UNIVERSIDADE DE SÃO PAULO
FFCLRP – DEPARTAMENTO DE BIOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA COMPARADA

Família dos girassóis no espaço e no tempo: taxonomia, filogenômica, biogeografia histórica e macroevolução de Barnadesioideae

Sunflower family in space and time: taxonomy, phylogenomics, historical biogeography and macroevolution of Barnadesioideae

Paola de Lima Ferreira

Tese apresentada à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da USP, como parte das exigências para obtenção do título de Doutor em Ciências, Área: Biologia Comparada

RIBEIRÃO PRETO - SP
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Paola de Lima Ferreira

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.

Magno amore in familiam Synantherearum captus. . . .
(Lessing, 1829)

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RESUMO

A subfamília Barnadesioideae (Compositae) compreende dez gêneros e 84 espécies endêmicos da América do Sul, distribuídos da Venezuela até a Argentina, sendo principalmente encontrados em áreas xeromórficas ao longo dos Andes e da Patagônia. O interesse em Barnadesioideae vem aumentando consideravelmente desde a descoberta que constituem o grupo irmão para todo o restante das Compostas. Hipóteses filogenéticas robustas associadas a estudos biogeográficos, morfológicos e macroevolutivos podem elucidar questões relativas à origem e diversificação da família como um todo. Estudos filogenéticos prévios baseados em dados morfológicos, moleculares ou combinadas têm sido propostas para Barnadesioideae nos últimos 20 anos, porém seus resultados são incongruentes e não possuem uma extensa amostragem de marcadores moleculares ou taxonômica. Por outro lado, hipóteses biogeográficas para o grupo nunca foram propostas utilizandos os fósseis descritos para o grupo como método de calibração e estudos macroevolutivos nunca foram investigados. Neste trabalho, propusemos uma hipótese filogenética baseada em dados de sequenciamento de Nova Geração que incluem quase mil marcadores nucleares e genomas plastidiais quase que completos para todas as espécies. Nossa hipótese filogenética compreende 9 dos 10 gêneros e cerca de 60% das espécies atualmente circunscritas para a subfamília e resolve as relações com alto suporte nos ramos, esclarecendo seus clados contenciosos, embora as relações no clado *Chuquiraga*, *Doniophyton* e *Duseniella* ainda sejam duvidosas devido ao baixo suporte nos ramos. A árvore filogenética inferida neste trabalho foi utilizada pela primeira vez para inferir os tempos de divergência das linhagens utilizando os fósseis descritos para a subfamília como método de calibração. A reconstrução biogeográfica propõe que Barnadesioideae originou-se no Eoceno há c. 49 Milhões de anos e a diversificação do ancestral comum mais recente dos gêneros teria iniciado há cerca de 20 milhões de anos durante o Mioceno. Estudos de diversificação propõe que as taxas de extinção e especiação foram homogêneas e constantes através do tempo, não sendo detectada nenhuma mudança na filogenia. Além dos estudos sistemáticos, biogeográficos e macroevolutivos, também foi realizada uma sinopse genérica para Barnadesioideae, atualizando a circunscrição dos gêneros frente as novas mudanças nomenclaturais desenvolvidas ao longo dos anos de estudos na subfamília. A sinopse inclui uma chave de identificação, descrições morfológicas atualizadas e expandidas, mapas de distribuição geográfica, fotografias de todos os gêneros bem como da diversidade morfológica dos apêndices apicais e basais das anteras. Paralelamente aos objetivos e resultados principais apresentados nesta tese, importantes colaborações foram estabelecidas resultando em publicações de revisão e descrição de compostos químicos que certamente contribuem o conhecimento evolutivo a cerca do grupo além de auxiliar em sua delimitação.

Palavras-chave: Barnadesieae, Biogeografia, Compositae, Filogenia, Macroevolução, Taxonomia.

ABSTRACT

The subfamily Barnadesioideae (Compositae) comprises ten genera and 84 species endemics to South America, distributed from Venezuela to Argentina, being mainly found in xeromorphic areas along the Andes and Patagonia. The interest in Barnadesioideae has considerably increased since they were recovered as sister to the rest of Compositae. Robust phylogenetic hypotheses allied to biogeographic, morphological and macroevolutionary studies can provide insights into the origin and diversification of the family as a whole. Previous phylogenetic studies based on morphological, molecular or combined datasets have been proposed for Barnadesioideae in the last 20 years, but their results were incongruent and did not have extensive molecular markers or taxonomic sampling. On the other hand, biogeographic hypothesis for the group has never been proposed using the fossils described for the group as calibration points and macroevolutionary studies have never been investigated. In this work, we proposed a phylogenetic hypothesis based on Next-Generation sequencing data that includes nearly 1,000 nuclear markers and almost complete plastid genomes for all those species. Our phylogenetic hypothesis comprises 9 of the 10 genera and about 60% of the species, and resolves the relationships with high support in the branches, clarifying their contentious clades, although the relationships in the *Chuquiraga*, *Doniophyton* and *Duseniella* clade remain unresolved due to low support in the branches. The phylogenetic tree inferred here was the first study to infer the divergence times using the fossils described for the subfamily as a calibration method. The biogeographic reconstruction proposes that Barnadesioideae originated in the Eocene at 49 million years ago, and the diversification of the most recent common ancestor of the genera would have started about 20 million years ago during the Miocene. Diversification studies propose that extinction and speciation rates were homogeneous and constant though time, and any shift was detected in the phylogeny. In addition to systematics, biogeographic and macroevolutionary studies, a generic synopsis for Barnadesioideae was also performed, updating the genera circumscription in the light of the new nomenclatural changes developed during the years studying the subfamily. The synopsis includes a key, updated and expanded morphological descriptions, geographical distribution maps, photographs of all genera as well as the morphological diversity of the apical and basal anther appendages. Together with the objectives and main results presented here, important collaborations were established resulting in a review and description of chemical compounds that were already published and certainly contribute to the evolutionary insights into the group and support its delimitation.

Keywords: Barnadesieae, Biogeography, Compositae, Phylogeny, Macroevolution, Taxonomy.

Introduction

"Botany – the science of the vegetable kingdom, is one of the most attractive, most useful, and most extensive departments of human knowledge. It is, above every other, the science of beauty"
(Joseph Paxton, 1838)

Barnadesia pycnophylla
© Gari Ccana-Ccapatinta

Compositae (or Asteraceae) is one of the largest angiosperm families, comprising 1600-1700 genera and 25,000 - 33,000 species, distributed on all continents, except in Antartica (Panero and Funk, 2008; Funk et al., 2009; Mandel et al., 2017). The family can be easily recognized by its flowers arranged on a receptacle in centripetal heads and surrounded by bracts, by its anthers laterally connate that enclose the style and the stigma with a mechanism of secondary pollen presentation, by its bicarpellate inferior ovary with a basal and erect ovule, and by the presence of cypsela usually with a pappus (Bremer, 1994, Funk et al., 2009, Jeffrey, 2007).

The monophyly of Compositae has never been in question (Jasen & Palmer, 1987, Jansen et al., 1991a, Panero and Funk, 2002, Funk et al., 2005, Bonifacino et al., 2009, Panero, 2016). Since the 17th centuries, renowned botanists have been proposing infra-familiar classifications in order to understand its great diversity in morphology, chemistry compounds, habits, life forms, and others allied to its wide distribution (Bonifacino et al., 2009). However, the problem is the family is too large and diverse with many evolutionary parallelisms in numerous characters which turns difficult to infer the relationships based on morphological data (Stuessy et al., 1996).

In the last three decades, synantherologists (the botanists specialized in Compositae) have spent most of their efforts to elucidate the evolutionary history of Compositae (Bremer 1987, Jasen et al., 1991a, b, Kim and Jasen 1995, Panero and Funk, 2002;2008, Funk et al., 2005; 2009, Panero et al., 2014, Panero and Crozier, 2016). Currently, Compositae is classified in 13 subfamilies and 44 tribes (Panero et al., 2014). Within the 13 subfamilies recognized in Compositae, Barnadesioideae is an interesting branch and has attracted much attention due to its phylogenetic position as sister group to the rest of the family (Jasen and Palmer, 1987, Bremer, 1987, Jasen et al., 1991a, b, Bremer and Jasen, 1992, Bremer, 1994, Jasen and Kim, 1996, Funk et al. 2005, Funk et al., 2009, Panero and Crozier, 2016). Therefore, evolutionary studies including a well-supported phylogeny with an extensive taxonomic sampling allied to other studies such as historical biogeography and morphology can provide insights into the early evolution of the family.

Barnadesioideae

Barnadesioideae comprises 10 genera and 84 species (Ferreira et al., 2019; Fig. 1) endemic to South America, from northern Venezuela to southern Argentina, which most of the species are found in dry areas along to the Andes and Patagonia (Stuessy et al., 1996, Urtubey and Stuessy 2001, Stuessy et al. 2009; Fig. 2). Seven of the 10 genera are small in species number and represented by up to 3 species: *Duseniella* K. Schum (Fig. 1C), *Schlechtendalia Less.* (Fig.1F), *Huarpea* Cabrera (Fig. 1H) are monotypic and distributed in narrow areas in Argentina, Uruguay and Brazil (Fig. 2F). *Archidasyphyllum* (Cabrera) P.L. Ferreira, Saavedra & Groppo (Ferreira et al., 2019; Fig. E), *Doniophyton* Wedd. (Katinas and Stuessy, 1997; Fig. 1D), and *Fulcaldea* Poir. (Roque and Funk, 2011; Fig. 1J) consisting of two species distributed Chile and Argentina (Fig. 2C, E), and a remarkable disjunct distribution of *Fulcaldea* in Southern Ecuador, Northern Peru and Northeastern Brazil (Fig. 2D). *Arnaldoa* Cabrera (Stuessy and Sagástegui, 1993, Ulloa et al., 2002; Fig. 1G) comprises three species distributed in Ecuador and Peru (Fig. 2D). The three other genera comprise more than

10 species: *Barnadesia* Mutis ex. L. F. (Urtubey et al., 1999, Hind, 2001; Fig. 1I) comprises 19 species circumscribed into two subgenera distributed along to the Andes, from Colombia to northern Argentina, and one species (*B. caryophylla*) is found in Brazil. *Chuquiraga* Juss. comprises 22 species (Ezcurra, 1985, Harling, 1991, Sagástegui and Sánchez, 1991, Granda, 1997; Fig. 1B) circumscribed into two sections and two series distributed along to the Andes and Patagonia from Colombia to Argentina (Fig. 2B). *Dasyphyllum* Kunth is the largest genus of Barnadesioideae comprising 31 species (Saavedra, 2011, Saavedra et al., 2014, Saavedra et al., 2018, Ferreira et al. 2019; Fig. 1A) distributed from Venezuela to Northwestern Argentina, but absent in Amazon region (Cabrera, 1959; Saavedra, 2011; Saavedra et al., 2014, Saavedra et al., 2018, Ferreira et al., 2019)

Although Barnadesioideae comprises less than 1% of the species circumscribed in Compositae, it has a great morphology diversity ranging from perennial or annual herbs to large trees reaching 30 meters high. The leaves can be opposite, alternate or fasciculate leaves. The plants have axillary spines that can be found sometimes solitary, in pairs or fasciculate. The involucre ranges from cylindrical to widely campanulate. The capitula can be homogamous or heterogamous, discoid, radiate or disciform, sessile or pedunculate. The number of flowers is highly variable ranging from 1-135, actinomorphic or zygomorphic, white, pink, yellow, purple, red, orange corolla (Stuessy et al., 2006). The number of stamens ranges from 3-5, and the filaments can be free or rarely fused (only in *Barnadesia*), inserted at base to throat. The anthers can be ecaudate to tailed, calcarate or ecalcarate and the apical appendages can be acute, apiculate, emarginated. The pappus is commonly plumose, but can be also barbellate, scaly or rarely absent (Stuessy et al., 2009). Despite the great morphology diversity in Barnadesioideae, it is clearly distinguished from the other Compositae by its axillary spines, and by its "barnadesioid trichomes" a pubescence of unbranched three-celled hairs on the corollas, cypselas and pappus (Cabrera, 1959, Urtubey, 1999, Erbar and Leins, 2000). Moreover, another diagnostic feature is the lack of two DNA chloroplast inversions located in the Large single copy region (LSC) that it is found in all other Compositae (Jansen and Palmer, 1987, Kim et al., 2005).

Barnadesioideae is recovered as a monophyletic group in all phylogenetic hypotheses based on morphology, molecular data and combined of them (Jansen et al., 1991a,b, Jansen and Kim 1996, Gustafsson et al., 2001, Funk et al., 2005, Panero and Funk, 2008, Funk et al., 2009, Gruenstaeudl et al., 2009, Panero et al., 2014, Ferreira et al. 2019, Mandel et al., 2017). However, the relationships within the subfamily are controversial and remain unclear, specially regarding the genera relationships and the monophyly of genera, infrageneric classification and even species (Fig. 3.; Bremer, 1994, Stuessy et al., 1996, Gustafsson et al., 2001, Urtubey and Stuessy, 2001, Gruenstaeudl et al., 2009, Ferreira et al. 2019, Padin et al., 2015; Fig. 3).

Previous phylogenetic hypotheses based on molecular data (Gustafsson et al., 2001, Gruenstaeudl et al., 2009) did not comprise an extensive taxonomic sampling (35% of the total species; Gruenstaeudl et al., 2009) or were inferred using few markers (*trn*L intron and ITS, Gustafsson et al., 2001). On the other hand, most of the phylogenetic hypotheses based on morphological data only included genera or infrageneric classification as taxa (Bremer et al., 1994, Stuessy et al., 1996). Up to date, the most complete species-level phylogenetic hypothesis for Barnadesioideae is based on two molecular markers (*trn*L intron and ITS) and included 54 of

the 84 species (Gustaffson et al., 2001). However, many branches of the tree were poorly understood due to the lack of resolution and support.

Historical biogeographical hypotheses in Barnadesioideae are scarce (Ezcurra, 2002, Stuessy et al., 1996, Gruenstauedl et al., 2009). According to Stuessy et al. (1996), during the Miocene (25-5 mya) the Andean orogeny created new ecological and habitat opportunities for the common ancestor of Barnadesioideae. Moreover, the authors described how the "*proto*-genera" arose based on the climatic and vegetational data, and the history of each genus through the time. Despite the effort of the authors to explain the historical biogeography of Barnadesiodieae, the authors did not provide any method to refuse/corroborate their historical biogeographic hypothesis.

A comprehensive phylogenetic hypothesis for Barnadesioideae including a well-supported tree allied to an extensive taxonomic sampling is necessary to clarify the relationships within the subfamily. Moreover, a comprehensive phylogeny allied to historical biogeographical studies such as divergence times using the three fossils described for the subfamily (Palazzesi et al., 2009) as calibration points, and estimate the rates of speciation, extinction and net species diversification are necessary to understand the evolutionary history of Barnadesioideae, as well as to shed light on the early evolution of Compositae.

Figure 1. Genera of Barnadesioideae. A) *Dasyphyllum*; B) *Chuquiraga*; C) *Duseniella*; D) *Doniophyton*; E) *Archidasyphyllum*, F) *Schlechtendalia*; G) *Arnaldoa*; H) *Huarpea*; I) *Barnadesia*; and J) *Fulcaldea*.

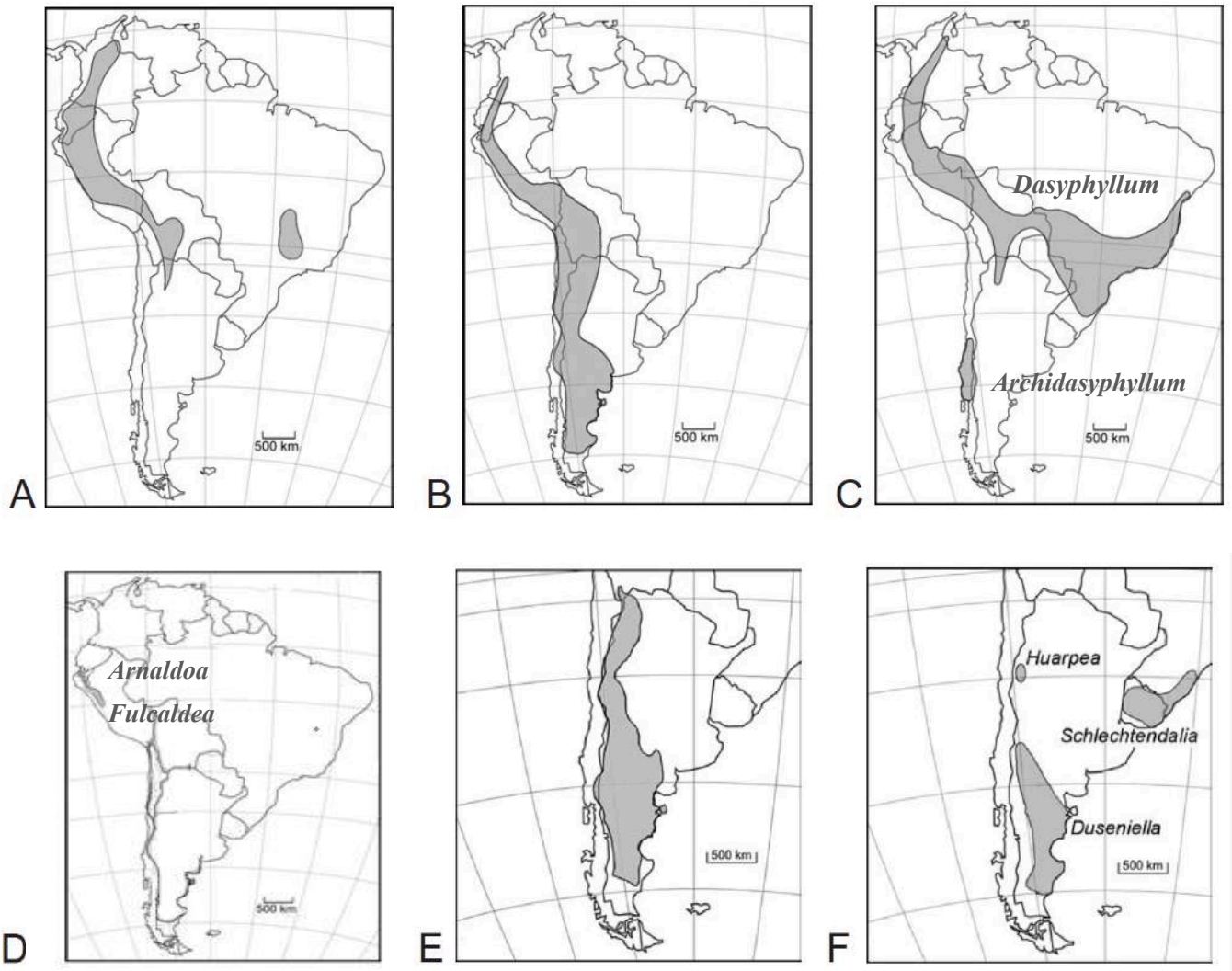

Figure 2. Distribution of Barnadesioideae genera extracted and adapted from Stuessy et al. (2009). A) *Barnadesia*, B) *Chuquiraga*, C) *Dasyphyllum* and *Archidasyphyllum*, D) *Arnaldoa* and *Fulcaldea*, E) *Doniophyton*, F) *Duseniella*, *Huarpea*, and *Schlechtendalia*.

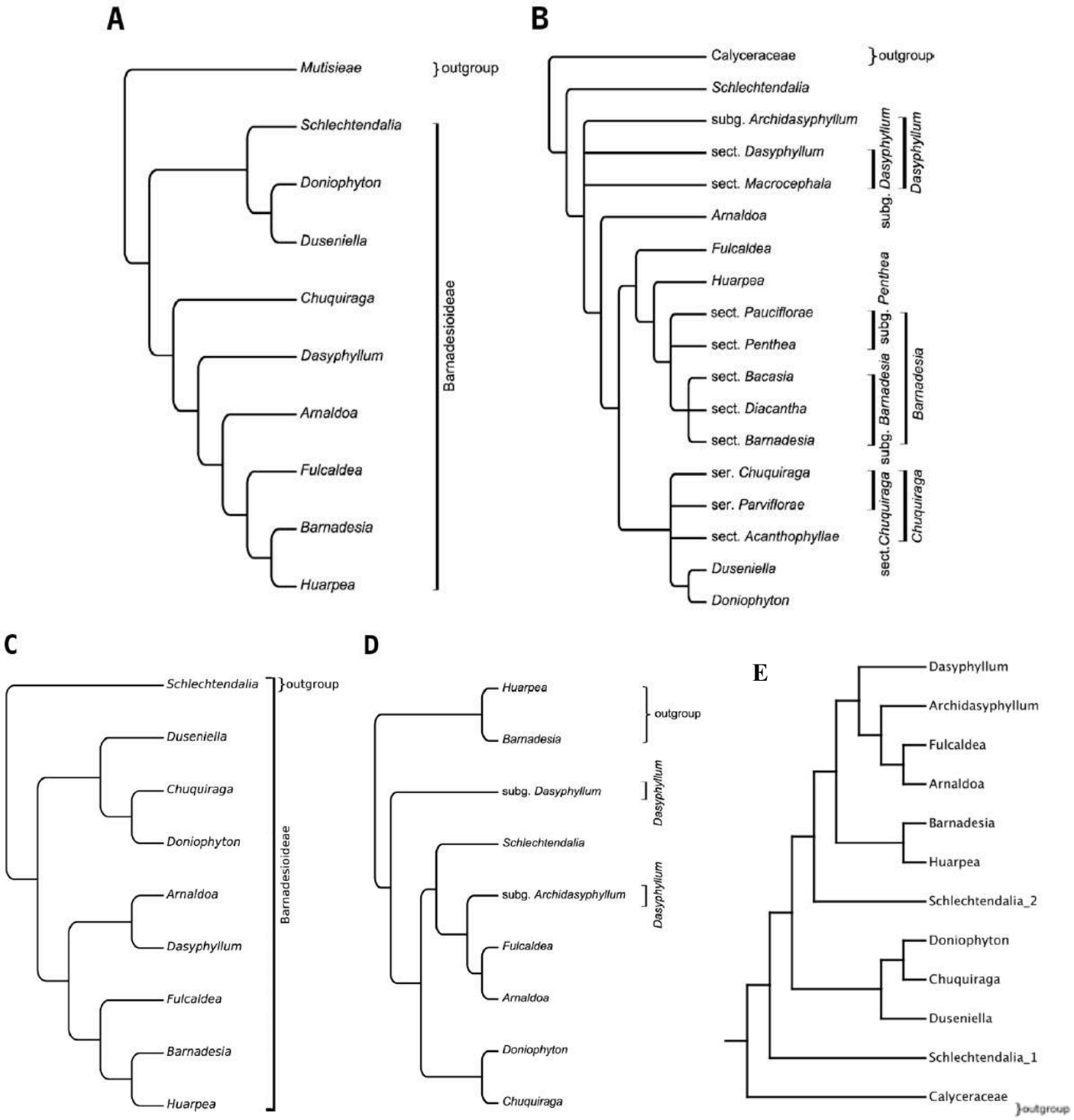

Figure 3. Previous phylogenetic relationships among genera and/or infrageneric classification of Barnadesioideae. A) Bremer (1994); B) Stuessy et al. (1996); C) Urtubey and Stuessy (2001); D) Gustafsson et al. (2001); E) Gruenstaeudl et al. (2009).

Goals

The goals of the present thesis, therefore, are:

- propose a phylogenetic hypothesis of Barnadesioideae based on molecular data, including an extensive taxonomic sampling, investigating the relationships within the subfamily and test the monophyly of all genera and the infrageneric classification of *Barnadesia* (Urtubey, 1999, Hind, 2001) and *Chuquiraga* (Ezcurra, 1985, Harling, 1991, Sagástegui and Sánchez, 1991, Granda, 1997);
- propose historical biogeographic and macroevolutionary studies to investigate temporally and spacially perspectives on the evolution of Barnadesioideae, combining a phylogenetic framework allied to divergence times using fossil as calibration points, ancestral areas reconstruction, and diversification analyses;
- present a generic synopsis of Barnadesioideae, providing a morphological description of the subfamily, a generic taxonomic key, as well photos, distribution and habitat, maps, and taxonomic notes for each genus.

Structure of the thesis

The present thesis is divided into Introduction, three chapters, conclusion and three appendices.

- **Chapter 1** presents a phylogenetic hypothesis, reevaluating the relationships within Barnadesioideae, and testing the monophyly of all genera and infrageneric classification based on robust phylogenomics datasets (nuclear and plastidial). This article is planned to be submitted to the *Systematic Biology* journal and is co-authored by Romina Batista, Tobias Adermann, Milton Groppo, Christine Bacon & Alexandre Antonelli.

- **Chapter 2** presents a historical biogeographic study using a phylogenetic tree of *Chapter 1* as a framework to investigate the divergence times calibrating it using fossil data. Additionally, macroevolutionary studies were proposed to understand the patterns that shape the diversification of Barnadesioideae. This article is planned to be submitted to the *Journal of Biogeography* journal and is co-authored by Romina Batista, Christine Bacon, Milton Groppo & Alexandre Antonelli.

- **Chapter 3** presents a generic synopsis of Barnadesioideae updating the genera circumscription based on the taxonomic and systematic studies proposed here. It also comprises a morphological description and pictures of each genus, notes about geographical distribution and taxonomy. This chapter is planned to be submitted to the *Phytotaxa* and is co-authored by Alexandre Antonelli and Milton Groppo.

- **Appendix 1** comprises a published paper entitled “Phylogeny and circumscription of *Dasyphyllum* (Asteraceae: Barnadesioideae) based on molecular data with the recognition of a new genus, *Archidasyphyllum*”. This paper comprises an important piece of the evolutionary puzzle of Barnadesioideae

including a new circumscription for the largest genus os the subfamily based on molecular, morphological and distributional data. This appendix was published in *PeerJ*.

- **Appendix 2** comprises a published paper entitled "Chemistry and medicinal uses of the subfamily Barnadesioideae (Asteraceae)". Although this paper is not directly related to systematics and historical biogeography, we decided to include this paper as an appendix because it summarizes the current knowledge of chemistry and also discuss the absence of sesquiterpene lactones in Barnadesioideae, that it is another diagnostic feature that supports the separated position of the subfamily within Compositae. Moreover, the article describes the medicinal uses of Barnadesioideae, which constitutes an important part of traditional medicine with numerous medical indications in several South American countries. This appendix was published in *Phytochemistry Reviews*.

- **Appendix 3** comprises a published paper entitled "Caffeic Acid Ester Derivatives and Flavonoids of genus *Arnaldoa* (Asteraceae, Barnadesioideae)". This paper describes the phytochemical composition in *Arnaldoa*, and discuss the chemotaxonomic implication of caffeic acid ester derivates and flavonoids glycosides, as well as the absence of lactone sesquiterpenes which are important molecular markers diagnostics in Asteraceae. This appendix was published in *Biochemical Systematics and Ecology*.

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Chapter 01

How much data do we need to build a reliable phylogeny?

An example in Barnadesioideae (Compositae)

Dasyphyllum sprengelianum
© Paola Ferreira

"*The affinities of all the beings of the same class have sometimes been represented by a great tree... As buds give rise by growth to fresh buds, and these if vigorous, branch out and overtop on all sides many a feebler branch, so by generation I believe it has been with the great Tree of Life, which fills with its dead and broken branches the crust of the earth, and covers the surface with its ever branching and beautiful ramification.*"

(Charles Darwin, 1859)

Abstract

Target enrichment has emerged as a powerful sequencing tool by gathering hundreds to thousand genomic regions of interest in a cost- and time-efficient approach. Towards reconstructing the tree of life, evolutionary biologists have to decide if all the genomic regions recovered should be included or which regions should be excluded based on a criterion. Filtering regions comprising large amounts of missing data is a common practice in phylogenomics, nonetheless, empirical studies assessing its impact on plant phylogenies are scarce. Here, we investigated the impact of missing data by excluding loci with large among of missing nucleotides and also shed light into the generic relationships in a contentious group of sunflowers (Compositae), the subfamily Barnadesioideae. We generated molecular data for 49 Barnadesioideae taxa plus 12 outgroups using the probes designed for the family Compositae. We accessed the impact of missing data by allowing three matrices of taxonomic completeness under concatenation and coalescent approaches. Our phylogenies provided congruent and well-supported relationships considering different matrices of taxonomic completeness and inference methods. Our phylogenetic analyses provided further evidence that big data science can resolve with strong support previously contentious generic relationships such as the position of *Schlechtendalia.* Conversely, conflicting generic relationships and species remain for the clade *Chuquiraga* and *Doniophyton*. Further, our results provide further evidence that the exclusion of molecular regions can be problematic and affect branch support. Therefore, researchers should explore their data under different threshold of taxonomic completeness in order to investigate the impact of missing data in their topologies and branch support.

Keywords: Asteraceae, Evolution, Missing data, Next-Generation sequencing, Sequence Capture.

1. Introduction

Understanding how organisms are related is one of the most important tools to explain Earth's biodiversity and test hypotheses about its origin and evolution. In the last decades, systematics has improved radically through the use of molecular data, new mathematical models and advances in computer science letting us closer to the dream to reconstruct the tree of life (Liu et al., 2015, Hug et al., 2016, Bravo et al., 2019). Particularly in the field of molecular data, one of the biggest advances has been the advent of various new sequencing technologies (Schuster, 2008; Shendure & Ji, 2008; Metzker, 2010; Harrison & Kidner, 2011; Goodwin et al., 2016; Mardis, 2016; Slatko et al., 2018).

High-throughput DNA sequencing entails various powerful, less expensive and fast approaches, allowing the generation of data from hundreds to thousands of loci to complete genomes derived from both fresh or historical samples (Harrison & Kidner, 2011, Godden et al., 2012, McCormack et al., 2013). Numerous applications have been used for evolutionary studies such as restriction-digest (Andrews et al., 2016, Nazareno et al., 2017, Tripp et al., 2017), transcriptome (Wang et al., 2017, Roodt et al., 2017, Valderrama et al., 2018), and target enrichment (Mandel et al., 2014, Mandel et al., 2015, Carlsen et al., 2018, Johnson et al., 2018) showing that NGS contains enough genetic information to clarify several contentious clades in animals (Hackett et al., 2008, Quattrini et al., 2018) and plants (Moore et al., 2007, Parks et al., 2009, Ruhfel et al., 2014, Zeng et al., 2014, Gitzendanner et al., 2018).

Despite the remarkable advantages of high-throughput sequencing, researchers also face numerous computational (*e.g.* assembly and mapping of short reads, Pop & Salzberg, 2008, Bao et al., 2011, Treangen & Salzberg, 2011) and biological challenges (*e.g.* horizontal gene transfer, incomplete lineage sorting, gene duplication; Maddison, 1997, Cronn et al., 2012, Liu et al., 2015). After overcoming certain challenges, users also need to decide which data recovered can and should be included, bringing the field back to a persistent question in evolutionary biology - what is the impact of incomplete sequences and taxonomic sampling in phylogenetic analysis (Wiens, 2003, 2006). Particularly, standard procedures to accommodate missing data are not well-established, and researchers either test use all the data recovered (Mandel et al., 2014) or filter data based on set criteria (Gernandt et al., 2018). To filter data, the most commonly used approach is to exclude loci for species under a sampling threshold to avoid large amount of missing data and systematic bias (Faircloth et al., 2015, Bryson et al., 2016, Alfaro et al., 2018).

Although the exclusion of loci for species with high amounts of missing data is common practice, empirical studies accessing the impact of missing data has been investigating using ultraconserved elements in birds (Hosner et al., 2015), lizards (Streicher et al., 2015), and reptiles (Zheng & Weins, 2016). However, no such investigation has been made in plants placing us in a new avenue for investigations.

The land plants (Embriophyte) is a diverse clade estimated by 391,000 species, and its representatives are an essential component of terrestrial ecosystem and biodiversity (Kersey, 2019, Knapp, 2019). Land plants (or simply “plants”) have different structure, function, and plastic genomes thus leading to different evolutionary histories compared to animals (Kejnovsky et al., 2009, Murat et al., 2012, Kersey, 2019).

Here, we assess the impact of missing data using empirical datasets for a morphologically diverse and highly contentious clade of the sunflowers family (Compositae), the subfamily Barnadesioideae (Fig. 1). This subfamily is endemic to South America and comprises ten genera and 84 species distributed from Venezuela to Argentina in dry areas along the Andes (Stuessy et al., 2009, Ferreira et al., 2019), and can be distinguished from the rest the family by the axillary spines, straight or curved, rarely solitary, in pairs or fasciculated, and by the “barnadesioid trichomes” a pubescence of unbranched three-celled hairs on the corollas, cypselas and pappus (Cabrera, 1959, Urtubey, 1999, Erbar and Leins, 2000).

Here, we assess the impact of missing data using empirical datasets for a morphologically diverse and highly contentious clade of the sunflowers family (Compositae), the subfamily Barnadesioideae (Fig. 1). This subfamily is endemic to South America and comprises ten genera and 84 species distributed from Venezuela to Argentina in dry areas along the Andes (Stuessy et al., 2009, Ferreira et al., 2019). The representatives of this subfamily can be distinguished from the rest the family by the axillary spines, straight or curved, rarely solitary, in pairs or fasciculated, and by the “barnadesioid trichomes” a pubescence of unbranched three-celled hairs on the corollas, cypselas and pappus (Cabrera, 1959, Urtubey, 1999, Erbar and Leins, 2000). Understanding the relationships within Barnadesioideae is challenging due to conflicting inferences of generic relationships and the monophyly of genera as well as species (Hasen 1991, Bremer, 1994, Stuessy et al., 1996, Gustafsson et al., 2001, Urtubey & Stuessy, 2001, Gruenstaeudl et al., 2009, Padin et al., 2015, Ferreira et al., 2019). Previous molecular studies resolved Barnadesioideae as the sister group to the rest of the family (Bremer, 1987, Jansen et al., 1992), and a well-supported phylogeny allied to existing, robust morphological and biogeographical data are fundamental to understanding the evolution of the family as a whole.

Accordingly, we gathered a representative sample of Barnadesioideae for this study, together with several species of Compositae and Calyceraceae based on target enrichment data using baits designed explicitly for the family (Mandel et al., 2014). The two major goals were to: (1) explore the impact of missing data by employing matrices allowing different number of taxa and amount of missing data using coalescent-methods and concatenation approaches, and (2) clarify the inter- and intrageneric relationships within the subfamily and test the monophyly of all genera.

2. Materials and Methods

2.1 Taxon sampling

We selected 49 Barnadesioideae taxa representing nine genera, 44 species, and five subspecies to propose a phylogenetic hypothesis. The only genus missing is *Duseniella*, which we could not include due to our old samples (<1980's) with insufficient amount of DNA template for this study. Given the disjunct distribution and morphological variability of *Chuquiraga jussieui,* individuals from two populations were sampled. Within the three largest genera, taxa were selected to span their infrageneric classification (Ezcurra, 1985, Urtubey, 1999) and/or recover the major internal clades based on previous molecular phylogenies (Gustafsson et al., 2001, Gruenstaeudl et al., 2009, Padin et al., 2015, Ferreira et al., 2019). Additionally, we included 12 outgroups: 11 from Asteraceae and one Calyceraceae species, the sister group of Compositae, all available from NCBI (Mandel et al., 2014). Sample information and its accession numbers can be found in Supplementary Table S1.

2.2 DNA extraction, library preparation and sequencing

Total genomic DNA was extracted from 3-5 mg of silica gel dried or herbarium leaves using the Qiagen DNeasy Plant Mini Kit (Qiagen, California, U.S.A.) according to the manufacturer's specifications. The amount of DNA template was verified using Qubit Fluorometer 3.0 Fluorometer (High sensitivy kit; Life Technologies). The DNA was mechanically sheared to an average size of 300 bp. Illumina libraries were constructed by repairing the ends of the sheared fragments followed by the ligation of an adenine residue to the 3'-end of the blunt-end fragments. Barcoded adapters suited for Illumina Sequencing platform were ligated to the libraries then PCR-amplified for using standard cycling protocols (e.g. Mamanova et al., 2010). Samples were pooled 16 barcoded libraries with equimolar amounts to a total of 500 ng for hybridization.

Target enrichment was performed using the "COS Compositae/Asteraceae 1Kv1" baits set (Mandel et al., 2014) consisting of 9,678 baits targeting 1061 orthologous genes following the MYbaits Version 2.3.1 user manual (MYcroarray, Ann Arbor, Michigan, USA). After enrichment, samples were re-amplified for additional 6-12 cycles and sequenced using an Illumina HiSeq 3000 with paired-end 100 bp reads.

2.3 Bioinformatic analyses

2.3.1 Nuclear conserved ortology loci

A nuclear conserved orthologue loci set (herafter COS) was analyzed using the "Sequence Capture Processor workflow" (SECAPR, available at https://github.com/AntonelliLab/seqcap_processor/blob/master/documentation.ipynb; Andermann et al., 2018). A workflow for the bioinformatic analyses is illustrated in Fig. 2

Raw data were trimmed and quality-filtered using Trimmomatic 0.35 (Bolger et al., 2014) with the parameters: simpleClipThreshold 5, palindromeClipThreshold 20, seedMismathes 5 and cropToLength 93. Cleaned reads were quality-checked using a plotting function that summarizes the FastqC results as implemented in the SECAPR (Andermann et al., 2018). Cleaned reads for each sample were *de novo* assembled into contigs using ABySS (Simpson et al., 2009) testing the k-mer size lengths: 25, 35, and 50 in order to keep the highest number of contig matches and discarding those contigs shorter than 90 bp avoiding ambiguous reconstructions (Koren et al., 2017). Contigs were mapped against to the "COS Compositae/Asteraceae 1Kv1" baits (Mandel et al., 2014) in order to identify those sequences that were enriched during the library preparations using the minimum coverage and identity values of 70 in LASTZ (Harris, 2007).

We performed multiple sequence alignments for each target locus identified in the mapping using MAFFT version 7 (Katoh & Standley, 2013). We used the additional parameters: "--no trim", in order to keep full contig sequences and to avoid cutting the alignments at the ends, and "--no ambiguous", allowing the inclusion of Ns into the alignment.

2.3.2 Phylogenetic analyses

In order to evaluate the impact of missing data on the phylogenetic reconstruction, we constructed three sets of taxonomic completeness using "phyluce_align_get_only_loci_with_min_taxa.py" in Phyluce (Faircloth, 2016). The alignments generated were used in downstream phylogenetic analyses using concatenation and coalescent approaches considering three thresholds:

1) allowing all loci recovered for four or more taxa (COS 100%);

2) greater than 50% of the taxa for each locus (COS 50%);

3) greater than 75% of the taxa for each locus (COS 25%).

Summary statistics of each dataset was evaluated using the "phyluce_align_get_align_summary_data.py" in Phyluce. Sequence alignments for each taxonomic completeness and the phylogenetic trees are available from the Dryad Digital Repository (Ferreira et al., in prep.).

Concatenation analyses were performed under the Maximum-likelihood estimation (ML) using RAxML version 8.2.9 (Stamatakis, 2014) by conducting 20 searches with the GTR model with gamma rate substitution model and evaluated branch support using the autoMRE function implemented in RAxML (Pattengale et al., 2010).

Coalescent analyses were performed using summary and site-based methods (Warnow, 2017). Summary method was estimated in ASTRAL-III 5.6.3 (Zhang et al., 2018) using unrooted gene trees estimated by ML searches conducted in RAxML (Stamatakis, 2014). Branch support was evaluated using local posterior probabilities, as suggested to be more accurate measure of support than multi-

bootstrapping (Sayyari & Mirarab, 2016). Site-based method was estimated in SVDquartets (Chifman & Kubatko, 2014) with exhaustive sampling all the possible quartets and branch support was accessed using 1,000 nonparametric bootstrap replicates.

2.3.3 Chloroplast dataset

Although the libraries were enriched for COS probe set, we were able to use the off-target reads to capture chloroplast sequences. Cleaned reads (see 2.3) were mapped against to *Centaurea diffusa* (NC_024286; Turner & Grassa, 2014) and *Helianthus annus* (NC_007977; Timme et al., 2007), using default parameters in Bowtie2 plugin (Langmead & Salzberg, 2012) as implemented in Geneious 11.0.4 (Kearse et al., 2012). Due to the lack of 22kb in the large single copy region (LSC) and in order to correctly determine gene orientation in Barnadesioideae, the consensus sequences from mapped reads were initially annotated using the Dual Organellar Genome Annotator (DOGMA; Wyman *et al*., 2004) that uses a BLAST search against a custom database. Here, we compared with the results from Geneious using *Centaurea diffusa* (NC_024286) and *Helianthus annus* (NC_007977) as references. We performed multiple sequence alignments for each gene (coding regions and/or introns) using MAFFT version 7 (Katoh & Standley, 2013) as implemented in Geneious, excluding one Inverted Repeat (IR) to avoid duplication of data. Phylogenetic analysis of the chloroplast dataset was inferred using the same concatenation approach described in 2.3.2 for the all loci recovered for four or more taxa.

3. Results

3.1 Summary statistics

Our sequencing obtained an average of 1,177,351 raw reads (range: 371,716 – 2,043,963) per sample, but we found a statistical difference in the herbarium compared to the silica gel materials ($p < 0.05$; Supplementary Figure 1A), in which we trimmed and quality-filtered 11% (7,11 – 29.08%). Interesting we did not find a statistically difference between the quality of herbarium *vs.* silica gel ($p > 0.05$; Supplementary Figure 1B). Across the 61 enriched samples, we captured an average of 301 COS loci (218 – 366) with a statistical difference between the materials ($p < 0.05$; Supplementary Figure 1C). Using the off-target reads, we were able to recover an average of 107 chloroplast genes (84 – 111) with no statistical difference between materials preservation ($p < 0.05$; Supplementary Figure 1D). Summary statistics for each taxon can be found in the Supplementary Material S1.

3.2 Impact of missing data on different matrices of taxonomic completeness

Our results show that missing data greatly affected the general statistics and phylogenetic

reconstructions (Fig. 3; Table 1). Considering the loci enriched for four or more taxa, we recovered a total of 942 COS generating an alignment comprising 747,821 bp of 87.8% of missing nucleotides (Table 1). Our approach to filter loci for species comprising less than 50% and 75% of the taxa for each locus progressively decreased the matrix length (166,488 and 45,868) and the amount of missing nucleotide (78,1 and 58,2%). On the other hand, these matrices increased the number of variable and informative sites. Based on the number of missing nucleotides, we expected that the 942 COS alignment would retrieve a topology with the lowest number of unsupported nodes. However, concatenation and one coalescent approach (ASTRAL) showed that the 942 COS matrix comprises the largest number of nodes supported with 100% (Fig. 3). On the other hand, excluding loci allowing the lowest number of missing data possible, it is not considered the best approach since it increases the number of unsupported nodes (< 49%), especially in the coalescent reconstruction.

3.3 Phylogenetic trees

Our COS concatenated and coalescent analyses were well-resolved, and mostly well-supported and congruent in major clades (Fig. 4 - 5; Supplementary Figures 2 - 7). In all phylogenetic analyses, Barnadesioideae is recovered as monophyletic and sister to the rest of Compositae with strong support (Fig. 4 - 5; Supplementary Figures 2 - 8). Moreover, the clades comprising *Barnadesia-Huarpea*; *Archidasyphyllum-Arnaldoa-Fulcaldea;* and *Chuquiraga-Doniophyton* were retrieved consistently and supported in our phylogenetic analyses. On the other hand, we found topological incongruences in the generic relationships of *Dasyphyllum* and *Schlechtendalia* under different phylogenetic approaches. For instance, ASTRAL trees yielded both genera are sister groups, but this clade was not supported in any analyses (<60%; Fig. 5A, Supplementary Fig. 4 and 7). In contrast, RAxML and SVD-quartets trees recovered *Dasyphyllum* as sister to the clade comprising *Archidasyphyllum-Arnaldoa-Barnadesia-Fulcaldea-Huarpea* with strong support (99-100%; Fig. 4 - 5; Supplementary Figure 2-3, 5-6). Regarding *Schlechtendalia*, RAxML and SVD-QUARTETS analyses recovered the genus as sister group to the rest of the subfamily (Fig. 4, 5B, Supplementary Figures 3, 5, and 6), except the analysis comprising 145 COS that proposed *Schlechtendalia* as sister to *Chuquiraga-Doniophyton* clade with low support (48% BP; Supplementary Figure 2). Analyses confirm the monophyly of all genera, except *Chuquiraga* which is sometimes paraphyletic by positioning of *Doniophyton* (Fig. 4 – 5A, Supplementary Figure 8).

At the infrageneric level, all phylogenetic analyses corroborate the classification of *Barnadesia* into two subgenera (*Barnadesia* and *Bacasia*). In addition, our analyses also confirm the infrageneric classification of *Chuquiraga* into two sections (*Acanthophylla* and *Chuquiraga*), but the monophyly of both series (*Chuquiraga* and *Parviflorae*) are not clear outline due to the lower support.

Finally, the chloroplast topology was highly congruent with the COS results, except that *Dasyphyllum* is recovered as sister to *Barnadesia-Huapea* clade with high support (100%;

Supplementary Figure 8), *Schlechtendalia* is recovered as sister to *Archidasyphyllum-Arnaldoa-Barnadesia-Fulcaldea-Dasyphyllum-Huarpea* clade (97% BP); the monophyly of *Arnaldoa* and *Fulcaldea* were rejected.

4. Discussion

Impact of missing data

It is doubtless that molecular data has revolutionized the field of evolutionary biology by gathering a great number of informative characters in a relatively short time. Since all the organism information is written in their genetic code, molecular phylogenies can be used to elucidate relationships of distant taxa and shed light into the dream to reconstruct the tree of life. Nevertheless, obtain data from whole-genomic sequencing are not feasible for most evolutionary studies since it requires a considerable number of taxa and the costs and time are still too great (Mammova et al., 2010). Therefore, researchers have focused on genomic reduction techniques as the target enrichment method (McComarck & Faircloth, 2013).

Target enrichment is a common method used to capture orthologous genomics markers via hybridization (Mayer et al., 2016). The desirous results are when all the probes designed hybridizes in all target loci for all species studied. In spite of that, several library factors (*e.g.* probes did not identify and hybridize with the target regions), biological reasons (*e.g.* species with divergent molecular sequence) and bioinformatic analyses (*e.g.* remove of potential paralogous or sequences with low quality) can generate large amounts of missing data bringing us to rhetorical questions in evolutionary biology (Weins, 2003, 2006).

