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 of 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.

Keywords: identification key - histochemistry- taxonomy - Valdivian forest - xeromorphic

1. INTRODUCTION

Myrtaceae Juss. (Myrtales; APGIII, 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 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 25º and 47º 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

100 *Eugenia* and shown to be important for taxonomic purposes (Fontenelle et al., 1994; Haron
101 and Moore, 1996).
102
103 The leaf anatomy and micromorphology of the Chilean Myrtaceae has not been documented
104 in much detail (P.G. Wilson, pers. comm.), other than a few species, namely *Luma apiculata*,
105 *Myrceugenia parvifolia* (Retamales and Scharaschkin, 2014) and *Ugni molinae* (Retamales et
106 al., 2014). There has never been a comprehensive study of the Chilean Myrtaceae other than
107 taxonomic revisions based on gross morphological characters (Kausel, 1942; Landrum, 1981,
108 1986, 1988a; McVaugh, 1968; Reiche, 1897). The Chilean Myrtaceae show high variation in
109 gross morphology of leaves between species (Fig. 1) and also within same species, which
110 precludes diagnosis and species identification (McVaugh, 1968). A complete anatomical
111 investigation of these taxa could provide relevant information by identifying reliable
112 characters with taxonomic and ecologic significance. In this investigation we present the
113 outcome of extensive research on the anatomical and micromorphological characters of all
114 the species of Myrtaceae occurring in Chile.
115
116 **2. MATERIAL AND METHODS**
117 2.1 MATERIAL EXAMINED
118 All 26 species of Chilean Myrtaceae were examined in this study. Wherever possible, fresh
119 leaf material was collected but in a few cases herbarium specimens (CONC) were used.
120 Sampling was conducted between January 2006 and February 2014 and included a number of
121 different natural populations in Chile. Mature leaves were randomly sampled from sun-
122 exposed branches from a number of typical and healthy individuals. Young leaves were also
123 collected as trichomes and certain other structures are reported to be early caduceus
124 (Landrum 1988a). Young leaves were also used to describe early ontogenetic stages of

125 secretory cavities and epidermis. Fresh leaf material was fixed in formalin-acetic acid-alcohol
126 (FAA) for 24-48 h depending upon the thickness of the leaves and subsequently stored in
127 70% ethanol. Herbarium specimens were rehydrated in boiling water for 10 min to recover
128 the leaf shape before being fixed in FAA (Haron and Moore, 1996). Herbarium accessions
129 are currently housed in the Queensland Herbarium, Brisbane, Australia (BRI) with duplicates
130 in the Forestry Sciences Herbarium, University of Chile (EIF). Details about specimens
131 studied, vouchers, localities and habitat are presented in the Appendix 1.
132
133 2.2 SCANNING ELECTRON MICROSCOPY (SEM)
134 Leaf material fixed in FAA was dehydrated using a graded ethanol series and then critical
135 point dried (Anderson, 1951) in an Autosamdri-815 automatic critical point drier (Tousimis,
136 Rockville, USA). Samples were mounted on stubs with self-adhesive double-sided carbon
137 discs and sputter-coated with gold palladium for 175 sec using a Leica EM SCD005 Gold
138 Coater (Leica Microsystems, Macquarie Park, NSW, Australia). Examination and
139 documentation of images was conducted using a FEI Quanta 200 SEM/ESEM (FEI,
140 Hillsboro, Oregon, USA) operated at 10kV.
141
142 2.3 LIGHT MICROSCOPY (LM)
143 FAA-fixed material was dehydrated through a graded ethanol series and embedded in
144 paraffin wax (Johansen, 1940; Ruzin, 1999). Transverse sections of leaves were cut using a
145 Leica RM2245 rotary microtome (Leica Microsystems, Macquarie Park, NSW, Australia) at
146 5µm. Staining of sections was performed using the stains ruthenium red (0.05% aqueous
147 solution), toluidine blue (TBO) (0.1% aqueous solution), safranin O (1% alcoholic solution)
148 and alcian blue, alone or combined according to standard staining protocols (Ruzin, 1999;
149 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 1µl of acetic acid and 1µl 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 *Nothomyrcia 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, 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 1; therefore these have been omitted from the text henceforth.

174 The five types of stomatal complexes studied here were anomocytic, paracytic, actinocytic,
175 anisocytic and laterocytic. When more than one type of stomatal complex was identified in
176 some species, the less frequent type is indicated in parentheses (Table 1). The description and
177 interpretation of the different stomatal types in Chilean Myrtaceae are as follows: (1)
178 Anomocytic: the guard cells are surrounded by unspecialized subsidiary cells without any
179 consistent pattern and are indistinguishable in shape from other epidermal cells. (2) Paracytic:
180 the guard cells are surrounded by two subsidiary cells, which are relatively specialized. These
181 two cells are normally parallel with the long axis of the guard cells and are generally similar
182 in size. (3) Actinocytic: the guard cells are surrounded by four or more, usually radially
183 elongated, subsidiary cells. (4) Anisocytic: the guard cells are surrounded by three cells that
184 are usually unequal in size. One of the three cells is usually much smaller than the other two.
185 (5) Laterocytic: the guard cells are surrounded by six irregularly shaped subsidiary cells.
186
187 In order to reliably identify different types of secretory cavities, we observed ontogenetic
188 stages in young leaves. Secretory cavities initially formed by dissolution of cells are
189 classified as lysigenous, while those formed by initial separation of epithelial cells are
190 classified either as schizogenous or schizolysigenous (Cicarelli et al. 2008). Multiple layers
191 of epidermis are classified as hypodermis or multiple epidermis depending upon ontogenetic
192 development of this character (Martins et al. 2012).
193
194 Terminology for describing leaf micromorphology (mainly stomata) was based on previous
195 descriptions of van Wyk et al. (1982), Fontenelle et al., (1994), Haron and Moore (1996) and
196 Soh and Parnell (2011). Terminology for leaf anatomy was based on Schmid (1980), Schmid
197 and Baas (1984), Keating (1984), Cardoso et al., (2009), Soh and Parnell (2011) and

