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

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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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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,

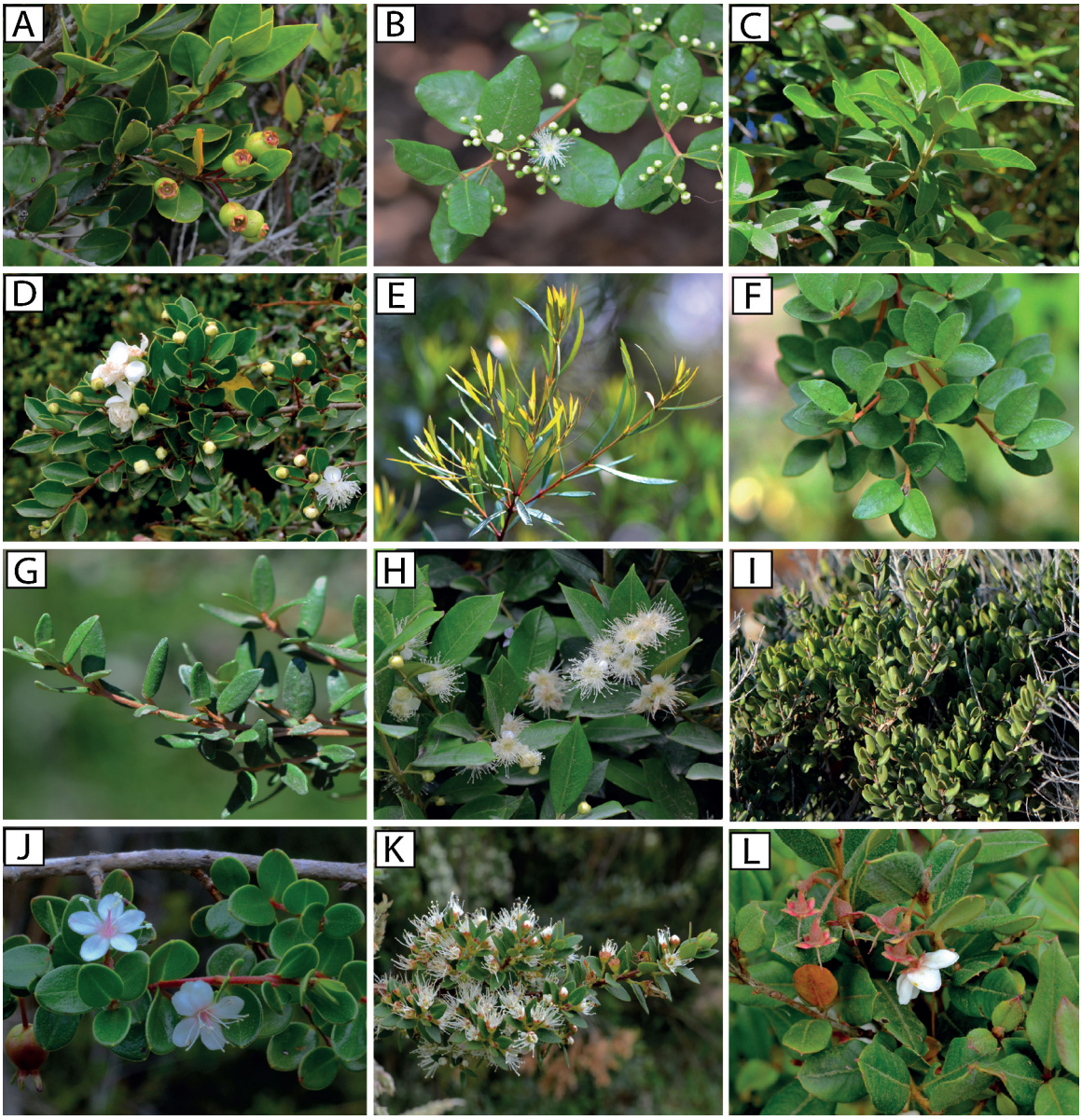

Fig. 1. Gross morphology of Chilean species of Myrtaceae. (A) *Amomyrtus meli*. (B) *Blepharocalyx cruckshanksii*. (C) *Legrandia concinna*. (D) *Luma apiculata*. (E) *Myrceugenia lanceolata*. (F) *Myrceugenia obtusa*. (G) *Myrceugenia rufa*. (H) *Myrceugenia planipes*. (I) *Myrcianthes coquimbensis*. (J) *Myrteola nummularia*. (K) *Tepualia stipularis*. (L) *Ugni candollei*.

the genera with the root *Myr—* (*Myrceugenia*, *Myrcianthes*, *Myrteola*) will not be abbreviated in the text other than in the anatomical synopsis of genera. Taxonomic authorities of species are shown in Appendix A; therefore these have been omitted from the text henceforth.

The five types of stomatal complexes studied here were anomocytic, paracytic, actinocytic, anisocytic and laterocytic. When more than one type of stomatal complex was identified in some species, the less frequent type is indicated in parentheses (Table 1). The description and interpretation of the different stomatal types in Chilean Myrtaceae are as follows: (1) Anomocytic: the guard cells are surrounded by unspecialized subsidiary cells without any consistent pattern and are indistinguishable in shape from other epidermal cells. (2) Paracytic: the guard cells are surrounded by two subsidiary cells, which are relatively specialized. These two cells are normally parallel with the long axis of the guard cells and are generally similar in size. (3) Actinocytic: the guard cells are surrounded by four or more, usually radially elongated, subsidiary cells. (4) Anisocytic: the guard cells are surrounded by three cells that are usually unequal in size. One of the three cells is usually much smaller than the other two. (5) Laterocytic: the guard cells are surrounded by six irregularly shaped subsidiary cells.

In order to reliably identify different types of secretory cavities, we observed ontogenetic stages in young leaves. Secretory cavities initially formed by dissolution of cells are classified as lysigenous, while those formed by initial separation of epithelial cells are classified either as schizogenous or schizolysigenous (Ciccarelli et al., 2008). Multiple layers of epidermis are classified as hypodermis or multiple epidermis depending upon ontogenetic development of this character (Martins et al., 2012).

Terminology for describing leaf micromorphology (mainly stomata) was based on previous descriptions of van Wyk et al. (1982), Fontenelle et al. (1994), Haron and Moore (1996) and Soh and Parnell (2011). Terminology for leaf anatomy was based on Schmid (1980), Schmid and Baas (1984), Keating (1984), Cardoso et al. (2009), Soh and Parnell (2011) and Retamales et al. (2014).

Table 1
Leaf anatomical and micromorphological characters in epidermis of Chilean Myrtaceae.

Taxon	Epidermis and papillae			Stomata	Indumentum
	Epidermis	Sinuosity of abaxial anticinal walls	Papillae	Stomatal type	Type of hairs
Amomyrtus luma (Molina) D. Legrand and Kausel	Single	High	Absent	Anomocytic	Simple
Amomyrtus meli (Phil.) D. Legrand and Kausel	Single	High	Absent	Anomocytic	Simple
Blepharocalyx cruckshanksii (Hook. and Arn.) Nied.	Single	High	Absent	Anomocytic	Absent
Legrandia concinna (Phil.) Kausel	Single	High	Conical	Anomocytic	Simple
Luma apiculata (DC.) Burret	Single	High	Absent	Paracytic	Simple
Luma chequen (Feuillée ex Molina) Gray	Single	Slight	Absent	Paracytic	Simple and glandular
Myrceugenia chrysocarpa (O.Berg) Kausel	Single	Slight	Absent	Anomocytic	Dibrachiate
Myrceugenia colchaguensis (Phil.) Navas	Single	Straight	Absent	Actinocytic (anomocytic)	Dibrachiate and glandular
Myrceugenia correifolia (Hook. and Arn.) O.Berg	Single	Straight	Conical	Anomocytic	Dibrachiate
Myrceugenia exsucca (DC.) O.Berg	Single	Slight	Absent	Anomocytic	Dibrachiate
Myrceugenia lanceolata (Juss. ex J. St.-Hil.) Kausel	Single	Slight	Absent	Anomocytic	Simple and dibrachiate
Myrceugenia leptospermoides (DC.) Kausel	Single	Slight	Absent	Anomocytic	Simple and dibrachiate
Myrceugenia obtusa (DC.) O.Berg	Single	Slight	Absent	Anomocytic	Simple, dibrachiate and glandular
Myrceugenia ovata (Hook. and Arn.) O.Berg	Single	Slight	Absent	Anomocytic	Simple and dibrachiate
Myrceugenia ovata var. nannophylla (Burret) L.R. Landrum	Single	Slight	Absent	Anomocytic	Simple and dibrachiate
Myrceugenia parvifolia (DC.) Kausel	Single	Slight	Absent	Anomocytic	Simple and dibrachiate
Myrceugenia pinifolia (F. Phil.) Kausel	Single	High	Absent	Anomocytic	Simple and dibrachiate
Myrceugenia planipes (Hook. and Arn.) O.Berg	Single	Slight	Absent	Anomocytic	Dibrachiate
Myrceugenia rufa (Colla) Skottsb. ex Kausel	Double	Straight	Absent	Anomocytic	Dibrachiate
Myrceugenia schulzei Johow	Single	Slight	Conical	Anomocytic	Dibrachiate
Myrcianthes coquimbensis (Barnéoud) Landrum and Grifo	Single	Slight	Absent	Laterocytic (paracytic)	Simple and glandular
Myrteola nummularia (Poir.) O.Berg	Single	Slight	Absent	Anomocytic	Simple
Nothomyrcia fernandeziana (Hook. and Arn.) Kausel	Hypodermis	Slight	Absent	Anomocytic	Simple
Ugni candollei (Barnéoud) O.Berg	Hypodermis	Slight	Conical	Anisocytic (anomocytic)	Simple and dibrachiate
Ugni molinae Turcz.	Single	Slight	Absent	Anomocytic	Simple
Ugni selkirkii (Hook. and Arn.) O.Berg	Single	Slight	Absent	Anomocytic	Simple
Tepualia stipularis (Hook. and Arn.) Griseb.	Single	Slight	Absent	Anomocytic	Absent

Other general references consulted for anatomical terminology were Gifford and Foster (1989), Dickison (2000), Evert (2006) and Pole (2012).

3. Results

The results will be presented in three parts: (1) A survey of the leaf anatomical and micromorphological characters, (2) a synopsis of the leaf anatomy of each genus and (3) an identification key of Chilean species of Myrtaceae using anatomical and micromorphological characters. Leaf characters are summarized in Tables 1 and 2.

3.1. Survey of leaf characters

3.1.1. Epidermis, cuticle and epicuticular waxes

Different types of anticlinal walls of abaxial epidermal cells are observed. The most common type is the slightly sinuous with thin walls, present in the majority of taxa. Some species possess sinuous cell walls (Fig. 2A–C) while others have straight and thick walls (Fig. 2D). Adaxial epidermal cells have straight or straight-sinuous anticlinal walls in all cases.

The epidermal cell walls are mucilaginous (evidenced by test with ruthenium red), single layered in most of the species (Fig. 3A) and generally thicker on the adaxial side of the leaf. The species *M. correifolia*, *Myrceugenia obtusa* and *M. coquimbensis* possess a very thick adaxial epidermis, sometimes with a diffuse second layer beneath. Adaxial epidermal cells have thin primary cell walls and are plano-convex and mainly isodiametric in shape in the majority of taxa. Some species have enlarged-rectangular epidermal cells. Both species of *Luma* have isodiametric and enlarged-rectangular epidermal cells distributed equally on the adaxial surface. *Myrceugenia colchaguensis* possesses irregularly shaped epidermal cells. The pattern of the epidermal cells (shape and size) changes above the main vascular bundle in *Amomyrtus* spp. and *Legrandia concinna* but remains unchanged in the majority of the species. The species *N. fernandeziana*, *U. candollei* and *M. rufa* possess extra subepidermal cell layers. Observations in young leaves showed that the subepidermal layer in *N. fernandeziana* and *U. candollei* possibly correspond to hypodermis as this tissue is related to ground meristem in origin (Fig. 3B). On the other hand, the homogenous subepidermal layer observed in *M. rufa* is originated from the protodermis, which suggests that the species has a multiple (double) epidermis (Fig. 3C). Abaxial epidermal cells are small, rounded and isodiametric in nearly all the species. *M. obtusa* and *M. coquimbensis* have larger abaxial epidermal cells, with nearly 1:1 relative size to adaxial epidermal cells. Conical papillae can be observed on both adaxial and abaxial surfaces in some species. When present, papillae are combined with cuticular striations.

