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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MACROEVOLUTION OF BARNADESIOIDEAE**

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MACROEVOLUÇÃO DE BARNADESIOIDEAE**

Orientada: Paola de Lima Ferreira

Orientador: Milton Groppo Júnior

Coorientador: Alexandre Antonelli

Tese apresentada à Faculdade de Filosofia,
Ciências e Letras de Ribeirão Preto-USP, como
parte das exigências para obtenção do título de
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Comparada.

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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 utilizando 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 *Dusenilla* 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 *Dusenilla* 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 through 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
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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 Antarctica (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: *Dusenilla* 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 (*trnL* 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 (*trnL* 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, Gruenstaudl 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 Barnadesioideae, 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) Gruenstaedl 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 of 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 derivatives 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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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)

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 (*trnL-trnF*, *psbA-trnH*, 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 !\[\]\(95c552df6353b48e62ab71c0e20270ca_img.jpg\) DOI: 10.7717/peerj.6475/fig-1](https://doi.org/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 *trnL-trnF* and *psbA-trnH*, 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: *trnL-trnF* using primers “c” and “f” (Taberlet et al., 1991); *psbA-trnH* 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 *psbA-trnH* and *trnL-trnF*.

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 ≥ 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 (*psbA-trnH* and *trnL-trnF*) and the combined marker datasets generated in this study (*psbA-trnH*, *trnL-trnF*, 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 (*trnL-trnF*, *psbA-trnH*, 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 & Gruenstaedl, 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 (*trnL-trnF* = 912 bp; *psbA-trnH* = 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
<i>Arnaldoa macbrideana</i>	0	0	0	0
<i>Arnaldoa weberbaueri</i>	0	0	0	0
<i>Dasyphyllum argenteum</i>	0	1	1	1
<i>Dasyphyllum armatum</i>	0	1	1	1
<i>Dasyphyllum brasiliense</i>	0	1	1	1
<i>Dasyphyllum brevispinum</i>	0	1	1	1
<i>Dasyphyllum colombianum</i>	0	1	1	1
<i>Dasyphyllum diacanthoides</i>	1	2	1	0
<i>Dasyphyllum diamantinense</i>	0	1	1	1
<i>Dasyphyllum donianum</i>	0	1	0	0
<i>Dasyphyllum excelsum</i>	1	2	1	1
<i>Dasyphyllum ferox</i>	0	1	1	1
<i>Dasyphyllum flagellare</i>	0	1	1	1
<i>Dasyphyllum floribundum</i>	0	1	1	1
<i>Dasyphyllum fodinarum</i>	0	1	0	0
<i>Dasyphyllum hystrix</i>	0	1	1	0
<i>Dasyphyllum inerme</i>	0	1	1	1
<i>Dasyphyllum lanceolatum</i>	0	1	1	1
<i>Dasyphyllum leptacanthum</i>	0	1	1	0
<i>Dasyphyllum popayanense</i>	0	1	1	1
<i>Dasyphyllum reticulatum</i>	0	1	0	0
<i>Dasyphyllum spinescens</i>	0	1	1	1
<i>Dasyphyllum sprengelianum</i>	0	1	0	0
<i>Dasyphyllum trichophyllum</i>	0	1	0	0
<i>Dasyphyllum vagans</i>	0	1	1	1
<i>Dasyphyllum</i> sp. nov. (1)	0	1	0	0
<i>Dasyphyllum</i> sp. nov. (2)	0	1	1	1
<i>Dasyphyllum</i> sp. nov. (3)	0	1	1	1
<i>Dasyphyllum</i> sp. nov. (4)	0	1	1	1
<i>Fulcaldea laurifolia</i>	0	0	1	1
<i>Fulcaldea stuessy</i>	0	0	1	1

Note:

Leaf venation: (0) Acrodromous, (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.

