxandra martiana* and *Pseudoxandra spiritus-sancti*. It is unlike that androdioecy has appeared before than node 9, indicating that there were here three independent origins (Figure 5C).

Concave to flat midrib is the ancestral condition in the tribe (PP 0.90, figure 6A, node 6). Convex midrib is present in (*Unonopsis* (*Bocageopsis*, *Onychopetalum*)), *Mosanonna* and oxandra euneura group. Their more recent common ancestor has convex primary vein (with PP 0.73, figure 6B, node 8). In the same manner, all species of oxandra euneura group, but *O. espintana*, show convex midrib, the ancestral state estimate for this group (PP 0.96, figure 6C, node 15). In *Pseudoxandra*, just *P. spiritus-sancti* shows convex midrib, the most recent common ancestor was estimated to have concave midrib with 0.70 PP (Figure 6A, node 29).

Solitary flowers were estimated to be ancestral in the tribe, with 0.95 PP (Figure 7A, node 6), and the character has changed five times in Malmeeae history. The ancestral that includes all tribe members but *Malmea*, *Crematosperma* and *Pseudoxandra* was estimated as possessing uncertain character state (node 7, figure 7B). Many flowers are the synapomorphy of (*Unonopsis* (*Bocageopsis*, *Onychopetalum*)), with PP 0.99 (Figure 7A, node 24).

Imbricate petals are ancestral in Malmeeae (PP 0.99, figure 8, node 6), and (*Unonopsis* (*Bocageopsis*, *Onychopetalum*)) has valvate petals (PP 0.99, figure 8, node 24). It is unlike that valvate petals have arisen before in Malmeeae, once the posterior probability of this state in the node just below is around zero (Figure 8, node 7).

One ovule per carpels and capitate stigma are ancestral in Malmeeae (PP 0.99 and 0.95, respectively, figures 9A and 10A, node 6). Two to five ovules per carpel are only found in *Bocageopsis* and *Onychopetalum* (Figure 9A, node 25). One ovule per carpel remained ancestral up to node 7 (0.93 PP, figure 9B). Inside the tribe, cylindrical stigma is a synapomorphy of (*Unonopsis* (*Bocageopsis*, *Onychopetalum*)) (PP 0.98, figure 10A, node 24), with a reversion in *Bocageopsis*. Lobed stigma in *Crematosperma* (Figure 10A, node 34) was estimate to have occurred in the ancestral of the genus (PP 0.99) and not before, because the ancestral state was estimated to be capitate stigma (PP 0.84, figure 10B, node 28).

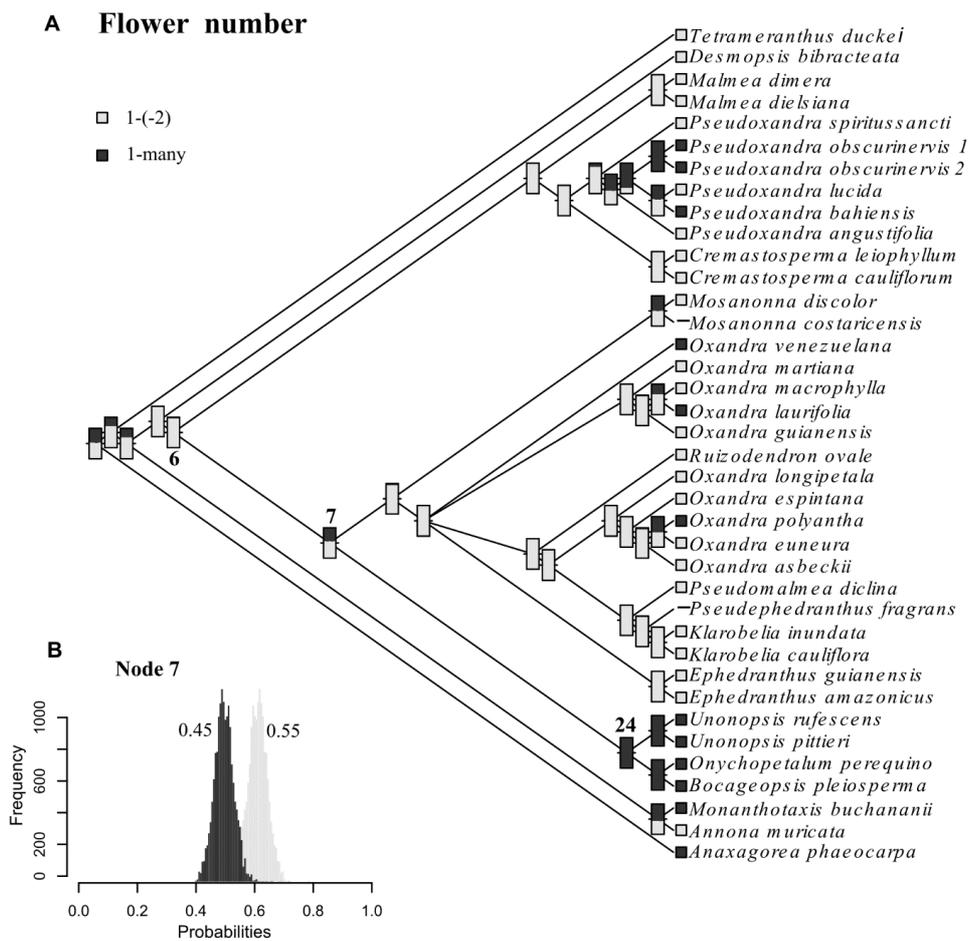


**Figure 5.** Consensus tree from BI. Bars show estimation of the posterior probability (PP) for each state; panels depict distribution of PP for selected nodes and show the mean values of PP for each state. **A.** Ancestral state analysis for sexual system. **B.** Node 11, distribution of states PP. **C.** Node 9, distribution of states PP.

In the tribe, stamens can be narrow, with tongue-like connective, or peltate-truncate. The first is the ancestral state (PP 0.99, figure 11, node 6). Peltate-truncate stamens have evolved independently seven times in the tribe, in *Crematosperma*, *Ephedranthus*, *Klarobelia*, *Malmea*, *Mosanonna*, *Pseudomalmea*, and *Unonopsis*. In

(*Unonopsis* (*Bocageopsis*, *Onychopetalum*)) stamen with tongue-like connective was estimated as ancestral (PP 0.82, figure 11, node 26), with reversion in *Unonopsis*.

Absence of staminodes is an ancestral state in Malmeeae (PP 0.99, figure 12A, node 6). In the whole tribe, staminodes have been reported only in *P. spiritus-sancti*. The probability of this state has arisen in the ancestral of *Pseudoxandra* lineage is only PP 0.30 (Figure 12B, node 29).



**Figure 6.** Consensus tree from BI. Bars show estimation of the posterior probability (PP) for each state; panels depict distribution of PP for selected nodes and show the mean values of PP for each state. **A.** Ancestral state analysis for midrib. **B.** Node 8, distribution of states PP. **C.** Node 15, distribution of states PP.

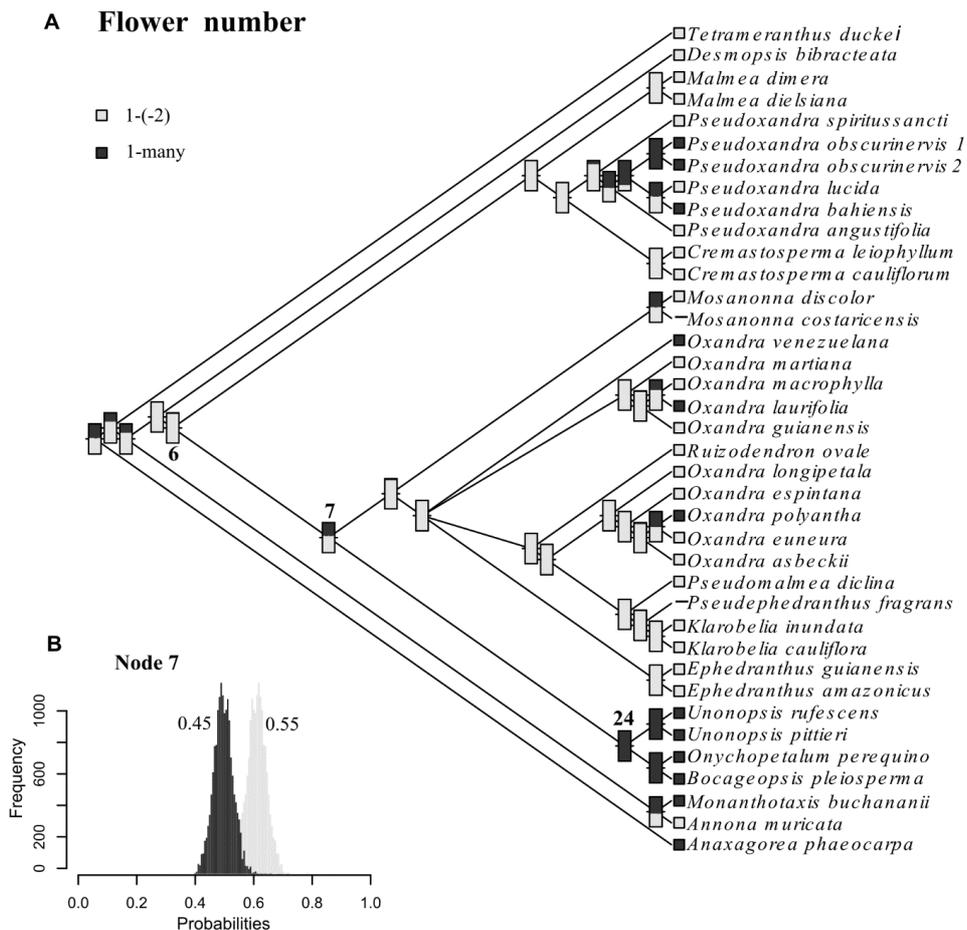
## Discussion

### *Phylogenetic relationship*

The phylogenetic relationships among the genera of tribe Malmeeae have suffered some improvements when compared with previous phylogenies (Chatrou et al. 2012, Pirie et al. 2006). Instead of a polytomy at the base of the tribe, a dichotomy with (*Malmea* (*Crematosperma*, *Pseudoxandra*)) sister to the rest of the tribe (1.0 BI, 86% ML, 87% MP) has emerged. (*Unonopsis* (*Bocageopsis*, *Onychopetalum*)) is sister to *Mosannonna*, *Pseudephedranthus*, *Klarobelia*, *Ephedranthus*, *Oxandra*, *Ruizodendron* and *Pseudomalmea*, as in Pirie et al. (2006) and Chatrou et al. (2012). In addition, *Crematosperma* and *Pseudoxandra* are confirmed as sister groups (Chatrou et al. 2012, Pirie et al. 2006), and the monophyly of *Pseudoxandra* is highly supported, the increasing of data providing a better result in this aspect than in Pirie et al. (2006). *Klarobelia* sister to *Pseudephedranthus* has been recovered (Chatrou et al. 2012, Pirie et al. 2006). *Pseudomalmea*, of uncertain position in Chatrou et al. (2012), and in Pirie et al. (2006), is now sister to *Klarobelia* and *Pseudephedranthus*, with high support in BI analysis.

Despite these improvements, the use of 66 chloroplast molecular markers was not enough to resolve the position of *Oxandra venezuelana*. Thus, other strategies, such as the use of low-copy nuclear genes, which normally show to be rich in phylogenetic information (Sang 2002), could be a solution to phylogenetic problems like that.

A successful strategy adopted in our analysis was the inclusion of six species with only three out of 66 molecular markers sampled. Adding taxa with incomplete data is a main issue in phylogenetic analysis (Wiens & Tiu 2012). Computational simulations with the addition of taxa with up to 90% of missing data have proven to benefit the accuracy of analyses in Bayesian, maximum likelihood and parsimony approaches (Wiens & Tiu 2012). Bayesian and Likelihood analyses showed better results in accuracy than Parsimony when adding 75% more samples with only 10% of complete data to the analyses (Wiens & Tiu 2012). However, the paucity of data for *Klarobelia cauliflora* and *Oxandra venezuelana*, with only 5% of complete data, could explain their problematic placements (Figure 2, 3 and 4).



**Figure 7.** Consensus tree from BI. Bars show estimation of the posterior probability (PP) for each state; panels depict distribution of PP for selected nodes and show the mean values of PP for each state. **A.** Ancestral state analysis for Flower number. **B.** Node 7, distribution of states PP.

### *Ancestral state evolution analysis*

#### *Evolution of androdioecy*

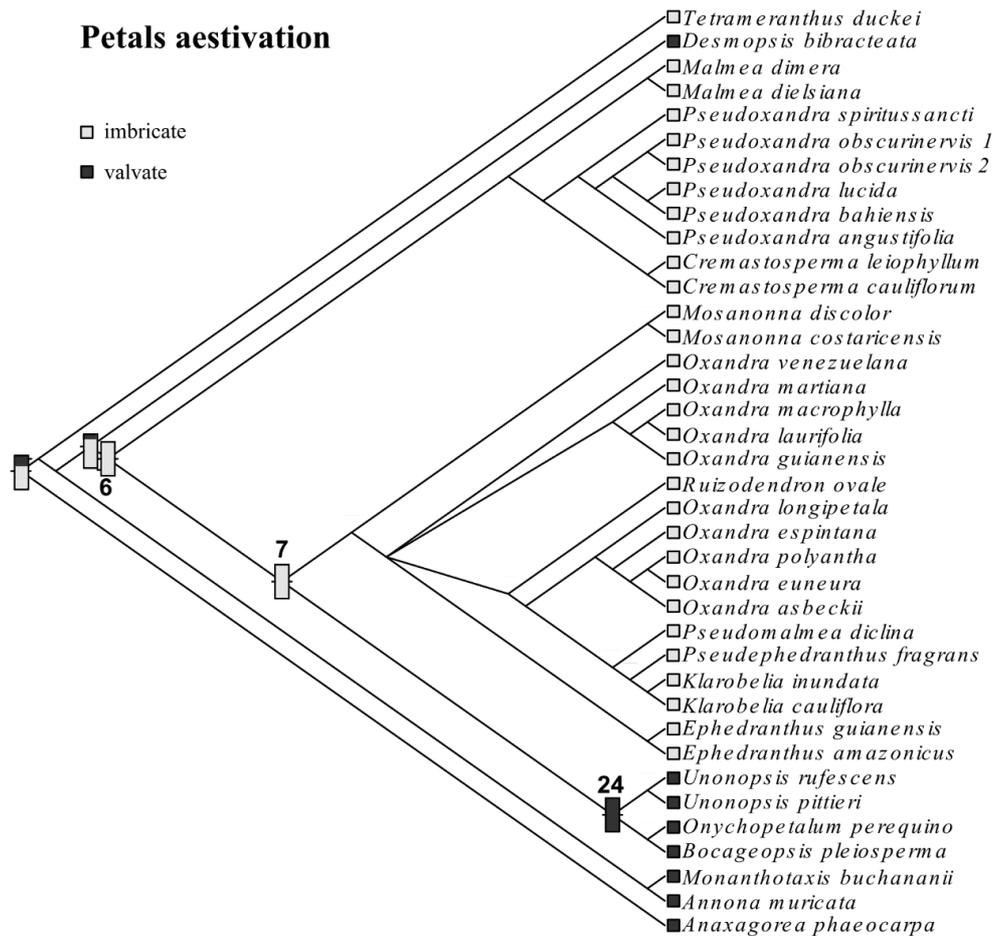
Hermaphroditism is ancestral in Malmeeae as in many taxa of Annonaceae (Saunders 2010). Presence of male flowers is homoplastic in the tribe and has appeared four or five independent times depending on the analysis considered (Figure 4 and 5A). Nevertheless, androdioecy is a synapomorphy of at least two groups, the one formed by *Klarobelia*, *Pseudephedranthus* and *Pseudomalmea*, with 14 species, and of *Ephedranthus*, with seven species (Chatrou 1998, Lopes & Mello-Silva *in prep*). Independent occurrence of androdioecy has been observed also in *Pseuduvaria*, an Asian dioecious genus of Annonaceae, in which androdioecy has evolved five times (Su

et al. 2008), although autapomorphically, and not as synapomorphies like in Malmeeae. Despite *Pseuduvaria* and Malmeeae belonging to Malmeoideae, the evolution of androdioecy has different origins in both groups. In *Pseuduvaria* it is derived from dioecy and in Malmeeae from hermaphroditism. In *Fraxinus*, Oleaceae, a group of woody plants in which the plesiomorphic condition is hermaphroditism, as in Malmeeae, evolution to androdioecy occurred just once and has acted as an intermediate step to the evolution of dioecy in one lineage (Wallander 2008). Some authors (Bawa 1980, Charlesworth 1984, Wolf & Takebayashi 2004) have hypothesized the evolution of androdioecy as an intermediate step to dioecy, as illustrated by *Fraxinus*, but well-authenticated cases has not been reported (Barrett 2002). However, this is not the case in Malmeeae, in which dioecy has not been reported.

Thus, the evolution of androdioecy in Malmeeae is an exception, since it is not derived from dioecy neither an intermediate step to the evolution of dioecy, but rather derived from hermaphroditism. An explanation that cannot be discarded is that bisexual flowers are here acting as female flowers, with androdioecious species being functionally dioecious (Lloyd 1979). Our results with *Pseudoxandra spiritus-sancti* show that pollen from bisexual flowers are viable, being unlike that *P. spiritus-sancti* acts as a functional dioecious species (Lopes et al. *in prep.*).

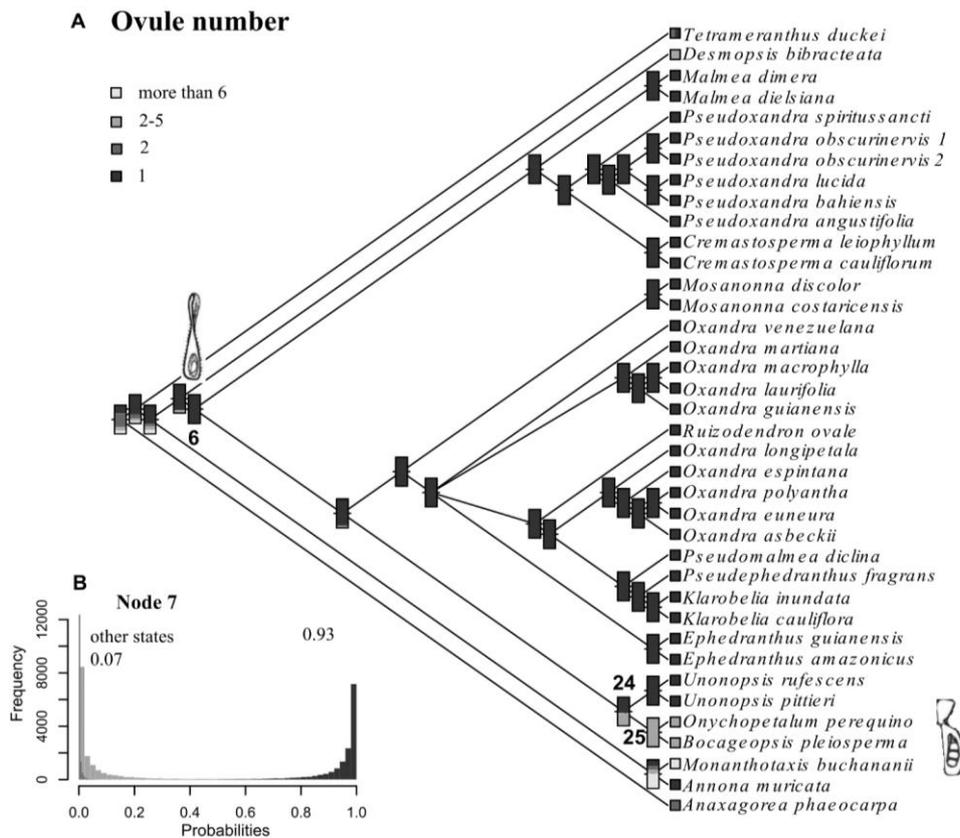
#### *Evolution of morphological features*

Characters just found in (*Malmea* (*Cresmastosperma*, *Pseudoxandra*)) are the lobed stigma (7), an autapomorphy of *Cresmastosperma* (Pirie 2005, Figures 4 and 10A) and the presence of staminodes (8), in a single species of *Pseudoxandra* (Lopes et al. *in prep.*, figures 4 ad 12A). Staminodes are found in few genera of Annonaceae, notably in *Anaxagorea*, the sister genus of the rest of the family (Chatrou et al. 2012, van Heusden 1992). Staminodes are also present in Eupomatiaceae, the sister family of Annonaceae (APG III 2009, Endress 1984). Our ancestral state analysis is limited in relation to outgroups and has estimated the absence of staminodes as ancestral in Malmeeae. But there are three alternative optimizations of this character within Magnoliales, and the presence of staminodes in *Pseudoxandra spiritus-sancti* could be either a reversion or a convergence.



**Figure 8.** Consensus tree from BI. Bars show estimation of the posterior probability (PP) for each state. Ancestral state analysis for petal aestivation.

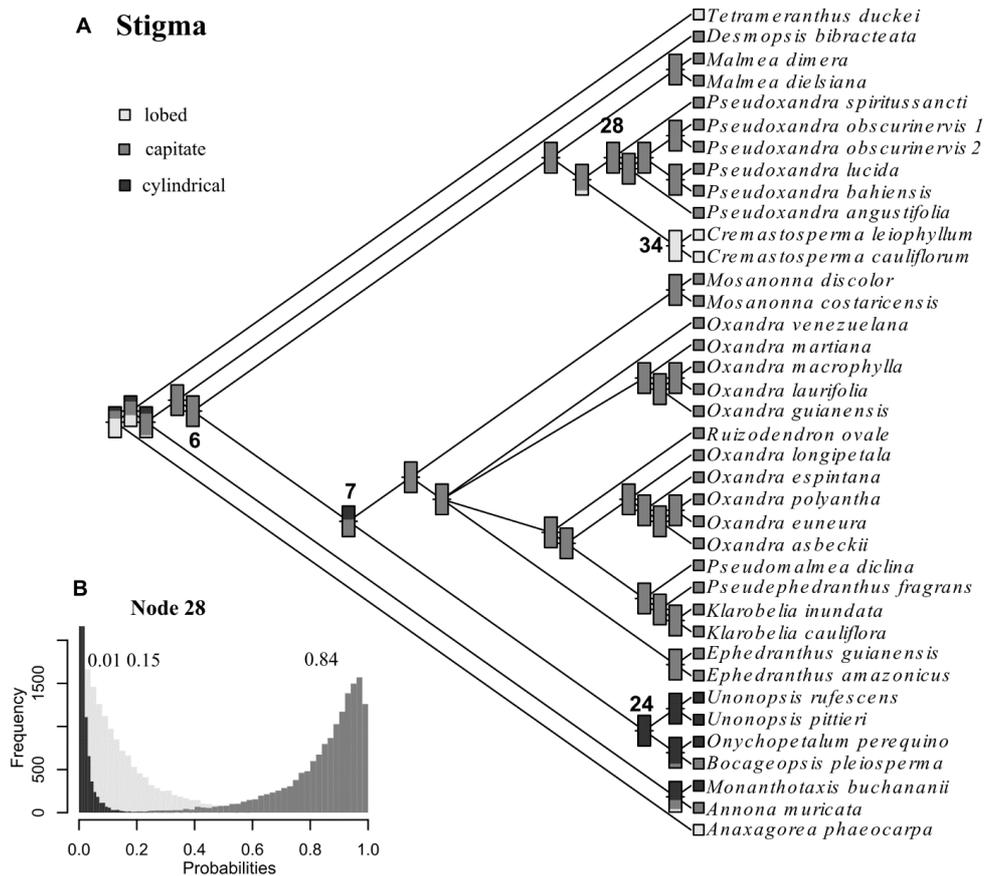
Valvate petals are just found in *Unonopsis*, *Bocageopsis* and *Onychopetalum*. The petals aestivation is one of the main characteristics used to delimit groups in classifications of Annonaceae, (Koek-Noorman et al. 1990). For this reason, these genera with valvate petals were not grouped together with *Crematosperma*, *Ephedranthus*, *Malmea s.l.*, *Oxandra*, *Pseudoxandra*, and *Ruizodendron*, genera with imbricate petals. In Malmeeae, imbricate petals (character 4) are the ancestral state, and the change to valvate petals is a synapomorphy of *Unonopsis*, *Bocageopsis* and *Onychopetalum* group (Figures 4 and 8). Besides that, many flowers (3) and cylindrical stigma (6) are also synapomorphies of that group (Figures 4, 7A and 10A). *Bocageopsis* and *Onychopetalum* are sister groups that share carpels with two to five ovules (5), a unique novelty within the tribe (Maas et al. 2007, figures 4 and 9A).