The cuticle is thicker on the adaxial surface than the abaxial surface in all species. The cuticular layer is either thin (3 μm or less) in a majority of the species, but in some (such as *M. correifolia*, *M. rufa* and *M. coquimbensis*) it is thick (>5 μm, up to 8 μm). The cuticle has ornamentations of epicuticular waxes in some taxa (Fig. 3D). Epicuticular waxes, as observed by SEM are granules or flakes. *Myrceugenia lanceolata* has very abundant epicuticular waxes on the abaxial surface, which gives a whitish colour to this side of the leaves.

3.1.2. Stomata

All species have hypostomatic leaves, except for *Myrteola nummularia*, which has amphistomatic leaves (stomata on both adaxial and abaxial surfaces). Stomata protrude slightly above the level of the epidermis (Fig. 4G–I). Anomocytic stomata were observed in *Amomyrtus*spp., *B. cruckshanksii*, *L. concinna*, most of the *Myrceugenia* species, *U. molinae*. and *U. selkirkii* (Fig. 2E). Paracytic stomata

Table 2
Leaf anatomical characters in the mesophyll and vascular system of Chilean Myrtaceae.

Taxon	Mesophyll			Vascular system			
	P.p. layers	Type of crystals	Type of cavities	Shape	Ad. Phloem partition	Phoem confluence	Amount of ad. phloem
Amomyrtus luma (Molina) D. Legrand and Kausel	2–3	Druses	Schizogenous	Ellipsoid	Absent	Confluent	Abundant
Amomyrtus meli (Phil.) D. Legrand and Kausel	2–3	Druses	Schizogenous	Ellipsoid	Strong	Confluent	Medium
Blepharocalyx cruckshanksii (Hook. and Arn.) Nied.	2–3	Druses	Schizolysigenous	Ellipsoid	Weak	Not confluent	Abundant
Legrandia concinna (Phil.) Kausel	3	Druses	Schizogenous	Slight arc	Absent	Confluent	Medium
Luma apiculata (DC.) Burret	2–3	Druses	Schizogenous	Ellipsoid	Strong	Confluent	Medium
Luma chequen (Feuillée ex Molina) Gray	2	Druses	Schizolysigenous	Ellipsoid	Weak	Not confluent	Scarce
Myrceugenia chrysocarpa (O.Berg) Kausel	2–3	Spherical	Schizogenous	Circular	Strong	Confluent	Scarce
Myrceugenia colchaguensis (Phil.) Navas	2	Rhombohedral	Schizogenous	Arc	Weak	Confluent	Medium
Myrceugenia correifolia (Hook. and Arn.) O.Berg	2–3	Druses	Schizolysigenous	Arc	Weak	Confluent	Medium
Myrceugenia exsucca (DC.) O.Berg	2–3	Druses	Schizogenous	Arc	Weak	Confluent	Abundant
Myrceugenia lanceolata (Juss. ex J. St.-Hil.) Kausel	3	Druses	Schizogenous	Arc	Strong	Not confluent	Abundant
Myrceugenia leptospermoides (DC.) Kausel	2–3	Druses	Schizogenous	Arc	Weak	Confluent	Medium
Myrceugenia obtusa (DC.) O.Berg	2	Druses	Schizolysigenous	Arc	Strong	Confluent	Medium
Myrceugenia ovata (Hook. and Arn.) O.Berg	2–3	Druses	Schizolysigenous	Arc	Strong	Not confluent	Abundant
Myrceugenia ovata var. nannophylla (Burret) L.R. Landrum	2	Druses	Schizogenous	Arc	Strong	Confluent	Abundant
Myrceugenia parvifolia (DC.) Kausel	1	Druses	Schizogenous	Arc	Weak	Confluent	Medium
Myrceugenia pinifolia (F. Phil.) Kausel	2–3	Druses	Schizogenous	Arc	Strong	Confluent	Abundant
Myrceugenia planipes (Hook. and Arn.) O.Berg	2	Spherical	Schizogenous	Arc	Strong	Confluent	Medium
Myrceugenia rufa (Colla) Skottsb. ex Kausel	4	Druses	Schizogenous	Arc	Slight	Not confluent	Scarce
Myrceugenia schulzei Johow	2	Rhombohedral	Schizogenous	Arc	Strong	Not confluent	Medium
Myrcianthes coquimbensis (Barnéoud) Landrum and Grifo	2	Druses	Schizogenous	Circular	Strong	Not confluent	Medium
Myrteola nummularia (Poir.) O.Berg	2–3	Druses	Schizolysigenous	Circular	Absent	Confluent	Scarce
Nothomyrcia fernandeziana (Hook. and Arn.) Kausel	2–3	Druses	Schizogenous	Ellipsoid	Strong	Not confluent	Abundant
Ugni candollei (Barnéoud) O.Berg	2–3	Druses	Schizogenous	Circular	Strong	Not confluent	Medium
Ugni molinae Turcz.	3	Druses	Schizogenous	Arc	Strong	Not confluent	Medium
Ugni selkirkii (Hook. and Arn.) O.Berg	2–3	Rhombohedral	Schizogenous	Arc	Weak	Confluent	Medium
Tepualia stipularis (Hook. and Arn.) Griseb.	2	Absent	Schizogenous	Circular	Weak	Not confluent	Scarce

P.p.: palisade parenchyma; Ad: adaxial.

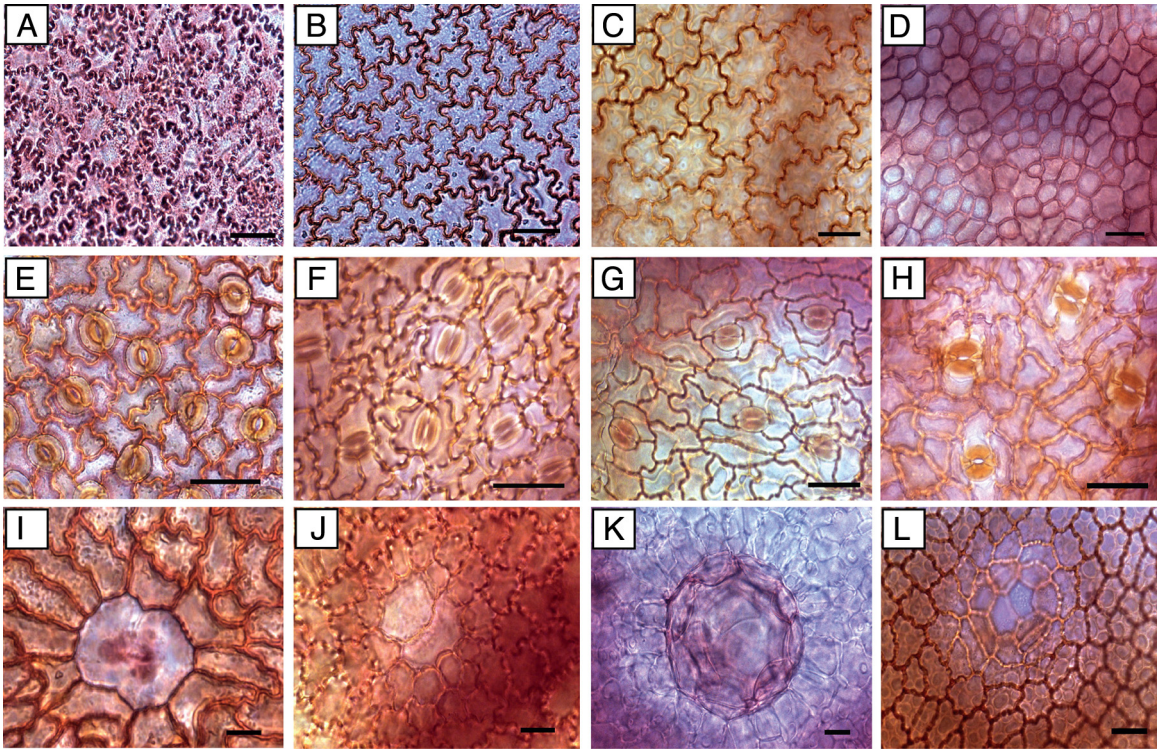

Fig. 2. Light micrographs (LM) of leaf clearings of Chilean Myrtaceae. (A–D) Shape of abaxial epidermal anticlinal walls: (A) highly sinuous in *Amomyrtus meli*. (B) Highly sinuous in *Blepharocalyx cruckshanksii*. (C) Slightly sinuous in *Myrteola nummularia*. (D) Straight walls in *Myrceugenia correifolia*. (E–H) Stomatal types: (E) anomocytic in *B. cruckshanksii*. (F) Paracytic in *Luma apiculata*. (G) Anisocytic in *Ugni candollei*. (H) Laterocytic in *Myrcianthes coquimbensis*. (I–L) Secretory cavities: (I) cavity showing ca. 10 irregular cells surrounding the two cap cells in *B. cruckshanksii*. (J) Cavity surrounded by ca. 14 isodiametric cells in *L. concinna*. (K) Cavity showing eight epithelial cells in *Myrceugenia leptospermoides*. (L) Cavity surrounded by ca. 7 cells in *Tepualia stipularis*. Scale bars = 25 μm (A–H), 10 μm (I–L). Stain used: Safranin O.