198 Retamales et al. (2014). Other general references consulted for anatomical terminology were
199 Gifford and Foster (1989), Dickison (2000), Evert (2006) and Pole (2012).
200
201 **3. RESULTS**
202 The results will be presented in three parts: (1) A survey of the leaf anatomical and
203 micromorphological characters, (2) a synopsis of the leaf anatomy of each genus and (3) an
204 identification key of Chilean species of Myrtaceae using anatomical and micromorphological
205 characters. Leaf characters are summarized in Table 1 and Table 2.
206
207 3.1 SURVEY OF LEAF CHARACTERS
208 *3.1.1 Epidermis, cuticle and epicuticular waxes*
209 Different types of anticlinal walls of abaxial epidermal cells are observed. The most common
210 type is the slightly sinuous with thin walls, present in the majority of taxa. Some species
211 possess sinuous cell walls (Figs 2A, 2B, 2C) while others have straight and thick walls (Fig.
212 2D). Adaxial epidermal cells have straight or straight-sinuous anticlinal walls in all cases.
213
214 The epidermal cell walls are mucilaginous (evidenced by test with ruthenium red), single
215 layered in most of the species (Fig. 3A) and generally thicker on the adaxial side of the leaf.
216 The species *Myrceugenia correifolia*, *Myrceugenia obtusa* and *Myrcianthes coquimbensis*
217 possess a very thick adaxial epidermis, sometimes with a diffuse second layer beneath.
218 Adaxial epidermal cells have thin primary cell walls and are plano-convex and mainly
219 isodiametric in shape in the majority of taxa. Some species have enlarged-rectangular
220 epidermal cells. Both species of *Luma* have isodiametric and enlarged-rectangular epidermal
221 cells distributed equally on the adaxial surface. *Myrceugenia colchaguensis* possesses
222 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 *Myrceugenia 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 *Myrceugenia 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. *Myrceugenia obtusa* and *Myrcianthes 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 (3µm or less) in a majority of the species, but in some (such as *Myrceugenia correifolia*, *Myrceugenia rufa* and *Myrcianthes 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 (Figs 4G, 4H, 4I). Anomocytic stomata were

248 observed in *Amomyrtus spp.*, *B. cruckshanksii*, *L. concinna*, most of the *Myrceugenia* species,
249 *U. molinae*. and *U. selkirkii* (Fig. 2E). Paracytic stomata were observed in in *L. apiculata* and
250 *L. chequen* (Fig. 2F). Actinocytic stomata are common in *Myrceugenia colchaguensis.*
251 Anisocytic stomata are the most common type in *U. candollei* (Fig. 2G). Laterocytic stomata
252 are common in *Myrcianthes coquimbensis* (Fig. 2H). In transverse section, differences in the
253 shape of guard cells and the degree of cutinized thickenings on the outer periclinal cell walls
254 of guard cells can be observed. Guard cells are triangular and have cutinized thickenings of
255 outer periclinal walls in some species (*Myrceugenia lanceolata* and *Myrceugenia planipes*
256 (Fig. 3E)). Ovate guard cells without cutinized thickenings were observed in *L. chequen* (Fig.
257 3F), while *L. apiculata* shows ovate guard cells with heavy cutinized thickenings (Fig. 3G).
258 Irregular thickenings were observed in *U. selkirkii* (Fig. 3H).
259
260 *3.1.3 Indumentum*
261 The majority of the species have sparsely pubescent leaves on both adaxial and abaxial
262 surfaces (Fig. 5A). The leaves in most of the species become glabrescent with age. Only two
263 species (*T. stipularis* and *B. cruckshanksii)* have completely glabrous leaves, where hairs
264 were not observed in either young or mature leaves. Four species, namely *U. candollei* (Fig.
265 5F), *Myrceugenia correifolia* (Fig. 5B), *Myrceugenia exsucca* and *Myrceugenia planipes*,
266 have sparse to moderately pubescent indument, particularly on the abaxial surface. Abaxially
267 lanate (densely hairy) leaves were observed in *Myrceugenia colchaguensis*, *Myrceugenia*
268 *rufa* (Fig. 5C) and *Myrceugenia schulzei* (Fig. 5E). Abaxially and adaxially lanate leaves
269 were observed in *Myrcianthes coquimbensis* (Fig. 5D).
270
271 Three types of unicellular hairs were observed: simple (straight, curved, hooked, twisted or
272 ciliate) (Figs 6A, 6C), dibrachiate (symmetrically or asymmetrically dibrachiated) (Fig. 6B)

and glandular (Figs 6D, 6E, 6F). Simple hairs are observed in *L. concinna*, *Myrcianthes coquimbensis*, *Myrteola 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*, *Myrceugenia colchaguensis, Myrceugenia obtusa* and *Myrcianthes coquimbensis.* A distinctive staining reaction to TBO is detected around some glandular hairs of *M. obtusa,* which probably indicated the presence of sesquiterpenes.