**Figure 9.** Consensus tree from BI. Bars show estimation of the posterior probability (PP) for each state; panels depict distribution of PP for selected nodes and show the mean values of PP for each state. **A.** Ancestral state analysis for number of ovules per carpel. **B.** Node 7, distribution of states PP. Drawings from van Heusden (1992).

Convex or raised midrib (2), other feature that has been traditionally used to recognize *Bocageopsis*, *Onychopetalum*, and *Unonopsis* (Maas et al. 2007), emerges as a synapomorphy of the clade that includes all genera except *Malmea*, *Crematosperma* and *Pseudoxandra* (Chatrou 1998, Maas et al. 2007, Oliveira & Sales 1999, Figures 4 and 6A). However, the evolution of this feature in the tribe is complex, with many reversions. *Oxandra euneura* group has convex midrib, the only exception being *O. espintana*, and *oxandra laurifolia* group show impressed to flat midrib (Figures 4 and 6A, nodes 15 and 20). Other species of *Oxandra* not analysed here follow the same division in Pirie et al. (2006). *Oxandra nitida* R.E.Fr., included in the *oxandra euneura* group, has convex midrib, and *O. sphaerocarpa* R.E.Fr. and *O. xylopioides* Diels, belonging to the *oxandra laurifolia* group, show impressed to flat midrib. Thus, the evolution to convex midrib is a synapomorphy of *oxandra euneura* group. Another

traditionally important feature in previous classifications of Annonaceae, and in the recognition of *Oxandra* is the stamen shape (Prantl 1891, van Heusden 1992). Narrow stamen with tongue-like connective, found in *Oxandra* and other genera of Malmeeae, has proven to be ancestral in the tribe.



**Figure 10.** Consensus tree from BI. Bars show estimation of the posterior probability (PP) for each state; panels depict distribution of PP for selected nodes and show the mean values of PP for each state. **A.** Ancestral state analysis for stigma. **B.** Node 28, distribution of states PP.

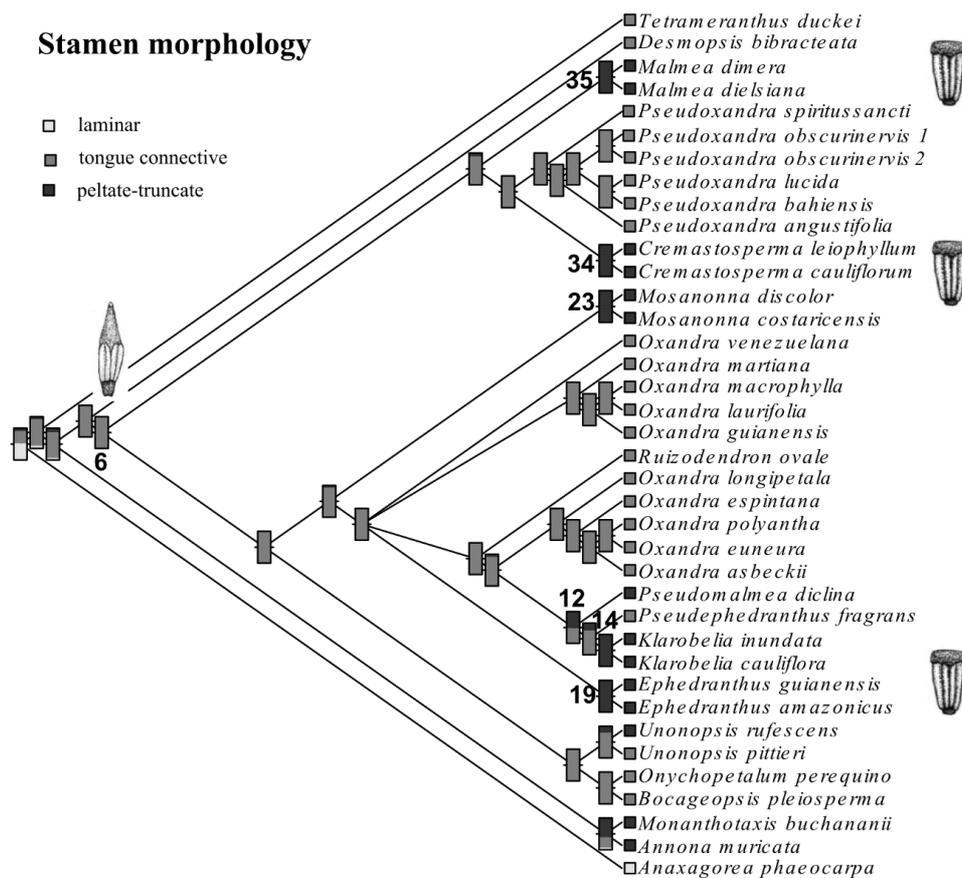
The two groups of *Oxandra* are also supported by the seed rumination, which is spiniform in the *oxandra laurifolia* group, and peg-shaped in the *oxandra euneura* group, except in *O. polyantha*, with spiniform rumination (character 9:n 2; figure 4). The group that includes the *oxandra euneura* group, ((*Ruizodendron ovale*, *Oxandra venezuelana*) ((*Pseudomalmea diclina* (*Pseudephedranthus fragrans*, *Klarobelia inundata*)) *oxandra*

euneura group)), has lamellate rumination as the ancestral state (Figure 4). This group includes two monotypic genera, *Pseudephedranthus* and *Ruizodendron*, one with only three species, *Pseudomalmea*, and *Klarobelia*, with 12 species. *Klarobelia*, *Pseudephedranthus*, and *Pseudomalmea* share the androdioecy (Chatrou 1998, Oliveira & Sales 1999). *Ruizodendron* is hermaphrodite and its excentrically stipitate monocarps are unique in the tribe (Paul Maas unpublished data). An isolated of the genus *Oxandra*, *O. venezuelana*, has uncertain position. Since *Oxandra* is polyphyletic and the oldest genus in the tribe, nomenclatural changes would be needed in order to recover the monophyletism of *Oxandra*. However, *Oxandra lanceolata* (Sw.) Baill., the type of the genus, has not been sampled.

#### *Groups and classification*

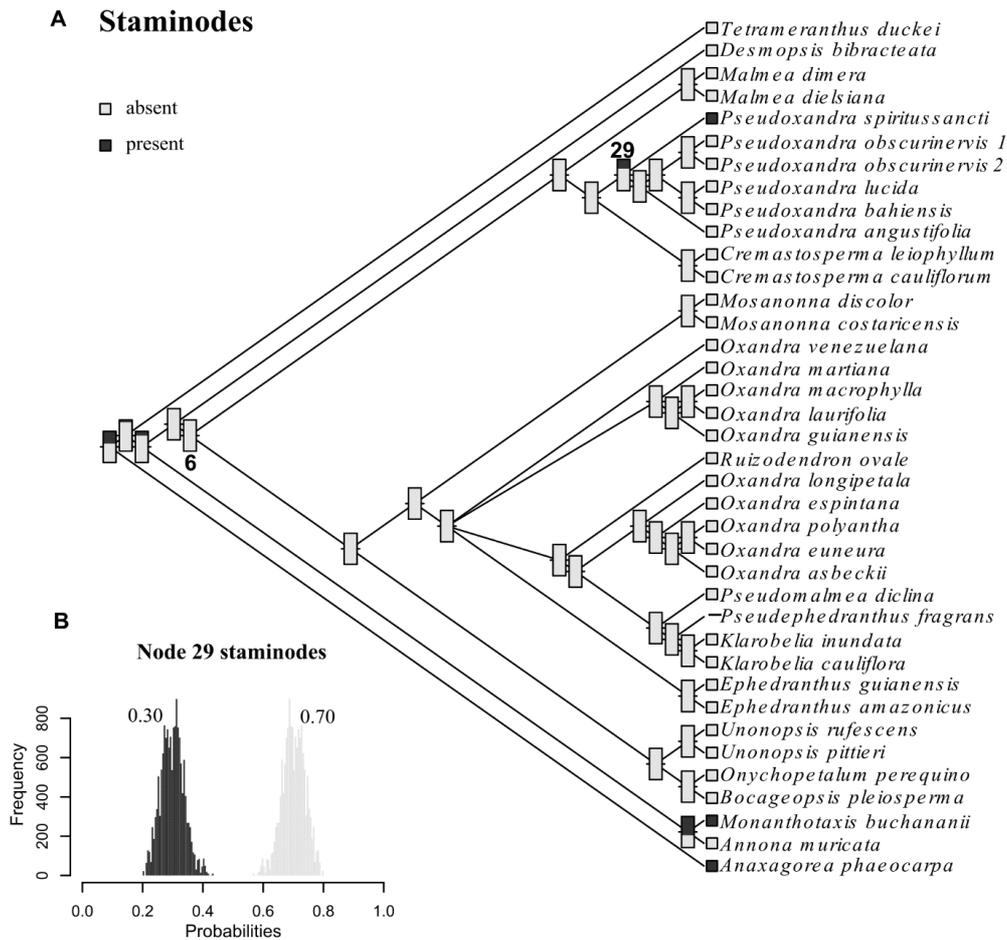
Most genera in Malmeeae have been described in the 20th century. *Oxandra* A. Richard (1845) was the first to be described. The remaining genera, *Ephedranthus* S. Moore (1895), *Unonopsis* R.E. Fries (1900), *Malmea* R.E. Fries (1905), *Bocageopsis* R.E. Fries, *Crematosperma* R.E. Fries, and *Onychopetalum* R.E. Fries (1931), *Ruizodendron* R.E. Fries (1936), *Pseudoxandra* R.E. Fries (1937), *Pseudephedranthus* Aristeguieta (1969), *Klarobelia* Chatrou, *Mosannonna* Chatrou, and *Pseudomalmea* Chatrou (1998) were described later, almost all of them in 20<sup>th</sup> century.

*Klarobelia*, *Mosannonna*, and *Pseudomalmea* have been dismembered from *Malmea s.l.* (Chatrou 1998), and *Pseudephedranthus fragrans* was at first classified as *Ephedranthus* (Aristeguieta 1969). *Ephedranthus* and *Oxandra* are included in the group Uvarieae in Engler & Diels's (1900) classification. However, Prantl (1891) placed them in different groups, *Oxandra* in Miliusae, a tribe characterized by prolonged connectives, and *Ephedranthus* in Uvarieae, which includes taxa mostly with imbricate petals (Koek-Noorman et al. 1990).



**Figure 11.** Consensus tree from BI. Bars show estimation of the posterior probability (PP) for each state. Ancestral state analysis for stamen morphology. Drawings from van Heusden (1992).

In the most recent classifications, *Crematosperma*, *Ephedranthus*, *Malmea* s.l., *Oxandra*, *Pseudoxandra*, and *Ruizodendron* were classified in the Uvarieae tribe (Fries 1959, Hutchinson 1964). Uvarieae sensu Fries (1959) is subdivided into informal groups, being *Malmea* s.l. included in the Duguetia group, and the remaining genera in the Assimina group. One of the differences between these two groups is the leaf-opposed inflorescence in Duguetia group and the axillary one in the Assimina group (van Heusden 1992). van Heusden (1992) placed *Crematosperma*, *Ephedranthus*, *Malmea* s.l., *Oxandra*, *Pseudoxandra* e *Ruizodendron* in the Crematosperma group, based on floral characteristics. They share imbricate petals and sepals, and a basal or lateral single ovule per carpel.



**Figure 12.** Consensus tree from BI. Bars show estimation of the posterior probability (PP) for each state; panels depict distribution of PP for selected nodes and show the mean values of PP for each state. **A.** Ancestral state analysis for staminodes. **B.** Node 29, distribution of states PP.

*Bocageopsis*, *Onychopetalum* and *Unonopsis* were classified by Fries (1959) in tribe Unoneae, and by Hutchinson (1964) in Xylopiineae, both groups including the genera with valvate petals. Fries (1959) assigned these genera, together with other African genera, to the *Unonopsis* group, inside tribe Unoneae. Even though, Unoneae sensu Fries (1959) formed a heterogeneous assemblage due to the selection of characters (Koek-Noorman et al. 1990). *Bocageopsis*, *Onychopetalum* and *Unonopsis* were placed together also by van Heusden (1992) in the *Unonopsis* group.

Walker (1971) was the first to place together *Bocageopsis*, *Onychopetalum*, and *Unonopsis* (Unoneae, *Unonopsis* group, Xylopiineae), with *Crematosperma*,

*Ephedranthus*, *Malmea s.l.*, *Oxandra*, *Pseudoxandra*, *Ruizodendron*, (Uvarieae, Crematosperma group), in a new tribe called Malmea. His classification was based in pollen features, having all these genera columellar monosulcate pollen grains. Except for *Annickia*, an African genus also placed in tribe Malmea, this is the current delimitation of tribe Malmeeae (Chatrou et al. 2012). *Annickia*, by its turn, has been placed in the tribe Piptostigmateae, also in subfamily Malmeoideae (Chatrou et al. 2012).

## Conclusions

Malmeeae has three main groups, (*Malmea (Crematosperma, Pseudoxandra)*) sister to the rest of the tribe, and (*Unonopsis (Bocageopsis, Onychopetalum)*) sister to (*Mosanonna (Ephedranthus, Klarobelia cauliflora (oxandra laurifolia group (((Pseudephedranthus, Klarobelia inundata) Pseudomalmea) oxandra eunera group (Ruizodendron, Oxandra venezuelana))))*). *Oxandra* is polyphyletic and composed by two main groups of species plus *Oxandra venezuelana*, sister either to *Ruizodendron* or to *Ephedranthus*. Thus, a potential taxonomic solution for that would be sinking all those small and weakly delimited genera of (oxandra laurifolia group (((Pseudephedranthus, Klarobelia inundata) Pseudomalmea) oxandra eunera group (Ruizodendron, Oxandra venezuelana))) into a broad delimited *Oxandra* (Backlund & Bremer 1998). A taxonomic revision and a phylogenetic reconstruction, including a broader sample of *Oxandra* species, have been initiated (Maas & Junikka unpublished data, Zwartsenberg et al. unpublished data). Both taxonomic and phylogenetic results will bring new information for more reasoned decision.

Hermaphroditism is plesiomorphic in the tribe, with four independent evolutions to androdioecy, which is a synapomorphy of (*Pseudephedranthus, Klarobelia Pseudomalmea*), that includes 14 species, and of *Ephedranthus*, with seven species. The other two independent origins are autapomorphies of *Oxandra martiana* and *Pseudoxandra spiritus-sancti*. The possibility of Androdioecy being in fact a functional dioecy should be consider with caution in Malmeeae, since *P. spiritus-sancti* is not a functional dioecious. Nevertheless, the discover of the two main androdioecious clades in Malmeeae calls for further comparative investigation to understand how androdioecy

is displayed in each case. In addition, it brings crucial information that could shed more light on the evolution of androdioecy in woody plants with hermaphrodite ancestors.

## References

- Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410.
- A.P.G. III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.
- Aristeguieta, L. 1969. Annonaceae. Pp.43. In: Maguire and collaborators. *Botany of the Guayana Highlands. Part VIII. Memoirs of The New York Botanical Garden* 18(2): 1–290.
- Backlund, A. & Bremer, K. 1998. To be or not to be – principles of classification and monotypic plant families. *Taxon* 47: 391–400.
- Bakker, F.T., Lei, D., Yu, J., Mohammadin, S., Wei, Z., van de Kerke, S., Gravendeel, B., Nieuwenhuis, M., Staats, M., Alquezar-Planas, D.E. & Holmer, R. 2016. Herbarium genomics: plastome sequence assembly from a range of herbarium specimens using an Iterative Organelle Genome Assembly pipeline. *Biological Journal of the Linnean Society* 117(1): 33–43.
- Barrett, S.C.H. 2002. The evolution of plant sexual diversity. *Nature Reviews Genetics* 3: 274–284.
- Bawa K.S. 1980. Evolution of dioecy in flowering plants. *Annual Review of Ecology Evolution and Systematics* 11: 15–39.
- Bawa, K.S. & Beach, J.H. 1981. Evolution of sexual systems in flowering plants. *Annals of the Missouri Botanical Garden* 68: 254–274.
- Charlesworth, D. 1984. Androdioecy and the evolution of dioecy. *Botanical Journal of the Linnean Society* 22: 333–48.
- Chatrou, L.W. 1998. Changing genera. Systematic studies in Neotropical and West African Annonaceae. Ph.D. Thesis, Utrecht University, Utrecht.
- Chatrou, L.W., Pirie, M.D., Erkens, R.H.J., Couvreur, T.L.P., Neubig, K.M.J., Abbott, R., Mols, J.B., Maas, J.W., Saunders, R.M.K. & Chase, M.W. 2012. A new subfamilial and tribal classification of the pantropical flowering plant family

- Annonaceae informed by molecular phylogenetics. *Botanical Journal of the Linnean Society* 169(1): 5–40.
- Chen, B.L. & Nootboom, H.P. 1993. Notes on Magnoliaceae III: The Magnoliaceae of China. *Annals of the Missouri Botanical Garden* 80: 999–1104.
- Clark, S.C., Egan, R., Frazier, P.I. & Wang, Z. 2013. ALE: a generic assembly likelihood evaluation framework for assessing the accuracy of genome and metagenome assemblies. *Bioinformatics* 29: 435–443.
- Doyle, J.J. & Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- Doyle, J.A. & Le Thomas, A. 1996. Phylogenetic analysis and character evolution in Annonaceae. *Bulletin du Muséum National d'Histoire Naturelle, Section B, Adansonia* 18: 279–334.
- Endress, P.K. 1984. The role of inner staminodes in the floral display of some relic Magnoliales. *Plant Systematics and Evolution* 146: 269–282.
- Engler, A. & Diels, L. 1900. I. Übersicht über die bekannten Gattungen der Anonaceen und Beschreibung einiger neuen Gattungen dieser Familie aus dem tropischen Afrika. *Notizblatt des Botanischen Gartens und Museums zu Berlin-Dahlem* 3(23): 45–59.
- Fries, R.E. 1900. Beiträge zur Kenntniss der Süd-Amerikanischen Anonaceen. *Kongliga Svenska Vetenskaps Academiens Handlingar, n.s.*, 34, 5: 1–59.
- Fries, R.E. 1905. Studien in der Riedel'schen Anonaceen-Sammlung. *Arkiv för Botanik utgivet av K. Svenska Vetenskapsakademien* 5(4): 1–24.
- Fries, R.E. 1931. Revision der Arten einiger Annonaceen-Gattungen II. *Acta Horti Bergiani* 10(2): 129–341.
- Fries, R.E. 1931. Revision der Arten einiger Annonaceen-Gattungen II. *Acta Horti Bergiani* 10(2): 129–341.
- Fries, R.E. 1936. *Ruizodendron*, eine neue Annonaceen-Gattung. *Arkiv för Botanik utgivet av K. Svenska Vetenskapsakademien* 28B: 1–4.
- Fries, R.E. 1937. Revision der Arten einiger Annonaceen-Gattungen IV. *Acta Horti Bergiani* 12: 222–231.
- Fries, R.E. 1959. Annonaceae. Pp. 1–171. In: Engler, A. & Prantl, K. (eds.). *Die natürlichen Pflanzenfamilien*, ed. 2, Band 17a, II. Duncker und Humblot, Berlin.
- Goloboff, P., Farris, J. & Nixon, K. 2003. T.N.T.: Tree Analysis Using New Technology. [lillo.org.ar/phylogeny/tnt](http://lillo.org.ar/phylogeny/tnt).

- Hutchinson, J. 1964. The genera of flowering plants, Angiospermae. Clarendon Press, Oxford.
- Ishida, K. & Hiura, T. 1998. Pollen fertility and flowering phenology in an androdioecious tree, *Fraxinus lanuginosa* (Oleaceae), in Hokkaido, Japan. *International Journal of Plant Sciences* 159: 941–47.
- Kainer, D. & Lanfear, R. 2015. The effects of partitioning on phylogenetic inference. *Molecular Biology and Evolution* 32(6): 1611–1627.
- Katoh, K., Misawa, K., Kuma, K. & Miyata, T. 2002 MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* 30: 3059–3066.
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12): 1647–1649.
- Koek-Noorman, J., Westra, L.Y.T. & Maas, P.J.M. 1990. Studies in Annonaceae. XIII. The role of morphological characters in subsequent classifications of Annonaceae: A comparative survey. *Taxon* 39(1): 16–32.
- Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701.
- Lanfear, R., Calcott, B., Kainer, D., Mayer, C. & Stamatakis, A. 2014. Selecting optimal partitioning schemes for phylogenomic datasets. *BMC Evolutionary Biology* 14: 82.
- Lepart, J. & Dommée, B. 1992. Is *Phillyrea angustifolia* L. (Oleaceae) an androdioecious species? *Botanical Journal of the Linnean Society* 108: 375–87.
- Lloyd, D.G. 1975. The maintenance of gynodioecy and androdioecy in angiosperms. *Genetica* 45: 325–339.
- Lloyd, D.G. 1979. Some reproductive factors affecting the selection of self-fertilization in plants. *American Naturalist* 113: 67–79.
- Lopes, J.C. & Mello-Silva, R. 2014. Annonaceae da Reserva Natural Vale, Linhares Espírito Santo. *Rodriguésia* 65(3): 599–635.
- Lopes, J.C. & Mello-Silva, R. *in prep.* Morphological phylogenetic analysis and revision of *Ephedranthus* (Annonaceae).