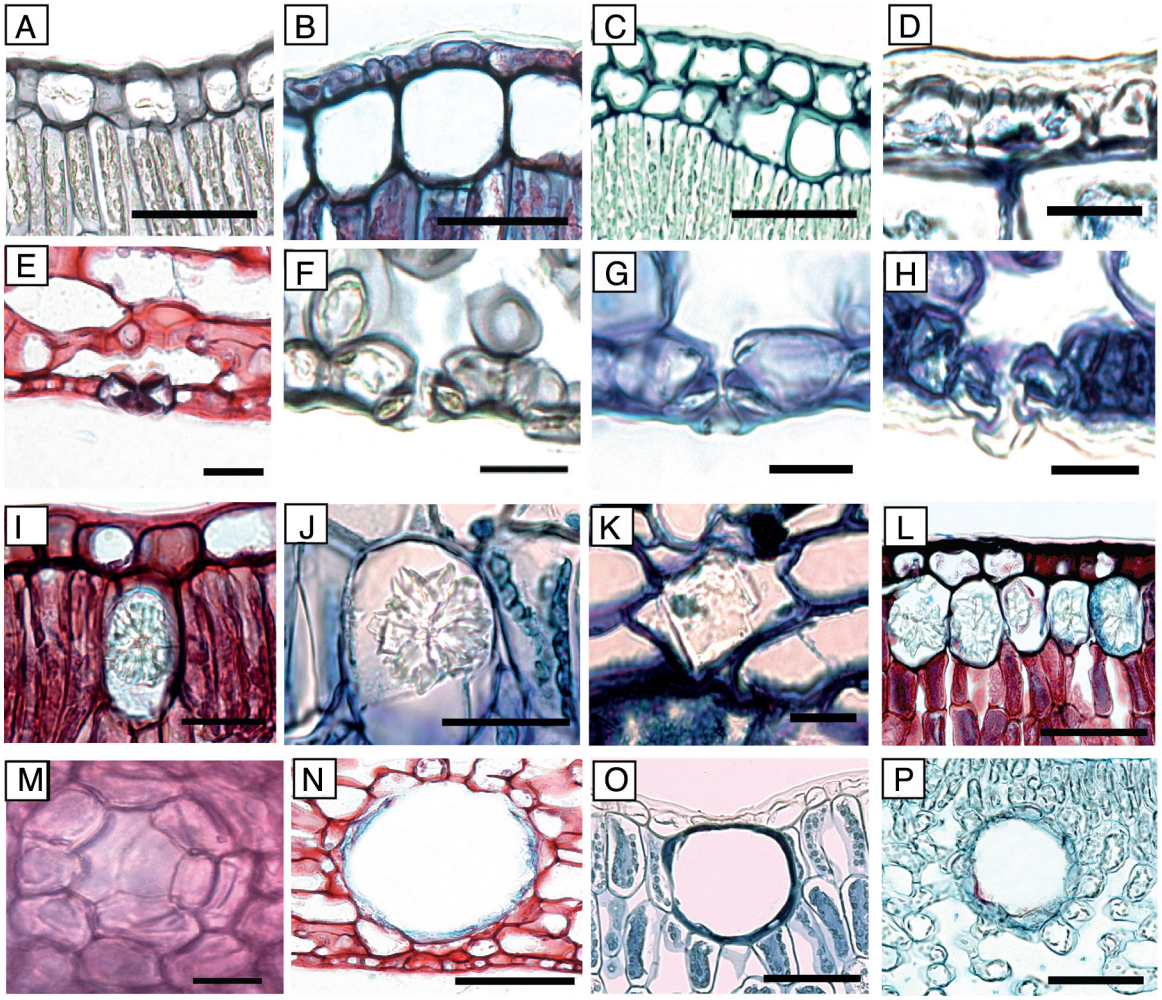

Fig. 3. Transverse light micrographs (LM) of leaf showing epidermis, stomata and mesophyll elements in Chilean Myrtaceae. (A–D) Epidermis and cuticle: (A) single layered epidermis with thin cuticle in *Luma chequen*. (B) Thick hypodermis with simple thick cuticle in *Ugni candollei*. (C) Double epidermis with thick cuticle in *Myrceugenia rufa*. (D) Single layered epidermis with ornamented cuticle in *Amomyrtus meli*. (E–H) Transverse view of stomata at equatorial level: (E) triangular guard cells with cutinized thickening of outer periclinal walls in *Myrceugenia planipes*. (F) Ovate guard cells without thickenings in *Luma chequen*. (G) Heavy cutinized thickenings of outer periclinal walls of guard cells in *Luma apiculata*. (H) Irregular thickenings in *Ugni selkirkii*. (I–L) crystals: (I) spherical crystal in *Myrceugenia planipes*. (J) Druse in *Amomyrtus luma*. (K) Rhombohedral crystal in *Ugni selkirkii*. (L) Several grouped druses in *Legrandia concinna*. (M–P) Secretory cavities: (M) early stage of schizogenous secretory cavity showing small and isodiametrical epithelial cells with thin primary walls in *Ugni molinae*. (N) Schizogenous cavity in spongy parenchyma of *Myrceugenia planipes*. (O) schizolysigenous cavity in palisade parenchyma of *Myrteola nummularia*. (P) Schizolysigenous cavity in the mesophyll of *Luma chequen*. Scale bars = 10 μm (A–D, G, L), 25 μm (E–F, I–K), 50 μm (H, M–P). Stains used: chlorazol black E (A, C, F), TBO (B, D, G, H, J, K, O, P), safranin O—alcian blue (E, I, L, M, N).

were observed in in *L. apiculata* and *L. chequen* (Fig. 2F). Actinocytic stomata are common in *M. colchaguensis*. Anisocytic stomata are the most common type in *U. candollei* (Fig. 2G). Laterocytic stomata are common in *M. coquimbensis* (Fig. 2H). In transverse section, differences in the shape of guard cells and the degree of cutinized thickenings on the outer periclinal cell walls of guard cells can be observed. Guard cells are triangular and have cutinized thickenings of outer periclinal walls in some species (*M. lanceolata* and *Myrceugenia planipes* (Fig. 3E)). Ovate guard cells without cutinized thickenings were observed in *L. chequen* (Fig. 3F), while *L. apiculata* shows ovate guard cells with heavy cutinized thickenings (Fig. 3G). Irregular thickenings were observed in *U. selkirkii* (Fig. 3H).

3.1.3. Indumentum

The majority of the species have sparsely pubescent leaves on both adaxial and abaxial surfaces (Fig. 5A). The leaves in most of the species become glabrescent with age. Only two species (*T. stipularis* and *B. cruckshanksii*) have completely glabrous leaves, where hairs were not observed in either young or mature leaves. Four species, namely *U. candollei* (Fig. 5F), *M. correifolia* (Fig. 5B), *Myrceugenia exsucca* and *M. planipes*, have sparse to moderately pubescent indument, particularly on the abaxial surface. Abaxially lanate (densely hairy) leaves were observed in *M. colchaguensis*, *M. rufa* (Fig. 5C) and *M. schulzei* (Fig. 5E). Abaxially and adaxially lanate leaves were observed in *M. coquimbensis* (Fig. 5D).

Three types of unicellular hairs were observed: simple (straight, curved, hooked, twisted or ciliate) (Fig. 6A and C), dibrachiate (symmetrically or asymmetrically dibrachiated) (Fig. 6B) and glandular (Fig. 6D–F). Simple hairs are observed in *L. concinna*, *M. coquimbensis*, *M. nummularia*, *N. fernandeziana*,*U. molinae* and *U. selkirkii*. Dibrachiate hairs were observed in *Myrceugenia*spp., with some species also possessing simple hairs. Hairs are appressed in some species, especially in the case of dibrachiate hairs. Glandular hairs were observed in *L. chequen*, *M. colchaguensis*, *M. obtusa* and *M. coquimbensis*. A distinctive staining reaction to TBO is detected around some glandular hairs of *M. obtusa*, which probably indicated the presence of sesquiterpenes.

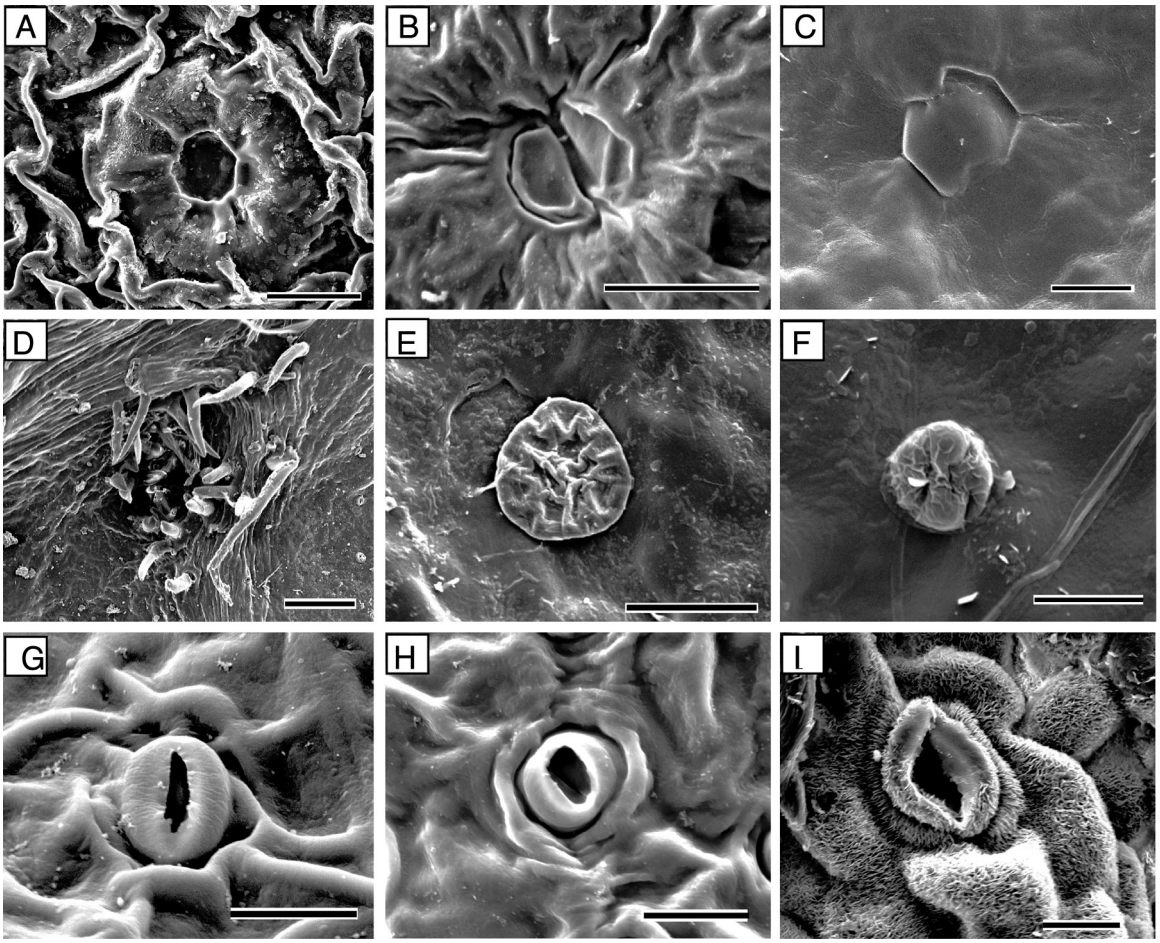

Fig. 4. Scanning electron micrographs (SEM) of leaf adaxial and abaxial elements of Chilean Myrtaceae. (A–C) Secretory cavities: (A) raised cavity in *Myrceugenia leptospermoides*. (B) Cavity with two clear overlying cells in *Myrceugenia exsucca*. (C) Deep secretory cavity with two barely visible overlying cells in *Myrteola nummularia*. (D) Domatium in *L. concinna* covered with ciliate hairs. (E) Extrafloral nectary on adaxial surface of *Myrceugenia planipes*. (F) Extrafloral nectary on adaxial surface of *Tepualia stipularis*. (G–H) Stomata with subsidiary cells in (G) *Ugni candollei* and (H) *Myrceugenia exsucca*. (I) Stomatal complex surrounded by epidermal cells with hairs and epicuticular waxes in *Myrceugenia colchaguensis*. Scale bars = 25 μm (A–C), 100 μm (D), 10 μm (E–I).

3.1.4. Mesophyll

All taxa have dorsiventral mesophyll with palisade parenchyma composed of rectangular, attenuated and vertical cells. The number of cell layers of the palisade parenchyma varies from a single layer in *M. parvifolia* (Fig. 7A) to three distinct layers in *M. lanceolata* (Fig. 7B) and *L. concinna* (Fig. 7C). Four compressed layers were observed in *M. rufa* (Fig. 7D). The remaining taxa have two layers of palisade parenchyma, usually with a diffuse and poorly developed third layer (Fig. 7E). The spongy parenchyma is composed of irregularly shaped cells that vary from rounded to polygonal. Intercellular spaces do not vary considerably between taxa. The staining reaction to ruthenium red confirms the presence of mucilage and pectins in the mesophyll of all the species. The mesophyll of *M. coquimbensis* (Fig. 7F) and *U. selkirkii* is rich in tannins and polyphenols. *L. concinna* possesses domatia covered with ciliate hairs on the abaxial side of leaves, which are originated from the mesophyll. Domatia are easily observed in the axils of the midrib and the secondary veins of *L. concinna* (Fig. 4D).

3.1.5. Crystals

Intracellular crystals are present in most of the species. Two main types of crystals were found, namely druses (aggregated individual crystals) and prismatic crystals (rhombohedral and spherical). Crystals were dissolved after testing with acetic acid and hydrochloric acid, discarding silica composition. Differential solubility indicates that crystals are composed of CaOx (calcium oxalates). Druses are mainly contained in idioblasts and present in the palisade parenchyma below the adaxial epidermis (Fig. 3J). In some species, druses are also present around the leaf phloem and contained in bundle sheath cells. *M. colchaguensis, M. schulzei* and *U. selkirkii* exhibit prismatic rhombohedral crystals, mainly around the vascular system (Fig. 3K). Two species (*Myrceugenia chrysocarpa* and *M. planipes*) possess spherical crystals located below the epidermis and also throughout the spongy parenchyma (Fig. 3I). Idioblasts with druses are mainly solitary or occur in pairs, however in some species (e.g., *L. concinna*) several idioblasts are grouped together (Fig. 3L). Druses were not observed in the leaves of *T. stipularis* and appear to be rare in *M. coquimbensis*.