Our empirical study provides further evidence that the exclusion of molecular regions can be problematic and affect branch support (Fig. 3). Our missing data investigation comprised three different approaches under concatenation and coalescent phylogenetic reconstruction: Firstly, we proposed a phylogeny based on a concatenated matrix comprising all the loci recovered that comprises four or more species (hereafter COS 100%); secondly, we excluded all the loci that were recovered less than 50% of the species (COS 25%); thirdly, we excluded all the loci that were recovered for 75% species (COS 25%; Fig. 2). Consequently, our “COS 100 matrix” comprised the largest number of nuclear loci (942 COS) and missing nucleotides (87.8%; Table 1). It is important to mention that the phylogenetic relationships between the approaches were largely congruent. None of the generic relationships that differed between the trees were well supported, and the major phylogenetic incongruences are generally restricted to closely related taxa (Fig. 4 and 5, Supplementary Figures 1-8).

Phylogenetic analyses based on concatenation (RAxML) and species tree based on summary methods (ASTRAL-II) are the most common reconstruction methods in phylogenomics, and quantitative results inferred in both approaches show that the number of clades recovered with 100% of support is largest in the "COS 100% matrix". On the other hand, if researchers limit their dataset and exclude the loci considering a taxonomic threshold, the number of support nodes in a phylogeny will considerably decrease (Fig. 3).

We, therefore, reinforced the conclusion of previous studies performed in animals (Hosner et al., 2015, Streicher et al., 2016) and advised that researchers should see the results with cautions and always explored their datasets in order to guarantee that they have congruent topologies under different phylogenetic reconstructions and datasets.

Efectivesseness of Herbarium Material in the Phylogenomic Era

Museum collections are a remarkable and irreplaceable source of information for the whole society being considered as "biological library" (Suarez & Tsutsui, 2004), holding several specimens and playing a critical role in different fields. In biological sciences, researchers investigate the drivers of biodiversity and its loss by looking into the past and predict and step towards the future. Scientific collections are also important sources of DNA materials for all species, particularly valuable for rare, microendemic species with difficult localities access and also for extinct taxa, saving time and financial resources. However, accessing genetic information in plants has been provided difficult due to the often highly degraded DNA (Staats et al., 2011, Hart et al., 2016).

Next-generation sequencing has revolutionized the field of evolutionary studies since many sequencing approaches actually require sharing DNA into small fragments (Dodsworth, 2015, Hart et al., 2016). Our results further corroborate the effectiveness of museum and often degraded samples for Next-Generation sequencing studies (Supplementary Figure 1; Bakker et al., 2015, Beck & Semple, 2015). Even though the number of silica gel raw reads are statistically higher compared to the museum samples, they were recovered in the same quality (Supplementary Figure 1A, B). Nonetheless, our results found a statistical difference in the number of regions captured according to the genome analyzed (Supplementary Figure 1C, D). The number of nuclear loci recovered in the silica-gel preservation was statistically significant high compared to the herbarium materials (Supplementary Figure 1C). On the other hand, we did not find any difference in the number of plastid genes (Supplementary Figure 1D).

In this study, we hypothesized that the statistic differences in the nuclear and plastid may be explained by their genomic structure and by the conditions that the samples were preserved. It is well-known that plastomes (chloroplast genomes) are found in multiple identical copies in each cell, and their structure, gene content, and order are highly conserved among flowering plants in which

facilitate the capture and sequencing (Moore et al., 2006, Bock, 2007, Moore et al., 2010, Fonseca & Lohmann, 2017). By contrast, two explanations are plausible regarding the nuclear data. Firstly, in the target enrichment studies, the capture of the nuclear loci is performed by hybridization of oligonucleotides probes (or baits; Mayer et al., 2016). The baits used to capture the nuclear data here were designed based on expressed sequence tags (ESTs) of three non-closely related Compositae subfamilies (Asteroideae, Cichorioideae e Carduoideae; Mandel et al., 2014). Nonetheless, the efficiency of the nuclear locus enriched relies on sequence similarity between the probe and the targeted sequencing region (Chau et al., 2018, Mayer et al., 2016). Due to the divergent morphology and unique chloroplast features in Barnadesioideae (Chapter 3, Jasen & Palmer 1987a, Jasen & Palmer 1987b, Gruenstaeudl et al., in prep.), it is notwithstanding to hypothesize that our materials significantly differ from the baits developed for Compositae posing a challenge for the hybridization. (Mayer et al., 2016). Secondly, the herbarium materials are often comprising degraded DNA, and therefore, it is necessary for some adjustments during the library preparation, for an efficient hybridization (Paijmans et al., 2015). Because of our library preparation and sequencing were performed in a outsource, we can not guarantee that it was performed such adjustments focusing on better results.

We recommend further comparative studies comprising the whole-genome sequencing of Barnadesioideae as well as Asteraceae species could provide insights into its genomic structure and shed light into why Barnadesioideae have a considerable divergent morphology.

Phylogenetic relationships in Barnadesioideae

The target conserved orthologous sequences designed for Compositae have placed the family in the new molecular era providing enthusiasm by shedding light into major clades (Mandel et al., 2014; 2015; 2019). The baits set has also been demonstrated useful to resolve challenge close relationships (Herrando-Moraira et al., 2018). Here, we confirm the utility of the 9,678 baits to elucidate the phylogenetic history of contentious subfamily Barnadesioideae with certainly in high and shallow taxonomic levels, although few relationships exceptions were found and will be discussed later.

At the generic relationships, our results confirm previous phylogenetic studies regarding the close relationship of *Barnadesia* and *Huarpea* which is supported by the heterogamous capitula, radiate, ray flower subbilabiate, anthers with basal appendages slightly sagittate or decurrent, lophate pollen, and the gynoecium atrophy (only in *Barnadesia* subgenus *Bacasia*; Cabrera 1951, Urtubey, 1999, Gustafsson et al., 2001, Gruenstaeudl et al., 2009, Stuessy et al., 2009).

Another congruent clade is *Archidasyphyllum* as sister to *Fulcaldea* and *Arnaldoa* (Gustafsson et al., 2001, Gruenstaeudl et al., 2009; Ferreira et al., 2019). Although this clade has been recovered in several phylogenetic studies based on molecular data, this clade is relatively small and comprises

seven species with a narrow geographic distribution but fairly morphological diverse. *Archidasyphyllum* comprises the largest trees in the subfamily reaching 30 meters high, with discoid capitula, pinnate leaves, monoecious or gynodioecious breed system, distributed in the *Nothofagus* forest in Chile and adjacent areas in Argentina (Ferreira et al., 2019). *Fulcaldea* comprises two species of small trees or shrubs, with one single flower per capitulum with swollen style below the branch point (Roque & Funk, 2011). Interesting, this genus has a remarkable disjunction distribution being *F. laurifolia* found in Northern Peru and Southern Ecuador and *F. stuessyi* found in Chapada diamantina, Brazil. *Arnaldoa* comprises three shrub species with monoecious capitula, discoid, trinerved leaves, 30-95-flowered and share the geographical distribution with *F. laurifolia* (Gustaffson et al., 2001; Stuessy et al., 2009; Ferreira et al., 2019). Lastly, a phylogenetic incongruence in this clade needs to be pointed out. All phylogenetic analyses based on nuclear data corroborate *Fulcaldea* and *Arnaldoa* as sister and well-defined genera, nevertheless, plastid tree rejected their monophyly (Supplementary Figure 8). Topological incongruences between plastid and nuclear markers are well documented in plant phylogenies that may be explained by DNA inheritance, convergence, long branch attraction, phylogenetic sorting, hybridization/introgression chloroplast capture, and lower number of informative characters (Table 1; Soltis and Kuzoff, 1995, Fehrer et al., 2007). Despite the chloroplast results, we did not find reasonable arguments to propose taxonomic changes since both genera are well-supported in all nuclear analyses, morphological distinguishable, and they have never been proposed as a taxonomic unit.

The third well-supported and one of the most enigmatic clades of the subfamily is *Chuquiraga* and *Doniophyton*. Earlier studies proposed *Chuquiraga* in a clade with two small genera *Duseniella* and *Doniophyton* (Stuessy et al., 1996; Gustafsson et al., 2001; Urtubey & Stuessy, 2001; Gruenstauedl et al., 2009; Padin et al., 2015) by sharing the dry areas in the Andes and Patagonia, yellow flowers, long caudate anthers and pollen without mesocolpal depression (Hasen 1991; Gustafsson et al., 2001). However, previous phylogenetic hypotheses failed to resolve the relationships in this clade due to the limited taxonomic sampling or molecular markers. The most comprehensive phylogenetic study developed in this clade was performed by Padin et al. (2015). The authors argued that the ambiguous relationships may be the result of few informative characters, artifacts of the methods/data used or biological questions. Even though our study increased the number of nuclear loci for the genus in approximately 294% (mean 294 *vs* 1) and 27,5% chloroplast data markers (mean 110 *vs* 4), our results are still topology incongruent with the relationship of *Chuquiraga* which is sometimes paraphyletic by the position of *Doniophyton* (Fig 4, 5A and Supplementary Figure 8). Possible explanations for these results may be that even we used a large number of data, those markers did not have a sufficient amount of phylogenetic informativeness to resolve the relationships in this clade, or the group could be evolved from a common ancestor in short periods of time (Padin et al., 2015). Furthermore, the inclusion of *Duseniella*, a monotypic

genus endemic to Patagonia Argentinean with several autapomorphies is also necessary to shed light into this clade.

Interesting, our phylogenomics results resolved the long-standing incongruent placement of *Schlechtendalia* and *Dasyphyllum* within the subfamily (Bremer 1994; Gustafsson et al., 2001, Urtubey and Stuessy 2001, Gruenstaeudl et al., 2009; Padin et al., 2015). Here, *Schlechtendalia* is a recovered as sister to the rest of Barnadesioideae with strong support (Fig 4, 5B; Supplementary figures 3, 5-6) or placed in different phylogenetic relationships, however, these results did not receive strong support in any analyses (Fig. 5A; Supplementary Figures 2, 4 and 7). *Schlechtendalia* is a monoespecific genus endemic to Pampas biome in Argentina, Uruguay and Southern Brazil with many morphological features that differ the genus from the rest of the subfamily (Gustafsson et al., 2001; Stuessy et al., 2009). Furthermore, the placement of the genus as sister to the rest of Barnadesioideae is corroborated by the chromosome numbers (Ciadella and López de Kiesling, 1981; Gruenstauedl et al., 2009).

Dasyphyllum is proposed as sister to *Archidasyphyllum-Arnaldoa-Barnadesia-Huarpea-Fulcaldea* clade with strong support (Fig. 4, 5B, Supplementary Figures 2, 3, 5, and 6). Furthermore, our phylogenomics dataset supports the recent taxonomic re-circumscription (Ferreira et al., 2019). *Dasyphyllum* is the largest genus of the subfamily comprising 31 morphologically diverse species found in many biomes distributed from Venezuela to Northwestern Argentina.

Regarding the infrageneric classification, our results support the taxonomic classification of *Barnadesia* into two subgenera (Urtubey, 1999), and *Chuquiraga* into two sections (Ezcurra, 1985). Furthermore, our phylogenomics analyses corroborate previous studies by rejecting the classification of *Chuquiraga* sect. *Chuquiraga* into two series (Gustafsson et al., 2001; Urtubey and Stuessy, 2001, Gruenstaeudl et al., 2009, Padin et al., 2015). The series *Parviflorae* and *Chuquiraga* were distinguished by the capitula, corollas and anther length, and presumably different pollinators that have been suggested as selective pressure of hummingbird pollination and evolved at least three times in Barnadesioideae (Ezcurra, 1985, Ezcurra 2002, Gruenstaeudl et al., 2009).

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Table 1. Summary statistic across three nuclear conserved orthologue loci datasets (COS) and the chloroplast dataset.

	Aligments			
Matrix	**COS (100%)**	**COS (50%)**	**COS (25%)**	**Chloroplast**
Number of loci	942	145	40	111
Alignment length in bp	736,996	166,488	45,868	88,446
Average locus length in bp (min – max)	782,37 (113 - 3,670)	1147,92 (558 - 3,670)	1146,70 (828 - 2,2161)	796,81 (70 - 7,387)
Average of species recovered per loci (min – max)	19,50 (4 – 56)	39,51 (30 – 56)	49,05 (45 – 56)	61,63 (50 – 61)
Missing nucleotide	87.8%	78.1%	58.2%	8.5%
Constant sites	469,990 (63,8%)	89,613 (53,8%)	22,616 (49,3%)	76,989 (87%)
Variable sites	151,235 (20,5%)	34,884 (21%)	9,839 (21,5%)	6,352 (7,2%)
Informative sites	115,771 (15,7%)	41,951 (25,2%)	13,413 (29,2%)	5,105 (5,8%)

Figure 1. Some representative species of Barnadesioideae. A. *Schlechtendalia luzulifolia* Less. B. *Fulcaldea stuessyi* Roque & V.A. Funk. C. *Barnadesia odorata* Griseb. D. *Chuquiraga jussieui* J.F. Gmel. E. *Dasyphyllum sprengelianum* (Gardner) Cabrera. Photo credits: A. Gustavo Heiden, B. Ivan Abreu, C. Danilo Marques, D and E. Paola Ferreira.

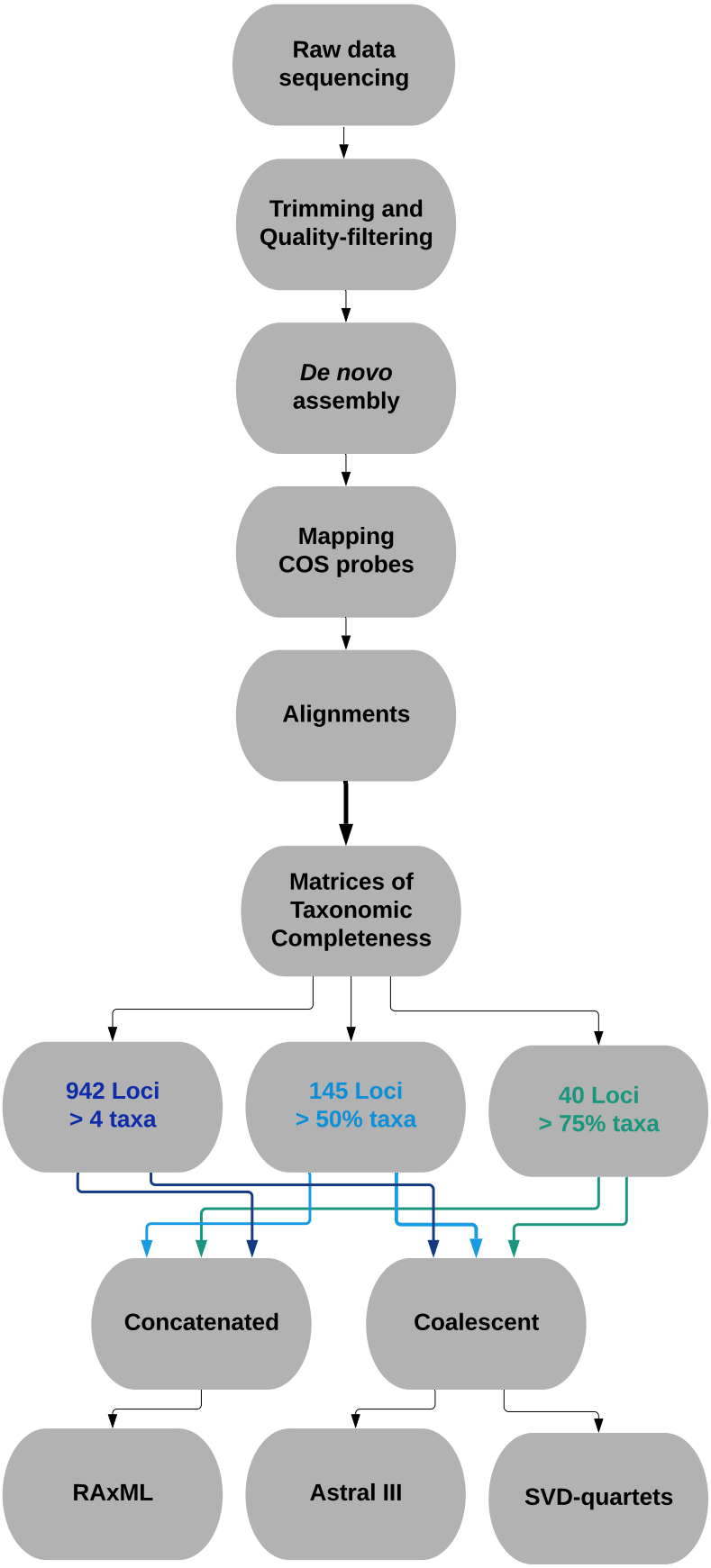


Figure 2. Phylogenomics Workflow. Schematic of the bioinformatics, and the datasets used in the phylogenetic analyses for this study.

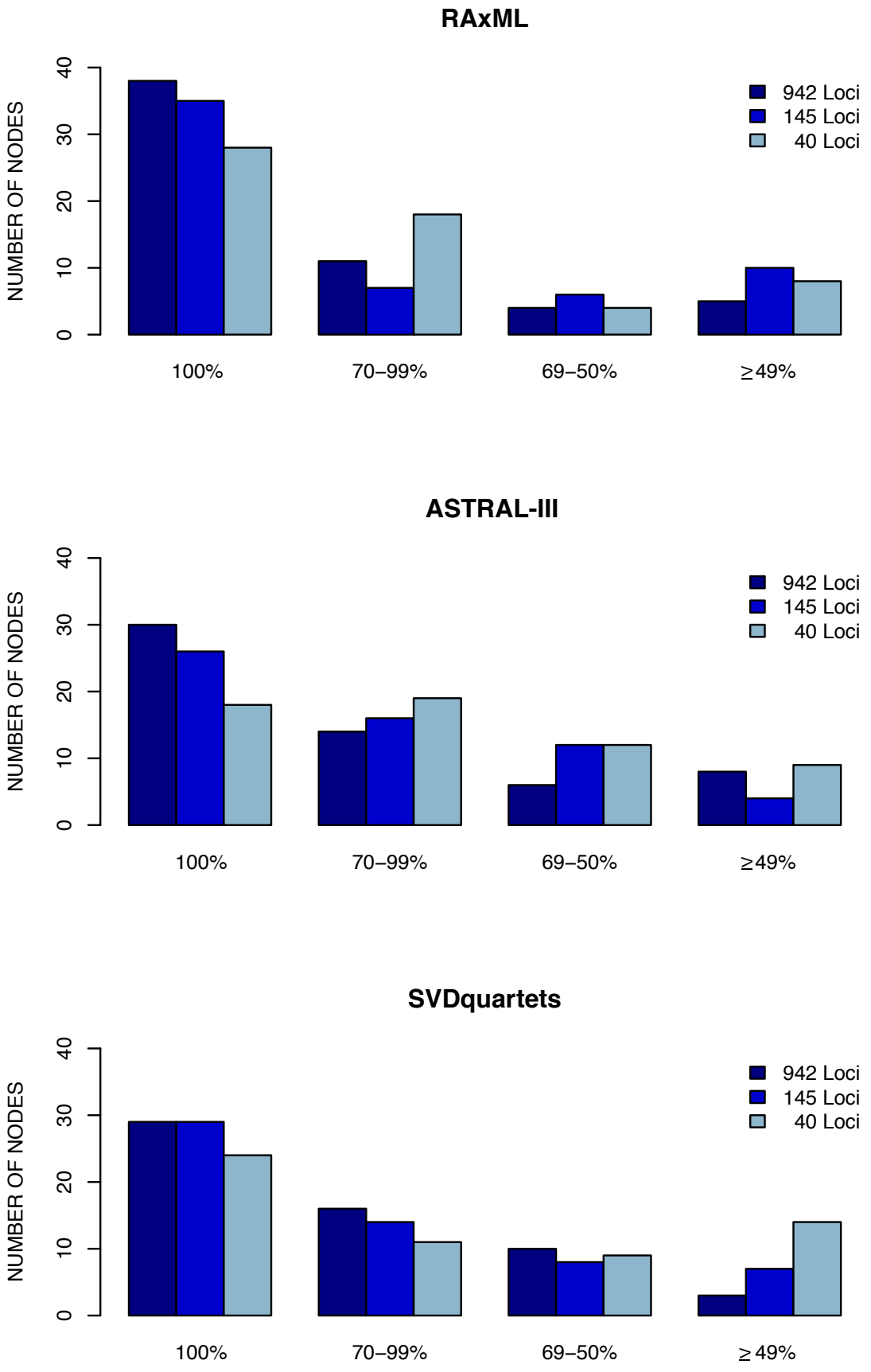


Figure 3. Number of nodes in four support thresholds inferred from Concatenated (RAxML) and Coalescent approach (Astral-III and SVDquartets) across different matrices of taxonomic completeness.

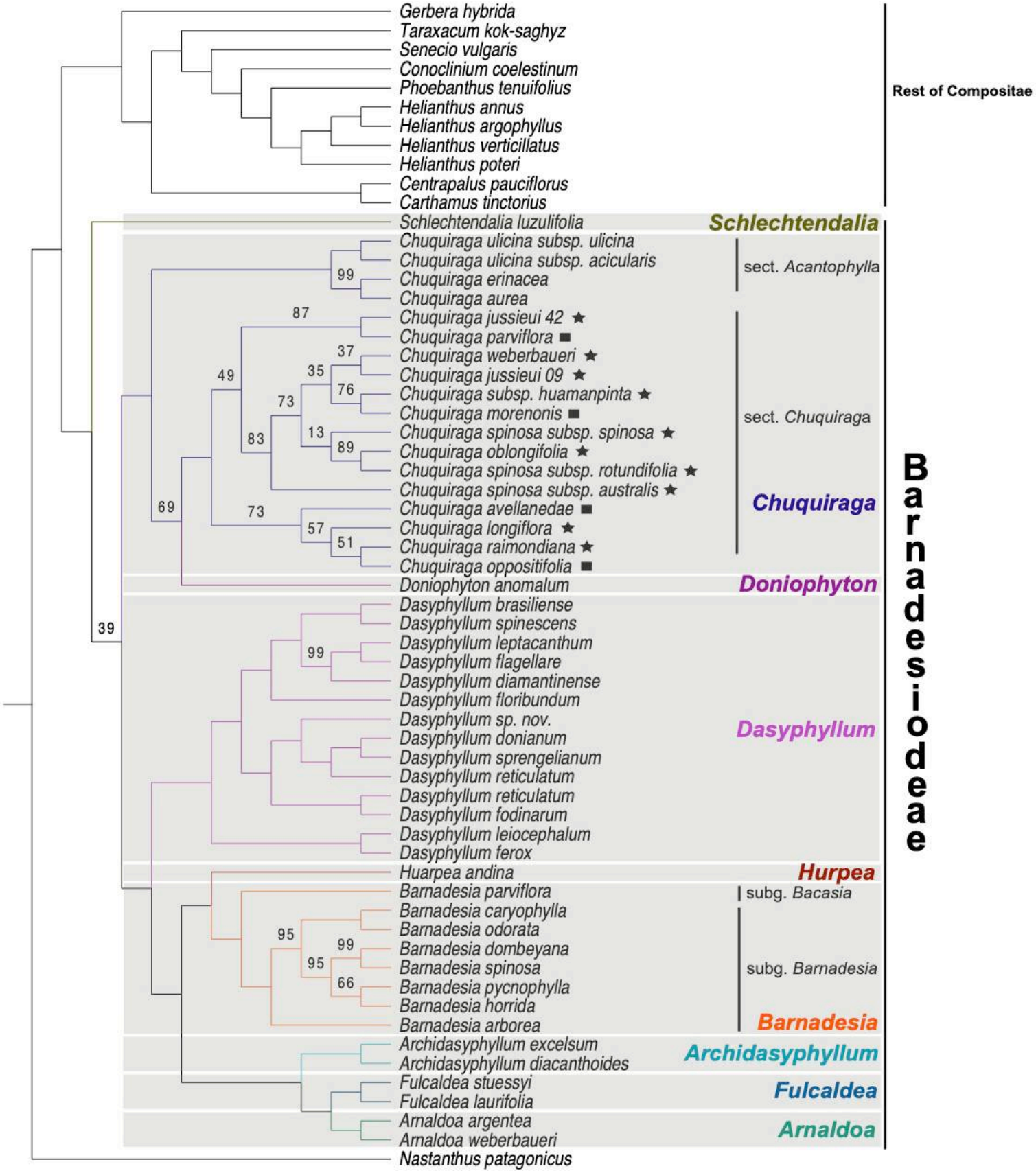


Figure 4. Phylogenetic hypothesis of Barnadesioideae based on 942 Loci inferred from the concatenation approach (RAxML). All nodes recovered 100% bootstrap supported unless specified. Black stars and squares indicate the species currently classified in *Chuquiraga* sect. *Chuquiraga* ser. *Chuquiraga* and *Chuquiraga* sect. *Chuquiraga* ser. *Parviflorae*, respectively.

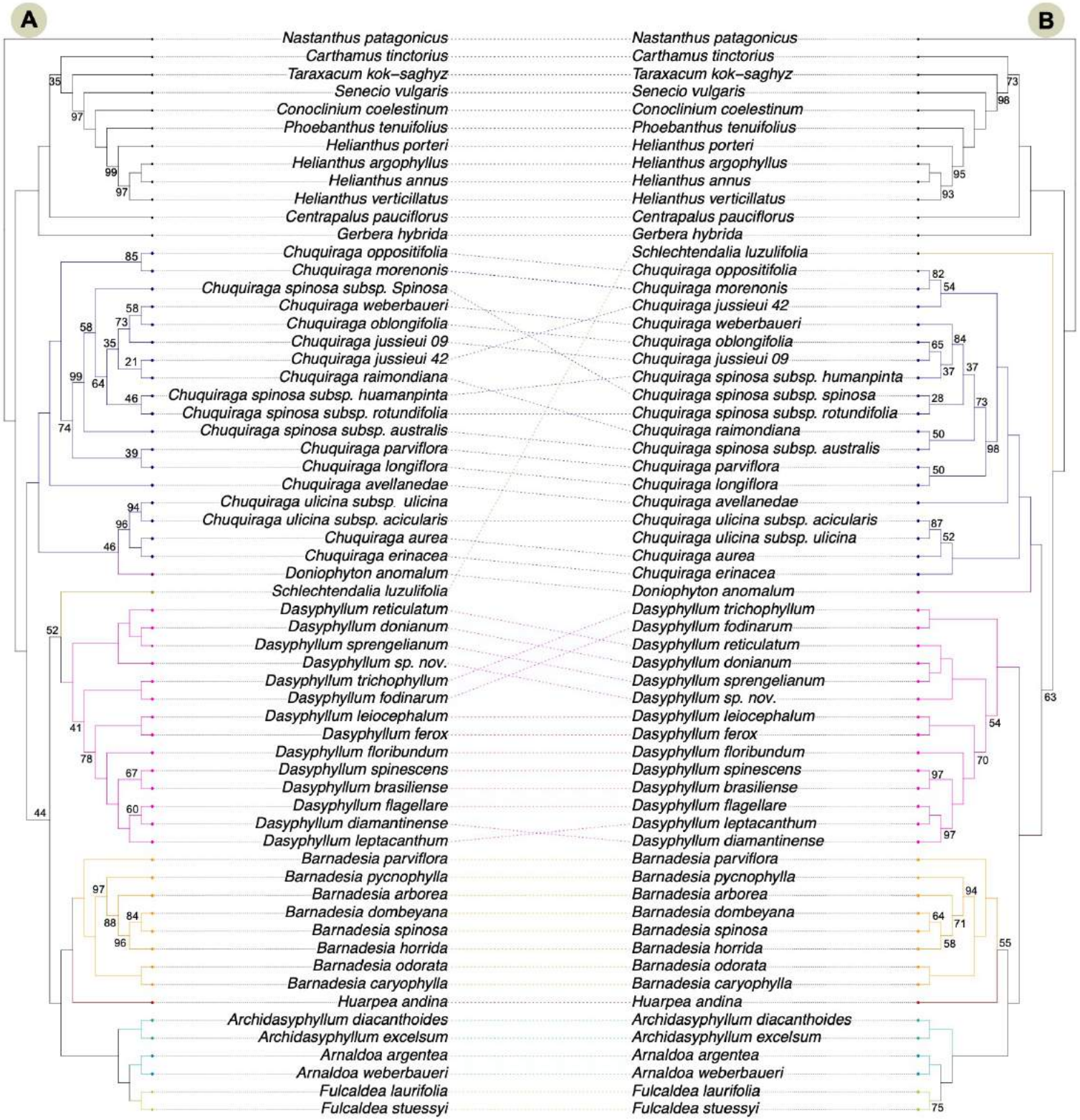
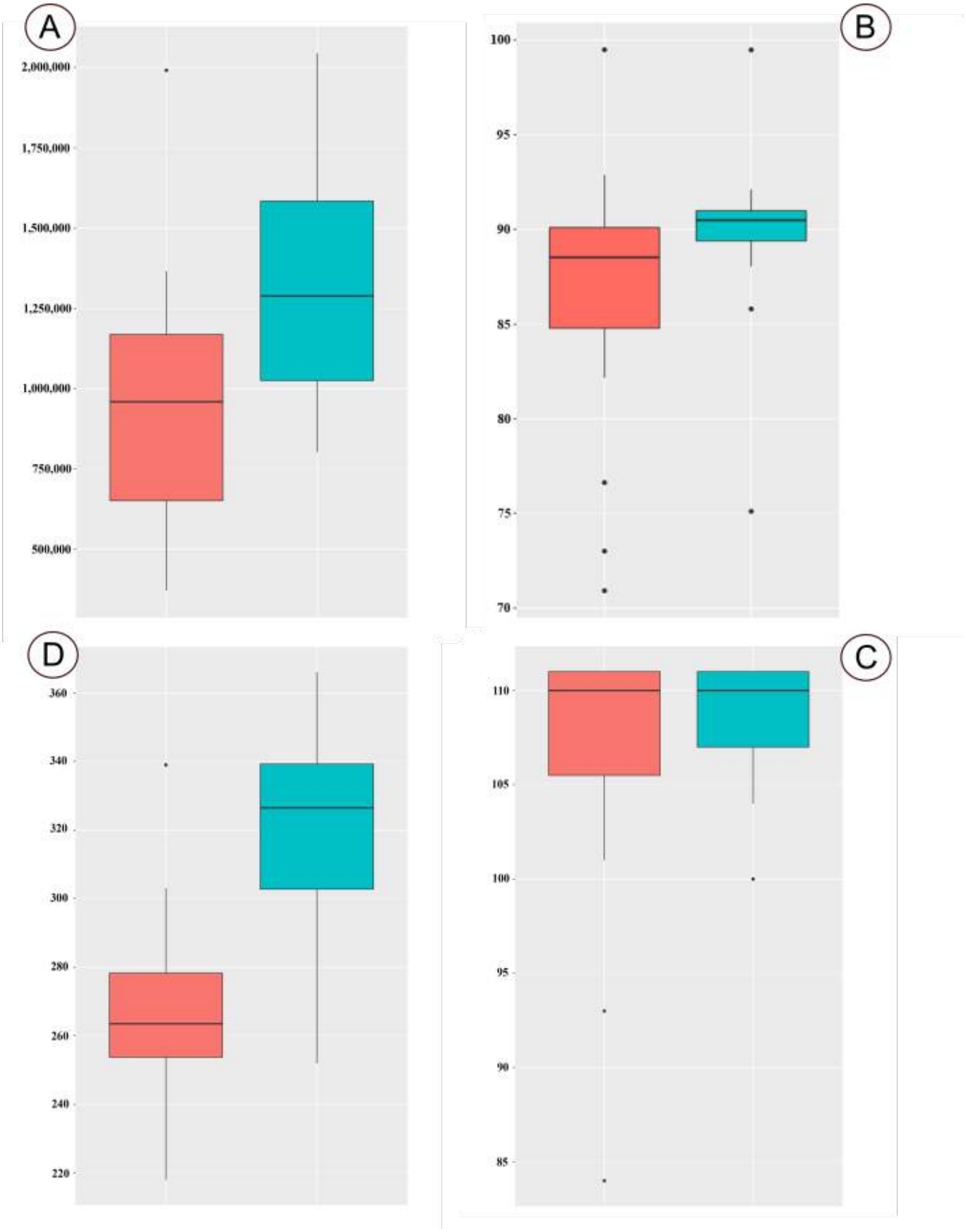
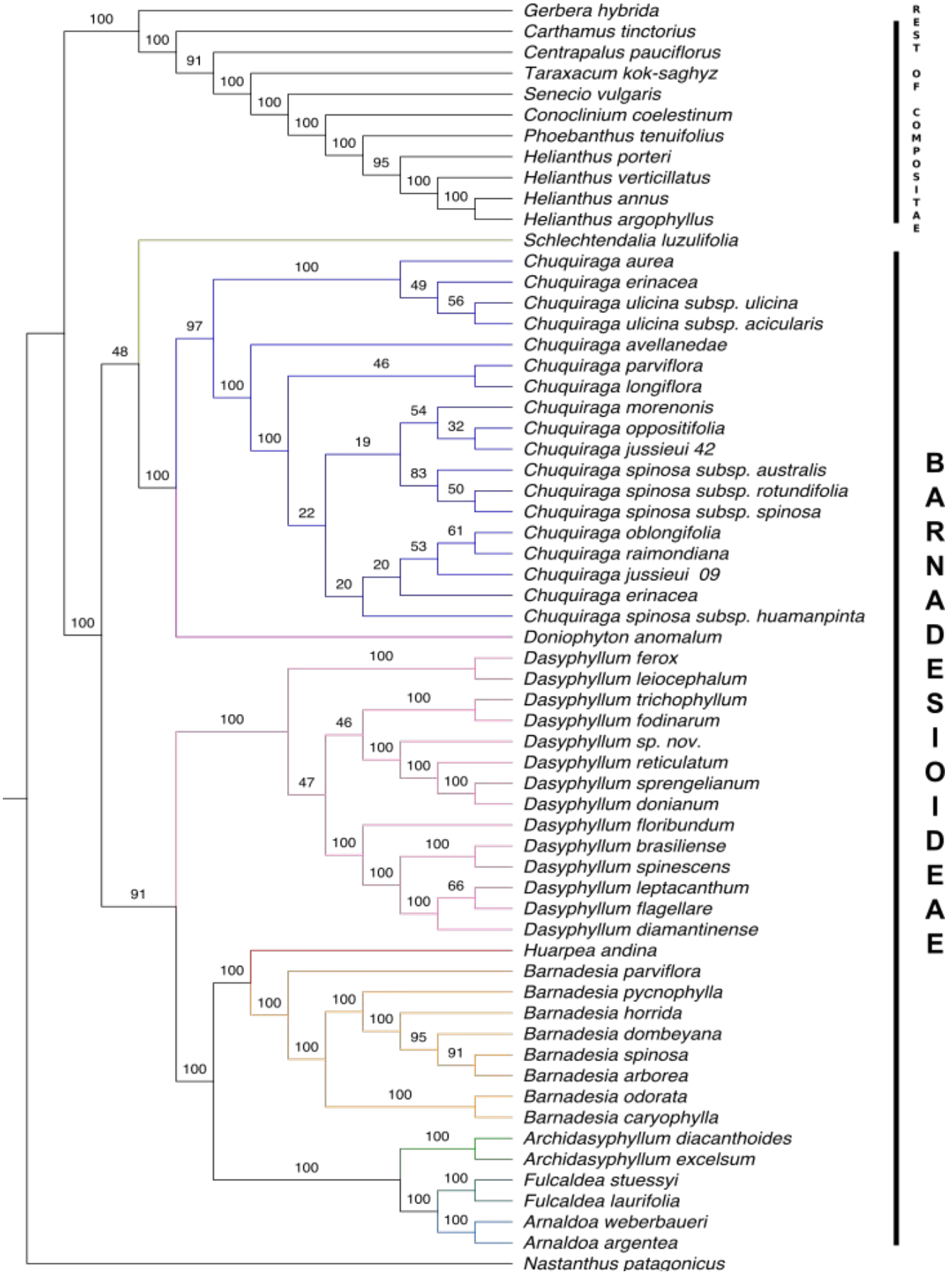


Figure 5. A tanglegram of Barnadesioideae phylogenetic hypotheses based on 942 COS loci inferred from coalescent approaches. A) ASTRAL-III. B) SVD-Quartets. All nodes recovered 100% support unless specified.

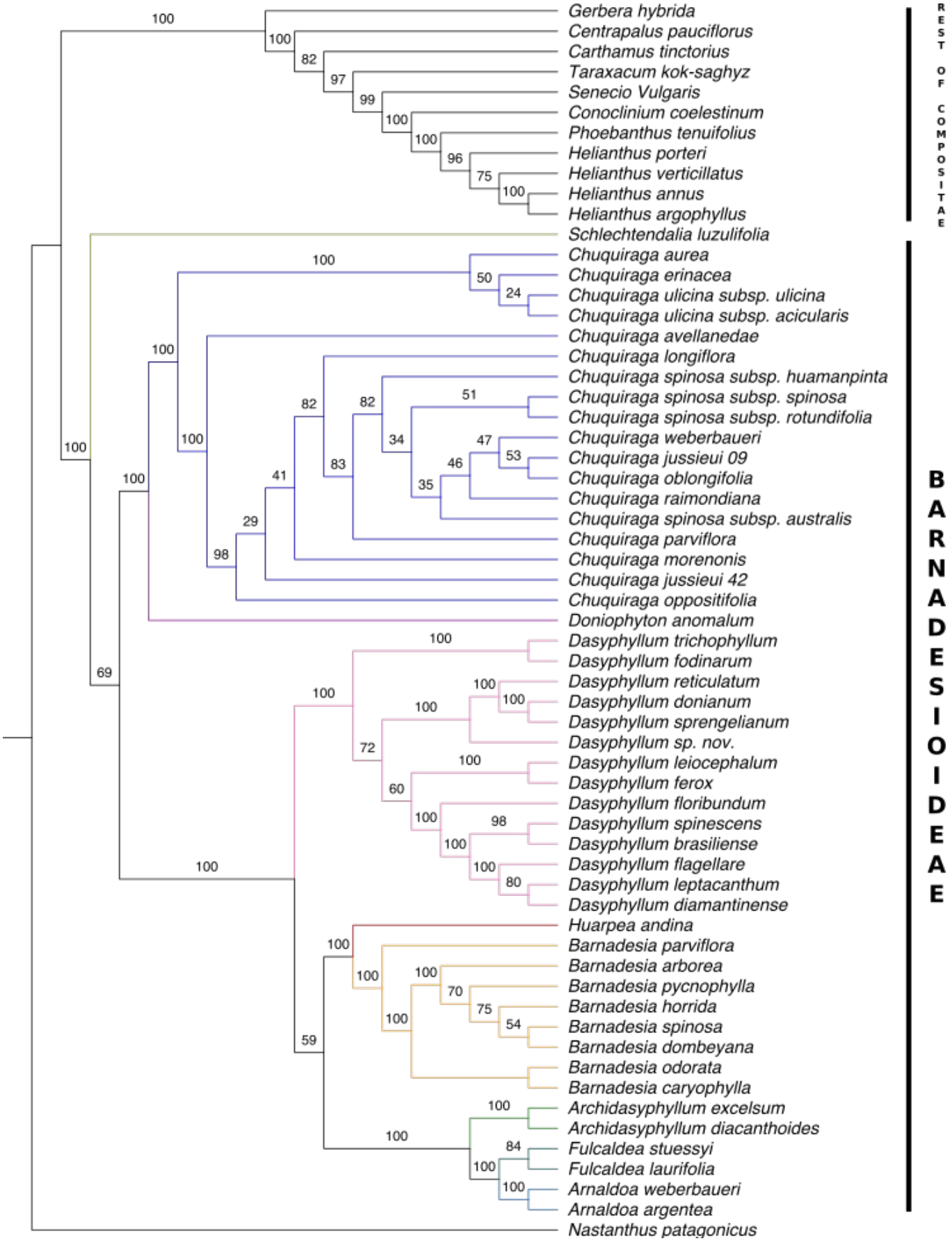
Supplementary Material



Supplementary Figure 1. Boxplots comparing summary statistics of the sequence capture by the material preservation type. A) Number of raw reads; B) Percent of cleaned reads after the trimming and quality-filtering; C) Number of nuclear conserved orthologue loci set; D) Number of chloroplast genes. Orange boxplots = herbarium materials. Blue boxplots = silica gel materials.

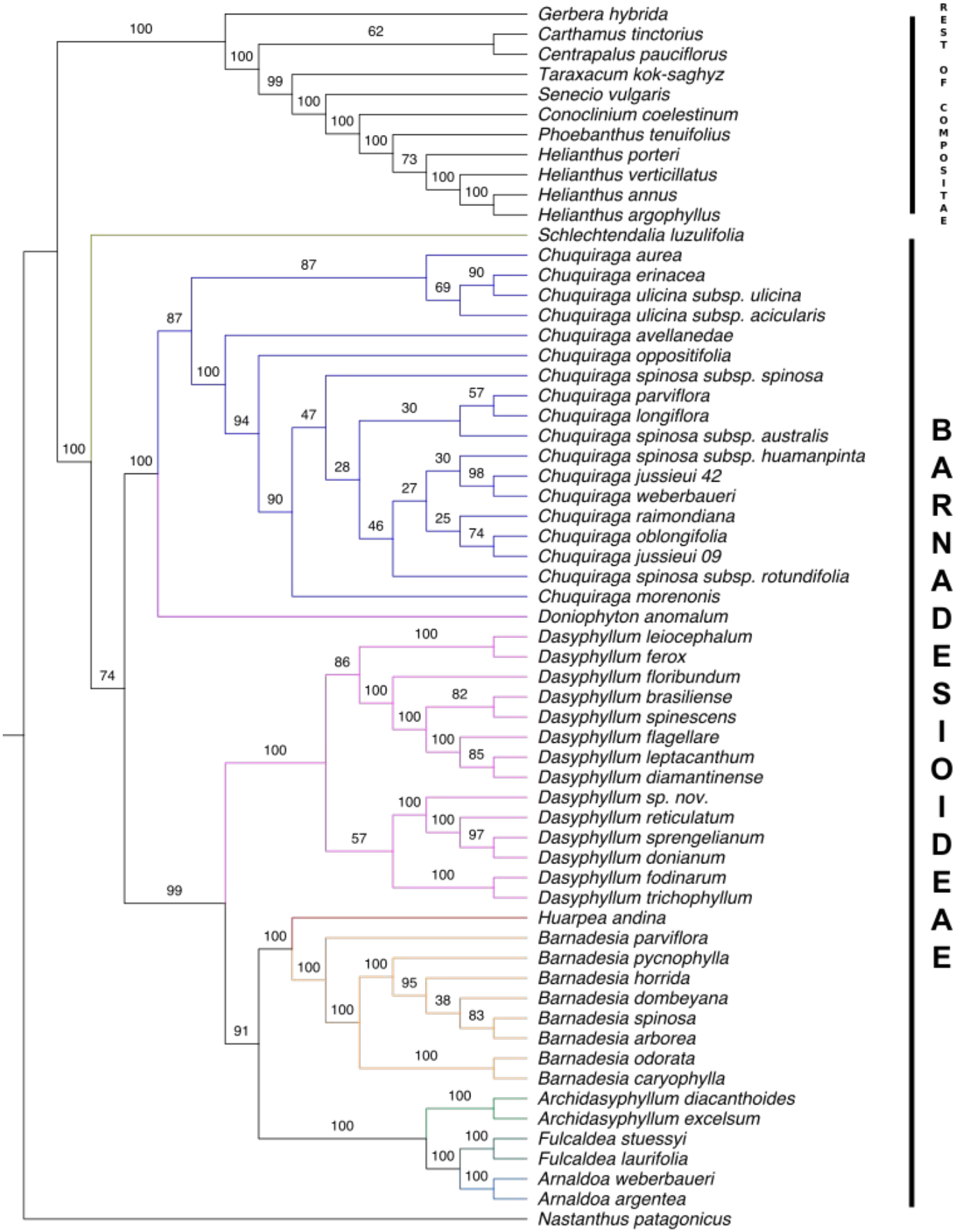


Supplementary Figure 2. Phylogenetic hypothesis of Barnadesioideae based on 145 COS inferred from the concatenation approach (RAxML). Numbers above branches indicate bootstrap support.



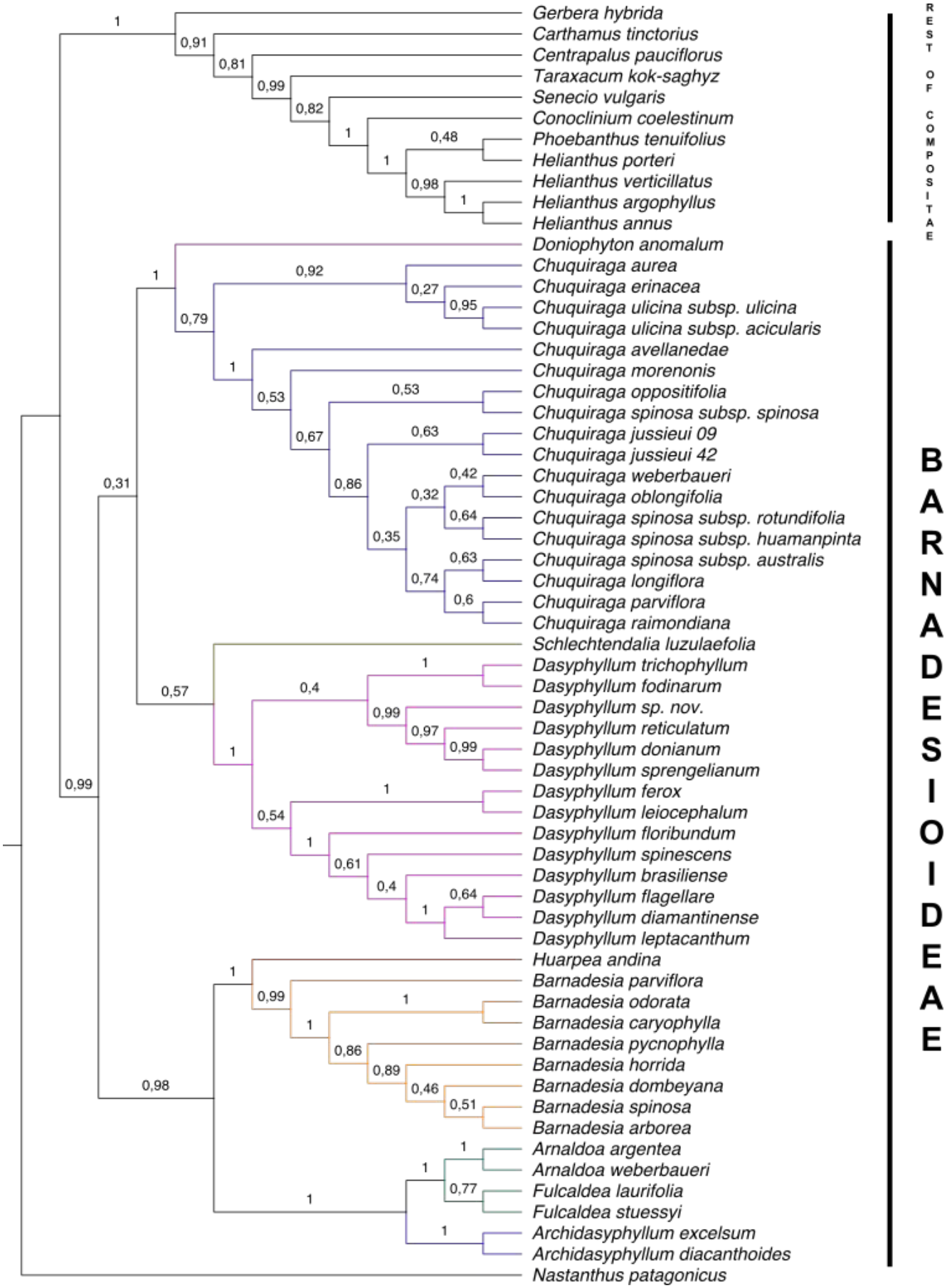
Supplementary Figure 3. Phylogenetic hypothesis of Barnadesioideae based on 145 COS inferred from the coalescent approach (SVD-quartets). Numbers above branches indicate bootstrap support.