- Lopes, J.C., Sajo, M.G., Rudall, P. & Mello-Silva, R. *in prep.* Is *Pseudoxandra spiritus-sancti* (Annonaceae), with its male and bisexual flowers, an androdioecious species?
- Maas, P.J.M. & Westra, L.Y.T. 1984. Studies in Annonaceae II: A monograph of the genus *Anaxagorea* A.St.-Hil. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 105: 73–134.
- Maas, P.J.M. & Westra, L.Y.T. 1985. Studies in Annonaceae II: A monograph of the genus *Anaxagorea* A.St.-Hil. Part 2. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 105: 145–204.
- Maas, P.J.M., van de Kamer, H.M., Junikka, L., Mello-Silva, R. & Rainer, H. 2002. Annonaceae from Central-eastern Brazil. *Rodriguésia* 52: 61–94. (“2001”).
- Maas, P.J.M. & Westra, L.Y.T. 2003. Revision of the Neotropical genus *Pseudoxandra*. *Blumea* 48(2): 201–259.
- Maas, P.J.M. & Westra, L.Y.T. 2005 A new species of *Pseudoxandra* (Annonaceae). *Blumea* 50: 61–64.
- Maas, P.J.M., Westra, L.Y.T. & Vermeer, M. 2007. Revision of the Neotropical genera *Bocageopsis*, *Onychopetalum*, and *Unonopsis* (Annonaceae). *Blumea* 52(3): 413–554.
- Maddison, W.P. & Maddison, D.R. 2015. Mesquite: a modular system for evolutionary analysis. Version 3.04, <http://mesquiteproject.org>
- Miller, M.A., Pfeiffer, W. & Schwartz, T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, 14 Nov. 2010, New Orleans.
- Moore, S.M. 1895. The Phanerogamic Botany of the Matto Grosso Expedition, 1891–92. *Transactions of the Linnean Society of London*, 2nd series: Botany 4 (1894–1986): 265–516.
- Nixon, K.C. 1999a. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 415–428.
- Nixon, K. C. 1999b. Winclada (BETA) ver. 0.9.9. Published by the author, Ithaca.
- Oliveira, J. & Sales, M.F. 1999. Estudos taxonômicos dos gêneros *Ephedranthus* e *Pseudephedranthus* Aristeg.: Annonaceae. *Boletim do Museu Paraense “Emílio Goeldi”*. Nova série, Botânica 15(2): 117–166.
- Pagel, M., Meade, A. & Barker, D. 2004. Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology* 53: 673–684.

- Pagel, M. & Meade, A. 2014. BayesTraits v. 2.0. Reading: University of Reading. <http://www.evolution.rdg.ac.uk>.
- Pannell, J.R. 2001. A hypothesis for the evolution of androdioecy: the joint influence of reproductive assurance and local mate competition in a metapopulation. *Evolutionary Ecology* 14: 195–211.
- Pannell, J.R. 2002. The evolution and maintenance of androdioecy. *Annual Review of Ecology and Systematics* 33: 397–425.
- Paulino-Neto, H.F. & Teixeira, R.C. 2006. Florivory and sex ratio in *Annona dioica* St. Hil. (Annonaceae) in the Pantanal at Nhecolândia, southwestern Brazil. *Acta Botanica Brasilica* 20: 405–409.
- Pirie, M.D. 2005. *Crematosperma* (and other evolutionary digressions). Molecular phylogenetic, biogeographic, and taxonomic studies in Neotropical Annonaceae. PhD thesis. Universiteit Utrecht, Utrecht.
- Pirie, M.D., Chatrou, L.W. Mols, J. B., Erkens, R.H. J. & Oosterhof, J. 2006. ‘Andean-centred’ genera in the short-branch clade of Annonaceae: Testing biogeographical hypotheses using phylogeny reconstruction and molecular dating. *Journal of Biogeography* 33: 31–46.
- Pontes, A.F., Barbosa, M.R.V. & Maas, P.J.M. 2004. Flora Paraibana: Annonaceae Juss. *Acta Botanica Brasilica* 18(2): 281–293.
- Prantl, K. 1891. Anonaceae. Pp. 23–39. In: A. Engler & K. Prantl. Die natürlichen Pflanzenfamilien, III. Teil, 2. Abteilung. Wilhelm Engelmann, Leipzig.
- R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org>.
- Richard, A. 1845. Histoire Physique, Politique et Naturelle de l’Ile de Cuba - Botanique. Plantes Vasculaires. Arthus Bertrand, Paris.
- Ross, M.D. 1982. Five evolutionary pathways to subdioecy. *The American Naturalist* 119: 297–318.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542.
- Safford, W.E. 1916. *Desmopsis*, a new genus of Annonaceae. *Bulletin of the Torrey Botanical Club* 43(4): 183–193.

- Sang, T. 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical Reviews in Biochemistry and Molecular Biology* 37:121–147.
- Saunders, R.M.K. 2010. Floral evolution in the Annonaceae: hypotheses of homeotic mutations and functional convergence. *Biological Reviews* 85: 571–591.
- Simmons, M.P. 2014. Limitations of locally sampled characters in phylogenetic analyses of sparse supermatrices. *Molecular Phylogenetics and Evolution*: 1–14.
- Stamatakis, A., Hoover, P. & Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57: 758–771.
- Stamatakis, A. 2014. RAxML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30(9): 1312–1313.
- Su, Y.C.F. & Saunders, R.M.K. 2006. Monograph of *Pseuduvaria* (Annonaceae). *Systematic Botany Monographs* 79: 1–204.
- Su, Y.C.F., Smith, G.J.D. & Saunders, R.M.K. 2008. Phylogeny of the basal angiosperm genus *Pseuduvaria* (Annonaceae) inferred from five chloroplast DNA regions, with interpretation of morphological character evolution. *Molecular Phylogenetics and Evolution* 48: 188–206.
- Sugiura, N. 1978. Further analysis of the data by Akaike's information criterion and the finite corrections. *Communications in Statistics - Theory and Methods* A7: 13–26.
- Vaidya, G., Lohman, D.J. & Meier, R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27: 171–180.
- van Heusden, E.C.H. 1992. Flowers of Annonaceae: morphology, classification, and evolution. *Blumea* 7 (Supplement): 1–218.
- van Heusden, E.C.H. 1997. Revision of the Southeast Asian genus *Trivalvaria* (Annonaceae). *Nordic Journal of Botany* 17(2): 169–180.
- Wallander, E. 2008. Systematics of *Fraxinus* (Oleaceae) and evolution of dioecy. *Plant Systematics and Evolution* 273: 25–49.
- Walker, J.W. 1971. Pollen morphology, phytogeography, and phylogeny of the Annonaceae. *Contributions from the Gray Herbarium of Harvard University* 202: 1–132.
- Westra, L.Y.T. 1995. Studies in Annonaceae. XXIV. A taxonomic revision of *Raimondia* Safford. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 117: 273–297.

- Westra, L.Y.T. & Maas, J.P.M. 2012. *Tetrameranthus* (Annonaceae) revisited including a new species. *PhytoKeys* 12: 1–21.
- Wiens, J.J. & Tiu, J. 2012. Highly incomplete taxa can rescue phylogenetic analyses from the negative impacts of limited taxon sampling. *PLoS ONE* 7(8): e42925.
- Wolf, D.E. & Takebayashi, N. 2004. Pollen limitation and the evolution of androdioecy from dioecy. *American Naturalist* 163: 122–137.
- Zhou, X.-J., Ma, L. & Liu, W-Z. 2016. Functional androdioecy in the rare endemic tree *Tapiscia sinensis*. *Botanical Journal of the Linnean Society* 180(4): 504–514.

## Capítulo 2

### **Is *Pseudoxandra spiritus-sancti* (Annonaceae), with its male and bisexual flowers, an androdioecious species?**

#### **Abstract**

The majority of flowers in angiosperms are bisexual and approximately only 10% are unisexual, that can be originated either by abortion or from inception. Unisexual flowers occur in a wide range of sexual systems, and androdioecy, in which plants produce exclusively male or bisexual flowers, is the rarest one. It is supposed that, in some cases, androdioecy is functionally dioecious, with the bisexual flower acting only as a female flower. In spite of its rareness, androdioecy occurs in several genera and lineages of Annonaceae. Most of androdioecious species of Annonaceae belongs to Malmeeae tribe, a Neotropical group. Besides the genera already report as androdioecious, *Pseudoxandra spiritus-sancti* was recently confirmed to possess male and bisexual flowers in different individuals. The aim of this paper is to investigate whether *Pseudoxandra spiritus-sancti* is androdioecious or functional dioecious. Buds and flowers of *Pseudoxandra spiritus-sancti* were fixed in 70% FAA solution. The material was critical-point dried and examined by SEM. For anatomical analysis, buds were dehydrated in ethanol and embedded in Paraplast, sectioned at *ca* 13  $\mu\text{m}$  thickness, stained in safranin and Alcian blue and observed in LM. Pollen viability was assessed using a fluorochromatic reaction. Sex ratio was analysed from *Ephedranthus*, *Klarobelia*, *Pseudomalmea* and *Pseudoxandra spiritus-sancti* herbarium collection. The results presents the morphological descriptions of the male and bisexual flowers. The sex ratio do not deviates from 0.5 male and bisexual flowers in *P. spiritus-sancti*. Male flowers are unisexual from inception and bisexual flowers presents staminodes and functional stamens with viable pollen. For this reason, *Pseudoxandra spiritus-sancti* is structurally androdioecious and it is unlikely to be functionally dioecious.

**Key words:** Malmeeae, sex ratio, staminodes.

## Introduction

The majority of flowers in angiosperms are bisexual and approximately only 10% are unisexual (Barret 2002). Unisexual flowers can be originated either by abortion, with the gynoecium or androecium primordia early stopping their development (type I), or from inception, when only androecium or gynoecium primordia initiate (type II, Mitchell & Diggle 2005). Unisexual flowers occur in a wide range of sexual systems, like monoecy, andro- and gynodioecy, dioecy and andro- and gynodioecy. In the monoecy, male and female flowers grow on the same plant; in the andromonoecy, plants possess bisexual and male flowers and, in the gynodioecy, they possess bisexual and female flowers. In dioecy, some plants produce exclusively female flowers and others only male ones; in gynodioecy, female and bisexual flowers grow in different plants and, in androdioecy, plants produce exclusively male or bisexual flowers (Bawa & Beach 1981).

Androdioecy is supposed to be, in some cases, functionally dioecious, with the bisexual flower acting only as a female one (Charlesworth 1984). Thus, species bearing this sexual system cannot be discarded as being functionally dioecious rather than androdioecious. Androdioecy is a rare sexual system, being confirmed only for few species (Pannell 2002). Its scarcity can be due to the fact that male individuals must produce twice more descendants than the hermaphrodites to persist in the population (Charlesworth 1984, Lloyd 1975). Most of the androdioecious species have evolved from dioecious ancestors (Pannell 2002) and they are usually herbaceous plants whose populations suffer recurrent local extinction followed by re-colonization (Pannell 2001). Nevertheless, some androdioecious species descend from hermaphrodite ancestors, such as the woody group Oleaceae (Ishida & Hiura 1998, Lepart & Dommée 1992). However, more studies are needed for a better understanding of these cases (Pannell 2002).

Androdioecy descending from hermaphroditism also occurs in several genera and lineages of Annonaceae (Chatrou *et al.* 2012, Saunders 2010). Annonaceae are a pantropical family with approximately 2,440 species and 109 genera. Within the family, *Anaxagorea* is the sister group of all Annonaceae, with the ambavioid clade (nine genera) emerging in sequence. The remaining genera are included in two large clades, the subfamilies Annonoideae and Malmeoideae (Chatrou *et al.* 2012), with androdioecy

found in both. Within Annonoideae, two genera with androdioecious species are *Annona* and *Anonidium*, which forms the Annonae tribe (van Heusden 1992, Westra 1995). Within the subfamily Malmeoideae, nine androdioecious genera are distributed into three distinct lineages: *Greenwayodendron* and *Polyceratocarpus* form the Piptostigmateae tribe (van Heusden 1992), *Pseuduvaria* and *Trivalvaria*, the Miliuseae tribe (Su & Saunders 2006, van Heusden 1997), and *Ephedranthus*, *Klarobelia*, *Oxandra* (some species), *Pseudephedranthus* and *Pseudomalmea*, Malmeeae tribe (Chatrou 1998, Fries 1931, Oliveira & Sales 1999, van Heusden 1992). Thus, most of androdioecious species of Annonaceae belongs to tribe Malmeeae.

Also within the tribe Malmeeae, *Pseudoxandra* has only recently reported to be androdioecious (Lopes & Mello-Silva 2014). It is a Neotropical genus with 24 species mainly from the Amazon basin, and with few representatives in the Atlantic Forest (Maas & Westra 2003). More than half of its species, 14, were described recently (Maas & Westra 2003, 2005, 2010), ten of them with unknown flower morphology. One of those species, *Pseudoxandra spiritus-sancti* Maas, possesses male and bisexual flowers in different individuals. The aim of this paper is to investigate whether it is another case of androdioecy or a functional dioecy.

## Material & Methods

### *Taxon sampling*

*Pseudoxandra spiritus-sancti* (Figure 1) was collected in its natural habitat. Trees ranging from 5 up to 15 m height occur through the Atlantic Forest in the Espírito Santo State, Brazil. The vouchers *Lopes 319*, male flower, and *Lopes 436*, bisexual flower, are deposited in CVRD and SPF herbaria (acronyms according to Thiers 2016).

### *Light and Scanning Electron Microscopy*

Buds and flowers were fixed using 70% formalin-acetic alcohol (FAA) and subsequently stored in 70% ethanol. For the floral morphological studies, buds were dissected in 70% alcohol under a stereomicroscope, critical-point dried using an Autosamdri-815B critical-point dryer, mounted on aluminium stubs with carbon discs and coated with platinum in a Quorum Q150T sputter-coater. Samples were examined

and images were taken using a *Hitachi S-4700* cold field emission scanning electron microscope (SEM).

Analyses of flower morphology were complemented examining floral anatomy. Floral buds were dehydrated in ethanol and embedded in Paraplast using standard methods. Prepared material were serially sectioned at *ca* 13  $\mu\text{m}$  thickness, stained in safranin and Alcian blue, dehydrated through an ethanol series to 100% ethanol, transferred to HistoClear, and mounted in DPX mounting medium (distrene, with dibutyl phthalate and xylene). Slides were examined using a Leica DMLB photomicroscope fitted with a Zeiss Axiocam digital camera.

Images were processed and illustrations prepared using Adobe Photoshop (Redwood City, CA).



**Figure 1.** Bisexual flowers of *Pseudoxandra spiritus-sancti*. Photo Alexandre Zuntini. Bar = 1.0 cm.

### *Pollen viability*

Thirteen stamens were selected from four floral FAA fixed buds in late developmental stages. They were then smashed onto the slides to release pollen grains.

Pollen viability was assessed using a fluorochromatic reaction (Heslop-Harrison & Heslop-Harrison 1970). The substrate solution was prepared adding drop by drop of fluorescein diacetate to a 0.5 M sucrose solution until the appearance of a persistent milkiness. Slides with the released pollen grains were covered with this substrate solution and mounted for observation and analysis. Pollen total content was counted twice, first under light microscope and later under a fluorescence microscope (Leica DMA6000B). When the pollen membrane permeability is intact, pollen grains will present a shiny green fluorescence, indicating they are viable. The percentage of viable and unviable pollen was measured by examination of 125 pollen grains.

### *Sex ratio measurements*

Material from 19 species of *Ephedranthus*, *Klarobelia*, *Pseudomalmea* and *Pseudoxandra spiritus-sancti*, deposited in the U and SPF herbaria (acronyms according to Thiers 2016) were analyzed with respect to the possession of male, bisexual flowers and fruits. *Pseudephedranthus fragrans*, the only species of this genus, was excluded because of its rareness and consequent scarcity of material. First, 59 sheets of *Ephedranthus*, 120 of *Klarobelia*, 85 of *Pseudomalmea*, and 24 of *Pseudoxandra spiritus-sancti*, totalizing 264 sheets, were examined. Then, due to the number of material with fruits in the collections were greater than that with flowers, fruit material as well as sterile sheets have been excluded from the analyses, remaining 184 out of 264 sheets analysed.

For hypothesis testing was used an exact binomial test applied on BINONDIST formula with the argument TRUE on Excel (Microsoft Windows). It was used a two-tailed test and the value obtained by the binomial test was multiplied by two to get the *P* value. The null hypothesis was that the proportion between male and bisexual individuals is 1:1 and the alternative hypothesis was that the proportion between them is different from 1:1. The significance level value used was 0.05 and *P* values below this was used to reject the null hypothesis.

## Results

### *Flower morphology and pollen viability*

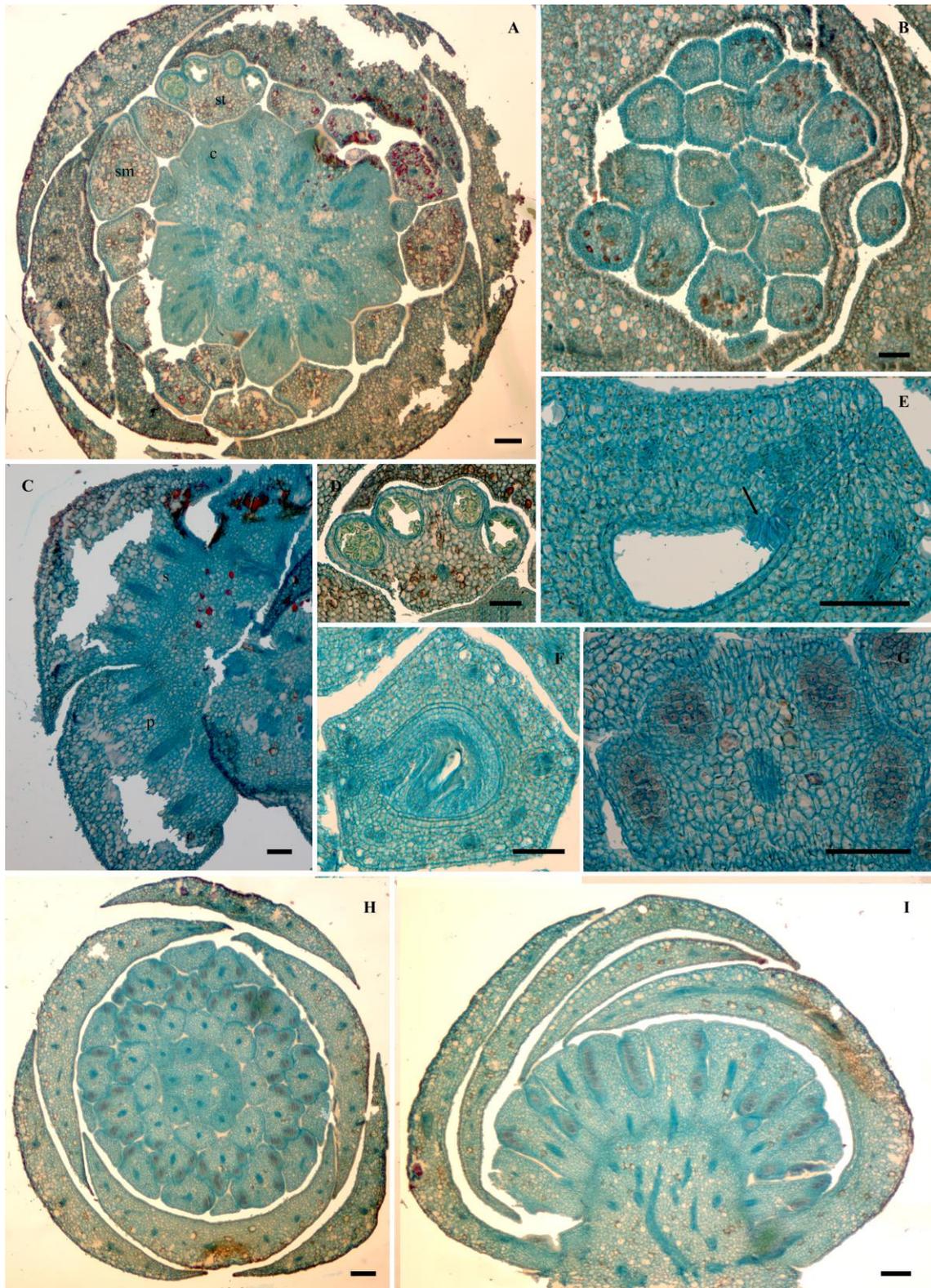
*Pseudoxandra spiritus-sancti* produces male and bisexual flowers in distinct individuals. Male flowers are of type II, in which the single gender results from inception with no differentiation of carpel primordium (Figure 2I).

Both bisexual (Figure 3A) and male flowers (Figure 3B) are protected by 3–5 bracts and present three perianth whorls with three members each. The outer elements are smaller than those of the inner whorls and possess longer marginal trichomes. The elements of the two inner whorls possess shorter trichomes (Figure 3A–B). All perianth members are supplied by three vascular bundles (Figure 2C).

### *Bisexual flower*

In bisexual flowers, stamens are arranged irregularly in a single outer whorl while carpels, also without regular organization, are usually present in three whorls (Figure 2A, 3I). Occasionally, some stamens do not completely develop and remain non-functional, originating staminodes (Figure 2A, 3D–E). Each stamen is tetrasporangiate and supplied by a single vascular bundle (Figure 2A, D), with anthers twice as long as the filament, and a truncate apex connective (Figure 3C).

Internally to the stamen layer, there are around 20 pistils of a cylindrical concave shape with a constriction at the stigma base. The stigma, long and of irregular shape (Figure 3G), is covered by anticlinally developed cells of glandular aspect. These cells also cover the merged region of carpels, the style (Figure 2B, E–F, 3F–G), and constitute the pollen tube transmitting tissue that extends to the ovule micropile (Figure 2F). Each carpel possesses an adaxial slit that differentiates earlier and persists until the carpel is mature (Figure 3F–G, I). The carpel margin junction results from the epidermal cells juxtaposition only (Figure 2E). Each carpel is supplied with one dorsal vascular bundle and two ventral ones, both branching within the ovary wall (Figure 4). The ovule is anatropous and bitegmic. The inner integument is two-layered and the outer one is 6–7 layered at the region opposite to the funiculus (Figure 2F).



**Figure 2.** *Pseudoxandra spiritus-sancti*. A–G. Bisexual bud. A. transverse section (c) carpel, (sm) staminode, (st) stamen. B. stigmatic region. C. (s) sepaloid tepal and (p) petaloid tepal. D. stamen with pollen. E. style region, arrow in the pollen transmitting tissue. F. carpel and ovule. G–I. Male bud. G. stamen. H. transverse section. I. longitudinal section. Bars = 100  $\mu$ m.

In male flowers, around 40 stamens are arranged in a 3–5 irregular whorls (Figure 2H–I, 3J). Each stamen possesses two thecae and a broad filament and receives a single vascular bundle (Figure 2G–H, 3H). The anther is three times as long as the filament and the connective apex is truncate (Figure 2I).

Amongst 125 analysed pollen grains, 104 (83.2%) were detected as viable (Figure 5). This result should be considered as an indication about the viability of the pollen from bisexual flowers, once the fluorochromatic reaction has some limitations.

### *Sex ratio*

The proportion of male and bisexual flowers in *Ephedranthus* and *Klarobelia* clearly deviates from 1:1 ( $P$  value 0.002). However, it is not possible to reject the null hypothesis for *Pseudomalmea* and *Pseudoxandra spiritus-sancti* (Table1).