3.1.6. Secretory cavities

Leaf secretory cavities are generally located in the palisade parenchyma, usually in contact with the adaxial epidermis (Fig. 3O) but in some species they are located below both adaxial and abaxial surfaces. In young leaves, all cavities are initially formed by separation of epithelial cells (Fig. 3M), which confirms that secretory cavities in Chilean Myrtaceae are not lysigenous (cavities formed by dissolution of cells). Species have either schizogenous or schizolysigenous cavities (a mixture of schizogenous and lysige-

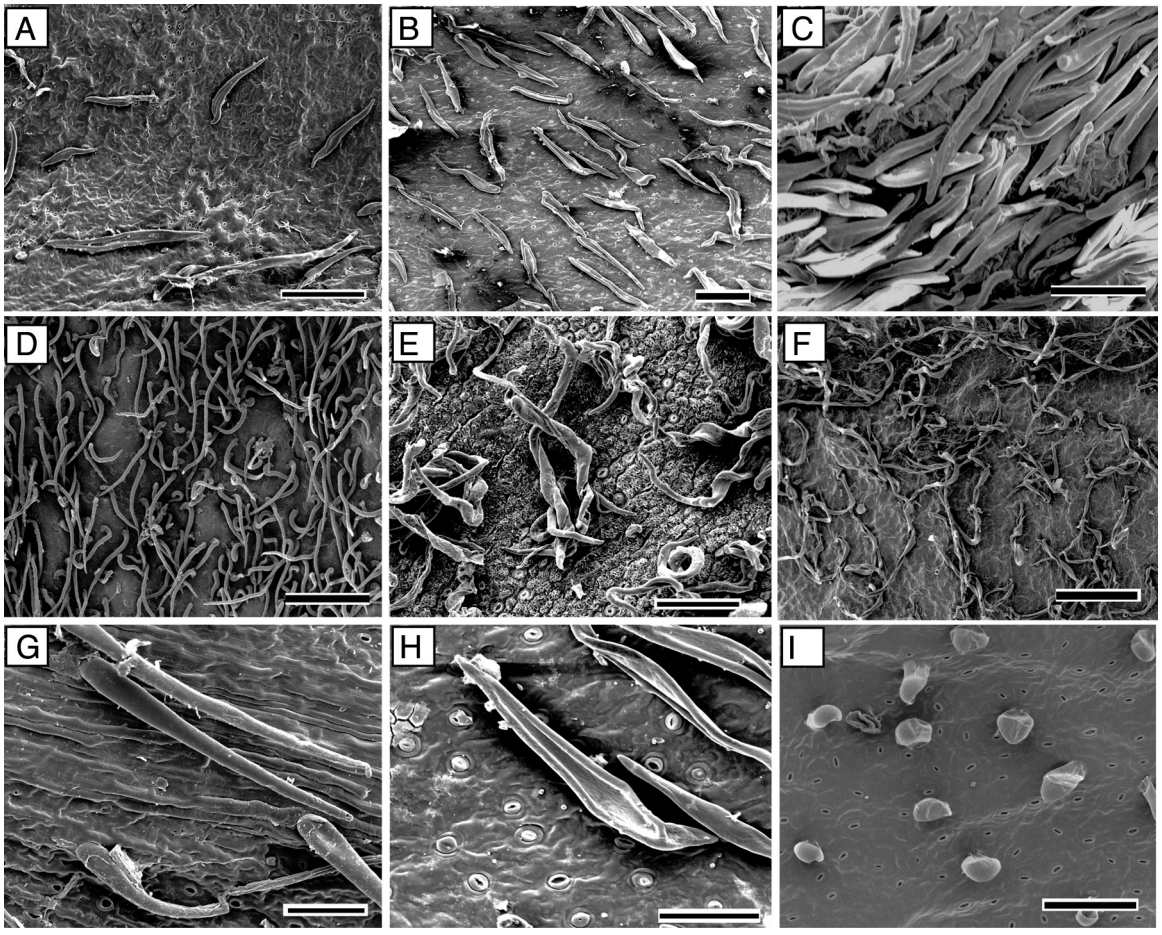

Fig. 5. Scanning electron micrographs (SEM) of leaf hairs of Chilean Myrtaceae. (A–C) Abundance of hairs: (A) sparsely hairy abaxial surface in *Myrceugenia ovata* var. *ovata*. (B) Slightly pubescent abaxial surface in *Myrceugenia correifolia*. (C) Densely hairy abaxial surface in *Myrceugenia rufa*. (D–F) Distribution of hairs in some pubescent species: (D) strongly pubescent leaves with straight hairs in *Myrcianthes coquimbensis*. (E) Pubescent leaves with twisted hairs in *Myrceugenia schultzei*. (F) Pubescent leaf with hooked hairs in *Ugni candollei*. (G–I) Different types of hairs: (G) simple hairs in *Amomyrtus luma*. (H) Symmetrically dibrachiate hairs in *Myrceugenia correifolia*. (I) Glandular hairs in *Luma chequen*. Scale bars = 50 μm (A–B, G–I), 250 μm (C–D, F), 100 μm (E).

nous cavities). In early developmental stages, epithelial cells of schizogenous cavities are small, isodiametrical and have very thin primary cell walls (Fig. 3M). At maturity, schizogenous cavities have a layer of epithelial cells surrounding a wide lumen, while schizolysigenous cavities only have a lumen without secretory epithelial cells. Epithelial cells in schizolysigenous cavities have collapsed at some developmental stage and show secretions around the lumen. Secretory cavities are schizogenous in most of the species (e.g., *Amomyrtus* spp., *M. coquimbensis*, *Myrceugenia* spp., *T. stipularis*, *Ugni* spp.) (Fig. 3N) and schizolysigenous in others (e.g., *B. cruckshanksii*, *L. chequen*, *M. nummularia,*) (Fig. 3O and P). A number of species (e.g., *L. chequen*, *M. coquimbensis*) have additional secretory cavities throughout palisade and spongy parenchyma (Fig. 3P). In surface view, two overlying cells (epidermal cells above secretory cavities) can be observed. These cells vary in shape and are surrounded by a variable number of epidermal cells (Fig. 2I–L). The cavities and overlying cells can be clearly differentiated as polyhedral in shape in *M. exsucca* (Fig. 4B) and *Myrceugenia leptospermoides* (Fig. 4A). The overlying cells are barely visible in *Ugni* spp. and *M. nummularia* (Fig. 4C). Histochemical reaction with Sudan IV suggests the presence of lipophilic substances in the epithelial cells lining the cavity. Extrafloral nectaries are observed on the adaxial surface of *M. planipes* (Fig. 4E) and *T. stipularis* (Fig. 4F).

3.1.7. Vascular system

Most taxa have a flattened or slightly grooved adaxial leaf surface above the vascular region, but some species possess a noticeable depression (e.g., *L. concinna*, *M. exsucca*, *M. planipes*, *U. molinae*). A prominent swelling on the adaxial side of the leaf over the main vascular system is observed in *B. cruckshanksii* and *N. fernandeziana*, species that morphologically do not have impressed midribs as the remaining Chilean Myrtaceae.

The vascular system occupies the half of the lamina in cross section in almost all the species, but it is particularly small in *M. rufa* (Fig. 8H), *M. nummularia* (Fig. 8J), *T. stipularis* (Fig. 8K) and *U. candollei*. The shape of vascular systems varies from circular (*M. chrysocarpa*,*M. coquimbensis*,*M. nummularia, T. stipularis* Fig. 8E, I, J, K) to arc-shaped vascular systems (e.g., *M. correifolia*, *M. planipes*, *U. molinae* and *U. selkirkii*—Fig. 8G and L). The vascular system is composed of a central region of xylem with bicollateral phloem (adaxial and abaxial) in all the species. The adaxial phloem may be confluent with the abaxial phloem, i.e., merged together to form an arc of continuous phloem, or could be discontinuous and not connected to the abaxial phloem. The adaxial phloem itself could be a single patch (continuous) or it could form two islands of phloem due to the presence of a partition, composed of fibres, vessels or parenchymatous cells. The adaxial phloem partition can be considered either weak or strong depending on the degree of separation

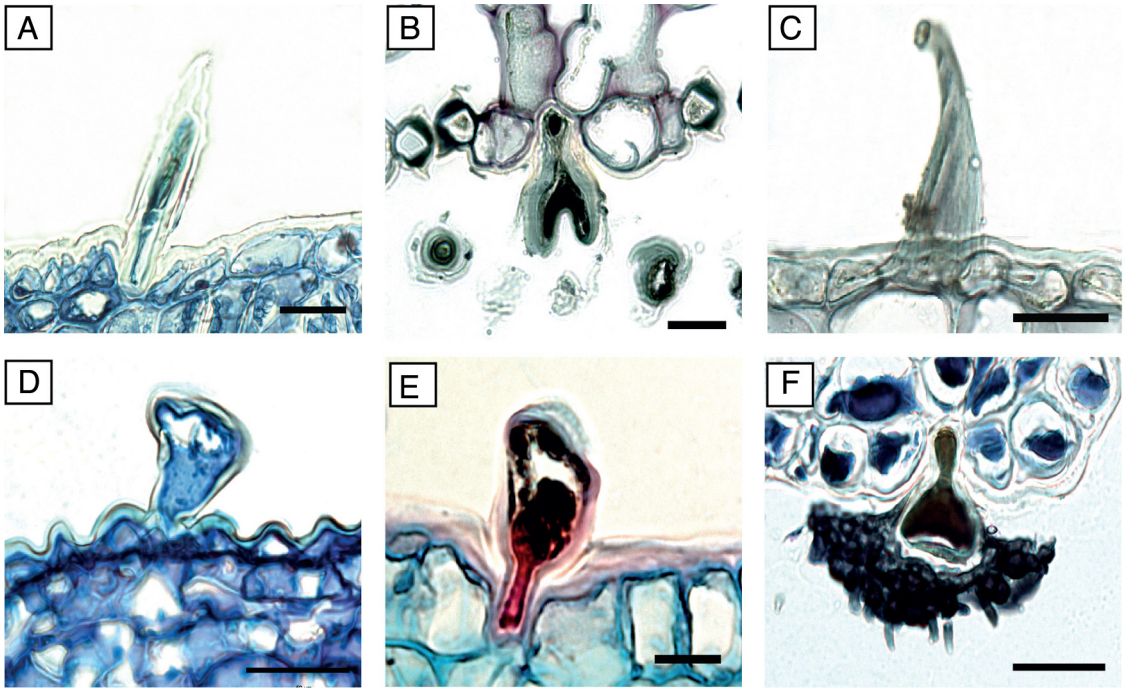

Fig. 6. Transverse light micrographs (LM) of leaf hairs of Chilean Myrtaceae. (A) Simple hair in *Amomyrtus luma*. (B) Symmetrically dibrachiate hair in *Myrceugenia rufa*. (C) Simple hooked hair in *Ugni candollei*. (D–F) Glandular hairs: (D) *Myrceugenia colchaguensis*. (E) *Myrcianthes coquimbensis*. (F) *Myrceugenia obtusa* with dark stained secretions around the hair. Scale bars = 40 μm (A), 10 μm (B–F). Stains used: TBO (A, D, F), ruthenium red (B, C), ruthenium red-TBO (E).