Supplementary Figure 4. Phylogenetic hypothesis of Barnadesioideae based on 145 COS inferred from the coalescent approach (ASTRAL). Numbers above branches indicate the local posterior probability (PP).

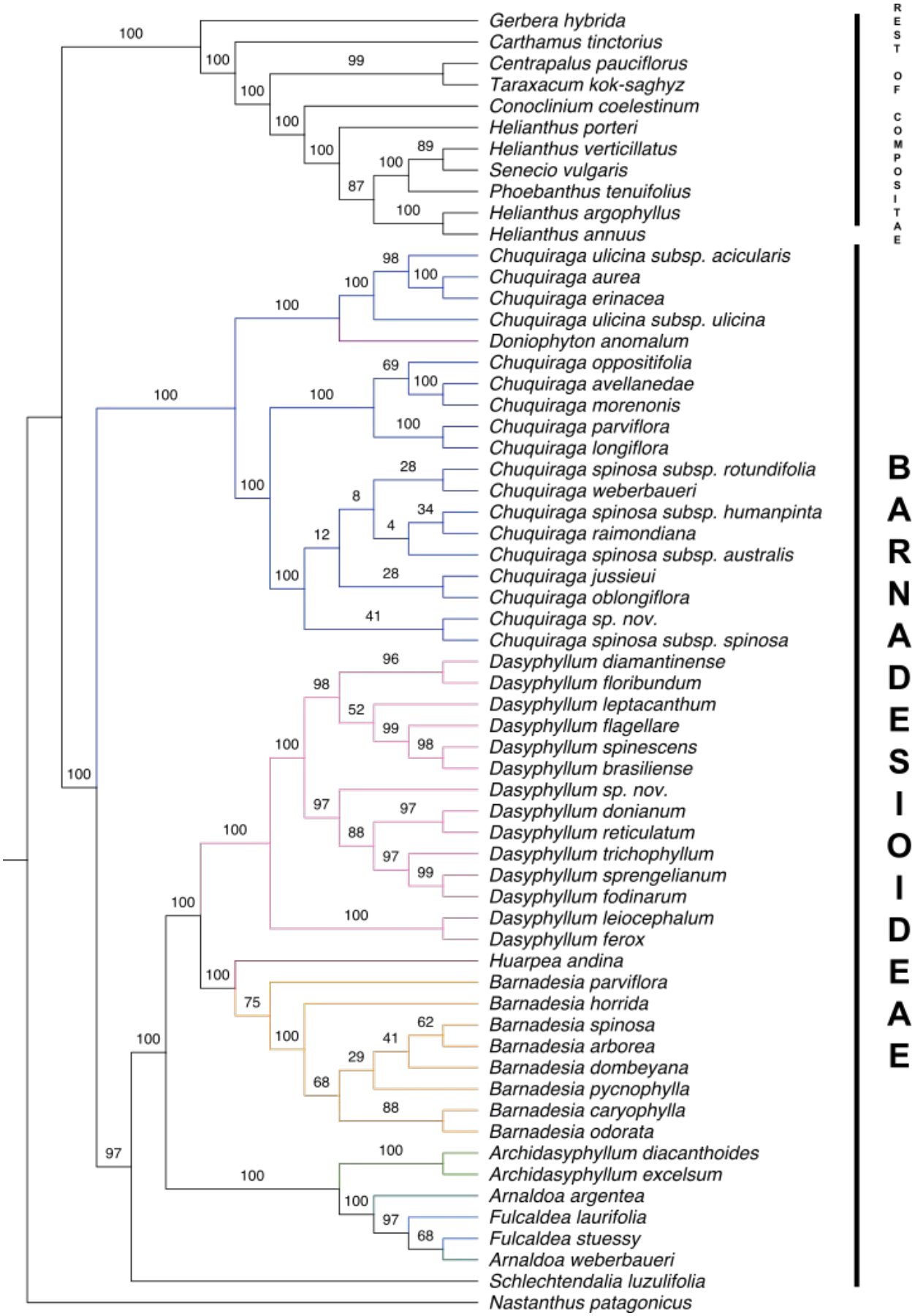


Supplementary Figure 5. Phylogenetic hypothesis of Barnadesioideae based on 40 COS inferred from the concatenation approach (RAxML). Numbers above branches indicate bootstrap support.

Supplementary Figure 6. Phylogenetic hypothesis of Barnadesioideae based on 40 COS inferred from the coalescent approach (SVD-quartets). Numbers above branches indicate bootstrap support.



Supplementary Figure 7. Phylogenetic hypothesis of Barnadesioideae based on 40 COS inferred from the coalescent approach (ASTRAL). Numbers above branches indicate the local posterior probability (PP).



Supplementary Figure 8. Phylogenetic hypothesis of Barnadesioideae based on 111 chloroplast genes inferred from the concatenation approach. Numbers above branches indicate bootstrap.

Supplementary Table S1. Taxon sampled, voucher information and Genbank accession numbers used this study. Taxa used under the PRJNA Genbank accession numbers.

Family	Tribe	Infrageneric classification	Taxon	Collector(s) and number	Sample ID	BioProject	Conservation type	Collection date	Location	Herbarium acronym	Raw reads	Cleaned reads (%)	COS recovered	Percent of COS recovered	Plastid Genes recovered	Percent of Plastid genes recovered
Calyceraceae			*Nastanthus patagonicus* Speg.	Bonifacino & Funk 4016	WI11	PRJNA236448	-	14 Dec 2009	Argentina. Santa Cruz. Rio Chico.	US	7,483,947	6,791,620 (90.75%)	353	33,27	111	100,00
Asteraceae	Barnadesieae	-	*Archidasyphyllum diacanthoides* P.L. Ferreira, Saavedra & Groppo	M. Monge 2273	WE02	XXX	silica	22 Oct 2012	Chile. La Araucanía. Caraucatín.	SPFR	1,070,276	955,941 (89.32%)	288	27,14	109	98,20
Asteraceae	Barnadesieae	-	*Archidasyphyllum excelsum* P.L. Ferreira, Saavedra & Groppo	C. Luz 195	WE03	XXX	silica	21 Sep 2013	Chile. Valparaíso. Olmué.	SPFR	1,678,177	1,530,715 (91.21%)	261	24,60	111	100,00
Asteraceae	Barnadesieae	-	*Arnaldoa argentea* C. Ulloa, P. Jørg. & M.O. Dillon	J. Madsen 8341	WD12	XXX	silica	14 Aug 2001	Ecuador. Loja.	AAU	1,399,621	1,261,063 (90.10%)	298	28,09	107	96,40
Asteraceae	Barnadesieae	-	*Arnaldoa weberbaueri* (Muschl.) Ferreyra	G. Ccana-Ccapatinta 44	WE01	XXX	silica	24 Jan 2016	Peru. Cajamarca. Celendín.	SPFR	1,614,457	1,463,315 (90.64%)	275	25,92	111	100,00
Asteraceae	Barnadesieae	*Barnadesia* subg. *Barnadesia*	*Barnadesia arborea* Kunth	L. Delgado 114	WH11	XXX	herbarium	28-30 Jun 2011	Ecuador. Pichincha.	QCA	962,801	682,848 (70.92%)	305	28,75	93	83,78
Asteraceae	Barnadesieae	*Barnadesia* subg. *Barnadesia*	*Barnadesia caryophylla* (Vell.) S.F. Blake	P. Ferreira 7	WE09	XXX	silica	05 May 2013	Brazil. Rio de Janeiro. Teresópolis.	SPFR	1,000,049	888,875 (88.88%)	382	36,00	100	90,09
Asteraceae	Barnadesieae	*Barnadesia* subg. *Barnadesia*	*Barnadesia dombeyana* Less.	G. Ccana-Ccapatinta 47	WF03	XXX	silica	12 Feb 2016	Peru. Huaraz. Ezuay.	SPFR	1,583,189	1,421,172 (89.77%)	348	32,80	111	100,00
Asteraceae	Barnadesieae	*Barnadesia* subg. *Barnadesia*	*Barnadesia horrida* Muschl.	G. Ccana-Ccapatinta 54	WE10	XXX	silica	11 Jan 2016	Peru. Cusco. Urubamba.	SPFR	1,656,890	1,478,429 (89.23%)	345	32,52	107	96,40
Asteraceae	Barnadesieae	*Barnadesia* subg. *Barnadesia*	*Barnadesia odorata* Griseb.	C. Ferreira 1	WE11	XXX	silica	26 Sep 2015	Argentina.	SPFR	1,424,137	1,288,610 (90.48%)	343	32,33	109	98,20
Asteraceae	Barnadesieae	*Barnadesia* subg. *Barnadesia*	*Barnadesia pycnophylla* Muschl.	G. Ccana-Ccapatinta 49	WF01	XXX	silica	27 Jan 2016	Peru. Huánuco. Ambo.	SPFR	1,523,999	1,385,103 (90.89%)	342	32,23	110	99,10
Asteraceae	Barnadesieae	*Barnadesia* subg. *Barnadesia*	*Barnadesia spinosa* Less. ex Urtubey	M. Gutierrez 611	WF02	XXX	silica	19 Nov 2011	Colombia. Bogotá	JBB	1,285,574	1,148,524 (89.34%)	360	33,93	100	90,09
Asteraceae	Barnadesieae	*Barnadesia* subg. *Bacasia*	*Barnadesia parviflora* Spruce ex Benth. & Hook. f.	J. Jaramillo 12253	WE12	XXX	herbarium	26 Ago 1990	Ecuador. Napo.	QCA	1,193,410	871,288 (73.01%)	253	23,85	84	75,68
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Acanthophylla*	*Chuquiraga aurea* Skottsb.	T. Stuessy 12931	WG05	XXX	herbarium	17 Feb 1993	Argentina. Chubut.	WU	390,108	347,152 (88.99%)	282	26,58	104	93,69
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Acanthophylla*	*Chuquiraga erinacea* D. Don	T. Stuessy 12882	WG07	XXX	herbarium	13 Feb 1993	Argentina. Chubut.	WU	371,716	337,906 (90.90%)	265	24,98	106	95,50
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Acanthophylla*	*Chuquiraga ulicina* subsp. *acicularis* (D. Don) C. Ezcurra	T. Stuessy 12751	WH06	XXX	herbarium	18 Jan 1993	Chile. Coquimbo.	WU	409,922	366,493 (89.41%)	275	25,92	110	99,10
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Acanthophylla*	*Chuquiraga ulicina* (Hook. & Arn.) Hook. & Arn. subsp. *ulicina*	T. Stuessy 12799	WH05	XXX	herbarium	21 Jan 1993	Chile. Coquimbo.	WU	704,517	627,235 (89.03%)	256	24,13	111	100,00
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Chuquiraga*	*Chuquiraga jussieui* J.F. Gmel.	G. Ccana-Ccapatinta 9	WG08	XXX	silica	Jan 2015	Ecuador. Loja. Loja.	SPFR	1,298,886	1,153,669 (88.82%)	310	29,22	111	100,00
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Chuquiraga*	*Chuquiraga jussieui* J.F. Gmel.	G. Ccana-Ccapatinta 42	WH12	XXX	silica	2016		SPFR	1,291,417	970,027 (75.11%)	282	26,58	108	97,30

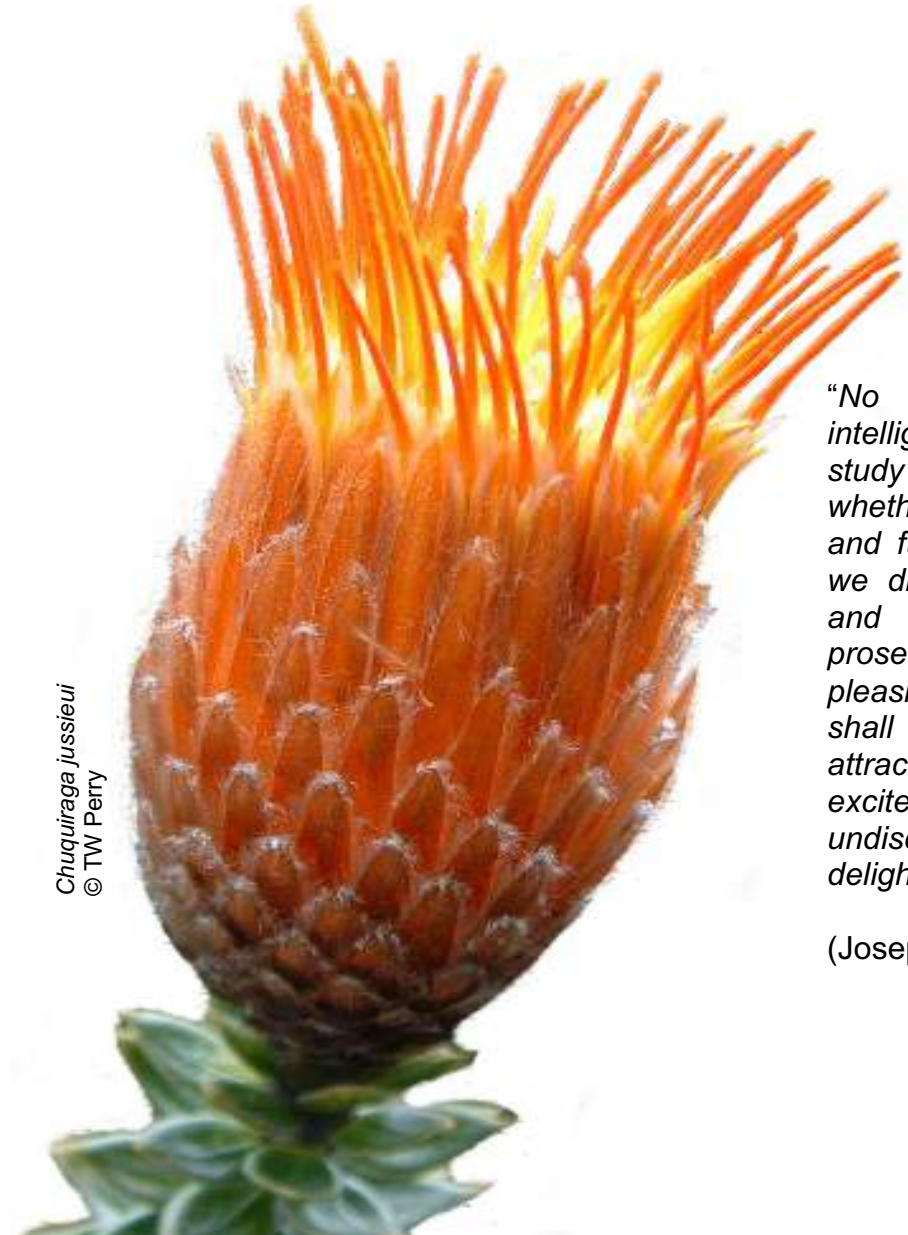
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Chuquiraga*	*Chuquiraga longiflora* (Griseb.) Hieron.	M. Schulte 21	WG09	XXX	silica	19 Nov 1987	Bolivia. Potosi.	M	546,325	491,739 (90.01%)	313	29,50	111	100,00
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Chuquiraga*	*Chuquiraga oblongifolia* Sagást. & Sánchez Vega	T. Stuessy 12625	WG11	XXX	herbarium	17 Jul 1992	Peru. Cajamarca	WU	1,160,239	1,048,439 (90.36%)	295	27,80	110	99,10
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Chuquiraga*	*Chuquiraga raimondiana* A. Granda	G. Ccana-Ccapatinta 48	WH10	XXX	silica	27 Jan 2016	Peru. Huánuco. Ambo.	SPFR	1,065,173	947,323 (88.94%)	302	28,46	107	96,40
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Chuquiraga*	*Chuquiraga spinosa* subsp. *australis* C. Ezcurra	P. Simon 522	WH01	XXX	herbarium	08 Jan 2001	Argentina. Jujuy.	WU	686,621	588,166 (85.66%)	309	29,12	111	100,00
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Chuquiraga*	*Chuquiraga spinosa* subsp. *huamanpinta* C. Ezcurra	G. Ccana-Ccapatinta 15	WH02	XXX	silica	Jan 2015	Peru. Lima. Lima.	SPFR	802,229	711,849 (88.73%)	283	26,67	111	100,00
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Chuquiraga*	*Chuquiraga spinosa* subsp. *rotundifolia* (Wedd.) C. Ezcurra	G. Ccana-Ccapatinta 22	WH04	XXX	silica	Jan 2015	Peru. Júnin. Huancayo.	SPFR	1,616,731	1,478,900 (91.47%)	300	28,28	111	100,00
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Chuquiraga*	*Chuquiraga spinosa* Less. subsp. *spinosa*	G. Ccana-Ccapatinta 30	WH03	XXX	silica	Jan 2015	Peru. Huacanvelica. Huacanvelica.	SPFR	1,447,792	1,242,145 (85.80%)	293	27,62	110	99,10
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Chuquiraga*	*Chuquiraga weberbaueri* Tovar	G. Ccana-Ccapatinta 43	WH07	XXX	silica	2016		SPFR	1,710,805	1,550,937 (90.66%)	311	29,31	111	100,00
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Parviflorae*	*Chuquiraga avellanedae* Lorentz	T. Stuessy 12920	WG06	XXX	herbarium	15 Feb 1993	Argentina. Chubut.	WU	1,329,849	1,235,268 (92.89%)	316	29,78	111	100,00
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Parviflorae*	*Chuquiraga morenonis* (Kuntze) C. Ezcurra	T. Stuessy 12940	WG10	XXX	herbarium	17 Feb 1993	Argentina. Chubut.	WU	1,138,959	1,030,637 (90.49%)	287	27,05	110	99,10
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Parviflorae*	*Chuquiraga oppositifolia* D. Don	T. Stuessy 12726	WG12	XXX	herbarium	16 Jan 1993	Chile.	WU	955,694	841,568 (88.06%)	325	30,63	111	100,00
Asteraceae	Barnadesieae	*Chuquiraga* sect. *Chuquiraga* ser. *Parviflorae*	*Chuquiraga parviflora* (Griseb.) Hieron.	J. Wood 7781	WH09	XXX	herbarium	30 Dec 1993	Bolivia. Chuquisaca.	SPF	1,990,852	1,635,915 (82.17%)	283	26,67	111	100,00
Asteraceae	Barnadesieae	-	*Dasyphyllum brasiliense* (Spreng.) Cabrera	M. Saavedra 520B	WF04	XXX	silica	09 Sep 2007	Brazil. Espírito Santo. Afonso Claúdio.	RB	810,913	734,949 (90.63%)	305	28,75	104	93,69
Asteraceae	Barnadesieae	-	*Dasyphyllum diamantinense*Saavedra & M. Monge	P.L. Ferreira 8	WF06	XXX	silica	17 May 2013	Brazil. Bahia. Palmeiras.	SPFR	1,578,363	1,453,975 (92.12%)	318	29,97	100	90,09
Asteraceae	Barnadesieae	-	*Dasyphyllum donianum* (Gardner) Cabrera	M. Saavedra 995	WF07	XXX	silica	19 Jul 2009	Brazil. Bahia. Correntina.	RB	1,660,983	1,525,041 (91.82%)	345	32,52	111	100,00
Asteraceae	Barnadesieae	-	*Dasyphyllum ferox* (Wedd.) Cabrera	G. Ccana-Ccapatinta 58	WF09	XXX	silica	06 Feb 2016		SPFR	1,064,270	966,345 (90.80%)	318	29,97	111	100,00
Asteraceae	Barnadesieae	-	*Dasyphyllum flagellare* (Casar.) Cabrera	M. Saavedra 796	WF08	XXX	silica	18 Jul 2008	Brazil. Espírito Santo. Castelo.	RB	1,108,634	1,010,975 (91.19%)	316	29,78	106	95,50
Asteraceae	Barnadesieae	-	*Dasyphyllum floribundum* (Gardner) Cabrera	M. Saavedra 997	WF10	XXX	silica	20 Jul 2009	Brazil. Bahia. Coribe.	RB	1,186,265	1,066,570 (89.91%)	336	31,67	110	99,10
Asteraceae	Barnadesieae	-	*Dasyphyllum fodinarum* (Gardner) Cabrera	C. Fraga 3330	WF11	XXX	silica	02 Jun 2011	Brazil. Minas Gerais.	RB	1,274,341	1,155,312 (90.66%)	316	29,78	108	97,30
Asteraceae	Barnadesieae	-	*Dasyphyllum leiocephalum* (Wedd.) Cabrera	G. Ccana-Ccapatinta 55	WG01	XXX	silica	10 Jan 2016	Peru. Cusco. Urubamba.	SPFR	968,487	871,889 (90.03%)	312	29,41	111	100,00

Asteraceae	Barnadesieae	-	*Dasyphyllum leptacanthum* (Gardner) Cabrera	P.L. Ferreira 1	WF12	XXX	silica	03 May 2013	Brazil. Rio de Janeiro. Petrópolis	SPFR	938,572	841,220 (89.63%)	305	28,75	111	100,00
Asteraceae	Barnadesieae	-	*Dasyphyllum spinescens* (Less.) Cabrera	M. Saavedra 1018	WG03	XXX	silica	19 May 2010	Brazil. Rio de Janeiro. Itatiaia.	RB	914,845	827,766 (90.48%)	317	29,88	110	99,10
Asteraceae	Barnadesieae	-	*Dasyphyllum reticulatum* (DC.) Cabrera	C. Fraga 3352	WG02	XXX	silica	04 Jun 2011	Brazil. Minas Gerais.	RB	1,033,888	934,684 (90.40%)	326	30,73	110	99,10
Asteraceae	Barnadesieae	-	*Dasyphyllum sprengelianum* (Gardner) Cabrera	M. Saavedra 998	WG04	XXX	silica	23 Jul 2009	Brazil. Bahia. Érico Cardoso.	RB	998,817	903,833 (90.49%)	317	29,88	111	100,00
Asteraceae	Barnadesieae	-	*Dasyphyllum trichophyllum*(Baker) Cabrera	M. Saavedra 578	WH08	XXX	silica	20 Nov 2007	Brazil. Minas Gerais. Botumirim.	RB	1,584,879	1,441,837 (90.97%)	308	29,03	111	100,00
Asteraceae	Barnadesieae	-	*Dasyphyllum* sp. nov.	M. Saavedra 1035	WF05	XXX	herbarium	02 Aug 2010	Brazil. Espírito Santo. Águia Branca.	RB	911,072	829,511 (91.05%)	329	31,01	108	97,30
Asteraceae	Barnadesieae	-	*Doniophyton anomalum*(D. Don) Kurtz	M. Bonifacino 96	WE06	XXX	herbarium	12 Jan 2000		LP	991,819	860,443 (86.75%)	304	28,65	111	100,00
Asteraceae	Barnadesieae	-	*Fulcaldea laurifolia* (Bonpl.) Poir.	G. Lewis 3497	WE05	XXX	herbarium	19 Aug1997	Ecuador. Loja. Sozoranga.	QCA	1,366,028	1,175,240 (86.03%)	312	29,41	109	98,20
Asteraceae	Barnadesieae	-	*Fulcaldea stuessyi* Roque & V.A. Funk	I. Abreu 123	WI04	PRJNA236448	silica	19 Aug 2010	Brazil. Bahia. Rio das Contas	US	8,999,244	7,637,382 (84.87%)	200	18,85	111	100,00
Asteraceae	Barnadesieae	-	*Huarpea andina* Cabrera	Nicora 8573	WE07	XXX	herbarium	18 Jan 1983	Argentina. Rio Negro. Iglesia	LP	766,273	587,205 (76.63%)	327	30,82	101	90,99
Asteraceae	Barnadesieae	-	*Schlechtendalia luzulifolia* Less.	G. Heiden 2008	WE04	XXX	silica	25 Oct 2012	Brazil. Rio Grande do Sul. Alegrete.	SPF	2,043,963	1,860,455 (91.02%)	276	26,01	106	95,50
Asteraceae	Mutiseae	-	*Gerbera hybrida*	J. Mandel 105	WI05	PRJNA236448	-	22 Oct 2013	Greenhouse grown cutting, Terra Nigra, USA	GA	16,000,000	14,188,644 (88.68%)	271	25,54	111	100,00
Asteraceae	Cardueae	-	*Carthamus tinctorius* L.	N/A	WI01	PRJNA236448	-	N/A	N/A	N/A	10,436,332	7,819,453 (74.93%)	363	34,21	111	100,00
Asteraceae	Cichorieae	-	*Taraxacum kok-saghyz* L.E. Rodin	J. Mandel 102	WJ03	PRJNA236448	-	27 Aug 2013	Greenhouse cultivated	GA	12,996,414	9,923,192 (76.35%)	283	26,67	110	99,10
Asteraceae	Vernonieae	-	*Centrapalus pauciflorus* (Willd.) H. Rob.	J. Mandel 104	WI02	PRJNA236448	-	22 Oct 2013	Greenhouse grown seed USDA, PI 312852	GA	7,797,810	6,843,698 (87.76%)	399	37,61	111	100,00
Asteraceae	Eupatorieae	-	*Conoclinium coelestinum*(L.) DC.	V. Funk 12769	WI03	PRJNA236448	-	11 Sep 2001	USA. Virginia. Falls Church.	US	11,532,082	10,201,289 (88.46%)	331	31,20	107	96,40
Asteraceae	Heliantheae	-	*Phoebanthus tenuifolius* (Torr. & A. Gray) S.F. Blake	M. Mason 101	WI12	PRJNA236448	-	10 Sep 2010	Greenhouse grown seed collected USA. Liberty Co. Florida.	GA	9,845,420	7,387,912 (75.04%)	236	22,24	109	98,20
Asteraceae	Senecionae	-	*Senecio vulgaris* L.	J. Mandel 12774	WI09	PRJNA236448	-	07 Nov 2011	USA. Washington, D.C.	NMNH	10,342,788	7,702,867 (74.48%)	293	27,62	109	98,20
Asteraceae	Heliantheae	-	*Helianthus annus* L.	N/A	WI06	PRJNA236448	-	N/A	Voucher n/a, USDA, PI 603989	N/A	16,000,000	14,172,138 (88.58%)	257	24,22	108	97,30
Asteraceae	Heliantheae	-	*Helianthus porteri* (A. Gray) Pruski	J. Mandel 103	WI08	PRJNA236448	-	22 Oct 2013	Greenhouse grown seed collected	GA	12,369,548	9,123,427 (73.76%)	234	22,05	109	98,20

								USA. Liberty Co. Florida.							
Asteraceae	Heliantheae	-	*Helianthus verticillatus* Small	J. Mandel 101	WI10	PRJNA236448	-	01 Sep 2004	Greenhouse grown seed collected USA. Madison Co., Tennesee	GA	6,804,526	5,073,815 (74.57%)	218	20,55	109	98,20
Asteraceae	Heliantheae	-	*Helianthus argophyllus* Torr. & A. Gray	N/A	WI07	PRJNA236448	-	N/A	Voucher n/a, USDA, PI 435623	N/A	14,548,246	12,763,376 (87.73%)	239	22,53	108	97,30

Chapter 02

Understanding the early evolution of the South American Compositae: Historical biogeographic and diversification insights into Barnadesioideae



Chuquiraga jussieui
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"*No occupation is more worthy of an intelligent and enlightened mind than the study of Nature and natural objects; and whether we labour to investigate the structure and function of the human system, whether we direct our attention to the classification and habitats of the animal kingdom, or prosecute our researches in the more pleasing and varied field of vegetable life, we shall constantly find some new object to attract our attention, some fresh beauties to excite our imagination, and some previously undiscovered source of gratification and delight*"

(Joseph Paxton, 1838)

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Abstract

Aim: The high biodiversity of South America has instigated scientists for centuries. However, phylogenies including an extensive taxonomic and molecular sampling allied to historical and macroevolutionary studies are still scarce for most of the organisms, limiting our understanding of biodiversity. Here, we investigate processes and patterns that have shaped the biodiversity in South America by studying the divergence times, biogeographical history, and species diversification of the largest angiosperm family, Compositae, with focus on the subfamily Barnadesioideae.

Location: South America.

Methods: We used a phylogenomic dataset based on 942 nuclear loci to propose the first dated phylogeny of Barmadesioideae calibrated with the fossils described for the group. We also inferred the geographical range evolution of lineages and estimated the speciation and extinction rates over the evolutionary history.

Results: Our results suggest that the ancestor of Barnadesioideae was probably distributed in southern South America in the early Eocene at 49.1 Mya. During the Miocene, the most common ancestor of the major clades started to diversify and occupied the Central and North areas. The speciation and extinction rates were inferred very low and constant through time, and any shift was detected in the phylogeny.

Main Conclusion: We show that phylogenomic data when combined with dense species-level sampling and analyzed under probabilistic methods, can produce robust results shedding light on historical biogeography, systematics, and species diversification.

KEYWORDS

Asteraceae, biogeography, diversification, Next-generation sequencing, South America.

1. Introduction

Biodiversity is not equally distributed across the globe. Within the 34 richest regions on earth, South America comprises more than 32,107 endemic species of plants distributed in five hotspots (Mittermeier et al., 2004). Understanding why there are so many plants there and the factors that have shaped its biodiversity remains one of the biggest questions in biology (Antonelli & Sanmartín, 2011). Several processes have been proposed to explain its evolutionary history which undoubtedly is a product of abiotic and biotic factors in space and time (Antonelli et al., 2009, Graham 2010, Horn et al., 2010, Antonelli & Sanmartín, 2011, Hughes et al., 2012, Antonelli et al., 2015). However, understanding such factors can be compared to assembly a complex puzzle in which many pieces still need to be inserted. The family Compositae (the sunflower or daisy group) can reveal interesting missing pieces.

Compositae is one of the three largest flowering plant families comprising 25,000-35,000 species classified in 13 subfamilies (Panero et al., 2014). The family is worldwide distributed being the South America the richest region with 6316 species in 9 subfamilies (Panero & Crozier, 2016). Within these nine subfamilies, Barnadesioideae is an interesting and morphologically diverse group comprising ten genera and 84 species distributed from Venezuela to Argentina (Figure 1, Stuessy et al., 2009; see Chapter 3 for maps of distribution). Most species occur in xeromorphic areas in western South America along to the Andes and Patagonia, some extend to the east in Cerrado, Caatinga and Chaco, and few members are found in humid areas such as Yungas and Atlantic Forest (Stuessy et al., 1996, Stuessy et al., 2009).

Understanding the spatially and temporally aspects of Barnadesioideae would bring interesting insights into South America evolution but it can also contribute to the early evolution of Compositae since its members are recovered as sister to the rest of the family (Jasen & Palmer, 1987a, 1987b). According to Stuessy et al. (1996), the origin of Barnadesioideae was placed in Miocene (25-5 Mya) and its diversification was influenced by the Andes uplift. Nevertheless, this study was relied upon a bibliographical review of Compositae fossil record, weather and geological changes. Due to the

advances in the field of historical biogeography, phylogenies become essential components to test hypotheses and provide robust results. However, previous molecular studies in Barnadesioideae have hampered our knowledge since those studies were based on few markers, limited taxonomic sampling and incongruent topologies (Gustafsson et al., 2001; Gruenstaeudl et al., 2009). With the advances of high-throughput DNA sequencing, Ferreira et al. (see Chapter 1) overcome all the previous challenges producing a well-supported phylogeny and congruent topologies under several probabilistic inferences by gathering data from 942 nuclear loci and almost completed chloroplast genomes for 49 species of Barnadesioideae.

Here, we use a phylogeny based on robust phylogenomics dataset as a framework to infer the first time-calibrated phylogeny using the fossil described for Barnadesioideae (Palazzesi et al., 2009), and the biogeographical scenario that best explain their current distribution and diversity. We addressed the following questions: (1) When and where did Barnadesioideae most likely originate?; (2) How and when did it attain its current distribution?; (3) What is the pattern of speciation, extinction and net diversification rates through the time in Barnadesioideae?. Our study sheds further light on the geographical and temporal origins and composition of the highly diverse South American Compositae flora.

2. Material and Methods

2.1 Taxon sampling

Our taxonomic sampling includes almost 60% of Barnadesioideae species, including nine of the 10 genera (*Duseniella* is missing), 44 species and five subspecies currently circumscribed in the subfamily (Ferreira et al., 2019), and recovers great part of the morphological variation, major clades, and infrageneric classification found in previous molecular studies (Gustafsson et al., 2001, Gruenstaeudl et al., 2009; Chapter 1). Eleven representatives of Asteraceae and one Calyceraceae species were used as outgroups (Table 1)

2.2 Divergence times estimation

We used a concatenated phylogeny of 942 nuclear conserved orthologue dataset (Chapter 1) which comprises the highest bootstrap support values as a framework to time-calibrate under penalized likelihood approach using treePL (Smith & O'Meara, 2012). The rate smoothing parameter was set to 100 and established using the cross-validation parameter available in treePL. Four fossil points were used as calibration points based on synapomorphies allowing confidence to specific node clades of the phylogeny: 1) *Quilembaypollis tayuoides* Barreda and Palazzesi fossil pollen was used to assign the most recent common ancestor (MRCA) of *Dasyphyllum* with maximum age at 23 Mya and minimum age at 20 Mya (Palazzesi et al., 2009); 2) *Quilembaypollis gamerroi* Barreda and Palazzesi fossil pollen was used to assign the MRCA of *Chuquiraga-Doniophyton* clade with maximum age at 23 Mya and minimum age at 20 Mya (Palazzesi et al., 2009); 3) *Mutisiapollis telleriae* Barreda, Katinas, Passalia & Palazzesi fossil pollen and the co-fossilized macrofossil *Raiguenrayun cura* with the minimum age at 47.5 Mya (Barreda et al., 2012) were used to assign the MRCA of Mutisioideae-Asteroideae clade; 4) *Ambrosia*-type pollen was used to assign the MRCA of Asterioideae clade with the maximum age at 35 Mya and minimum age at 25 Mya (Graham 1996).

2.3 Biogeographical history

Distribution data for each species were extracted from the literature (Cabrera 1959, Ezcurra 1985, Hansen, 1991, Harling 1991, Sagástegui & Sánchez 1991, Stuessy & Sagástegui 1993, Ferreyra 1995, Bremer 1994, Granda 1997, Katinas & Stuessy 1997, Urtubey 1999, Hind 2001, Ulloa et al. 2002, Stuessy & Urtubey 2006, Stuessy & Urtubey 2007, Stuessy et al. 2009, Funk & Roque 2011, Saavedra 2011, Saavedra et al. 2014, Saavedra et al. 2018), examination of ca. 3,500 herbarium specimens (ALCB, B, CEPEC, ESA, GB, HRCB, HUEFS, QCNE, QCA, K, MO, RB, SPF, SPFR, UEC, UETC, UFU, WU; acronyms according to Thiers 2019), field work and public sources when was possible to check the taxonomic inaccuracies (GBIF, Flora del ConoSur, Tropicos, Smithsonian, Species link, and Jabot). A total of 3,867 records for the extant distribution were used to bind into six operational areas using the biogeographic regionalization of South America (Morrone, 2014; 2015) in SpeciesGeoCoder (Töpel et al., 2017) with some adjustuments: (1) Dry

Diagonal; (2) Atlantic forest; (3) Pampas; (4) Patagonia and South Andes (PaSa); (5) Central Andes and Amazonia (CA); (6) North Andes.

Biogeographic analyses were performed by using the Dispersal-Extinction-Cladogenesis model (Ree and Smith 2008) implemented in the R package BioGeoBEARS (Matzke 2018), pruning the outgroups in order to avoid the widely and globally distribution species patterns (e.g. *Lactuca sativa*). Our final taxonomic sampling included all 49 species of Barnadesioideae used in the divergence times analyses (Table 1).

2.4 Diversification analyses

We used BAMM version 2.5.0 (Bayesian Analysis of Macroevolutionary Mixtures; Rabosky et al. (2014) in order to estimate the speciation and extinction rates through the time and identify the shifts in diversification rate. We accounted for incomplete taxon sampled by applying sampling fractions for each clade using the most recent taxonomic treatments (Supplementary Table 1). BAMM analyses were run for 10,000,000 million generation of Markov chain Monte Carlo (MCMC) with four chains, sampling evolutionary parameters every 1,000 generation, and priors were estimated using *setBAMMpriors* function in BAMMtools package version 2.1.6 in R (Rabosky et al., 2015). The first 10% of the MCMC run were discarded as burn-in, and the remaining samples were assessed for convergence by computing the ESS values >200. Results were visualized and analyzed using BAMMtools (Rabosky et al., 2015).

3. Results

3.1 Divergence time estimates and range evolution

The crown node of Barnadesioideae was inferred during the Eocene (49.1 Mya) and the ancestral range reflected a low probability to occur in Central, PaSa and Pampas (20% probability for the areas 3, 4 and 5; 14% probability for the areas 3 and 4; Figure 1). By the Miocene, the MRCA of the *Chuquiraga*, *Dasyphyllum* and *Barnadesia* (the three largest genera of the subfamily) started to

diversify. The MRCA of *Chuquiraga* was primarily found in PaSa (around 21 Mya) where *C.* section *Acanthophylla* is currently distributed, while the sect. *Chuquiraga* dispersed to the South-Central Andes plus Amazonia areas, and one species (*C. jussieui* 09) reached the North Andes. *Dasyphyllum* has most likely originated in the Atlantic Forest and Central Andes/Amazonia areas (around 20 Mya). Within *Dasyphyllum*, two patterns of range evolution could be inferred: clade 1 in Western South America being distributed in Central Andes/Amazonia, and clade 2 was distributed in Eastern South America in Dry Diagonal and Atlantic Forest areas. The common ancestor of *Barnadesia* is estimated to have originated in Central Andes/Amazonia and North Andes around 17 Mya. The ancestral range of *Archidasyphyllum*, *Arnaldoa* and *Fulcaldea* clade was largely ambiguous (Fig. 2).

Diversification Rates

Results from BAMM analysis shows that diversification in Barnadesiodeae was homogeneous and constant along the branches and any diversification shift was detected (Fig. 3d; Supplementary Figure 1). Regarding the rates, our results show a constant increasing speciation rates along the branches (average 0.11 events per Myr, 0.09-0.14; Fig. 3a). The extinction rates were also inferred as constant and very low with 0.02 events per Myr (0.001-0.058; Fig. 3b).

4. Discussion

Based on the phylogeny comprising multi-loci, we estimate the divergence times, ancestral range and species dynamics of the Barnadesioideae. Our results further shed light and the origin and the ancestral evolution of Compositae in South America.

4.1 Early evolution of Compositae in space and time

Many studies have tried to trace the ancestral characters, and estimate the age and the place of origin in Compositae. Estimates varied from Miocene (20 Mya) to Cretaceous (100 Mya; Turner 1977, Funk et al. 2005), numerous morphological features were proposed as plesiomorphies for the

group (Stuessy et al. 1996, Bremer 1994), and different regions in the American continent was suggested as the most likely origin (Cronquist 1977, Jeffrey 1977, Turner 1977, Bremer 1994). Nonetheless, conflicting hypotheses have hampered our understanding since Compositae is a large and morphologically diverse group with many parallelisms in most features (Stuessy et al. 1996). In the molecular era, Jasen & Palmer (1987a, 1987b) provided new insights into the early evolution by recovering the small subtribe Barnadesiinae (tribe Mutiseae) endemic to South America as sister group to the rest. Although they brought new findings on character evolution, the place and the time of the orign were still in debate.

Previous historical biogeographic studies based on the distribution data of the extant species proposed southern South America as the ancestral area of Compositae (Funk et al. 2005) in which it also shares with its sister group family, Calyceraceae (Palazzesi et al. 2010). In the last decade, reliable fossil pollen grains close related to Barnadesioideae and Calyceraceae from Miocene were found in Patagonia (Palazzesi et al., 2009, Palazzesi et al., 2010), suggesting a young and fast diversification. Nonetheless, a new fossil preserved in dinosaur-bearing deposits drastically pushes back the age of the family. *Tubulifloridites lilliei* type A is a fossil described from the Late Cretaceous and it was found in a place that any extant species of Compositae is found, Antarctica (Barreda et al. 2015). Based on the results inferred from a parsimony analysis using morphological data for 26 pollen characters and a low taxonomic sampling in Asterales, the authors concluded that the fossil pollen was ascribed to extant *Dasyphyllum* taxa (Barnadesioideae). However, *Tubulifloridites lillei* entails various fossil pollen morphotypes that have been assigned to other angiosperms families (*e.g.* Ranunculaceae, Dettmann 1994; Euphorbiaceae, Macphail et al. 2014). Besides the uncertain systematic placement, the findings of Barreda et al. (2015) has been critically contested concerning the methodology, and biases in the characters and their states codification used in the analyses (Panero 2016; Panero and Crozier 2016).

Our divergence time results placed the origin of Asteraceae in the Paleocene (57.6 Mya; Supplementary Figure 2) corroborating with previous age estimations (64.75 Mya; 55.1 – 74.4 95% HPD) using the relaxed molecular clock method (Panero & Crozier 2016) and refusing the origin of

Compositae in the Cretaceous (Barreda et al. 2015). Further, we recommended more studies including a large taxonomic sampling within and outside Asterales and a revision of the morphological characters before assigning *Tubulifloridites lilliei* type A as an Asteraceae member and change all the historical biogeography of the family.

4.1 Evolution Range and species dynamics of Barnadesioideae

For the first time, a phylogenetic hypothesis based on robust phylogenomics dataset was used to infer the divergence times in Barnadesioideae using fossil information as calibration points (Palazzesi et al., 2009). Because this subfamily is recovered as sister group to the rest of Compositae, historical biogeographic and macroevolutionary studies of Barnadesioideae can also provide insights into the early evolution range in South America.

Our evolution range results were similar to the hypothesis performed by Stuessy et al. (1996), except by the divergence time estimations. The origin of Barnadesioideae was inferred at 49.1 Mya and older than previous estimates (25-5 Mya - Stuessy et al., 1996, 39-36 Mya - Kim et al., 2005, Panero & Crozier, 2016). During the evolutionary history of Barnadesioideae, several processes occurred in South America specially the Andean uplift that may explain its biodiversity (Zachos et al. 2001, Jaramillo 2002, Ortiz-Jaureguizar & Cladera 2006, Posadas & Ortiz-Jaureguizar 2016). As a general distribution pattern, the history of Barnadesioideae could be explained by a southern South American origin in the early Eocene followed by Northwestern to east Northeastern occupation during the Miocene.

During the early Eocene, the southern South America was mostly continental inundated by marine incursions (Pascual et al. 1996, Ortiz-Jaureguizar & Cladera 2006), and the surface temperature was being affected by the Paleocene-Eocene Thermal Maximum (Zeebe et al., 2009). Paleoflora reconstructions support the diverse and tropical or subtropical moist forests, although some angiosperms species found in arid areas have been reported (Barreda & Palazzesi 2007, Barreda et al. 2010). Considering the distribution of the extant members of Barnadesioideae (see Chapter 3), we suggested that the MRCA of the subfamily may have inhabit those arid areas or

transitional zones. During the Oligocene, the global and ocean temperatures progressively decreased, a pronounced drop in sea levels, and the convergence between the Nazca and South American plates activated the magmatic responses and igneous activities expanded larger in west-central Argentina, Bolivia and Peru giving the rise of the central Andes (Ortiz-Jaureguizar & Cladera 2006).

In the Miocene, the MRCA of the extant genera started to diversify around 20 Mya in agreement with the previous age estimattion for *Chuquiraga* and *Dasyphyllum* (Stuessy et al., 1996). Our results suggested that *Chuquiraga* originated in the southern South America at 21 Mya, where its section *Acanthophylla* remains distributed in xeromorphic areas along to Chilean and Argentinean Patagonia (Ezcurra 1985). Meanwhile, *C.* sect. *Chuquiraga* dispersal towards the Northeastern in Central Andes agreeing with the ages implied for its rising (Ortiz-Jaureguizar & Cladera 2006). In the same epoch, the MRCA of *Dasyphyllum* originated at 20 Mya most probably in the humid areas like the Yungas and Atlantic Forest. Within *Dasyphyllum*, two main biogeographic patterns can be inferred: the clade 1 is a monophyletic group originated in the late Miocene at 9 Mya, comprising two endemic species found in the humid mountain forests (Yungas province); and the clade 2 is a monophyletic group originated in the middle Miocene at 13 Mya, distributed eastern South America being mostly comprised by Brazilian species found in dry areas along to the Caatinga, Cerrado and Chaco or in humid areas along to the Atlantic forest (Ferreira et al. 2019).

Here, the MRCA of *Barnadesia* is inferred to originate at 13 Mya in the middle Eocene. *Barnadesia* is a genus mostly found in the mountains along to the Central Andes being Peru with the highest number of species (Urtubey 1999). Due to the extant distribution of *Barnadesia*, Stuessy et al. (1996) suggested that the genus originated near to the central Andean range and the montane forest developed on the eastern slopes. Our historical biogeographical reconstruction proposed that *Barnadesia* originated in Central and North Andes and refused the hypothesis of a younger origin for the genus during the Pliocene present by Stuessy et al. (1996).

In the last 10 Mya, a clade comprising the three most enigmatic genera of the Barnadesioideae history arose. *Archidasyphyllum* is a genus comprising the two largest tree species endemics to Chilean and Argentinean *Nothofagus* forests (Ferreira et al. 2019). Our results placed the MRCA in

the southern South America at 4 Mya during the Pliocene. *Arnaldoa* is a genus comprising three shrubby species endemics to xeromorphic areas from Northern Peru to Southern Ecuador (Stuessy & Sagástegui 1993, Ulloa et al. 2002). Our biogeographic studies inferred that the MRCA of the genus was in the Central or North Andes at 7 Mya during the Miocene. *Fulcaldea* is a genus comprising two tree or shrubby species with a remarkable disjunct distribution (Funk and Roque 2011). *Fulcaldea stuessyi* is found in Chapada diamantina (Brazil) and *F. laurifolia* is found in southern Ecuador to northwestern Peru (Ferreyra 1995). Funk and Roque (2011) postulated that the disjunct distribution in *Fulcaldea* can be explained by a large dispersal event since they have well-developed pappus. We recommend futher studies in this clade since our historical biogeographic reconstructions were largely unambiously.

