Flora 217 (2015) 138–154

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Comparative leaf anatomy and micromorphology of the Chilean Myrtaceae: Taxonomic and ecological implications

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ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form 5 October 2015
Accepted 15 October 2015
Edited by Alessio Papini
Available online 19 October 2015

Keywords:
Identification key
Histochemistry
Taxonomy
Valdivian forest
Xeromorphic

ABSTRACT

The family Myrtaceae in Chile comprises 26 species in 10 genera. The species occur in a diverse range of environments including humid temperate forests, swamps, riparian habitats and coastal xeromorphic shrublands. Most of these species are either endemic to Chile or endemic to the humid temperate forests of Chile and Argentina. Although many taxa have very restricted distributions and are of conservation concern, little is known about their biology and vegetative anatomy. In this investigation, we describe and compare the leaf anatomy and micromorphology of all Chilean Myrtaceae using standard protocols for light and scanning electron microscopy. Leaf characters described here are related to epidermis, cuticle, papillae, stomata, hairs, mesophyll, crystals, secretory cavities and vascular system. Nearly all the species have a typical mesophytic leaf anatomy, but some species possess xerophytic characters such as double epidermis, hypodermis, pubescent leaves, thick adaxial epidermis and straight epidermal anticlinal walls, which correlate with the ecological distribution of the species. This is the first report on leaf anatomy and micromorphology in most of these species. We identified several leaf characters with potential taxonomic and ecological significance. Some combinations of leaf characters can reliably delimitate genera, while others are unique to some species. An identification key using micromorphological and anatomical characters is provided to distinguish genera and species.

1. Introduction

Myrtaceae Juss. (Myrtales; Angiosperm Phylogeny Group, 2009) is a large family of angiosperms with approximately 5500 species, divided into two subfamilies, 17 tribes and ca. 140 genera (Biffin et al., 2010 Wilson, 2011). It is a predominantly southern hemisphere family with a high diversity in South America and Australasia (Snow, 2000). In Chile, the family is represented by 26 species in 10 genera distributed from the north-centre to the southern tip of the mainland region and in the Juan Fernandez Islands (Landrum, 1988a; Murillo and Ruiz, 2011). All Chilean species of Myrtaceae belong to the tribe Myrteae, with the exception of *Tepualia stipularis* (Hook. and Arn.) Griseb. which is in the tribe Metrosidereae (*sensu* Wilson et al., 2005).

Five genera (*Amomyrtus*, *Legrandia*, *Luma*, *Tepualia* and *Nothomyrcia*) are endemic to the humid temperate forests of Chile and Argentina. *Amomyrtus* (Burret) D. Legrand and Kausel and *Luma* A. Gray possess two species each, while *Legrandia* Kausel, *Nothomyrcia* Kausel and *Tepualia* Griseb. are monospecific genera (Landrum, 1988a). *Nothomyrcia* is endemic to the Robinson Crusoe Island, Juan Fernandez Islands (Murillo and Ruiz, 2011). The remaining five genera have a wider distribution range and also occur outside of Chilean–Argentinian forests. The genus *Ugni* Turcz. comprises four species, two of which are native to the forests of mainland Chile, one is endemic to Juan Fernandez Islands and one occurs in Mexico and Central America (Wilson, 2011). The genus *Myrceugenia* O. Berg. has ca. 40 species, of which 10 species occur exclusively in Chile, two species occur in Central-Southern Chile and Argentina, one species is endemic to the Juan Fernandez Islands and ca. 17 species occur in southeast Brazil (Landrum, 1981). *Blepharocalyx* O. Berg has three species, of which one occurs in Chile and the remaining occur in the Caribbean, Brazil, Paraguay, Uruguay and Argentina. *Myrcianthes* O. Berg has around 30 species, with one species in Chile and the remaining mainly distributed in the Andes from Mexico to Perú (Wilson, 2011). *Myrteola* O. Berg has

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http://dx.doi.org/10.1016/j.flora.2015.10.005
0367-2530/

three species, of which one occurs in Chile and the remaining occur in Colombia, Venezuela and Argentina (Landrum, 1986, 1988b; Landrum and Griffo, 1988).

The majority of the Chilean Myrtaceae occur in the "Chilean Winter Rainfall-Valdivian Forest Hotspot", an area located in between 25° and 47° south latitude. This region is known for a high level of plant endemism (Arroyo et al., 2004). Part of this area is considered as a priority for plant conservation at global scale (Myers et al., 2000). This biogeographic region encompasses the Juan Fernandez Islands, where three species of Myrtaceae are endemic, namely *Myrceugenia schulzei* Johow, *Nothomyrcia fernandeziana* (Hook. and Arn.) Kausel and *Ugni selkirkii* (Hook. and Arn.) O. Berg. (Landrum, 1988a). Most species of Chilean Myrtaceae occur in humid temperate forests or flooded environments, usually wet gullies or streams (Kausel, 1942, 1956). The Chilean Myrtaceae are an abundant component in the upper, middle and even lower strata of these forests (Hildebrand-Vogel, 2002). A few species, such as *Myrceugenia rufa* (Colla) Skottsb. ex Kausel and *Myrcianthes coquimbensis* (Barnéoud) Landrum and Grifo, occur exclusively in dry habitats with the water supply limited to fog and ocean breeze (Serra et al., 1986; Landrum and Grifo, 1988). *Myrceugenia correifolia* occurs in coastal xeromorphic habitats in central Chile, with some populations in cloud forests (Landrum, 1981).

