ANATOMICAL STUDIES OF THE FLOWER, FRUIT AND SEEDS OF *MYRCEUGENIA RUFA* (MYRTACEAE)

Hernán A. Retamales[A,B], Angel Cabello[C], María Teresa Serra[D] and Tanya Scharaschkin [A]
[A]School of Earth, Environmental and Biological Sciences, Science and Engineering Faculty. Queensland University of Technology. Brisbane, QLD 4001, Australia
[B]Plant Biology Laboratory, Faculty of Forest Sciences and Nature Conservation, University of Chile, P.O. Box 9206, Santiago, Chile
[C]Chagual Botanic Garden, Santiago, Chile
[D]Forestry Herbarium (EIF), Faculty of Forest Sciences and Nature Conservation, University of Chile, P.O. Box 9206, Santiago, Chile
[A,B]Corresponding author: hernanalfonso.retamales@student.qut.edu.au

RESUMEN

Myceugenia rufa es una rara y endémica especie de la costa de Chile central. A la fecha, no hay estudios publicados que describan la anatomía de flor, fruto o semilla del taxón. Cuarenta y dos especímenes fueron muestreados a lo largo del rango geográfico de la especie. Las estructuras reproductivas fueron fijadas, deshidratadas, incluidas en parafina, cortadas y teñidas con Safranina O y Fast Green. La anatomía de los botones florales, flores maduras, frutos y semillas fue descrita. La anatomía reproductiva es afín a la de otras especies de la familia Myrtaceae, como por ejemplo la presencia de cristales, floema interno y cavidades secretoras esquizógenas. El conocimiento acerca de la anatomía y el desarrollo anatómico de las estructuras reproductivas de *M. rufa* podría ser de utilidad para estudios futuros acerca de la reproducción natural y programas de conservación para la especie.

Palabras clave: *Myrceugenia rufa*, anatomía reproductiva, flor, fruto, semillas

ABSTRACT

Myceugenia rufa is a rare and endemic species from the coast of central Chile. There are no published studies describing flower, fruit or seed anatomy. Forty-two accessions were collected from across the geographic range of the species. Reproductive structures were fixed, dehydrated, embedded in paraffin, sectioned and stained with Safranin O and Fast green. Anatomy of floral buds, mature flowers, fruits and seeds was described. Reproductive anatomy matches that of other Myrtaceae, such as presence of druses, internal phloem and schizogenous secretory cavities in buds, flowers, fruits and seeds. The anatomy and development of reproductive structures of *M. rufa* might enhance the understanding for future studies regarding natural reproduction and conservation programs.

Keywords: *Myrceugenia rufa*, reproductive anatomy, flower, fruit, seeds

INTRODUCTION

Myrceugenia Berg is a monophyletic group that comprises 40 species, 14 of which occur in Chile and the remaining in southeastern Brazil (Landrum 1981a; Murillo-A *et al.* 2012). The Chilean species of *Myrceugenia* are distributed from the semi-arid centre-northern region to the humid temperate forests in the southern tip of South America, as well as the Juan Fernández Islands (Landrum 1981a; 1988). These species are an important component in the upper and middle strata of the temperate forests (Hildebrand-Vogel 2002). *Myrceugenia rufa* (Colla) Skottsberg ex Kaussel (Myrtaceae: Myrteae) is a shrub 1-2 m high, with reddish-brown hairs on leaves, peduncles, sepals and fruits. The leaves are small, thick, coriaceous, densely pubescent beneath and puberulent above. Peduncles are uniflorous, densely pubescent, solitary or 2-3 in a row in the axils of leaves. Flowers are bisexual, with suborbicular and pubescent calyx lobes and petals, and numerous stamens. The fruits are fleshy, from green to orange-yellowish and pubescent

(Landrum 1981a; 1988). The seeds are poorly known as they are frequently eaten by insects (Kausel 1944; Landrum 1981a; 1988), usually leading to the destruction of *ca*. 95% of the seeds in any one plant (Cortés *et al.* 2006).

The species only occurs in the coast of central-north of Chile, in fragmented and open bushlands along *ca.* 200 km in the coastline and only few hundreds of meters inland (Landrum 1981a; Serra *et al.* 1986). *Myrceugenia rufa* is considered "Rare" by the Chilean legislation (Benoit 1989), due to its scarcity (Kausel 1957) and fragmented habitat. The main threats to the populations stem from urban development and fires (Hechenleitner 2005).

Even though several anatomical studies have been conducted in Myrtaceae, information about *Myrceugenia* is scarce and mainly limited to wood anatomy (Janssonius *et al.* 1908; Landrum 1981a; Metcalfe and Chalk 1950; Ragonese 1978; Record and Hess 1943; Schmid and Baas 1984). Reproductive anatomy is still unknown in the genus, as is the case with most of Myrtaceae genera (Lughadha and Proenca 1996). A reproductive anatomical study of any single species of *Myrceugenia* has never been undertaken.

A complete anatomical study of the species could enhance the understanding of the reproductive biology in the species, which is under human threat and has a very low regeneration and germination rates.

This investigation has two aims: (1) Undertake a complete anatomical description of the reproductive structures of *M. rufa* for the first time and (2) determine if the reproductive anatomy of *M. rufa* matches or differs from that of other South American Myrtaceae.

MATERIAL AND METHODS

Specimen sampling

Floral buds, mature flowers, fruits and seeds were collected between November 2008 and February of 2014. Sampling was conducted to represent the entire distribution range of the species. Specimens were collected from the following localities: Quebrada de Córdoba (El Tabo, Region of Valparaíso; 33°20'S), Los Molles (Region of Valparaíso; 32°40'S), Cerro de la Cruz (Zapallar, Region of Valparaíso; 32°33'S), Rodelillo (Viña del Mar, Region of Valparaíso; 33°00') and Cerro Talinay, Region of Coquimbo; 30°50'S – 71°40'O) (Figure 1a, b). Samples from cultivated specimens were obtained from the National Botanic Garden (Viña del Mar). A total of 42 accessions represent all known populations were examined. The material was identified according to Kausel (1944) and Landrum (1981a; 1988). Specimen identity was confirmed by comparison with vouchers (EIF-09992, EIF-08881, EIF-11238, EIF-08879, EIF-06644, EIF-03601, EIF-08878, EIF-08875, EIF-06201) identified as those of *M. rufa* in the herbarium collection from EIF (Forestry School, University of Chile).