**Table 1.** Number of male and bisexual flower in *Ephedranthus*, *Klarobelia*, *Pseudomalmea* and *Pseudoxandra spiritus sancti*.

Genera	Male Flower	Bisexual flower	Total	$P$ value
<i>Ephedranthus</i>	15	2	17	0,00235
<i>Klarobelia</i>	31	11	42	0,002887
<i>Pseudomalmea</i>	12	9	21	0,663624
<i>Pseudoxandra spiritus-sancti</i>	6	4	10	0,663624

## **Discussion**

### *Flower morphology and pollen viability*

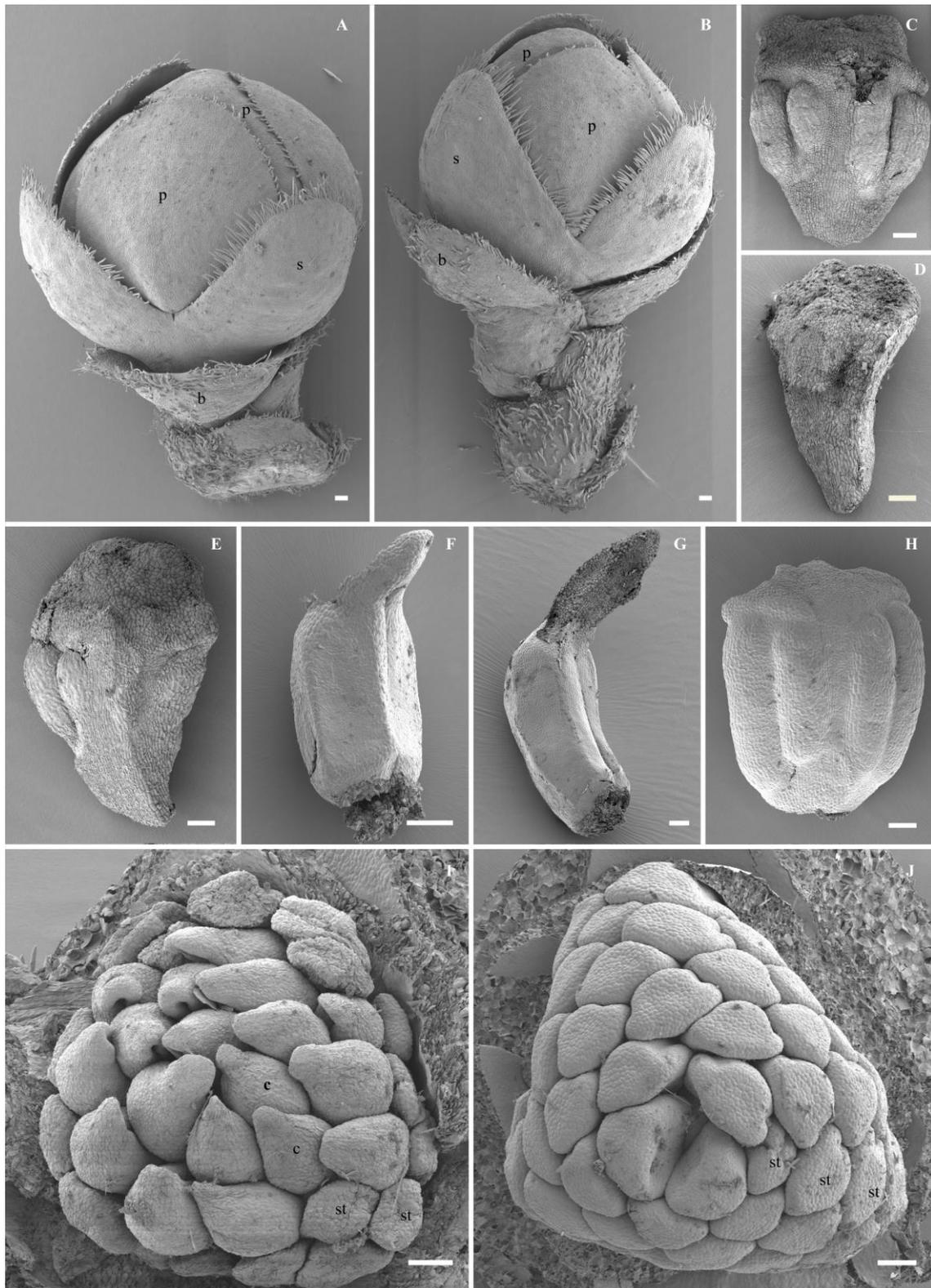
Sepals and petals are supposed to have different origin with sepals developing from bracts and petals from staminodes. Sepals resemble leaves in having chlorophyll and being supplied by three vascular bundles while petals are similar to staminodes as they are not green and receive a single vascular bundle (Celakosvsky 1896/1900, Hiepko 1965, Endress 1984). Regarding this interpretation, both the outer and the two inner whorls of *P. spiritus-sancti* could be recognized as sepal-like structures, despite their difference in shape, size and colour that often have lead to the interpretation that they are formed by sepals and petals (van Heusden 1992). However, all these elements are irrigated by three vascular bundles reinforcing the idea that they actually represent sepals (Endress & Armstrong 2011) with no significant differences. However,

differences from this pattern can be found in *Fenerivia*, a genus with a very complex perianth structure, in which the flowers present four whorls, where three of them are connected by the vascular tissue and petals are instead served by a unique vascular trace (Deroin 2007). Nonetheless, we hypothesize that this exception in the family may be related to the fusion of the perianth presented in *Fenerivia*.

Staminodes are rarely found in Annonaceae and do not display the same function as in other basal angiosperms, such as pollinator attraction, glandular secretion or prevention of selfing (Endress 1984). These structures are sometimes present in *Anaxagorea*, *Atapostema*, *Fusaea*, *Orophea* (a few species), *Uvaria* and *Xylophia*, genera with bisexual flowers (van Heusden 1992), and in the female flowers of *Pseuduvaria* (Su & Saunders 2006). In this latter, the pollen grains produced by the staminodes of female flowers are smaller and apparently sterile comparing to those of the male flowers (Su & Saunders 2006). In *Pseudoxandra spiritus-sancti*, the pollen of bisexual flowers has around 43  $\mu\text{m}$  in its polar axis (medium-size grain; Figure 5), while this measurement average is around 71  $\mu\text{m}$  in large grains (Walker 1971). This result suggests that they could be ‘staminode-like-pollen’, although it is known that the pollen size varies depending on the technique used to fix it (Walker 1971). Moreover, despite being smaller the pollen-grains from bisexual flowers of *P. spiritus-sancti* are apparently viable (Figure 5).

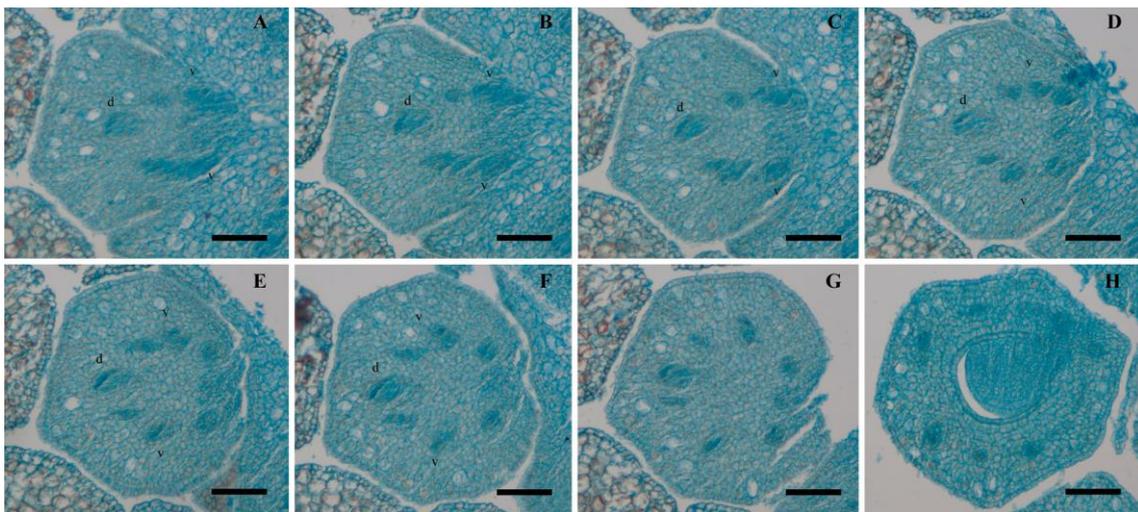
On the other hand, there is no report on pistillodes in Annonaceae male flowers either for dioecious or monoecious species, such as in *Pseuduvaria* (Su & Saunders 2006, van Heusden 1992). Similarly, in male flowers of *Pseudoxandra spiritus-sancti*, pistillodes have not been found (Figure 2I). The absence of pistillodes in Annonaceae could be explained by the lack of synorganization in the flower that allow carpels to be replaced by stamens (Xu & Ronse De Craene 2010).

Because there are no vestiges of pistillodes in the male flower, it is classified as unisexual from inception (type II flower). In addition, the pollen viability in bisexual flower, discard the possibility of this flower being unisexual by abortion (type I flower). Even though, only 9% of dioecious taxa presents flower of different flower type, which makes unlikely that *P. spiritus-sancti* is a functional dioecious species (Mitchell & Diggle 2005).



**Figure 3.** *Pseudoxandra spiritus-sancti*. Scanning electron microscopy micrographs of bisexual and male buds. A. bisexual bud. B. male bud, (b) bract, (s) sepaloid tepal, (p) petaloid tepal. C. Stamen from bisexual bud. D. Staminode from bisexual bud. E. Stamen with one theca from bisexual bud. F. Young carpel. G. Developed carpel. H. Stamen from male bud. I. Bisexual bud (c) carpel, (st) stamen. J. Male bud (st) stamen. Bars = 100  $\mu$ m.

In both male and bisexual flowers of *P. spiritus-sancti*, the gynoecium and androecium elements are not clearly organized in whorls, particularly closer to the receptacle centre (Figure 3I–J). This pattern of irregular or chaotic phyllotaxis in Annonaceae probably is derived developmentally from whorled phyllotaxis. Its origin in the family is due to organ doubling, i.e. two structures appeared instead of a single one (Endress & Doyle 2007). Doubling has been reported in many genera of Annonaceae, such as *Anaxagorea*, *Cananga*, *Desmos*, *Fissistigma*, *Mezzettiopsis*, *Mitrephora Miliusa*, *Polyalthia*, *Popowia* and *Pseuduvaria* (Endress & Armstrong 2011, Ronse De Craene & Smets 1990, Xu & Ronse De Craene 2010). As already described for other Annonaceae (Endress & Armstrong 2011, Persimamy & Swamy 1956), the carpels of *P. spiritus-sancti* are supplied with three vascular bundles, being one dorsal bundle and two ventral. The ventral bundles branch and merge with the dorsal bundle, as can be seen in cross-section (Figure 4).

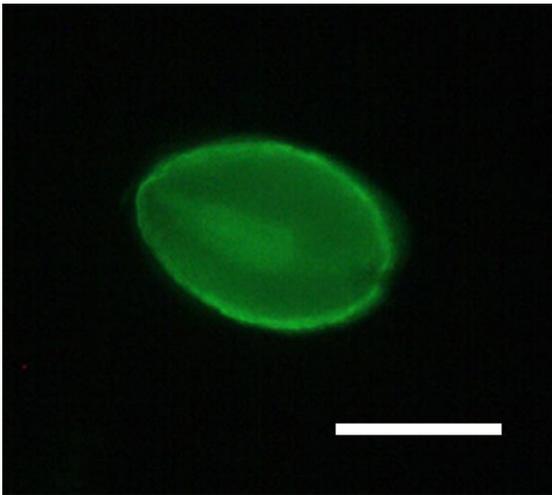


**Figure 4.** *Pseudoxandra spiritus-sancti* A–H Transverse serial sections of bisexual bud, the ramification of ventral bundle (d) dorsal bundle, (v) ventral bundle. Bars = 100  $\mu$ m.

#### *Sex ratio*

It has been hypothesised that the male individuals must produce twice more descendants than the hermaphrodites to persist in the population (Charlesworth 1984, Lloyd 1975,). In *Pseudoxandra spiritus-sancti* and *Pseudomalmea* the proportion of 1:1 of flowers from both sexes could not be rejected. It might be due to the low number of samples analysed, ten and 21, respectively. On the other hand, the results of *Klarobelia*

and *Ephedranthus* analyses were clearly deviated from this proportion. In *Annona dioica*, an androdioecious species, the analysis of more than 200 individuals has found the proportion 2:1 male flower to bisexual flower (Paulino-Neto & Teixeira 2006). The results for *Klarobelia*, *Ephedranthus* and *Annona dioica* suggest that the increase of data should reveal the true proportion of flowers from both sexes.



**Figure 5.** *Pseudoxandra spiritus-sancti*. Pollen viability, example of viable pollen using the fluorochromatic reaction. Bars = 25  $\mu$ m.

Although the sex ratio is not different from 0.5 in *Pseudoxandra spiritus-sancti*, other factors should be taken into account for the number of decedents generated by each kind of individual. One of these factors is the pollen grain production per flower. Bisexual flowers of *P. spiritus-sancti* have relatively few stamens and just a small proportion of them are functional and consequently produce pollen. On the other hand, male flowers have a much greater number of stamens and they all produce pollen. Thus, although the proportion of the distinct flowers observed so far are not different, the proportion of pollen coming from male flowers seems to be greater than that coming from bisexual flowers.

## Conclusions

*Pseudoxandra spiritus-sancti* is structurally androdioecious and it is unlikely to be functionally dioecious. Bisexual flowers are not functionally female, because there are stamens producing pollen. In spite of the limitations of the test of viability applied in

this study, the pollen from bisexual flowers seems to be viable. In addition, the male flowers do not show any vestiges of pistillodes, which would be expected from a functional dioecious species. More studies need to be done in regard to the sex ratio, production of pollen and pollen viability.

## References

- Barrett, S.C.H. 2002. The evolution of plant sexual diversity. *Nature Reviews Genetics* 3: 274–284.
- Bawa, K.S. & Beach, J.H. 1981. Evolution of sexual systems in flowering plants. *Annals of the Missouri Botanical Garden* 68: 254–274.
- Čelakovský, L.J. 1896/1900. Über den phylogenetischen Entwicklungsgang der Blüte und über den Ursprung der Blumenkrone 1/2. *Sitzungsberichte der Königlich böhmischen Gesellschaft der Wissenschaften. Mathematisch-naturwissenschaftliche Classe*, 1896(40): 1–91/1900(3): 1–221.
- Chatrou, L.W. 1998. Changing genera. Systematic studies in Neotropical and West African Annonaceae. Ph.D. Thesis, Utrecht University, Utrecht.
- Chatrou, L.W., Pirie, M.D., Erkens, R.H.J., Couvreur, T.L.P., Neubig, K.M.J., Abbott, R., Mols, J.B., Maas, J.W., Saunders, R.M.K. & Chase, M.W. 2012. A new subfamilial and tribal classification of the pantropical flowering plant family Annonaceae informed by molecular phylogenetics. *Botanical Journal of the Linnean Society* 169(1): 5–40.
- Charlesworth, D. 1984. Androdioecy and the evolution of dioecy. *Botanical Journal of the Linnean Society* 22: 333–48.
- Derooin, T. 2007. Floral vascular pattern of the endemic Malagasy genus *Fenerivia* Diels (Annonaceae). *Adansonia*, serie 3, 29: 7–12.
- Endress, P.K. 1984. The role of inner staminodes in the floral display of some relic Magnoliales. *Plant Systematics and Evolution* 146: 269–282.
- Endress, P.K. & Doyle J.A. 2007. Floral phyllotaxis in basal angiosperms: development and evolution. *Current Opinion in Plant Biology* 10: 52–57.
- Endress, P.K. & Armstrong, J.E. 2011. Floral development and floral phyllotaxis in *Anaxagorea* (Annonaceae). *Annals of Botany* 108: 835–845
- Fries, R.E. 1931. Revision der Arten einiger Annonaceen-Gattungen II. *Acta Horti*

- Bergiani 10(2): 129–341.
- Hiepkotter, P. 1965. Vergleichend-morphologische und entwicklungsgeschichtliche Untersuchungen über das Perianth bei den Polycarpicae. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie*. 84: 359–508.
- Heslop-Harrison, J. & Heslop-Harrison, Y. 1970. Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. *Stain Technology* 45: 115–120.
- Ishida, K. & Hiura, T. 1998. Pollen fertility and flowering phenology in an androdioecious tree, *Fraxinus lanuginosa* (Oleaceae), in Hokkaido, Japan. *International Journal of Plant Sciences* 159: 941–47.
- Lepart, J. & Dommée, B. 1992. Is *Phillyrea angustifolia* L. (Oleaceae) an androdioecious species? *Botanical Journal of the Linnean Society* 108: 375–87.
- Lopes, J.C. & Mello-Silva, R. 2014. Annonaceae da Reserva Natural Vale, Linhares Espírito Santo. *Rodriguésia* 65(3): 599–635
- Lloyd, D.G. 1975. The maintenance of gynodioecy and androdioecy in angiosperms. *Genetica* 45: 325–339.
- Maas, P.J.M. & Westra, L.Y.T. 2003. Revision of the neotropical genus *Pseudoxandra*. *Blumea* 48(2): 201–259.
- Maas, P.J.M. & Westra, L.Y.T. 2005. A new species of *Pseudoxandra* (Annonaceae). *Blumea* 50(1): 61–64.
- Maas, P.J.M. & Westra, L.Y.T. 2010. New species of Annonaceae from the Neotropics and miscellaneous notes. *Blumea* 55(3): 259–275.
- Mitchell, C.H. & Diggle, P.K. 2005. The evolution of unisexual flowers: Morphological and functional convergence Results from diverse developmental transitions *American Journal of Botany* 92(7): 1068–1076
- Oliveira, J. & Sales, M.F. 1999. Estudos taxonômicos dos gêneros *Ephedranthus* e *Pseudephedranthus* Aristeg.: Annonaceae. *Boletim do Museu Paraense “Emílio Goeldi”*. Nova série, Botânica 15(2): 117–166.
- Pannell, J.R. 2002. The evolution and maintenance of androdioecy. *Annual Review of Ecology and Systematics* 33: 397–425.
- Paulino Neto, H.F. & Teixeira, R.C. 2006. Florivory and sex ratio in *Annona dioica* St. Hil. (Annonaceae) in the Pantanal at Nhecolândia, southwestern Brazil. *Acta Botanica Brasilica* 20: 405–409.

- Periasamy, K. & Swamy, B.G.L. 1956. The conduplicate carpel of *Cananga odorata*. Journal of the Arnold Arboretum 37: 366–372.
- Ronse De Craene L.P. & Smets E.F. 1990. The floral development of *Popowia whitei* (Annonaceae). Nordic Journal of Botany 10: 411–420 [correction in Nordic Journal of Botany 11 (1991): 420].
- Saunders, R.M.K. 2010. Floral evolution in the Annonaceae: hypotheses of homeotic mutations and functional convergence. Biological Reviews 85: 571–591.
- Su, Y.C.F. & Saunders, R.M.K. 2006. Monograph of *Pseuduvaria* (Annonaceae). Systematic Botany Monographs 79: 1–204, pl. 1–3.
- Thiers, B. [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/ih/>
- van Heusden, E.C.H. 1992. Flowers of Annonaceae: morphology, classification, and evolution. Blumea 7 (Supplement): 1–218.
- van Heusden, E.C.H. 1997. Revision of the Southeast Asian genus *Trivalvaria* (Annonaceae). Nordic Journal of Botany 17(2): 169–180.
- Xu, F. & De Craene, L.R. 2010. Floral ontogeny of Annonaceae: evidence for high variability in floral form. Annals of Botany 106: 591–605.
- Walker, J.W. 1971. Pollen morphology, phytogeography and, phylogeny of the Annonaceae. Contributions from the Gray Herbarium 202: 1–131.
- Westra, L.Y.T. 1995. Studies in Annonaceae. XXIV. A taxonomic revision of *Raimondia* Safford. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 117: 273–297.

# Capítulo 3

## Morphological phylogenetic analysis and revision of *Ephedranthus* (Annonaceae)

**Abstract.** *Ephedranthus* is a Neotropical genus distributed from Colombia and Guyanas to Bolivia and South-Eastern Brazil, with seven species related as ((*E. colombianus*, *E. boliviensis*) ((*E. guianensis*, *E. amazonicus*) (*E. dimerus* (*E. parviflorus*, *E. pisocarpus*)))) and supported by presence of male flowers and ovules with basal placentation. The genus is recognized by the combination of leaves with distinct secondary veins and impressed primary vein above, short pedicels with generally up to five bracts, androdioecy, i.e., flowers with distinct sexes, male and bisexual flowers in different individuals, and one-seed monocarps. Androdioecy also occurs in other members of tribe Malmeeae, in which *Ephedranthus* is included. This revision aims to update the knowledge about the genus, providing a morphological phylogenetic analysis of *Ephedranthus* species, keys, descriptions, distribution maps and illustrations of the diagnostics characters of the species. The current subdivision of *Ephedranthus* is not monophyletic, and the sections are here synonymized.

**Key words:** Androdioecy, *Ephedranthus* sect. *Ephedranthus*, *Ephedranthus* sect. *Sphaeranthema*, phylogeny, systematics, taxonomy.

## Introduction

*Ephedranthus* is a Neotropical genus distributed from Colombia and Guyanas to Bolivia and South-Eastern Brazil. Its species occur in almost all forested regions of South America, in the Amazon Forest, in the *caatinga*, *cerrado* and Atlantic Forest, in Brazil, in the *chiquitanos* forest in Bolivia, and in the Andean region of Colombia. There are seven known species, three of them endemic to Brazil, *E. dimerus*, *E. parviflorus*, and *E. pisocarpus*, one endemic to Colombia, *E. colombianus*, one endemic to the Guyanas, *E. guianensis*, and two relatively widely distributed in the Amazon region, *E. amazonicus* and *E. boliviensis* that, despite its name, occurs also in Brazil.

The genus can be recognized by the combination of leaves with distinct secondary veins and impressed primary vein above, short pedicels with generally up to five bracts, flowers with distinct sexes, male and bisexual flowers in different individuals, and one-seed monocarps. Other related genera with numerous bracts are *Oxandra* and *Pseudoxandra*. These genera are distinguished from *Ephedranthus* by the non-distinct secondary vein on the leaf, by having only bisexual flowers, with few exceptions in both genera, and stamen shape, narrower with tongue-like connective extension versus stamen peltate with truncate connective in *Ephedranthus*.

*Ephedranthus* was described by Moore (1895), with one species, *E. parviflorus*. Fries (1931) describes *E. guianensis* with two varieties, *E. guianensis* var. *oligantha* and var. *guianensis*. Later, the same author describes *E. amazonicus* and divides the genus in two sections, *Ephedranthus* sect. *Ephedranthus* ("Eu-*Ephedranthus*"), which includes *E. guianensis* and *E. parviflorus*, and *Ephedranthus* sect. *Sphaeranthema*, including solely *E. amazonicus* (Fries 1934). Section *Ephedranthus* was characterized by long hairs in pedicels, oblong, erect-patent petals, and ovoid ovary, with long stigma. Section *Sphaeranthema* was defined by short hairs in pedicels, orbicular-concave petals, and prismatic ovary, with truncate stigma (Fries 1934). Fries (1941) describes one more species, *E. pisocarpus*, and includes it in the section *Ephedranthus*. More than 40 years later, Maas et al. (1988) describe *E. colombianus*, and 11 years after that a first taxonomic revision is published (Oliveira & Sales 1999). In the following 15 years, two new species were described, *E. boliviensis* (Chatrou & Pirie 2003), and *E. dimerus* (Lopes et al. 2014).