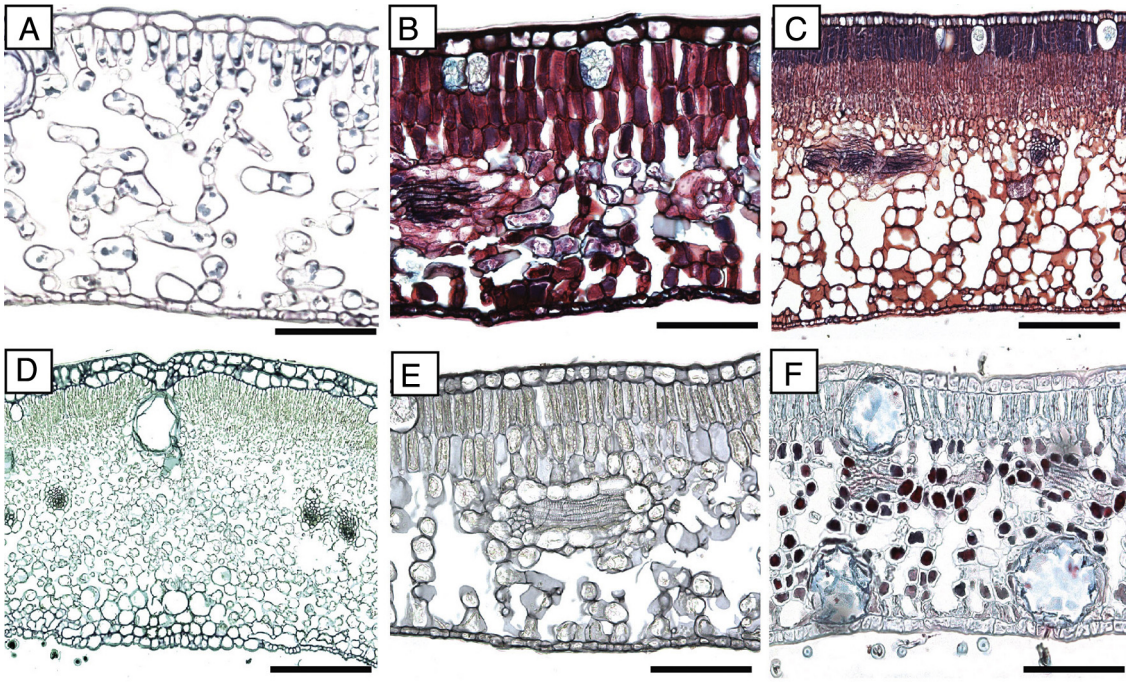

Fig. 7. Transverse light micrographs (LM) of epidermis and mesophyll of Chilean Myrtaceae. (A) Single layered epidermis, single layered palisade parenchyma and loose spongy parenchyma in *Myrceugenia parvifolia*. (B) Single layered epidermis and palisade parenchyma with three layers in *Legrandia concinna*. (C) Single layered epidermis and compacted palisade parenchyma with three-four layers in *Myrceugenia lanceolata*. (D) Multiple epidermis and compacted spongy and palisade parenchyma with three layers in *Myrceugenia rufa*. (E) Single layered epidermis and palisade parenchyma with two layers in *Luma chequen*. (F) Single layered epidermis, palisade parenchyma with two layers and spongy parenchyma rich in tannins in *Myrcianthes coquimbensis*. Stains used: TBO (A) safranin O—alcian blue (B, C), ruthenium red (D), chlorazol black (E), ruthenium red-TBO (F).

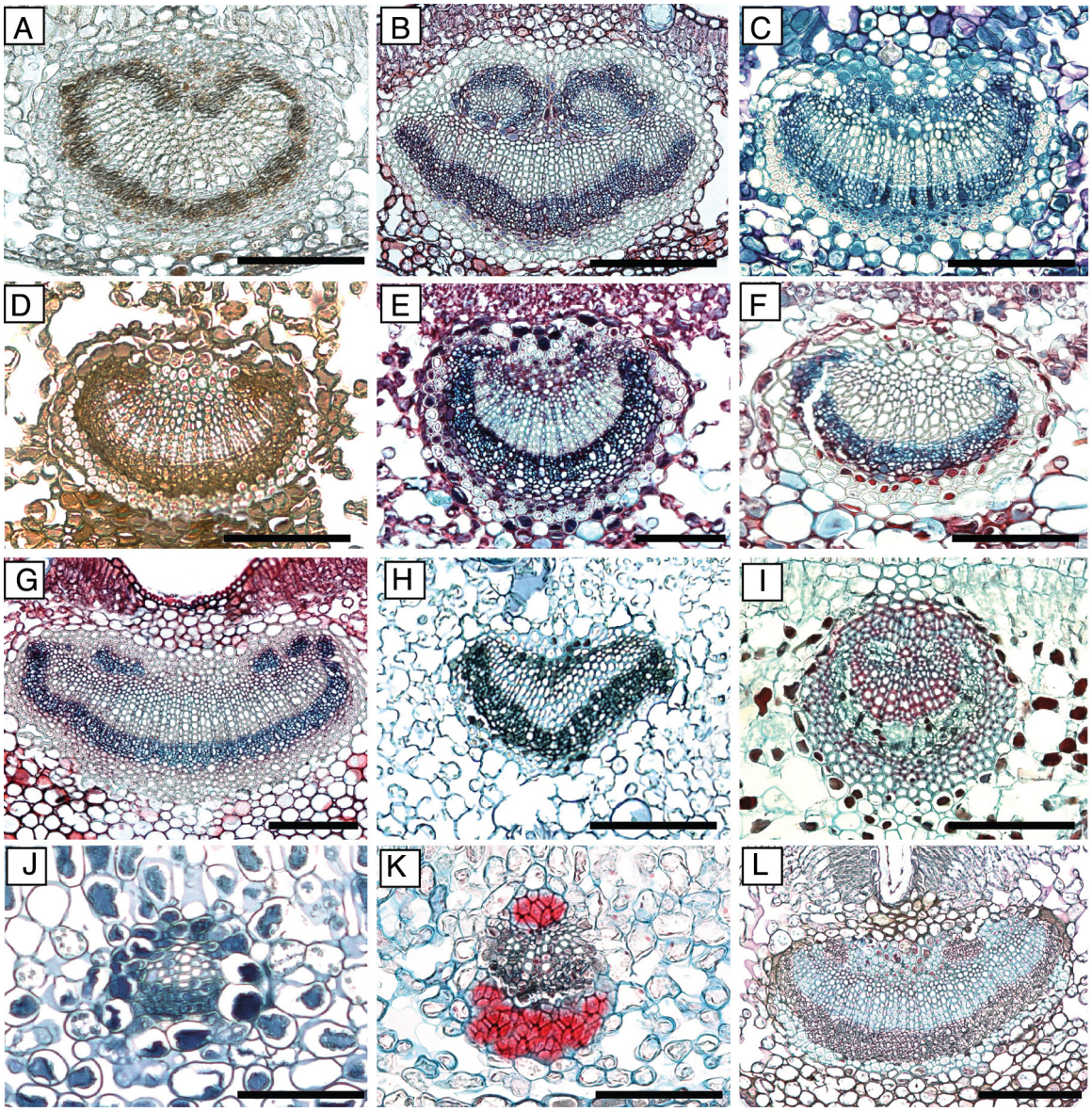

Fig. 8. Transverse light micrographs (LM) through the leaf vascular system of Chilean Myrtaceae. (A) Circular vascular system with continuous phloem in *Amomyrtus luma*. (B) Ellipsoid vascular system with adaxial phloem surrounding two isolated groups of xylem in *Blepharocalyx cruckshanksii*. (C) Arc-shaped vascular system with abaxial and adaxial confluent phloem in *Legrandia concinna*. (D) Ellipsoid vascular system with scarce adaxial phloem with strong partition in *Luma apiculata*. (E) Circular vascular system with strong adaxial phloem partition in *Myrceugenia chrysocarpa*. (F) arc-shaped vascular system with adaxial and abaxial confluent phloem and strong adaxial partition in *Myrceugenia obtusa*. (G) Arc-shaped vascular system with strong phloem partition in *Myrceugenia planipes*. (H) Reduced arc-shaped vascular system in *Myrceugenia rufa*. (I) Circular midrib with scarce adaxial phloem in *Myrcianthes coquimbensis*. (J) Reduced circular vascular system with scarce adaxial phloem in *Myrteola nummularia*. (K) Reduced circular vascular system with scarce adaxial phloem and deeply stained fibres with very thick walls in *Tepualia stipularis*. (L) Arc-shaped vascular system with strong adaxial phloem partition in *Ugni molinae*. Scale bars = 100 μm. Stains used: chlorazol black E (A), safranin O—alcian blue (B, E, F, G), TBO (C, J, K), ruthenium red (D, H), ruthenium red—TBO (I, L).

between the two patches of adaxial phloem. The amount of adaxial phloem can vary from scarce to abundant, which can be interpreted as poorly and well developed respectively. In the vascular system of *A. luma* and *L. concinna* the adaxial and abaxial phloem is confluent and the adaxial phloem does not have partition, forming a continuous ring that surrounds the xylem (Fig. 8A and C). In some species, the adaxial phloem has a weak partition and there is confluence between the adaxial and abaxial phloem (e.g., *M. correifolia*, *M. exsucca*, *M. leptospermoides*). In the remaining species, the adaxial phloem has a strong partition. Some of these have a confluent adaxial and abaxial phloem, such as *A. meli*, *Luma* spp., *M. chrysocarpa*, *M. obtusa* (Fig. 8D–F). Species with a strong adaxial partition and without adaxial-abaxial confluence (vascular system with open extremities) include *M. rufa*, *M. coquimbensis*, *N. fernandeziana*, *U. candollei* and *U. molinae* (Fig. 8H,I,L). In these species, the adaxial phloem usually forms two islands of phloem that are disconnected from the abaxial phloem. In the vascular system of *L. chequen*, *M. chrysocarpa*, *M. rufa*, *M. nummularia* and *T. stipularis* the adaxial phloem is scarce, unlike the majority of taxa, which have abundant and well developed adaxial phloem (Fig. 8). The vascular system of *B. cruckshanksii* has the adaxial phloem curved inward forming two isolated groups of xylem surrounded by adaxial phloem (Fig. 8B), while in the case of *N. fernandeziana* the xylem surrounds two islands of adaxial phloem. The latter can be classified as a vas-

cular system with adaxial phloem with weak partition and without adaxial and abaxial phloem confluence. Sclerenchyma fibres form a continuous ring around the vascular system in the majority of species (Fig. 8), but they are discontinuous and form an abaxial arc in *A. meli*, *M. colchaguensis* and *M. schulzei.* There are no fibres around the vascular system of *M. nummularia* (Fig. 8J) and they are very abundant around the midri of *M. coquimbensis* (Fig. 8I). Histochemical reaction to phloroglucinol + 20% HCl was observed in all sclerenchymatous tissues, especially fibres in the vascular system.

3.2. Synopsis of leaf anatomical characters in genera of chilean myrtaceae

The following section is a synopsis of the salient anatomical and micromorphological characters of each genus of Chilean Myrtaceae. For each genus, the species studied are indicated, as well as the total number of accepted species for that genus. Vouchers and herbarium are indicated in parentheses. In this summary, only those characters that were present have been included and the absence of characters is only reported in cases where our observation contradict those already published.

1. *Amomyrtus* (Burret) D. Legrand and Kausel (Figs. 1A, 2A, 3D,J, 5G, 6A, 8A).

Number of species in genus: Two

Species studied: *Amomyrtus luma* (Reta-07.1/07.2, BRI), *A. meli* (Reta-25.1/25.2, BRI).

The leaves are hypostomatic with anomocytic stomatal complexes. The epidermis is single-layered, mucilaginous and has a thin cuticle layer (3 μm thick or less). The adaxial epidermis is slightly thicker than the abaxial epidermis. Epidermal anticlinal walls are highly sinuous and thin. The leaves are glabrous to sparsely pubescent on midrib and margins, but more pubescent in *A. luma* than *A. meli*. The hairs are simple and straight-curved. The mesophyll is dorsiventral with two-three layers of palisade parenchyma. The spongy parenchyma is composed of large, isodiametric cells. Idioblasts containing druses are distributed only below the adaxial epidermis. The secretory cavities are schizogenous and are mainly located below the adaxial epidermis. The shape of the vascular system is circular. The adaxial phloem is abundant and continuous (without partition) in *A. luma*, while it is partitioned into two clear clusters in *A. meli*. The adaxial and abaxial phloem is confluent in *A. luma* but not so in *A. meli*. Fibres form a continuous ring around the vascular system.

2. *Blepharocalyx* O. Berg (Figs. 1B, 2E,I, 8B).