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 *Myrceugenia parvifolia* (Fig. 7A) to three distinct layers in *Myrceugenia lanceolata* (Fig. 7B) and *L. concinna* (Fig. 7C). Four compressed layers were observed in *Myrceugenia 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 *Myrcianthes coquimbensis* (Fig. 7F) and *U. selkirkii* is rich in tannins and polyphenols. *Legrandia 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

298 Intracellular crystals are present in most of the species. Two main types of crystals were
299 found, namely druses (aggregated individual crystals) and prismatic crystals (rhombohedral
300 and spherical). Crystals were dissolved after testing with acetic acid and hydrochloric acid,
301 discarding silica composition. Differential solubility indicates that crystals are composed of
302 CaOx (calcium oxalates). Druses are mainly contained in idioblasts and present in the
303 palisade parenchyma below the adaxial epidermis (Fig. 3J). In some species, druses are also
304 present around the leaf phloem and contained in bundle sheath cells. *Myrceugenia*
305 *colchaguensis, Myrceugenia schulzei* and *U. selkirkii* exhibit prismatic rhombohedral
306 crystals, mainly around the vascular system (Fig. 3K). Two species (*Myrceugenia*
307 *chrysocarpa* and *Myrceugenia planipes*) possess spherical crystals located below the
308 epidermis and also throughout the spongy parenchyma (Fig. 3I). Idioblasts with druses are
309 mainly solitary or occur in pairs, however in some species (e.g., *L. concinna*) several
310 idioblasts are grouped together (Fig. 3L). Druses were not observed in the leaves of *T.*
311 *stipulari*s and appear to be rare in *Myrcianthes coquimbensis.*
312
313 *3.1.6 Secretory cavities*
314 Leaf secretory cavities are generally located in the palisade parenchyma, usually in contact
315 with the adaxial epidermis (Fig. 3O) but in some species they are located below both adaxial
316 and abaxial surfaces. In young leaves, all cavities are initially formed by separation of
317 epithelial cells (Fig. 3M), which confirms that secretory cavities in Chilean Myrtaceae are not
318 lysigenous (cavities formed by dissolution of cells). Species have either schizogenous or
319 schizolysigenous cavities (a mixture of schizogenous and lysigenous cavities). In early
320 developmental stages, epithelial cells of schizogenous cavities are small, isodiametrical and
321 have very thin primary cell walls (Fig. 3M). At maturity, schizogenous cavities have a layer
322 of epithelial cells surrounding a wide lumen, while schizolysigenous cavities only have a

323 lumen without secretory epithelial cells. Epithelial cells in schizolysigenous cavities have
324 collapsed at some developmental stage and show secretions around the lumen. Secretory
325 cavities are schizogenous in most of the species (e.g., *Amomyrtus* spp., *Myrcianthes*
326 *coquimbensis*, *Myrceugenia* spp., *T. stipularis*, *Ugni* spp.) (Fig. 3N) and schizolysigenous in
327 others (e.g., *B. cruckshanksii*, *L. chequen*, *Myrteola nummularia,*) (Figs 3O, 3P). A number
328 of species (e.g., *L. chequen*, *Myrcianthes coquimbensis*) have additional secretory cavities
329 throughout palisade and spongy parenchyma (Fig. 3P). In surface view, two overlying cells
330 (epidermal cells above secretory cavities) can be observed. These cells vary in shape and are
331 surrounded by a variable number of epidermal cells (Figs 2I, 2J, 2K, 2L). The cavities and
332 overlying cells can be clearly differentiated as polyhedral in shape in *Myrceugenia exsucca*
333 (Fig. 4B) and *Myrceugenia leptospermoides* (Fig. 4A). The overlying cells are barely visible
334 in *Ugni* spp. and *Myrteola nummularia* (Fig. 4C). Histochemical reaction with Sudan IV
335 suggests the presence of lipophilic substances in the epithelial cells lining the cavity.
336 Extrafloral nectaries are observed on the adaxial surface of *Myrceugenia planipes* (Fig. 4E)
337 and *T. stipularis* (Fig. 4F).
338
339 *3.1.7 Vascular system*
340 Most taxa have a flattened or slightly grooved adaxial leaf surface above the vascular region,
341 but some species possess a noticeable depression (e.g., *L. concinna*, *Myrceugenia exsucca*,
342 *Myrceugenia planipes*, *Ugni molinae*). A prominent swelling on the adaxial side of the leaf
343 over the main vascular system is observed in *B. cruckshanksii* and *N. fernandeziana,* species
344 that morphologically do not have impressed midribs as the remaining Chilean Myrtaceae.
345 The vascular system occupies the half of the lamina in cross section in almost all the species,
346 but it is particularly small in *Myrceugenia rufa* (Fig. 8H), *Myrteola nummularia* (Fig. 8J), *T.*
347 *stipularis* (Fig. 8K) and *U. candollei*. The shape of vascular systems varies from circular

(*Myrceugenia chrysocarpa, Myrcianthes coquimbensis, Myrteola nummularia, T. stipularis* Figs 8E, 8I, 8J, 8K) to arc-shaped vascular systems (e.g., *Myrceugenia correifolia*, *Myrceugenia planipes*, *U. molinae* and *U. selkirkii*- Figs 8G, 8L). 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 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 (Figs 8A, 8C). In some species, the adaxial phloem has a weak partition and there is confluence between the adaxial and abaxial phloem (e.g., *Myrceugenia correifolia*, *Myrceugenia exsucca*, *Myrceugenia 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., *Myrceugenia chrysocarpa*, *Myrceugenia obtusa* (Figs 8D, 8E, 8F). Species with a strong adaxial partition and without adaxial-abaxial confluence (vascular system with open extremities) include *Myrceugenia rufa*, *Myrcianthes coquimbensis*, *N. fernandeziana*, *U. candollei* and *U. molinae* (Figs 8H, 8I, 8L). 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*, *Myrceugenia chrysocarpa*, *Myrceugenia rufa*, *Myrteola nummularia* and *T. stipularis* the adaxial phloem is scarce, unlike the majority of taxa, which have