*Ephedranthus* is a monophyletic assemblage (Pirie et al. 2006, Chatrou et al. 2012, Lopes et al. in prep). However, the phylogenetic relationship between its species

has not been recovered till now. *Ephedranthus* belongs to tribe Malmeeae (subfamily Malmeoideae), which in its turn also form a monophyletic group of 13 genera (Pirie et al. 2006, Chatrou et al. 2012, Lopes et al. in prep). The position of *Ephedranthus* within the tribe remain unclear. It seemed to be related to *Ruizodendron*, a monotypic genus (Chatrou et al. 2012, Pirie et al 2006), but this has not been confirmed by posterior analyses (Lopes et al. in prep).

Together with *Oxandra*, whose revision has been already initiated (Junnika & Maas unpubl. data), *Ephedranthus* is the only Neotropical genus from tribe Malmeeae not fully revised. Here we present the taxonomic revision of *Ephedranthus*, as part of the phylogenetic studies in tribe Malmeeae (Lopes et al. *in prep.*), and the first phylogenetic analysis of its species based on morphological data.

## Material & Methods

### *Taxonomic treatment*

A total of 500 *Ephedranthus* specimens from the BM, BR, INPA, IAN, K, MG, S, SPF, U and WAG herbaria were analysed (acronyms according to Thiers 2016). Material from Re flora (2016) have been also analysed. Measurements of vegetative and reproductive structures were taken and variation in the morphology was observed using stereomicroscope. Nomenclature of shapes and leaf structure followed Hickey (1979).

Maps were prepared using Quantum Gis (QGIS Development Team 2009). Shape files with Mundial and Brazilian politic divisions were downloaded from <http://www.forest-gis.com/p/download-de-shapefiles.html>. Illustrations were prepared by Laura Montserrat and based on photos and herbarium material cited in the legends of the figures.

### *Cladistic analysis*

The cladistic analysis was undertaken using all species of *Ephedranthus*, seven, and four species as outgroup, three members of tribe Malmeeae, *Bocageopsis pleiosperma* Maas, *Pseudoxandra obscurinervis* Maas, and *Ruizodendron ovale* (Ruiz & Pav.) R.E.Fr., plus *Anaxagorea phaeocarpa* Mart. taken as the ultimate outbroup (Farris 1972, Nixon & Carpenter 1993), based on the Annonaceae analysis by Chatrou et al. (2012). Morphological characters are listed on Table 1 and the matrix on Table 2,

totalizing 26 informative characters. Some characters were taken from Doyle & Le Thomas (1996), mainly leaf, floral and pollen characters. Character states of leaf anatomy, pollen, and seeds were taken from van Setten & Koek-Noorman (1986, 1992) and Walker (1971). Maas & Westra (1984, 1985, 2003), Maas et al. (2007), and Paul Maas (unpublished data) were consulted for character states of the outgroup taxa. Voucher specimens are in material examined in the taxonomic treatment. Below a brief description of each character and states.

*Characters 0–5. Leaf.*

Simple hairs on the leaves is an important character in the distinction of the species in *Ephedranthus*. Papillae are unspecialized epidermal cells and are found in some genera of Malmeeae and few species of *Annona* (van Setten & Koek-Noorman 1986). Oil cells have been reported to occur in the lamina in species of Annonaceae. These cells occur mostly in the sponge parenchyma, or in the intermediate zone between the palisade and sponge parenchyma. However, oil cells also occur in the palisade parenchyma and less often immediately below the epidermis (van Setten & Koek-Noorman 1986). The relative position of primary and tertiary veins are informative characters in Annonaceae (Doyle & Le Thomas 1996). The outline of primary veins are also an important feature in the recognition of genera in Malmeeae (Maas & Westra 2003, Maas et al 2007).

*Characters 6–14. Flower.*

Flowers are either always solitary or rarely in pairs, or born in determinate inflorescences that varies in the same species from one to many, normally with many flowers (Doyle & Le Thomas 1996). Length of flower pedicel is an important feature in the recognition of species in *Ephedranthus*. Petals aestivation in Annonaceae are valvate, reduplicate-valvate, imbricate, transversely folded and apert (van Heusden 1992). Valvate and imbricate petals are the most common aestivation condition and the only states found in the group analysed. This character was important in previous classifications (Koek-Noorman et al. 1990). Petals in Annonaceae are usually fleshy (van Heusden 1992), becoming coriaceous in dried material. Chartaceous petals are less common, and are an important trait of *Ephedranthus*. The shape of the petals is variable, usually also between inner and outer ones, being the inner ones narrower (van Heusden 1992). Petals shape is one of the characteristics used in the classification of sections in *Ephedranthus* (Fires 1934). In general, flowers in Annonaceae are bisexual, but male and female flowers, in monoecious, dioecious or androdioecious species can be found

(van Heusden 1992, Saunders 2010). In Malmeeae, androdioecy, presence of individuals with bisexual flowers and others with male ones, is an important feature (Lopes et al. in prep). The stamens of Annonaceae are mainly narrowly oblong to oblanceoloid, with a very short filament, two linear thecae and a connective with shield-like prolongation (van Heusden 1992). Stigma shape varies from lobed; capitate, those with a distinct basal constriction; and cylindrical (Doyle & Le Thomas 1996). Number of ovules in Annonaceae varies from one to numerous; *Anaxagorea* has generally two ovules per carpel (Chatrou et al. 2012, Maas & Westra 1984). In the rest of the taxa analysed, there are groups either with a single ovule, or with two to five ovules.

*Character 15. Pollen.*

The pollen grain wall is formed by two layers. The outer layer, the exine, is also divided in inner and outer layers. The exine outer layer, the sexine, is usually sculptured. In the sexine, the tectum may be granular or columellar (Punt et al. 2006, Walker 1971).

*Characters 16–21. Fruit.*

All fruit characters are important for recognizing the species of *Ephedranthus*, and have been used in taxonomic treatments. The monocarp is usually ellipsoid, but in few species, its shape ranges from globose, ovoid to cylindrical; club-shaped monocarps are exclusively found in *Anaxagorea* (Maas & Westra 1984). The monocarp apex is or rounded or apiculate or umbonate, the latter with a long apicule. The stipe, the structure that connects the monocarp to the receptacle, varies from 5.0 mm to 40.0 mm long in *Ephedranthus*. Despite of the arbitrary division of states of this character, 0–11 mm × 20–40 mm long, all species of *Ephedranthus* except by two are aggregate in the first interval of stipe length.

*Characters 22–25. Seeds.*

Apocarpous fruits have usually marginal seeds; those with one seed show mainly basal placentation (van Setten & Koek-Noorman 1992). Rumination in seeds, a synapomorphy of Annonaceae, is due to the infolding of dark inner layer of the seed coat into the lighter coloured endosperm (van Setten & Koek-Noorman 1992). The ruminations are categorized in two main cross section divisions, spiniform and lamellate, ribbon-shaped rumination being found only in *Anaxagorea*.

**Table 1.** Characters analysis and coding. D&LT = Doyle & Le Thomas (1996), vS&K = van Setten & Koek-Noorman (1986, 1992), and W = Walker (1971).

- 
0. Indument on abaxial side of lamina: absent 0 present 1
  1. Papillae (vS&K): absent (0); present (1)
  2. Oil cell in palisade parenchyma (vS&K): absent (0); present (1)
  3. Idioblasts with silica bodies (vS&K): absent (0); present (1)
  4. Midrib (D&LT): concave or flat (0); convex (1)
  5. Tertiary veins in abaxial side of lamina: immersed to slightly raised (0); distinctly raised-reticulate (1)
  6. Flower number: always 1(-2): 0 (0); 1-many: 1 (1)
  7. Length of flower pedicel: more than 3.0 mm (0); 0–2.0 mm (1)
  8. Petals aestivation (D&LT): valvate (0); imbricate (1)
  9. Petals texture: coriaceous (0); chartaceous (1)
  10. Outer petals shape: narrowly obovate to oblanceolate to elliptic (0) ovate, orbiculate to suborbiculate (1); narrowly oblong to oblong (2)
  11. Male flower: absent (0); present (1)
  12. Stamen shape (D&LT): laminar (0); narrow with tongue-like connective (1); peltate-truncate (2)
  13. Stigma shape (D&LT): lobed (0); capitate (1); cylindrical (2)
  14. Number of ovules: one (0); two (1); 2–5 (2)
  15. Infratectal structure(W): granular (0); columellar (1)
  16. Indument on fruit pedicel: absent (0); yellowish hairs (1)
  17. Veins on monocarp wall: absent (0); present (1)
  18. Texture of monocarp wall: smooth (0); verrucose (1)
  19. Monocarp shape (D&LT): club-shape (0); ellipsoid (1) globose to ovoid (2); cylindrical (3)
  20. Monocarp apex: rounded (0); apiculate (1) umbonate (2)
  21. Stipe length: 0–11.0 mm (0); 12.0–19.0 mm (1); 20.0–40.0 mm (2)
  22. Number of seeds (vS&K): one (0); two (1); more than 2 (2)
  23. Placentation (vS&K): basal (0); lateral (1)
  24. Raphe (vS&K): indistinct (0); ribbed (1); grooved (2)
  25. Rumination (vS&K): ribbon-shaped (0); spiniform (1); lamellate (2)
-

**Table 2.** Morphological matrix.

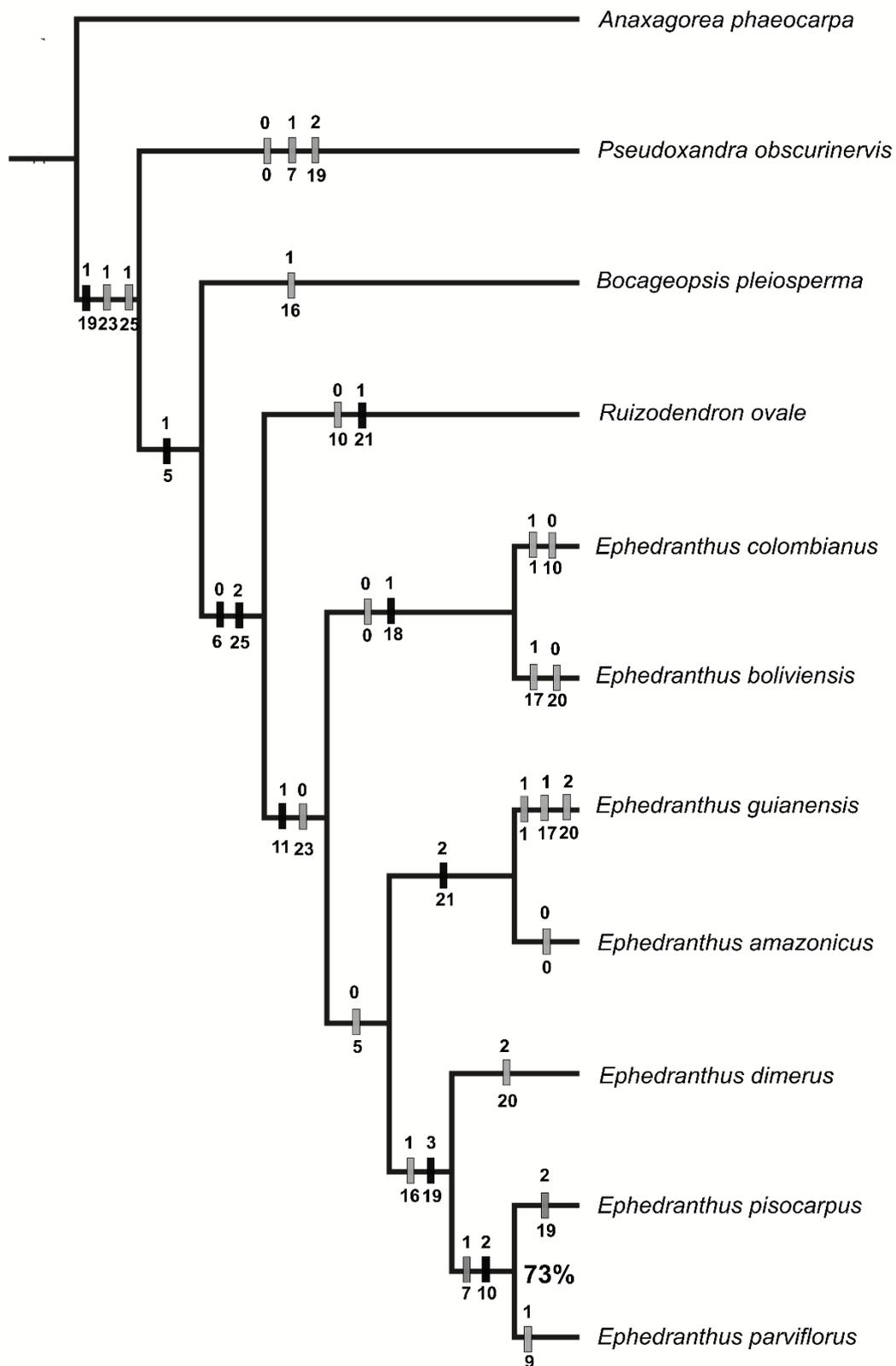
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
	0	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
<i>Anaxagorea phaeocarpa</i>	1	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	?	0	0	--	1	0	0						
<i>Bocageopsis pleiosperma</i>	1	1	0	1	1	1	0	0	0	1	0	1	2	2	1	1	0	0	1	0	0	2	1	1						
<i>Pseudoxandra obscurinervis</i>	0	0	1	1	0	1	1	1	0	1	0	2	1	0	1	0	0	0	2	1	0	0	1	2	1					
<i>Ruizodendron ovale</i>	1	0	0	0	1	0	0	1	1	0	0	2	1	0	1	0	0	0	1	1	1	0	1	2	2					
<i>Ephedranthus amazonicus</i>	0	0	0	1	0	0	0	1	0	1	1	2	1	0	1	0	0	0	1	1	2	0	0	2	2					
<i>Ephedranthus boliviensis</i>	0	0	0	1	0	1	0	1	0	1	1	2	1	0	1	1	1	0	0	0	0	2	2							
<i>Ephedranthus colombianus</i>	0	1	0	1	0	0	1	0	0	1	2	1	0	1	0	0	1	1	1	0	0	0	2	2						
<i>Ephedranthus dimerus</i>	1	0	0	1	0	0	0	1	0	1	1	2	1	0	1	1	0	0	3	2	0	0	0	2	2					
<i>Ephedranthus guianensis</i>	1	1	0	1	0	0	0	1	0	1	1	2	1	0	1	0	1	0	1	2	2	0	0	2	2					
<i>Ephedranthus parviflorus</i>	1	0	0	1	1	1	2	1	2	1	0	1	1	0	0	3	1	0	0	0	2	2								
<i>Ephedranthus pisocarpus</i>	1	0	0	0	1	1	-	2	1	2	1	0	1	1	0	0	2	1	0	0	0	2	2							

Parsimony analysis was conducted on TNT (Goloboff et al 2003). The analysis used the RATCHET, an algorithm that reduces the risk of the analysis stuck in a suboptimal tree island (Nixon 1999), with 5 seed and 100 replications. Standard bootstrap analysis was conducted with 100 replications.

## Results

### Phylogenetic relationships in *Ephedranthus*

One most parsimonious tree, with 55 steps, was recovered. Bootstrap values were 73% for *Ephedranthus pisocarpus* sister to *E. parviflorus*. Bootstrap values for all the other nodes were below 50% (Figure 1).



**Figure 1.** Most parsimonious tree. Bars represent character states changes, black = unique, grey = homoplasy. Numbers below bars are characters numbers, and those above are characters states. Characters states changes in outgroups are only those cited in the text. Percentage in the nodes are bootstrap values, nodes without any indication have bootstrap values below 50%.

(*Ruizodedron ovale*, *Ephedranthus*) is supported by solitary flowers (character 6) and lamellate rumination (25). ((*E. colombianus*, *E. boliviensis*) ((*E. guianensis*, *E. amazonicus*) (*E. dimerus* (*E. parviflorus*, *E. pisocarpus*)))) is supported by male flowers (11), and basal seed placentation (23). (*E. colombianus*, *E. boliviensis*) is supported by glabrous leaves in the adaxial surface (0) and verrucose monocarp wall (18). Leaves with immersed to slightly raised tertiary veins (5, reversion) supports ((*E. guianensis*, *E. amazonicus*) (*E. dimerus* (*E. parviflorus*, *E. pisocarpus*))). In this clade, stipes 20–40 mm long (21) supports (*E. guianensis*, *E. amazonicus*), cylindrical monocarps (19, reversion to ovoid or globose monocarps in *E. pisocarpus*), and fruit pedicel covered by yellowish hairs (16, convergence in *Bocageopsis pleiosperma*) support (*E. dimerus* (*E. parviflorus*, *E. pisocarpus*)). Within this group, narrowly oblong to oblong petals (10), and pedicel 0–2 mm long (7, convergence in *Pseudoxandra obscurinervis*) support (*E. parviflorus*, *E. pisocarpus*) (Figure 1).

## Taxonomic Treatment

*Ephedranthus* S. Moore, Trans. Linn. Soc. Lond. Bot. Ser. 2. 4: 296. pl. 21, 38. 1895.  
TYPE: *Ephedranthus parviflorus* S.Moore, *S.M. Moore 310* (Holotype, BM!; Isotype, NY).

*Ephedranthus* sect. *Sphaeranthema* R.E.Fr., Acta Horti Berg. 12(1): 201. 1934.  
Type: *Ephedranthus amazonicus* R.E.Fr., Acta Horti Berg. 12(1): 200. 1934. **syn. nov.**

Trees or shrubs 5–30 m tall. *Leaves* distichous, venation brochidodromous, primary vein impressed to flat above, raised below, secondary veins mostly distinct, impressed to flat above, raised below. Inflorescence axillary, on older branches in the leafless

zone, 1–2-flowered; bracts 2–5, 1.5–5.0 × 4 mm, ovate to widely ovate. *Flowers* actinomorphic, bisexual or male; plant androdioecious; perianth one whorl of sepals and two whorls of petals; sepals (2–)3, imbricate, free, smaller than the petals; petals (4–)6, imbricate, free; bisexual flower with 9–75 carpels, ovary with 1 basal ovule, stigma capitate, stamens 20–30, extrorse, connective shield truncate; male flower 50–190 stamen; androecium and gynoecium arranged in irregular whorls. *Fruit* apocarpous, 2–80 stipitate monocarps; monocarps ellipsoid, ovoid, globose to cylindrical, wall papyraceous to woody. *Seeds* 1 per monocarp, transversally ellipsoid, transversely striate, ruminations lamellate, divided in 4 equal parts in cross section.

### Key to the species

1. Leaves glabrous below
  2. Leaves with tertiary veins immersed below; stipe of fruit 20–30 mm long (Amazonian region) ..... *E. amazonicus*
  2. Leaves with tertiary veins distinctly raised below; stipe of fruit 6–10 mm long (Colombian Andes, Bolivia, and Brazil, AC)
    3. Petals narrowly obovate to narrowly elliptic; pedicel in fruit 12–20 mm long; monocarps ca. 28 mm long (Colombian Andes) ..... *E. colombianus*
    3. Petals ovate; pedicel in fruit 7–8 mm long; monocarps ca. 18 mm long (Bolivia and Brazil, AC) ..... *E. boliviensis*
1. Leaves sparsely to densely covered by hairs below
  4. Pedicel in fruit glabrous; monocarps 30–80; stipe 30–40 mm long (Guyanas and Surinam) ..... *E. guianensis*
  4. Pedicel in fruit covered by yellowish hairs; monocarps 2–20; stipe 5–10 mm long (Brazil)
    5. Pedicel in flower 5.0–10.0 mm long; perianth with 2 sepals and 4 petals; petals orbiculate to oblanceolate, coriaceous (E Brazil, BA, ES, MG) ..... *E. dimerus*
    5. Pedicel in flower absent; perianth with 3 sepals and 6 petals; petals narrowly oblong to oblong, chartaceous
      6. Mature monocarp cylindrical, 15–20 mm long (Central Brazil, GO, MS, MT, TO) ..... *E. parviflorus*

6. Mature monocarp globose, ovoid to ellipsoid, 11–13 mm long (NE Brazil, CE, MA, PI) ..... *E. pisocarpus*

**1. *Ephedranthus amazonicus* R.E.Fr.**, Acta Horti Berg. 12(1): 200. 1934. Type: Brazil, Amazonas, Manaus, Capoeirão na Estrada da Raiz, 9 Apr 1932 (fl, fr) *Ducke s.n.* (Holotype, S!, 2 sheets; isotypes, HB, RB 23891, 2 sheets, K!) Figure 2A–B, Map 1.

Tree 5.0–30.0 m high; branchlets, petioles and buds densely covered by appressed, straight, yellowish hairs to glabrescent. Leaves coriaceous to chartaceous, glabrous above, glabrous to glabrescent, rarely densely covered by appressed, straight hairs below; petiole 5.0–8.0 mm long; lamina 7.0–32.0 × 3.0–11.0 cm, narrowly elliptic to elliptic, oblanceolate to narrowly oblong; apex acute to acuminate, base acute, obtuse to slightly asymmetric; primary vein impressed, forming a sulcus near to the base above, prominent below, secondary veins flat to impressed above, raised below, 9–16 secondary veins pairs, tertiary veins immersed in both sides, angles between primary and secondary veins 55–70°. Inflorescence axillary, 1-flowered; bract 3–4. Flower pedicel 4.0–5.0 mm, fruiting pedicel 8.0–15.0 mm long, glabrous; flower buds globose-flattened; sepals 3, 2.0–4.0 × 3.0–6.0 mm, widely ovate, densely covered by appressed, crisped, yellowish hairs; petals 6, in 2 whorl of 3 petals each, outer petals 9.0–25.0 × 10.0–16.0 mm, ovate to suborbiculate; inner petals 10.0–15.0 × 10.0 mm, orbiculate to elliptic with obtuse apex, coriaceous, sparsely to densely covered by appressed, crisped, yellowish hairs; bisexual flower with ca. 75 carpels, ovary cylindrical prismatic, densely covered by appressed, straight and goldish hairs, stamens not seen; male flower with 120–150 stamens, 2.0 mm long. Fruit ca. 23 monocarps, 15.0–40.0 × 8.0–20.0 mm, ellipsoid, rarely globose, shortly apiculate, immature yellow, mature red, stipe 20.0–30.0 mm long; fruit wall smooth. Seeds 18.0 mm long, 10.0 mm in diameter, ellipsoid.