Anatomy

Preparation of histological samples for light microscopy followed Johansen (1940), Feder and O'Brien (1968), Ruzin (1999) and protocols already used for Myrtaceae (Belsham and Orlovich 2003; Donato and Morretes 2009; Schmid and Baas 1984). Floral buds at different stages and mature flowers (Figure 1c) were fixed in FAA for 48 h, dehydrated through ethanol series, followed by xylene-ethanol combinations (1:3, 1:2, 1:1, 1:0) 2 h each, infiltrated and embedded in paraffin wax. Flowers were longitudinally cut and seeds were carefully removed from the fruits before fixation (Figure 1d, e). Sections of 5 µm thick were cut with an E. Leitz Wetzlar rotary microtome. Sections were stained with safranine O (1%), fast green, ferric chloride and tannic acid for various periods of time (Johansen 1940). The slides were sealed with Eukitt mounting medium.

Observation and photography

Slides were examined with a Carl Zeiss Axiostar 10-031 microscope equipped with a Canon Power Shot A640 digital camera. Cells were measured and counted on digital micrographs with the UTHSCSA ImageTool 3.0 software (Wilcox *et al.* 2009). Cells and structures dimensions were calculated with 35

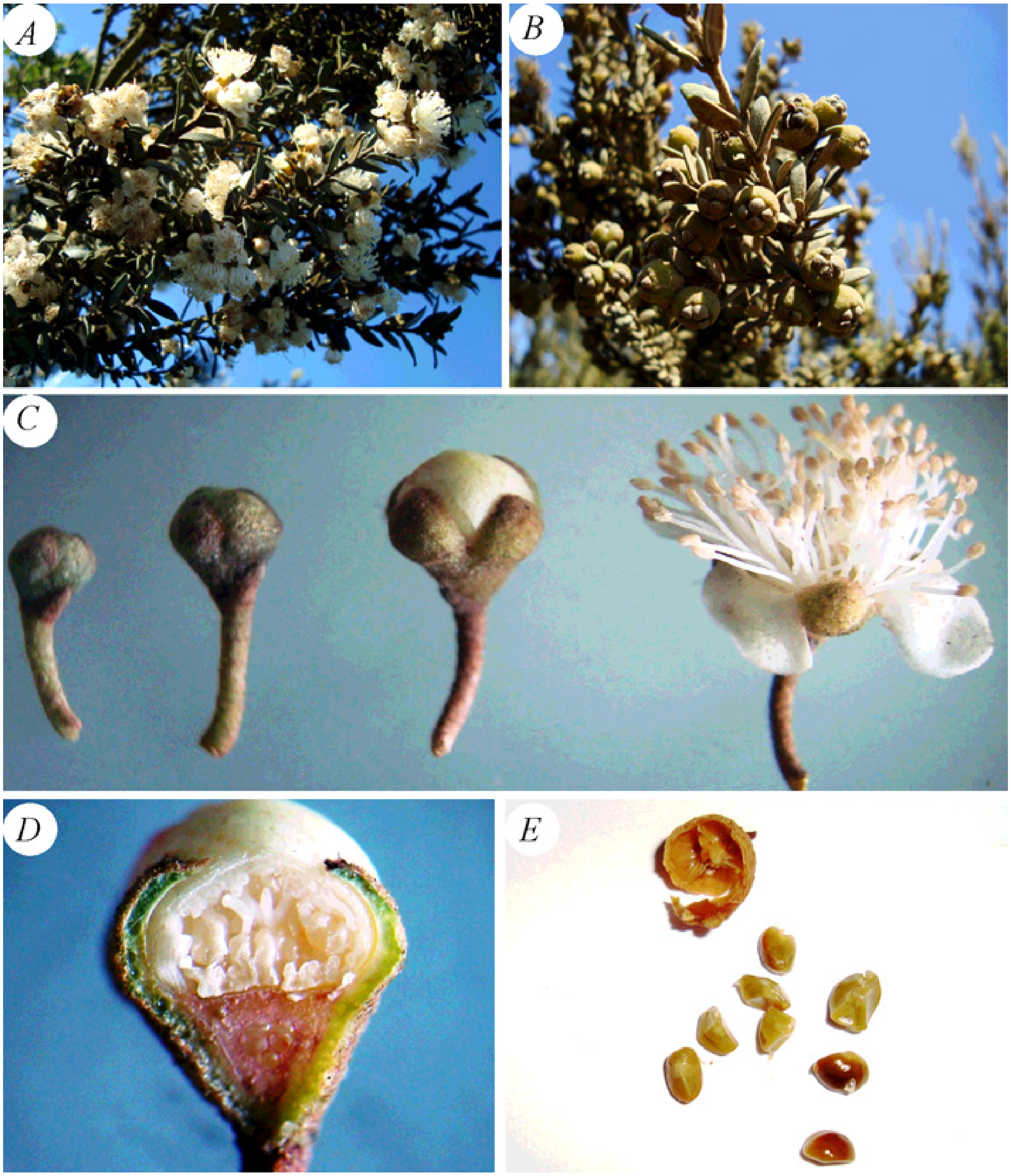

FIGURE 1: Sampling of *Myrceugenia rufa*, a,b) Flowering and fruiting specimen in Rodelillo (Viña del Mar, V Región); c) Different stages of floral buds and open flower; d) Longitudinally sectioned flower bud showing ovary, stamens and perianth; e) Open fruit showing a number of seeds.

random repetitions in different samples of 42 specimens, in order to obtain representative mean values. Measurements were calculated in micrometers (µm) and millimetres (mm) depending upon structure or cell type. Botanical terminology was based on Esau (1953) and Raven *et al.* (2005). Myrtaceae-specific anatomical descriptions and terminology were based on Schmid (1980; 1984), Cardoso *et al.* (2009) and da Silva *et al.* (2012).