373 abundant and well developed adaxial phloem (Fig. 8). The vascular system of *B.*
374 *cruckshanksii* has the adaxial phloem curved inward forming two isolated groups of xylem
375 surrounded by adaxial phloem (Fig. 8B), while in the case of *N. fernandeziana* the xylem
376 surrounds two islands of adaxial phloem. The latter can be classified as a vascular system
377 with adaxial phloem with weak partition and without adaxial and abaxial phloem confluence.
378 Sclerenchyma fibres form a continuous ring around the vascular system in the majority of
379 species (Fig. 8), but they are discontinuous and form an abaxial arc in *A. meli*, *Myrceugenia*
380 *colchaguensis* and *Myrceugenia schulzei.* There are no fibres around the vascular system of
381 *Myrteola nummularia* (Fig. 8J) and they are very abundant around the midri of *Myrcianthes*
382 *coquimbensis* (Fig. 8I). Histochemical reaction to phloroglucinol+ 20% HCl was observed in
383 all sclerenchymatous tissues, especially fibres in the vascular system.
384
385 **3.2 SYNOPSIS OF LEAF ANATOMICAL CHARACTERS IN GENERA OF**
386 **CHILEAN MYRTACEAE**
387 The following section is a synopsis of the salient anatomical and micromorphological
388 characters of each genus of Chilean Myrtaceae. For each genus, the species studied are
389 indicated, as well as the total number of accepted species for that genus. Vouchers and
390 herbarium are indicated in parentheses. In this summary, only those characters that were
391 present have been included and the absence of characters is only reported in cases where our
392 observation contradict those already published.
393
394 1. *Amomyrtus* (Burret) D.Legrand and Kausel (Figs 1A, 2A, 3F, 3L, 5G, 6A, 7A).
395 Number of species in genus: Two
396 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 (3µm 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, 2I, 7B).

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 (3-5µm 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, 2N, 3H, 7C).

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 (3-5µm 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, 3B, 3C, 3I, 3P, 5I, 7D).

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 (3-5µm thick). The adaxial epidermis is

447 slightly thicker than the abaxial epidermis. The epidermal cells are isodiametric with slightly
448 sinuous and thin anticlinal walls. The leaves are glabrous to sparsely pubescent on midrib and
449 margins. The hairs are simple and straight-curved in *L. apiculata*, while *L. chequen* also has
450 glandular hairs on both surfaces. The mesophyll is dorsiventral with two-three layers of
451 palisade parenchyma in *L. apiculata* and two layers in *L. chequen*. The spongy parenchyma is
452 composed of small, isodiametric cells. Idioblasts containing druses are distributed only below
453 the adaxial epidermis. The secretory cavities are schizogenous and mainly located below the
454 adaxial epidermis in *L. apiculata* and schizolysigenous and located throughout the mesophyll
455 in *L. chequen*. The shape of the vascular system is ellipsoidal. The adaxial phloem is
456 abundant with a strong partition in *L. apiculata* and scarce with a weak partition in *L.*
457 *chequen*. The adaxial and abaxial phloem is confluent in *L. apiculata* and not confluent in *L.*
458 *chequen*. Fibres are discontinuous around the vascular system.
459
460 5. *Myrceugenia* O.Berg (Figs 1E, 1F, 1G, 1H, 2K, 3A, 3E, 3K, 3N, 4A, 4B, 4E, 4H, 4I, 5A,
461 5B, 5C, 5E, 5H, 6B, 6D, 6F, 7F, 7G, 7H).
462 Number of species in genus: ca. 40
463 Species studied: *M. chrysocarpa* (Reta-01.1/01.2, BRI), *M. colchaguensis* (CONC 121491,
464 CONC), *M. correifolia* (Reta-16.1/16.2, BRI), *M. exsucca* (Reta-11.1/11.2, BRI), *M.*
465 *lanceolata* (Reta-22.1/22.2, BRI), *M. leptospermoides* (Reta-12.1/12.2, BRI), *M. obtusa*
466 (Reta-19.1/19.2, BRI), M. ovata (Reta-18.1/18.2, BRI), *M. ovata* var. *nanophylla* (Reta-
467 15.1/15.2, BRI), *M. parvifolia* (Reta-21.1/21.2, BRI), *M. pinifolia* (Reta-27.1/27.2, BRI), *M.*
468 *planipes* (Reta-02.1/02.2, BRI), *M. rufa* (Reta-10.1/10.2, BRI), *M. schulzei* (CONC 157850,
469 CONC).
470 The leaves are hypostomatic in all species. The stomatal complexes are anomocytic in most
471 of the species, but actinocytic (and anomocytic) in *M. colchaguensis*. The epidermis is single-