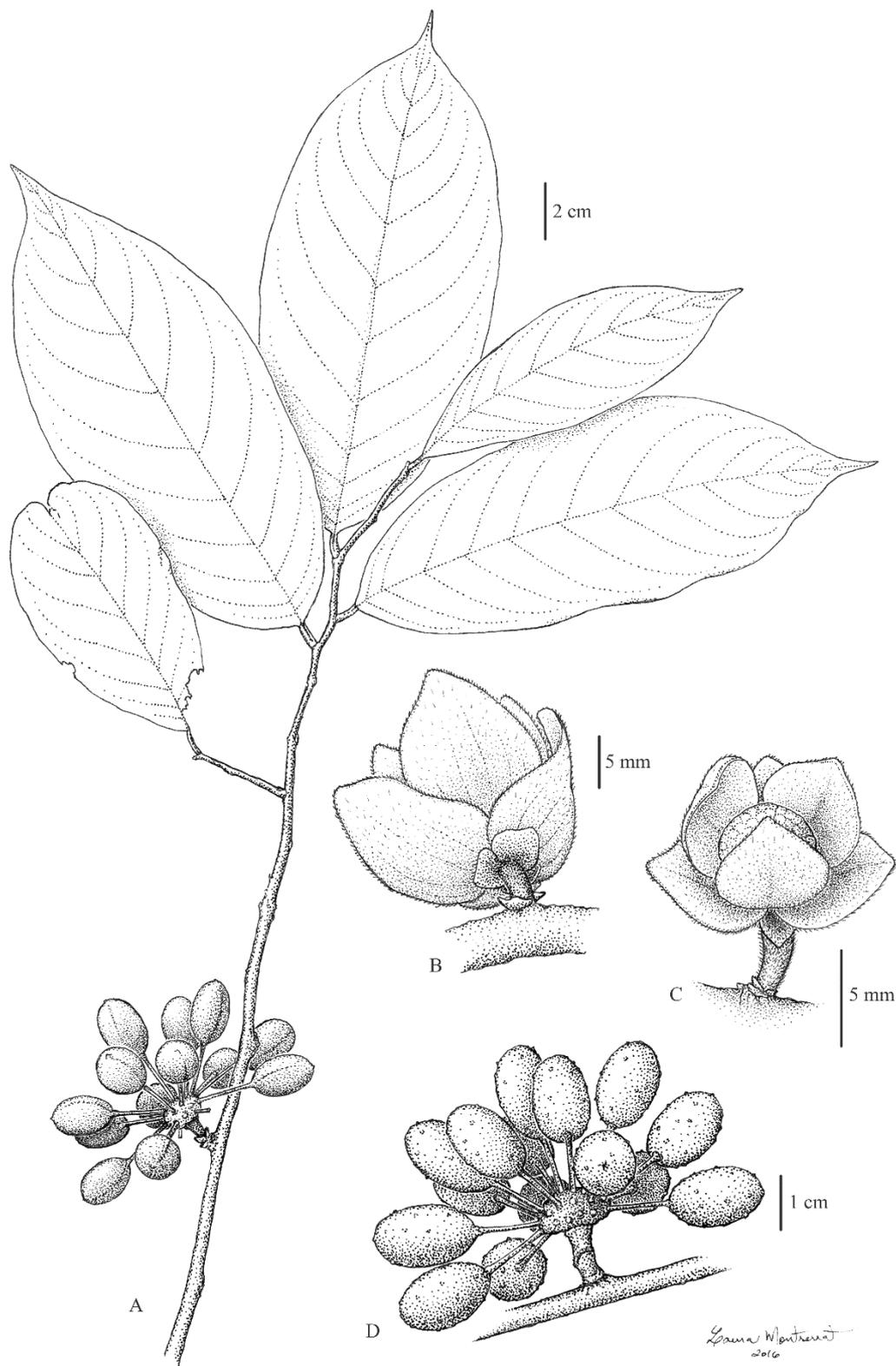
*Habitat and distribution.* *Ephedranthus amazonicus* is widely spread in the Amazon region of Brazil, Colombia and Peru. There is a single known collection from Venezuela. It occurs in non-inundated forest on lateritic soil, at elevations from 50 to

200 m. Flowers from April to June, rarely on October, and fruits throughout the year, mainly from April to August.

*Vernacular names:* cargero (Spanish, Colombia), envira, envira-preta (Portuguese, Brazil), pari (Yurí, Colombia).

*Ephedranthus amazonicus* is distinguished from *E. guianensis* by the fruits with 23 monocarps, with smooth wall. Both species are phylogenetically related and are supported by stipe 20–40 mm long (Figure 1, character 21). Leaves of *E. amazonicus* are in general very long, up to 32 cm long. The also large leaves of *E. colombianus* are instead reticulate below.

*Additional specimen examined.* BRAZIL. **Acre:** Xapuri, rio Acre, 3 hours by boat downstream from Xapuri and 1 hour walking inland from left bank, *Daly et al. 7164* (INPA, NY, U, UFACPZ); Cut-off to Esperança on Brasília-Assis Brasil road, ca. 6



**Figure 2.** A–B. *Ephedranthus amazonicus* A. Twig with fruit. B. Flower. C–D. *Ephedranthus boliviensis*. C. Flower. D. Fruit. A. Vicentini 991 (U); B. Van Dulmen 270 (U); C. Solomon 6484 (U); D. Jardim 251 (U).

km from Brasília, *Lowrie et al.* 722 (INPA, K, MG, NY, U, UFACPZ); Seringal Boa Água,

*Pires* 13745 (IAN). **Amazonas:** Atalaia do Norte, rio Javari, localidade Estirão do Equador, a 3 km da estrada do campo de pouso, *Cid Ferreira et al.* 9893 and 9896 (CEN); Borba, BR 320, estrada Transamazônica, 3 km E of Sucundri, *Henderson* 321 (U, UB); Itacoatiara, *Kinupp* 1370 (INPA); Manaus, along road to Aleixo, *Krukoff* 7981 (K, NY, U); margem do igarapé Bindá, *Chagas s.n* (INPA 1310); Manaus, Itacotiara road km 146, roadside forest, *Bisby et al.* P18068 (INPA, MG); Reserva Florestal Walter Egler, road Manaus-Itacoatiara, km 64, *Prance et al.* 9048 (INPA, NY, U); Estrada do Aleixo, grounds of Companhia das Plantações, *Prance et al.* 18787 (INPA, K, MG, NY, U); Reserva Florestal Ducke, Manaus-Itacoatiara, km 26, *Coelho D13* (HFSL, NPA, K, L, MG, U, ULM); *Assunção & Pereira* 203 (INPA K, L, MG, U, UB, ULM); *Costa & Silva* 318 (HAMAB, INPA, K, L, U, UFACPZ); *Hopkins et al.* 1434 (INPA, K, L, MG, MO, U, UFMT, ULM); *Prance et al.* 4692 (INPA, K, MG, NY, U); *Ribeiro et al.* 1617 (BM, INPA, K, L, MG, R, RB, U, UB, ULM, US, VEN); *Ribeiro et al.* 1671 (INPA, L, U); *Ribeiro & Silva* 1383 (INPA, K, L, U); Em frente à meteorologia, *Assunção et al.* 223 (IAN, INPA, U); *Vicentini et al.* 991 (COL, F, INPA, K, L, MG, R, U, UB, ULM, VEN); igarapé Ipiranga, *Ribeiro et al.* 938 (INPA, L, U); Igarapé da Bolívia, sentido bairro Sabiá, *Ribeiro et al.* 856 (INPA, K, L, U); Trilha à esquerda do km 0,35, estrada alojamento-torre, *Vicentini et al.* 467 (INPA, K, L, MG, U, ULM); Estrada do Acará, *Coelho & Lima D26* (INPA, K, L, MG, U, ULM); Distrito Agropecuário, Reserva 1501, km 41 da Smithsonian/INPA, *Freitas et al.* 22 (U); *Freitas et al.* 856 (U); *Mori et al.* 19527 and 21371 (U); Rio Cuieiras, igarapé Lobisomem, *Mori & Gracie* 21811 (INPA, NY, U); upstream, farm of sr. Nemério, *Campbell P21815* (INPA); Rio Negro, próximo ao rio Arara, *Loureiro s.n.* (INPA 37883, MG); Rio Tarumã, mata alta central, *Froés* 24940 (IAN); Rio Urubu, between cachoeira Iracema and Natal, at Manaus-Caracará road, *Prance et al.* 4987 (INPA, NY, U); Novo Airão, área indígena Waimiri Atroari, rio Camanaú, vicinity of aldeia Maré, *Miller* 356 and 590 (INPA); Presidente Figueiredo, estrada do Pau Rosa, cerca de 5 km da margem da estrada, *Silva et al.* 797 (INPA); **Mato Grosso:** Aripuanã, margem da Bahia, ao lado das picadas da encanação de água, *Gomes* 458 (INPA); Serra do Cachimbo, *Nascimento* 463 (MG). **Pará:** Alto Tapajós, Vila Nova, perto da Cachoeira do Cachorrão, *Pires* 4028 (IAN); Conceição do Araguaia, fazenda São José, *Lobato et*

al. 2823 (MG); Estudo Ecológico entre Estreito e Marabá, *Pires 13636* (IAN); Oriximiná, rio Trombetas, mineração Santa Patrícia, estrada para Porto Trombetas, Serra da Onça, *Martinelli 7299* (INPA, MG, U); Tucuruí, *Silva et al. 424* (MG). **Rondônia:** Buritis, fazenda do sr. José Vespal, *Carlos et al. 1226A* (MG); Forte Príncipe da Beira, *Rodrigues et al. 4279* (NY, INPA); Porto Velho, usina hidrelétrica de energia Jirau, *Pereira-Silva 13596* (INPA); Machadinho d'Oeste, near Tabajara, upper Machado river region, *Krukoff 1358 and 1597* (K, U); Serra dos Parecis, a 27 km de Alta Floresta, na linha 65 da Topografia BASE VI, *Lisboa et al. 2512* (MG).

COLOMBIA. **Amazonas:** Casaquiare, río Casaquiare, Capihuara, *Colella & Guayamare 1740* (U); río Caquetá, frente a Villa Azul, terraza baja, *van Andel et al. 495* (U); al sur de la desembocadura de la quebrada Bocaduche y el río Caquetá, frente a la isla Sumaeta, *Dulmen 209* (COAH, U); *Dulmen 270* (U); *Dulmen 345* (L, U); **Caquetá:** trocha al Yará, *Murillo & Román 590* (U); *Murillo & Román 606* (U).

PERU. **Loreto:** Maynas, acampamento “Explorama Lodge”, zona Bushmaster, *Díaz et al. 1182* (U); Explornapa Camp, on río Sucurasi afluente of río Napo, 75 km of Iquitos, *Chatrou et al. 173* (U); Yanomono, Explorama Tourist Camp, río Amazonas, halfway between Indiana and mouth of río Napo, *Gentry & Vasquez 42408* (MO, U); *Gentry et al. 29075* (MO, U); above mouth of río Napo on río Amazonas, *Gentry et al. 27769* (MO, U); Quebrada Sucursari, río Napo, *Gentry et al. 42687* (MO, U).

VENEZUELA: **Amazonas,** Casiquiare, rio Casiquiare, Capihuana, *Collela 1740* (U).

**2. *Ephedranthus boliviensis* Chatrou & Pirie**, Revista Soc. Bol. Bot. 4(1):25, f. 1, 2. 2003. Bolivia. Beni, Vaca Diez, Cachuela Esperanza, along río Beni, 10°32'S 65°38' W, alt. ca. 200 m, 9 Nov 2001 (fl), *Chatrou et al. 420* (Holotype, LPB; isotypes, AAU, K!, MO, U!, USZ, WU). Figure 2C–D, Map 2.

Tree 3.0–19.0 m high; branchlets, petioles and buds sparsely to densely covered by appressed, straight to crisped, yellowish to ferruginous hairs to glabrescent. Leaves chartaceous, glabrous with appressed, crisped, yellowish hairs along the primary vein above, glabrescent, below; petiole 3.0–6.0 mm long; lamina 6.0–17.0 × 2.5–7.0 cm, narrowly elliptic to elliptic, elliptic-obovate, oblong, ovate; apex acute to acuminate,

base obtuse, acute to slightly asymmetric; primary vein flat, sometimes with a sulcus near to the base above, prominent below, secondary veins flat to impressed above, raised below, 8–14 secondary veins pairs, indistinct above, distinctly raised-reticulate below, angles between primary and secondary veins 50–70°. Inflorescence axillary, 1-flowered; bract 4. Flower pedicel ca. 3.0 mm, fruiting pedicel 7.0–8.0 mm long, glabrous; flower buds flattened-globose; sepals 3, 1.5–3.0 × 2.0–3.0 mm, widely ovate to triangular, densely covered by appressed, crisped, yellowish hairs; petals 6, in 2 whorl of 3 petals each, coriaceous, sparsely to densely covered by appressed, crisped, yellowish hairs, outer petals 5.0–10.0 × 5.0–9.0 mm, ovate; inner petals 6.0–10.0 × 5.0–6.0 mm, ovate; bisexual flower not seen; male flower with 50 stamens, 1.5 mm long. Fruit 10–30 monocarps, 18.0 × 7.0–11.0 mm, ellipsoid, apex rounded, yellow *in vivo*, stipe 10.0 mm long; fruit wall verrucose. Seeds 14.0 mm long, 8.0 mm in diameter, ellipsoid.

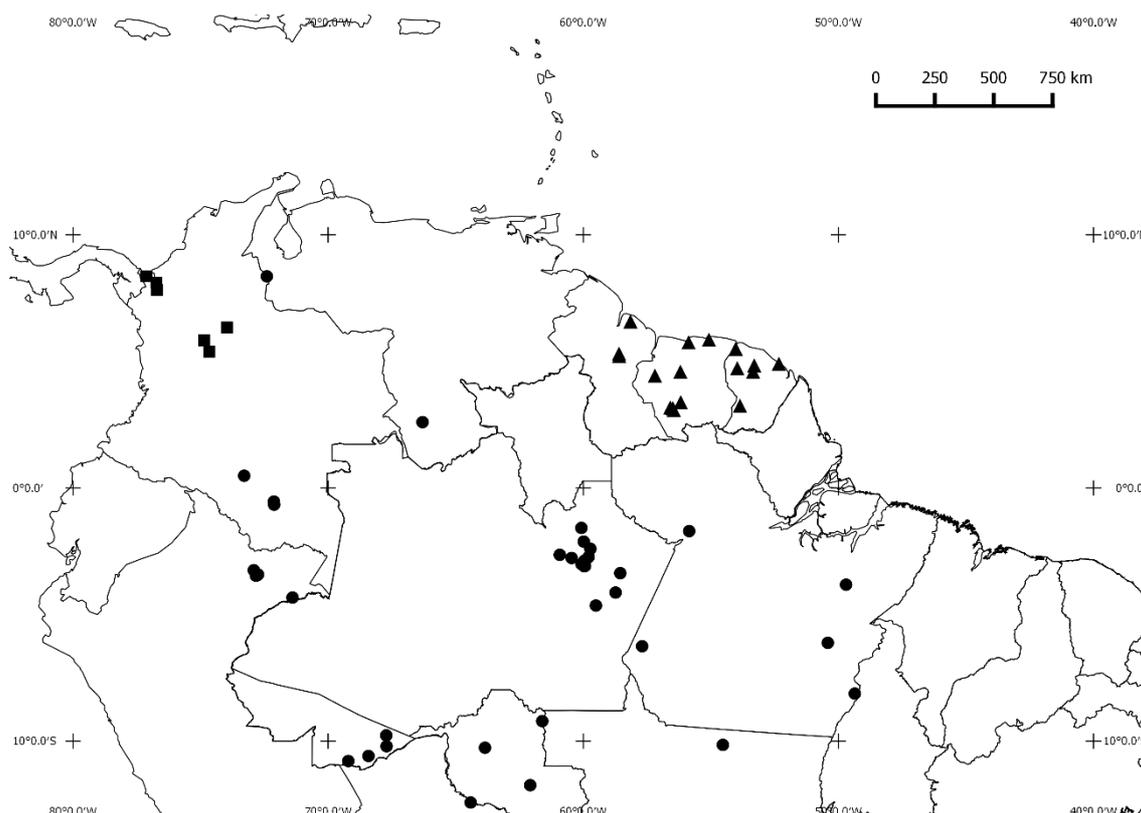
*Habitat and distribution.* *Ephedranthus boliviensis* occurs in Bolivia and Brazil, in dry *chiquitanos* forest, and disturbed forest, at elevations from 125 to 300 m. Flowers from September to November and fruits on November.

*Vernacular names:* josis (Chacobo, Bolivia), peraquina (Spanish, Bolivia).

*Ephedranthus boliviensis* is distinguished from *E. parviflorus* by the flower pedicel, 3.0 mm long (vs sessile in *E. parviflorus*), widely ovate to triangular sepals 1.5 to 3.0 mm long (vs ovate sepals 3.0–5.0 mm long) and ellipsoid and verrucose monocarps (vs cylindrical and smooth).

*Additional specimen examined.* BOLIVIA. **Beni:** Cachuela Esperanza, río Yuta, Meyer *s.n.* U14968 (U); Meyer 112 (INPA, U); Vaca Diez, vicinity of the Chacóbo village, Alto Ivon, Boom 4396 (U); Boom 4460 (INPA,U); Tumi Chucua, 30 km south of Riberalta, Solomon 6484 (U); **Pando:** Madre de Dios, along río Madre de Dios, at Puerto Candelaria, Nee 31829 (U); **Santa Cruz,** Velasco, Parque Nacional Noel Kempff Mercado, Jardim 222, 251 and 3079 (U); Chatrou *et al.* 301 and 336 (U).

BRAZIL. **Acre:** Rio Branco, Parque Zoobotânico, trail behind herbarium HPZ, Maas *et al.* 9254 (HPZ, NY, U)



**Map 1.** Northern region of South America showing distribution of *Ephedranthus amazonicus* (●), *E. colombianus* (■), and *E. guianensis* (▲).

**3. *Ephedranthus colombianus* Maas & Setten**, Proc. Kon. Ned. Akad. Wetensch. C 91(3): 248, f. 5, 6, 7. 1988. Type: Colombia, Antioquia, San Luís, Cañón del río Claro, NW sector, left bank, alt. 375–600 m, 9 Mar 1984 (fl), *Cogollo 1448* (Holotype, JAUM; isotypes, NY, US). Figure 3A–D, Map 1.

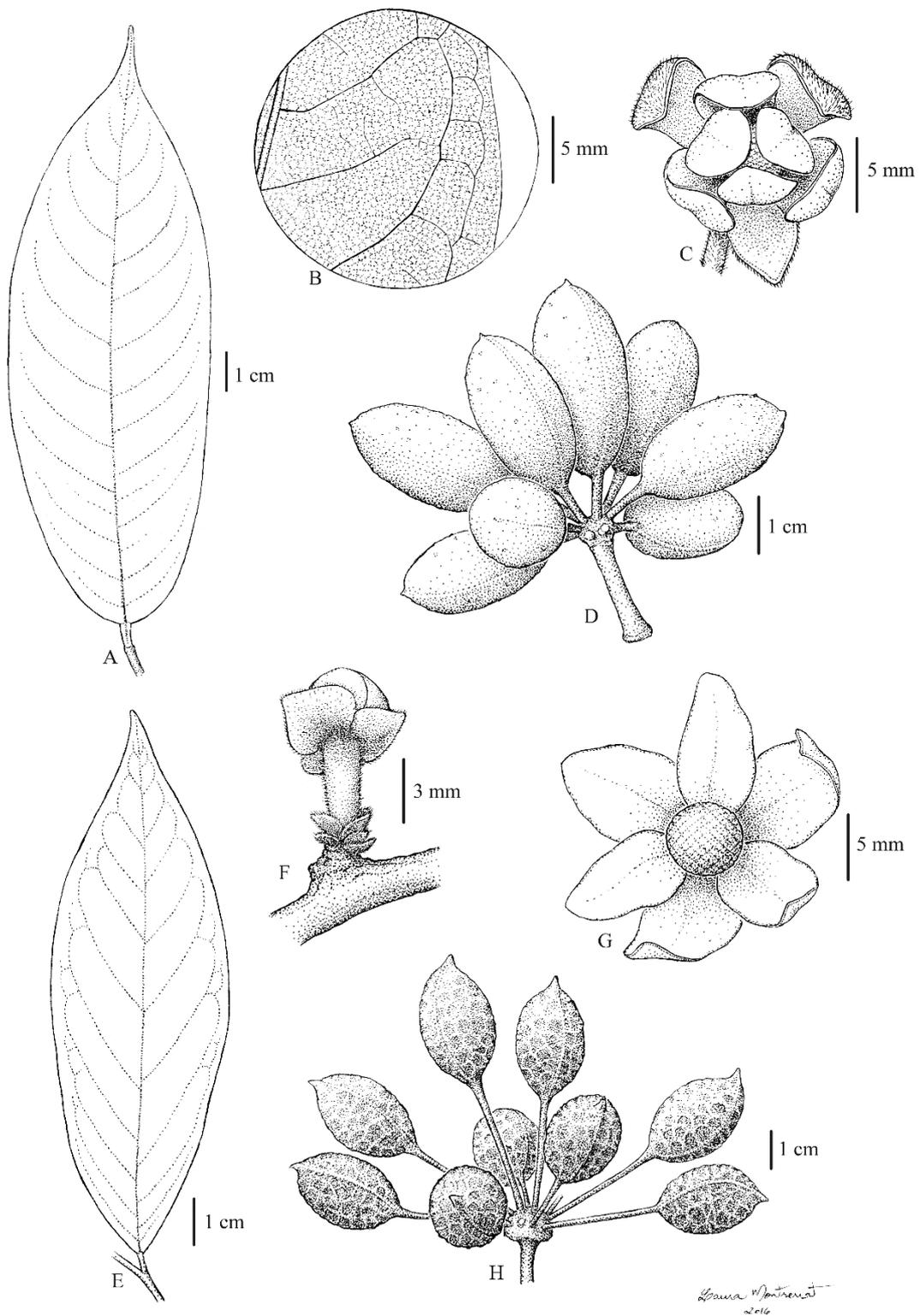
Tree 5.0–20.0 m high; branchlets, petioles and buds densely covered by appressed, straight, ferrugineous hairs or covered by papillae. Leaves chartaceous to coriaceous, glabrous with appressed, crisped, yellowish hairs along the primary vein above, glabrous, below; petiole 3.0–8.0 mm long; lamina 9.5–24.0 × 3.0–9.0 cm, narrowly oblong to oblong, narrowly elliptic; apex acute to acuminate, base obtuse; primary vein impressed, above, prominent below, secondary veins impressed above, raised below, 8–14 secondary veins pairs, tertiary veins indistinct above, distinctly raised-reticulate below, angles between primary and secondary veins 60–80°. Inflorescence axillary, 1–2-flowered; bract 2. Flower pedicel 6.0–10.0 mm, fruiting pedicel 12.0–20.0 mm long, glabrous to covered by papillae; sepals 3, ca. 7.0 × 4.0 mm, lanceolate, densely covered

by crisped, yellowish hairs; petals 6, in 2 whorls of 3 petals each,  $15.0 \times 4.0\text{--}5.0$  mm, coriaceous, densely covered by appressed, crisped, brownish hairs, narrowly obovate to narrowly elliptic; bisexual flower with ca. 15 carpels, ovary densely covered by appressed, straight, brownish hairs, stamens not seen; male flower not seen. Fruit ca. 6 monocarps,  $28.0 \times 11.0$  mm, ellipsoid, apiculate, immature green, mature purple to dark purple, stipe 6.0–7.0 mm long; fruit wall verrucose, with apparent veins. Seeds 15.0–19.0 mm long, 7.0–8.0 mm in diameter, ellipsoid to ovoid.