RESULTS

Anatomy of floral buds

Sepals have a single epidermis, similar on adaxial and abaxial surfaces, composed of rectangular cells with thickened primary cell walls (Figure 2a). The cuticle is prominent on the abaxial epidermis but in the adaxial surface is thinner or barely visible (Figure 2e). Short multicellular hairs are abundant on both surfaces of the petals. Stomata are not observed on either side of sepals. Sepal mesophyll is composed of 6-8 layers of isodiametrical parenchymous cells, which contain abundant chloroplasts and some intercellular spaces. Subepidermal idioblasts containing druses are abundant throughout the mesophyll. Schizogenous secretory cavities are observed in the mesophyll of sepals (Figure 2c). These cavities are composed of large spaces surrounded by a sheath of peripheral epithelial cells, which are almost degenerated. Secretory cavities are abundant in all unlignified tissues and have variable dimensions (68 ± 21 µm).

Petals are anatomically similar to sepals other than being thinner and possessing conspicuous vacuoles with pigments (Figure 2d). Druses, secretory cavities and epithelial cells are also present in petals, with similar dimensions to the ones found in sepals.

The stamens are deflected towards the centre of the flower, whereas the parts of the perianth are imbricated in the same direction and surrounding them. Stamens occupy most of the space in the floral bud, being densely grouped between the hypanthium and the perianth (Figure 2b). The ovary has 2-4 locules (Figure 2f) with 6-10 ovules in locule. The style and stigma are not clearly observed.

Anatomy of mature flowers

In mature flowers, the androecium, gynoecium and perianth were fully developed following the typical pattern of a Myrtaceae flower, with numerous stamens in the center surrounded by the perianth (Figure 3a). The hypanthium is composed of parenchymous tissue, with abundant secretory cavities and epithelial cells.

Sepals have a unicellular epidermis, composed of rounded-polygonal cells. The cuticle of the abaxial epidermis is thick, while that of the adaxial surface is thinner (table 1). Multicellular glandular hairs are abundant on abaxial surface, forming a dense layer (Figure 3b), but are absent from the adaxial side.

The mesophyll is composed of parenchymous cells with thin primary cell walls. This tissue has numerous chloroplasts, which contribute to the colour of the sepals. The mesophyll possesses several secretory cavities and druses.

Petals have a unicellular epidermis but lack a continuous cuticle. The cuticle is present only in some sectors and is never more than 4 µm thick. The mesophyll is composed of isodiametric parenchymous cells. Numerous vacuoles containing pigments are seen in the cells of the epidermis and mesophyll (Figure 3c). Secretory cavities and epithelial cells are also observed in petals.

Stamens consist of a filament and a two lobed anther with four pollen sacs. The filament has a thin unicellular epidermis (<5 µm thick) containing vacuoles with pigments. The internal tissue of the filament is parenchymous, and it has a bicollateral vascular bundle with internal phloem. The pollen sacs are united by connective tissue, which is composed of parenchymous cells and bicollateral vascular bundles with scarce xylem. The anther wall has a unicellular epidermis and an endothecium composed of large rectangular cells. Middle layers or tapetum are not observed. The pollen grains are mainly rounded and slightly oblate and triangular (Figure 3d)

The ovary is composed of two carpels with two to four locules and central placentation with