472 layered in all the species except for the adaxial epidermis of *M. rufa*, where the epidermis is
473 double-layered. The epidermis is mucilaginous and has a regular cuticle layer (3-5µm thick)
474 in most of the species, but thick (>5µm, up to 8µm) in *M. correifolia* and *M. rufa*. The
475 adaxial epidermis is slightly thicker than the abaxial in most of the cases but thicker and
476 equally thick in *M. obtusa*. The epidermal cells are isodiametric with highly sinuous and thin
477 anticlinal walls on the abaxial surface in most of the species but highly sinuous in *M.*
478 *pinifolia* and straight and thick in *M. colchaguensis*, *M. correifolia* and *M. rufa.* Conical
479 papillae present on the abaxial surface of *M. correifolia* and *M. schulzei*. All the species
480 possess dibrachiated hairs. Simple hairs were observed in *M. lanceolata*, *M. leptospermoides*,
481 *M. obtusa*, *M. ovata*, *M. parvifolia* and *M. pinifolia*. Glandular hairs were only seen in *M.*
482 *colchaguensis* and *M. obtusa*. The mesophyll is dorsiventral with two-three layers of palisade
483 parenchyma, except for *M. parvifolia* that possess only one layer. The spongy parenchyma
484 has isodiametric or irregularly shaped cells, with abundant intercellular spaces in most
485 species and scarce in *M. correifolia* and *M. rufa*. Idioblasts containing druses are observable
486 below the adaxial epidermis in most species, but also occur around the vascular system in *M.*
487 *colchaguensis* and *M. schulzei.* Most of the species have druses, but spherical crystals are
488 observed in *M. chrysocarpa* and rhombohedral in *M. colchaguensis* and *M. schulzei*. The
489 secretory cavities are shizogenous in all the species, except for *M. correifolia*, *M. obtusa* and
490 *M. ovata*, which have shizolysigenous cavities. The vascular system is arc-shaped in all the
491 species other than *M. chrysocarpa* in which it is circular. The adaxial phloem is either scarce
492 or abundant and the partition weak or strong depending upon species (Table 2). The adaxial
493 and abaxial phloem is confluent in most of taxa, but not confluent in *M. lanceolata*, *M. ovata*,
494 *M. rufa* and *M. schulzei*. Fibres are discontinuous around the vascular system.
495
496 6. *Myrcianthes* Berg (Figs 1I, 2D, 5D, 6E, 7I).

497 Number of species in genus: 30

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

499

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

512

513 7. *Myrteola* Berg (Figs 1J, 2C, 3O, 4C, 5D, 7J).

514 Number of species in genus: 3

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

516

517 The leaves are amphistomatic with anomocytic stomatal complexes. The epidermis is single-
518 layered, mucilaginous and has a thin cuticle layer (3µm thick or less). The adaxial epidermis
519 is thicker than the abaxial epidermis. The epidermal cells are isodiametric with highly
520 sinuous and thin anticlinal walls on the abaxial surface. The leaves are glabrous to sparsely
521 pubescent on midrib and margins. The hairs are simple and straight-curved. The mesophyll is

522 dorsiventral with two-three layers of palisade parenchyma. The spongy parenchyma is
523 composed of large, isodiametric cells. Idioblasts containing druses are distributed below the
524 adaxial epidermis. The secretory cavities are schizolysigenous and are mainly located below
525 the adaxial epidermis. The shape for the vascular system is circular. The adaxial phloem is
526 scarce and continuous (without partitions). The adaxial and abaxial phloem is confluent.
527 There are no fibres around the vascular system.
528
529 8. *Nothomyrcia* Kausel
530 Number of species in genus: 1
531 Species studied: *N. fernandeziana* (Reta-20.1/20.2, BRI).
532
533 The leaves are hypostomatic with anomocytic stomatal complexes. The epidermis is single-
534 layered, mucilaginous and has a regular cuticle layer (3-5µm thick). The adaxial epidermis is
535 slightly thicker than the abaxial epidermis. The epidermal cells are isodiametric with slightly
536 sinuous and thin anticlinal walls on the abaxial surface. A hypodermis is observed under the
537 adaxial epidermis. The leaves are glabrous to sparsely pubescent on midrib and margins. The
538 hairs are simple and straight-curved. The mesophyll is dorsiventral with two-three layers of
539 palisade parenchyma. The spongy parenchyma is composed of large, irregularly shaped cells.
540 Idioblasts containing druses are distributed below the adaxial epidermis. The secretory
541 cavities are schizogenous and are mainly located below the adaxial epidermis. The shape of
542 the vascular system is ellipsoidal. The adaxial phloem is abundant with a weak partition.
543 Xylem and fibers surrounds two islands of adaxial phloem. The adaxial and abaxial phloem is
544 not confluent. Fibres form a continuous ring around the vascular system.
545
546 9. *Ugni* Turcz. (Figs 1L, 2G, 3D, 3G, 3J, 3M, 4G, 5F, 6C, 7L)

547 Number of species in genus: 4

548 Species studied: *U. candollei* (Reta-14.1/14.2, BRI), *U. molinae* (Reta-04.1/04.2, BRI), *U.*

549 *selkirkii* (CONC 116898, CONC).