	<i>trnL-trnF</i>	<i>psbA-trnH</i>	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 involucre (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](#); [Gruenstaedl 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](https://doi.org/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 acrodromous 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](#); [Gruenstaedl et al., 2009](#); [Stuessy, Urtubey & Gruenstaedl, 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](https://doi.org/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.** $\equiv$ *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.** $\equiv$ *Flotovia diacanthoides* Less, *Syn. Gen. Compos.*: 95. 1832. $\equiv$ *Piptocarpha diacanthoides* (Less.) Hook. & Arn., *Comp. Bot. Mag.* 1: 110. 1835. $\equiv$ *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 leucoxylon* 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.** $\equiv$ *Chuquiraga excelsa* D. Don, Phil. Mag. 11: 392. 1832. $\equiv$ *Piptocarpha excelsa* (D. Don) Hook. & Arn., Comp. Bot. Mag. 1:110. 1835. $\equiv$ *Dasyphyllum excelsum* (D. Don) Cabrera, *Revista Mus. La Plata, Secc. Bot.*, 9(38): 46. 1959. *Typus*: Chile, Valparaíso, 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)

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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)

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 jussieu*, *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.

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Keywords Barnadesioideae · Flavonoids ·
 Triterpenoids · Traditional medicine

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

Table 1 continued

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

Table 1 continued

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

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

^aUpdated 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), Juárez 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 H₂O: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–Et₂O–petrol, 1:1:1; MeOH–EtOAc–hexane, 1:1:1; CH₂Cl₂–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 the 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 (Yakovlev 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
<i>C. acanthophylla</i>	AR	<i>Espina amarilla</i>	Cold, cough and fever. Stomachache. Urinary tract infections	Barbarán (2008)
<i>C. atacamensis</i>	AR	<i>Hierba de san Pedro, san Pedro, kishka tola</i>	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	<i>San Gerónimo, fundición, kutu kutu, chajllampa</i>	Cold, cough, fever. Urinary tract infections, cystitis, prostatitis. Relief of postpartum symptoms. Not recommended in pregnant women	Zamora (2008)
	CH	<i>Lengua de gallo, tastará, quebrolla, killokisca, chana chaklamba</i>	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)
<i>C. avellanadae</i>	AR	<i>Quilimbay-trayao, tratrakcha, trayau</i>	Cough. Headache and fever, boiled leaves are chewed in a mixture with sugar	Richeri et al. (2013)
<i>C. erinacea</i>	AR	<i>Romerillo, falsa uña de gato, trifrif mamull</i>	Stomachache and liver disease. Kidney disease. Strengthens the brain and nerves	Ladio and Lozada (2009)
<i>C. jussieui</i>	CO	<i>Chuquiragua, vela de páramo</i>	Febrifuge, diuretic, kidney stones	Díaz-Piedrahita and Vélez-Nauer (1993)
	EC	<i>Chuquiraga, chuquiragua</i>	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	<i>Chiquiragua</i> (northern Peru). <i>Inca llauilli, kentayllauilli, quishuara, kiswaara, kiswaara tiutumpi, qharisirviy</i> (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)
<i>C. longiflora</i>	AR	<i>Azafrán de la puna</i>	The plant is added to water for personal washing	Giberti (1983)
<i>C. oppositifolia</i>	AR	<i>Azafrán del campo</i>	Hypoglycaemic, hypocholesterolaemic. Antifungal	Raad (2012)
<i>C. parviflora</i>	BO	<i>Chiñi michi michi</i>	Against curse	Vandebroek et al. (2003)

Table 2 continued

Species	Country	Common names	Indications ^a	References
<i>C. spinosa</i>	AR	<i>Charkoma</i>	Regulation of the menstrual cycle	Giberti (1983)
	BO	<i>Huamanpinta</i>	Kidney stones and cystitis	Ceuterick et al. (2011)
	EC	<i>Chuquiragua</i>	Cold, cough and fever. Pain of the bones. Malaria	Bussmann and Sharon (2006b)
	PE	<i>Huamanpinta, huancapita, huancaspita, laulinco, pucacasha, pazpapamaquin, qharisirviy, cjari sirvi</i>	Respiratory affections. Antibleorrhagic 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)
<i>C. weberbaueri</i>	PE	<i>Amaro amaro</i>	Cough, bronchitis, asthma. Liver disease. Diuretic and depurative	Brack (1999)