Number of species in genus: Three

Species studied: *B. cruckshanksii* (Reta-24.1/24.2, BRI)

The leaves are hypostomatic with anomocytic stomatal complexes. The epidermis is single-layered, mucilaginous and has a regular cuticle layer (3–5 μm thick). The adaxial epidermis is slightly thicker than the abaxial epidermis. The epidermal cells are isodiametric with highly sinuous and thin anticlinal walls on the abaxial surface. The leaves are glabrous. The mesophyll is dorsiventral with two-three layers of palisade parenchyma. The spongy parenchyma is composed of small, irregularly shaped cells. Idioblasts containing druses are distributed below the adaxial epidermis. The secretory cavities are schizolysigenous and are mainly located below the adaxial epidermis. The shape of the vascular system is ellipsoidal. The adaxial phloem is abundant with a weak partition and surrounds two islands of xylem. The adaxial and abaxial phloem is not confluent. Fibres are discontinuous around the vascular system.

3. *Legrandia* Kausel (Figs. 1C, 3L, 4D, 7B, 8C).

Number of species in genus: 1

Species studied: *L. concinna* (Reta-09.1/09.2, BRI)

The leaves are hypostomatic with anomocytic stomatal complexes. The epidermis is single-layered, mucilaginous and has a regular cuticle layer (3–5 μm thick). The adaxial epidermis is slightly thicker than the abaxial epidermis. The epidermal cells are isodiametric with highly sinuous and thin anticlinal walls on the abaxial surface. Domatia are observed in the axils of veins on the abaxial surface. Conical papillae are present on the abaxial surface. The leaves are glabrous to sparsely pubescent on midrib and margins. The hairs are simple and straight-curved. The mesophyll is dorsiventral with three layers of palisade parenchyma. The spongy parenchyma is composed of large, isodiametric cells. Idioblasts containing druses are distributed below the adaxial epidermis, sometimes forming clusters of six-seven. The secretory cavities are schizogenous and are mainly located below the adaxial epidermis. The shape of the vascular system is arc-shaped. The adaxial phloem is abundant and continuous (without partition). The adaxial and abaxial phloem is confluent. Fibres are discontinuous around the vascular system.

4. *Luma* Gray (Figs. 1D, 2F, 3A,F,G,P, 5I, 8D).

Number of species in genus: 2

Species studied: *L. apiculata* (Reta-26.1/26.2, BRI), *L. chequen* (Reta-05.1/05.2, BRI).

The leaves are hypostomatic with paracytic stomatal complexes. The epidermis is single-layered, mucilaginous and has a regular cuticle layer (3–5 μm thick). The adaxial epidermis is slightly thicker than the abaxial epidermis. The epidermal cells are isodiametric with slightly sinuous and thin anticlinal walls. The leaves are glabrous to sparsely pubescent on midrib and margins. The hairs are simple and straight-curved in *L. apiculata*, while *L. chequen* also has glandular hairs on both surfaces. The mesophyll is dorsiventral with two-three layers of palisade parenchyma in *L. apiculata* and two layers in *L. chequen*. The spongy parenchyma is composed of small, isodiametric cells. Idioblasts containing druses are distributed only below the adaxial epidermis. The secretory cavities are schizogenous and mainly located below the adaxial epidermis in *L. apiculata* and schizolysigenous and located throughout the mesophyll in *L. chequen*. The shape of the vascular system is ellipsoidal. The adaxial phloem is abundant with a strong partition in *L. apiculata* and scarce with a weak partition in *L. chequen*. The adaxial and abaxial phloem is confluent in *L. apiculata* and not confluent in *L. chequen*. Fibres are discontinuous around the vascular system.

5. *Myrceugenia* O. Berg (Figs. 1E–H, 2D,K, 3C,E,I,N, 4A,B,E,H,I, 5A–C,E,H, 6B,D,F, 7A,C,D, 8E–H).

Number of species in genus: ca. 40

Species studied: *M. chrysocarpa* (Reta-01.1/01.2, BRI), *M. colchaguensis* (CONC 121491, CONC), *M. correifolia* (Reta-16.1/16.2, BRI), *M. exsucca* (Reta-11.1/11.2, BRI), *M. lanceolata* (Reta-22.1/22.2, BRI), *M. leptospermoides* (Reta-12.1/12.2, BRI), *M. obtusa* (Reta-19.1/19.2, BRI), M. ovata (Reta-18.1/18.2, BRI), *M. ovata* var. *nanophylla* (Reta-15.1/15.2, BRI), *M. parvifolia* (Reta-21.1/21.2, BRI), *M. pinifolia* (Reta-27.1/27.2, BRI), *M. planipes* (Reta-02.1/02.2, BRI), *M. rufa* (Reta-10.1/10.2, BRI), *M. schulzei* (CONC 157850, CONC).

The leaves are hypostomatic in all species. The stomatal complexes are anomocytic in most of the species, but actinocytic (and anomocytic) in *M. colchaguensis*. The epidermis is single-layered in all the species except for the adaxial epidermis of *M. rufa*, where the epidermis is double-layered. The epidermis is mucilaginous and has a regular cuticle layer (3–5 μm thick) in most of the species, but thick (>5 μm, up to 8 μm) in *M. correifolia* and *M. rufa*. The adaxial epidermis is slightly thicker than the abaxial in most of the cases but thicker and equally thick in *M. obtusa*. The epidermal cells are isodiametric with highly sinuous and thin anticlinal walls on the abaxial surface in most of the species but highly sinuous in *M. pinifolia* and straight and thick in *M. colchaguensis*, *M. correifolia* and *M. rufa*. Conical papillae present on the abaxial surface of *M. correifolia* and *M. schulzei*. All the species possess dibrachiated hairs. Simple hairs were observed in *M. lanceolata*, *M. leptospermoides*, *M. obtusa*, *M. ovata*, *M. parvifolia* and *M. pinifolia*. Glandular hairs were only

seen in *M. colchaguensis* and *M. obtusa*. The mesophyll is dorsiventral with two-three layers of palisade parenchyma, except for *M. parvifolia* that possess only one layer. The spongy parenchyma has isodiametric or irregularly shaped cells, with abundant intercellular spaces in most species and scarce in *M. correifolia* and *M. rufa*. Idioblasts containing druses are observable below the adaxial epidermis in most species, but also occur around the vascular system in *M. colchaguensis* and *M. schulzei.* Most of the species have druses, but spherical crystals are observed in *M. chrysocarpa* and rhombohedral in *M. colchaguensis* and *M. schulzei*. The secretory cavities are shizogenous in all the species, except for *M. correifolia*, *M. obtusa* and *M. ovata*, which have shizolysigenous cavities. The vascular system is arc-shaped in all the species other than *M. chrysocarpa* in which it is circular. The adaxial phloem is either scarce or abundant and the partition weak or strong depending upon species (Table 2). The adaxial and abaxial phloem is confluent in most of taxa, but not confluent in *M. lanceolata*, *M. ovata*, *M. rufa* and *M. schulzei*. Fibres are discontinuous around the vascular system.

6. *Myrcianthes* Berg (Figs. 1I, 2H, 5D, 6E, 7F, 8I).

Number of species in genus: 30

Species studied: *M. coquimbensis* (Reta-08.1/08.2, BRI).

The leaves are hypostomatic with laterocytic (and paracytic) stomatal complexes. The epidermis is single-layered, mucilaginous and has a tick cuticle layer (>5 μm, up to 8 μm thick). The adaxial and abaxial epidermises are tick and have the same thickness. The epidermal cells are isodiametric with slightly sinuous anticlinal walls on the abaxial surface. The leaves are densely covered by hairs in both abaxial and adaxial surfaces. The hairs are simple and straight-curved. Glandular hairs are also observed. The mesophyll is dorsiventral with two layers of palisade parenchyma. The spongy parenchyma is composed of large, irregularly shaped cells. Idioblasts containing druses are distributed below the adaxial epidermis. The secretory cavities are schizogenous and are located below the adaxial epidermis and throughout the mesophyll. The shape of the vascular system is circular. The adaxial phloem has a medium development (abundance) and a strong partition. The adaxial and abaxial phloem is confluent. Fibres form a continuous ring around the vascular system.

7. *Myrteola* Berg (Figs. 1J, 2C, 3O, 4C, 8J).

Number of species in genus: 3

Species studied: *M. nummularia* (Reta-03.1/03.2, BRI).

The leaves are amphistomatic with anomocytic stomatal complexes. The epidermis is single-layered, mucilaginous and has a thin cuticle layer (3 μm thick or less). The adaxial epidermis is thicker than the abaxial epidermis. The epidermal cells are isodiametric with highly sinuous and thin anticlinal walls on the abaxial surface. The leaves are glabrous to sparsely pubescent on midrib and margins. The hairs are simple and straight-curved. The mesophyll is dorsiventral with two-three layers of palisade parenchyma. The spongy parenchyma is composed of large, isodiametric cells. Idioblasts containing druses are distributed below the adaxial epidermis. The secretory cavities are schizolysigenous and are mainly located below the adaxial epidermis. The shape for the vascular system is circular. The adaxial phloem is scarce and continuous (without partitions). The adaxial and abaxial phloem is confluent. There are no fibres around the vascular system.

8. *Nothomyrcia* Kausel

Number of species in genus: 1

Species studied: *N. fernandeziana* (Reta-20.1/20.2, BRI).

The leaves are hypostomatic with anomocytic stomatal complexes. The epidermis is single-layered, mucilaginous and has a regular cuticle layer (3–5 μm thick). The adaxial epidermis is slightly thicker than the abaxial epidermis. The epidermal cells are isodiametric with slightly sinuous and thin anticlinal walls on the abaxial surface. A hypodermis is observed under the adaxial epidermis. The leaves are glabrous to sparsely pubescent on midrib and margins. The hairs are simple and straight-curved. The mesophyll is dorsiventral with two-three layers of palisade parenchyma. The spongy parenchyma is composed of large, irregularly shaped cells. Idioblasts containing druses are distributed below the adaxial epidermis. The secretory cavities are schizogenous and are mainly located below the adaxial epidermis. The shape of the vascular system is ellipsoidal. The adaxial phloem is abundant with a weak partition. Xylem and fibers surrounds two islands of adaxial phloem. The adaxial and abaxial phloem is not confluent. Fibres form a continuous ring around the vascular system.

9. *Ugni* Turcz. (Figs. 1L, 2G, 3K,M, 4G, 5F, 6C, 8L)

Number of species in genus: 4

Species studied: *U. candollei* (Reta-14.1/14.2, BRI), *U. molinae* (Reta-04.1/04.2, BRI), *U. selkirkii* (CONC 116,898, CONC).

The leaves are hypostomatic with anomocytic stomatal complexes in *U. molinae* and *U. selkirkii*, but anisocytic (and anomocytic) in *U. candollei*. The epidermis is single-layered, mucilaginous and has a regular cuticle layer (3–5 μm thick). The adaxial epidermis is slightly thicker than the abaxial epidermis. The epidermal cells are isodiametric with slightly sinuous and thin anticlinal walls. A hypodermis is observed under the adaxial epidermis in *U. candollei*. The leaves are glabrous to sparsely pubescent on midrib and margins in *U. molinae* and *U. selkirkii*. *Ugni candollei* have sparse to moderately pubescent leaves particularly on midribs. The hairs are simple and straight-curved in *U. molinae* and *U. selkirkii*, while *U. candollei* also has dibrachiate hairs. The mesophyll is dorsiventral with two-three layers of palisade parenchyma in *U. candollei and U. selkirkii*, while in *U. molinae* three layers are observed. The spongy parenchyma is composed of small, irregularly shaped cells. Idioblasts containing druses are observable below the adaxial epidermis in *U. candollei* and *U. molinae*, but also occur around the vascular system in *U. selkirkii.* Rhombohedral crystals are observed in *U. selkirkii*. The secretory cavities are schizogenous and mainly located below the adaxial epidermis. The vascular system is arc-shaped with strong curvature in *U. molinae* and *U. selkirkii*, while it is circular in *U. candollei*. The adaxial phloem has a medium development and has a strong partition in *U. candollei* and *U. molinae*, while there is a weak partition in *U. selkirkii*. The adaxial and abaxial phloem is not confluent in *U. candollei* and *U. molinae,* but it is confluent in *U. selkirkii*. Fibres are discontinuous around the vascular system.