550

551 The leaves are hypostomatic with anomocytic stomatal complexes in *U. molinae* and *U.*

552 *selkirkii*, but anisocytic (and anomocytic) in *U. candollei*. The epidermis is single-layered,

553 mucilaginous and has a regular cuticle layer (3-5µm thick). The adaxial epidermis is slightly

554 thicker than the abaxial epidermis. The epidermal cells are isodiametric with slightly sinuous

555 and thin anticlinal walls. A hypodermis is observed under the adaxial epidermis in *U.*

556 *candollei*. The leaves are glabrous to sparsely pubescent on midrib and margins in *U. molinae*

557 and *U. selkirkii*. *Ugni candollei* have sparse to moderately pubescent leaves particularly on

558 midribs. The hairs are simple and straight-curved in *U. molinae* and *U. selkirkii*, while *U.*

559 *candollei* also has dibrachiate hairs. The mesophyll is dorsiventral with two-three layers of

560 palisade parenchyma in *U. candollei and U. selkirkii*, while in *U. molinae* three layers are

561 observed. The spongy parenchyma is composed of small, irregularly shaped cells. Idioblasts

562 containing druses are observable below the adaxial epidermis in *U. candollei* and *U. molinae*,

563 but also occur around the vascular system in *U. selkirkii.* Rhombohedral crystals are observed

564 in *U. selkirkii*. The secretory cavities are schizogenous and mainly located below the adaxial

565 epidermis. The vascular system is arc-shaped with strong curvature in *U. molinae* and *U.*

566 *selkirkii*, while it is circular in *U. candollei*. The adaxial phloem has a medium development

567 and has a strong partition in *U. candollei* and *U. molinae*, while there is a weak partition in *U.*

568 *selkirkii*. The adaxial and abaxial phloem is not confluent in *U. candollei* and *U. molinae,* but

569 it is confluent in *U. selkirkii*. Fibres are discontinuous around the vascular system.

570

571 10. *Tepualia* Griseb (Figs. 1K, 2L, 4F, 7K).

572 Number of species in genus: 1

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

574

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

587

588 **3.3 IDENTIFICATION KEY**

589 The following identification key is based on leaf morpho-anatomical characters for genera
590 and species of Chilean Myrtaceae (*Myrceugenia* species not included).

591

592 1. Amphistomatic leaves…….……..……***Myrteola nummularia***
593 1. Hypostomatic leaves……..2
594 2. Presence of domatia on abaxial surface……..***Legrandia concinna***
595 2. Absence of domatia on abaxial surface…….…….3
596 3. Transverse section of leaf ellipsoid-shaped, no crystals in leaves……....***Tepualia stipularis***
597 3. Transverse section of leaf other than above, crystals in leaves …….……4
598 4. Leaves with a pronounced parenchymatous swelling over midrib……..……..5
599 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.

631 Potential links between anatomical characters and environmental conditions are also
632 discussed.
633
634 *4.1 Epidermis and indumentum*
635 Here we have interpreted the hypodermis as a layer of large cells located below a single layer
636 of smaller epidermal cells and mainly originated from the ground meristem (Martins et al.,
637 2012). On the other hand, two or more layers of aligned cells and originated from the
638 protodermis were considered a multiple epidermis (Dickison 2000; Sharma and Mehra, 1972;
639 Martins et al. 2012). The hypodermis and multiple epidermis are regarded as two non-
640 homologous anatomical features, therefore ontogenetic observations are always
641 recommended to avoid misinterpretations (Martins et al., 2012). The presence of a multiple
642 epidermis or hypodermis has been considered an ecological adaptation of xerophytic plants to
643 arid environments, which prevents water loss due to excessive evapotranspiration and
644 protects the lamina from high solar radiation (Dickison 2000; Metcalfe and Chalk 1979;
645 Evert, 2006). A single epidermis is commonly associated with mesophytic and hydrophytic
646 species and is considered the normal type of epidermis in vascular plants (Dickison, 2000).
647 The presence of a single epidermis has been reported for most species of the family
648 Myrtaceae (Metcalfe and Chalk, 1979). Genera with single-layered epidermis include
649 *Eugenia* (Armstrong et al., 2012; Donato and Morretes, 2009; Fontenelle et al., 1994),
650 *Myrcia*, *Campomanesia* (Gomes et al., 2009), *Callistemon, Eucalyptus, Melaleuca* (Tantawy,
651 2004), *Acmena*, *Syzygium*, *Heteropyxis,* and *Tristania* (Keating, 1984, Soh and Parnell,
652 2011). The presence of a hypodermis has been identified in *Campomanesia*, *Myrcianthes*,
653 *Psidium* and *Pimenta* (Cardoso et al., 2009; Gomes et al., 2009). Cardoso et al. (2009)
654 reported the presence of hypodermis in the Brazilian species *Myrceugenia euosma*.
655 *Myrceugenia rufa* is the only species of Chilean Myrtaceae with adaxial double epidermis

656 and can be reliably identified using this anatomical character. The main habitat of
657 *Myrceugenia rufa* is the xeromorphic shrublands of north-central Chile, where rainfall is
658 restricted to few days of the year (Serra et al., 1986). The presence of double epidermis in this
659 species supports this ecological association. The occurrence of an adaxial hypodermis was
660 observed only in *N. fernandeziana* and *U. candollei*, species that mainly occur in wet forests
661 and open vegetation in humid regions of Chile. In this case, the presence of hypodermis
662 might not be associated with a xerophytic habitat. *Nothomyrcia fernandeziana* is
663 phylogenetically positioned within a clade that is closely related to the "*Pimenta* group"
664 (Murillo et al., 2013), which includes genera known to have hypodermis, such as *Pimenta*
665 and *Psidium* (Cardoso et al., 2009). Consequently, the presence of hypodermis in
666 *Nothomyrcia*, *Pimenta* and *Psidium* could be due to phylogenetic history and not
667 environment. As the systematic position of *U. candollei* is unknown, the presence of
668 hypodermis cannot yet be linked to phylogenetic constraints.
669
670 Papillae have been reported as projections of the epidermal cells in some Myrtaceae,
671 including South American species such as *Gomidesia nitida* and *Myrceugenia euosma*
672 (Cardoso et al., 2009; Metcalfe and Chalk, 1979). Here we observed papillae on the leaf
673 surface of *L. concinna*, *Myrceugenia correifolia*, *Myrceugenia schulzei* and *U. candollei*,
674 species that occur in distinct environments (mesophytic and xerophytic). The role of papillae
675 needs more investigation, but might be related to plant defence against pathogens and
676 herbivory (Voigt, 2014).
677
678 The anticlinal epidermal walls correspond to the outline of the primary walls between
679 adjacent cells and depend on the cellulose microfibril organization and deposition (Panteris et
680 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 (*Myrceugenia correifolia*, *Myrceugenia rufa*, *Myrcianthes 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