*Habitat and distribution.* *Ephedranthus colombianus* occurs in the Colombian departments of Antioquia, Santander and Sucre. It is present mainly in forests from the Andean region, at elevations from 30 to 410 m, reaching up to 2,300 m in La Ceja, Antioquia. In Sucre occurs also in limestone formations. Flowers on April and fruits from June to September.

*Ephedranthus colombianus* differs from the other species by the reticulate leaves and verrucose monocarps, with apparent veins. Verrucose monocarps (character 18), and leaves glabrous at abaxial side (character 0, homoplastic) are the synapomorphies that support the sister relationship of *E. colombianus* and *E. boliviensis* (Figure 1). Collections bearing flowers are less common than that with fruits, and in general, flowering herbarium sheets bear old flowers, from which stamens had already fell. Male flowers have not been observed in the specimens examined.

*Additional specimen examined.* COLOMBIA. **Antioquia:** La Ceja, vereda El Tabor, *Alzate & Sierra 875* (HUA, U); Puerto Berrío, vereda Alicante, finca El Rebaño, en la via San Juan de Bedout-La Cabaña, *Callejas 9333* (U); San Luis, cañon del río Claro, sector Sur, *Sánchez 1058* (U); Turbo, Corregimiento Lomas Aisladas, Finca las Aisladas, *Alzate & Cardona 1259* (HUA, U), *Alzate & Cardona 1262* (HUA, K); Caldas, La Dourada, *Espintal 268* and *1196* (COL); **Santander:** Cimitarra, Corregimiento de Puerto Olaya, hacienda El Bosque, *Alzate 1266* (HUA, U); Vicinity to Cimitarra, road north from airport between río Guasqualito and 5 km beyond the bridge, *Gentry & Forero 15471* (COL, MO, U); Río Segovia, *Rentería 2364* (COL); San Luís, cañon del río Claro, *Cogollo 503, 631, and 1262* (COL); Alrededores de la vereda



**Figure 3.** A–D. *Ephedranthus colombianus*. A. Leaf; B. Detail of the leaf reticulation; C. Flower. D. Fruit. E–H. *Ephedranthus guianensis* E. Leaf; F. Flowering pedicel with bracts. G. flower. H. Fruit. A–B Gentry 15471 (U); C. Photo: Andrés Link; D Sánchez 1058 (U); E–F Irwin 55720 (U); G Schulz 8568 (U); H Sabatier 3389 (U).

Chorro de Oro, *Cogollo 4594* (COL); Turbo, carretera Panamericana, Tapón del Darién, sector Río León, lomas Aisladas,

*Brand 1146* (COL); **Sucre**, Colosó, Estación de Primatos, *Gentry & Cuadros 68213* (MO, U).

**4. *Ephedranthus dimerus* J.C.Lopes, Chatrou & Mello-Silva**, *Brittonia* 66(1): 71, f. 1, 2. 2014. Type: Brazil. Espírito Santo, Linhares, Reserva Florestal da Companhia Vale do Rio Doce, aceiro Lasa, G. Santos, km 0,658, lado direito, 23 Nov 1982 (fl), *Folli 414* (Holotype, SPF!; isotypes, CVRD!, K!, MO, NY, U!). Figure available on Lopes et al. (2014), Map 2.

Tree 2.0–30.0 m tall; branchlets, petioles and buds covered with simple, straight to crisped, erect hairs, sometimes glabrescent, branches glabrous. Leaves chartaceous, glabrous or sparsely covered with erect, straight hairs on primary vein above, sparsely to densely covered with straight to crisped, erect hairs below; petiole 3.5–5.5 mm long; lamina 7.0–14.5 × 3.0–6.5 cm, oblanceolate to obovate or elliptic, apex acute, rarely obtuse or emarginated; base cuneate to acute; primary vein impressed above and raised below, secondary vein impressed above and raised below, 9–14 pairs of secondary veins, tertiary veins immersed in both sides, angles between primary and secondary veins 50–60°. Inflorescence axillary, 1-flowered; bract 4–5. Flower pedicel 5.0–10.0 mm long, fruiting pedicel 1.0–2.0 cm long, glabrous to densely covered by yellowish hairs; flower bud globose; sepals 2, 3.0–4.5 × 5.0–5.5 mm, widely ovate, densely covered with appressed, straight, goldish hairs; petals 4, in 2 whorls of 2 petals each, 7.0–10.0 × 5.0–9.5 mm, the outer ones orbiculate, the inner ones oblanceolate, coriaceous, densely covered with appressed, crisped hairs below, almost glabrous above; bisexual flowers with ca. 40 carpels, ovary glabrescent, stamens ca. 20; male flowers with ca. 80 stamens, 1.5 mm long. Monocarps 2–20, 15.0–25.0 × 10.0–15.0 mm, ellipsoid to cylindrical, apex rounded to umbonate, immature green, mature orange to dark red, stipe 6.5–11.0 mm long; fruit wall smooth. Seeds 1.5 cm long, 1.0 cm diameter, ellipsoid.

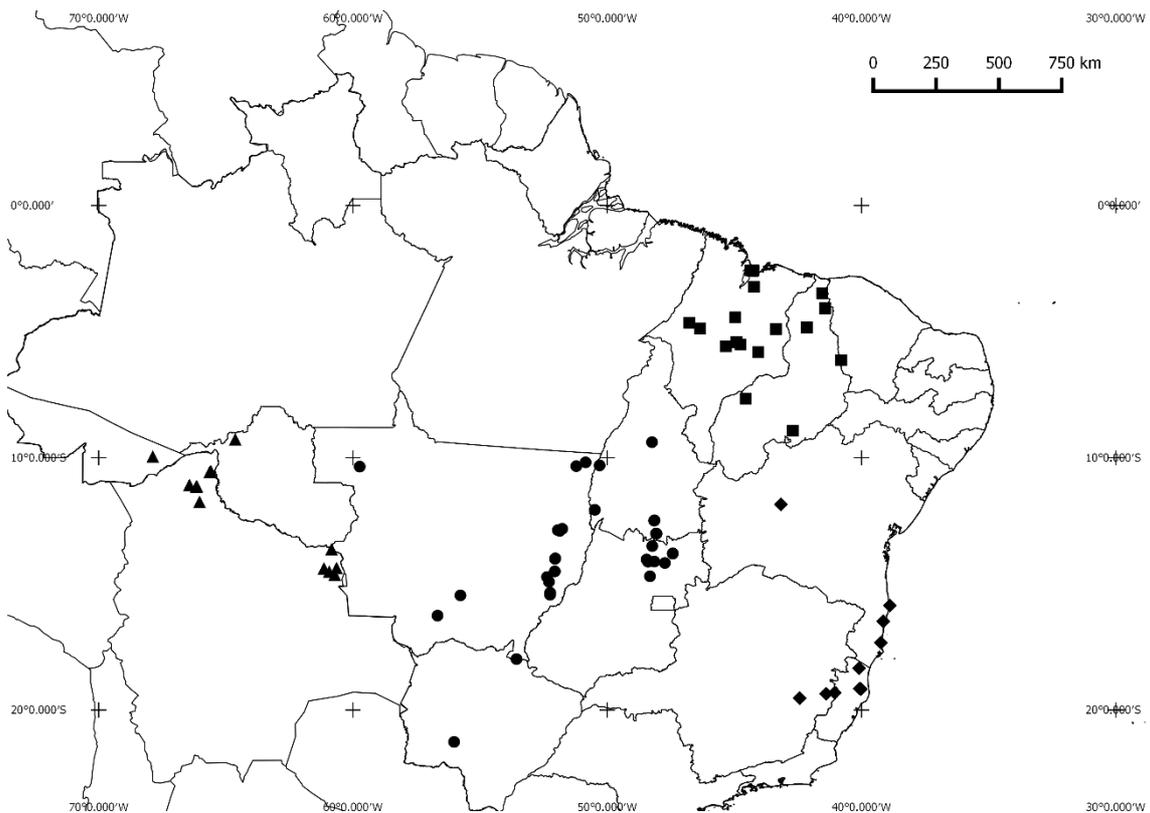
*Habitat and distribution:* *Ephedranthus dimerus* occurs in the Atlantic Forest of Brazil, along the Rio Doce basin, in Minas Gerais and Espírito Santo, and near to the coast in Bahia, with a single inland collection in Barra municipality. Flowers from October to November and fruits from February to June.

*Vernacular names:* pindaíba-preta (Portuguese, Brazil).

*Ephedranthus dimerus* is the only species in the genus with flowers showing two whorls of two petals each and one whorl with two sepals. It is also the only *Ephedranthus* from the Brazilian Atlantic Forest. Moreover, it has the longest pedicel among the species of the genus, with up to 1.0 cm long. *Ephedranthus dimerus* is sister to *E. parviflorus* and *E. pisocarpus*, and the group is supported by yellowish hairs covering the fruit pedicel and cylindrical monocarps (Figure 1, characters 16 and 19).

*Additional specimen examined:* BRAZIL. **Bahia:** Barra, brejos Olhos d'Água, Buracão, Costa & Nascimento Júnior 679 (ASE); Belmonte, Estação Experimental de Belmonte, CEPLAC, Santos 1105 (CEPEC, U); Prado, Reserva Florestal da Brasil de Holanda Indústrias S.A., entrance at km 18 E of Itamaraju on road to Prado, 8 km from entrance, Thomas et al. 10132 (K, NY, RB, U); Santa Cruz Cabralia, área da Estação Ecológica do Pau-Brasil (ESPAB), cerca de 16 km a W. de Porto Seguro, BR 367 (Porto Seguro–Eunápolis), Santos 732 (CEPEC, U); Santos 904 (U). **Espírito Santo:** Linhares, Reserva Florestal da Companhia Vale do Rio Doce, estrada Macanaíba-Pele-de-Sapo, próximo à estrada Baba-de-Boi, Folli 2278 (CVRD, RB, SPF); Estrada Cinco-Folhas, atrás do laboratório, próximo ao Lago do Jacaré, Folli 465 (CVRD, RB); Estrada Flamengo, ca. de 900 m da BR 101, na borda da mata, Lopes et al. 145 (CVRD, SPF); Estrada Cinco-Folhas, próximo à Lagoa do Jacaré, atrás do Laboratório de Sementes, Lopes et al. 149 (CVRD, SPF); Próximo ao lago indo para o galpão de máquinas, Siqueira 677 (CVRD, SPF); Estrada Mantegueira, 1,4 km, próximo ao pomar, Lopes et al. 154 (CVRD, SPF); Estrada Mantegueira, 1.5 km from Flamengo, near orchard, Maas et al. 8826 (CVRD, K, U); Estrada Cinco-Folhas, 200 m, Folli 5998 (CVRD);

Estrada Flamengo, 1450 m, *Folli 5463* (CVRD); Pinheiros, Reserva Biológica Córrego do Veado, interior da floresta, *Leoni 5840* (RB, SPF). **Minas Gerais:** Caratinga, fazenda Montes Claros, Trilha do jequitibá, *Andrade & Lopes 155* (BHCB, SPF); Estação Biológica de Caratinga, Mata do Jaó, *Gomes 5* (BHCB, SPF); *Couto 181* (BHCB, SPF); *Andrade & Lopes 371* (BHCB, SPF); *Pereira 1022* (BHCB). Itueta, Usina Hidrelétrica de Aimorés, fazenda Adolfo Schumaker, *Tameirão Neto 2446* (BHCB, SPF). Santa Rita do Itueto, Quatituba à Cachoeira do Peão, *Luz 275* (CVRD).



**Map 2.** Central portion of South America showing distribution of *Ephedranthus boliviensis* (▲), *E. dimerus* (◆), *E. parviflorus* (●), and *E. pisocarpus* (■).

**5. *Ephedranthus guianensis* R.E.Fr.**, Acta Horti Berg. 10(2): 176. 1931. Type: Surinam, Copename, Raleighvallen, 17 Aug 1923 (fl), *Stahel & Gonggrijp BW6312* (Holotype, U!; isotypes AAU, BR, S! fragment). Figure 3E–H, Map 1.

*Ephedranthus guianensis* var. *oligantha* R.E.Fr., Acta Horti Berg. 10(2): 177. 1931.  
Type: French Guiana, S. Jean, 26 Apr 1914 (fl), *Benoist 1159* (Holotype, P; isotype S! fragment).

Tree to shrub 1.5–18.0 m high; branchlets, petioles and buds densely covered by erect, straight, yellowish hairs to glabrescent. Leaves chartaceous, glabrous, with erect, straight, yellowish hairs along the primary vein above to glabrescent, sparsely to densely covered by appressed, straight, whitish hairs and papillae below; petiole 4.0–8.0 mm long; lamina 6.5–19.0 × 2.5–6.0 cm, narrowly elliptic to elliptic, narrowly oblong to oblanceolate; apex acuminate to attenuate, acute, base acute to obtuse or slightly asymmetric; primary vein impressed above, prominent below, secondary veins flat to impressed above, raised below, 5–11 pairs of secondary veins, tertiary veins immersed in both sides, angles between primary and secondary veins 55–70°. Inflorescence axillary, 1-flowered; bract 3–5. Flower pedicel 5.0–8.0 mm, fruiting pedicel 1.5–4.0 cm long, glabrous; flower buds ovoid; sepals 3, 2.0–4.0 × 3.0–6.0 mm, triangular, densely covered by appressed, crisped to straight, brownish hairs; petals 6, in 2 whorls of 3 petals each, outer petals 9.0–20.0 × 6.0–14.0 mm, widely ovate to oblong-ovate; inner petals 9.0–18.0 × 5.0–10.0 mm, elliptic, oblanceolate to narrowly obovate, coriaceous, densely covered by appressed to erect, crisped, brownish hairs; bisexual flower with ca. 100 carpels, cylindrical, densely covered by appressed, straight hairs, stamens ca. 30; male flower with 75–190 stamens, 1.5–3.0 mm long. Fruit (6–)30–80 monocarps, 17.0–30.0 × 12.0–20.0 mm, ellipsoid, apiculate to umbonate, immature green, mature red-purple to red-orange, stipe 30.0–40.0 mm long; fruit wall fleshy, smooth, with apparent veins, transversal raised line around the monocarp and longitudinal intrusions apparent on dried monocarp. Seeds 18.0–22.0 mm long, 10.0–12.0 mm in diameter, ellipsoid.

*Habitat and distribution.* *Ephedranthus guianensis* occurs in Guyana, French Guyana and Surinam, in forest on lateritic soil, at elevations from 200 to 900 m. Flowers from January to February and from August to October, fruits from June to September, rarely on April.

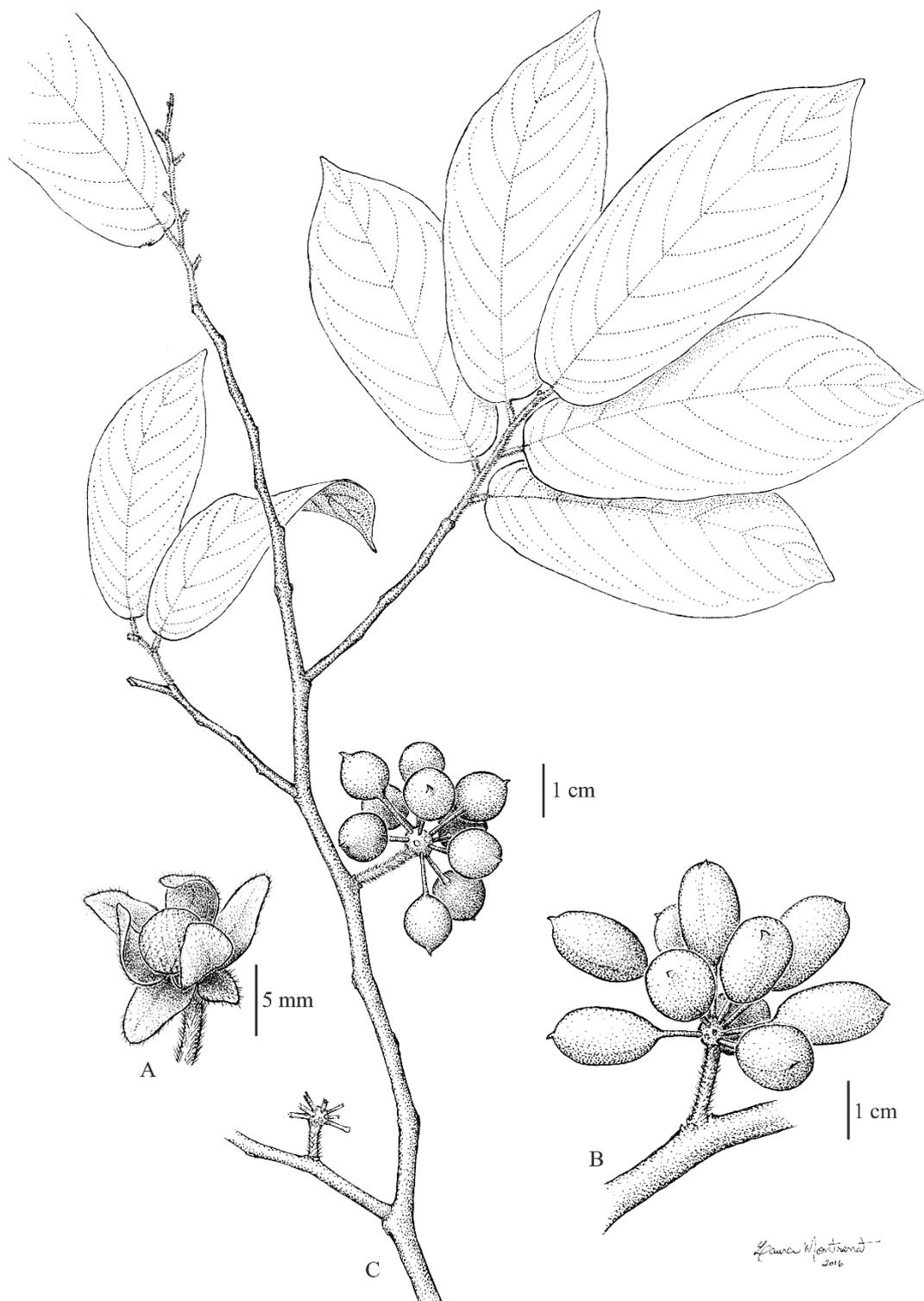
*Vernacular name:* karishiri (Creole, Guyana), man-pika-pika (Sranan, Surinam), mamayawé (Creole, French Guyana), mouamba (Paramaka, French Guyana).

*Ephedranthus guianensis* is recognizable by the medium sized, usually, oblanceolate leaves, fruits with very long stipes, from 3.0 to 4.0 cm long, and up to 80 monocarps with apparent veins on the wall. *Ephedranthus guianensis* var. *oligantha* R.E.Fr. has been distinguished from the typical variety by the smaller flowers (sepal 1.5–2.0 mm vs 2.0–2.5 mm; outer petal 6.0 × 4.0 mm vs 10.0–13.0 × 7.0–8.0 mm, Fries 1931), but it was based on a single collection with atypical flowers. For this reason, Oliveira & Sales (1999) took this variety as a synonym of *E. guianensis*.

*Additional specimen examined.* FRENCH GUYANA. **Saint-Laurent-du-Maroni**, BAFOG (*Service Forestier*) 7478 (U); Entre Citron et le Mont Décou Décou, *Cremers* 8235 (CAY, U); Banafokondre, *Sauvain* 134 (CAY, U); Rivière Grand Inini, bassin du Maroni, *Sabatier & Prévost* 3389 (CAY, NY, P, U); Montagnes de la Trinité, Inselberg Nord Ouest, *Granville et al.* 6112 (BR, CAY, G, K, MG, P, U); Piste de Saint-Elie, interfluve Sinnamary-Counamama, *Prévost & Sabatier* 4130 (CAY, U); km 25, bord de zone defrichés, *Riéra* 466 (U); Station des Nouragues, bassin de l'Arataye, *Sabatier & Prevost* 2782 (K).

GUYANA. **Upper Demerara-Berbice**, Mabura Hill, near Ecological Reserve guest house, *Maas et al.* 7135 (K, U); 180 km SSE of Georgetown, near Reserve house, *Steege et al.* 280 (U); **West Demerara**, Hill area, Tropenbos reserve, *Scharf* 64 (BRG, LZ, U); Takutu Ck. Puruni R., Mazaruni Rivier, *Fanshawe* 2052 (U); *Forest Department of British Guiana* 4788 (K).

SURINAME. **Nickerie**, area of Kabalebo Dam project, *Lindeman* 333 (K, U); *Lindeman* 753 (BBS, U); Zuid Rivier, 2 km above confluence with Lucie Rivier, *Irwin* 55720 (U); Jodensavanne-Mapane kreek area, *Schulz* 8568 (U); *Schulz* 7518 (U); *LBB* 9888 (U); *Lindeman* 6749 (U); Boven-Suriname Rivier bij Goddo, *Stahel* 44 (U); Juliana top, 14 km north of Lucie Rivier, *Irwin* 54720 (U); Paris Jacob-kreek, *Maas et al.* *LBB11029* (U); Haleighvallen, Voltzberg Natuurreservaat, *De Jong* *LBB15778* (U); Coppename R, Raleigh Falls, *Stahel* *BW6269* (IAN, K, U); *Boon* 1126 (U); Sipaliwini, Central Suriname Nature Reserve, ca. 2 km SE of E and of Kayserberg Airstrip, *Rosário et al.* 1793 (K, MG, MO); Vicinity of camp on W bank of Zuid river, *Rosário et al.* 2064 (MG, MO)



**Figure 4.** A–B. *Ephedranthus parviflorus*. A. Flower; B. Fruit; C. *Ephedranthus pisocarpus*. C. Twig with fruit. A Solórzano 93 (UB); B Pereira-Silva 5776 (SPF); C Daly 500 (U).

6. *Ephedranthus parviflorus* S.Moore, Trans. Linn. Soc. Lond. Bot. Ser. 2. 4: 296. pl. 21, 38. 1895. Type: Brazil, Mato Grosso, Barra do Bugres (Santa Cruz), in sylvulis juxta Santa Cruz, Sep 1891 (fl), *Moore 310* (Holotype, BM!; isotype, NY). Figure 4A–B, Map 2.