Lastly, our macroevolutionary studies inferred that the diversification rates are constant and homogenous through the time (Fig. 3, Supplementary Figure 1), being the speciation and extinction rates inferred as very low (Fig. 3). However, we are aware that our results could be biased by the low number of tips since BAMM has been critically criticized (Cooper et al., 2016). Nonetheless, our results agree with previous estimations that the diversification rates are low in the early branches of Compositae (Panero & Crozier, 2016, Mandel et al., 2019).

5. Conclusions

This study presents the first macroevolutionary and historical biogeographic inferences of Barnadesioideae based fossil time-calibrated tree. We proposed that Barnadesioideae originated in southern South America at 49.1 Mya during the Eocene. Also, we suggested that the Andes uplift is a possible vicariant event that may have influenced the divergence of the major clades during the Miocene. Macroevolutionary studies propose that the speciation and extinction rates are homogeneous, constant and very low through the time, and did not indicate any shift in the phylogeny. An expanded sampling of the taxa in addition to paleoclimatic and trait-dependent

analyses are needed to continue to shed light on the evolutionary history of the subfamily Barnadesioideae.

Data Availability statement

All geographic records and scripts produced in this project is freely available on Dryad Digital Repository (Ferreira et al., in prep.).

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Biosketch

Paola de Lima Ferreira is interested in understanding how biodiversity has been shaped in space and time. She is mainly focused on South America in general, where she uses the sunflower family (Compositae) with focus on basal lineages as a model to unravel the historical assembly of their biotas. She conducted her PhD at the University of São Paulo (FFCLRP, Brazil), as part of the 'Comparative Biology' programme, in collaboration with the Antonelli Lab at the University of Gothenburg, Sweden, and Missouri Botanical Garden, the United States of America.

Author contributions: P.L.F., R.B., M.G., C.D.B. and A.A. conceived the ideas; P.L.F. collected the data; P.L.F, R.B., and C.D.B. analyzed the data; P.L.F. led the writing with contributions from all authors.

Figure 1. Representative species of Barnadesioideae. A) *Barnadesia arborea* Kunth. B) *Chuquiraga weberbaueri* Tovar. C) *Schlechtendalia luzulifolia* Less. D) *Dasyphyllum reticulatum* (DC.) Cabrera. Photo Credits: A. Carmen Ulloa. B. Gari Ccana-Ccapatinta. C. Gustavo Heiden. D. Mauricio Mercadante.

Figure 2. Dated molecular phylogeny of Barnadesioideae and node calibrated with four fossils pruning the outgroups. The colours and the numbers at the tips represent the biogeographical regions of the extant species used for the ancestral range reconstruction according to the map. Ancestral nodes might be estimated in multiple regions (legend). White sections of the pie charts represent all ranges combined with a probability of <.05% inferred for that node. Qua = Quaternary, Pli = Pliocene, Ple = Pleistocene.

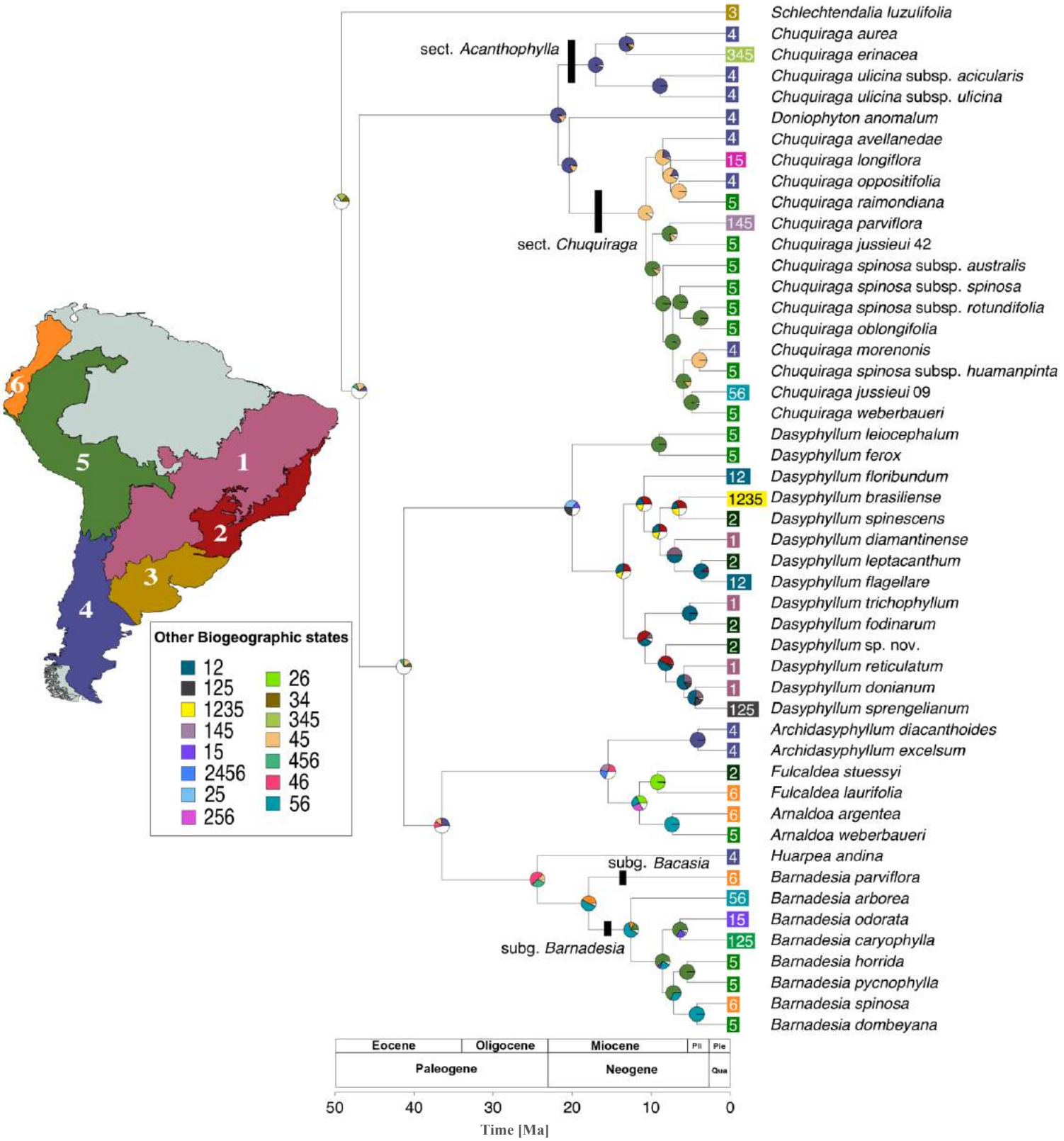
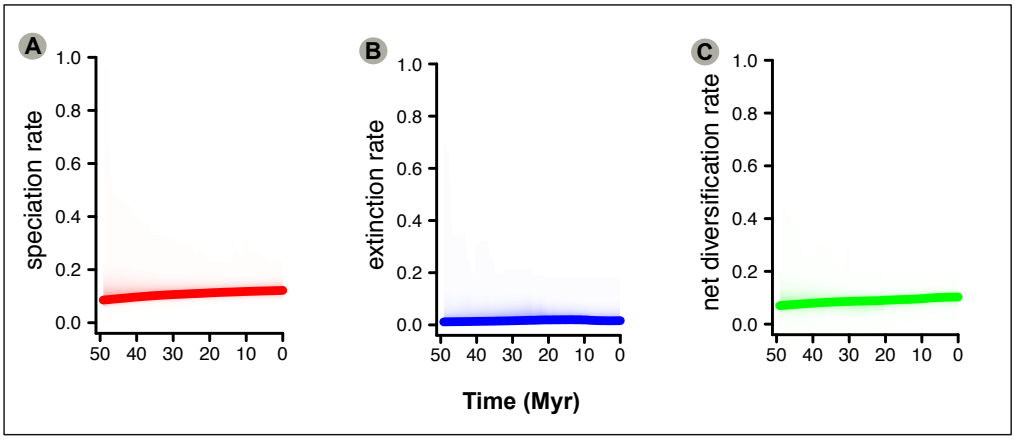


Figure 3. Phylogenetic pattern of Barnadesioideae diversification. Rates-through-time rates per million years plots with curved lines represent the median values with 95% confidence intervals showing: (A) Speciation, (B) Exctintion (B), and (C) Net-diversification: speciation (λ) - extinction (μ). (D) Time calibrated phylogenetic tree with branches coloured in proportion to the marginal density of specific evolutionary rates showing homogeneous pattern and do not indicate shifts in the phylogeny.



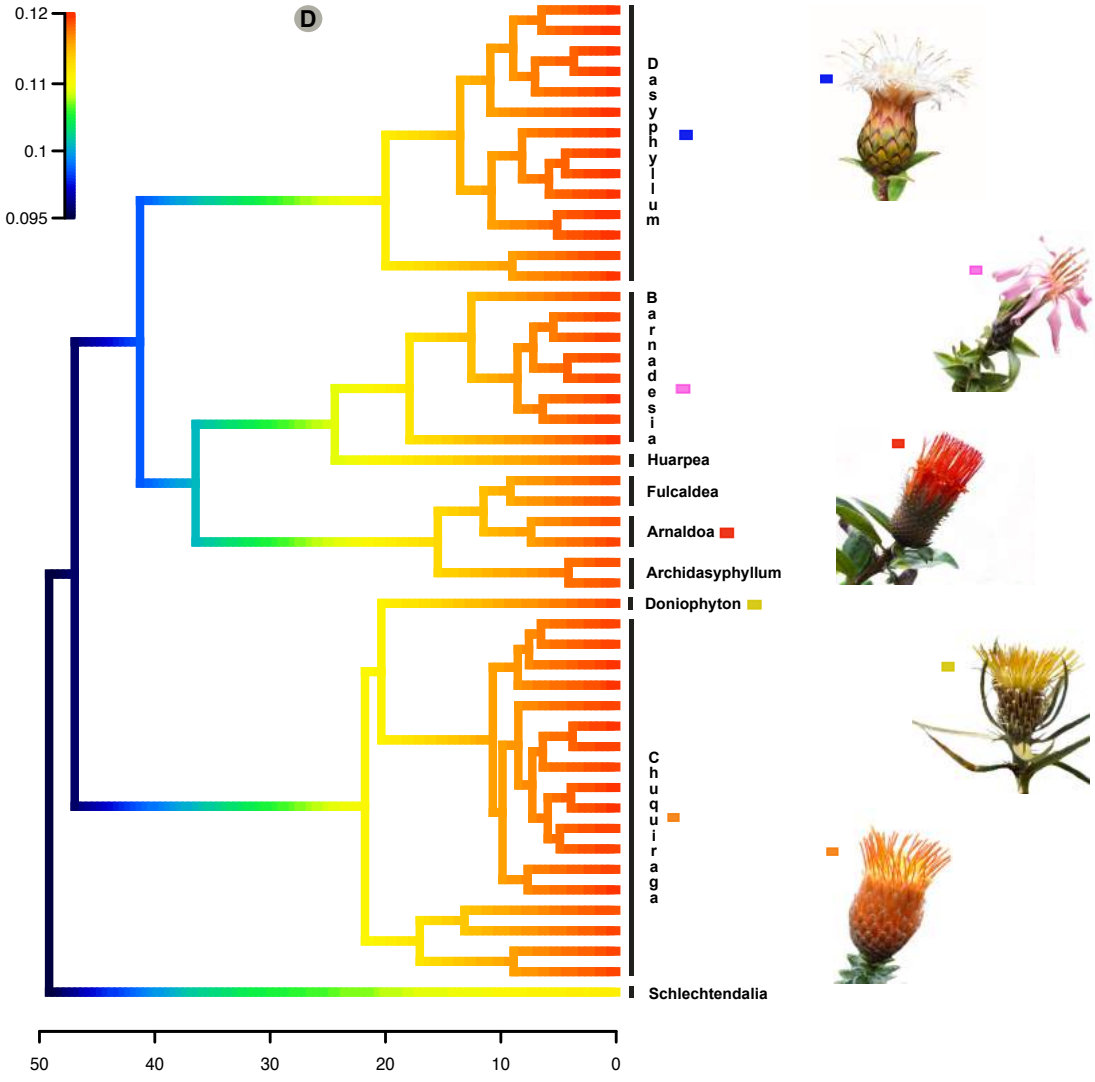


Table 1. Taxonomic sampling using in the historical biogeographic and macroevolutionary analyses.

TAXA
Asteraceae
Subfamily Barnadesioideae
Archidasyphyllum diacanthoides P.L. Ferreira, Saavedra & Groppo
Archidasyphyllum excelsum P.L. Ferreira, Saavedra & Groppo
Arnaldoa argentea C. Ulloa, P. Jørg. & M.O. Dillon
Arnaldoa weberbaueri (Muschl.) Ferreyra
Barnadesia arborea Kunth
Barnadesia caryophylla (Vell.) S.F. Blake
Barnadesia dombeyana Less.
Barnadesia horrida Muschl.
Barnadesia odorata Griseb.
Barnadesia parviflora Spruce ex Benth. & Hook. f.
Barnadesia pycnophylla Muschl.
Barnadesia spinosa Less. ex Urtubey
Chuquiraga aurea Skottsb.
Chuquiraga avellanedae Lorentz
Chuquiraga erinacea (D. Don) C. Ezcurra
Chuquiraga jussieui 09 Gmel.
Chuquiraga jussieui 42 Gmel.
Chuquiraga longiflora (Griseb.) Hieron.
Chuquiraga morenonis (Kuntze) C. Ezcurra
Chuquiraga oblongifolia Sagást. & Sánchez Vega
Chuquiraga oppositifolia D. Don
Chuquiraga parviflora (Griseb.) Hieron.
Chuquiraga raimondiana A. Granda
Chuquiraga spinosa subsp. australis C. Ezcurra
Chuquiraga spinosa subsp. huamanpinta C. Ezcurra
Chuquiraga spinosa subsp. rotundifolia (Wedd.) C. Ezcurra
Chuquiraga spinosa Less. *subsp. spinosa*
Chuquiraga ulicina subsp. acicularis (D. Don) C. Ezcurra
Chuquiraga ulicina subsp. ulicina Hook. & Arn. subsp. *ulicina*
Chuquiraga weberbaueri Tovar
Dasyphyllum brasiliense (Spreng.) Cabrera
Dasyphyllum diamantinense Saavedra & M. Monge
Dasyphyllum donianum (Gardner) Cabrera
Dasyphyllum ferox (Wedd.) Cabrera
Dasyphyllum flagellare (Casar.) Cabrera
Dasyphyllum floribundum (Gardner) Cabrera
Dasyphyllum fodinarum (Gardner) Cabrera
Dasyphyllum leiocephalum (Wedd.) Cabrera
Dasyphyllum leptacanthum (Gardner) Cabrera
Dasyphyllum reticulatum (DC.) Cabrera

Dasyphyllum sp. nov.
Dasyphyllum sprengelianum (Gardner) Cabrera
Dasyphyllum spinescens (Less.) Cabrera
Dasyphyllum trichophyllum (Baker) Cabrera
Doniophyton anomalum (D. Don) Kurtz
Fulcaldea laurifolia (Bonpl.) Poir.
Fulcaldea stuessyi Roque & V.A. Funk
Huarpea andina Cabrera
Schlechtendalia luzulifolia Less.

Tribe Cardueae
Carthamus tinctorius L.

Tribe Cichorieae
Taraxacum kok-saghyz L.E. Rodin

Tribe Vernonieae
Centrapalus pauciflorus (Willd.) H. Rob.

Tribe Eupatorieae
Conoclinium coelestinum(L.) DC.

Tribe Heliantheae
Phoebanthus tenuifolius (Torr. & A. Gray) S.F. Blake
Helianthus annus L.
Helianthus porteri (A. Gray) Pruski
Helianthus verticillatus Small
Helianthus argophyllus Torr. & A. Gray

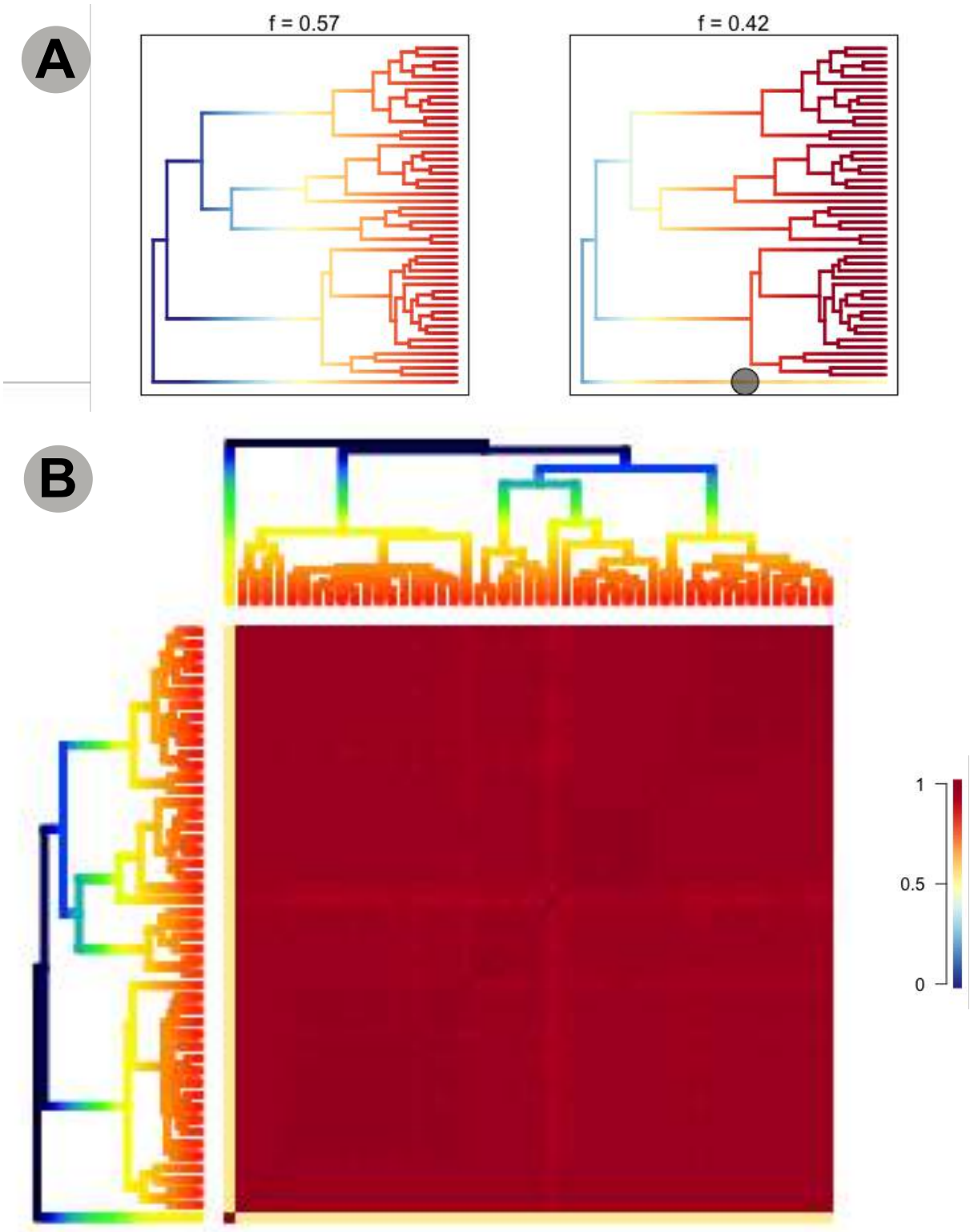
Tribe *Senecioneae*
Senecio vulgaris L.

Calyceraceae
Nastanthus patagonicus Speg.

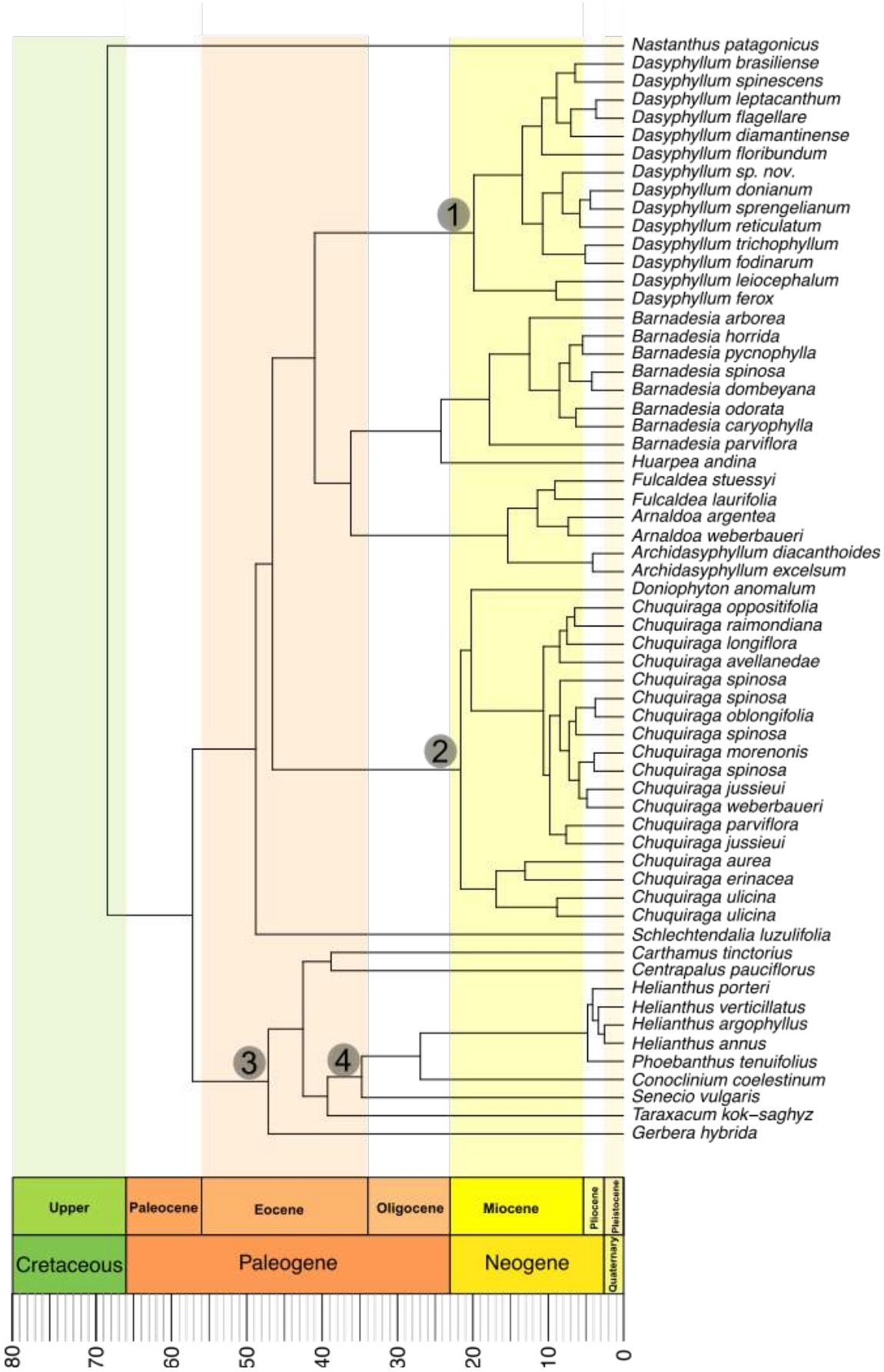
Supplementary Material

Supplementary Table 1. Total diversity and proportional representation in phylogeny for each Barnadesioideae clade.

Clade	Total Clade Species Diversity	# Representatives in tree	Proportion sampled	References
Arnaldoa	3	2	0.67	Stuessy & Sagástegui (1993); Ulloa et al. (2002)
Barnadesia	19	8	0.42	Urtubey (1999); Hind (2001)
Chuquiraga *Doniophyton* *Duseniella*	25	14	0.56	Ezcurra (1985); Harling (1991); Sagástegui & Sánchez (1991); Granda (1997); Katinas & Stuessy (1997)
Dasyphyllum	31	14	0.45	Saavedra (2011); Ferreira et al. (2019)



Supplementary Figure 1. (A) Time-calibrated phylogenetic tree with branches coloured in proportion to the marginal density of specific evolutionary rates and the probabilliy of distinct rate shift configurations in the posterior density simulated with BAMM. (B) Macroevolutionary cohort matrix displaying the pairwise probability of any two species share a common macroevolutionary pattern, indicating in this case evidence of a homogenous pattern with no shifts in any location in the phylogeny.



Supplementary Figure 2. Dated molecular phylogeny of Barnadesioideae including outgroups. Numbers above the branches indicated the four node calibrations used in this study.

Chapter 03

A generic synopsis of Barnadesioideae (Compositae)

Arnaldoa weberbaueri
© Marco Rabanal

"'If you do not know the names of things, the knowledge of them is lost too"
(Linnaeus, 1751)

Abstract

Barnadesioideae comprises 10 genera and 84 species endemic to South America, which most of the species found in xeromorphic areas along to the Andean and Patagonian region. Previous phylogenetic hypotheses have clarified the relationships within the subfamily showing that there are many non-monophyletic groups in different taxonomic ranks. As a result, taxonomic changes have been proposed over the last decades in order to reflect classifications only comprising monophyletic groups. In the present study, we propose a generic synopsis of the subfamily Barnadesioideae based on the most recent generic circumscriptions including a key, expanded morphological descriptions, information on geographical distribution, habitat, photographs and taxonomic notes of all genera.

Keywords

Asteraceae, distribution, Neotropical flora, morphology, taxonomy.

Resumo

Barnadesioideae compreende 10 gêneros e 84 espécies endêmicos da América do Sul, no qual a maioria das espécies são encontradas em áreas xeromórficas ao longo da região Andina e Patagônica. Hipóteses filogenéticas prévias têm clarificado as relações dentro da subfamília mostrando que há vários grupos não monofiléticos em diferentes níveis taxonômicos. Como resultado, mudanças taxonômicas têm sido propostas ao longo das últimas décadas a fim de refletir classificações que compreendem apenas grupos monofiléticos. No presente estudo, nós propomos uma sinopse genérica para a subfamília Barnadesioideae baseada nas mais recentes circunscrições incluindo uma chave de identificação, descrições morfológicas expandidas, informações sobre distribuições geográficas, habitat, fotografias e notas taxonômicas de todos os gêneros.

Palavras-chave

Asteraceae, distribuição, flora Neotropical, morfologia, taxonomia.

Introduction

The subfamily Barnadesioideae (D.Don) K.Bremer & R.K.Jansen (Bremer & Jansen 1992: 415) consists of ten genera and 84 South American species, distributed from Venezuela to Argentina (Figure 1), which most of them are found in xeromorphic areas in the Andean and Patagonian region (Bremer 1994, Stuessy et al. 2009). The subfamily is characterized by having axillary spines, rarely solitary, in pairs or fascicles, straight or curve, convergent (when the spines follow the same direction, Figure 2A-B) or divergent (when the spines follow different directions, Figure 2C), and by the "barnadesioid trichomes" a pubescence of unbranched three-celled hairs on the corollas, cypselae and pappus (Fig. 2D; Cabrera 1959, Urtubey 1999, Erbar & Leins 2000, Stuessy et al. 2009).

Barnadesioideae were first recognized as a group by Don (1830), who described the tribe Barnadeseae (1830: 273) classifying 12 species in two genera, *Barnadesia* and *Chuquiraga*. Later, the genera of Barnadesiodieae were recognized in different taxonomic ranks (Lessing 1832, Candolle 1838, Bentham 1873, Hoffmann 1893, Cabrera 1961). In the beginning of the molecular phylogenies, phylogenetic studies recovered the subtribe Barnadesiinae as sister group to the rest of the family (Jansen & Palmer 1987, Bremer 1987, Jansen et al. 1992), and so, the subtribe was elevated to the subfamilial rank, as Barnadesioideae (Bremer & Jansen 1992).

Although Barnadesioideae comprises less than 1% of the species currently circumscribed in Asteraceae, the subfamily presents a great diversity in many aspects such as anatomy (Ezcurra 1985, Urtubey 1999, Padin et al. 2015a), chemistry compounds (Bohm & Stuessy 1995, Mediondo et al. 1997, 2000, Bohm & Stuessy 2001, Ccana-Ccapatina et al. 2018), pollen (Skvarla et al. 1977, Hasen 1991, Urtubey 1997, Urtubey & Telléria 1998, DeVore et al. 2000, Zhao et al. 2000, Telléria et al. 2015) chromosome counts (Diers 1961, Heiser 1963, Olsen 1980, Ciadella & López 1981, Wulff 1984, Cristóbal 1986, Wulff 1990, Stuessy & Sagástegui 1993, Strother & Panero 1994), morphology (Stuessy & Urtubey 2006, Stuessy et al. 2009), and habitats (Gruenstaeudl et al. 2009, Stuessy et al. 2009), which turns the subfamily an interesting group to study evolutionary trends in the Neotropics. Moreover, since Barnadesioideae is proposed as the sister group to the rest of the Asteraceae (Jasen

& Palmer 1987, 1988), evolutionary studies allied to morphology, taxonomy, nomenclature and historical biogeography can shed light into the early evolution of the family.

Despite the fact of the previous efforts to shed light on the evolution of Barnadesioideae, understanding the relationships of the subfamily can be considered a challenge. Nowadays, there is no doubt that the subfamily is monophyletic; however, previous phylogenetic analyses have provided different hypotheses regarding the monophyly and between the relationships of genera, infrageneric classification and also species (Bremer 1994, Stuessy et al. 1996, Gustafsson et al. 2001, Urtubey & Stuessy 2001, Gruenstaeudl et al. 2009, Padin et al. 2015b, Ferreira et al. 2019). Probably, previous phylogenetic results could be affected by the taxa selected, choose of the molecular regions, and phylogenetic reconstruction (Nabhan & Sarkar 2012). With the advance of next-generation sequencing, phylogenies including thousands of molecular markers and species allied to the new mathematical models and coalescence methods could provide better insights into this group (see Chapter 1 or Ferreira et al. in prep.).

The present work provides a taxonomical contribution which aims to increase the understanding of this diverse subfamily, providing updated generic circumscriptions and also a key, morphological descriptions, photographs, geographical distribution, maps, habitat, and notes for all genera currently circumscribed in Barnadesioideae.

Material and Methods

This study was based on a bibliographic review of Barnadesioideae (Cabrera 1959, Ezcurra 1985, Hansen 1991, Harling 1991, Sagástegui & Sánchez 1991, Stuessy & Sagástegui 1993, Ferreyra 1995, Bremer 1994, Granda 1997, Katinas & Stuessy 1997, Urtubey 1999, Hind 2001, Ulloa et al. 2002, Urtubey & Stuessy 2001, Stuessy & Urtubey 2006, Stuessy et al. 2009, Funk & Roque 2011, Saavedra 2011, Saavedra et al. 2014, Saavedra et al. 2018, Ferreira et al. 2019), examination of ca. 3,500 voucher materials from the herbaria: ALCB, B, CEPEC, ESA, GB, HRCB, HUEFS, QCNE, QCA, K, MO, RB, SPF, SPFR, UEC, UETC, UFU, WU (acronyms according to Thiers 2019), and

observations of living plants during field works conducted in Brazil and Ecuador between 2012 and 2018. Information on pollen and chromosome number present in the taxonomic treatment were directly extracted from the literature (Diers 1961, Heiser 1963, Skvarla et al. 1977, Olsen 1980, Cialdella & López 1981, Hansen 1991, Wulff 1984, Cristóbal 1986, Wulff 1990, Stuessy & Sagástegui 1993, Strother & Panero 1994, Stuessy et al. 1996, Urtubey 1997, Urtubey & Tellería 1998).

Anther appendages exhibit a great morphological variation in Barnadesiodieae. Therefore, we examined the variation from herbarium material or from 70% ethanol-preserved flowers with scanning electron microscopy (SEM). Flowers from herbaria material were rehydrated with hot water, and processed in the same manner as the ethanol-preserved. Anthers were critically point dried, sputter coated with gold and analyzed using an EVO 50 scanning electron microscope (Carl Zeiss, Cambridge, UK).

They key was proposed in order to reflect the recent modifications on generic circumscriptions and its morphological diversity within Barnadesioideae. Morphological terms follow Radford et al. (1974) for indument and shapes, whereas corolla types follow Stuessy & Urtubey (2006), except for the genus *Chuquiraga* following Ezcurra (1985). Geographical distribution was obtained from herbarium specimens and bibliographical review cited above. For the maps, when the coordinates were not available, we georeferenced using the municipality coordinate provided by NASA (https://mynasadata.larc.nasa.gov/), last accessed December 2018) and the records were plotted using QGIS version 3.2.1 (QGIS Development Team 2019).

Results and Discussion

Taxonomic treatment

Barnadesioideae (D.Don) K.Bremer & R.K.Jansen (1992: 415). **Type:** *Barnadesia* Mutis ex. L.f.

Annual or perennial herbs, subshrubs, shrubs, trees, or woody vines, up to 30m tall, usually multi-stemmed with nodal spines in pairs or fascicles, straight or curved, convergent or divergent.

Stems erect or decumbent, single- to much-branched, striated, with or without lenticels, cylindrical or rarely flat, with or without scales at base, glabrous or with diverse types of trichomes, unarmed or armed with axillary spines rarely solitary, in pairs or fascicles, straight or curve, divergent or convergent, glabrous, pubescent or villous at base and becoming glabrous towards the apex. *Leaves* simple, alternate, opposite, fasciculate rarely rosulate or opposite at base, alternate, opposite on stems or amplexicaul, sessile to petiolate, persistent to deciduous, blade elliptic to orbicular, chartaceous to coriaceous, rarely carnose, 1-, 3- or 5-nerved, pale or lustrous, glabrous or with diverse types of trichomes, apex unarmed, mucronate or spiny, leaf margin entire, flat, revolute, involute, rarely plicate, glabrous or commonly ciliate. *Capitulescence* (the secondary arrangement of capitula) terminal or axillary, monocephalous, cymose, corymbose, panicle, racemose, speciform or umbellate. *Capitula* homogamous or heterogamous, discoid, disciform or radiate, 5—100-flowered, sessile to pedunculate, involucre cylindrical to campanulate, 3—14 seriate, phyllaries imbricate, scarious to coriaceous, commonly corful, erect to reflexed, glabrous to densely villous, triangular-ovate to linear, apex unarmed, mucronate or spiny, margin flat or rarely reflexed, glabrous or commonly ciliate. Receptacule flat, pilose or rarely convex, glabrous. *Flowers* isomorphic or dimorphic, bissexual or pistillate by the adroecium atrophy or staminate by the suppression of the style, corolla bilabilate, ligulate, pseudobilabiate, tubular, ligulate, 3—5-lobed, cream, white, yellow, orange, red, pink to purple, glabrous to villous on both surfaces. *Anthers* 5, rarely 3 or 4, apical appendage acute, bifid, apiculate, emarginated, obtuse, basal appendage ecaudate or caudate, ecalcarate or calcarate, stamens inserted from the base to the throat of corolla, filaments free, glabrous, rarely partial connate or connate into a staminal tube, villous. *Style* cylindrical or rarely swollen bellow the branching point, cream, white, yellow, red, purple or orange. *Cypsela* cylindrical, fusiform or turbinate, villous or rarely glabrous. *Papus* barbellate, plumose, scale, setaceous, rarely absent, 1-seriate, connate at base, shorter to equal to the corolla length, glabrous or with bristles with, pink or red. *Pollen* with or without intercorpal depressions, psilate, lophate, microechinate, sparsely microechinate, scabrate-microechinate. *Chromossome numbe*r (haloploid numbers) = 8, 12, 24, 25, 27, 31, 48, 50, 54.

Key to Genera of Barnadesioideae

1. Herbs or subshrubs ……2

- Shrubs, trees or woody vines.....5

2. Axillary spines present.....*Doniophyton*

- Axillary spines absence.....3

3. Capitula discoid.....*Schlechtendalia*

- Capitula disciform or radiate.....4

4. Leaves carnose; capitula with marginal flowers female and corollas tubular 10-40, disc flowers hermaphroditic and corollas tubular 30-95, anthers caudate.....*Duseniella*

- Leaves not carnose; capitula with marginal flowers female and corollas subbillabiate 5, disc flower hermaphroditic and corolla tubular 1, anthers ecaudate……..…….....*Huarpea*

5. Capitula uni-flowered; style swollen below the branching point .....*Fulcaldea*

- Capitula pluri-flowered; style not swollen below the branching point 6

6. Capitula radiate.....*Barnadesia*

- Capitula discoid.....7

7. Leaves with unninerved venation; anthers with apical appendage obtuse.....*Archidasyphyllum*

- Leaves with pinnatinerved or rarely unninerved (only in *Chuquiraga*) venation; anthers with apical appendage acute, rounded or bilobed.....8

8 Corolla orange, red, purple, yellow, or rarely white (*A. argentea*); anthers with apical appendage acute or rounded.....9

- Corolla white or yellow; apical appendage bilobed.....*Dasyphyllum*

9. Corolla subbillabiate; anthers with fillaments inserted at the corolla throat.....*Arnaldoa*

- Corolla tubular; anthers with filaments inserted at the base of the corolla.....*Chuquiraga*

1. *Archidasyphyllum* (Cabrera) P.L.Ferreira, Saavedra & Groppo (2019)

Type: *Archidasyphyllum diacanthoides* (Less.) P.L.Ferreira, Saavedra & Groppo

Trees, up to 30 m tall, multi-stemmed. *Stems* erect or decumbent, much-branched, lenticelate, cylindrical, scales imbricate at base, glabrous, strigose, villous, or velutinous, unamerd or with axillary spines in pairs, rarely fascicles, straight, convergent or rarely divergent, glabrous or rarely sparse pubescent at base and becoming glabrous the apex. *Leaves* alternate-spiralate, sessile to petiolate, persistent to deciduous, blade narrow eliptic to orbiculate, coriaceous, uninerved, pale or lustrous, glabrous or pubescent in both surfaces, apex unarmed, mucronate or spiny, leaf margin flat, ciliate. *Capitulescence* terminal or axillary, monocephalous or speciform. *Capitula* homogamous, discoid, 20—35-flowered, sessile to pedunculate, involucre campanulate, 3—4 seriate, phyllaries coriaceous, green or brown, erect or rarely slightly reflexed, glabrous or densely villous, ovate-triangulate grading to lanceolate, apex unarmed or mucronate, margin flat, ciliate. *Receptacle* flat, pilose. *Flowers* isomorphic, bisexual, or pistillate by the gynoecium athrophy, corolla tubular, ligulate, subbilabiate or bilabiate, 5-lobed, white to cream, externally sericeous at lobes apex. *Anthers* 5, apical appendage obtuse or emarginated, basal appendage caudate, calcarate, stamens inserted on the corolla tube, filaments free, glabrous. *Style* cylindrical, cream to yellow. *Cypsela* fusiforms, densely villous. *Pappus* plumose, slight shorter or equal corolla length, britlesh withish. *Pollen* with intercolpar depressions. *Chromosome number* = unknow.

Fig. 3; 11A; 12A and 13E.

Distribution and habitat:- *Archidasyphyllum* comprises two species restricted to the *Nothofagus* forest of central Chile and adjacent central-western areas of Argentina above 1200m high (Cabrera 1959, Ferreira et al. 2019).

Notes:- *Archidasyphyllum* can be distinguished from the rest of Barnadesioideae by a set of characters: monoecious or *gynodioecious* trees reaching 30m tall, discoid capitula with obtuse or emarginated apical appendages. Cabrera (1959) in the taxonomic revision of *Dasyphyllum* considered *Archidasyphyllum* as a subgenus due to the high morphological similarity (both genera have discoid

capitula, monoecious or *gynodioecious* breed system, pollen with intercolpar depressions, corolla shape and color). Moreover, Cabrera (1959) believed that *Dasyphyllum*, especially the subgenus *Archidasyphyllum* (*Archi* – from the ancient Greek ἀρχι- chief, early + *Dasyphyllum* – a genus of Barnadesioideae) was the most primitive group and should be derivated the other genera within the subfamily. However, molecular phylogenies recovered *Dasyphyllum* subgenus *Archidasyphyllum* as sister to *Arnaldoa* and *Fulcaldea* clade (Gustafsson et al. 2001, Gruenstaeudl et al. 2009, Ferreira et al. 2019, see Chapter 1). Therefore, Ferreira et al. (2019), elevated the subgenus *Archidasyphyllum* as a genus rank, *Archidasyphyllum* based on molecular phylogenetic, morphology, and biogeographical evidence. Although all phylogenetic analyses based on molecular data recover *Archidasyphyllum* as sister to *Fulcaldea* and *Arnaldoa*, this clade is morphologically diverse and synapomorphies that support the relationships are still unknown. (Funk & Roque 2011, Ferreira et al. 2019).

2. ***Arnaldoa*** Cabrera (1962: 39)

Type: *Arnaldoa weberbaueri* (Muschl.) Ferreyra

Arching shrubs, up to 4 m tall, multi-stemed. *Stems* erect or decumbent, much-branched, lenticelate, glabrous, velutinous or densely tomentose, cylindrical, axillary spines in pairs, straight, convergent or divergent, glabrous or tomentose at base and becoming glabrous towards the apex. *Leaves* alternate, peciolate, persistent or deciduos, blade elliptic to oblate, coriaceous, trinerved, pale or lustrous, adaxially glabrous to tomentose, abaxially glabrous to densely tomentose, villous, floccose, lanose, apex unarmed, mucronate or spiny, leaf margin flat or slightly revolute, glabrous or ciliate. *Capitulescence* terminal, monocephalous. *Capitula* homogamous, discoid, 30—95-flowered, sessile, involucre campanulate, 8—15 series, phylaries coriaceous, brown or black, erect or reflexed, glabrous to densely tomentose, ovate-triangulate grading to lanceolate, apex mucronate or spiny, margin flat, ciliate. *Receptacle* flat, pilose. *Flowers* isomorphic, bisexual, corolla subbilabiate, 5-lobed, red, purple, orange or white, villous. *Anthers* 5, apical appendage obtuse, basal appendage caudate, calcarate, stamens inserted on corolla throat, filaments free, glabrous or villous. *Style*

cylindrical, red, purple, orange, white to cream. *Cypselae* turbinate or cylindrical, densely villous. *Pappus* plumose, slightly shorter or equal corolla length, britlesh withish. *Pollen* with intercolpar depression, microechinate. *Chromosome number* = 24-27.

Fig. 4; 11B and 13F.

Distribution and habitat:- *Arnaldoa* comprises three shrubby species distributed in xeromorphic areas of Northern Peru and Southern Ecuador (Stuessy & Sagástegui-Alva 1993, Ulloa et al. 2002).

Notes:- *Arnaldoa* is morphologically closed to *Chuquiraga* by sharing the shrubby habit, discoid capitula, colorful corollas and stigma, anthers long caudate, and microechinate pollen. However, *Arnaldoa* is distinguished by the subbilate corolla (*vs.* tubular in *Chuquiraga* sensu Ezcurra 1985) and stamens inserted on the throat of corolla tube (*vs.* base of corolla in *Chuquiraga*). Phylogenetic hypotheses based on molecular data recovered *Arnaldoa* as close related to *Fulcadea* (Gustafsson et al. 2001, Gruenstaeudl et al. 2009, Funk & Roque 2011, Ferreira et al. 2019, see Chapter 1). Although the morphological differences between *Arnaldoa* and *Fulcaldea* are remarkable (*Arnaldoa* being solitary capitula with 30-95 flowered, subbilabiate corollas, caudate anthers, microechinate pollen, cylindrical style *vs* capitula arranged in synflorescence with 1-flowered, tubular corollas, ecaudate anthers, spinulose pollen, swollen style bellow the branching point in *Fulcaldea*), they share the arching shrubby habit, red corollas, and geographical distribution.

3. ***Barnadesia*** Mutis ex L. f. (1782: 55)

Type: *Barnadesia spinosa* L. f.

Arching shrubs or trees, up to 20 m tall, multi-stemed. *Stems* erect, decumbent or sometimes scandent, much-branched, lenticelate, cylindrical or rarely flat, with or without scales imbricate at base, glabrous, tomentose, villous, velutinous, sericeous, rarely dendritic, unarmed or with axillary spines in pairs or fascicles, straight or curved, convergent or divergent, glabrous, villous or pubescent at base and becoming glabrous towards the apex. *Leaves* alternate or in fascicles, sessile to pedunculate, persistent to deciduous, blade elliptical to obovate, chartaceous to coriaceous, uni- or

trinerved, pale or lustrous, glabrous to densely villous, tomentose or sericeous on both surfaces, apex unarmed, mucronate or spiny, leaf margin flat, glabrous or ciliate. Capitulescence terminal or axillary, monocephalous, corymbose or racemose. *Capitula* heterogamous or homogamous, radiate, 9- or 16-flowered, sessile to pedunculate, involucre cylindrical, turbinate or campanulate, 6—14 seriate, phyllaries scarious to coriaceous, green, brown, reddish brown to purple, erect or reflexed, glabrous or pubescent, ovate grading to lanceolate, apex unarmed, mucronate or spiny, margin flat, ciliate. *Receptacle* flat, pilose. *Flowers* dimorphic or isomorphic. *Ray flowers* 8 or 13, bisexual, corolla subbilabiate, 5-lobed, red to purple, pink or rarely white, externally villous, internally glabrous or sericeous on throat, rarely sericeous on tube. *Anthers* 5, apical appendage obtuse, basal appendage ecaudate, ecalcarate, stamens inserted on corolla throat, filaments free, partial connate or connate into a staminal tube, glabrous. *Style* cylindrical, purple. *Cypsela* cylindric or rarely turbinate, villous. *Pappus* plumose, shorter than the corolla length. *Disc flowers* 1 to 3, bisexual or pistillate by the adroecium atrophy or staminate by the suppression of the style (only in *Barnadesia* subgenus *Bacasia*), corolla tubular, subbilabiate or ligulate, 3—5-lobed, purple, pink or rarely white, externally and internally glabrous or villous. *Anthers* 3—5, apical appendage obtuse, basal appendage ecaudate, ecalcarate, stamens inserted at base, tube or throat, filaments free or rarely connate into a staminal tube, glabrous. *Style* cylindrical, purple. *Cypsela* turbinate, densely villous. *Pappus* plumose, barbellate or setaceous, when barbellate or setaceous usually recurved at maturity, glabrous to villous, equal or shorter than the corolla, britlesh withish. *Pollen* lophate, psilate. *Cromossome number* = 12, 24, 25, 26, 31, ca. 48, ca. 50.

Fig. 5; 11C; 12 B and 13C.

Distribution and habitat:- *Barnadesia* is the third largest genus of Barnadesioideae comprising 19 species, distributed along to the Andes from Colombia to Argentina, with the highest number of species found Peru (Urtubey 1999, Stuessy et al. 2009).