706 (armed biramous hairs) were observed in all the species of Chilean *Myrceugenia* and also in
707 *U. candollei*. Most of the species of *Myrceugenia* are reported to possess dibrachiate hairs, as
708 well as *Calyptranthes*, *Eugenia, Marlierea* and some species of *Myrcia* (Landrum and
709 Kawasaki, 1997). The presence of glandular or secretory hairs is not widely reported in South
710 American Myrtaceae. Secretory hairs have been reported on the abaxial leaf surface of the
711 Brazilian species *Myrceugenia euosma*, formed by papillose cells with thick cell walls
712 (Cardoso et al. 2009). Wilson (2011) refers to infundibular hairs (funnel-shaped) in a group
713 of South American *Eugenia*, but such hairs were not observed in any Chilean species. The
714 dense layer of hairs on the abaxial leaf surface was observed in a number of Chilean species
715 from arid environments (*Myrcianthes coquimbensis*, *Myrceugenia colchaguensis*,
716 *Myrceugenia correifolia* and *Myrceugenia rufa*). Most of these species occur in coastal
717 shrublands in the north-centre of Chile (Landrum, 1988a), where rainfall and humidity are
718 much lower compared to the typical mesophytic habitat of Chilean Myrtaceae. *Myrceugenia*
719 *euosma* is a South American species that occurs in Mata Atlântica, States of São Paulo,
720 Paraná, Santa Catarina and Rio Grande do Sul (Sobral et al., 2015) and that has been
721 considered one of the most xerophytic species of the genus (Landrum, 1981). Although
722 *Myrceugenia euosma* resembles the xerophytic *Myrceugenia rufa*, the first species has been
723 reported to occur also in flooded environments (Cardoso et al. 2009). In order to confirm the
724 consistency of some anatomical characters/character states related to ecological and
725 environmental associations (e.g., hypodermis, epidermal anticlinal walls, hairs),
726 comprehensive sampling of more populations is recommended. The phylogenetic position of
727 the most pubescent species of Chilean *Myrceugenia* is either basal to the genus (*Myrceugenia*
728 *rufa*) or part of a monophyletic group near the base (*Myrceugenia colchaguensis* +
729 *Myrceugenia schulzei*) (Murillo et al., 2013). In order to infer whether trichome characters

730 have a common phylogenetic origin or are product of convergent evolution, further
731 investigation is required.
732
733 *4.2 Stomata*
734 Although distribution of stomata and types of stomatal complexes are considered important
735 for taxonomic delimitation, there are a number of different classifications, each with a
736 particular terminology (Dressler, 1993). For a better understanding of stomatal complexes,
737 ontogenetic studies are critically important (Carpenter, 2005; Pole, 2012). Developmental or
738 ontogenetic studies are also necessary to find out if different types of mature stomata are
739 homologous (Pole, 2012).
740
741 Amphistomatic leaves (stomata distributed on both abaxial and adaxial leaf surfaces) are
742 commonly observed in hydrophytes and creeping species from wet habitats (Evert, 2006;
743 Gifford and Foster, 1989). The presence of amphistomatous leaves in *Myrteola nummularia*
744 suggests an environmental correlation with the habitat of the species. In Chile, *Myrteola*
745 *nummularia* is mainly a creeping shrub or subshrub that occurs in wet habitats such as
746 swamps, peatlands and the lower strata of humid forests (Landrum, 1988b).
747
748 *Eugenia* is one of the most widely studied genera of Myrtaceae and paracytic stomatal
749 complexes the most common type in the genus (Fontenelle et al., 1994; Haron and Moore,
750 1996; Hussin et al., 1992). Anomocytic stomata have also been reported as a common type at
751 family level (Gomes et al., 2009; Metcalfe and Chalk, 1979). Paracytic stomata were
752 observed only in the two species of the genus *Luma*, while the anomocytic type was observed
753 in a number of genera (*Amomyrtus*, *Legrandia*, *Myrceugenia*, *Ugni*). The different types of