10. *Tepualia* Griseb (Figs. 1K, 2L, 4F, 8K).

Number of species in genus: 1

Species studied: *T. stipularis* (Reta-06.1/06.2, BRI).

The leaves are hypostomatic with anomocytic stomatal complexes. The transverse section of the leaf is ellipsoid-shaped, The epidermis is single-layered, mucilaginous and has a thin cuticle layer (3 μm thick or less). The adaxial epidermis is thicker than the abaxial epidermis. The epidermal cells are isodiametric with slightly sinuous and thin anticlinal walls on the abaxial surface. The leaves are glabrous to sparsely pubescent on midrib and margins. The hairs are simple and straight-curved. The mesophyll is dorsiventral with two-three layers of palisade parenchyma. The spongy parenchyma is composed of large, irregularly shaped cells. Crystals were not found in the species. The secretory cavities are schizolysigenous and are mainly located below the adaxial epidermis. The shape of the vascular system is circular. The adaxial phloem is scarce, with a weak partition. The adaxial and abaxial phloem is not confluent. Fibres are discontinuous around the vascular system, forming a prominent plate under the abaxial phloem.

3.3. Identification key

The following identification key is based on leaf morpho-anatomical characters for genera and species of Chile*c* species not included).

1. Amphistomatic leaves……*Myrteola nummularia*

1. Hypostomatic leaves……..2

2. Presence of domatia on abaxial surface……..*Legrandia concinna*

2. Absence of domatia on abaxial surface……3

3. Transverse section of leaf ellipsoid-shaped, no crystals in leaves……...*Tepualia stipularis*

3. Transverse section of leaf other than above, crystals in leaves ……4

4. Leaves with a pronounced parenchymatous swelling over midrib……..5

4. Leaves with depression above midrib……...6

5. Hypodermis present, adaxial xylem surrounding two islands of phloem……..*Nothomyrcia fernandeziana*

5. Hypodermis absent, adaxial phloem surrounding two islands of xylem……*Blepharocalyx cruckshanksii*

6. Leaves with paracytic stomata……7 (*Luma*)

6. Leaves with stomata other than paracytic……8

7. Glandular hairs present, schizolysigenous cavities throughout mesophyll.....*Luma chequen*

7. Glandular hairs absent, shizogenous cavities under adaxial epidermis…….*Luma apiculata*

8. Arc-shaped vascular system……9

8. Shape of vascular system other than arc……10

9. Dibrachiate hairs present…… *Myrceugenia*

9. Dibrachiate hairs absent……..13

10. Laterocytic stomata, glandular hairs present, epidermis thick on both surfaces, epidermal cells with 1:1 size ratio…… *Myrcianthes coquimbensis*

10. Stomata other than laterocytic, glandular hairs absent, epidermis thin, usually thicker on the adaxial surface……...11

11 Hypodermis present, conical papillae present, anisocytic stomata……*Ugni candollei*

11. Hypodermis absent, papillae absent, anomocytic stomata ……12 (*Amomyrtus*)

12. Continuous adaxial phloem in vascular systems……*A. luma*

12. Partitioned adaxial phloem in vascular systems……*A. meli*

13. Druses under adaxial epidermis, strong adaxial phloem partition……*U. molinae*

13. Prismatic rhombohedral crystals around vascular system, weak adaxial phloem partition.....*U. selkirkii*

4. Discussion

A number of the leaf anatomical and micromorphological characters observed here can be used to identify genera or species. The anatomical results of this investigation largely agree with those for South American Myrtaceae in Fontenelle et al. (1994), Donato and Morretes (2009, 2011), Cardoso et al. (2009), Gomes et al. (2009) and Soh and Parnell (2011). Differences in some characters were observed and will be pointed out in this discussion. Potential links between anatomical characters and environmental conditions are also discussed.

4.1. Epidermis and indumentum

Here we have interpreted the hypodermis as a layer of large cells located below a single layer of smaller epidermal cells and mainly originated from the ground meristem (Martins et al., 2012). On the other hand, two or more layers of aligned cells and originated from the protodermis were considered a multiple epidermis (Sharma and Mehra, 1972; Dickison, 2000; Martins et al., 2012). The hypodermis and multiple epidermis are regarded as two non-homologous anatomical features, therefore ontogenetic observations are always recommended to avoid misinterpretations (Martins et al., 2012). The presence of a multiple epidermis or hypodermis has been considered an ecological adaptation of xerophytic plants to arid environments, which prevents water loss due to excessive evapotranspiration and protects the lamina from high solar radiation (Metcalfe and Chalk, 1979; Dickison, 2000; Evert, 2006). A single epidermis is commonly associated with mesophytic and hydrophytic species and is considered the normal type of epidermis in vascular plants (Dickison, 2000). The presence of a single epidermis has been reported for most species of the family Myrtaceae (Metcalfe and Chalk, 1979). Genera with

single-layered epidermis include *Eugenia* (Fontenelle et al., 1994; Donato and Morretes, 2009; Armstrong et al., 2012), *Myrcia*, *Campomanesia* (Gomes et al., 2009), *Callistemon*,*Eucalyptus*,*Melaleuca* (Tantawy, 2004), *Acmena*, *Syzygium*, *Heteropyxis*, and *Tristania* (Keating, 1984; Soh and Parnell, 2011). The presence of a hypodermis has been identified in *Campomanesia*, *Myrcianthes*, *Psidium* and *Pimenta* (Cardoso et al., 2009; Gomes et al., 2009). Cardoso et al. (2009) reported the presence of hypodermis in the Brazilian species *Myrceugenia euosma*. *M. rufa* is the only species of Chilean Myrtaceae with adaxial double epidermis and can be reliably identified using this anatomical character. The main habitat of *M. rufa* is the xeromorphic shrublands of north-central Chile, where rainfall is restricted to few days of the year (Serra et al., 1986). The presence of double epidermis in this species supports this ecological association. The occurrence of an adaxial hypodermis was observed only in *N. fernandeziana* and *U. candollei*, species that mainly occur in wet forests and open vegetation in humid regions of Chile. In this case, the presence of hypodermis might not be associated with a xerophytic habitat. *N. fernandeziana* is phylogenetically positioned within a clade that is closely related to the "*Pimenta* group" (Murillo et al., 2013), which includes genera known to have hypodermis, such as *Pimenta* and *Psidium* (Cardoso et al., 2009). Consequently, the presence of hypodermis in *Nothomyrcia*, *Pimenta* and *Psidium* could be due to phylogenetic history and not environment. As the systematic position of *U. candollei* is unknown, the presence of hypodermis cannot yet be linked to phylogenetic constraints.

Papillae have been reported as projections of the epidermal cells in some Myrtaceae, including South American species such as *Gomidesia nitida* and *M. euosma* (Metcalfe and Chalk, 1979; Cardoso et al., 2009). Here we observed papillae on the leaf surface of *L. concinna*, *M. correifolia*, *M. schulzei* and *U. candollei*, species that occur in distinct environments (mesophytic and xerophytic). The role of papillae needs more investigation, but might be related to plant defence against pathogens and herbivory (Voigt, 2014).

The anticlinal epidermal walls correspond to the outline of the primary walls between adjacent cells and depend on the cellulose microfibril organization and deposition (Panteris et al., 1993). Epidermal anticlinal walls have low intraspecific variation in Myrtaceae (Carr et al., 1971) and can be regarded as a taxonomically stable character (Pole, 2012). The shape of anticlinal epidermal walls is considered an environmental adaptation, as mesophytic species usually have sinuous walls while xerophytic have straight walls (Gifford and Foster, 1989). Fontenelle et al. (1994) have reported straight and thick epidermal anticlinal walls in xerophytic species of *Eugenia*. Our observations of the Chilean Myrtaceae support these environmental associations as those species occurring in xerophytic habitats (*M. correifolia*, *M. rufa*, *M. coquimbensis*) have straight anticlinal walls, while mesophytic species possess slightly sinuous or highly sinuous walls. Epidermal anticlinal walls (mainly abaxial) are a suitable character for delimiting a number of species of Chilean Myrtaceae.

The occurrence of hairs in plants is regarded as a xerophytic adaptation, especially when the hair covering is dense (Evert, 2006). Hairs extend the boundary layer in a leaf which creates a stable microclimate on the surface and reduces water losses due to excessive solar radiation (Ehleringer, 1985). Fontenelle et al. (1994) suggest that some xerophytic characters in Myrtaceae (straight anticlinal walls, hairs, waxes) are not strictly associated with environmental conditions, as species from different geographic zones and habitats, encompassing xerophytic and mesophytic habitats, possess these features. Leaves of Myrtaceae are often glabrous or possess scattered hairs on midribs and leaf blades (Wilson, 2011). Unicellular hairs are the main type of trichome present in Myrtaceae (Briggs and Johnson, 1979; Metcalfe and Chalk, 1979) and the only type found in Chilean species. Trichomes observed in Chilean Myrtaceae largely agree with the results reported by Landrum (1981, 1986, 1988a). Simple hairs are widely present in South American Myrtaceae (Cardoso et al., 2009; Gomes et al., 2009) and were observed here in *Amomyrtus*, *Legrandia*, *Luma*, *Myrcianthes*, *Myrteola*, *Nothomyrcia* and two species of *Ugni*. Dibrachiate hairs (armed biramous hairs) were observed in all the species of Chilean *Myrceugenia* and also in *U. candollei*. Most of the species of *Myrceugenia* are reported to possess dibrachiate hairs, as well as *Calyptranthes*, *Eugenia*, *Marlierea* and some species of *Myrcia* (Landrum and Kawasaki, 1997). The presence of glandular or secretory hairs is not widely reported in South American Myrtaceae. Secretory hairs have been reported on the abaxial leaf surface of the Brazilian species *M. euosma*, formed by papillose cells with thick cell walls (Cardoso et al., 2009). Wilson (2011) refers to infundibular hairs (funnel-shaped) in a group of South American *Eugenia*, but such hairs were not observed in any Chilean species. The dense layer of hairs on the abaxial leaf surface was observed in a number of Chilean species from arid environments (*M. coquimbensis*, *M. colchaguensis*, *M. correifolia* and *M. rufa*). Most of these species occur in coastal shrublands in the north-centre of Chile (Landrum, 1988a), where rainfall and humidity are much lower compared to the typical mesophytic habitat of Chilean Myrtaceae. *M. euosma* is a South American species that occurs in Mata Atlântica, States of São Paulo, Paraná, Santa Catarina and Rio Grande do Sul (Sobral et al., 2015) and that has been considered one of the most xerophytic species of the genus (Landrum, 1981). Although *M. euosma* resembles the xerophytic *M. rufa*, the first species has been reported to occur also in flooded environments (Cardoso et al., 2009). In order to confirm the consistency of some anatomical characters/character states related to ecological and environmental associations (e.g., hypodermis, epidermal anticlinal walls, hairs), comprehensive sampling of more populations is recommended. The phylogenetic position of the most pubescent species of Chilean *Myrceugenia* is either basal to the genus (*M. rufa*) or part of a monophyletic group near the base (*M. colchaguensis* + *M. schulzei*) (Murillo et al., 2013). In order to infer whether trichome characters have a common phylogenetic origin or are product of convergent evolution, further investigation is required.

4.2. Stomata

Although distribution of stomata and types of stomatal complexes are considered important for taxonomic delimitation, there are a number of different classifications, each with a particular terminology (Dressler, 1993). For a better understanding of stomatal complexes, ontogenetic studies are critically important (Carpenter, 2005; Pole, 2012). Developmental or ontogenetic studies are also necessary to find out if different types of mature stomata are homologous (Pole, 2012).