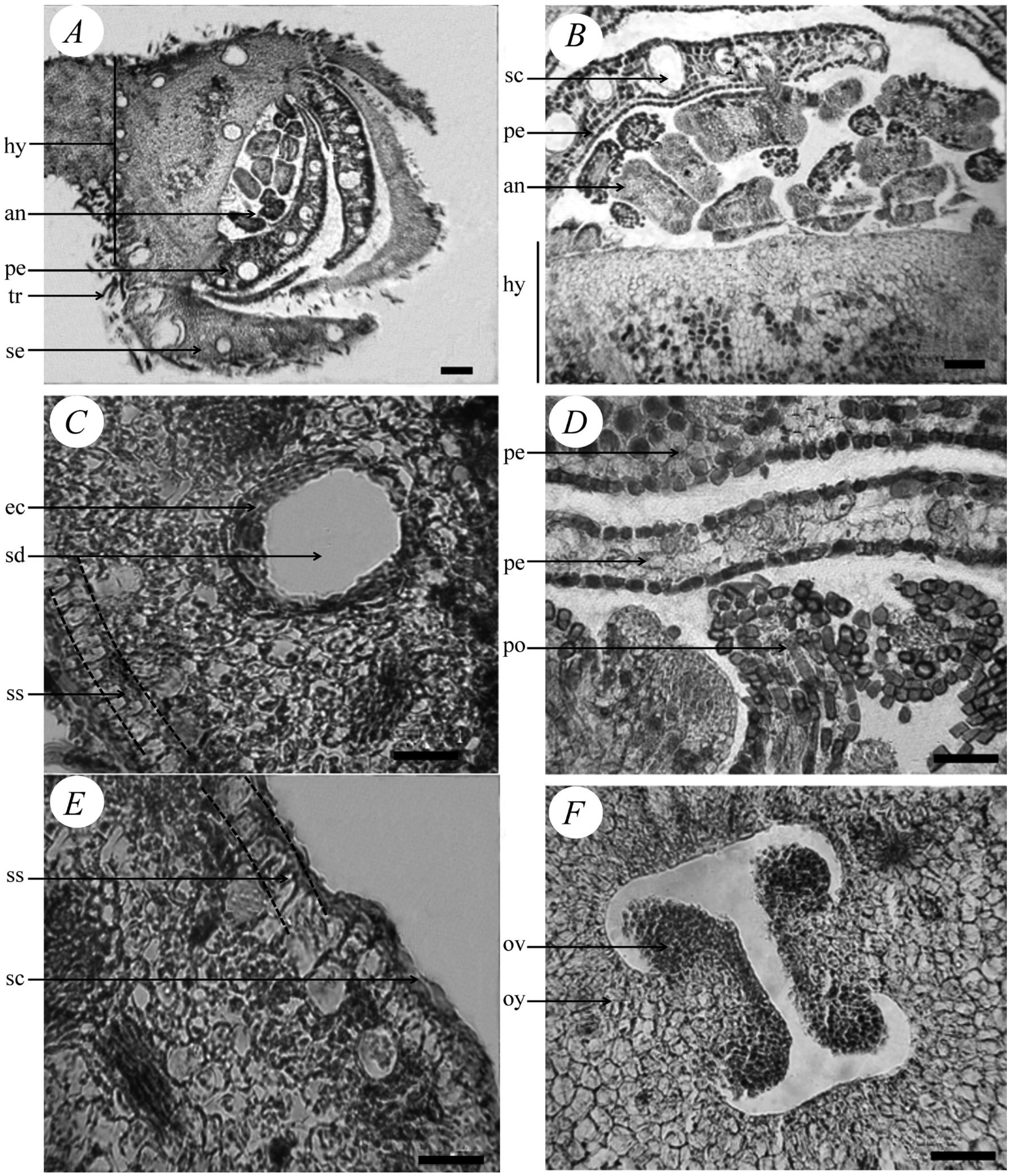

FIGURE 2: Light micrographs of sections through floral buds of *M. rufa*, a) Longitudinal section showing perianth, anthers and hypanthium; b) Longitudinal section with details of petals, hypanthium and anthers; c) Secretory cavity and epithelial cells in mesophyll of sepals; d) Section through petals showing mesophyll containing vacuoles with pigments. Also visible are developing pollen grains; e) Detail of epidermis and cuticle in sepals; f) bicarpelar ovary in development. Dotted lines in c) and e) show the epidermis of sepals. **an**- anthers, **ec**- epithelial cell, **hy**- hypanthium, **oy**- ovary wall, **ov**- ovules, **pe**- petals, **po**- pollen grains, **sc**- petal secretory cavity, **sc**-sepal cuticle, **se**- sepals, **ss**- sepal epidermis, **tr**- trichomes (hairs) Scale bars = 30 µm

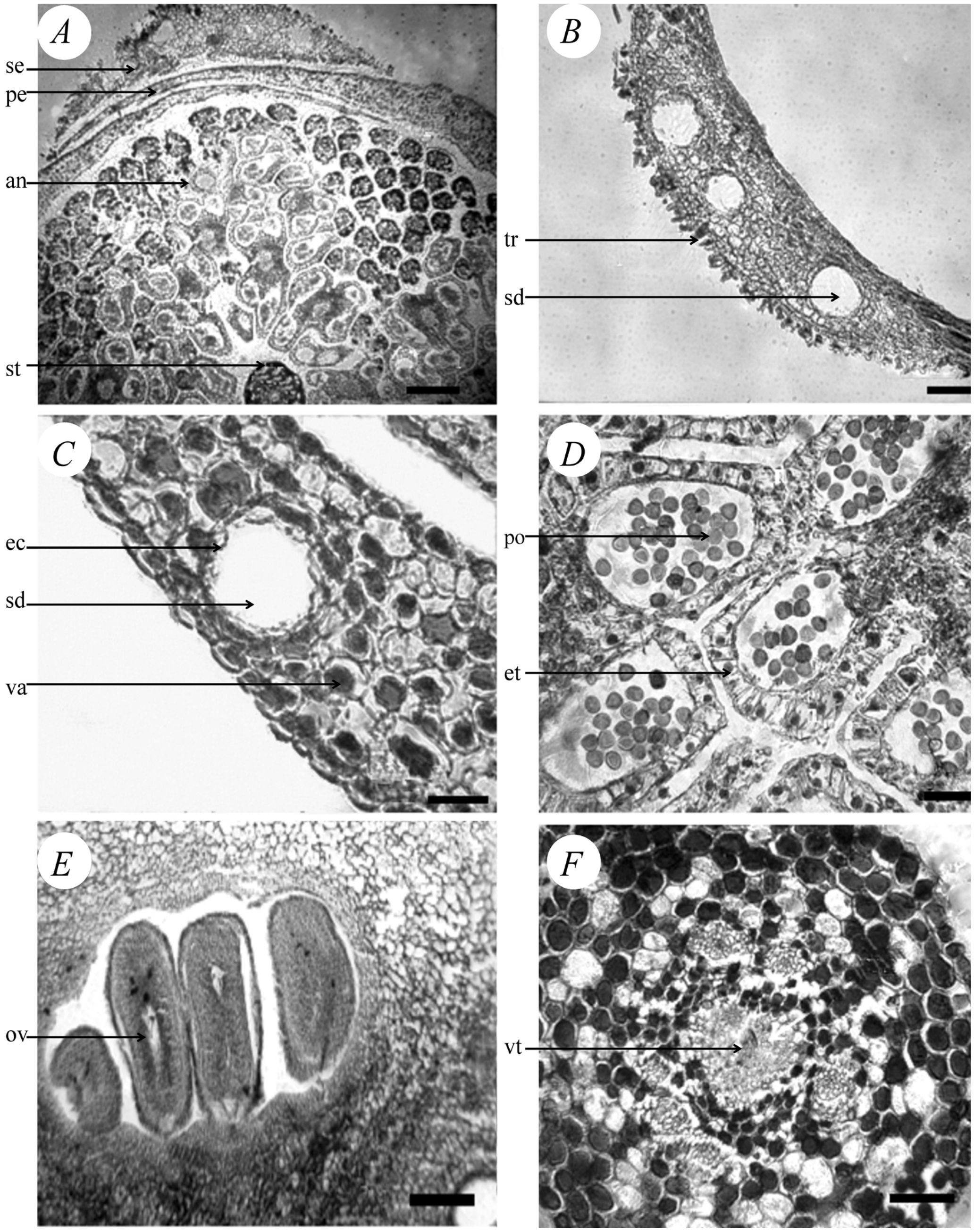

FIGURE 3. Light micrographs of mature flower of *Myrceugenia rufa*, a) Transverse section where fully developed sepals, petals, androecium and gynoecium can be observed (100x); b) Detail of sepal covered with multicellular hairs on the abaxial surface; c) Mesophyll of petal with vacuoles containing pigments. Secretory cavity with epithelial cells are visible; d) Transverse section of stamens, with detail of pollen sacs, anther walls and pollen grains; e) Longitudinal section through ovary showing flower ovary with four ovules in one locule; f) Transverse section of the style, with detail of parenchymous and vascular tissue. **an**- anthers, **ec**- epithelial cell, **et**- endothecium, **ov**- ovules, **pe**- petals **,** **po**- pollen grains, **sd**- secretory cavity**,** **se**- sepals, **st**- style **tr**- trichomes (hairs), **va**- pigmented vacuoles, **vt**- vascular tissue. Scale bars = 30 µm.