Tree to shrub 3.0–18.0 m high; branchlets, petioles and buds densely covered by appressed to erect, crisped, yellowish hairs. Leaves chartaceous, glabrous above, except the primary vein covered by hairs, sparsely covered by erect, straight hairs below; petiole 3.0–8.0 mm long; lamina 5.5–16.0 × 2.5–7.0 cm, narrowly elliptic, elliptic to widely elliptic, narrowly obovate to oblanceolate, suborbiculate; apex acute, rarely obtuse to attenuate, base acute, obtuse to slightly asymmetric, rarely slightly cordate; primary vein impressed, forming a sulcus near to the base above, raised below, secondary veins impressed above, raised below, 7–13 pairs of secondary veins, tertiary veins immersed above and slightly raised below, angles between primary and secondary veins 50–70°. Inflorescence axillary, 1-flowered; bract ca. 5. Flower sessile, fruiting pedicel 6.0–20.0 mm long, densely covered by yellowish hairs; flower buds globose; sepals 3, 3.0–5.0 × 4.0 mm, ovate, densely covered by appressed, straight, goldish hairs; petals 6, in 2 whorls of 3 petals each, outer petals 6.0–8.0 × 3.0–5.0 mm, ovate to narrowly oblong with acute to rounded apex; inner petals 8.5–9.0 × 3.0–4.0 mm, oblong to narrowly oblong with acute to rounded apex, chartaceous, densely covered by appressed, straight, goldish hairs; bisexual flower with 9–16 carpels, ovary ovoid, densely covered by appressed, straight hairs, stamens 27–30; male flower with ca. 100 stamens, 1.5–2.0 mm long. Fruit 4–14 monocarps, 15.0–20.0 × 8.0–10.0 mm, immature ellipsoid to globose, mature cylindrical, shortly apiculate, immature green, mature yellowish to red, stipe 5.0–8.0 mm long; fruit wall smooth. Seeds 16.0–19.0 mm long, 7.0–8.0 mm in diameter, ellipsoid.

*Habitat and distribution.* *Ephedranthus parviflorus* occurs in Central-Western Brazil in the states of Goiás, Mato Grosso, Mato Grosso do Sul, and Tocantins, in riparian forest in the *cerrado* domain, on clay to sandy soil, at elevations from 350 to 750 m. Flowers from June to August, fruits from October to December.

*Vernacular name:* adzo waihi (Aquém, Brazil), conduru, meju-do-porco (Portuguese, Brazil).

*Ephedranthus parviflorus* shares with *E. boliviensis*, *E. dimerus*, and *E. pisocarpus* the medium sized leaves with apparent secondary veins on the upper side. It can be distinguished from *E. boliviensis* and *E. pisocarpus* by the monocarps, ellipsoid or globose in *E. boliviensis* and *E. pisocarpus*, and cylindrical in *E. parviflorus*, and from *E. dimerus* by its dimerous flowers with 1 cm long pedicel. *Ephedranthus parviflorus* and *E. pisocarpus* are sister species supported by pedicel absent to 2 mm long, and narrowly oblong to oblong petals (Figure 1, characters 7 and 10). *Ephedranthus parviflorus* is usually confused with a new species of *Oxandra* (Alvarenga 1292). Both species occur in Goiás and show similar leaves, with brownish-yellow veins on the upper side. However, *Oxandra sp. nov.* shows reticulate venation, and one or two globose, glaucous monocarps, with very short stipes.

*Additional specimen examined:* BRAZIL. **Mato Grosso:** Água Boa, Reserva Indígena Areões, 8 km da aldeia Mutum, *Marimon 147* (SPF); *Marimon 79* (UB); Barra do Garça, Vale dos Sonhos, c. 10 km N of Aragarças on the Aragarças – Xavantina road, *Ratter et al. 406* (K, UB); *Ratter et al. 2199* (UB); Chapada dos Guimarães, base aérea do SINDACTA, *Hatschbach 63697* (MBM, SPF); vicinity of Buriti, *Prance 19246* (INPA, K, NY, U); Drainage of upper rio Araguaia, 25 km S. of Xavantina, *Irwin 17100* (IAN, NY, U); *Irwin 16794* (IAN, NY); Nova Xavantina, Reserva Biológica Mário Viana, mata de galeria do córrego Bacaba, *Marimon 169* (SPF, UB); Novo Santo Antônio, Parque Estadual do Araguaia, *Solórzano et al. 93* (UB); near the base camp of the expedition, ca. 270 km N of Xavantina, *Ramos et al. 140* (K, U); expedition base camp, *Harley & Souza 10082* (IAN, K, UB); *Harley et al. 11010* (K, UB); *Onishi et al. 473/1252* (K); *Philcox et al. 3082* (IAN, K, UB); *Castro 10553* (UB); Cabeceira do Guariba a 3 km sudeste do acampamento da expedição inglesa, *Sidney & Onishi 1252* (UB); Santa Terezinha, hillside forest along road to Santa Terezinha (MT 413), 7 km E of junction with BR 158, *Thomas et al. 4368* (MG, NY, SPF, U); BR 158, rodovia para Vila Rica, a 21 km S da cidade de Santa Terezinha, Serra da Cobrinha, *Cid Ferreira et al. 6433* (K, MG, SPF, U); São Félix do Araguaia, área da fazenda Jamaica, 28 km S do

cruzamento da estrada BR 158 e MT 242, *Cid Ferreira et al. 6486* (INPA, K, MG, SPF, U); Poconé, *Maciel 117* (INPA). **Mato Grosso do Sul:** Guia Lopes da Laguna, fazenda Trincheira, 9 km N of Guia Lopes da Laguna on the road to Nioaque (BR419), *Ratter et al. R7575V* (UB). **Goiás:** Alto Paraíso de Goiás, fazenda Antônio Sertão, a 20 km de Alto Paraíso, *Nóbrega 871* (HEPE, SPF); Anápolis, entre Araguaína e Colina, *Silva 57769* (K); Cavalcante, Rio do Carmo (rio Tocantins) km 4, *Pereira-Silva et al. 5741* (CEN, SPF); balsa do rio Tocantins (Serra Branca), rio Macacão, cerca de 800 m do rio Tocantins, *Pereira-Silva 5776* (CEN, SPF); Colinas do Sul, bacia da inundação da usina hidrelétrica Serra da Mesa, rio Tocantinzinho, *Santos et al. 38* (CEN, SPF); Estrada Colinas do Sul – Minaçu, à esquerda, 5 km após Vila Borba, *Santos 573* (CEN); Jussara, fazenda Santa Rita do Araguaia, *Silva 114* (IBGE); Minaçu, futuro reservatório do aproveitamento hidrelétrico Serra da Mesa, *Walter et al. 3571* (CEN, SPF); Niquelândia, Serra Negra, estrada paralela ao rio Bagagem, *Walter et al. 2491* (CEN, SPF); Fazenda Serra Negra, bacia da inundação da usina hidrelétrica Serra da Mesa, ao lado da linha de transmissão elétrica, *Walter et al. 1635* (CEN, SPF); Bacia da inundação da usina hidrelétrica Serra da Mesa, na Serra Negra, *Santos et al. 58* (CEN, SPF); A montante da balsa entre Niquelândia e Campinaçu, pelo rio a cerca de 5 km da balsa, mata ciliar do rio Maranhão (Tocantins), margem direita, *Walter 2837* (CEN, SPF); Santa Isabel, Ilha do Bananal, Parque Nacional do Araguaia, *Silva 369* (UB). **Tocantins:** Ilha do Bananal, Parque Nacional do Araguaia, ca 2 km of Macaúba, *Ratter et al. 4402* (K, UB); Novo Acordo, Usina Hidrelétrica Novo Acordo, Rio do Sono, *Haidar & Santos 1144* (IBGE); Paranã, entroncamento balsa do Coronel Valente – Rio Custódio, km 5, *Pereira-Silva & Moreira 11053* (CEN); balsa do Coronel – vila Rosário, km 1, ribeirão Funil, *Pereira-Silva et al 11984* (CEN); cerca de 500 m a leste da sede da fazenda do Coronel Valente, ribeirão Funil, *Pereira-Silva et al 12328* (CEN); São Salvador do Tocantins, córrego Obá, estrada para o córrego Mutum, *Pereira-Silva et al 10701* (CEN); canteiro de obras da Usina Hidrelétrica São Salvador, margem esquerda do rio Tocantins, *Pereira-Silva et al 10625* (CEN); fazenda Serrinha, *Pereira-Silva et al 12351* (CEN); Tocantínia, 16 km from Lajeado on the road to Tocantínia, Xerente Reserve, *Ratter et al. 7860* (K, UB).

**7. *Ephedranthus pisocarpus* R.E.Fr.**, Acta Horti Berg. 13(3): 108. f. 1a, b. 1941.  
Type: Brazil, Maranhão, São Luís, island of São Luiz, Feb–Mar 1939 (fr), *Froés 11592*  
(Holotype, S! 2 sheets; isotype K!). Figure 4C, Map 2.

Tree to shrub 4.5–22.0 m high; branchlets, petioles and buds densely covered by appressed to erect, crisped, yellowish hairs. Leaves papyraceous to chartaceous, glabrous above, except by the primary vein covered by hairs, densely covered by erect, crisped hairs below; petiole 2.0–5.0 mm long; lamina 5.0–11.0 × 2.5–5.0 cm, narrowly oblong to oblong, elliptic, narrowly obovate to obovate; apex acute to acuminate, rarely obtuse, base obtuse to slightly cordate, rarely acute; primary vein impressed, forming a sulcus near to the base above, raised below, secondary veins impressed above, raised below, 8–10 pairs of secondary veins, tertiary veins immersed above and slightly raised below, angles between primary and secondary veins 50–75°. Inflorescence axillary, 1–2-flowered, bracts ca. 5. Flower pedicel sessile, fruiting pedicel 7.0–10.0 mm, densely covered by yellowish hairs; sepals 3, 4.0–6.0 × 4.0–7.0 mm, ovate, densely covered by appressed, straight hairs; petals 6, in 2 whorls of 3 petals each, 15.0–20.0 × 6.0–10.0 mm, oblong, densely covered by appressed, straight hairs; bisexual flower with ca. 20 carpels; stamens not seen; male flower with ca. 100 stamens, ca. 1.0 mm long. Fruit 2–13 monocarps, 11.0–13.0 × 7.0–10.0 mm, ovoid, globose to ellipsoid, shortly apiculate, mature yellowish to dark red, stipe 5.0–7.0 mm long; fruit wall smooth, with longitudinal intrusions apparent in the dried monocarp. Seeds 10.0 mm long, 7.0 mm in diameter, ellipsoid.

*Habitat and distribution.* *Ephedranthus pisocarpus* occurs in the North Eastern Brazil, Maranhão, Piauí and Ceará, in *cerrado*, *caatinga* and forests. Flowers on August, fruits from January to March.

*Vernacular name:* conduru, cundurubrabo, cundurumarelo, envira-de-cocho (Portuguese, Brazil).

The mature monocarps of *Ephedranthus pisocarpus*, small and ellipsoid to globose, are similar to the unripe ones from *E. parviflorus*, and then the two species can

be confounded. However, they are always distinguishable by the leaf and fruit pedicel size, usually smaller in *E. pisocarpus*. Another species generally mistaken for *E. pisocarpus* is *Oxandra sessiliflora* R.E.Fr. Both are sympatric and share the small leaves with brown primary vein on the upper side. Nevertheless, *Oxandra sessiliflora* has narrowly elliptic leaves and almost sessile monocarps, whereas *E. pisocarpus* has oblong to obovate leaves and stipitate monocarps.

*Additional specimen examined:* BRAZIL. **Ceará:** Novo Oriente, Ibiapaba, Estrondo, Araújo 135 (EAC); São Benedito, Serra de Ibiapaba, mata à margem de uma vereda, Bezerra 409 (U). **Maranhão,** Buriticupu, Reserva Florestal da Companhia Vale do Rio Doce, Oliveira et al. 143 (MG, U); Barra do Corda, Canela Indian Village & Vicinity, ca. 50 km SW of Barra do Corda, Eiten 461 (UB); Carema, Cantagalo, Froés 25693 (IAN); Caxias, Ducke 792 (MG); Fortuna and Buriti-Bravo along the rio Itapecuru, the border between the two municipalities, 15 km SE of Fortuna, Schatz et al. 732 (K, MG); Santa Luzia, localidade de Bom Jesus, penetração à margem direita da BR 222 em frente ao km 100, a 12 km da rodovia, fazenda CODOMINAS, às margens do Rio dos Sonhos, Silva 1034 (IAN, INPA, MG, U); Cerrado near dirt road, approx. 40 km E of Barra do Corda toward Presidente Dutra, Daly et al. 557 (IAN, INPA, MG, NY, U); São Luís, Island of São Luis, Krukoff 11592 (U); Ilha de São Luís, Gottsberger 12-12283(U); São Luiz, Reserva Florestal do Sacavem, Muniz 2 (INPA); Fazenda Bacaba, Doctor Haroldo, 5 km S of MA 119 from entrance 3 km NW of Lago do Junco, Daly et al. 500 (IAN, INPA, K, MG, NY, U); Tuntum, Palmeirinhas, 46 km W of Presidente Dutra on the road to Barra do Corda, then 20 km S to Angelim, then 20 km E, Schatz 776 (MG). **Piauí:** Brasileira, baixa do Tinguizeiro, Parque Nacional de Sete Cidades, Alencar 468 (SPF, TEPB); Campo Maior, Alto do Comandante, Lopes et al. 52 (SPF, TEPB); Cocal, Chaves et al. 216 (SPF, TEPB); Piracuruca, Haidar et al. 43 (UB); São Raimundo Nonato, Boqueirão Grande, Emperaire 2311 (U); Uruçuí, fazenda Boa Vista, Miranda 4833 (INPA).

## Discussion and Conclusions

*Ruizodendron ovale* is sister to *Ephedranthus* (Figure 1). The same relationship was recovered by Chatrou et al. (2012) and Pirie et al. (2006). However, in a more recent phylogenetic reconstruction, *Ephedranthus* is in a polytomy that includes *Klarobelia*, *Oxandra*, *Pseudomalmea*, *Pseudephedranthus fragrans* and *Ruizodendron ovale* (Lopes et al in prep.).

The current subdivision of *Ephedranthus* as established by Fries (1934), *Ephedranthus* sect. *Ephedranthus*, including *E. guianensis*, *E. parviflorus* and *E. pisocarpus*, and *E. sect. Sphaeranthema*, including *E. amazonicus*, is not monophyletic (Figure 1), and its sections may not be recognized. One of the characters used to delimit these two sections is the shape of petals. *Ephedranthus* sect. *Ephedranthus* is characterized by oblong petals and *Ephedranthus* sect. *Sphaeranthema* by orbicular-concave petals (Fries 1934). Oblong petals is a synapomorphy of *E. parviflorus* and *E. pisocarpus*. However, *E. guianensis* are variable, ranging from widely ovate to oblong-ovate petals, better fitted as the character state 10:1, ovate, orbiculate to suborbiculate petals (Figure 1). In addition, *E. guianensis* is sister to *E. amazonicus*, sharing stipes with 20 to 40 mm long (Figure 1, character 21). Thus, sect. *Sphaeranthema* is nested in sect. *Ephedranthus*, rendering this last paraphyletic. For this reason, both sections are here synonymized under *Ephedranthus*.

Collections of *Ephedranthus boliviensis*, which occurs close to the border between Bolivia and Brazil, have been included in *E. parviflorus*, from the Central-Western Brazil, by Oliveira & Sales (1999).

The cladogenetic event separating the West species ((*E. colombianus*, *E. boliviensis*) ((*E. guianensis*, *E. amazonicus*) from the East species (*E. dimerus* (*E. parviflorus*, *E. pisocarpus*)) might be a consequence of forest contraction and expansion of open vegetation formations due to cooler and drier macroclimates (Brown Jr & Ab'Sáber 1979). These changes in climate were caused by glaciations during the Pleistocene, 2.6 Mya, and Holocene (Ab'Sáber 1979, 1980.). These ages are compatible with the age estimated to the crown group in Malmeeae, which includes *Ephedranthus*, 20 to 10 Mya (Pirie et al. 2006). The open vegetation corridor formed by *cerrado* and *caatinga* allowed the dispersion Southwest-Northeast observed in the distribution of *E. parviflorus* and *E. pisocarpus* (Oliveira & Ratter 1995).

## Excluded name

*Ephedranthus fragrans* R.E.Fr., Mem. New York Bot. Gard. 9: 327. 1957. Maguire & Wurdack 34954 (Holotype, S!) = *Pseudephedranthus fragrans* (R.E.Fr.) Aristeg.

## References

- Ab'Sáber, A.N. 1979. Os mecanismos da desintegração das paisagens tropicais no Pleistoceno: efeitos paleoclimáticos do período Würm-Wisconsin no Brasil. Inter-Facies escritos e documentos 4: 1–11.
- Ab'Sáber, A.N. 1980. Razões da retomada parcial da semi-aridês holocênica, por ocasião do optimum climaticum, primeiras idéias. Inter-Facies escritos e documentos 8: 1–7.
- Brown, K.S., Jr & Ab'Sáber, A.N. 1979. Ice-age forest refuges and evolution in the Neotropics: correlation of paleoclimatological, geomorphological and pedological data with modern biological endemism. Paleoclimas 5: 1–30.
- Chatrou, L.W. & Pirie, M.D. 2003. Two new species of Annonaceae from Bolivia. Revista de la Sociedad Boliviana de Botánica 4(1): 25–30.
- Chatrou, L.W., Pirie, M.D., Erkens, R.H.J., Couvreur, T.L.P., Neubig, K.M., Abbott, J.R., Mols, J.B., Maas, J.W., Saunders, R.M.K. & Chase, M.W. 2012. A new subfamilial and tribal classification of the pantropical flowering plant family Annonaceae informed by molecular phylogenetics. Botanical Journal of the Linnean Society 169(1): 5–40.
- Doyle, J.A. & Le Thomas, A. 1996. Phylogenetic analysis and character evolution in Annonaceae. Bulletin du Muséum National d'Histoire Naturelle, Section B, Adansonia 18: 279–334.
- Farris, J.S. 1972. Estimating phylogenetic trees from distance matrices. American Naturalist 106: 645–668.
- Fries, R.E. 1931. Revision der Arten einiger Annonaceen-Gattungen II. Acta Horti Bergiani 10(2): 129–341.
- Fries, R.E. 1934. Revision der Arten einiger Annonaceen-Gattungen III. Acta Horti Bergiani 12(1): 135–154.
- Fries, R.E. 1941. Nueu Amerikanische Annonaceen. Acta Horti Bergiani 13(3): 108–116.

- Goloboff, P., Farris, J. & Nixon, K. 2003. T.N.T.: Tre Analysis Using New Tecnology. Program and documentation available from the authors at [lillo.org.ar/phylogeny/tnt](http://lillo.org.ar/phylogeny/tnt)
- Koek-Noorman, J.; Westra, L.Y.T. & Maas, P.J.M. 1990. Studies in Annonaceae. XIII. The role of morphological characters in subsequent classifications of Annonaceae: A comparative survey. *Taxon* 39(1): 16–32.
- Lopes, J.C., Chatrou, L.W. & Mello-Silva, R. 2014. *Ephedranthus dimerus* (Annonaceae), a new species from the Atlantic Forest of Brazil, with a key to the species of *Ephedranthus*. *Brittonia* 66(1): 70–74.
- Lopes, J. C., Chatrou, L.W. & Mello-Silva, R. *in prep.* Phylogenomics and evolution of androdioecy in tribe Malmeeae (Annonaceae), a Neotropical woody group.
- Hickey, L.J. 1979. A revised classification of the architecture of dicotyledonous leaves. Pp. 25–39. In: Metcalfe, C.R. & Chalk, L. *Anatomy of the Dicotyledons. Volume I. Systematic anatomy of leaf and stem, with a brief history of the subject.* 2ed. Clarendon Press, Oxford.
- Nixon, K.C. & Carpenter, J.M. 1993. On outgroups. *Cladistics* 5: 275–289.
- Nixon, K.C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 415–428.
- Maas, P.J.M. & Westra, L.Y.T. 1984. Studies in Annonaceae. II A monograph of the genus *Anaxagorea* A.St.-Hil. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 105(1): 73–134.
- Maas, P.J.M. & Westra, L.Y.T. 1985. Studies in Annonaceae. II A monograph of the genus *Anaxagorea* A.St.-Hil. Part 2. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 105(2): 145–204.
- Maas, P.J.M., van Heusden, E.C.H., Koek-Noorman, J., van Setten, A.K. & Westra, L.Y.T. 1988. Studies in Annonaceae. IX. New species from the Neotropics and miscellaneous notes. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Series C: Biological and Medical Sciences* 91(3): 243–282.
- Maas, P.J.M. & Westra, L.Y.T. 2003. Revision of the neotropical genus *Pseudoxandra*. *Blumea* 48(2): 201–259.
- Maas, P.J.M.; Westra, L.Y.T. & Vermeer, M. 2007. Revision of the Neotropical genera *Bocageopsis*, *Onychopetalum*, and *Unonopsis* (Annonaceae). *Blumea* 52(3): 413–554.
- Moore, S. M. 1895. The Phanerogamic Botany of the Matto Grosso Expedition, 1891–

92. Transactions of the Linnean Society of London, 2nd series: Botany 4 (1894–1986): 265–516.
- Oliveira, J. & Sales, M.F. 1999. Estudos taxonômicos dos gêneros *Ephedranthus* e *Pseudephedranthus* Aristeg.: Annonaceae. Boletim do Museu Paraense “Emílio Goeldi”. Nova série, Botânica 15(2): 117–166.
- Oliveira-Filho, A.T. & Ratter, J.A. 1995. A study of the origin of central Brazilian forests by the analysis of plant species distribution patterns. Edinburgh Journal of Botany 52: 141–194.
- Pirie, M.D., Chatrou, L.W. Mols, J.B., Erkens, R.H.J. & Oosterhof, J. 2006. ‘Andean-centred’ genera in the short-branch clade of Annonaceae: Testing biogeographical hypotheses using phylogeny reconstruction and molecular dating. Journal of Biogeography 33: 31–46.
- Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S. & Le Thomas, A. 2006. Glossary of pollen and spore terminology. Review of Palaeobotany and Palynology 143: 1–81.
- QGIS. 2009. Development Team. QGIS Geographic Information System. Open Source Geospatial Foundation. URL <http://qgis.osgeo.org>.
- Goloboff, P. Farris, J. & Nixon, K. 2003. T.N.T.: Tre Analysis Using New Tecnology. Program and documentation available from the authors at [lillo.org.ar/phylogeny/tnt](http://lillo.org.ar/phylogeny/tnt).
- Reflora. 2016. Virtual Herbarium. Available at: <http://reflora.jbrj.gov.br/reflora/herbarioVirtual>. Accessed on 13/6/2016.
- Saunders, R.M.K. 2010. Floral evolution in the Annonaceae: hypotheses of homeotic mutations and functional convergence. Biological Reviews 85: 571–591.
- Thiers, B. [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/ih/>.
- van Heusden, E.C.H. 1992. Flowers of Annonaceae: morphology, classification, and evolution. Blumea 7 (Supplement): 1–218.
- van Setten, A.K. & Koek-Noorman, J. 1986. Studies in Annonaceae. VI. A leaf anatomical survey of genera of Annonaceae in the Neotropics. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 108(1): 17–50.
- van Setten, A.K. & Koek-Noorman, J. 1992. Studies in Annonaceae. XVII. Fruits and seeds of Annonaceae: morphology and its significance for classification and identification. Bibliotheca Botanica 142: 1–101.

Walker, J.W. 1971. Pollen morphology, phytogeography, and phylogeny of the Annonaceae. *Contributions from the Gray Herbarium of Harvard University* 202: 1–132.