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

^aCommonly, 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. avellanadae* 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 northern 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 (LD₅₀) > 10,000 µg/ml in the brine shrimp lethality assay, whereas the ethanolic extracts displayed LC₅₀ 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 stems (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-Capatinta

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, isorhamnetin, 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
<i>C. atacamensis</i>	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 ^{•-}	IC ₅₀ = 3.5–20 µg/ml	
	80% Ethanol (5 g/100 ml), 500 µg of GAE/ml	<i>Staphylococcus aureus</i> strains	MIC = 80–600 µg/ml	Zampini et al. (2009)
		<i>Enterococcus faecalis</i> strains	MIC = 150–300 µg/ml	
		<i>Escherichia coli</i> strain	MIC = 600 µg/ml	
		Other gram-negative bacteria	MIC = 300–600 µg/ml	
<i>C. erinacea</i>	Ethanol (dry extract)	Antioxidant, ABTS ⁺ assay	SC ₅₀ = 1.5 µg/ml	Zampini et al. (2010)
	Ethanol extract (dry extract)	In vitro AChE inhibitory activity	IC ₅₀ = 7.26 mg/ml	Gurovic et al. (2010)
<i>C. jussieui</i>	Water (2 g/100 ml)	Antioxidant	IC ₅₀ = 64.9 mg/l	Dueñas et al. (2014)
<i>C. spinosa</i>	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)
		<i>Candina albicans</i>	MIC = 2.5 µg on TLC plate	
		<i>Cladosporium cucumerinum</i>	MIC = 2.5 µg on TLC plate	
		<i>Rhizopus stolonifer</i>	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%	
		<i>Candina albicans</i>	MIC = 6.3 µg on TLC plate	
		<i>Rhizopus stolonifer</i>	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)
		<i>Rhizopus stolonifer</i>	MIC = 18.5 µg on TLC plate	
	Water (5 g/500 ml)	<i>Staphylococcus aureus</i> 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
	Hexane fraction (dry extract)	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	
		Cytotoxicity in DU-145 cell line	IC ₅₀ = 27.03 µg/ml	
		Cytotoxicity in DU-145 cell line	IC ₅₀ = 33.10 µg/ml	
		Cytotoxicity in DU-145 cell line	IC ₅₀ = 24.19 µg/ml	
		Cytotoxicity in DU-145 cell line	IC ₅₀ = 54.12 µg/ml	
		Cytotoxicity in DU-145 cell line	IC ₅₀ = 54.12 µg/ml	
<i>C. straminea</i>	80% Methanol (dry extract)	Antioxidant, DPPH, ABTS ⁺	SC ₅₀ = 14.5–34.9 µg/ml	Mendiondo et al. (2011)
		<i>Staphylococcus aureus</i> strains	MIC = 200–800 µg/ml	

GAE gallic acid equivalents

^aPlant/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 used 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 IC₅₀ 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 IC₅₀ values of 5.32–9.25 µg/ml. Interestingly, the lipophilic fractions (hexane, petrol, chloroform and ethyl acetate) obtained from the initial extract displayed IC₅₀ 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-Barnadesioideae.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-Barnadesioideae.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 (^1H - 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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SWGKAHCIOQPKFW-JTNORFRNSA-N
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CWVRJTMFETXNAD-JUHZACGLSA-N

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 Limón”, 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; Mendiando 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 H₂O: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 H₂O (with 0.1% HCO₂H) 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 SiO₂ 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 H₂O: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 H₂O:EtOH (100:0 → 0:100) to afford 7 subfractions: SF1 – SF7. These subfractions were submitted to semi-preparative HPLC on a C₁₈ Onyx monolithic column (2 µm macropore size, 10 × 100 mm, Phenomenex) using a linear gradient of H₂O:MeCN (100:0 → 70:30, in 30 min, 2.5 mL/min), both with 0.1% HCO₂H, 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-glucuronide (**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 caffeoyl-feruloyltartaric 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 (J). 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-glucuronide	+	-	-	-
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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