754 stomatal complexes observed in Chilean Myrtaceae can be used to some extent to delimit
755 genera.
756
757 *4.3 Mesophyll, crystals and secretory cavities*
758 Dorsiventral (bifacial) mesophyll is the most common type of mesophyll in Myrtales and
759 Myrtaceae (Keating, 1984; Wilson, 2011). Few genera, such as the Australasian *Corymbia*,
760 *Eucalyptus, Leptospermum* and *Melaleuca,* species with vertically oriented leaves, have
761 isobilateral mesophyll (Gomes et al., 2009; Wilson, 2011). All the Chilean Myrtaceae have
762 dorsiventral mesophyll and the leaves are generally horizontally positioned. Mucilage and
763 pectins were staned by ruthenium red as granules or red content in the mesophyll of all
764 species, as indicated by Jensen (1962).
765
766 Crystals composed of calcium oxalate are the most common biomineral occurring in plants
767 (Arnott, 1982). These structures have been related to the regulation of calcium activity in
768 tissues (Volk et al., 2002.), as well as protection against herbivores and pathogens
769 (Franceschi and Nakata, 2005). Calcium oxalate crystals are widely present in Myrtaceae and
770 have different shapes and structure (Metcalfe and Chalk, 1979). Druses are the most common
771 type of crystal in Chilean Myrtaceae and have been also reported in *Eugenia, Gomidesia*,
772 *Psidium* and *Myrcia*, among other South American genera (Cardoso et al., 2009; Gomes et
773 al., 2009). Rhombohedral crystals observed in *Myrceugenia colchaguensis*, *Myrceugenia*
774 *schulzei* and *Ugni selkirkii* are similar to those reported for the Australasian genus *Syzygium*
775 (Soh and Parnell, 2011) and other South American genera, such as *Calyptranthes*,
776 *Campomanesia*, *Gomidesia* and *Mosiera* (Cardoso et al., 2009).
777

778 Schizogenous secretory cavities are originated by separation of cells and are composed of a
779 layer of epithelial cells surrounding a wide lumen space at maturity (Ciccarelli et al., 2008).
780 Lysigenous secretory cavities arise by dissolution of cells and do not possess epithelial cells
781 at maturity (Evert, 2006). Schizolysigenous cavities occur when cavities arise due to the
782 separation of cells (schizogenous origin), but epithelial cells are dissolved at maturity by
783 autolysis (Evert, 2006). Secretory cavities are mainly located adjacent to the adaxial and/or
784 abaxial epidermis and are primarily protodermal in origin, with participation of the ground
785 meristem (Arruda and Fontenelle, 1994; Fahn, 1979). The role of compounds produced by
786 secretory cavities (mainly sesquiterpenes and flavonoids in Myrtaceae) has been associated to
787 a number of plant functions. These roles are related to direct defence responses, metabolism
788 of diverse chemicals (Banthorpe et al., 1972) and plant architecture, through inhibition of
789 shoot branching (Akiyama et al., 2008). Secretory cavities are one of the most distinctive
790 features of Myrtaceae (Wilson et al., 2011), and are often referred as oil dots in field guides
791 and keys. Schizogenous secretory cavities are the most common type observed in Myrtaceae
792 (Alves et al., 2008; Donato and Morretes, 2011; Gomes et al., 2009) and also in Chilean
793 species. Schizolysigenous cavities were observed in a few Chilean species.
794
795 *4.4 Vascular system*
796 All species of Chilean Myrtaceae, other than *Nothomyrcia fernandeziana,* have been
797 described as possessing leaves with impressed midribs (Landrum, 1988a). Anatomically, the
798 pronounced swelling above the midrib of *N. fernandeziana* is composed of large and
799 isodiametrical parenchymatous cells. *Blepharocalyx cruckshanksii* possess a slight swelling
800 above the midrib, which is not usually reported in morphological descriptions of the species.
801 Adaxial phloem in vascular system is regarded as a typical character in the order Myrtales
802 (Cronquist, 1981) and is widely present in Myrtaceae (Cardoso et al., 2009; Schmid, 1980).

803 Vascular system characters observed here, such as adaxial phloem partition, confluence of
804 adaxial and abaxial phloem and sclerenchyma (fibres) around the vascular system, largely
805 agree with what has been observed in other South American genera (Cardoso et al., 2009;
806 Donato and Morretes, 2009; Gomes et al., 2009). These features are considered suitable
807 characters to identify species in Myrtaceae (Cardoso et al., 2009; Soh and Parnell, 2011).
808 *Blepharocalyx cruckshanksii* and *N. fernandeziana* are the only species of Chilean Myrtaceae
809 with inwardly curved vascular tissues: phloem surrounding two islands of xylem in *B.*
810 *cruckshanksii* and xylem surrounding phloem in *N. fernandeziana*. This anatomical character
811 supports the close phylogenetic relationship suggested for these two species and the
812 recognition of *Nothomyrcia*, as a separate genus distinct from *Myrceugenia* (Murillo and
813 Ruiz, 2011; Murillo et al., 2013).
814

815 **5. CONCLUSION**
816
817 This is the first investigation that describes the leaf anatomy of the 26 species of Chilean
818 Myrtaceae, including all the accepted species of a number of genera (*Amomyrtus*, *Legrandia*,
819 *Luma*, *Tepualia*). Anatomical features described here largely agree with previous characters
820 found in other Myrtaceae. Most of the species possess a typical mesophytic leaf anatomy,
821 while others show a combination of xerophytic characters such as hairy leaves, hypodermis,
822 thick adaxial epidermis and straight epidermal anticlinal walls. Anatomical and
823 micromorphological characters described here have potential taxonomic, ecologic and
824 phylogenetic significance. Yet, anatomical descriptions of other South American and
825 Australasian genera of Myrteae are recommended in order to use these features in a broader
826 taxonomic and evolutionary context. Further anatomical studies from additional populations
827 are recommended in order to confirm the consistency of some characters at species level.
828
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7. COMPETING INTERESTS

The authors declare that they have no competing interests.

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TABLES

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

	Epidermis and papillae			Stomata	Indumentum
Taxon	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

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

	Mesophyll			Vascular system			
Taxon	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

APPENDIX 1. ***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)*	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)*	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)*	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
*Material obtained from herbarium specimens

FIGURE 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*.

FIGURE 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.

FIGURE 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).

FIGURE 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).

FIGURE 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).

FIGURE 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).

FIGURE 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).

FIGURE 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).