Notes:- The species of the genus are classified in two subgenera (following Urtubey 1999): subg. *Barnadesia* with 17 species from Colombia to Argentina and one species is found in Brazil (*B.*

caryophylla) being recognized by the sessile or subsessile leaves disposed in fascicles, sessile or shortly pedunculate capitula, disc flowers with subbilabiate or ligulate corolla with and pappus simple or barbellate; and the subg. *Bacasia* comprises two species from Colombia to Bolivia being recognized by the alternate and petiolate leaves, pedunculate capitula, disc flower with tubular corolla and plumose pappus.

Barnadesia is the type genus of the subfamily and can be distinguished by the other genera by a set of characters: arching shrub or trees, radiate capitula, 8 or 13 ray flowers, 1 or 3 disc flowers, colorful corolla (pink, red, purple, white), ecalcarate and ecaudate anthers, apical appendage obtuse, filaments free, partial or connate into a staminal tube, pappus of the disc flower usually setaceous or barbellate that recurved at maturity, and by the lophate pollen.

4. *Chuquiraga* Juss. (1789: 178)

Type: *Chuquiraga jussieui* J.F. Gmel.

Shrubs or dwarf cushion-forming shrubs, up to 2 m tall, much-stemed. *Stems* erect, much-branched, without lenticels, cylindrical, rarely scales imbricate at base, glabrous, tomentose, strigose, villous, sericeous, unarmed or with axillary spines in pairs or fascicles, straight, convergent or divergent, glabrous, villous or pubescent at base and becoming glabrous towards the apex. *Leaves* alternate or opposite, sessile to subsessile, persistent to deciduous, blade acicular to ovate, coriaceous, uni- or tri-nerved, pale or commonly lustrous, glabrous to densely sericeous on both surfaces, apex mucronate or spiny, leaf margin flat or involute, glabrous or ciliate, sometimes the abaxial with proeminent midvein**.** *Capitulescence* terminal, monocephalous. *Capitula* homogamous, discoid, 5—100-flowered, sessile, involucre cylindrical, turbinate or campanulate, 4—14 seriate, phyllaries scarious or commonly coriaceous, green, brownish, yellow to orange, erect or reflexed, glabrous to densely sericeous, triangular-ovate triangular-ovate to lanceolate,
apex mucronate or spiny, margin flat or reflexed, glabrous or ciliate. *Receptacle* flat, pilose. *Flowers isomorphic*, bisexual, tubular, 5-lobed with often separated from the other lobes by unequal and deep

incisions, yellow, orange**,** villous. *Anthers* 5, apical appendage acute, basal appendage caudate, calcarate, stamens inserted at corolla base, filaments free, glabrous. *Style* cylindrical, yellow to orange**.** *Cypselae* turbinate, densely villous. *Pappus* plumose*,* shorter to equal corolla length, britlesh withish. *Pollen* with intercolpar depression, microechinate. *Chromosome number* = 24-27.

Fig. 6; 11D; 12C and 13B.

Distribution and habitat:- *Chuquiraga* is the second largest genus of Barnadesioideae comprising 22 species (Ezcurra 1985, Harling 1991, Sagástegui & Sánchez 1991, Granda 1997) found in xeromorphic areas along to the Andes and Patagonia from Colombia to Argentina.

Notes:- Species of *Chuquiraga* display an important commercial factor comprising several medicinal proprietaries commonly used into the phytopharmaceutical industry and commercialized by European countries. In addition, *Chuquiraga* is largely commercialized in some medicinal marketplaces in Peru and Ecuador and it is indicated for several of healthy treatments (Ccana-Ccapatinta et al. 2018). Therefore, a correct species determination and circumscription is essential for medicinal and evolutionary studies.

The species of *Chuquiraga* are classified into two sections (following Ezcurra 1985): sect. *Acanthophylla* comprises nine species distributed from Northwest Bolivia to Chile and Argentina (Fig. 6A-B) being characterized by leaves with the margin involute resembling a boat-shape (Fig. 6B), abaxial surface without the prominent midvein, and absence of axillary spines; and section *Chuquiraga* with the largest number of species classified in the genus, comprising 13 species distributed from Colombia to Argentina (Fig. 6 C-E), being characterized by the flat leaves, and the abaxial surface with proeminent midvein and by the presence of axillary spines, rarely reduced or absent. Within the section *Chuquiraga*, two series are recognized: *Chuquiraga* and *Parviflorae*, being disctintive by the length of capitula (>3cm *vs.* 0—3cm), corolla (<16mm *vs.* >17mm) and anthers (15—20mm *vs.* 8—12mm), and by the geographical distribution (from Colombia to Northwest Argentina *vs.* Western Bolivia to Argentinean Patagonia).

The great morphological variation such as habit, leaf shape, venation, trichomes, spines, involucre shape, size, bract colors, and others, turns *Chuquiraga* on the most taxonomically complex group of Barnadesioideae. After analyzing hundreds of materials of this genus, we decided to follow the corolla classification *sensu* Ezcurra (1985), and do not use the classification *sensu* Urtubey & Stuessy (2006). Alhough Stuessy & Urtubey (2006) article comprises a significant step to understand the corolla evolution in Barnadesioideae, in *Chuquiraga* it is difficult to apply a corolla classification, since this genus has different length of corolla incisions splitting the lobes. Therefore, we prefer to classify the corolla as tubular with different incision lengths (see Sagástegui & Sánchez 1991, Figure 1.I; for the corolla line drawing).

A most comprehensive phylogeny at species-level for *Chuquiraga* was performed based on molecular data by Padin et al. (2015b). The results were incongruent regarding the monophyly of the genus by the position of *Doniophyton* or *Duseniella*. Within the genus, the sections were found to be monophyletic, but the results do not corroborate the series of *Chuquiraga* sect. *Chuquiraga*, a found that was also corroborated by phylogenomic studies (see Chapter 1). Moreover, Padin et al. (2015b) in some cases not justified by the authors, included more than one sample per species casting doubts on the species delimitation. It is needed further taxonomical and morphological studies in order to propose a new classification and species circumscription.

5. ***Dasyphyllum*** Kunth (1820: 17)

Type: *Dasyphyllum argenteum* Kunth

Shrubs, trees or woody vines, up to 15 m tall, multi-stemed. *Stems* erect, decumbent or scandent, much-branched, lenticelate, cylindrical, with or without scales imbricate at base, glabrous, villous, sericeous, tomentose or velutinous, unamerd or with axillary spines in pairs or fascicles, straight or curved, convergent or divergent, glabrous or sparse pubescent at base and becoming glabrous at the apex. *Leaves* alternate-spiralate, rarely in fascicles, subsessile to petiolate**,** persistent to deciduous, blade narrow elliptic to orbiculate, chartaceous to coriaceous, 3- or 5-nerved, glabrous or pubescent,

villous, tomentose, lanose in both surfaces, pale or lustrous, apex mucronate or spiny, leaf margin flat or revolute, glabrous or ciliate. *Capitulescence* terminal or axillary, monocephalous, racemose, panicle, corymbiform or umbellate. *Capitula* homogamous, discoid, 5—60-flowered, sessile to pedunculate, involucre cylindrical to campanulate, 6—14 seriate, phyllaries scarious or commonly coriaceous, green, yellow-golden, brown or black, erect or reflexed, glabrous or densely villous, ovate-triangulate grading to lanceolate, apex unarmed, mucronate or spiny, margin flat, ciliate. *Receptacle* flat, pilose. *Flowers* isomorphic, bisexual or pistilate by the gynoecium athrophy, corolla tubular, ligulate, subbilabiate or bilabiate, 5-lobed, white to cream, externally glabrous or sericeous, internally sericeous. *Anthers* 5, apical appendage bifid, basal appendage shortly caudate, calcarate**,** stamens inserted from the base to throat corolla, filaments free, glabrous. *Style* cylindrical, cream to yellow. *Cypsela* cylindrical or fusiform, densely villous or rarely glabrous. *Pappus* plumose, shorter to equal corolla length, britlesh withish. *Pollen* with or rarely without intercolpar depressions, sparsely microechinate. *Chromosome number* = 27.

Fig. 7; 11E; 12D and 13A.

Distribution and habitat:- *Dasyphyllum* is the largest genus of Barnadesioideae comprising 31 species that occur in Tropical Andes, Atlantic Forest, Caatinga, Cerrado, and Chaco from Venezuela to Argentina distributed from Venezuela to Northwestern Argentina, but absent in the Amazon region (Ferreira *et al.* 2019).

Notes:- *Dasyphyllum* can be distinguished by the other genera by being arching shrubs, trees, or woody vines, discoid capitula, cream to white corolla, bisexual or pistilate by the gynoecium athrophy flowers, and bifid apical appendage. Previous phylogenetic hypotheses are incongruent regarding the relationships of the genus with the rest of the subfamily. A phylogeny comprising more taxa and thousands of loci is necessary to elucidate its internal clades and generic relationships.

6. ***Doniophyton*** Wedd. (1855: 7)

Type: *Doniophyton anomalum* (D. Don) Kurtz

Subshrubs, up to 8 cm tall. *Stems* erect or decumbent, much-branched, lenticelate, cylindrical, scales imbricated at base, tomentose, velutinous, axillary spines in fascicles, straight, divergent or convergent, glabrous or rarely pubescent at base. *Leaves* alternate, sessile, persistent to deciduous, blade linear to linear-lanceolate, chartaceous, uninerved, pale or lustrous, glabrous to tomentose on both surfaces, apex spiny, leaf margin revolute or plicate, ciliate, abaxial with proeminent midvein. *Capitulescence* terminal, monocephalous. *Capitula* heterogamous, disciform, 40—135-flowered, sessile or shortly pedunculate, involucre hemispherical or campanulate, 4—7 seriate, phyllaries scarious, yellow or yellow and purple, erect or reflexed, hirsute or velutinous, lanceolate grading to linear, apex spiny, magin flat, ciliate. *Receptacle* flat or convex, alveolate or tuberculate, pubescent. *Flowers isomorphic*. *Ray flowers* 10—40, pistillate, corolla narrowly tubular, 5-lobed, yellow, villous. *Style* cylindrical, purple. *Disc flowers* 30—95, bisexual, corolla tubular, 5-lobed, yellow, villous. *Anthers* 5, apical appendage acute or rarely apiculate, basal appendage ecalcarate, caudate, stamens inserted at base of corolla, filaments free, glabrous. *Style* cylindrical, purple. *Cypsela* turbinate, densely villous. *Pappus* plumose, shorter or equal corolla length, britlesh withish. *Pollen* without intercolpal depression, scabrate-microechinate. *Cromossome number* = 24, 25.

Fig. 8C-D; 11F; 12E and 13D.

Distribution and habitat:- *Doniophyton* comprises two species found in dry open areas from Northern Chile to Patagonian Argentina up to 4000 meters high (Katinas & Stuessy 1997).

***Notes*:-** *Doniophyton* is a xeromorphic genus that have been always proposed as close related to *Chuquiraga* and *Duseniella* by sharing the inhabit drier areas, long caudate anthers, yellow corolla, and pollen withouth intercolpar depression. However, this clade has been long argued as enigmatic, since previous phylogenetic relationships were incongruent with the relationship within this clade recovering *Doniophyton* nested to *Chuquiraga* (Gruenstaeudl et al. 2009, Padin et al. 2015b, see Chapter 1), or as sister to *Chuquiraga* (Gustaffson et al. 2001; Chapter 1). Morphologically, *Doniophyton* can be distinguished from *Chuquiraga* by the subshrub habit (*vs.*

shrub), chartaceous leaves (*vs.* coriaceous), heterogamous and disciform capitula (*vs.* homogamous and discoid capitula), female marginal flowers (*vs.* all flowers in the capitula are hermaphroditic).

7. *Duseniella* K. Schum. (1902: 475)

***Type*:** *Duseniella patagonica* (O. Hoffm.) K. Schum.

Annual herbs, up to 10 cm tall. *Stems* erect, much-branched, lenticelate, cylindrical or flat, scales imbricated at base, sparsely sericeous, unamerd. *Leaves* opposite at base, alternate in the upper parts, sessile, persistent, blade linear, carnose, trinerved, lustrous, adaxially glabrous or sparsely sericeous, adaxially sericeous, apex mucronate, leaf margin flat, ciliate. *Capitulescence* terminal, monocephalous. *Capitula* heterogamous, disciform, 9—41-flowered, sessile, surrounded by leaves, involucre campanulate, 4—5 seriate, phyllaries scarious, erect, glabrous, ovate-oblong grading to linear, apex spiny, margin flat, ciliate. *Receptacle* convex, glabrous. *Flowers isomorphic*. *Ray flowers* 4—16, female, corolla tubular, 5-lobed, yellow, villous at apex. *Style* cylindrical, yellow. *Disc flowers* 30—95, bisexual, corolla tubular, 5-lobed, externally sericeous at base of corolla and apical lobes, internally sericeous at tube margin petals. *Anthers* 5, apical appendage acute, long caudate, calcarada, stamens inserted at base of corolla tube, filaments free, glabrous. *Style* cylindrical, yellow. *Cypsela* cylindrical to turbinate, densely villous. *Pappus scale,* lanceolate, overlapped, shorter than the corolla tube, sericeous, ciliate. *Pollen* without intercolpal depression, microechinate. *Cromossome number* = unknow.

Fig. 8A-B; 11G; 12F and 13E.

Distribution and habitat:- *Duseniella* is a monotypic genus endemic to xeromorphic areas in Patagonia Argentinean up to 1000 meters high.

***Notes*:-** *Duseniella* is a morphological distinctive genus in the subfamily Barnadesioideae by comprising the only annual herb with unarmed branches, fleshy leaves, disciform capitula with pappus scale overlapped and ciliate. Because of its distinctiveness morphology, Cabrera (1959, 1961) did not recognize the genus as a member of the subtribe Barnadesiinae (tribe Mutiseae); instead he

placed *Duseniella* in the subtribe Gochnatiinae (Cabrera 1959). Phylogenetically, *Duseniella* is recovered in a clade with *Doniophyton* and *Chuquiraga*, but its relationship with these two genera is still unclear. A phylogenomic study comprising a large number of data and taxa of this clade could provide better insights into the evolution of this group.

8. *Fulcaldea* Poir. (1817)

Type: *Fulcaldea laurifolia* (Bonpl.) Poir.

Arching shrubs or small trees, up to 10 m tall. *Stems* erect, much-branched, lenticelate, cylindrical or flat, scales imbricate at base, glabrous, pubescent or rarely strigose, unarmed or with axillary spines in pairs, straight, convergent or divergent, glabrous or rarely pubescent at base. *Leaves* alternate-spiralate, subssesile to petiolate, persistent to deciduous, blade elliptic to ovate, coriaceous, trinerved, pale or rarely lustrous, glabrous, apex unarmed or mucronate, leaf margin flat, glabrous. *Capitulescence* terminal or axillary, corymbose or paniculate cymes. *Capitula* homogamous, 1-flowered, sessile or subsessile, involucre narrow cylindrical to cylindrical, 5—13 seriate, phyllaries scarious, green or pale brown, apex purple, erect or reflexed, glabrous or villous, ovate-triangulate grading to lanceolate, apex mucronate, margin flat, ciliate. *Receptacle* convex, glabrous or pilose. *Flowers* bisexual, corolla tubular, 5-lobed, white, red, purple, externally densely villous. *Anthers* 5, apical appendage acute, basal appendage ecaudate, ecalcarate, stamens inserted on the corolla tube**,** filaments free, glabrous. *Style* cylindrical but with a swollen portion below the branching point, white to cream, apex purple. *Cypsela* cylindrical, densely sericeous. *Pappus* plumose, taller than the corolla length, britles white, pink or red. *Pollen* without intercopal depressions, spinulose. *Chromosome number* = unknown.

Fig. 9A-B; 11H; 12G and 13F.

Distribution and habitat:- *Fulcaldea* comprises two species with a remarkable 4000km disjunct distribution (Funk & Roque 2011). *Fulcaldea laurifolia* (Bonpl.) Poir. is restricted to the dry forest in intermontane regions of Southern Ecuador and Northern Peru (Ferreyra 1995), and

Fulcaldea stuessyi is restricted to seasonally deciduous forest in Northeastern Brazil (Funk & Roque 2011).

***Notes*:-** *Fulcaldea* is easily distinguished from the other Barnadesioideae genera by having a single flower per capitulum and by the swollen style below the branching point.

9. ***Huarpea*** Cabrera (1951: 129)

Type: *Huarpea andina* Cabrera

Rhizomatous subshrubs, up to 4.5 cm tall, unarmed. *Stems* erect, few to much-branched, striated, lenticelate, cylindrical, scales imbricated at base, tomentose, unarmed. *Leaves* alternate, subrosulate, sessile, persistent, blade linear, coriaceous, uninerved, lustrous, adaxial glabrous, abaxial lanate, apex spiny, leaf margin revolute, ciliate, abaxial with proeminent midvein. Capitulescence terminal, monocephalous. *Capitula* heterogamous, radiate, 6-flowered, sessile, hidden by the leaves, involucre cylindric-campanulate, 5-7 seriate, phyllaries coriaceous, lanceolate, erect, lanate, apex spiny, margin flat, ciliate. *Receptacle* flat, pilose. *Flowers dimorphic*. *Ray flowers* 5, bisexual, subbilabiate, 5-lobed, with the inner lobe shorter than the outer lobes, externally hirsute-sericeous, white, internally glabrous, yellow. *Anthers* 5, apical appendage obtuse, basal appendage ecalcarate, ecaudate, stamens inserted at base, filaments free, yellow. *Style* cylindrical, yellow. *Cypselae* turbinate, densely villous. *Pappus* plumose. *Disc flower* 1, male, tubular, externally sericeous-pilose. *Anthers* shortly sagittate at base, ecaudate, ecalcarate, stamens inserted at base, apex acute or rounded, filaments free. *Styles* not seen. *Cypselae* cylindrical, densely villous. *Pappus* single villous bristle or absent, equal to the corolla length. *Pollen* lophate. Cromossome number = unknow.

Fig. 9C-D and 13A.

Distribution and habitat:- *Huarpea* is a monotypic genus restricted to the department of Iglesia in San Juan province, Argentina (Cabrera 1951). This species is found in the dry Monte vegetation above 3300 meters high (Stuessy et al. 1996).

Notes:- *Huarpea* can be distinguished from the other Barnadesioideae genera by being unarmed subshrubs up to 4,5 cm tall, capitula hidden by the leaves, with 5 ray flowers, hermaphroditic and subbilabiate corollas and one disc flower with tubular coroolas and atrophied gynoecium (Cabrera 1951, Stuessy et al. 2009). Phylogenetically, *Huarpea* is always recovered as sister to *Barnadesia* (Bremer 1994, Stuessy et al. 1996, Gustafsson et al. 2001, Gruenstaeudl et al. 2009, Ferreira et al. 2019, see also Chapter 1). This clade is supported by the capitula heterogamous, radiate, anthers base shligtly sagittate or decurrent, lophate pollen, and the gynoecium atrophy (only in *Barnadesia* subgenus *Bacasia*).

10. ***Schlechtendalia*** Less. (1830: 242-243)

Type: *Schlechtendalia luzulifolia* Less.

Perennial herbs, up to 1 m talln unarmed. *Stems* erect, single-branched, lenticelate, cylindrical, without scales imbricated at base, densely sericeous, unarmed. *Leaves* rosulate at base, opposite on stems, amplexicaul, sessile, persistent, blade linear, chartaceous, parallel venation, pale or lustrous, sericeous or lanate, apex spiny, leaf margin flat or slightly revolute, ciliate. *Capitulescence* terminal or axillary, monocephalous, cymose, corymbifom, racemose, or umbel. *Capitula* homogamous, discoid, 50—100 flowered, pedunculate, involucre turbinate or hemispherical, 5—7 seriate, phyllaries chartaceous, green, brownish, erect or reflexed, sericeous, lanceolate, apex spiny margin flat, ciliate. *Receptacle* flat, pilose. *Flowers isomorphic*, bisexual, corolla subbilabiate, 5-lobed, yellow, externally and throat villous. *Anthers* 5, apical appendage emarginated, basal appendage shortly caudate, ecalcarate, stamens inserted near base of corolla, filaments free, glabrous, anther collar distinctive. *Style* cylindrical, yellow. *Cypselae* turbinate, densely villous. *Papus* scale, lanceolate, shorter than the corolla, scarious, glabrous. *Pollen* with one depression per mesocolpus, sparsely microechinate. *Cromossome number* = 8.

Fig. 10; 11I; 12H and 13D.

Distribution and habitat:- *Schlechtendalia* is a monotypic genus occurring in grassy areas in Southern Brazil, Uruguay, and adjacent areas of Argentina (Stuessy et al. 1996, Stuessy et al. 2009). In Brazil, *Schlechtendalia* is found in the *Pampas* biome and it is classified as an endangered species since *Pampas* has lost almost 54% of the original vegetation (Nakajima et al. 2013).

Notes:- *Schlechtendalia* is clearly a Barnadesioideae member since it shows the "barnadesioids trichomes" and subbilabilate corollas (Gustafsson et al. 2001). However, it displays a set of morphological features that diverge from the rest of the subfamily. Firstly, it is an unarmed species, although this feature seems to evolve independently in many lineages of Barnadesioideae. Secondly, the leaves of *Schlechtendalia* is disposed of basally rosulate, and alternated on stems, and parallelinerved venation. Thirdly, the pappus is narrow scale (also found in *Duseniella*) and glabrous without the pappus bristles (Gustafsson et al. 2001). Moreover, it is the only genus absent in xeromorphic areas (Stuessy et al. 2009).

The phylogenetic position of *Schlechtendalia* within Barnadesioideae remains unclear. It is proposed as the sister group to the rest of the subfamily (Stuessy et al. 1996, Urtubey & Stuessy, 2001, Gruenstaeudl et al. 2009, Ferreira et al. 2019, see Chapter 1), as sister to *Doniophyton* and *Duseniella* clade (Bremer 1994); *Chuquiraga* and *Doniophyton* (Gustafsson et al. 2001); *Archidasyphyllum*, *Arnaldoa* and *Fulcaldea* (Gustaffson et al. 2001); or *Barnadesia* and *Huarpea* (Gruenstauedl et al. 2009).

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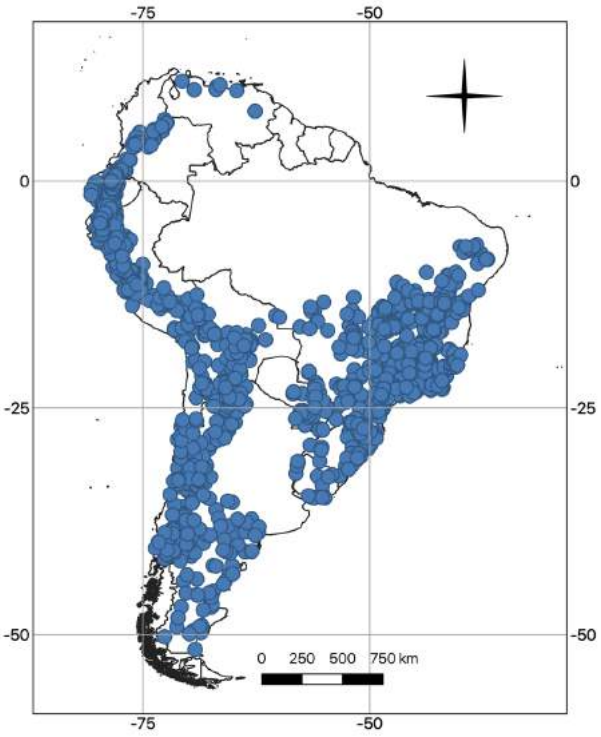

FIGURE 1. Distribution of the South American subfamily Barnadesioideae

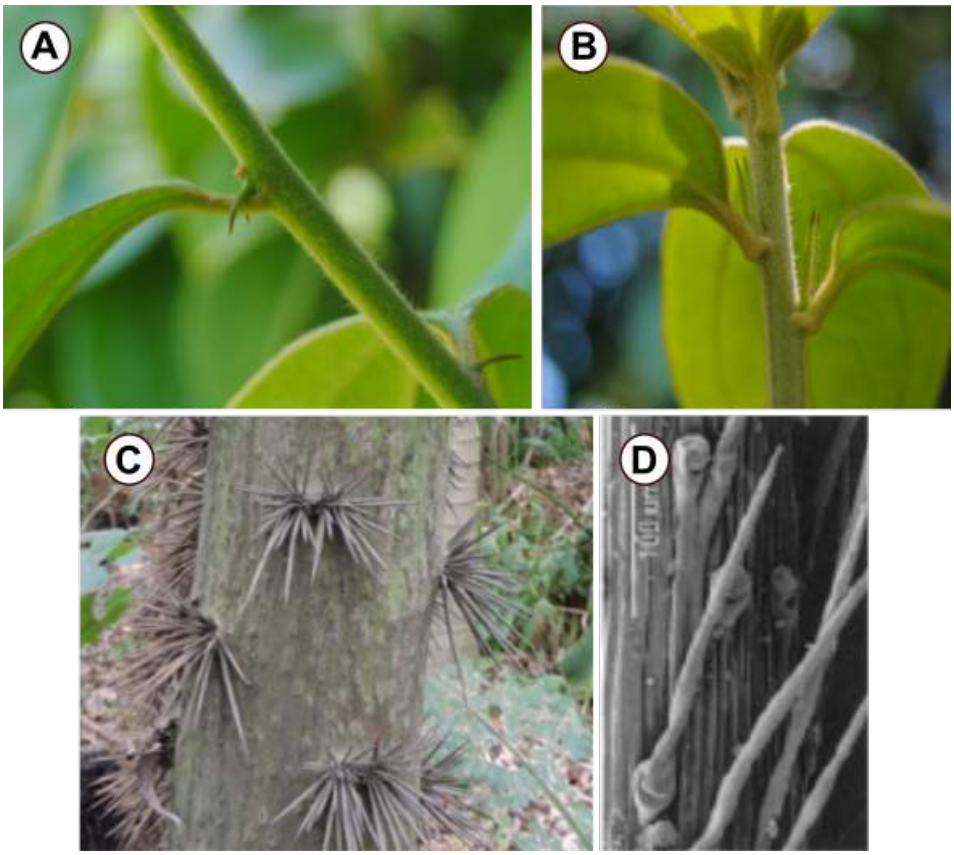

FIGURE 2. Synapomorphies of Barnadesioideae. A-C Diversity of spines. D Barnadesioid trichomes. A-B. *Dasyphyllum vagans*. A. Spines in pairs, curved, and convergent. B. Spines in pairs, straight, and convergent. C. *Barnadesia parviflora*. Spines in fascicles, straight, divergent. D. SEM photograph of the Barnadesioid trichomes extracted from Stuessy et al (2009).

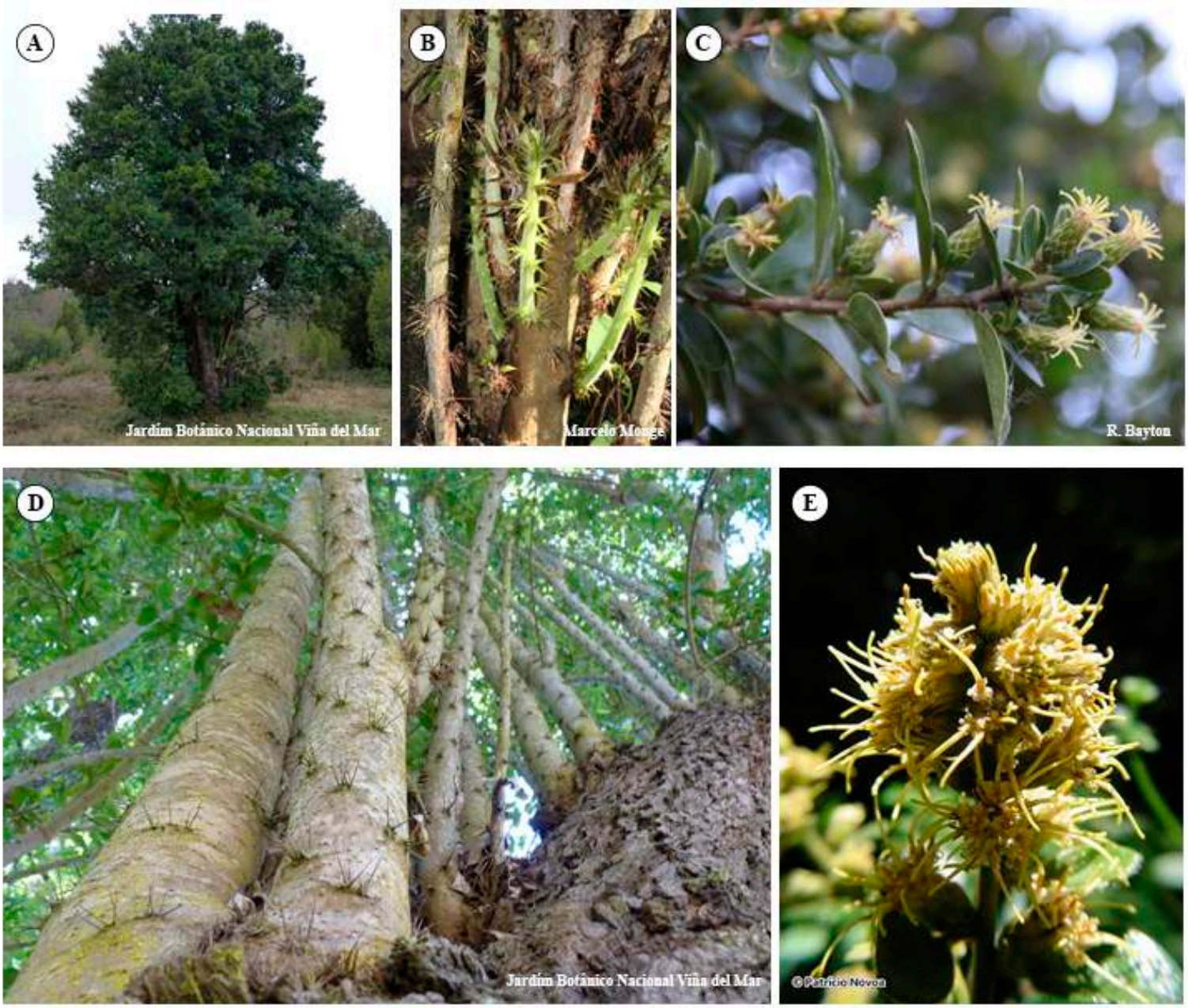

FIGURE 3. ***Archidasyphyllum***. A-C. ***Archidasyphyllum diacanthoides*****.** A. Habit. B. Multi-stem tree. C. Capitula. D-E. ***Archidasyphyllum excelsum***. D. Trunk with fasciculate spines. E. Capitula arranged into speciform synflorescences.

FIGURE 4. ***Arnaldoa.*** A-B ***Arnaldoa argentea.*** A. Habit. B. Branch with capitulum. C. ***Arnaldoa macbrideana***. Capitulum. D-E. ***Arnaldoa weberbaueri***. D. Capitulum. E. Habit.

FIGURE 5. ***Barnadesia.*** A-B. ***Barnadesia caryophylla***. A. Habit. B. Capitulum. C. ***Barnadesia odorata***. Capitulum. D. ***Barnadesia* cf. *spinosa***. Capitulum, white arrow showing the fillaments fused into a tube. E. ***Barnadesia polyacantha***. Divergent and fasciculate spines on stem.

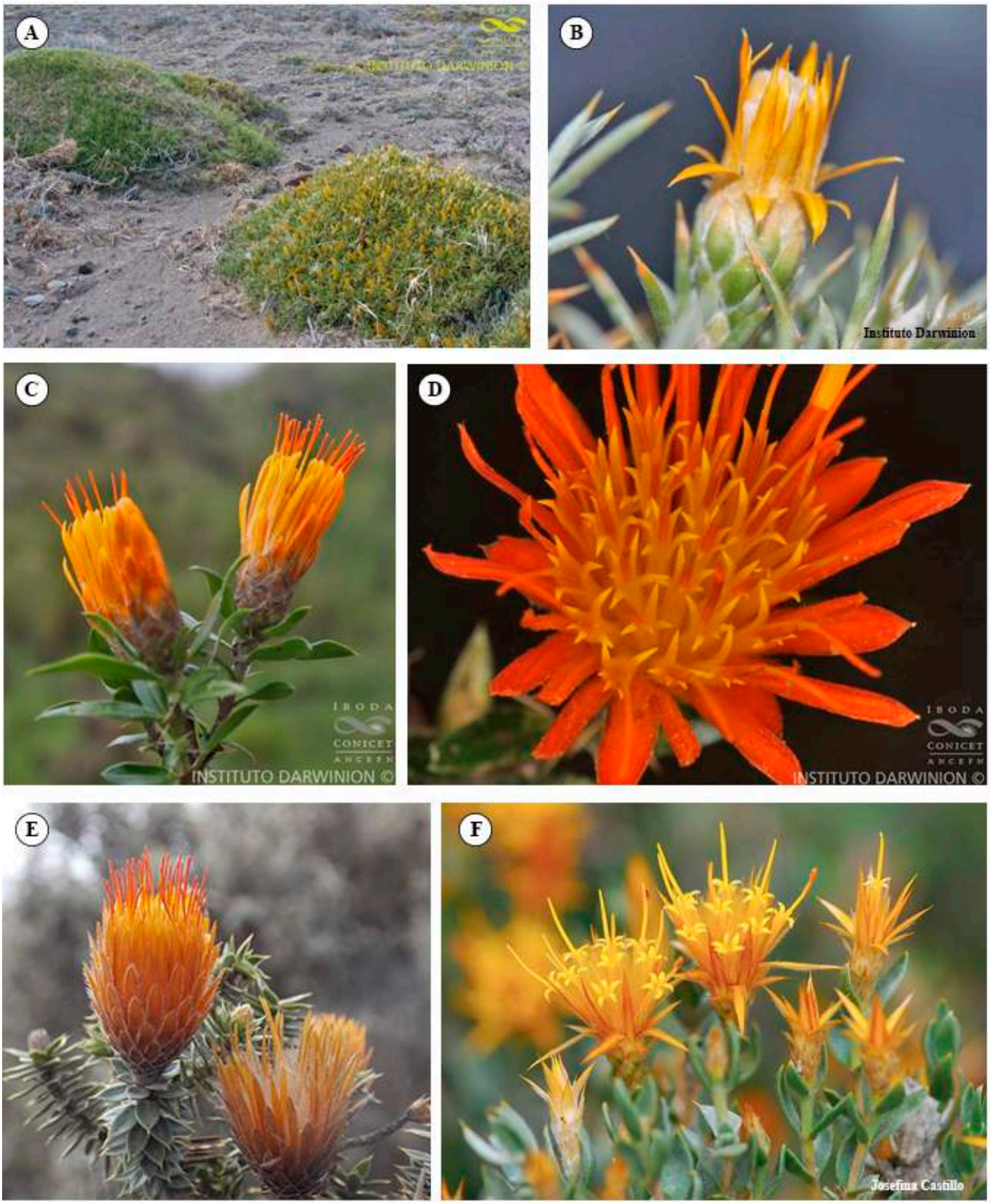

FIGURE 6. ***Chuquiraga.*** A-B. ***Chuquiraga aurea***. A. "Cushion" Habit. B. Capitulum. C. ***Chuquiraga calchaquina***. Capitulum. D. ***Chuquiraga longiflora.*** Capitulum and the tubular corolla. E. ***Chuquiraga jussieui***. Shrub habit. F. ***Chuquiraga oppositifolia***. Capitulum.

FIGURE 7. *Dasyphyllum*. A ***Dasyphyllum diamantinense***. Habitat. B. ***Dasyphyllum reticulatum***. Capitulum. C. ***Dasyphyllum sprengelianum***. Capitulum. D. ***Dasyphyllum vagans***. Capitula arranged into inflorescence with a white arrow showing a subbilabilate corolla. E. ***Dasyphyllum brasiliense***. Capitula.

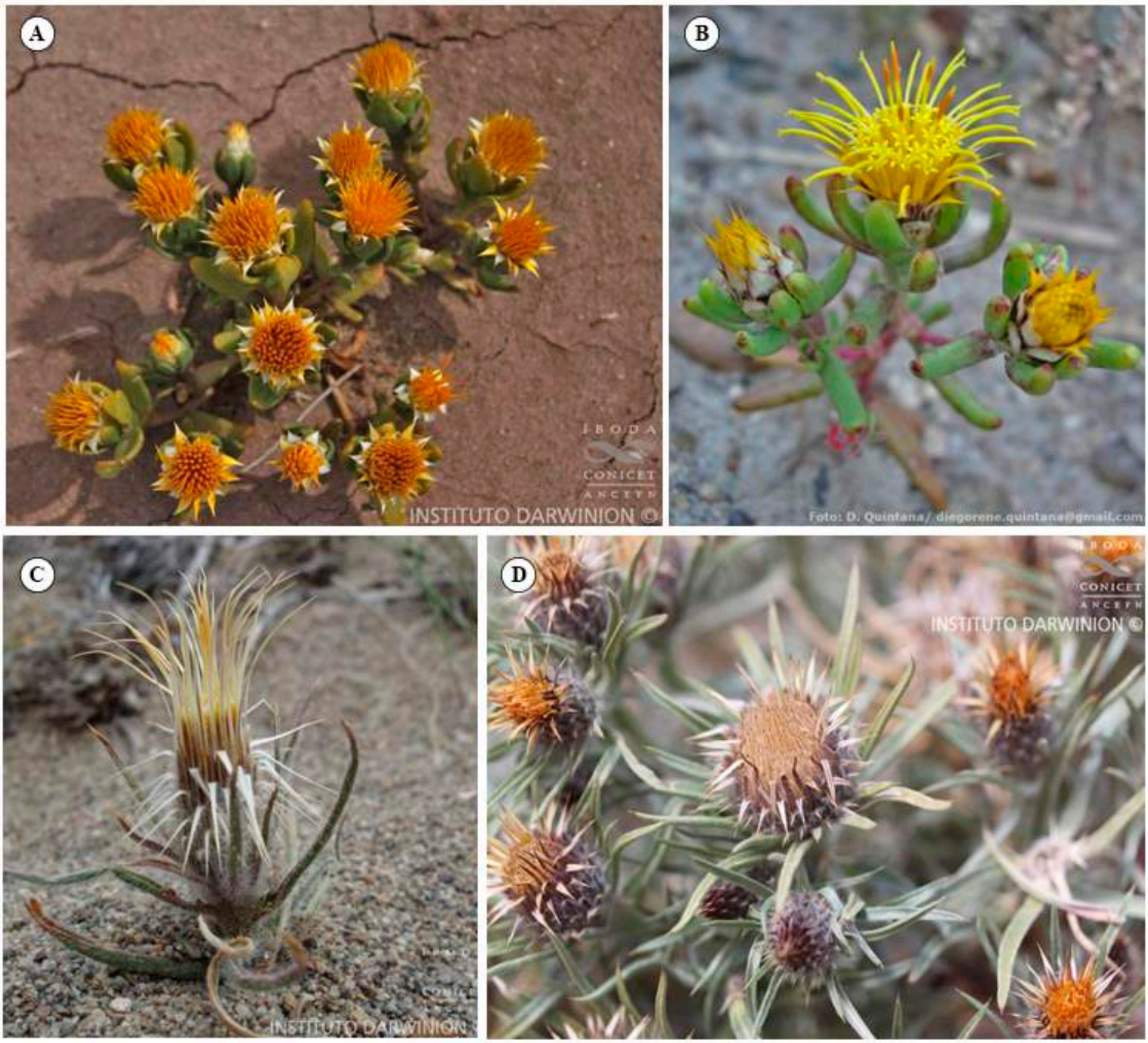

FIGURE 8. A-B ***Duseniella***. C-D ***Doniophyton***. A.-B ***Duseniella patagonica***. C. ***Doniophyton anomalum***. D. ***Doniophyton weddellii***.

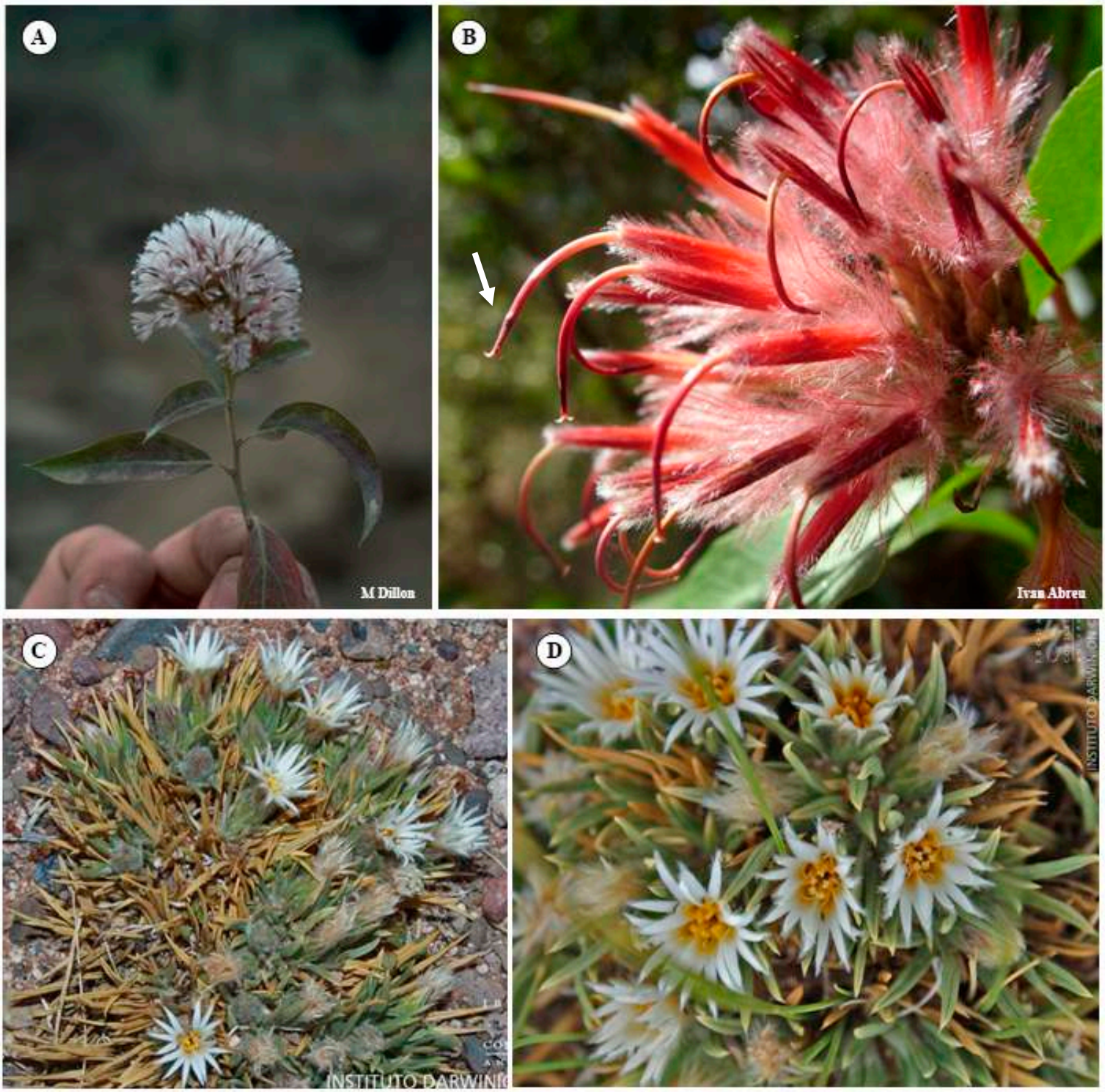

FIGURE 9. A-B ***Fulcaldea*** and C-D ***Huarpea***. A. ***Fulcaldea laurifolia***. Branch with inflorescence. B. ***Fulcaldea stuessyi***. Inflorescence with a white arrow showing swollen style below the branching point. C. ***Huarpea andina***. Habit. D. Capitulum showing five ray flowers.

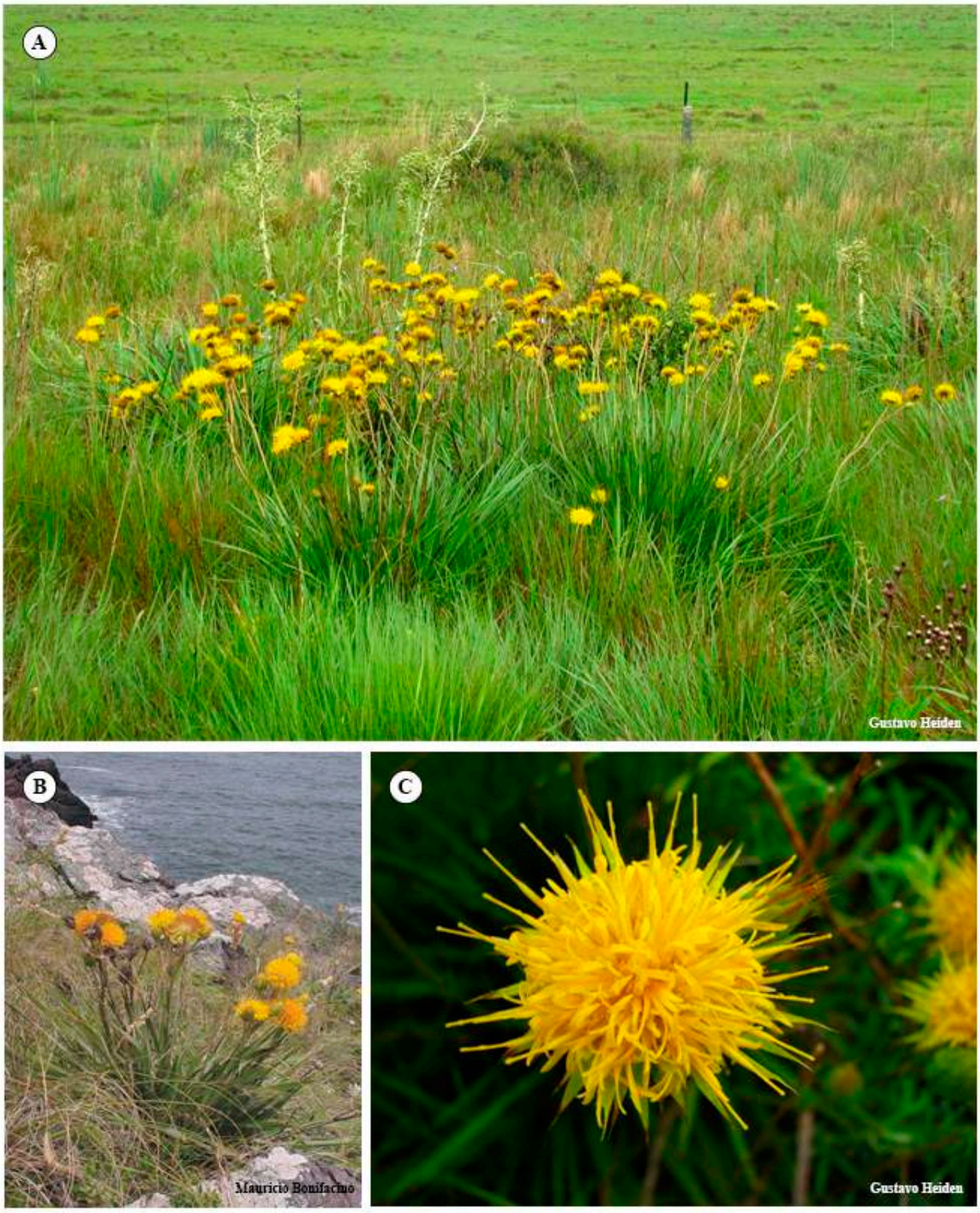

FIGURE 10. ***Schlechtendalia luzulifolia*****.** A. Brazilian Pampas. B. Grassy-like habit. C. Capitulum with subbilabiate corollas.