Leaf anatomical characters have provided valuable systematic and ecological information in Myrtaceae. Metcalfe and Chalk (1979), Schmid (1980) and Keating (1984) described leaf anatomical characters at family level with important taxonomic implications. Cardoso et al. (2009) and Gomes et al. (2009) conducted detailed leaf anatomical studies in several South American species, indicating that anatomical characters, alongside morphological features, can be used to identify species and genera. Based on leaf anatomical characters and DNA sequences, Soh and Parnell (2011) reconstructed the phylogeny of the Australasian genus *Syzygium* and found a number of characters useful in delimiting sections and species. Leaf micromorphology (using SEM) of South American Myrtaceae has been mainly studied in *Eugenia* and shown to be important for taxonomic purposes (Fontenelle et al., 1994; Haron and Moore, 1996).

The leaf anatomy and micromorphology of the Chilean Myrtaceae has not been documented in much detail (P.G. Wilson, pers. comm.), other than a few species, namely *Luma apiculata*, *Myrceugenia parvifolia* (Retamales and Scharaschkin, 2014) and *Ugni molinae* (Retamales et al., 2014). There has never been a comprehensive study of the Chilean Myrtaceae other than taxonomic revisions based on gross morphological characters (Kausel,1942; McVaugh, 1968; Landrum, 1981, 1986, 1988a; Reiche, 1897). The Chilean Myrtaceae show high variation in gross morphology of leaves between species (Fig. 1) and also within same species, which precludes diagnosis and species identification (McVaugh, 1968). A complete anatomical investigation of these taxa could provide relevant information by identifying reliable characters with taxonomic and ecologic significance. In this investigation we present the outcome of extensive research on the anatomical and micromorphological characters of all the species of Myrtaceae occurring in Chile.

2. Materials and methods

2.1. Material examined

All 26 species of Chilean Myrtaceae were examined in this study. Wherever possible, fresh leaf material was collected but in a few cases herbarium specimens (CONC) were used. Sampling was conducted between January 2006 and February 2014 and included a number of different natural populations in Chile. Mature leaves were randomly sampled from sun-exposed branches from a number of typical and healthy individuals. Young leaves were also collected as trichomes and certain other structures are reported to be early caduceus (Landrum, 1988a). Young leaves were also used to describe early ontogenetic stages of secretory cavities and epidermis. Fresh leaf material was fixed in formalin–acetic acid–alcohol (FAA) for 24–48 h depending upon the thickness of the leaves and subsequently stored in 70% ethanol. Herbarium specimens were rehydrated in boiling water for 10 min to recover the leaf shape before being fixed in FAA (Haron and Moore, 1996). Herbarium accessions are currently housed in the Queensland Herbarium, Brisbane, Australia (BRI) with duplicates in the Forestry Sciences Herbarium, University of Chile (EIF). Details about specimens studied, vouchers, localities and habitat are presented in the Appendix A.

2.2. Scanning electron microscopy (SEM)

Leaf material fixed in FAA was dehydrated using a graded ethanol series and then critical point dried (Anderson, 1951) in an Autosamdri-815 automatic critical point drier (Tousimis, Rockville, USA). Samples were mounted on stubs with self-adhesive double-sided carbon discs and sputter-coated with gold palladium for 175 s using a Leica EM SCD005 Gold Coater (Leica Microsystems, Macquarie Park, NSW, Australia). Examination and documentation of images was conducted using a FEI Quanta 200 SEM/ESEM (FEI, Hillsboro, Oregon, USA) operated at 10 kV.

2.3. Light microscopy (LM)

FAA-fixed material was dehydrated through a graded ethanol series and embedded in paraffin wax (Johansen, 1940; Ruzin, 1999). Transverse sections of leaves were cut using a Leica RM2245 rotary microtome (Leica Microsystems, Macquarie Park, NSW, Australia) at 5 μm. Staining of sections was performed using the stains ruthenium red (0.05% aqueous solution), toluidine blue (TBO) (0.1% aqueous solution), safranin O (1% alcoholic solution) and alcian blue, alone or combined according to standard staining protocols (Ruzin, 1999; Retamales and Scharaschkin, 2014). In order to reliably identify the chemical compounds in tissues, additional histochemical tests were performed in unstained leaves using the reagents sudan IV, chlorazol black E and phloroglucinol (20% HCl) to detect lipophilic substances and lignin. Chemical nature of leaf intracellular crystals was tested by adding 1 μl of acetic acid and 1 μl of hydrochloric acid to sections (Maclean and Ivimey-Cook, 1952). Sections were mounted using DPX (Sigma–Aldrich Co., St. Louis, Missouri, USA).

Leaf clearings were prepared by immersing 1–2 cm^2 pieces of leaf material in 10% KOH at room temperature for 48 h followed by 7% NaClO for 2 h or until leaves turned transparent (Gardner, 1975). Cleared leaves were washed five times with distilled water, stained with 1% safranin O and mounted with lactoglycerol (lactic acid-glycerol 1:1). Slides were observed using a Nikon eclipse 50i compound microscope and images captured using the Nikon NIS-Elements imaging software (Nikon Instruments Inc., Amsterdam, Netherlands).

2.4. Taxonomy and terminology

The taxonomy of Chilean Myrtaceae is based on Landrum (1988a) and follows the author abbreviations of International Plant Name Index (2015), with one exception. *Myrceugenia fernandeziana* (Hook. and Arn.) Johow is considered here as *N. fernandeziana* (Hook. and Arn.) Kausel based on Murillo and Ruiz (2011). The abbreviation spp. will be used for referring to all species included in this study from a particular genus. In order to avoid ambiguities,