9 ± 4 ovules per locule (Figure 3e). Ovules are attached to a placenta composed of isodiametric and large parenchymous cells. The style (Figure 3f) is long with a thin epidermis and well-developed parenchymous cells containing pigment-filled vacuoles. Bicollateral vascular tissue is located in the centre of the style. The stigma was not observed.

Anatomy of fruit and seeds

The pericarp of mature fruits is differentiated into three zones: exocarp, mesocarp and endocarp. The exocarp is a unicellular layer, composed of irregular and plano-convex cells. A thin cuticle and numerous hairs cover the surface of this tissue (Figure 4a). The mesocarp is composed of 7-8 layers of parenchymous cells, which are large and isodiametric, with thin walls. Abundant and large secretory cavities are observed throughout this tissue (Figure 4b). The endocarp is a thin tissue with sclerified cell walls and surrounding the seeds (Figure 4c).

Fully developed seeds are non-endospermic; hence the embryo occupies most of the internal area (Figure 4d). Two sclerified seed coats (testa) are observed. The embryo has elongated, thin and folded cotyledons, with a conspicuous rounded hypocotyl observed in transverse section. Isodiametric meristematic cells with large nuclei are observed. Hypocotyls as well as cotyledons possess abundant secretory cavities with epithelial cells (Figure 4 e,f). The number of seeds per fruit was determined as 5 ± 3.

DISCUSSION

Myrceugenia rufa shares a number of anatomical features with other Myrtaceae. These characters include druses (calcium oxalate crystals), internal phloem and secretory cells. Calcium oxalate crystals are abundant in flowers of *M rufa*, especially in sepals and petals. Druses are widely present in several genera of Myrtaceae, in diverse vegetative and reproductive structures. Donato and Morretes (2007) and Alves *et al.* (2008) described druses of calcium oxalate in South American species of *Eugenia.* Donato and Morretes (2011) reported the same structures for *Myrcia multiflora*. Poliedric crystals, including druses, have been reported in *Psidium*, *Eugenia*, *Gomidesia* and *Myrcia*, among others (Cardoso *et al.* 2009; Gomes *et al.* 2009). The function of these structures is not clear, but has been related to the regulation of calcium and other minerals (Volk *et al.* 2002), as well as protection against herbivores and pathogens (Franceschi and Nakata 2005; Korth *et al.* 2006).

Internal phloem was found in all vascular bundles of flowers, either as continuous tissue or strands. This character is regarded as a typical anatomical character in the order Myrtales (Cronquist 1981; Takhtajan 1980) and is widely present in Myrtaceae (Schmid 1980; Cardoso *et al.* 2009).

It is relevant to know whether internal phloem is derived from the procambium, procambially derived or mesophyll cells. This developmental difference may depend upon species or genera and is considered a potential taxonomic character (Patil *et al.* 2009). The ontogeny of this tissue has not been studied in *Myrceugenia*.

The secretory cavities follow the typical schizogenous pattern commonly observed in Myrtaceae with large spaces and almost degenerated epithelial cells (Alves *et al.* 2008; Donato and Morretes 2007; Gomes *et al.* 2009). Has been reported that the ontogeny of secretory cavities in *Myrtus communis* follows and schizolysigenous development, which is a combination of lysigenous and schizogenous (Cicarelli *et al*. 2008). These structures are extremely abundant in the reproductive structures of *M. rufa*. Volatile oils secreted by these cavities in Myrtaceae, have been identified as flavonoids (Wollenweber *et al.* 2000) and terpenoids (Judd *et al.* 1999; Lee 1998; Tanaka *et al.* 1996). Ruiz *et al* (1994) detected three types of flavonols and flavones in *M. rufa*, some of them unidentified however. The occurrence of three types of foliar colleters (petaloid, conic and euryform) has been recently reported in different tribes of Myrtaceae, including Myrteae (da Silva *et al.* 2012). These structures were not observed in this study on *M. rufa*.

The anatomy of flowers is consistent with other studies in Myrtaceae, showing similarities in terms of organization and maturation of certain floral elements such as hypanthium (Belsham and Orlovich 2003),