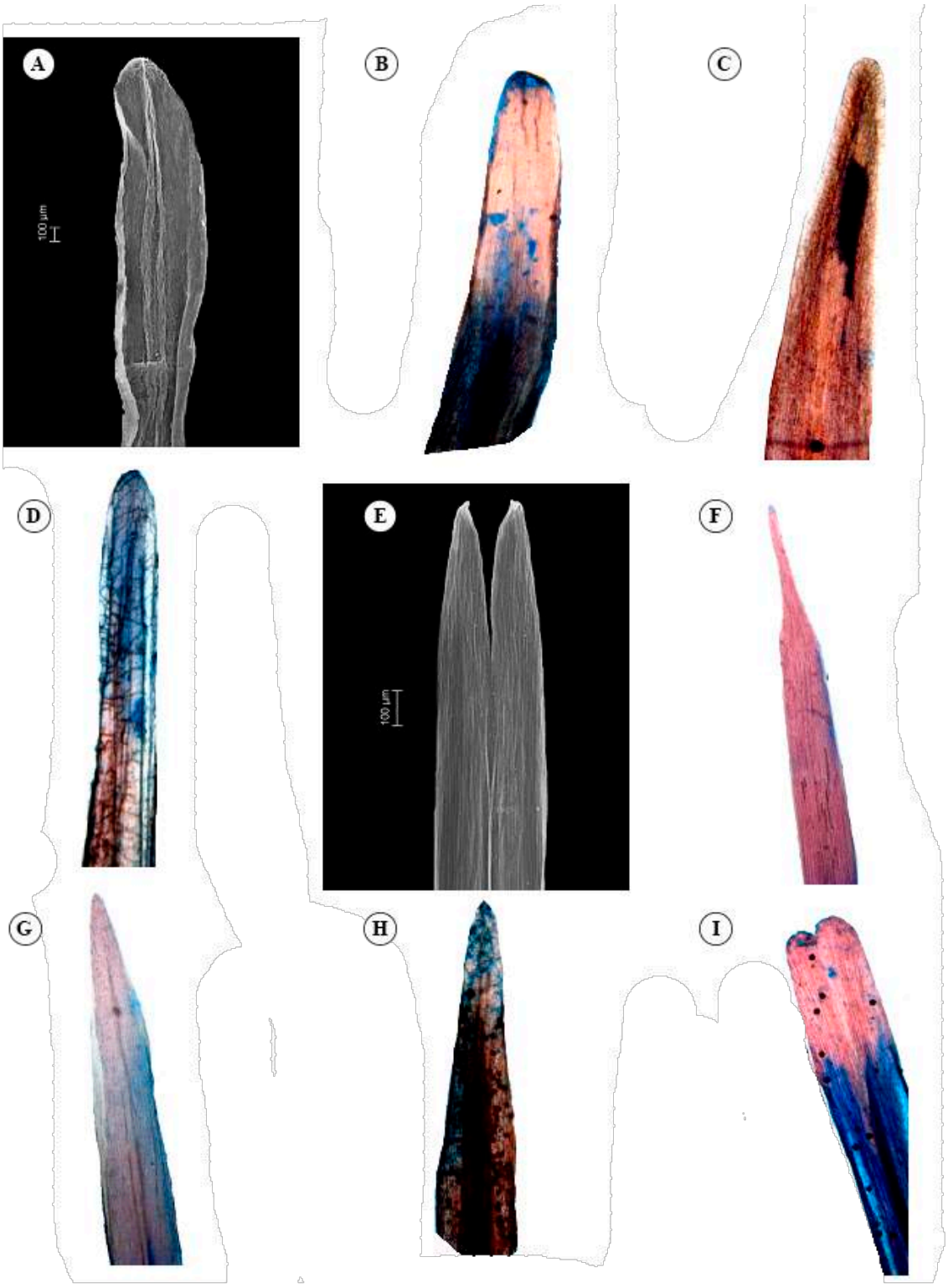

FIGURE 11. Protographs of anther apical appendages diversity in Barnadesioideae**.** A. ***Archidasyphyllum diacanthoides*** (M.Monge 2013, SPFR). B. ***Arnaldoa*** **argentea** (J.Madsen 8159, MO) C. ***Barnadesia pycnophylla*** (G.Ccana-Ccapatinta 53, SPFR). D. ***Chuquiraga jussieui*** (P.Ferreira 94, SPFR). E. ***Dasyphyllum trychophyllum*** (extracted from Ferreira *et al*. 2019). F. ***Doniophyton anomalum*** (T.Stuessy 12921, WU). G. ***Duseniella patagonica*** (W.Fischer 173, MO). H. ***Fulcaldea laurifolia*** (G.Lewis 3497, QCA). I. ***Schlechtendalia luzulifolia*** (G.Heiden s.n., ECTC)**.**

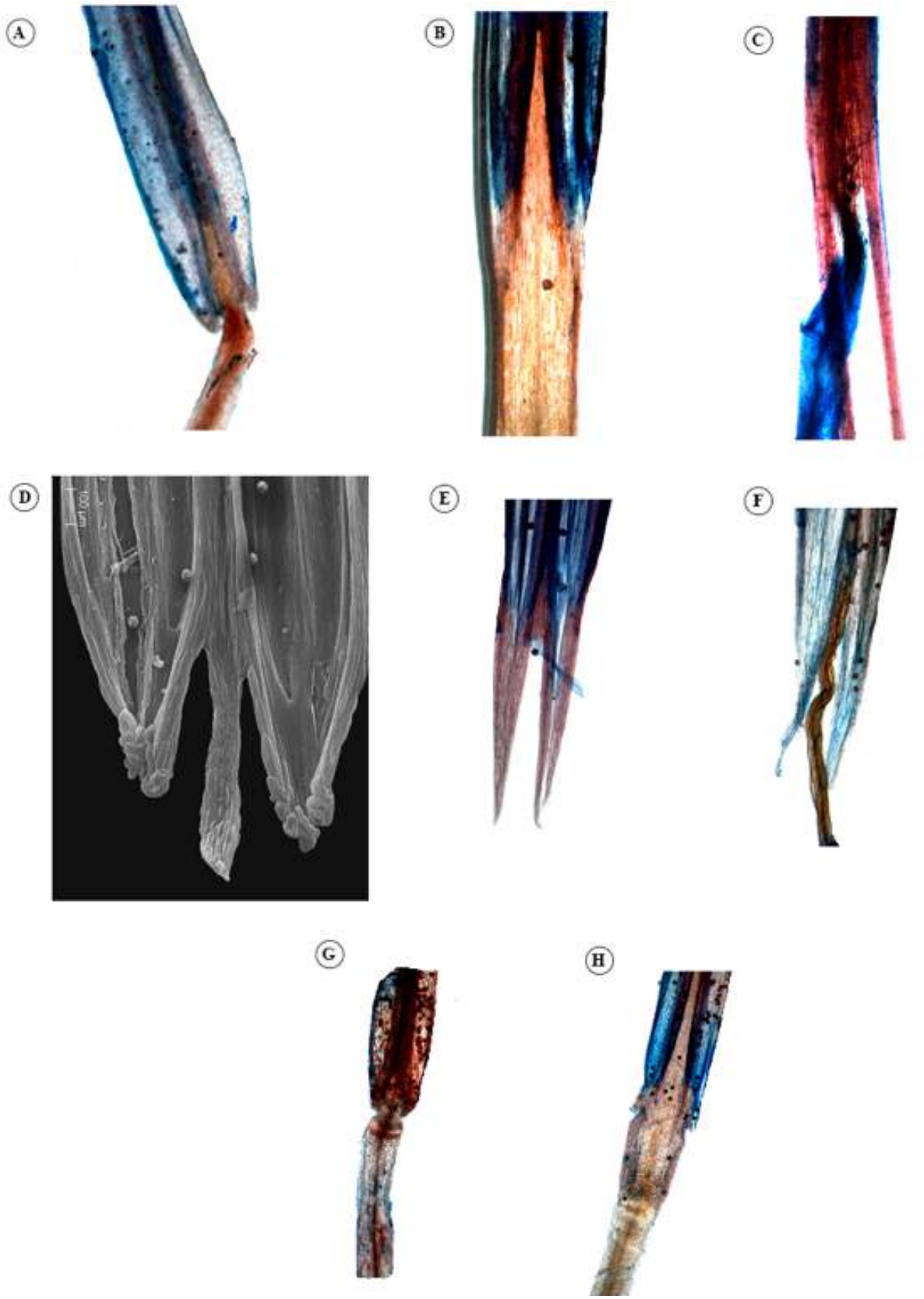

FIGURE 12. Protographs of anther base appendages in Barnadesioideae. A. ***Archidasyphyllum***. B. ***Barnadesia***. C. ***Chuquiraga***. D. ***Dasyphyllum***. E. ***Doniophyton***. F. ***Duseniella***. G. ***Fulcaldea***. H. ***Schlechtendalia***. Voucher information as same as used in the Figure 11.

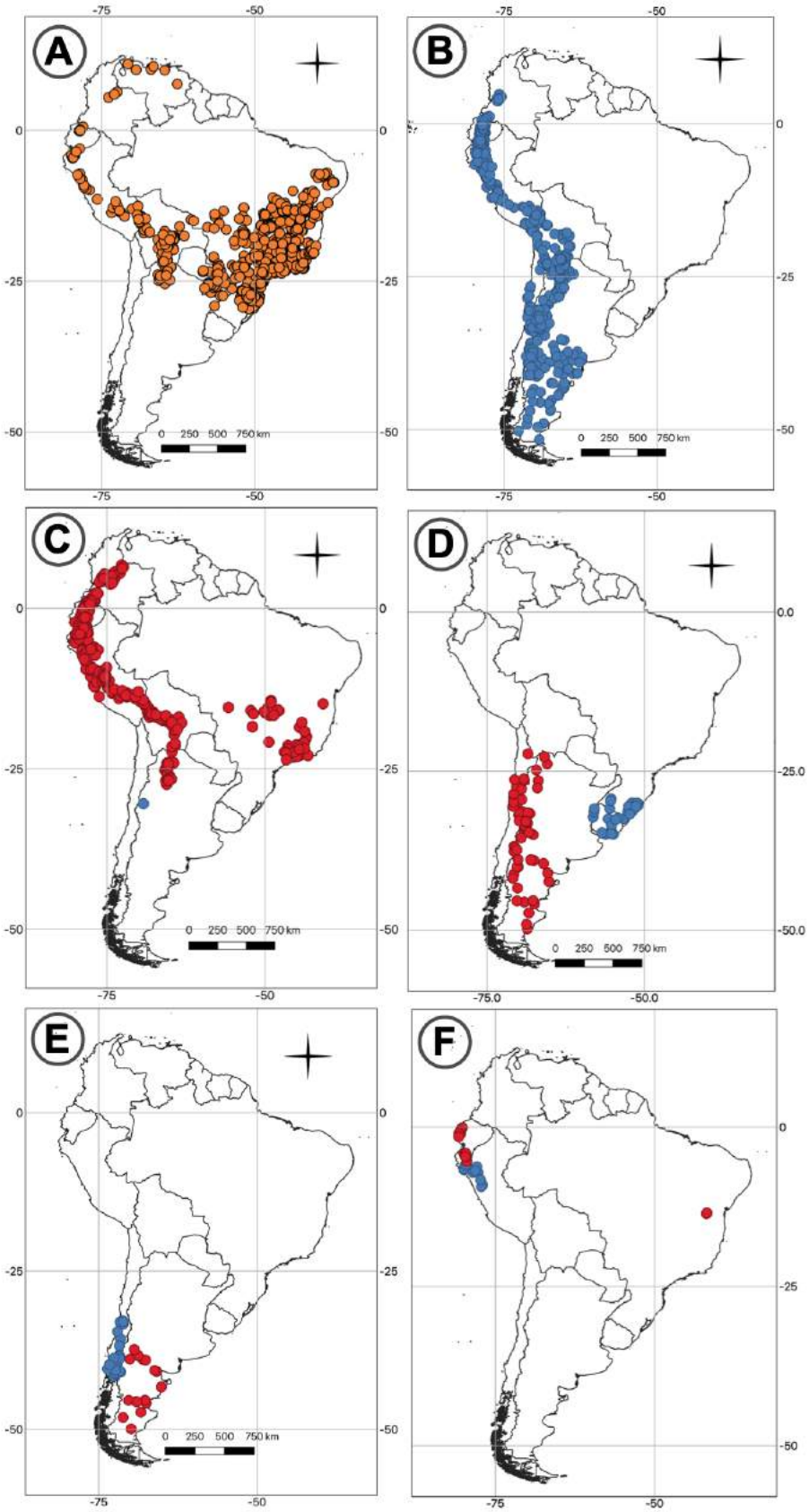

FIGURE 13. Geographical distribution for the genera of Barnadesioideae. A) ***Dasyphyllum*** (black dots) and ***Huarpea*** (red dots). B. ***Chuquiraga***. C. ***Barnadesia*** (red dots) and ***Huarpea*** (blue dots). D. ***Doniophyton*** (blue dots) and *Schlechtendalia* (red dots). E. ***Archidasyphyllum*** (blue dots) and ***Duseniella*** (red dots). F. ***Arnaldoa*** (blue dots) and ***Fulcaldea*** (red dots).

Final Conclusions

In the last decades, the substantial increase in biological data, including DNA sequences, species occurrence, and online databases allied to mathematical and technological advances has changed the study of biodiversity from a descriptive and often subjective to an integrated science with hypotheses that can be tested, providing robust results and discussion. The present thesis provides an example of how the advances in the study of biodiversity allied to big data science can shed light on a contentious group of plants, the subfamily Barnadesioideae (Compositae, the sunflower family).

Here, we proposed a phylogenetic hypothesis for Barnadesioideae using the next-generation sequencing technology assembling almost 300% more data than the previous molecular studies by gathering 736 kb from 942 nuclear loci plus almost completed chloroplast genomes for nearly 60% of the species currently circumscribed. Our results retrieved a well-supported and resolved phylogeny recovering Barnadesioideae and its genera as monophyletic groups, except *Chuquiraga* which is sometimes paraphyletic by the positioning of *Doniophyton*. Furthermore, *Chuquiraga* is inferred here as a contentious genus and evidently needs more taxonomic, morphological and molecular studies using a new sequencing method (for example RADseq). Furthermore, this chapter also has a methodological perspective by shedding light into a question in systematic: What is the impact of incomplete sequences and taxonomic sampling in phylogenetic analysis? Our results indicated that phylogenies have the highest support for the clades when researchers used all available data gathered.

We also used our phylogenetic hypothesis as a framework to investigate the historical biogeography and the speciation and extinction rates through time. We found that Barnadesioideae may appear in southern South America during the Eocene, and the MRCA of the largest clades started to diversify and occupied other regions during the Miocene. Our macroevolutionary studies inferred that the diversification in Barnadesioideae was constant and homogeneous through the time, and we did not find any evidence of shift during its evolutionary history.

Based on the phylogenetic results inferred here allied to the great morphological diversity in Barnadesioideae, we proposed a generic synopsis for the subfamily updating the genera circumscription. In the present work, Barnadesioideae comprises 10 genera and 84 species endemics to South America. Our work also includes a key, expanded morphological descriptions, maps of distribution, habitat, photographs and taxonomic notes.

We expect that this work contributes to shed light on the early evolution of Compositae as well as provide the first steps to investigate morphology, anatomy, macroevolution, ecology, niche

evolution as well as systematics and taxonomy of enigmatic clades as *Chuquiraga*/*Doniophyton*/*Duseniella*. Additionally, we hope that the present work may serve as a model to assembly the big data science with evolutionary studies in one of the richest regions on earth, the extraordinary South America.

Appendix 01

Phylogeny and circumscription of *Dasyphyllum* (Asteraceae: Barnadesioideae) based on molecular data with the recognition of a new genus, *Archidasyphyllum*

Paola de Lima Ferreira, Mariana Machado Saavedra, Milton Groppo

Archidasyphyllum diacanthoides
R. Bayton

""In England any person fond of natural history enjoys in his walks a great advantage, by always having something to attract his attention; but in these fertile lands teeming with life, the attractions are so numerous, that he is scarcely able to walk at all."
(Charles R. Darwin, 1839; after leaving Brazil on board of HMS Beagle)

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Phylogeny and circumscription of *Dasyphyllum* (Asteraceae: Barnadesioideae) based on molecular data with the recognition of a new genus, *Archidasyphyllum*

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ABSTRACT

Dasyphyllum Kunth is the most diverse genus of the South American subfamily Barnadesioideae (Asteraceae), comprising 33 species that occur in tropical Andes, Atlantic Forest, Caatinga, Cerrado, and Chaco. Based on distribution, variation in anther apical appendages, and leaf venation pattern, it has traditionally been divided into two subgenera, namely, *Archidasyphyllum* and *Dasyphyllum*. Further, based on involucre size and capitula arrangement, two sections have been recognized within subgenus *Dasyphyllum*: *Macrocephala* and *Microcephala* (=*Dasyphyllum*). Here, we report a phylogenetic analysis performed to test the monophyly of *Dasyphyllum* and its infrageneric classification based on molecular data from three non-coding regions (*trn*L-*trn*F, *psb*A-*trn*H, and ITS), using a broad taxonomic sampling of *Dasyphyllum* and representatives of all nine genera of Barnadesioideae. Moreover, we used a phylogenetic framework to investigate the evolution of the morphological characters traditionally used to recognize its infrageneric groups. Our results show that neither *Dasyphyllum* nor its infrageneric classification are currently monophyletic. Based on phylogenetic, morphological, and biogeographical evidence, we propose a new circumscription for *Dasyphyllum*, elevating subgenus *Archidasyphyllum* to generic rank and doing away with the infrageneric classification. Ancestral states reconstruction shows that the ancestor of *Dasyphyllum* probably had acrodromous leaf venation, bifid anther apical appendages, involucres up to 18 mm in length, and capitula arranged in synflorescence.

Subjects Biodiversity, Evolutionary Studies, Molecular Biology, Plant Science, Taxonomy
Keywords Asterids, Compositae, Character Evolution, South America, Systematics, Taxonomy

INTRODUCTION

Systematics of Asteraceae (Composite) has undergone major change over the last four decades, mainly due to the insights provided by molecular data. One of the pioneering

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molecular studies demonstrated an inversion of 22 kb in the chloroplast genome of all Asteraceae, except for the members of subtribe Barnadesiinae, tribe Mutiseae (*Jansen & Palmer, 1987*). Subsequent phylogenetic studies indicated that Barnadesiinae is the sister group to the rest of the family (*Bremer, 1987*; *Jansen et al., 1992*); therefore, the subtribe was elevated to the rank of subfamily as Barnadesioideae (*Bremer & Jansen, 1992*).

Barnadesioideae comprises nine genera and approximately 85 species, and is restricted to South America (*Bremer, 1987*, *1994*; *Jansen et al., 1992*; *Panero & Funk, 2002*; *Funk et al., 2005*, *2009*; *Panero et al., 2014*; *Panero & Crozier, 2016*; *Saavedra et al., 2018*). Its members are characterized by the presence of axillary spines arranged at the nodes, in pairs or in fascicles, and by the presence of unbranched three-celled hairs called "barnadesioid trichomes" on the corollas, cypselae, and pappus (*Cabrera, 1959*; *Ezcurra, 1985*; *Bremer & Jansen, 1992*; *Bremer, 1994*; *Urtubey, 1999*; *Erbar & Leins, 2000*; *Ulloa, Jørgensen & Dillon, 2002*; *Stuessy, Urtubey & Gruenstaeudl, 2009*).

Dasyphyllum is the largest genus in Barnadesioideae, comprising 33 species (*Saavedra, 2011*; *Saavedra et al., 2018*; Fig. 1) distributed from Venezuela to Northwestern Argentina, but absent in the Amazon region (*Cabrera, 1959*; *Saavedra, 2011*; *Saavedra, Monge & Guimarães, 2014*). The genus is morphologically diverse and can be distinguished from the other genera of Barnadesioideae by including trees, shrubs, and woody vines with pairs of straight, curved, or fasciculate spines, together with simple, alternate leaves; monoecious or gynodioecious capitula, comprising discoid heads with many types of corolla (*Stuessy & Urtubey, 2006*), and anthers with apical appendages that are either bifid or undivided (*Cabrera, 1959*; *Stuessy, Urtubey & Gruenstaeudl, 2009*; *Saavedra, 2011*).

Cabrera (1959) proposed the first infrageneric classification of *Dasyphyllum*, recognizing 36 species in two subgenera distinguished by several morphological characters and disjunct distributions. Subgenus *Archidasyphyllum* Cabrera comprised two tree-species and was characterized by the presence of leaves with pinnate venation and emarginate or obtuse anther apical appendages. Both species are restricted to the *Nothofagus* forests of central Chile and Argentina. In contrast, subgenus *Dasyphyllum* Cabrera comprised 34 tree or shrubs species, with acrodromous leaf venation and bifid anther apical appendages, distributed from the Andes eastward into tropical Argentina, Brazil, and Paraguay. Within subgenus *Dasyphyllum*, two sections are currently recognized: section *Microcephala* Cabrera (23 species) and section *Macrocephala* Cabrera (11 species). The two sections are distinguished by involucre size and capitula arrangement with section *Macrocephala* having involucre longer than 20 mm in length and arranged in a solitary or small group of heads (Figs. 1A and 1B) and section *Microcephala* having heads arranged in synflorescence (corymbiform cymes) smaller than 18 mm in length (Figs. 1C–1F).

Nonetheless, the treatment by *Cabrera (1959)* often relied on a single and narrow morphological concept to define the species. Due to the great morphological variation, floristic studies undertaken in Brazil have shown that many characteristics overlap; thus casting doubt on species delimitation (*Roque & Pirani, 1997*; *Saavedra et al., 2018*).

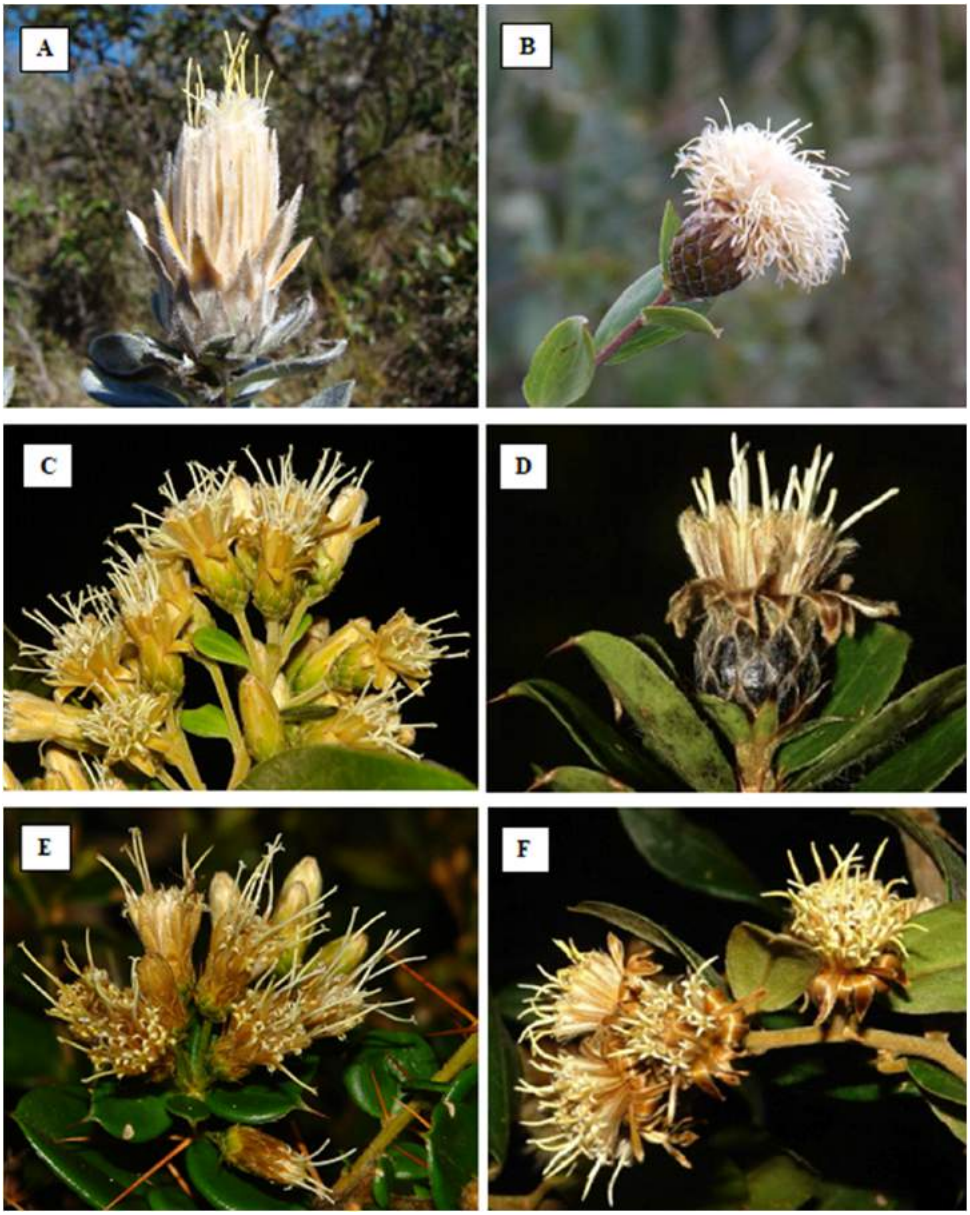

Figure 1 Photos of some *Dasyphyllum* species. (A) *Dasyphyllum reticulatum* (DC.) Cabrera. (B) *Dasyphyllum sprengelianum* (Gardner) Cabrera. (C) *Dasyphyllum brasiliense* (Spreng.) Cabrera. (D) *Dasyphyllum leptacanthum* (Gardner) Cabrera. (E) *Dasyphyllum diamantinense* Saavedra & M.Monge. (F) *Dasyphyllum flagellare* (Casar.) Cabrera. Photo credits: Photographs by Cláudio N. Fraga, except A (by Mariana M. Saavedra) and B (by Paola L. Ferreira). Full-size DOI: 10.7717/peerj.6475/fig-1

In this context, *Saavedra (2011)* and *Saavedra et al. (2018)* updated the taxonomy of *Dasyphyllum*, recognizing 33 species. Thirty of them were classified in two sections using the same morphological definition for sections provided by *Cabrera (1959)*, that is, *Dasyphyllum* Cabrera with 24 species, and *Macrocephala* Baker ex Saavedra with six species; and the remaining three species

(*D. diacanthoides, D. excelsum* belonging to *D.* subgenus *Archidasyphyllum*, and *D. hystrix*) were placed as *incertae sedis*.

Several phylogenetic studies aiming to clarify the phylogenetic relationships within Barnadesioideae have included species of *Dasyphyllum* (*Bremer, 1994*; *Stuessy, Sang & DeVore, 1996*; *Gustafsson et al., 2001*; *Urtubey & Stuessy, 2001*; *Gruenstaeudl et al., 2009*) but none of them representative of taxon sampling from each genus. Furthermore, these phylogenetic results proposed conflicting hypotheses for the relationships within the subfamily, especially regarding the monophyly of *Dasyphyllum* and its infrageneric classification.

Therefore, the main purposes of this work were to: (1) infer the intergeneric relationships of *Dasyphyllum* based on three molecular markers (plastid *trn*L-*trn*F and *psb*A-*trn*H, and nuclear ITS) using a broad taxonomic sampling of Barnadesioideae; (2) test the current circumscription of *Dasyphyllum* and its infrageneric classification according to *Saavedra (2011)* and *Saavedra et al. (2018)*, and update the taxonomy; and (3) investigate the character evolution of *Dasyphyllum*.

MATERIALS AND METHODS

Taxon sampling

A total of 60 out of the 85 species of Barnadesioideae, representing all nine genera, were sampled in this study. This included 27 of the 33 species (82%) from all sections of *Dasyphyllum* (*Saavedra, 2011*; *Saavedra et al., 2018*), covering most of its morphological diversity and geographical distribution. The six species missing in our analysis were not included due to unsuccessful DNA extractions or because we could not obtain voucher materials on loan for DNA extraction. A total of 61 accessions were newly sequenced and deposited in GenBank (Table S1); additionally, 125 accessions were obtained from previous studies (*Gustafsson et al., 2001*; *Gruenstaeudl et al., 2009*; *Katinas et al., 2008*; *Funk & Roque, 2011*; *Funk et al., 2014*; Table S2). Two species of *Mutisia* (Asteraceae: Mutisioideae) and one species of *Calycera* (Calyceraceae) were used as outgroups. All phylogenetic trees were rooted against to Calyceraceae, the sister family of Asteraceae (*Barker et al., 2016*; *Panero & Crozier, 2016*).

Molecular analysis

Total genomic DNA was extracted from three to five mg of silica-gel dried leaves using the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the instructions by the manufacturer. We selected and amplified three regions previously used to infer the phylogenetic relationships in Barnadesioideae: *trn*L-*trn*F using primers "c" and "f" (*Taberlet et al., 1991*); *psb*A-*trn*H using primers "psbAF" and "trnHR" (*Sang, Crawford & Stuessy, 1997*); and ITS using primers 18s F and 26s R (*Gruenstaeudl et al., 2009*). PCR reaction mixtures and purification were carried out after as per *Bruniera, Kallunki & Groppo (2015)*. Thermal cycling for plastid amplification was performed using initial denaturation at 94 °C (8 min), followed by 30 cycles at 94 °C (1 min), 54 °C (1 min), 72 °C, (2 min), ending with an elongation at 72 °C (3 min). Nuclear thermal cycling was performed according to *Barfuss et al. (2005)*, except for the annealing temperature of 62 °C

(used in this study). Sequencing of the amplified DNA regions was performed at CREBIO (Jaboticabal, São Paulo, Brazil) with the same primers used for PCR amplification.

Sequences were assembled and edited using the Biological Sequence Alignment Editor (BioEdit), version 7.2.5 (*Hall, 1999*). We performed sequence alignments using MAFFT version 7 (*Katoh & Standley, 2013*) with default parameters, followed by manual adjustments with Mesquite version 3.51 (*Maddison & Maddison, 2018*). All data matrices generated are included in Data S1.

Phylogenetic trees for each molecular region and the combined datasets were constructed under parsimony (PA), maximum likelihood (ML), and Bayesian inference (BI). PA analyses were performed in PAUP* version 4.0b10 (*Swofford, 2002*). Heuristics searches were performed with 10,000 random addition sequence replicates holding 10 trees at each step, tree-bisection-reconnection (TBR) branch swapping, with the "steepest descent" and "multrees" options off. All characters were unordered and equally weighted. Bootstrapping was implemented with 1,000 pseudoreplicates, 10,000 random taxon addition, and TBR branch-swapping algorithm. Bootstrap (BP) support values in the following ranges were considered strong (>88%), moderate (76–87%), weak (63–75%), and ambiguous (<63%) following *Bruniera, Kallunki & Groppo (2015)*.

Maximum likelihood and BI analyses were performed on the CIPRES Science Gateway (*Miller, Pfeiffer & Schwartz, 2010*). The most appropriate model of sequence evolution for each matrix was selected using the Akaike information criterion (*Akaike, 1973*) in jModelTest version 2.1.9 (*Posada, 2008*; *Darriba et al., 2012*). Selected models were GTR + I + G for ITS and GTR + G for both *psb*A-*trn*H and *trn*L-*trn*F.

Maximum likelihood analyses were performed using RaxML version 8 (*Stamatakis, 2014*) associated with a rapid BP analysis of 1,000 replicates under the GTRCAT model. ML BP were interpreted as in the PA analyses.

Bayesian inference analyses were performed in MrBayes version 3.2.6 (*Ronquist et al., 2012*) using two independent runs, each run with four simultaneous Markov chains (three heated chains and one cold chain) started from random trees. Analyses were run for 20 million generations, and values were sampled every 1,000 generations. The stationarity and convergence of runs, as the effective sample size $\geq$200 were ascertained using Tracer version 1.6 (*Rambaut et al., 2013*). The first 25% of the sample trees were discarded as burn-in and a 50% majority-rule consensus tree was calculated from the remaining trees using the sumt option. Posterior probabilities (PP) above 0.95 were considered as strong support.

The incongruence length difference test (ILD; *Farris et al., 1995*) was performed to test the congruence between the plastid marker datasets (*psb*A-*trn*H and *trn*L-*trn*F) and the combined marker datasets generated in this study (*psb*A-*trn*H, *trn*L-*trn*F, and ITS). The ILD test was performed using PAUP* version 4.0b10 (*Swofford, 2002*) with 1,000 replicates and the same parameters used for PA searches.

Taxonomy

The electronic version of this article in portable document format will represent a published work according to the international code of nomenclature for algae, fungi, and

plants (ICN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. In addition, new names contained in this work which have been issued with identifiers by IPNI will eventually be made available to the global names index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix "http://ipni.org/". The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central, and CLOCKSS.

Ancestral state reconstruction

In order to understand how the morphological features traditionally used to recognize the infrageneric groups have evolved in *Dasyphyllum*, we reconstructed ancestral character traits using the Bayesian majority-rule consensus tree based on the combined datasets (*trn*L-*trn*F, *psb*A-*trn*H, and ITS) and further ultrametrized using the chronopl function with default parameters in the R package "ape" (*Paradis, Claude & Strimmer, 2004*). Ancestral state reconstructions were estimated from 1,000 iterations of Bayesian stochastic character mapping (*Bollback, 2006*) using the function make.simmap in the R package phytools (*Revell, 2012*). Coding of morphological characters was extracted from the literature (*Cabrera, 1959*; *Stuessy, Urtubey & Gruenstaeudl, 2009*; *Funk & Roque, 2011*; *Saavedra, 2011*; *Saavedra, Monge & Guimarães, 2014*; *Saavedra et al., 2018*) and from examination of specimens from the following herbaria: ALCB, B, BAF, BHCB, BM, BOTU, BR, CEN, CEPEC, CESJ, CONC, CVRD, EAC, ESA, GFJP, GOET, GUYN, HB, HEPH, HPBR, HRCB, HST, HUEFS, HUFU, IBGE, ICN, IPA, JBP, K, LP, M, MBM, MBML, MO, MOSS, NY, OUPR, P, PACA, PEUFR, QCA, R, RB, S, SI, SP, SPF, SPFR, UB, UEC, UFG, UFMT, UFP, UFRN, UPCB, US, VIC (herbaria acronyms follow *Thiers, 2018*). A list of morphological characters and their character state coding used for the ancestral state reconstruction is detailed in Table 1.

Scanning electron microscopy was used to examine anther apical appendages in two species of *Dasyphyllum*. Dried florets were rehydrated with hot water and stored in 70% ethanol; then, anthers were critically point dried, sputter coated with gold and analyzed using an EVO 50 scanning electron microscope (Carl Zeiss, Cambridge, UK).

RESULTS

Phylogenetic analyses

The ILD test did not indicate incongruences between the plastid and combined datasets ($P > 0.05$), thus allowing both to be used for further phylogenetic analyses. Moreover, based on the results of BP and PP (>80), we did not find any evidence of significant incongruence among the relationships that differed between the trees (Fig. 2; Figs. S1–S4). Therefore, we decided to discuss our results based on the combined analysis of the three regions as it includes the largest number of taxa (Fig. 2). Our combined alignment consisted of 2,414 bp (*trn*l-*trn*F = 912 bp; *psb*A-*trn*H = 537; ITS = 965 bp) for 63 taxa (see summary statistics for each dataset in Table 2).

Table 1 Diagnostic feature coding used to infer the Bayesian stochastic character mapping analyses.

Taxon	Leaf venation	Anther apical appendage	Involucre size	Capitula arrangement
Arnaldoa macbrideana	0	0	0	0
Arnaldoa weberbaueri	0	0	0	0
Dasyphyllum argenteum	0	1	1	1
Dasyphyllum armatum	0	1	1	1
Dasyphyllum brasiliense	0	1	1	1
Dasyphyllum brevispinum	0	1	1	1
Dasyphyllum colombianum	0	1	1	1
Dasyphyllum diacanthoides	1	2	1	0
Dasyphyllum diamantinense	0	1	1	1
Dasyphyllum donianum	0	1	0	0
Dasyphyllum excelsum	1	2	1	1
Dasyphyllum ferox	0	1	1	1
Dasyphyllum flagellare	0	1	1	1
Dasyphyllum floribundum	0	1	1	1
Dasyphyllum fodinarum	0	1	0	0
Dasyphyllum hystrix	0	1	1	0
Dasyphyllum inerme	0	1	1	1
Dasyphyllum lanceolatum	0	1	1	1
Dasyphyllum leptacanthum	0	1	1	0
Dasyphyllum popayanense	0	1	1	1
Dasyphyllum reticulatum	0	1	0	0
Dasyphyllum spinescens	0	1	1	1
Dasyphyllum sprengelianum	0	1	0	0
Dasyphyllum trichophyllum	0	1	0	0
Dasyphyllum vagans	0	1	1	1
Dasyphyllum sp. nov. (1)	0	1	0	0
Dasyphyllum sp. nov. (2)	0	1	1	1
Dasyphyllum sp. nov. (3)	0	1	1	1
Dasyphyllum sp. nov. (4)	0	1	1	1
Fulcaldea laurifolia	0	0	1	1
Fulcaldea stuessy	0	0	1	1

Note:
Leaf venation: (0) Acrodomous, (1) Pinnate. Anther apical appendage: (0) Acute, (1) Bifid, (2) Obtuse. Involucre size: (0) ≥20 mm, (1) ≤18 mm. Capitula arrangement: (0) Solitary or few capitula (1) Capitula arranged in synflorescences (corymbiform cymes).

In all phylogenetic hypotheses, *Dasyphyllum* was found to be non-monophyletic due to the highly supported position of *D. diacanthoides* and *D. excelsum* (formely subgenus *Archidasyphyllum*) as sister clade to *Fulcaldea* and *Arnaldoa* (Fig. 2, Node 1, PA BP 99%, ML BP 100%, PP 1).

Dasyphyllum sensu stricto, defined here by excluding *D. diacanthoides* and *D. excelsum,* was recovered as monophyletic with moderate or strong support (Fig. 2; Node 2;

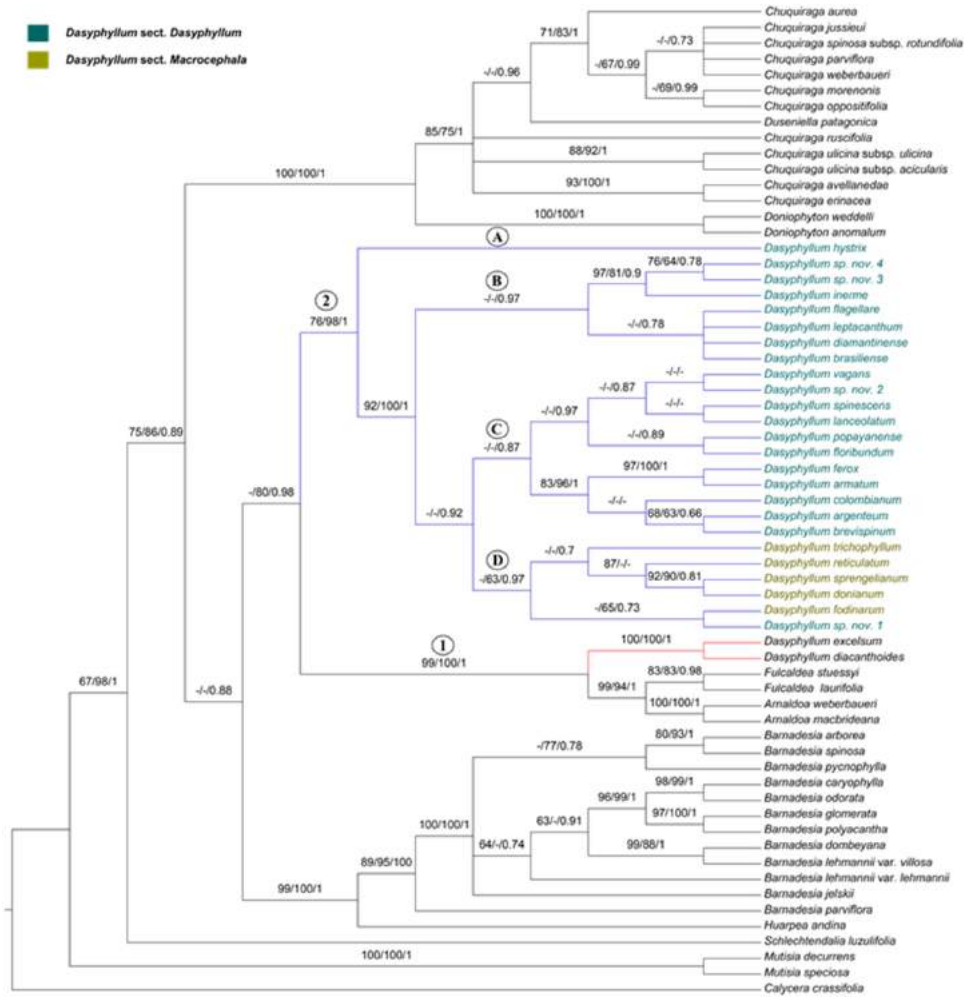

Figure 2 Phylogenetic relationships of *Dasyphyllum* based on combined datasets inferred from Bayesian inference. Support values are indicated above the branches in the order of parsimony, maximum likelihood, and Bayesian analyses. Support values less than 63% are indicated by a dash (–). Capital letters on internal clades of *Dasyphyllum* are discussed in the article.

Full-size DOI: 10.7717/peerj.6475/fig-2

PA BP 76%, ML BP 98%, PP 1). However, at the intrageneric level, both currently-accepted sections (*Dasyphyllum* and *Macrocephala*) were found to be non-monophyletic.

Members of *Dasyphyllum sensu stricto* are divided into four main lineages: (1) lineage "A" is composed only of *D. hystrix* and is sister to the rest of the genus (PA BP 76%, ML BP 98%, PP 1); (2) lineage "B" comprises seven species classified in section. *Dasyphyllum* of *Saavedra (2011)* and is only supported in the Bayesian analysis (PP 0.97); (3) lineage "C" is composed of 11 species, including approximately 46% of the species currently classified in sect. *Dasyphyllum* of *Saavedra (2011)*, with no strong support in any analysis; (4) lineage "D" is composed of five of the six species positioned in sect. *Macrocephala* of *Saavedra et al. (2018)*, plus one undescribed Brazilian species (*Dasyphyllum* sp. nov. 1)

Table 2 Summary statistics of the datasets used in this study.

	trnL-trnF	*psbA-trnH*	ITS	Plastid dataset	Combined dataset
Number of taxa included	53	49	60	53	63
Aligned length (BP)	912	537	965	1,449	2,414
Number of constant characters (%)	807 (88.49)	386 (71.88)	499 (51.71)	1,139 (78.61)	1,692 (70.09)
Number of variable characters (%)	105 (11.51)	151 (28.12)	466 (48.29)	310 (21.39)	722 (29.91)
Number of parsimony informative characters (%)	53 (5.81)	61 (11.36)	346 (35.85)	114 (7.87)	460 (19.06)
Tree length of best parsimony tree (steps)	120	222	1,375	348	1,743
Number of most parsimonious trees	20.251	3.120	309	11.337	3,475
Consistency index (CI)	0.9083	0.8018	0.4611	0.1753	0.4102
Retention index (RI)	0.9722	0.8739	0.4412	0.9181	0.8314

previously positioned in sect. *Dasyphyllum* of *Saavedra (2011)*, and it is only strongly supported in the Bayesian analysis (PP 0.97).

The phylogenetic analyses of individual (Figs. S1 and S2) and combined (Fig. S3) plastid marker datasets do not have good resolutions or supports and do not clarify the relationships of *Dasyphyllum sensu stricto* and the rest of the subfamily. On the other hand, in the ITS (Fig. S4) and combined phylogenies (Fig. 2), *Dasyphyllum* is placed as sister to the clade comprising *Arnaldoa*, *Fulcaldea, D. diacanthoides*, and *D. excelsum* ((PA BP 98%, ML BP 100%, PP1) support values for ITS; PA BP 99%, ML BP 100%, PP 1 support values for combined).

Ancestral state reconstruction analyses

Bayesian stochastic character mapping demonstrated that the ancestral condition in *Dasyphyllum sensu stricto* is acrodromous leaf venation (PP = 0.99; Fig. 3A), bifid anther apical appendages (PP = 0.96; Fig. 3B), and small involucres (PP = 0.99; Fig. 3C) with capitula arranged into an synflorescence (PP = 0.66; Fig. 3D). Pinnate venation (Fig. 3A) and obtuse anther apical appendages (Fig. 3B) evolved in the ancestor of the clade comprising *D. diacanthoides* and *D. excelsum* (PP 0.95 and PP 0.82, respectively). The larger involucre larger (≥20 mm) is inferred to have evolved twice, since it appears in the ancestor of lineage "D" (PP 0.98), and in the *Arnaldoa* clade (PP 0.95). Regarding capitula arrangement, solitary, or arranged in few inflorescences (2–4) is a derived state and appears at least five times over the evolutionary history of the group.

DISCUSSION

Previous molecular phylogenetic hypotheses aimed to clarify the intergeneric relationships within Barnadesioideae, but they only included a limited taxonomic sampling from each genus (*Gustafsson et al., 2001*; *Gruenstaeudl et al., 2009*). Our combined phylogeny greatly improves the taxonomic coverage by including almost 82% of the species recognized as belonging to *Dasyphyllum*. The results obtained here allowed us to review the generic taxonomy and to discuss the morphological features used to recognize the infrageneric groups within this genus.