Amphistomatic leaves (stomata distributed on both abaxial and adaxial leaf surfaces) are commonly observed in hydrophytes and creeping species from wet habitats (Evert, 2006; Gifford and Foster, 1989). The presence of amphistomatous leaves in *M. nummularia* suggests an environmental correlation with the habitat of the species. In Chile, *M. nummularia* is mainly a creeping shrub or subshrub that occurs in wet habitats such as swamps, peatlands and the lower strata of humid forests (Landrum, 1988b).

Eugenia is one of the most widely studied genera of Myrtaceae and paracytic stomatal complexes the most common type in the genus (Hussin et al., 1992; Fontenelle et al., 1994; Haron and Moore, 1996). Anomocytic stomata have also been reported as a common type at family level (Metcalfe and Chalk, 1979; Gomes et al., 2009). Paracytic stomata were observed only in the two species of the genus *Luma*, while the anomocytic type was observed in a number of genera (*Amomyrtus*, *Legrandia*, *Myrceugenia*, *Ugni*). The different

types of stomatal complexes observed in Chilean Myrtaceae can be used to some extent to delimit genera.

4.3. Mesophyll, crystals and secretory cavities

Dorsiventral (bifacial) mesophyll is the most common type of mesophyll in Myrtales and Myrtaceae (Keating, 1984 Wilson, 2011). Few genera, such as the Australasian *Corymbia*, *Eucalyptus, Leptospermum* and *Melaleuca,* species with vertically oriented leaves, have isobilateral mesophyll (Gomes et al., 2009 Wilson, 2011). All the Chilean Myrtaceae have dorsiventral mesophyll and the leaves are generally horizontally positioned. Mucilage and pectins were staned by ruthenium red as granules or red content in the mesophyll of all species, as indicated by Jensen (1962).

Crystals composed of calcium oxalate are the most common biomineral occurring in plants (Arnott, 1982). These structures have been related to the regulation of calcium activity in tissues (Volk et al., 2002), as well as protection against herbivores and pathogens (Franceschi and Nakata, 2005). Calcium oxalate crystals are widely present in Myrtaceae and have different shapes and structure (Metcalfe and Chalk, 1979). Druses are the most common type of crystal in Chilean Myrtaceae and have been also reported in *Eugenia, Gomidesia*, *Psidium* and *Myrcia*, among other South American genera (Cardoso et al., 2009; Gomes et al., 2009). Rhombohedral crystals observed in *M. colchaguensis*, *M. schulzei* and *U. selkirkii* are similar to those reported for the Australasian genus *Syzygium* (Soh and Parnell, 2011) and other South American genera, such as *Calyptranthes*, *Campomanesia*, *Gomidesia* and *Mosiera* (Cardoso et al., 2009).

Schizogenous secretory cavities are originated by separation of cells and are composed of a layer of epithelial cells surrounding a wide lumen space at maturity (Ciccarelli et al., 2008). Lysigenous secretory cavities arise by dissolution of cells and do not possess epithelial cells at maturity (Evert, 2006). Schizolysigenous cavities occur when cavities arise due to the separation of cells (schizogenous origin), but epithelial cells are dissolved at maturity by autolysis (Evert, 2006). Secretory cavities are mainly located adjacent to the adaxial and/or abaxial epidermis and are primarily protodermal in origin, with participation of the ground meristem (Fahn, 1979; Arruda and Fontenelle, 1994). The role of compounds produced by secretory cavities (mainly sesquiterpenes and flavonoids in Myrtaceae) has been associated to a number of plant functions. These roles are related to direct defence responses, metabolism of diverse chemicals (Banthorpe et al., 1972) and plant architecture, through inhibition of shoot branching (Akiyama et al., 2008). Secretory cavities are one of the most distinctive features of Myrtaceae (Wilson, 2011), and are often referred as oil dots in field guides and keys. Schizogenous secretory cavities are the most common type observed in Myrtaceae (Alves et al., 2008; Gomes et al., 2009; Donato and Morretes, 2011) and also in Chilean species. Schizolysigenous cavities were observed in a few Chilean species.

4.4. Vascular system

All species of Chilean Myrtaceae, other than *N. fernandeziana,* have been described as possessing leaves with impressed midribs (Landrum, 1988a). Anatomically, the pronounced swelling above the midrib of *N. fernandeziana* is composed of large and isodiametrical parenchymatous cells. *Blepharocalyx cruckshanksii* possess a slight swelling above the midrib, which is not usually reported in morphological descriptions of the species. Adaxial phloem in vascular system is regarded as a typical character in the order Myrtales (Cronquist, 1981) and is widely present in Myrtaceae (Schmid, 1980; Cardoso et al., 2009). Vascular system characters observed here, such as adaxial phloem partition, confluence of adaxial and abaxial phloem and sclerenchyma (fibres) around the vascular system, largely agree with what has been observed in other South American genera (Cardoso et al., 2009; Donato and Morretes, 2009; Gomes et al., 2009). These features are considered suitable characters to identify species in Myrtaceae (Cardoso et al., 2009; Soh and Parnell, 2011). *B. cruckshanksii* and *N. fernandeziana* are the only species of Chilean Myrtaceae with inwardly curved vascular tissues: phloem surrounding two islands of xylem in *B. cruckshanksii* and xylem surrounding phloem in *N. fernandeziana*. This anatomical character supports the close phylogenetic relationship suggested for these two species and the recognition of *Nothomyrcia*, as a separate genus distinct from *Myrceugenia* (Murillo and Ruiz, 2011; Murillo et al., 2013).

5. Conclusion

This is the first investigation that describes the leaf anatomy of the 26 species of Chilean Myrtaceae, including all the accepted species of a number of genera (*Amomyrtus*, *Legrandia*, *Luma*, *Tepualia*). Anatomical features described here largely agree with previous characters found in other Myrtaceae. Most of the species possess a typical mesophytic leaf anatomy, while others show a combination of xerophytic characters such as hairy leaves, hypodermis, thick adaxial epidermis and straight epidermal anticlinal walls. Anatomical and micromorphological characters described here have potential taxonomic, ecologic and phylogenetic significance. Yet, anatomical descriptions of other South American and Australasian genera of Myrteae are recommended in order to use these features in a broader taxonomic and evolutionary context. Further anatomical studies from additional populations are recommended in order to confirm the consistency of some characters at species level.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. : Species with taxonomic authority, vouchers, geographic locality and GPS coordinates of samples collected for this study

Taxon	Voucher (Herbarium)	Locality	Geographic coordinates	Habitat
Amomyrtus luma (Molina) D. Legrand and Kausel	Reta-07.1/07.2 (BRI)	Futrono	40° 7′ 28″ S/72° 22′ 51″ W	*Podocarpus-Nothofagus* forest
Amomyrtus meli (Phil.) D. Legrand and Kausel	Reta-25.1/25.2 (BRI)	Osorno	40° 34′ 0″ S/73° 9′ 0″ W	Closed *Nothofagus* forest
Blepharocalyx cruckshanksii (Hook. and Arn.) Nied.	Reta-24.1/24.2 (BRI)	Temuco	38° 44′ 0″ S/72° 36′ 0″ W	Swamp (“hualve”)
Legrandia concinna (Phil.) Kausel	Reta-09.1/09.2 (BRI)	Chillán	36° 36′ 0″ S/72° 7′ 0″ W	Open *Nothofagus* forest
Luma apiculata (DC.) Burret	Reta-26.1/26.2 (BRI)	Futrono	40° 7′ 28″ S/72° 22′ 51″ W	Closed *Nothofagus* forest
Luma chequen (Feuillée ex Molina) Gray	Reta-05.1/05.2 (BRI)	Los Vilos	31° 54′ 37″ S/71° 30′ 35″ W	Closed stream forest
Myrceugenia chrysocarpa (O.Berg) Kausel	Reta-01.1/01.2 (BRI)	Futrono	40° 7′ 28″ S/71° 5′ 50″ W	*Nothofagus* montane forest
Myrceugenia colchaguensis (Phil.) Navas	CONC 121491 (CONC)[a]	Colchagua	34° 40′ 34″ S/72° 22′ 51″ W	Sclerophyll forest
Myrceugenia correifolia (Hook. and Arn.) O.Berg	Reta-16.1/16.2 (BRI)	Pichidangui	32° 13′ 33″ S/71° 53′ 33″ W	Fog sclerophyll forest
Myrceugenia exsucca (DC.) O.Berg	Reta-11.1/11.2 (BRI)	Valdivia	39° 48′ 0″ S/73° 14′ 0″ W	Swamp (“hualve”)
Myrceugenia lanceolata (Juss. ex J. St.-Hil.) Kausel	Reta-22.1/22.2 (BRI)	Hualpén	36° 50′ 0″ S/73° 3′ 0″ W	Riparian forest
Myrceugenia leptospermoides (DC.) Kausel	Reta-12.1/12.2 (BRI)	Temuco	38° 44′ 0″ S/72° 36′ 0″ W	*Podocarpus* forest
Myrceugenia obtusa (DC.) O.Berg	Reta-19.1/19.2 (BRI)	Talcahuano	36° 43′ 0″ S/73° 7′ 0″ W	*M. obtusa* closed forest
Myrceugenia ovata (Hook. and Arn.) O.Berg	Reta-18.1/18.2 (BRI)	Puerto Montt	41° 28′ 18″ S/72° 56′ 12″ W	Evergreen mixed forest
Myrceugenia ovata var. nannophylla (Burret) L.R. Landrum	Reta-15.1/15.2 (BRI)	Neltume	39° 47′ 60″ S/71° 57′ 0″ W	Open *Nothofagus* forest
Myrceugenia parvifolia (DC.) Kausel	Reta-21.1/21.2 (BRI)	Puerto Montt	41° 28′ 18″ S/72° 56′ 12″ W	Evergreen mixed forest
Myrceugenia pinifolia (F. Phil.) Kausel	Reta-27.1/27.2 (BRI)	Laraquete	37° 9′ 45″ S/73° 10′ 52″ W	Riparian forest
Myrceugenia planipes (Hook. and Arn.) O.Berg	Reta-02.1/02.2 (BRI)	Futrono	40° 7′ 28″S/72° 22′ 51″ W	*Nothofagus* closed forest
Myrceugenia rufa (Colla) Skottsb. ex Kausel	Reta-10.1/10.2 (BRI)	Viña del Mar	33° 0′ 29″ S/71° 31′ 11″ W	Roadside sclerophyll bushland
Myrceugenia schulzei Johow	CONC 157850 (CONC)[a]	Masafuera, JF	33° 46′ 33″ S/80° 47′ 56″ W	*Myrceugenia* forest
Myrcianthes coquimbensis (Barnéoud) Landrum and Grifo	Reta-08.1/08.2 (BRI)	La Serena	29° 54′ 28″ S/71° 15′ 15″ W	Coastal shrubland
Myrteola nummularia (Poir.) O.Berg	Reta-03.1/03.2 (BRI)	Futrono	40° 7′ 28″ S/72° 22′ 51″ W	Peatland
Nothomyrcia fernandeziana (Hook. and Arn.) Kausel	Reta-20.1/20.2 (BRI)	Viña del Mar	29° 54′ 28″ S/71° 15′ 15″ W	Juan Fernandez plants Collection
Ugni candollei (Barnéoud) O.Berg	Reta-14.1/14.2 (BRI)	Puerto Montt	41° 28′ 18″ S/72° 56′ 12″ W	*Nothofagus*-Myrtaceae forest
Ugni molinae Turcz.	Reta-04.1/04.2 (BRI)	Futrono	40° 7′ 28″ S/72° 22′ 51″ W	Open *Nothofagus* forest
Ugni selkirkii (Hook. and Arn.) O.Berg	CONC 116898 (CONC)[a]	Masatierra, JF	33° 38′ 42″ S/78° 49′ 23″ W	*Myrceugenia* forest
Tepualia stipularis (Hook. and Arn.) Griseb.	Reta-06.1/06.2 (BRI)	Puerto Montt	41° 28′ 18″ S/72° 56′ 12″ W	*Tepualia* forest (tepual)

BRI: Queensland Herbarium.
CONC: University of Concepción Herbarium.
[a]Material obtained from herbarium specimens.

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