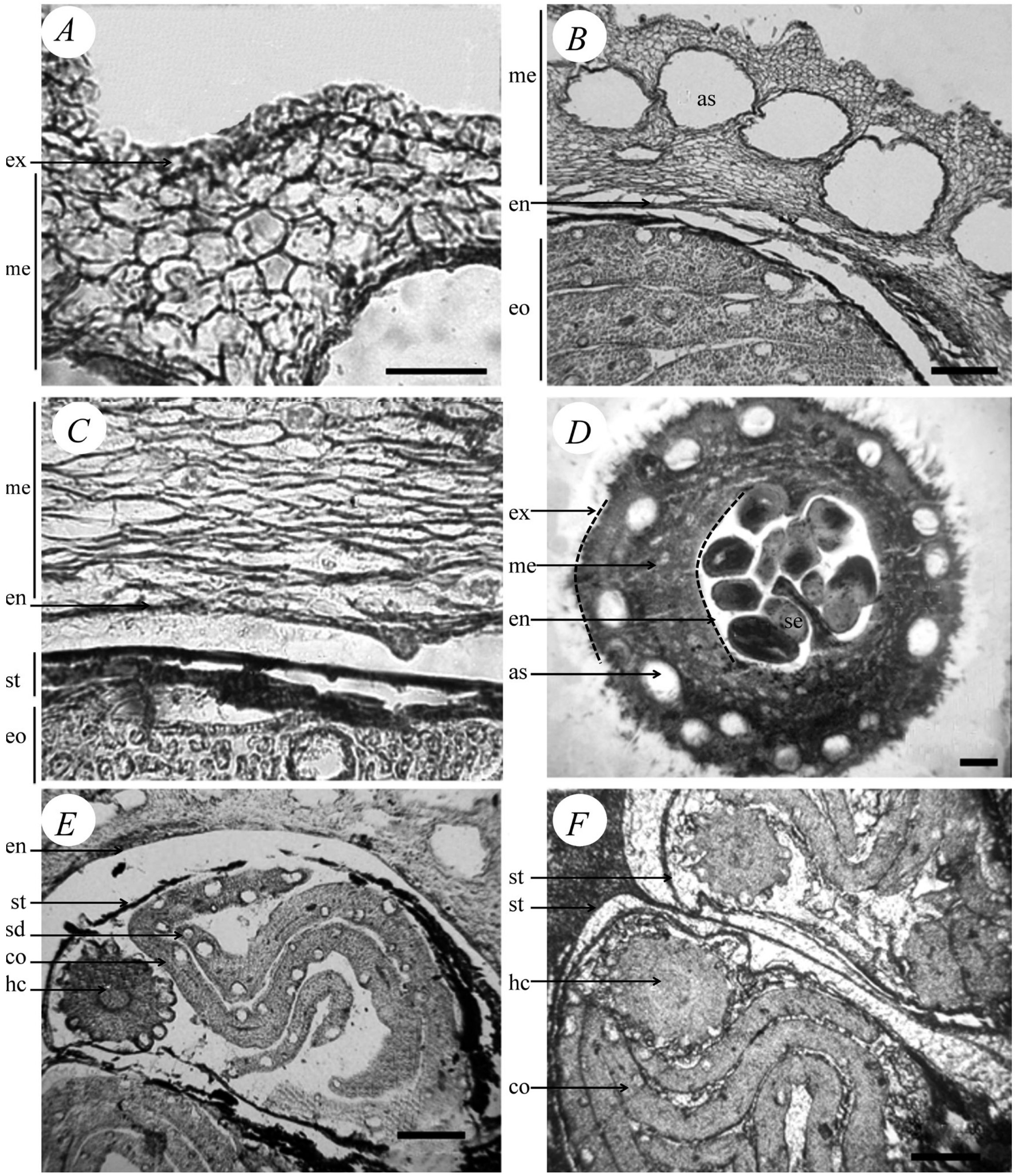

FIGURE 4. Light micrographs of fruit and seeds of *Myrceugenia rufa*, a) Transverse section of the fruit showing exocarp and mesocarp; b) Transverse section of the fruit, where mesocarp, endocarp and abundant secretory cavities are observed; c) Details of parenchymous cells of mesocarp and sclerified endocarp; d) Full transverse section of the fruit, where organization of pericarp (endo, meso and exocarp) and seeds is observed; e) Detail of an embryo with hypocotyl and long folded cotyledons; f) Two embryos covered by double seed coats. **as**- air spaces, **co**- cotyledon, **en**- endocarp, **eo**- embryo, **ex**- exocarp, **me**- mesocarp, **sd**- secretory cavity, **se**- seeds, **st**- seed coats, **hc**- hypocotyl. Scale bars = 50 µm.

perianth (Ciccarelli *et al.* 2008), stamens (Ladd *et al.* 1999) and gynoecium (Bohte and Drinnan 2005). The anatomy and internal organization of flower buds follows the pattern found in other Myrtaceae. The hypanthium is one of the first structures to develop in Myrtaceae, which allows early development of stamens in the bud (Belsham and Orlovich 2002). Our observations confirm the same developmental pattern in *M. rufa*, where the stamens and hypanthium are fully developed before the gynoecium. This might explain the presence of auto-incompatibility in *Myrceugenia* species before anthesis (Arroyo and Humaña 1999).

Floral anatomy was studied in detail by Schmid (1972a; 1972b; 1980) in the genera *Syzgium*, *Eugenia*, *Heteropyxis* and *Psiloxylon*. Anatomical similarities observed in *M. rufa* include bicollateral vascular bundles, druses and secretory cavities with epithelial cells. Muñoz (1959) described a central or subbasal placentation in the genus *Myrceugenia*, while Johow (1945) identified a central or pseudo-central placentation in Chilean Myrtaceae species. These descriptions agree with the placentation found in this study. The number of ovules in the ovary locules of *M. rufa* (9 ± 4), are similar to that reported by Johow (1945).

Myrceugenia rufa exhibits similarities in fruit anatomy with other South American species of Myrtaceae, namely *Eugenia punicifolia*, *Myrcia bella* and *Campomanesia pubescens*. Some of these shared traits include secretory cavities, idioblasts containing druses and 7-10 layers of parenchymous cells in the mesocarp Moreira (2007). Esemann-Quadros *et al.* (2008) describe druses in the mesocarp of fruits of the South American species *Acca sellowiana*. The thickness and structure of the endocarp in *M. rufa* is similar to that of *Eugenia*, *Myrcia*, *Campomanesia* and *Acca* as reported by Moreira (2007) and Esemann-Quadros *et al.* (2008). The number of seeds (5 ± 3) in *M. rufa* is consistent with that reported to the reported for the genus (Landrum 1981a). There is a difference between the number of ovules counted in the flower bud and the number of seeds. This change might be related to the usual attack of insects and/or the viability of the ovules. Non-endospermic seeds have been reported in fleshy-fruited Myrtaceae (Lughadha and Proenca 1996). Johow (1945) refers to Chilean Myrtaceae seeds as non-endospermic with endosperm traces. Species of *Myrceugenia* exhibit a myrcioid embryo, which correspond to membranous, thin and folded cotyledons and a horseshoe shaped hypocotyl, surrounding the cotyledons (Landrum 1981a; Landrum 1981b). The embryo of *M. rufa* matches with this description, except for the shape of the hypocotyl, which is more rounded. Secretory cavities in the embryo have not been described for the genus prior to this publication.