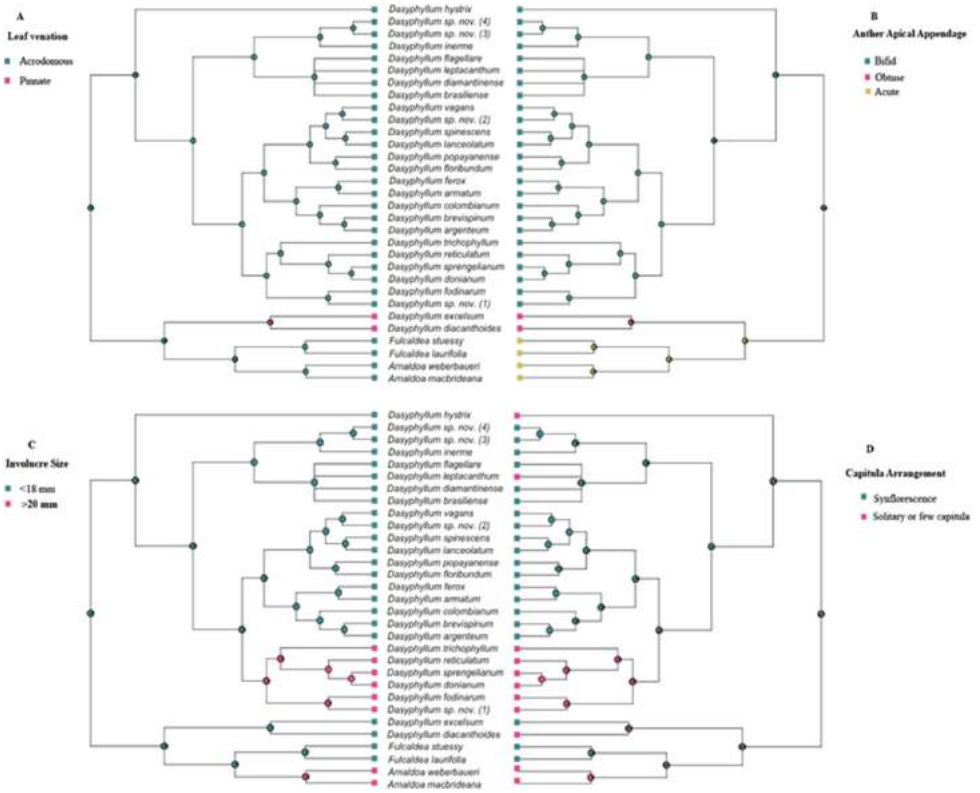

Figure 3 History of the morphological characters traditionally used to circumscribe infrageneric groups of *Dasyphyllum*. (A) Leaf venation. (B) Anther apical appendage. (C) Involucre size. (D) Capitula arrangement. Squares to the right and left of the phylogeny are color-coded according to each character state. Pie charts at nodes represent posterior probabilities of ancestral states using Bayesian inference. Full-size DOI: 10.7717/peerj.6475/fig-3

Re-circumscription of *Dasyphyllum*

All phylogenetic analyses show that, as traditionally circumscribed, *Dasyphyllum* is non-monophyletic due to the well-supported placement of *D. diacanthoides* and *D. excelsum*, which belong to *Dasyphyllum* subg. *Archidasyphyllum*, sensu *Cabrera (1959)*, in a clade sister to *Arnaldoa* and *Fulcaldea* (Fig. 2; Figs. S1–S4), a finding that confirms previous studies based on molecular data (*Gustafsson et al., 2001*; *Gruenstaeudl et al., 2009*; *Funk & Roque, 2011*; *Padin, Calviño & Ezcurra, 2015*). Despite their shared Andean distribution, the clade comprising *Arnaldoa*, *Fulcaldea*, *D. diacanthoides*, and *D. excelsum* is morphologically diverse and well-defined into distinct genera: *Fulcaldea* comprises two species of shrubs or small trees found in southern Ecuador, northern Peru, and Brazil; the species of this genus are distinguished by having single-flowered capitula, a style with subapical swelling, and villose pappus with red or pink bristles (*Gustafsson et al., 2001*; *Stuessy, Urtubey & Gruenstaeudl, 2009*; *Funk & Roque, 2011*). On the other hand, *Arnaldoa* comprises three shrubs species distributed in Ecuador and northern Peru; they are distinguished by their large and solitary

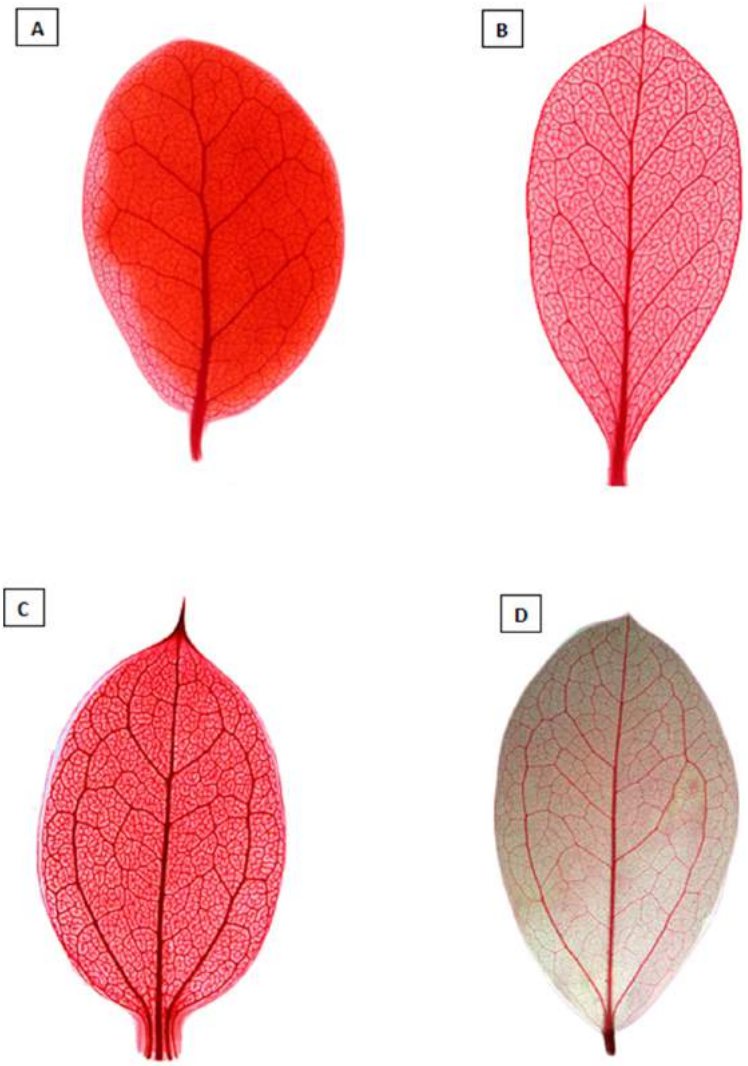

Figure 4 Diaphanized leaves showing the differences in venation. (A and B) show the pinnate venation of *Dasyphyllum* subgenus *Archidasyphyllum*. (C and D) show the acrodomous venation of *Dasyphyllum* sensu stricto. Photos: (A) *Dasyphyllum excelsum*. (B) *Dasyphyllum diacanthoides*. (C) *Dasyphyllum argenteum*. (D) *Dasyphyllum brasiliense*. All photographs were extracted from *Saavedra (2011)*. Full-size DOI: 10.7717/peerj.6475/fig-4

capitula with sub-bilabiate, white, orange, or purple corollas (*Stuessy & Sagástegui, 1993*; *Ulloa, Jørgensen & Dillon, 2002*). In contrast, *D. diacanthoides* and *D. excelsum* are restricted to the relict *Nothofagus* forests of central Chile and adjacent areas of Argentina (*Cabrera, 1959*; *Gustafsson et al., 2001*; *Gruenstaeudl et al., 2009*; *Stuessy, Urtubey & Gruenstaeudl, 2009*) and are easily distinguished from *Fulcaldea* and *Arnaldoa* because *D. diacanthoides* and *D. excelsum* are tall trees (up to 30 m) with leaves showing pinnate venation (Figs. 3A, 4A and 4B), solitary or spiciform (Fig. 3D), gynodioecious or monoecious capitula with more than one flower, and emarginated or obtuse anther apical

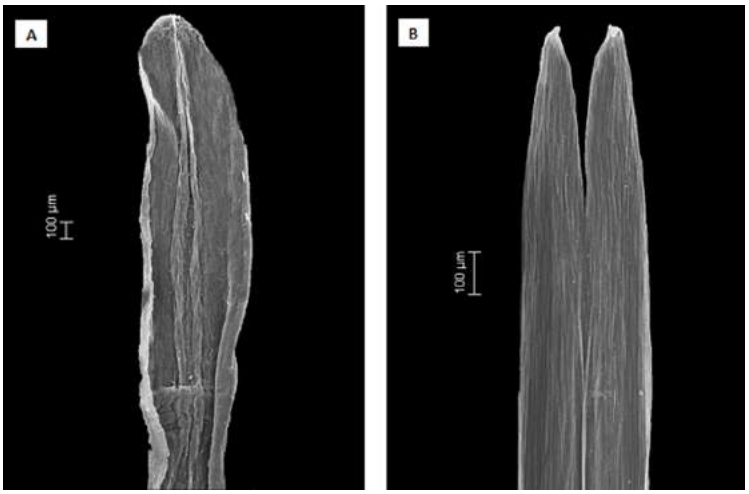

Figure 5 Scanning electron microscopy images showing the differences in anther apical appendages. (A) apical appendages obtuse of *Dasyphyllum diacanthoides* (*Dasyphyllum* subgenus *Archidasyphyllum*). (B) apical appendages bifid of *Dasyphyllum trichophyllum* (Baker) Cabrera (*Dasyphyllum* sensu stricto).
Full-size DOI: 10.7717/peerj.6475/fig-5

appendages (Figs. 3B and 5A; *Cabrera, 1959*; *Saavedra, 2011*). Due to the great morphological diversity, classifying *Arnaldoa*, *Fulcaldea*, and *Dasyphyllum* subg. *Archidasyphyllum* together in one single unit would result in several undesirable taxonomic changes and create a drastically broader genus concept with no obvious morphological support.

Instead, we propose a new circumscription of *Dasyphyllum* by elevating subg. *Archidasyphyllum* to the generic rank, *Archidasyphyllum*. This proposal is phylogenetically well-supported and consistent with leaf venation pattern (Fig. 4), anther apical appendage shape (Fig. 5), and distributional data (*Stuessy, Sang & DeVore, 1996*; *Gruenstaeudl et al., 2009*; *Saavedra, 2011*). New combinations and a key for this genus, as well as other commentaries about the distribution and phenology of the species, are presented at the end of the manuscript.

Dasyphyllum sensu stricto—intergeneric relationships and infrageneric classification

The phylogenetic relationships of *Dasyphyllum* with genera in Barnadesioideae remains unresolved. Our phylogenetic hypotheses are consistent with the placement of *Dasyphyllum* as a sister clade to the clade comprising *Arnaldoa*, *Fulcaldea*, and *Archidasyphyllum* (Fig. 2; Fig. S4). This relationship was also supported by previous molecular phylogenetic analyses (*Gustafsson et al., 2001*; *Gruenstaeudl et al., 2009*; *Funk & Roque, 2011*).

As stated in the introduction, *Dasyphyllum sensu stricto* (*D.* subgenus *Dasyphyllum*, sensu *Cabrera, 1959*) has been traditionally divided into two sections based on involucre size and capitula arrangement. Our results indicated that neither section is monophyletic (Fig. 2). Section *Macrocephala* comprises six species found in adjacent areas of Bolivia

and Paraguay (*Saavedra et al., 2018*) that share the presence of few large capitula, solitary or in small groups of heads (Figs. 1A and 1B), and it can be recognized as a monophyletic group by inclusion of *Dasyphyllum*. sp. nov. (1). Although these morphological features have evolved more than once over evolutionary history (Figs. 3C and 3D), they are useful to define this clade. Moreover, our Bayesian stochastic mapping analyses showed that the character states previously used to define section *Dasyphyllum* (involucre up to 18 mm in length and capitula arranged in synflorescences; Figs. 3C and 3D) are plesiomorphic, and therefore cannot be used to delimitate infrageneric groups as previously proposed by *Cabrera (1959)* and *Saavedra (2011)*.

Based on our taxonomic sampling, species of *Dasyphyllum sensu stricto* fall into four heterogeneous and poorly supported lineages (Fig. 2; lineages A–D). Therefore, the results of this work do not corroborate the subdivision of *Dasyphyllum* into sections and they should be abandoned.

Taxonomic treatment

Archidasyphyllum (Cabrera) P.L.Ferreira, Saavedra & Groppo, **stat. nov.** ≡ *Dasyphyllum* subgenus *Archidasyphyllum* Cabrera, *Revista Mus. de La Plata, Secc. Bot*., 9(38): 44. 1959. Type: *Archidasyphyllum diacanthoides* (Less.) P.L.Ferreira, Saavedra & Groppo.

Etymology. *Archi* (Greek) = First, Primitive; *Dasyphyllum* = genus that belongs to Barnadesioideae. *Cabrera (1959)* suggested that *Dasyphyllum* subgenus *Archidasyphyllum* is the earliest diverging group of the subfamily Barnadesioideae.

Key to species of *Archidasyphyllum*

1. Capitula solitary on the branches ..... ***A. diacanthoides***
1. Capitula arranged in spiciform synflorescences ..... ***A. excelsum***

New combinations:

Archidasyphyllum diacanthoides (Less.) P.L.Ferreira, Saavedra & Groppo **comb. nov.** ≡ *Flotovia diacanthoides* Less, *Syn. Gen. Compos.*: 95. 1832. ≡ *Piptocarpha diacanthoides* (Less.) Hook. & Arn., Comp. Bot. Mag. 1: 110. 1835. ≡ *Dasyphyllum diacanthoides* (Less.) Cabrera, *Revista Mus. La Plata, Secc. Bot*., 9(38): 44. 1959. - Type: Chile, Antuco, *E.F. Poeppig [Coll. pl. Chil. III, Syn. pl. Amer. austr. msc., Diar. 793]*, XII.1828 (*Lectotypus hic designatus*: P! [P00703408]; *Isolectotypi*: B † [photo F! [F0BN015834]], BM! [BM001010220], BR! [BR541864], M! [M-0030607], NY! [00169364, 00169365]).
Distribution and Habitat—Archidasyphyllum diacanthoides is distributed in southern Chile and adjacent areas of Argentina between 38° and 43°S. This species is found in forested areas ranging from 400 to 1,200 m in elevation.
Phenology—Flowering from November to April.
Note—Flotovia diacanthoides was described by *Lessing (1832)* based on the material "*Chuquiraga leucoxilon* Pöpp. mss. n. 793" (*nomen nudum*) collected by Poeppig. According to *Stafleu (1969)*, the plants collected by Poeppig in Chile were distributed by Kunze under the designation "Coll. pl. Chi.". Although all the type materials assigned to *Flotovia diacanthoides* are indicated with the phrase "Coll. pl. Chl.", we designated the

sheet deposited at P herbarium as the lectotype because it is the only material which also bears a handwritten label "N. 793 *Chuquiraga leucoxilon*".

Archidasyphyllum excelsum (D. Don) P.L.Ferreira, Saavedra & Groppo **comb. nov.** ≡ *Chuquiraga excelsa* D. Don, Phil. Mag. 11: 392. 1832. ≡ *Piptocarpha excelsa* (D. Don) Hook. & Arn., Comp. Bot. Mag. 1:110. 1835. ≡ *Dasyphyllum excelsum* (D. Don) Cabrera, *Revista Mus. La Plata, Secc. Bot.,* 9(38): 46. 1959. *Typus:* Chile, Valparaiso, *H. Cuming 328*, 1832 (*Lectotypus hic designatus*: K! [K000527920]; *Isolectotypi*: BM! [000522369], FI [107436 [image!]], GH [00006351 [image!]], P! [P00703407]).
Distribution and Habitat—*Archidasyphyllum excelsum* is endemic to central Chile between 32° and 34°S. This species is found in forested areas ranging from 350 to 900 m in elevation.
Phenology—Flowering from November to April.
Note—According to *Stafleu & Cowan (1976–1998)*, the herbarium of David Don was donated to the Linnean Society of London and should be conserved at the LINN herbarium. However, we have been unable to trace this material and we designated the lectotype in the K herbarium due to the specimen being well-represented in its reproductive and vegetative forms, besides the high preservation of the material.

CONCLUSIONS

This study comprises the most extensive molecular sampling for *Dasyphyllum* to date and provides a sound foundation for the re-circumscription of the genus. In so doing, it also sheds new light on the evolution of morphological features. Our phylogenetic analysis demonstrated that as currently circumscribed, *Dasyphyllum* is not monophyletic, because of *D. diacanthoides* and *D. excelsum* (*Dasyphyllum* subgenus *Archidasyphyllum*) being placed outside the genus, as sister to a clade comprising *Arnaldoa* and *Fulcaldea*. A well-supported phylogeny coupled with morphological and biogeographical data corroborate our taxonomic decision to elevate *Dasyphyllum* subgenus *Archidasyphyllum* to generic status as *Archidasyphyllum*. In addition, both sections of *D. sensu stricto* were also rejected. However, we prefer not to propose a new infrageneric classification until new data with unequivocal synapomorphies for the internal clades are available. Moreover, phylogenetic relationships between *Dasyphyllum* and other genera of Barnadesioideae remain to some extent unresolved. We suggest that future studies including additional characters from phylogenomics might better clarify the relationships of the internal clades in *Dasyphyllum*, as well as the relationships within the whole subfamily Barnadesioideae.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Paola de Lima Ferreira conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Mariana Machado Saavedra conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
- Milton Groppo conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The GenBank accession numbers are provided in Table S1 and Table S2. Molecular matrices are provided in the Supplemental Data S1.

New Species Registration

The following information was supplied regarding the registration of a newly described species:
Archidasyphyllum (Cabrera) P.L. Ferreira, Saavedra & Groppo LSID: 77194153-1.
Archidasyphyllum diacanthoides (Less.) P.L. Ferreira, Saavedra & Groppo LSID: 77194155-1.
Archidasyphyllum excelsum (D. Don) P.L. Ferreira, Saavedra & Groppo LSID: 77194156-1.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.6475#supplemental-information.

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Appendix 02

Chemistry and medicinal uses of the subfamily Barnadesioideae (Asteraceae)

Gari V. Ccana-Ccapatinta, Marcelo Monge, *Paola L. Ferreira*, Fernando B. Da Costa

Barnadesia odorata
D. Marques

"Without the gift of flowers and the infinite diversity of their fruits, man and bird, if they had continued to exist at all, would be today unrecognizable"
(Loren Eiseley, 1957)

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Chemistry and medicinal uses of the subfamily Barnadesioideae (Asteraceae)

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Abstract The subfamily Barnadesioideae (Asteraceae) constitutes a group of spiny plants that are entirely restricted to South America and currently encompasses 92 species distributed in nine genera. Barnadesioideae is particularly interesting because this subfamily constitutes the sister group of all other Asteraceae, and provides insights into the early evolution of Asteraceae. The present work summarizes the current knowledge of the chemistry and medicinal uses of Barnadesioideae. The up-to-date phytochemical profile of Barnadesioideae is composed of phenolic compounds, flavonoids, and triterpenoids, representing 39 different compounds described in 45 species of the subfamily. The presumable absence of sesquiterpene lactones—the typical Asteraceae taxonomical markers—in members of Barnadesioideae is also discussed. A few members of the genera *Barnadesia*, *Dasyphyllum*, and more frequently, *Chuquiraga*, are reported in the traditional medicine of Argentina, Brazil, Bolivia, Chile, Colombia, Ecuador, and Peru, where they are known for their antitussive, expectorant, anti-inflammatory, and many other properties. *Chuquiraga jussieui*, *Chuquiraga spinosa*, and *Chuquiraga weberbaueri* are species frequently sold in medicinal plant markets of Ecuador and Peru, where they are commonly recommended for the relief of genitourinary and reproductive disorders in women and men. Some phytopharmaceuticals containing *C. spinosa* are also marketed in Europe and North America. Further phytochemical studies on the members of Barnadesioideae would be of great interest for the chemotaxonomy of the family Asteraceae. Moreover, profiling the phytochemical composition of those medically important Barnadesioideae would support their uses in traditional medicine.

Keywords Barnadesioideae · Flavonoids · Triterpenoids · Traditional medicine

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Introduction

The subfamily Barnadesioideae (Benth. & Hook. f.) K. Bremer & R.K. Jansen comprises more than 90 species distributed in nine genera entirely restricted to South America (Bremer and Jansen 1992; Stuessy et al. 2009). Barnadesioideae members share a number of morphological and molecular features that support their position into a separate subfamily (Jansen and Palmer 1987; Bremer and Jansen 1992). The presence of axillary spines and barnadesioid trichomes (pubescences of unbranched three-celled hairs) on floral and vegetative structures constitute unique morphological characteristics within Asteraceae that distinguish Barnadesioideae from the rest of the family (Cabrera 1959; Ezcurra 1985, Bremer and Jansen 1992; Stuessy et al. 2009). Additionally, another feature of Barnadesioideae is the lack of two DNA inversions in their chloroplast genome, which are present in all other Asteraceae (Jansen and Palmer 1987; Kim et al. 2005).

Barnadesioideae has attracted increasing attention because of its well supported position as the sister group of all other Asteraceae (Funk et al. 2005; Panero and Funk 2008; Gruenstaeudl et al. 2009; Stuessy et al. 2009). Therefore, biogeographical, morphological, molecular genetics, phylogenetic and phytochemical studies of Barnadesioideae would be very important to understand the early history and evolution of Asteraceae (Gruenstaeudl et al. 2009; Stuessy et al. 2009). The present work summarizes the current knowledge of the chemistry and medicinal uses of the subfamily Barnadesioideae.

Data collection

The current taxonomic classification and geographic distributions of members of Barnadesioideae subfamily were summarized based on the most recent taxonomic treatment for each genus: Stuessy and Sagástegui (1993) and Ulloa Ulloa et al. (2002) for *Arnaldoa*; Urtubey (1999) and Hind (2001) for *Barnadesia*, Ezcurra (1985), Harling (1991), Sagástegui and Sánchez (1991) and Granada 1997 for *Chuquiraga*; Cabrera (1959, 1997), Sagástegui (1980), Sagástegui and Dillon (1985), Zardini and Soria (1994) and Saavedra et al. (2014) for *Dasyphyllum*; Katinas and Stuessy (1997) for *Doniophyton*; Funk and Roque (2011) for *Fulcaldea*, Cabrera (1951) for *Huarpea*, and Stuessy et al. (2009) for *Duseniella* and *Schlechtendalia*. Synonyms and updated geographic distributions were also consulted on TROPICOS database (www.tropicos.org).

Data collection on the chemistry and medicinal uses of the subfamily Barnadesioideae were compiled from scientific studies published in reports, theses, books and journals. A literature search covered several electronic databases (Science Direct, Scopus, SciFinder Scholar, and Google Scholar) using specific search terms such as "Barnadesioideae", "*Arnaldoa*", "*Barnadesia*", "*Chuquiraga*", "*Dasyphyllum*", "*Doniophyton*", "*Duseniella*", "*Huarpea*", "*Schlechtendalia*", and combined with specific names: e.g. "*Arnaldoa argentea*", "*Barnadesia spinosa*", "*Chuquiraga spinosa*", etc.

Chemical, spectroscopic and spectrometric data of secondary metabolites reported in Barnadesioideae were also compiled in an electronic spreadsheet file with the support of the Marvin Suite and JChem for Excel (2016, ChemAxon Ltd., www.chemaxon.com).

Barnadesioideae genera and their distribution

Despite the small number of species, Barnadesioideae genera display a broad range of habits and distinct geographic distributions. Table 1 summarizes the taxa of Barnadesioideae and their geographic distribution.

The monotypic genera *Duseniella* K.Schum., *Huarpea* Cabrera, and *Schlechtendalia* Less. are herbaceous/subshrubby plants distributed in isolated areas of Argentina, Brazil and Uruguay (Stuessy et al. 2009). The shrubby genus *Fulcaldea* Poir. was considered monotypic until 2011, when a second species was described in the "Chapada Diamantina", Bahia, Brazil (Funk and Roque 2011). The genus *Doniophyton* Wedd. includes two herbaceous species, which are found in xeric areas of Chile and Argentina (Katinas and Stuessy 1997). The three shrubby species of *Arnaldoa* Cabrera have a narrow distribution in southern Ecuador and northern Peru and grow in more or less xerophytic habitats (Stuessy and Sagástegui 1993; Ulloa Ulloa et al. 2002).

The genera *Barnadesia* Mutis ex L.f, *Chuquiraga* Juss, and *Dasyphyllum* Kunth constitute the largest and most representative taxa of Barnadesioideae

Table 1 Species of Barnadesioideae and their geographic distribution

Genus	Species	Distribution[a]
Arnaldoa Cabrera	*A. argentea* C. Ulloa, P. Jørg. & M.O.Dillon	EC
	A. macbrideana Ferreyra	PE
	A. weberbaueri (Muschl.) Ferreyra	PE
Barnadesia Mutis ex L.f	*B. aculeata* (Benth.) I.C.Chung	EC
	B. arborea Kunth	EC, PE
	B. blakeana Ferreyra	PE
	B. caryophylla (Vell.) S.F.Blake	BO, BR, PE
	B. corymbosa (Ruiz & Pav.) D.Don	BO, PE
	B. dombeyana Less.	PE
	B. glomerata var. *glomerata* Kuntze	BO
	B. glomerata var. *mucronata* I.C.Chung	BO
	B. horrida Muschl.	BO, PE
	B. jelskii Hieron.	EC, PE
	B. lehmannii var. *lehmannii* Hieron.	EC, PE
	B. lehmannii var. *angustifolia* I.C.Chung	PE
	B. lehmannii var. *ciliata* I.C.Chung	EC
	B. lehmannii var. *villosa* (I.C.Chung) Urtubey	EC, PE
	B. macbridei Ferreyra	PE
	B. macrocephala Kuntze	BO
	B. odorata Griseb.	AR, BO
	B. parviflora Spruce ex Benth. and Hook. f.	CO, EC, PE
	B. polyacantha Wedd.	BO, EC, PE
	B. pycnophylla Muschl.	BO, PE
	B. reticulata D.Don	PE
	B. spinosa L.f.	CO, EC
	B. woodii D.J.N.Hind	BO
Chuquiraga Juss	*C. acanthophylla* Wedd.	AR, BO
	C. atacamensis Kuntze	AR, BO, CH
	C. arcuata Harling	EC
	C. aurea Skottsb.	AR
	C. avellanedae Lorentz	AR
	C. calchaquina Cabrera	AR
	C. echegarayi Hieron.	AR
	C. erinacea subsp. *erinacea* D.Don	AR
	C. erinacea subsp. *hystrix* (D.Don) C.Ezcurra	AR
	C. jussieui J.F.Gmel.	BO, CO, EC, PE
	C. kuschelii Acevedo	CH
	C. longiflora (Griseb.) Hieron.	AR, BO
	C. oblongifolia Sagást. & Sánchez Vega	PE
	C. raimondiana A.Granda	PE
	C. morenonis (Kuntze) C.Ezcurra	AR
	C. oppositifolia D.Don	AR, BO, CH
	C. parviflora (Griseb.) Hieron.	AR, BO
	C. rosulata Gaspar	AR
	C. ruscifolia D.Don	AR

Table 1 continued

Genus	Species	Distribution[a]
	C. spinosa subsp. *spinosa* Less.	PE
	C. spinosa subsp. *australis* C.Ezcurra	AR, BO, CH
	C. spinosa subsp. *huamanpinta* C.Ezcurra	PE
	C. spinosa subsp. *rotundifolia* (Wedd.) C.Ezcurra	CH, PE
	C. straminea Sandwith	AR
	C. ulicina subsp. *ulicina* Hook.	CH
	C. ulicina subsp. *acicularis* (D.Don) C.Ezcurra	CH
	C. weberbaueri Tovar	PE
Dasyphyllum Kunth	*D. argenteum* Kunth	EC
	D. armatum (J.Kost.) Cabrera	AR, BO
	D. brasiliense var. *brasiliense* (Spreng.) Cabrera	AR, BR, PA
	D. brasiliense var. *barnadesioides* (Tovar) Cabrera	BO, PE
	D. brasiliense var. *divaricatum* (Griseb.) Cabrera	AR, BO
	D. brasiliense var. *latifolium* (Don.) Cabrera	BR
	D. brevispinum Sagást. & M.O.Dillon	PE
	D. cabrerae Sagást.	PE
	D. candolleanum (Gardner) Cabrera	BO, BR, PA
	D. colombianum (Cuatrec.) Cabrera	CO
	D. cryptocephalum (Baker) Cabrera	BR
	D. diacanthoides (Less.) Cabrera	CH, AR
	D. diamantinense Saavedra & M.Monge	BR
	D. donianum (Gardner) Cabrera	BR
	D. excelsum (D.Don) Cabrera	CH
	D. flagellare (Casar.) Cabrera	BR
	D. ferox (Wedd.) Cabrera	BO, PE
	D. floribundum (Gardner) Cabrera	BR, PR
	D. fodinarum (Gardner) Cabrera	BR
	D. horridum (Muschl.) Cabrera	PE
	D. hystrix var. *hystrix* (Wedd.) Cabrera	BO
	D. hystrix var. *peruvianum* (Wedd.) Cabrera	PE
	D. inerme (Rusby) Cabrera	AR, BO, PA
	D. infundibulare (Baker) Cabrera	BR
	D. lanosum Cabrera	BR
	D. lanceolatum (Less.) Cabrera	BR
	D. latifolium (Gardner) Cabrera	BO, BR, PA
	D. lehmannii (Hieron.) Cabrera	EC
	D. leiocephalum (Wedd.) Cabrera	BO, PE
	D. leptacanthum (Gardner) Cabrera	BR
	D. maria-lianae Zardini & Soria	PA
	D. orthacanthum (DC.) Cabrera	BR, PA
	D. popayanense (Hieron.) Cabrera	EC
	D. reticulatum var. *reticulatum* (DC.) Cabrera	BR
	D. reticulatum var. *robustum* Domke ex Cabrera	BR
	D. retinens (S.Moore) Cabrera	BR
	D. spinescens (Less.) Cabrera	BR

Table 1 continued

Genus	Species	Distribution[a]
	D. sprengelianum var. *sprengelianum* (Gardner) Cabrera	BR
	D. sprengelianum var. *inerme* (Gardner) Cabrera	BR
	D. synacanthum (Baker) Cabrera	BR
	D. tomentosum var. *tomentosum* (Spreng.) Cabrera	AR, BO, BR
	D. tomentosum var. *multiflorum* (Baker) Cabrera	BR
	D. trichophyllum (Baker) Cabrera	BR
	D. vagans (Gardner) Cabrera	BR
	D. varians (Gardner) Cabrera	PR
	D. velutinum (Baker) Cabrera	BR, BO
	D. vepreculatum (D.Don) Cabrera	VE
	D. weberbaueri (Tobar) Cabrera	EC, PE
Doniophyton Wedd.	*D. anomalum* (D.Don) Kurtz	AR, CH
	D. weddellii Katinas & Stuessy	AR, CH
Duseniella K.Schum.	*D. patagonica* (O.Hoffm.) K.Schum.	AR
Fulcaldea Poir.	*F. laurifolia* (Bonpl.) Poir.	EC, PE
	F. stuessyi Roque & V.A.Funk	BR
Huarpea Cabrera	*H. andina* Cabrera	AR
Schlechtendalia Less.	*S. luzulaefolia* Less.	AR, BR, UR

Taxonomy according to Stuessy and Sagástegui (1993) and Ulloa Ulloa et al. (2002) for *Arnaldoa*; Urtubey (1999) and Hind (2001) for *Barnadesia*, Ezcurra (1985), Harling (1991), Sagástegui and Sánchez (1991) and Granada (1997) for *Chuquiraga*; Cabrera (1959, 1997), Sagástegui (1980), Sagástegui and Dillon (1985), Zardini and Soria (1994) and Saavedra et al. (2014) for *Dasyphyllum*; Katinas and Stuessy (1997) for *Doniophyton*; Funk and Roque (2011) for *Fulcaldea*, Cabrera (1951) for *Huarpea*, and Stuessy et al. (2009) for *Duseniella* and *Schlechtendalia*

AR Argentina, *BO* Bolivia, *BR* Brazil, *CH* Chile, *CO* Colombia, *EC* Ecuador, *PA* Paraguay, *PE* Peru, *UR* Uruguay, *VE* Venezuela

[a]Updated distribution data were consulted on TROPICOS database (www.tropicos.org)

(Fig. 1). *Barnadesia* comprises 19 species of shrubs and trees, mainly distributed in the Andes from Colombia to Argentina, and one species is found in Brazil, mostly restricted to elevations of 1800–3400 m (Urtubey 1999; Hind 2001). *Chuquiraga* is a genus of 22 spiny evergreen shrubs that grow along the Andes and the Patagonia at high altitude habitats; however, some species are found at sea level areas in central Chile and Argentina (Ezcurra 1985; Harling 1991; Sagástegui and Sánchez 1991; Granada 1997). *Dasyphyllum* is a genus of deciduous shrubs or evergreen trees, which comprises 41 species distributed throughout the continent, with two centers of diversity, one in western South America, in Andean mountains from Venezuela to north-western Argentina, occupying arid regions such as the Puna, and the other in eastern South America, in Brazil, Bolivia, and Paraguay in Atlantic forest and savanna (Cabrera 1959, 1997; Sagástegui 1980; Sagástegui and Dillon 1985; Zardini and Soria 1994; Saavedra et al. 2014).

Secondary metabolite chemistry

The secondary metabolite chemistry of Barnadesioideae has been sometimes described as following a simple profile (Bohm and Stuessy 1995; Zdero et al. 1987). This possible simple chemistry profile is proposed and hypothesized as further evidence of the basal position of Barnadesioideae in the Asteraceae family (Bohm and Stuessy 1995, 2001; Calabria et al. 2007).

In total, two acetophenones (**1** and **2**) (Senatore 1996; Senatore et al. 1999), one benzaldehyde (**3**) (Hoeneisen et al. 2000), one benzoic acid (**4**) (Castelucci et al. 2007), one coumarin (**5**) (Hoeneisen

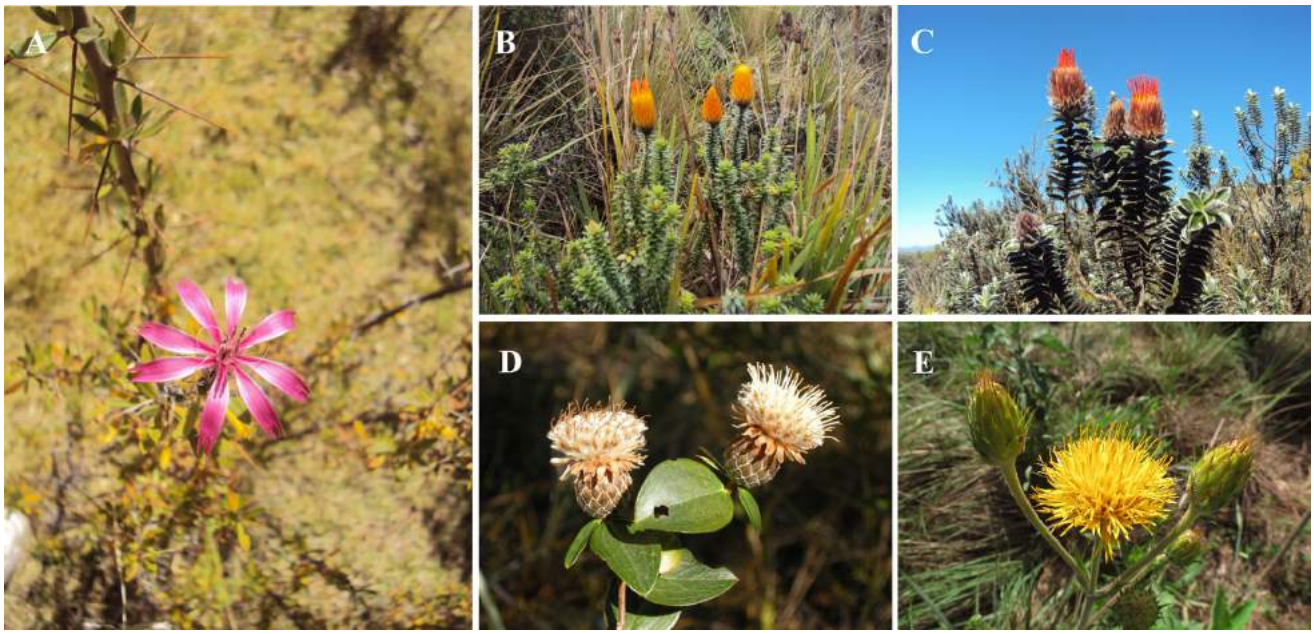

Fig. 1 Pictures of representative species of Barnadesioideae: **a** *Barnadesia horrida* (Q'orimark, Cusco, Peru), **b** *Chuquiraga jussieui* (Huancabamba, Piura, Peru), **c** *Chuquiraga weberbaueri* (Celendin, Cajamarca, Peru), **d** *Dasyphyllum sprengelianum* (Serra do Cipó, Minas Gerais, Brazil), **e** *Schlechtendalia luzulaefolia* (Cerro do Tigre, Manoel Viana, Brazil). Photos by: G. V. Ccana-Ccapatinta, G. Shimizu and G. Heiden

et al. 2000), 13 flavonoids (eriodictyol, kaempferol, quercetin, isorhammetin, and their 3-*O*-glycosides) (**6–18**) (Bohm and Stuessy 1995; Mendiondo et al. 1997, 2000; Senatore et al. 1999; Mendiondo and Juárez 2001; Juárez and Mendiondo 2002a, b, 2007; Landa et al. 2009), and 21 triterpenoids (taraxasterol, lupeol, ursane and oleanane derivatives) (**19–39**) (Zdero et al. 1987; Flagg et al. 1999; Hoeneisen et al. 2000; Gurovic et al. 2010) have been described to date in 45 species of Barnadesioideae (Fig. 2). Detailed information on the occurrence of these metabolites in species of Barnadesioideae is presented in **Table S1** (Supplementary material).

The flavonoid chemistry of Barnadesioideae has been explored by Bohm and Stuessy (1995), Mendiondo et al. (1997, 2000), Senatore et al. (1999), Mendiondo and Juárez (2001), Juarez and Mendiondo (2002a, b, 2007) and Landa et al. (2009). However, some reports suggest that other polar compounds may remain underinvestigated in Barnadesioideae. A partially elucidated acetophenone glycoside has been isolated from the leaves of *Chuquiraga spinosa* Less. (Gálvez and Pastor 1996). The HPLC–DAD chromatograms of H_2O:MeOH extracts of *C. spinosa* displayed several peaks with UV spectra characteristic of phenolic acids (Casado et al. 2011). Similarly, initial HPLC–DAD–MS analyses have demonstrated the presence of caffeoyl and feruloyl ester derivatives of quinic and shikimic acids in the polar fraction of *Dasyphyllum brasiliense* (Spreng.) Cabrera (Passoni et al. 2008). Additionally, a qualitative screening by the froth formation test suggested the presence of saponins in the alcoholic extract of *C. spinosa* (Arroyo-Acevedo et al. 2017; Herrera-Calderon et al. 2017).

Regarding more lipophilic compounds, Zdero et al. (1987) first reported on the triterpenoid chemistry of several members of *Barnadesia*, *Chuquiraga*, *Dasyphyllum* and *Schlechtendalia* genera, while more detailed surveys on selected *Chuquiraga* taxa have been conducted by Flagg et al. (1999), Hoeneisen et al. (2000) and Gurovic et al. (2010). It is interesting to notice that in these reports the plant materials were extracted with solvent mixtures capable of extracting middle/high lipophilic compounds (MeOH–Et_2O–petrol, 1:1:1; MeOH–EtOAc–hexane, 1:1:1; CH_2Cl_2–MeOH, 1:1). Under the same conditions of extraction and isolation, other related Asteraceae (*Gochnatia* Kunth, *Mutisia* L. f., and *Nausavia* Comm. ex Juss.) have afforded a diversity of coumarins, coumaranes, and methyl chromones derivatives as well as sesquiterpene lactones (Bohlmann et al. 1986; Zdero

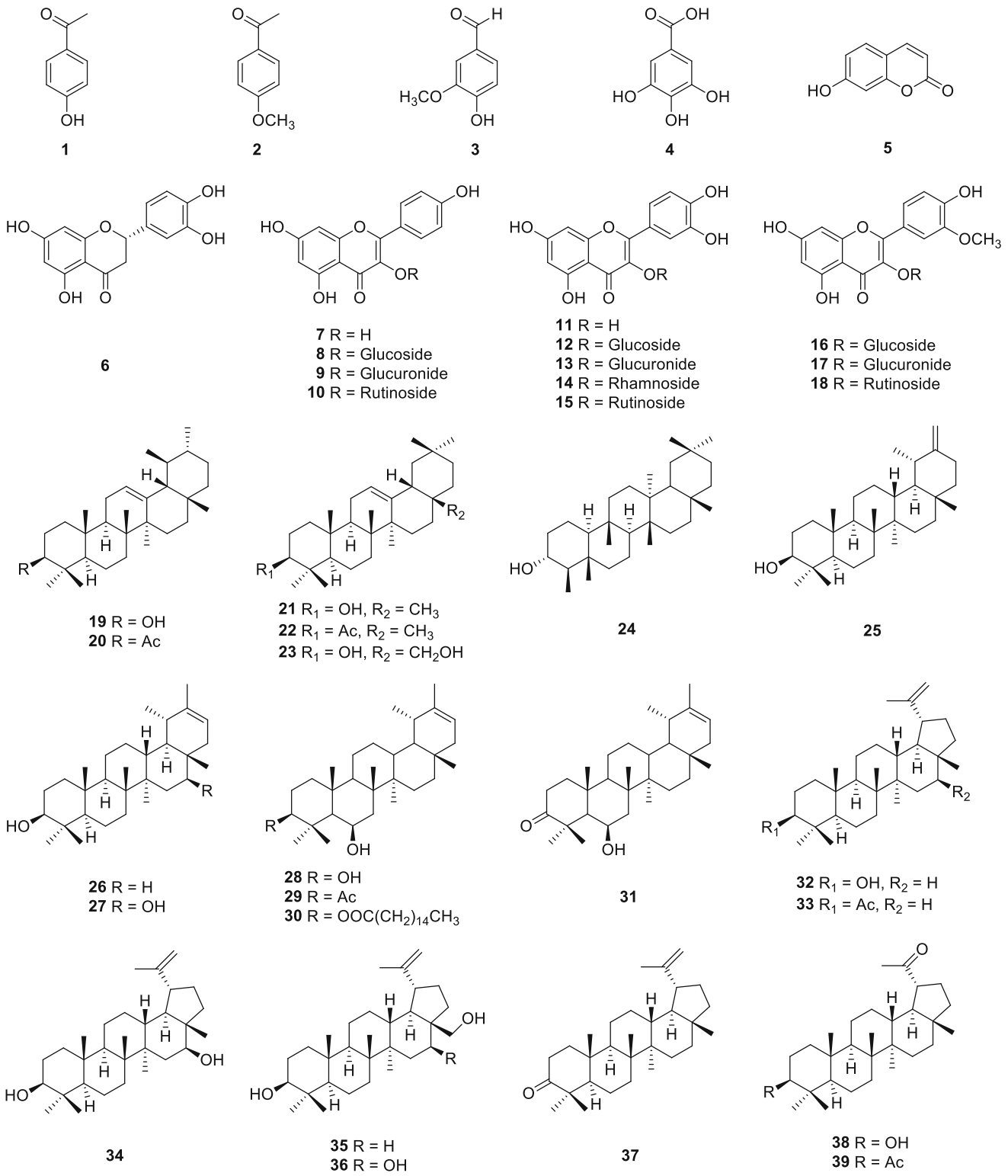

Fig. 2 Chemical constituents reported in members of Barnadesioideae

et al. 1986a, b). Sesquiterpene lactones are characteristic constituents of the Asteraceae family and, in addition to their toxic and biological properties, are considered taxonomic markers (Seamann 1982; Zdero and Bohlmann 1990; Spring 2000; Da Costa et al. 2005; Padilla-Gonzalez et al. 2016); however, no such report exists for Barnadesioideae (**Table S1**), therefore the possible absence of sesquiterpene lactones

may also corroborate the separate position of Barnadesioideae to the rest of Asteraceae. (Calabria et al. 2007; Lundberg 2009).

Remarkably, Nguyen et al. (2010, 2016) found germacrene A synthase (GAS) and germacrene A oxidase (GAO) activity when the cDNA of the homologous enzymes from *Barnadesia spinosa* (BsGAS, BsGAO) was expressed in yeast. These enzymes (GAS and GAO) are involved in the early steps of sesquiterpene lactones biosynthesis by sequentially cyclizing and oxidizing farnesyl diphosphate into the advanced intermediate germacrene A acid (Nguyen et al. 2010, 2016), which subsequently leads to the formation of costunolide, a framework in the biosynthesis of guaianolide, eudesmanolide and germacranolide sesquiterpene lactones (de Kraker et al. 2001, 2002). Additionally, the presence of germacrene A acid was confirmed by LC–ESI–MS in the ethyl acetate extract of leaves of *B. spinosa* (Nguyen et al. 2010). However, further investigations are required to identify the enzymes involved in the latter steps of sesquiterpene lactone biosynthesis (e.g. those involved in the oxidation of the C-6 carbon of germacrene A acid) to effectively correlate enzyme function to the presence or absence of sesquiterpene lactones in Barnadesioideae.

The localization of sesquiterpene lactones in glandular trichomes of numerous Asteraceae has facilitated the conduction of chemotaxonomy studies (Spring 1989, 2000; Da Costa et al. 2001). Chemical investigations on the content of Barnadesioideae trichomes (barnadesioid, malpighiaceous, and glandular) (Ezcurra 1985; Stuessy et al. 2009) could provide insights to elucidate de occurrence of sesquiterpene lactones in Barnadesioideae.

The possible occurrence of alkaloids in *Chuquiraga spinosa* has been suggested after positive reaction of an alcoholic extract with Dragendorff, Mayer, and Wagner reagents (Arroyo-Acevedo et al. 2017; Herrera-Calderon et al. 2017). In Asteraceae, alkaloids are restricted to Senecioneae, Eupatorieae, and Cardueae tribes (Calabria et al. 2009), therefore, further surveys and adequate procedures should be conducted to confirm the occurrence of alkaloids and other classes of metabolites in *C. spinosa* and other Barnadesioideae members.

Volatile metabolite chemistry

p-Methoxyacetophenone, *p*-hydroxyacetophenone, α-terpineol, linalol, nonanal, pulegone, apiol, β-humulene and spathulenol were described as main constituents of the essential oil of *Chuquiraga spinosa* (Senatore 1996).

Medicinal uses

A wealth of references supports the medicinal uses of Barnadesioideae (*Barnadesia*, *Dasyphyllum* and especially *Chuquiraga*). This pool of ethnobotanical information is summarized in the following sections.

Barnadesia Mutis ex L.f.