CONCLUSION

In this study, the reproductive anatomy of *Myrceugenia rufa* has been described for the first time. There are anatomical similarities between the species and other Myrtaceae taxa such as oil secretory cavities, idioblasts containing druses in all the organs and internal phloem in vascular bundles.

Incorporating these morpho-anatomical findings in future studies, particularly phylogenetic analyses, can provide a better understanding of the evolution of this species. The comprehensive use of morpho-anatomical characters in a broad phylogeny of Myrtaceae including all the species of *Myrceugenia* is recommended.

ACKNOWLEDGEMENTS

We thank the University of Chile, Plant Biology Laboratory, for the use of facilities and support for this investigation. Thanks to the National Botanic Garden of Chile (Viña del Mar) and the Chagual Botanic Garden (Santiago) for access to specimens and logistic support in sampling. We are very grateful to Paulette Naulin, Adelina Manriquez and Mila Arellano (University of Chile) for advice and guidance during the process of this publication. Thanks to QUT Plant Systematics Group and Rosa Scherson for valuable feedback.

BIBLIOGRAPHIC REFERENCES

ALVES, E., F. TRESMONDI and E. LONGUI
2008 Análise estrutural de folhas de *Eugenia uniflora* L. (Myrtaceae) coletadas em ambientes rural e urbano, SP, Brasil. Acta Botanica Brasilica 22: 241-248.

ARROYO, M. and A. HUMAÑA
1999 Sistema reproductivo de dos especies endémicas del bosque lluvioso del sur de Chile: *Amomyrtus meli* (Phil.) Legr. et Kaus.(Myrtaceae) y *Luzuriaga poliphylla* (Hook.) Macbr. (Philesiaceae). Gayana Botánica 56: 31-37.

BELSHAM, S. and D. ORLOVICH
2002 Development of the hypanthium and androecium in New Zealand Myrtoideae (Myrtaceae). New Zealand Journal of Botany 40: 687-695.

BELSHAM, S. and D. ORLOVICH
2003 Development of the hypanthium and androecium in South American Myrtoideae (Myrtaceae). New Zealand Journal of Botany 41(1): 161-169.

BENOIT, I.
1989 Libro rojo de la Flora terrestre de Chile. Corporación Nacional Forestal, Santiago, Chile.

BOHTE, A. and A. DRINNAN
2005 Ontogeny, anatomy and systematic significance of ovular structures in the eucalypt group (Eucalypteae, Myrtaceae). Plant Systematics and Evolution 255: 7-39.

CARDOSO, C., S. PROENCA and M. SAJO
2009 Foliar anatomy of the subfamily Myrtoideae (Myrtaceae). Australian Journal of Botany 57: 148-161.

CICCARELLI, D., F. GARBARI and A. PAGMI
2008 The flower of *Myrtus communis* (Myrtaceae): Secretory structures, unicellular papillae, and their ecological role. Flora - Morphology, Distribution, Functional Ecology of Plants 203: 85-93.

CORTES, F., L. RUZ and F. SQUEO
2006 Interacción entre el arbusto nativo *Myrceugenia rufa* (Myrtaceae) y la larva de un tineido (Lepidoptera) en un sector costero de la V Región de Valparaíso. In: XXIX Congreso Nacional de Entomología 28-29 Noviembre 2006, Temuco, CL.

CRONQUIST, A.
1981 An integrated system of classification of flowering plants. Columbia University Press, New York.

DA SILVA, C., L. BARBOSA, A., MARQUES, M., BARACAT-PEREIRA, A., PINHEIRO, A. and MEIRA, R.
2012 Anatomical characterisation of the foliar colleters in Myrtoideae (Myrtaceae). Australian Journal of Botany 60: 707-717.

DICKISON, W.
2000 Integrative plant anatomy. Academic Press, Massachusetts.

DONATO A. and B. MORRETES
2007 Anatomia foliar de *Eugenia brasiliensis* Lam. (Myrtaceae) proveniente de áreas de restinga e de floresta. Revista Brasileira de Farmacognosia 17: 426-443.

DONATO A. and B. MORRETES
2009 Anatomia foliar de *Eugenia florida* DC. (Myrtaceae). Revista Brasileira de Farmacognosia 19: 759-770.

DONATO A. and B. MORRETES
2011 Leaf morphoanatomy of *Myrcia multiflora* (Lam.) DC.-Myrtaceae. Revista brasileira de plantas medicinais 13: 43-51.

ESAU, K.
1953 Plant anatomy. John Wiley & Sons Inc, New York.

ESEMANN-QUADROS K., A. MOTA, G. KERBAUY, M. GUERRA, J. DUCROQUET and R. PESCADOR
2008 Estudo anatômico do crescimento do fruto em *Acca sellowiana* Berg. Revista Brasileira de Fruticultura 30: 296-302.

FEDER, N. and T. O'BRIEN
1968 Plant Microtechnique: Some Principles and New Methods. American Journal of Botany 55: 123-142.

FRANCESCHI, V. and P. NAKATA
2005 Calcium oxalate in plants: formation and function. Annual Review of Plant Biology 56: 41-71.