Barnadesia arborea Kunth, one of the most attractive shrubs/trees of the genus, is distributed among localities of Ecuador and Peru where it receives the following vernacular names: *chian, chuquirahua, espino, espino santo, espino de gato, espino de estrella, chivocaspi,* and *clavelillo* (Hind and Hall 2003). The infusion of *B. arborea* leaves is applied externally for the relief of spasms in children (Urtubey 1999). Additionally, the topical application of its flowers by rubbing is used in the treatment of dermatitis and influenza in Ecuador (Tene et al. 2007).

Barnadesia horrida Muschl., known in some localities of Peru as the Andean clove pink (*clavelina de tierra*), is distributed among the highlands of Bolivia and Peru (Herrera 1933). An infusion of its flowers is used for the treatment of common cold, bronchopneumonia, bronchitis, cough, headache, fever, and stomach ache in the traditional medicine of Cusco, Peru (Yakovleff and Herrera 1934; Herrera 1938; Roersch 1994), where it receives the vernacular names of *llaulli, llaulli–llaulli and kiska-llaulli*. This species is also cultivated as living walls in Peru.

Dasyphyllum Kunth

Dasyphyllum brasiliense is used for the treatment of inflammatory diseases, especially oral and oropharyngeal inflammatory diseases in São Paulo and central-west Minas Gerais states, Brazil, where is popularly

Table 2 Common names and medicinal uses of species of the genus *Chuquiraga*

Species	Country	Common names	Indications[a]	References
C. acanthophylla	AR	*Espina amarilla*	Cold, cough and fever. Stomachache. Urinary tract infections	Barbarán (2008)
C. atacamensis	AR	*Hierba de san Pedro, san Pedro, kishka tola*	Conjunctivitis, for which the plant is used to make a medicinal smoke. Rheumatic pain, where the plant infusion is used to wash rheumatic legs to relieve pain	Giberti (1983)
	BO	*San Gerónimo, fundición, kutu kutu, chajllampa*	Cold, cough, fever. Urinary tract infections, cystitis, prostatitis. Relief of postpartum symptoms. Not recommended in pregnant women	Zamora (2008)
	CH	*Lengua de gallo, tastará, quebrolla, killokisca, chana chaklamba*	The infusion is used as hot baths against colds. Productive and non-productive cough, fever. Genitourinary and reproductive disorders in women	Villagrán et al. (1998, 2003)
C. avellanedae	AR	*Quilimbay-trayao, tratrakcha, trayau*	Cough. Headache and fever, boiled leaves are chewed in a mixture with sugar	Richeri et al. (2013)
C. erinacea	AR	*Romerillo, falsa uña de gato, trifrif mamull*	Stomachache and liver disease. Kidney disease. Strengthens the brain and nerves	Ladio and Lozada (2009)
C. jussieui	CO	*Chuquiragua, vela de páramo*	Febrifuge, diuretic, kidney stones	Díaz-Piedrahita and Vélez-Nauer (1993)
	EC	*Chuquira, chuquiragua*	Liver disease, diabetes. Allergy and skin disorders. Pain of the bones, rheumatism and other inflammations. Toothache, stomachache and gastrointestinal disorders. Cold, fever, cough and respiratory disorders. Malaria, malarial fever, smallpox, internal infections. Urogenital disorders, diuretic. Relief of postpartum symptoms	Martínez (2006), Tene et al. (2007), Ansaloni et al. (2010)
	PE	*Chiquiragua* (northern Peru). *Inca llaulli*, *kentayllaulli*, *quishuara*, *kiswara*, *kiswara tiutumpi*, *qharisirviy* (southern Peru)	Stomachache and liver disease. Musculoskeletal pain. Skin eruptions, inflammations. Common cold, cough, sore throat, fever, respiratory disorders. Vaginitis and vaginal infections, as external washing. Urinary tract infections, kidney disease, stones, prostatitis. Postpartum symptoms. Endoparasiticide (intestinal worms), and ectoparasiticide (lice). Rheumatic pain, an infusion is used to wash the legs	Torres et al. (1992), Roersch (1994), De Feo (2003), Vásquez et al. (2010)
C. longiflora	AR	*Azafrán de la puna*	The plant is added to water for personal washing	Giberti (1983)
C. oppositifolia	AR	*Azafrán del campo*	Hypoglycaemic, hypocholesterolaemic. Antifungal	Raad (2012)
C. parviflora	BO	*Chiñi michi michi*	Against curse	Vandebroek et al. (2003)

Table 2 continued

Species	Country	Common names	Indications[a]	References
C. spinosa	AR	*Charkoma*	Regulation of the menstrual cycle	Giberti (1983)
	BO	*Huamanpinta*	Kidney stones and cystitis	Ceuterick et al. (2011)
	EC	*Chuquiragua*	Cold, cough and fever. Pain of the bones. Malaria	Bussmann and Sharon (2006b)
	PE	*Huamanpinta*, *huancapita*, *huancaspita, laulinco, pucacasha, pazpapamaquin, qharisirviy, cjari sirvi*	Respiratory affections. Antiblenorrhaegic and vermifuge. Conjunctivitis. Gonorrhoea. Urinary system disorders in women and men. Vaginitis and vaginal infection, the infusion of the plant is uses for external washing. Kidney and prostate inflammations. Prostate cancer. Diuretic. Sexual impotence	Brack (1999), Madaleno (2007), Rehecho et al. (2011)
C. weberbaueri	PE	*Amaro amaro*	Cough, bronchitis, asthma. Liver disease. Diuretic and depurative	Brack (1999)

AR Argentina, *BO* Bolivia, *CH* Chile, *CO* Colombia, *EC* Ecuador, *PE* Peru

[a]Commonly, aerial parts are used to make infusions or decoctions in water; other modes of use are detailed in the table text

known as *espinho-agulha* (Castelucci et al. 2007). The leaves and thorns are used to prepare a tea in boiling water that presents anti-inflammatory properties.

The cortex decoction of *D. diacanthoides* (Less.) Cabrera is used for the treatment of contusions and rheumatism in Mapuche traditional medicine in Chile, where it is known as *tayu, palo santo and palo blanco* (de Mösbach 1991). It is also used as an antitussive and as a remedy for stomach spasms (Estomba et al. 2006; Campos-Navarro and Scarpa 2013).

Chuquiraga Juss

Several species of the genus *Chuquiraga* are described as being used in the traditional medicine of Argentina, Bolivia, Chile, Colombia, Ecuador and Peru (Table 2). The medicinal uses of *Chuquiraga* can be traced to times of pre-Columbian South American cultures such as the Incas (Giberti 1983; Roersch 1994; Brack 1999; De-la-Cruz et al. 2007), Aymaras (Villagrán et al. 1998, 2003), and Tehuelches (Ramírez and Beloso 2002).

As a general trend, *Chuquiraga* medicinal species are used as infusions, alone or in mixture with other plants (Bussmann et al. 2010, 2015), for the treatment of respiratory, gastrointestinal, genitourinary and reproductive disorders. A small number of medicinal indications are reported for *C. acanthophylla* Wedd. (Barbarán 2008), *C. avellanedae* Lorentz (Ramírez and Beloso 2002), *C. erinacea* D.Don (Ramírez and Beloso 2002), *C. longiflora* (Griseb.) Hieron. (Giberti 1983), *C. oppositifolia* D.Don (Raad 2012), *C. parviflora* (Griseb.) Hieron. (Vandebroek et al. 2003), and *C. weberbaueri* Tobar (Bussmann and Sharon 2006a). On the other hand, *C. atacamensis* Kuntze (Camaqui 2007; Rondina et al. 2008), *C. jussieui* J.F.Gmel. (Bussmann and Sharon 2006b; de la Torre et al. 2008; Quattrocchi 2012), and *C. spinosa* (De-la-Cruz et al. 2007; Bussmann and Glenn 2010; Siura and Flores 2010; Monigatti et al. 2012) display a higher number of medicinal indications (Table 2).

Among the species of *Chuquiraga*, *C. jussieui* is the first botanically described species of the genus and certainly one of the most emblematic. Indeed, the genus name came from '*chuquiragua*', a vernacular name that is used to designate this species in southern Colombia, Ecuador, and northern Peru (Ezcurra 1985). A second area of occurrence of this species encompasses southern Peru and northwest Bolivia, where it is known as *kisaura, quishuará, ckentai,* and *ckentai-llaulli* (Ezcurra 1985). Even given this discontinuous area of distribution, the medicinal uses of *C. jussieui* in Ecuador, northern Peru, and southern Peru are quite similar. Historically, *C. jussieui* attracted particular attention when detailed descriptions of the use of this plant in Ecuador appeared in the scientific literature (Anderson 1867). Particularly, the

febrifuge property of *C. jussieui* was once compared to that of *Cinchona officinalis* L. (Rubiaceae) (Soubeiran 1868; Collins 1870; Ezcurra 1985). Currently, this species is still important in the traditional medicine of Ecuador and Peru (Duke et al. 2009; Quattrocchi 2012).

Chuquiraga spinosa is currently classified into four subspecies. These four subspecies are distributed principally along the central Andes of Peru to Bolivia, northern Chile and northwest Argentina. *Chuquiraga spinosa* is particularly appreciated in the treatment of inflammatory and infectious genitourinary illnesses. The infusions or decoctions of their aerial parts are taken alone but also mixed with other plants, most frequently potato skins (*Solanum tuberosum* L., Solanaceae), toasted grains of barley (*Hordeum vulgare* L., Poaceae), leaves of achiote (*Bixa orellana* L., Bixaceae), and horse tail (*Equisetum* L. spp, Equisetaceae) (Abad et al. 2009; Siura and Flores 2010; Madaleno 2007, 2012; Bussmann and Sharon 2015).

In norther Peru, *C. weberbaueri* is also used for the treatment of asthma, bronchitis and liver disease in mixture with *Eucalyptus globulus* Labill., *Piper aduncum* L., *Gaultheria erecta* Vent., *Desmodium molliculum* (Kunth) DC., *Minthostachys mollis* (Kunth) Griseb., and *Cordia lutea* Lam; it is also combined with *Malva sylvestris* L., *Picrosia longifolia* D. Don. for the treatment of intestinal complaints (Bussmann et al. 2010).

Ethnoveterinary uses

Chuquiraga weberbaueri has been reported as an endoparasiticide in the ethnoveterinary medicine of northern Peru. The decoction of the aerial parts is administered to cattle and sheep against internal parasites, particularly common liver fluke (*Fasciola hepatica*) infection and other helminthiases (Mostacero et al. 2011). However, careful administration of *C. weberbaueri* is recommended because excessive doses could kill animals (Mostacero et al. 2011).

Toxicity

There are few data about the toxicity or side effect of species of the genus *Chuquiraga*. The aqueous extracts of *C. spinosa* and *C. weberbaueri* displayed median lethal doses (LD_{50}) > 10,000 µg/ml in the brine shrimp lethality assay, whereas the ethanolic extracts displayed LC_{50} values of 1.1 and 0.25 µg/ml, respectively (Bussmann et al. 2011). Even though there is a report discouraging the administration of *C. atacamensis* infusions in pregnant women because it could cause miscarriage (Zamora 2008). Additional studies are required to reveal the possible toxicity and side effect of *Chuquiraga* species and other representatives of Barnadesioideae subfamily.

Commercialization and conservation concerns

The medicinal species of *Chuquiraga* are important and evident elements in medicinal plant markets of traditional cities of Ecuador and Peru but also in modern cities such as Guayaquil and Lima, and at least one species has been introduced in the international market. Differently to markets of Ecuador and Peru where commercialization of *Chuquiraga* species is frequent, the commercialization of *Chuquiraga* species in Markets of Bolivia seems to be absent (Macía et al. 2005; Bussmann et al. 2016).

Chuquiraga jussieui is one of the most popular medicinal plants in Ecuador and has been noted as a plant with promising industrial potential (Buitron 1999; Martínez 2006; Gupta 2006). The flowering parts of this species are also found in markets of northern Peru together with *C. weberbaueri* (Bussmann et al. 2007). In the markets of southern Peru, the inflorescences of *C. jussieui* are frequently commercialized separately from leaves and steams (Fig. 3a).

The aerial parts of *C. spinosa* are sold along the main cities of Peru (Madaleno 2007; Ceuterick et al. 2011; Huamantupa et al. 2011; Fig. 3b). This species is also distributed as a dietary supplement in Europe (Huamanpinta, Esparta GmcH, www.paracelmed.com; Fig. 3c) and North America (Huamanpinta, Alpha Omega Labs, www.alphaomegalabs.com). Products that contain *C. spinosa*, mixed with other plants, can also be found, for example, Women's Care Blend (Amazon, www.amazon.com), Prostate Care Blend and Kidney Cleanser Blend (Fito Global Inc., www.fitoglobal.com).

Despite the ornamental potential, medicinal uses, and commercialization of *Chuquiraga* species, there is no evidence that these species are currently cultivated in their natural habitats. Additionally, there are no data

Fig. 3 Commercial samples of *Chuquiraga* species: **a** flowers of *C. jussieui* (market in Puno City, Peru); **b** aerial parts of *C. spinosa* (market in La Oroya City, Peru); **c** capsules containing *C. spinosa* powder (commercialized in Austria and Germany). Photos by: G. V. Ccana-Ccapatinta

whether *Chuquiraga* species would be cultivable outside their natural habitats. The increasing demand for *Chuquiraga* medicinal species, which are currently collected in the wild, causes concerns about their conservation and sustainability. Therefore, the cultivation and transplantation of these species is an issue that must be further explored (Ezcurra 1985). In this context, Jadán et al. (2014) published an in vitro culture protocol of *C. jussieui* from apical and axillary buds. More studies are required to establish the *ex vitro* acclimatization of these micropropagated plants and its application for the conservation of other *Chuquiraga* species.

Biological activities

The aqueous extract of *D. brasiliense* demonstrated anti-inflammatory activity in β-glucan-induced peritonitis and mouse paw edema assays (Castelucci et al. 2007). Alcoholic extracts from *D. diacanthoides* and *D. tomentosum* (Spreng.) Cabrera displayed no antimicrobial activity on selected bacteria (Zampini et al. 2007; Paula et al. 2013).

The biological activities reported for *C. atacamensis* (Alberto et al. 2009; Zampini et al. 2009, 2010), *C. erinacea* (Gurovic et al. 2010), *C. jussieui* (Dueñas et al. 2014), *C. spinosa* (Bussmann et al. 2008; Casado et al. 2011; Arroyo-Acevedo et al. 2017; Herrera-Calderon et al. 2017) and *C. straminea* Sandwith (Mendiondo et al. 2011) are detailed in Table 3.

The alcoholic extracts of *C. atacamensis* (Alberto et al. 2009), *C. jussieui* (Dueñas et al. 2014), *C. spinosa* (Casado et al. 2011), and *C. straminea* (Mendiondo et al. 2011) displayed free-radical scavenging capacity and antioxidant activity. Additionally, the alcoholic extract of *C. spinosa* considerably reverted the paw and ear edema in rats and mice (Casado et al. 2011), while *C. atacamensis* displayed COX-1 and COX-2 inhibition (Alberto et al. 2009). Even though these studies did not comprise bioassay-guided identification of the biologically active metabolites, the reported occurrence of kaempferol, quercetin, isorhammetin, and their 3-*O*-glycosides could explain the antioxidant and anti-inflammatory properties observed for *Chuquiraga* species. For reviews on the antioxidant and anti-inflammatory activity of quercetin and derivatives, see Pietta (2000) and Carullo et al. (2017). The presence of flavonoids can also explain the COX-1 and COX-2 inhibition by *C. atacamensis* alcoholic extract (Ribeiro et al. 2015). Caffeic acid derivatives, *p*-hydroxyacetophenone, and acetophenone glycoside are other constituents with reported antioxidant and anti-inflammatory activities (Sala et al. 2001; Shahidi and Chandrasekara 2010; Ching-Wen et al. 2017), however their occurrence and

Table 3 Biological activities reported for species of the genus *Chuquiraga*

Plant	Extracting solvent[a], standardization	Bioactivity	Results	References
C. atacamensis	80% Ethanol (5 g/100 ml), 500 µg of GAE/ml	In vitro COX-1 inhibition	IC_{50} = 2 µg/ml	Alberto et al. (2009)
		In vitro COX-2 inhibition	IC_{50} = 4.7 µg/ml	
		Antioxidant, DPPH·, $ABTS^{+}$, $O_2^{\cdot -}$	IC_{50} = 3.5–20 µg/ml	
	80% Ethanol (5 g/100 ml), 500 µg of GAE/ml	*Staphylococcus aureus* strains	MIC = 80–600 µg/ml	Zampini et al. (2009)
		Enterococcus faecalis strains	MIC = 150–300 µg/ml	
		Escherichia coli strain	MIC = 600 µg/ml	
		Other gram-negative bacteria	MIC = 300–600 µg/ml	
	Ethanol (dry extract)	Antioxidant, $ABTS^{+}$ assay	SC_{50} = 1.5 µg/ml	Zampini et al. (2010)
C. erinacea	Ethanolic extract (dry extract)	In vitro AChE inhibitory activity	IC_{50} = 7.26 mg/ml	Gurovic et al. (2010)
C. jussieui	Water (2 g/100 ml)	Antioxidant	IC_{50} = 64.9 mg/l	Dueñas et al. (2014)
C. spinosa	Water (dry extract), 5.4 mg GAE/mg	Antioxidant, DPPH·, $ABTS^{+}$, O_2	IC_{50} = 9.6–30.5 µg/ml	Casado et al. (2011)
		Candina albicans	MIC = 2.5 µg on TLC plate	
		Cladosporium cucumerinum	MIC = 2.5 µg on TLC plate	
		Rhizopus stolonifer	MIC = 4.6 µg on TLC plate	
	50% Methanol (dry extract), 6.3 mg GAE/mg	Antioxidant: DPPH·, $ABTS^{+}$, O_2	IC_{50} = 8.5–21.7 µg/ml	Casado et al. (2011)
		Antiinflammatory, paw edema in rats	Maximal inhibition = 52.5%	
		Antiinflammatory, ear edema in mice	Inhibition = 88.1%	
		Candina albicans	MIC = 6.3 µg on TLC plate	
		Rhizopus stolonifer	MIC = 13.5 µg on TLC plate	
	Methanol (dry extract), 12.6 mg GAE/mg	Antioxidant, DPPH·, $ABTS^{+}$, O_2	IC_{50} = 10.5–36.5 µg/ml	Casado et al. (2011)
		Rhizopus stolonifer	MIC = 18.5 µg on TLC plate	
	Water (5 g/500 ml)	*Staphylococcus aureus* strain	13 mm, agar diffusion test	Bussmann et al. (2008)
	96% Ethanol (dry extract)	Cytotoxicity in DU-145 cell line	IC_{50} = 2.98 µg/ml	Arroyo-Acevedo et al. (2017)
	96% Ethanol (dry extract)	Cytotoxicity in MCF-7 cell line	IC_{50} = 9.25 µg/ml	Herrera-Calderon et al. (2017)
		Cytotoxicity in K-562 cell line	IC_{50} = 7.34 µg/ml	
		Cytotoxicity in HT-29 cell line	IC_{50} = 8.52 µg/ml	
		Cytotoxicity in H-460 cell line	IC_{50} = 5.32 µg/ml	

Table 3 continued

Plant	Extracting solvent[a], standardization	Bioactivity	Results	References
		Cytotoxicity in M-14 cell line	IC_{50} = 8.30 µg/ml	
		Cytotoxicity in HUTU-80 cell line	IC_{50} = 6.20 µg/ml	
		Cytotoxicity in DU-145 cell line	IC_{50} = 7.09 µg/ml	
	Hexane fraction (dry extract)	Cytotoxicity in DU-145 cell line	IC_{50} = 27.03 µg/ml	
	Petroleum ether fraction (dry extract)	Cytotoxicity in DU-145 cell line	IC_{50} = 33.10 µg/ml	
	Chloroform fraction (dry extract)	Cytotoxicity in DU-145 cell line	IC_{50} = 24.19 µg/ml	
	Ethyl acetate fraction (dry extract)	Cytotoxicity in DU-145 cell line	IC_{50} = 54.12 µg/ml	
C. straminea	80% Methanol (dry extract)	Antioxidant, DPPḢ, $ABTS^{\dot{+}}$	SC_{50} = 14.5–34.9 µg/ml	Mendiondo et al. (2011)
		Staphylococcus aureus strains	MIC = 200–800 µg/ml	

GAE gallic acid equivalents

[a]Plant/solvent ratio

contributions to bioactivity in Barnadesioideae needs further investigation.

One of the most popular uses of *Chuquiraga* species is in the treatment of urinary tract infections. Alcoholic extracts of *C. atacamensis*, *C. spinosa,* and *C. straminea* displayed antibacterial and antifungal activity on selected strains (Table 3) that could support their traditional uses in genitourinary infections. However, deeper studies are necessary to establish the antibacterial activity of *Chuquiraga* species, especially against *Escherichia coli* strains, and the corresponding bioactive constituents.

Chuquiraga jussieui and *C. spinosa* are also frequently uses in the treatment of prostatitis and prostate cancer (Table 2). In this context, Arroyo-Acevedo et al. (2017) described for the first time the protective effect of the administration of *C. spinosa* alcoholic extract on *N*-methyl nitrosourea (NMU)-induced prostate cancer in rats. The same extract displayed cytotoxicity in the DU-145 (prostate carcinoma) cell line with a IC_{50} of 2.98 µg/ml (Arroyo-Acevedo et al. 2017). Additionally, Herrera-Calderon et al. (2017) investigated the cytotoxicity of *C. spinosa* ethanolic extract on the MCF-7 (breast adenocarcinoma), K-562 (chronic myelogenous leukemia), HT-29 (colon adenocarcinoma), H-460 (lung large cell carcinoma), M-14 (amelanotic melanoma), HUTU-80 (duodenum adenocarcinoma), and DU-145 cell lines, obtaining IC_{50} values of 5.32–9.25 µg/ml. Interestingly, the lipophilic fractions (hexane, petrol, chloroform and ethyl acetate) obtained from the initial extract displayed IC_{50} values of 24.19–54.12 µg/ml, suggesting that the active constituents may remain in the polar fractions. Flavonoids and other phenolic compounds are known for their chemotherapeutic and chemopreventive effects (Gioti and Tenta 2015; Yang et al. 2015).

Final remarks

The subfamily Barnadesioideae constitutes an important group of the Asteraceae family. The secondary metabolite chemistry of the group requires further surveys to confirm the occurrence/absence of sesquiterpene lactones, acetophenone glycosides, caffeic acid derivatives, alkaloids, saponins, etc., which would be of great interest for the chemotaxonomy of the Asteraceae family. Species of the three genera *Barnadesia*, *Dasyphyllum*, and especially *Chuquiraga*

are part of the traditional medicine of several South American countries displaying a diversity of medical indications. Even though the widely commercialization of some species in medical plant markets of Peru and Ecuador, few is known about their toxicity or possible side effects. Initial studies have demonstrated the antioxidant, anti-inflammatory, antifungal, antimicrobial and chemopreventive activities of *Chuquiraga* species, however further phytochemical and biological/pharmacological studies are necessary to determine the biologically active phytochemical constituents and consequently support their uses by traditional medicine.

Supplementary material

The occurrence of the secondary metabolites reported in members of the Barnadesioideae subfamily is displayed in **Table S1** and in the AsterDB-Barnadesioiedae.sdf database. This database is embedded in a larger project, the AsterDB, an in-house database of the AsterBioChem research group containing chemical structures of Asteraceae (www.asterbiochem.org/asterdb). The AsterDB-Barnadesioiedae.sdf file, built with the Marvin Suite 16.2.15 (Academic Teaching License) and JChem for Excel 16.2.8 (2016, ChemAxon Ltd., www.chemaxon.com), contains 294 entries of 39 2D chemical structures (with assigned stereochemistry) in the .MOL file format together with their respective CAS numbers, common and scientific names, as well as their .smiles, .inchi, and .inchikey chemical file formats (**Figure S1**). It also contains essential spectrometric (molecular and monoisotopic mass, **Figure S2**) and spectroscopic (^{1}H- and ^{13}C-NMR, **Figure S3**) data of the secondary metabolites reported to date in Barnadesioideae. This database, available upon request, constitutes the initial step for dereplication and further phytochemical studies on the Barnadesioideae subfamily.

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Appendix 03

Caffeic Acid Ester Derivatives and Flavonoides of genus *Arnaldoa* (Asteraceae, Barnadesioideae)

Gari V. Ccana-Ccapatinta, *Paola L. Ferreira*, Milton Groppo & Fernando B. Da Costa

Arnaldoa macbrideana Roland

"Modern chemistry, with its far-reaching generalizations and hypotheses, is a fine example of how far the human mind can go in exploring the unknown beyond the limits of human sense"
(Horace Deming, 1923)

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Caffeic acid ester derivatives and flavonoids of genus *Arnaldoa* (Asteraceae, Barnadesioideae)

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ABSTRACT

The phytochemical composition of *Arnaldoa* species is barely known. In this work, the occurrence of caffeic acid ester derivatives and flavonoids in *A. argentea, A. macbrideana* and *A. weberbaueri* was established by liquid chromatography associated to high-resolution mass spectrometry analyses and comparison with data from isolated compounds. The distribution of chlorogenic acids in the genus *Arnaldoa* is herein described for the first time. The metabolite profile of *Arnaldoa* species was compared to that of *Tithonia diversifolia,* a known and rich source of chlorogenic acids and sesquiterpene lactones. In addition to the mono- and dicaffeoyl quinic acids present in *T. diversifolia*, *Arnaldoa* species exhibited the mono- and dicaffeoyl tartaric acids. Furthermore, mass features correspondent to that of sesquiterpene lactones present in *T. diversifolia* were not observed in *Arnaldoa* species. The chemotaxonomic implications of caffeic acid ester derivatives and flavonoid glycosides, as well as the potential absence of sesquiterpene lactones in the genus *Arnaldoa* and subfamily Barnadesioideae are discussed.

1. Subject and source

The genus *Arnaldoa* Cabrera (Asteraceae, Barnadesioideae) consists of three shrubby species that have a narrow distribution in Southern Ecuador and Northern Peru (Stuessy and Sagástegui, 1993; Ulloa et al., 2002). In this work, aerial parts of *A. weberbaueri* (Muschl.) Ferreyra were collected nearby "El Limon", district of Celendin, Department of Cajamarca, Peru (6°52′15.6″S 78°04′59.3″W), in January 2016. A voucher specimen (G.V. Ccana-Ccapatinta 44) was deposited at the Herbarium SPFR (Department of Biology, Faculty of Philosophy, Sciences and Letters at Ribeirão Preto – FFCLRP – University of São Paulo, USP) and identified by the taxonomist P.L. Ferreira (FFCLRP-USP). Leaves of *A. argentea* C. Ulloa, P. Jørg. & M.O. Dillon (J.E. Madsen 8341) and *A. macbrideana* Ferreyra (R. Ferreyra 13628) were obtained from the Herbarium MO (Missouri Botanical Garden Herbarium, Saint Louis, USA). Leaves of *Tithonia diversifolia* (Hemsl.) A. Gray were obtained from the Medicinal Plants Garden of the School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (FCFRP-USP). A voucher specimen (Sampaio #02) was deposited in the Herbarium SPF, Institute of Biosciences, University of São Paulo.

2. Previous work

The flavonoids kaempferol-3-*O*-glucoside, kaempferol-3-*O*-glucuronide, kaempferol-3-*O*-rutinoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-glucuronide, and quercetin-3-*O*-rutinoside were identified in *A. weberbaueri* by Bohm and Stuessy (1995). Other Barnadesioideae members (*Barnadesia*, *Chuquiraga* and *Dasyphyllum*) had also displayed similar flavonoid profiles consisting of 3-*O*-glucosides, 3-*O*-rutinosides and 3-*O*-glucuronides of kaempferol and quercetin (Bohm and Stuessy, 1995; Mendiondo et al., 1997; 2000). This flavonoid profile of the subfamily Barnadesioideae was stated as a "simple flavonoid profile" when compared to other Asteraceae taxa and proposed as further evidence of the basal position of Barnadesioideae within Asteraceae (Bohm and Stuessy, 1995).

3. Present work

3.1. Isolation of constituents from A. weberbaueri and T. diversifolia

Air-dried leaves (55 g) of *A. weberbaueri* were powdered in a knife mill and sieved (1 mm pore size). The powdered leaves were extracted

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by maceration with 70% ethanol (3 times, 1:10 plant/solvent ratio - g/mL). The solvent was evaporated under reduced pressure and then lyophilized to yield 16 g of a dry extract. This extract was further mixed with microcrystalline cellulose (48 g) and then submitted to solid-liquid partition with solvents of increasing polarity: hexane (Hex, 3 times of 250 ml), dichloromethane (DCM, 3 times of 250 ml), ethyl acetate (EtOAc, 3 times of 250 ml), and 50% ethanol (EtOH, 3 times of 250 ml). The 50% EtOH fraction yielded 10 g of an extract that was chromatographed on a Sephadex LH-20 column (100 g, 35 × 350 mm) by using mixtures of decreasing polarity of H_2O:EtOH (100:0 → 0:100). The eluted fractions were monitored by TLC and pooled by similarity generating three new fractions: F1 (2.6 g), F2 (400 mg) and F3 (180 mg). Fractions F1 – F3 were submitted to semi-preparative HPLC on a C18 Shim-pack column (15 μm, 20 × 250 mm, Shimadzu) using a linear gradient of H_2O (with 0.1% HCO_2H) and MeCN (95:5 → 70:30, in 50 min, 10.0 ml/min) as mobile phase, to afford the compounds **1** (8 mg, Rt = 14.0 min), **3** (20 mg, Rt = 19.9 min), **4** (2 mg, Rt = 23.5 min), **9** (70 mg, Rt = 31.6 min), **10** (15 mg, Rt = 34.7 min), **11** (9 mg, Rt = 36.4 min), and **14** (11 mg, Rt = 39.1 min). The Hex, DCM and EtOAc fractions were also processed by normal phase SiO_2 column chromatography and afforded complex terpenoid mixtures, which were not worked out.

Air-dried leaves (200 g) of *T. diversifolia* were rinsed with 2 L of absolute ethanol for 20 s to remove glandular trichomes and other compounds with low polarity. The gland-free material was dried once more (24 h, 40 °C) before to be powdered and sieved. The powdered leaves were extracted by maceration with 70% EtOH (3 times, 1:5 plant/solvent ratio - g/mL). The solvent was evaporated under reduced pressure and then lyophilized to yield 80 g of dry extract. This extract was further mixed with microcrystalline cellulose (240 g) and then submitted to solid-liquid partition with solvents of increasing polarity: Hex (3 times of 500 ml), DCM (3 times of 500 ml), EtOAc (3 times of 500 ml), and 50% EtOH (3 times of 500 ml). The 50% EtOH fraction yielded 60 g of an extract that was chromatographed 3 times (20 g each time) on a Sephadex LH-20 column (250 g, 50 × 500 mm) using mixtures of decreasing polarity of H_2O:EtOH (100:0 → 0:100). The eluted fractions were monitored by TLC and pooled by similarity generating 3 new fractions: A (40 g), B (10 g), and C (8 g). Fraction A was not processed because it did not display spots with UV absorption at 254 and 366 nm on TLC. Fractions B and C were combined and chromatographed on a Sephadex LH-20 column (250 g, 50 × 500 mm) using mixtures of decreasing polarity of H_2O:EtOH (100:0 → 0:100) to afford 7 subfractions: SF1 – SF7. These subfractions were submitted to semi-preparative HPLC on a C_{18} Onyx monolithic column (2 μm macropore size, 10 × 100 mm, Phenomenex) using a linear gradient of H_2O:MeCN (100:0 → 70:30, in 30 min, 2.5 mL/min), both with 0.1% HCO_2H, as mobile phase, to afford the compounds **2** (15 mg, Rt = 8.55 min), **3** (90 mg, Rt = 9.75 min), **5** (16 mg, Rt = 10.05 min), **7** (18 mg, Rt = 12.52 min), **8** (20 mg, Rt = 11.33 min), **12** (30 mg, Rt = 12.75 min), **13** (25 mg, Rt = 15.05), **15** (27 mg, Rt = 16.02), **16** (30 mg, Rt = 16.15 min), **17** (10 mg, Rt = 16.02), **19** (15 mg, Rt = 19.82 min) and **20** (10 mg, Rt = 16.51). The Hex, DCM and EtOAc fractions were not worked out.

The structures of the isolated compounds were established based on 1D and 2D-NMR spectroscopy, high-resolution MS and MS/MS analyses, and comparison with literature data. 2-*O*-Caffeoyltartaric acid (**1**) (Singleton et al., 1978), 5-*O*-caffeoylquinic acid (**3**) (Forino et al., 2015), caffeic acid (**4**) (Flamini et al., 2001), 2,3-*O*-dicaffeoyltartaric acid (**9**) (Scarpati and Oriente, 1958), quercetin-3-*O*-rutinoside (**10**) (Beck and Häberlein., 1999), quercetin-3-*O*-glucoside (**11**) (Manguro et al., 2003), and the putative quercetin-3-*O*-(6-*O*-malonyl)-glucoside (**14**) (Katsube et al., 2006) were isolated from *A. weberbaueri*. Additionally, 3-*O*-caffeoylquinic acid (**2**), 4-*O*-caffeoylquinic acid (**5**), 5-*O*-caffeoylquinic acid (**3**) (Pauli et al., 1998; Forino et al., 2015), 5-*O*-feruloylquinic acid (**7**), 3,4-*O*-dicaffeoylquinic acid (**13**), 1,5-*O*-dicaffeoylquinic acid (**15**), 3,5-*O*-dicaffeoylquinic acid (**16**), 4,5-*O*-dicaffeoylquinic acid (**19**) (Pauli et al., 1998, Pantoja Pulido et al., 2017), 4-*O*-caffeoyl-2-*C*-methyl-*D*-erythronic acid (**8**), 3-*O*-caffeoyl-2-(2-propyl)-2-hydroxybutanedioic acid (**17**), 3-*O*-caffeoyl-2-(2-butyl)-2-hydroxybutanedioic acid (**20**) (Ccana-Ccapatinta et al., 2017) and quercetin-3-*O*-glucoronide (**12**) (Bouktaib et al., 2002) were isolated from *T. diversifolia*. The identity of the chlorogenic acids was further confirmed by comparison of their MS/MS spectra with those from fragmentation patterns published by Clifford et al. (2003, 2005). The structures described herein for the chlorogenic acids follow the IUPAC numbering rules (Abrankó and Clifford, 2017; Clifford et al., 2017).

3.2. Liquid chromatography associated to high-resolution mass spectrometry analyses

The leaves of *A. argentea*, *A. macbrideana*, *A. weberbaueri* and *T. diversifolia* were powdered in an analytic mill and sieved (aperture of 0.355 mm and 42 mesh). Thirty milligrams (30 mg) of each sample were weighed and transferred to Eppendorf tubes, where 3 mL of hydroalcoholic solvent (50% methanol) were added and then submitted to ultrasonic bath for 15 min. The samples were filtered using modified cellulose syringe filters (0.22 μm pore size, Sartorius) and conditioned for chromatographic analysis.

Liquid chromatography analyses associated to high-resolution mass spectrometry (LC-ESI-MS) and ultraviolet detection were carried out in a setup of Thermo Scientific (USA) equipment, composed by two Accela 1250 quaternary pumps, coupled to an Accela diode array detector, and a Thermo Scientific Exactive Plus mass spectrometer equipped with an Orbitrap ion trap mass analyzer synchronized by Xcalibur 2.2 software (Thermo Scientific). Water (A) and acetonitrile (B), both with 0.1% formic acid, were used as mobile phases. Chromatographic separations were conducted in a Kinetex XB-C18 (1.7 μm, 150 × 2.1 mm) column coupled to a compatible guard column and an oven temperature of 35 °C. Four microliters of each sample were injected, and the mobile phase flow rate was set to 400 μL/min, with the following elution program: 2% B to 30% B in 30 min, then 100% B in 33 min, isocratic 100% until 35 min, and equilibration to 2% B until 40 min. The DAD scan wavelength was set from 190 to 600 nm. The mass spectrometer operated under fast scan-to-scan polarity switching (one full positive mode scan and one full negative mode scan at a resolution setting of 70,000) in both MS and MS/MS modes with the following conditions: electrospray ionization mode (ESI), pulverization voltage of 3.6 kV in positive and 3.2 kV in negative mode, capillary temperature of 320 °C, and a scan windows of 100 to 1,500 *m/z*. Higher energy collisional dissociation (HCD) fragmentation were conducted for MS/MS in negative and positive modes.

Fig. 1 displays the LC-ESI-MS chromatograms, in negative mode, of *Arnaldoa* species and *T. diversifolia*. In addition to the isolated compounds (**1**, **3**, **4**, **9–11** and **14**), the occurrence of 3-*O*-caffeoylquinic acid (**2**), 4-*O*-caffeoylquinic acid (**5**), 5-*O*-feruloylquinic acid (**7**), as well as 3,4-*O*-dicaffeoylquinic acid (**13**), 3,5-*O*-dicaffeoylquinic acid (**16**), 4,5-*O*-dicaffeoylquinic acid (**19**) were identified in *Arnaldoa* species by comparison with data from compounds isolated from *T. diversifolia*. Additionally, 1,3-*O*-dicaffeoylquinic acid (**6**) and caffeoylferuloyltartaric acid (**18**) were identified in *A. weberbaueri* by comparison of their MS/MS spectra with literature data provided by Clifford et al. (2005) and Jaiswal et al. (2011). The diversity of chlorogenic acids present in *T. diversifolia* resembles those previously published by Pantoja Pulido et al. (2017). A list of caffeic acid ester derivatives and flavonoids identified in the species surveyed in this work are shown in Table 1.

In the chromatogram of *T. diversifolia*, seven mass features (**21–27**)

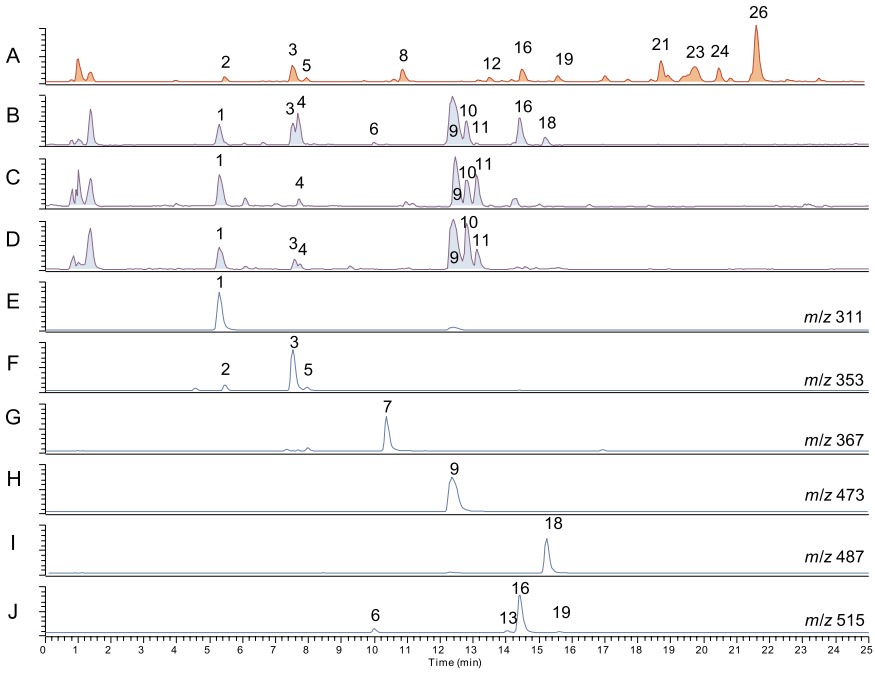

Fig. 1. LC-ESI-MS chromatograms, in negative mode, of *Arnaldoa* species and *T. diversifolia*. Full scan chromatograms of *T. diversifolia* (**A**), *A. weberbaueri* (**B**), *A. macbrideana* (**C**) and *A. argentea* (**D**). Selected ion monitoring chromatograms of caffeic acid ester derivatives in *A. weberbaueri* at *m/z* 311 (**E**), *m/z* 353 (**F**), *m/z* 367 (**G**), *m/z* 473 (**H**), *m/z* 487 (**I**) and *m/z* 515 (**I**). Constituents numbering according to Table 1.

Table 1
Constituents identified in *T. diversifolia* and *Arnaldoa* species.

No.	Rt (min)	Identity	TD	AW	AM	AA
1	5.28	2-*O*-Caffeoyltartaric acid	–	+	+	+
2	5.44	3-*O*-Caffeoylquinic acid	+	+	+	+
3	7.52	5-*O*-Caffeoylquinic acid	+	+	+	+
4	7.67	Caffeic acid	+	+	+	+
5	7.96	4-*O*-Caffeoylquinic acid	+	+	+	+
6	9.99	1,3-*O*-Dicaffeoylquinic acid	–	+	+	+
7	10.37	5-*O*-Feruloylquinic acid	+	+	lc	lc
8	10.90	4-*O*-caffeoyl-2-*C*-methyl-D-erythronic acid	+	–	–	–
9	12.36	2,3-*O*-Dicaffeoyltartaric acid	–	+	+	+
10	12.80	Quercetin-3-*O*-rutinoside	+	+	+	+
11	13.12	Quercetin-3-*O*-glucoside	–	+	+	+
12	13.36	Quercetin-3-*O*-glucoronide	+	–	–	–
13	14.02	3,4-*O*-Dicaffeoylquinic acid	+	+	lc	lc
14	14.08	Quercetin-3-*O*-(6-*O*-malonyl)-glucoside	–	+	lc	lc
15	14.22	1,5-*O*-Dicaffeoylquinic acid	lc	–	–	–
16	14.42	3,5-*O*-Dicaffeoylquinic acid	+	+	+	+
17	15.01	3-*O*-Caffeoyl-2-(2-propyl)-2-hydroxybutanedioic acid	lc	–	–	–
18	15.19	Caffeoyl-feruloyltartaric acid	–	+	lc	lc
19	15.59	4,5-*O*-Dicaffeoylquinic acid	+	+	+	+
20	17.16	3-*O*-Caffeoyl-2-(2-butyl)-2-hydroxybutanedioic acid	lc	–	–	–
21	18.80	Putative sesquiterpene lactone	+	–	–	–
22	19.67	Putative sesquiterpene lactone	+	–	–	–
23	19.83	Putative sesquiterpene lactone	+	–	–	–
24	20.55	Putative sesquiterpene lactone	+	–	–	–
25	21.58	Putative sesquiterpene lactone	+	–	–	–
26	21.70	Putative sesquiterpene lactone	+	–	–	–
27	23.02	Putative sesquiterpene lactone	+	–	–	–

TD, *T. diversifolia*. AW, *A. weberbaueri*. AM, *A. macbrideana*. AA, *A. argentea*. +, presence. -, absence. lc, low concentration.

were assigned as putative sesquiterpene lactones by inspecting their accurate mass and corresponding molecular formulas in our in house chemical structures database of the Asteraceae family (AsterDB, www.asterbiochem.org/asterdb). These putative sesquiterpene lactones were absent in the investigated *Arnaldoa* species.

4. Chemotaxonomic significance

Barnadesioideae comprises 10 genera and 85 species endemic to South America (Ferreira et al., 2019). Members of this group were previously classified in the subtribe Barnadesiinae, tribe Mutiseae, until Jansen and Palmer (1987, 1988) demonstrated that they lack an inversion of 22 kb in the chloroplast genome, a synapomorphy for all other Asteraceae. Later, this evidence supported the basal position of this group of plants in the family Asteraceae (Bremer and Jansen, 1992). The occurrence of ubiquitous triterpenoids, quercetin and kaempferol glycosides as well as the presumable absence of sesquiterpene lactones (the typical Asteraceae taxonomical markers) in members of Barnadesioideae may constitute further evidence regarding the basal position of this subfamily inside Asteraceae (Zdero et al., 1987; Bohm and Stuessy, 1995; Calabria et al., 2007).

Fig. 2 displays the chemical structures of the compounds identified in *Arnaldoa* species. Even though caffeic acid ester derivatives are widespread in Asteraceae, the occurrence of 11 compounds of this class are here described for the first-time in *A. weberbaueri*, *A. argentea* and *A. macbrideana*, with a possible chemotaxonomic value at the genus level. Although the occurrence of phenylpropanoid in *Dasyphyllum brasiliense* and *Chuquiraga spinosa* has been suggested (Ccana-Ccapatinta et al., 2018), no other member of the subfamily Barnadesioideae has records of this kind of compound. Additionally, the flavonoids **10** and **11** were previously identified in *A. weberbaueri* (Bohm and Stuessy, 1995), while **14** constitutes a putative analog of quercetin-3-*O*-(6-*O*-malonyl)-glucoside in this species. Nevertheless this is the first report on the occurrence of **10**, **11** and **14** in *A. argentea* and *A. macbrideana*, results that resemble previous observation on the occurrence of glycosides of quercetin in *A. weberbaueri* and Barnadesioideae (Bohm and Stuessy, 1995).

The inspection of the LC-ESI-MS chromatograms of *A. weberbaueri*, *A. argentea* and *A. macbrideana* showed the absence of mass features correspondent to that of sesquiterpene lactones that were present in *T. diversifolia*. The potential absence of sesquiterpene lactones in *Arnaldoa* species is in line with previous observations that highlight the absence of sesquiterpene lactone records in members of Barnadesioideae as an additional feature that support its basal position in Asteraceae (Calabria et al., 2007; Ccana-Ccapatinta et al., 2018).

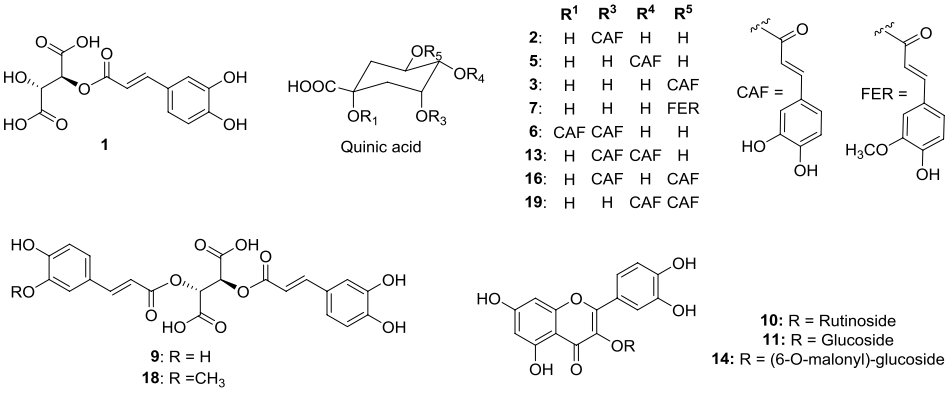

Fig. 2. Chemical structures of compounds identified in *Arnaldoa* species.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.bse.2019.103911. These data include MOL file and InChiKeys of the most important compounds reported in this article.

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