GOMES S, N. SOMAVILLA, S. GOMES-BEZERRA, S. MIRANDA, P. DE-CARVALHO and D. GRACIANO-

RIBEIRO
2009 Anatomia foliar de espécies de Myrtaceae: contribuições à taxonomia e filogenia. Acta Botanica Brasilica 23: 223-238.
HECHENLEITNER, P.
2005 Plantas amenazadas del centro-sur de Chile: distribución, conservación y propagación. Universidad Austral de Chile-Real Jardín Botánico de Edimburgo, Santiago
HILDEBRAND-VOGEL, R.
2002 Structure and dynamics of southern Chilean natural forests with special reference to the relation of evergreen versus deciduous elements. Folia Geobotanica 37: 107-128.
JANSEN, S., P. BAAS, P. GASSON, F. LENS and E. SMETS
2004 Variation in xylem structure from tropics to tundra: evidence from vestured pits. Proceedings of the National Academy of Sciences of the United States of America 101: 8833-8837.
JANSSONIUS, H., J. MOLL and S. KOORDERS
1908 Mikrographie des holzes der auf java vorkommenden baumarten, im auftrage des kolonial-ministeriums. EJ Brill, Berlin
JOHANSEN, D.
1940 Plant microtechnique. Mc Graw Hill, London
JOHOW, F.
1945 Flora de las plantas vasculares de Zapallar. Revista Chilena de Historia Natural 49: 8-364.
JUDD W., C. CAMPBELL, E. KELLOGG and P. STEVENS
1999 Plant systematics: a phylogenetic approach. Sinauer Associates: Massachusetts.
KAUSEL, E.
1944 Contribución al estudio de las Mirtáceas chilenas. Revista Argentina de Agronomía 11: 320-327.
KAUSEL, E.
1957 Beitrag zur Systematik der Myrtaceen. Arkiv för Botanik 3: 491-516.
KORTH, K., S. DOEGE, S. PARK, F. GOGGIN, Q. WANG, S. GOMEZ, G. LIU, L. JIA AND P. NAKATA
2006 Medicago truncatula mutants demonstrate the role of plant calcium oxalate crystals as an effective defense against chewing insects. Plant Physiology 141: 188-195.
LADD, P., J. PARNELL and G. THOMSON
1999 Anther diversity and function in *Verticordia* DC. (Myrtaceae). Plant Systematics and Evolution 219: 79-97.
LANDRUM, L.
1981a A Monograph of the Genus *Myrceugenia* (Myrtaceae). Flora Neotropica 29: 1-135.
LANDRUM, L.
1981b The phylogeny and geography of *Myrceugenia* (Myrtaceae). Brittonia 33: 105-129.
LANDRUM, L.
1988 The myrtle family (Myrtaceae) in Chile. Proceedings of the California Academy of Sciences 45: 277-317.
LEE, C.
1998 Ursane triterpenoids from leaves of *Melaleuca leucadendron*. Phytochemistry 49: 1119-1122.
LUGHADHA, E. and C. PROENCA
1996 A Survey of the Reproductive Biology of the Myrtoideae (Myrtaceae). Annals of the Missouri Botanical Garden 83: 480-503.
METCALFE, C. and L. CHALK
1950 Anatomy of the dicotyledons. Clarendron Press, Oxford
MOREIRA, I.
2007 Morfologia e ontogenese do pericarpo e semente de *Eugenia punicifolia, Myrcia bella* e *Campomanesia pubescens* (Myrtaceae). MSc diss. Universidade Estadual de Campinas, Campinas
MUÑOZ, C.
1959 Sinopsis de la flora chilena. Claves para la identificación de familias y géneros. Ediciones Universidad de Chile, Santiago
MURILLO-A, J., E. RUIZ-P, L. LANDRUM, T. STUESSY and M. BARFUSS
2012 Phylogenetic relationships in *Myrceugenia* (Myrtaceae) based on plastid and nuclear DNA sequences. Molecular Phylogenetics and Evolution 62: 764-776.

PATIL, V., K. RAO and K. RAJPUT
2009 Development of intraxylary phloem and internal cambium in *Ipomoea hederifolia* (Convolvulaceae). Journal of the Torrey Botanical Society 136: 423-432.
RAGONESE, A.
1978 Caracteres anatomicos del parenquima radial y axial en el leño de las Mirtaceas. Darwiniana 21: 27-41.
RAVEN P., R. EVERT and S. EICHORN
2005 Biology of plants. WH Freeman & Company, New York
RECORD S. and R. HESS
1943 Timbers of the new world. New Haven, Columbia
RUIZ, E., J. BECERRA, M. SSILVA, D. CRAWFORD and T. STUESSY
1994 Flavonoid chemistry of the endemic species of *Myrceugenia* (Myrtaceae) of the Juan Fernandez islands and relatives in continental South America. Brittonia 46: 187-193.
RUZIN, S.
1999 Plant microtechnique and microscopy. Oxford University Press, New York
SCHMID, R.
1972a Floral anatomy of Myrtaceae: 1. *Syzygium*. Botanische Jahrbücher für Systematik 92: 433-489.
SCHMID, R.
1972b Floral anatomy of Myrtaceae: 2. *Eugenia*. Journal of the Arnold Arboretum 53: 336-363.
SCHMID, R.
1980 Comparative Anatomy and Morphology of *Psiloxylon* and *Heteropyxis,* and the subfamilial and tribal classification of Myrtaceae. Taxon 29: 559-595.
SCHMID, R. and P. BAAS
1984 The occurrence of scalariform perforation plates and helical vessel wall thickenings in wood of Myrtaceae. IAWA Bulletin 5: 197-215.
SERRA, M., R. GAJARDO and A. CABELLO
1986 Programa de protección y recuperación de la flora nativa de Chile. Ficha técnica de especies amenazadas II: Especies raras. Departamento de Silvicultura y Manejo. Facultad de Ciencias Agrarias y Forestales, Universidad de Chile – Departamento de Áreas Silvestres Protegidas, CONAF, Santiago
TAKHTAJAN, A.
1980 Outline of the classification of flowering plants (Magnoliophyta). The Botanical Review 46: 225-359.
TANAKA, T., Y. ORII, G. NONAKA, I. NISHIOKA and I. KOUNO
1996 Syzyginins A and B, two ellagitannins from *Syzygium aromaticum*. Phytochemistry 43: 1345-1348.
VOLK, G., V. LYNCH-HOLM, T. KOSTMAN, L. GOSS and V. FRANCESCHI
2002 The role of druse and raphide calcium oxalate crystals in tissue calcium regulation in *Pistia stratiotes* leaves. *Plant biology* **4**, 34-45.
WILCOX, C., S. DOVE, W. MCDAVID and D. GREER
2009 ImageTool 3.0. Free image processing and analysis program. Department of Dental Diagnostic Science, University of Texas, Texas.
WOLLENWEBER, E., R. WEHDE, M. DÖRR, G. LANG and J. STEVENS
2000 C-Methyl-flavonoids from the leaf waxes of some Myrtaceae. Phytochemistry 55, 965-970.

Received, 27/May/2014; Accepted: 27/August/2014; Supervisor: Rocío Jaña.