DOI:10.21608/TAEC.2023.212293.1050 TAECKHOLMIA 43 (2023): 88-119

Taxonomic treatment of Myrtaceae based on leaf morphology, architecture, foliar oil glands and molecular characteristics

Al-Safa H. Mohamed*, Nareman K. Hosney, Karima A. Hamed and Soad A. Hassan

Botany Department, Faculty of science, Ain Shams University, Cairo, Egypt

*Corresponding authors: alsafa.alsherif@sci.asu.edu.eg

Abstract

The macromorphological characters and lamina vein architecture were examined by the aid of stero microscope. In addition, molecular study using SCoT markers were made on 26 species belonging to 15 genera of family Myrtaceae (nine genera belonging to subfamily Leptospermoideae and six genera belonging to subfamily Myrtoideae) to evaluate such characters in taxa delimitation. The taxa were collected from Mazhar Botanical Garden, Al-Baragil, Giza, Egypt. The obtained results revealed that, all studied taxa of Myrtaceae are distinguished by a combination of the following features: simple leaves with entire margin and glandular -punctate or pellucid leaves, while the leaf arrangement, lamina shape, apex and base showed great variation among them. Pinnate simple brochidodromous leaf venation is the most common, although other patterns were also recorded *viz*. cladodromous, eucamptodromous and craspedodromous. The irregular reticulate tertiary and quaternary vein fabric, presence or absence of an intramarginal vein were frequently observed. Eight SCoT primers were established to assess the genetic diversity between the studied taxa. The total number of amplified fragments was 125 from which 119 were polymorphic, and six were monomorphic. The total number of specific markers produced were 19 one of them scored for the absence of the band while the other 18 markers recorded for the presence of unique band. Only ten taxa revealed specific markers. Seven primers produced specific markers with largest number generated by primer SCoT 5 (six markers) and the lowest number generated by SCoT 2 and SCoT 5 (one marker for each), while primer SCoT 4 didn't reveal any specific markers. The obtained results were analyzed numerically (by PAST4 software) to construct three dendrograms; the leaf morphological based characters, the molecular based analysis and combined one. The combined data resulted dendrogram grouped all the 13-studied berry fruited Myrtoideae with seven capsular fruited Leptospermoideae, while the remaining five capsular fruited taxa grouped together. The combined and the morphological-based dendrograms recommend the maintenance of two subfamilies based on fruit type, despite both dendrograms showed deviation from the original system based on morphology.

Keywords: Lamina venation; Leptospermoideae; Myrtoideae; Numerical analysis; Polymorphism; SCoT markers

Introduction

Myrtaceae Juss. is one of the most widespread angiospermic families, also known as the myrtle family. There are around 132 genera and 5950 species (Christenhusz & Byng, 2016). According to Cronquist (1981) and Heywood (1993), the distribution of the family is found in tropical America and Australia. The family

Received on 20.05.2023 and accepted on 12/07/2023

is highly represented in many ecosystems according to Biffin *et al.* (2010), although Wilson (2011) claimed that Myrtaceae is diminished in Africa. The family have a high economic value as edible fruits, found in the majority of the genera in the subfamily Myrtoideae include *Psidium guajava* (guava) and *Syzygium aqueum* (Rose apple); raw material for oils can be obtained from *Backhousia citriodora* and *Eucalyptus* spp. (Wilson, 2011). Many species are locally used as spices, wood, and some species are cultivated as ornamentals. A number of Myrtoideae produce gums (Lambert *et al*. 2013) especially terpenes (Keszei *et al*. 2010), while some have high silica content in their leaves (Westbrook *et al.* 2009). The *Eucalyptus* group is rich in tannins, especially ellagic acid (Marsh *et al.* 2017).

Traditionally, Myrtaceae is classified into two subfamilies: Leptospermoideae found in Asia and Africa, and Myrtoideae found in tropical America, Asia, Australia, and the Pacific. The division of Myrtaceae into Leptospermoideae and Myrtoideae was challenged by a number of authors (Wilson *et al.* 2001, 2005 & 2011 and Biffin *et al.* 2010).

The subfamily Myrtoideae (60 genera) characterized by having bisexual flowers inferior or semi inferior ovary, fleshy fruits and opposite, entire leaves, (Wagner *et al*. 1990, Lucas 2007)**,** while the subfamily Leptospermoideae (72 genera) characterized by its unisexual flowers, female flowers with superior ovary, dry, dehiscent fruits (capsules) and spirally or alternately leaves (Cronquist, 1981, Lucas 2007).

Johnson & Briggs (1984) proposed to divide the Myrtaceae into two subfamilies: Leptospermoideae and Myrtoideae, of which Myrtoideae claimed to be polyphyletic. A study by Wilson *et al*. (2001) regarding the molecular and non-molecular data were used as a starting point for further analysis of the family, they stated that the classification of the family to Leptospermoideae and Myrtoideae was invalid. Tantawy (2004) pointed out that the examined species of the tribes Myrteae and Leptospermeae exhibit more or less constant macro-morphological characteristics of the vegetative and floral organs. However, Wilson *et al.* (2005) and Wilson (2011) classified the family into two subfamilies: Myrtoideae and Psiloxyloideae, based on plastid DNA phylogeny.

The most recent classification recognizes 17 tribes in two subfamilies; Myrtoideae (including 15 tribes) and Heteropyxidoideae (Psiloxyloideae) with two tribes and one genus in each mainly from Southern Africa (Lucas 2007; Stevens, 2017)

The family characterized by having opposite leaves tilled with aromatic essential oils in translucent 'gland dots', and often with a secondary marginal vein. Brooker & Nicolle (2013) and Wilson (2011) showed that the presence of pellucid glands observable as oil dots on the surface of the leaves is an important diagnostic feature of the Myrtaceae family. Niedenzu (1893) used macromorphological features *viz*. leaf arrangement, inflorescence, and fruit type to distinguish between the genera

of Myrtoideae and Leptospermoideae and the concept of two subfamilies was also applied by Hora (1978); Lughadha & Proenca (1996).

Leaf architecture characteristics are regarded as excellent tools that are widely utilized to solve taxonomic difficulties in Myrtaceae (Larano & Buot, 2010 and Ellis et al. 2009). Many authors analyzed the leaf venation patterns of Myrtaceae (Klucking, 1988; Costa et al. 1995; Farmacopeia Brasileira, 2002; Cardos & Sajo, 2004 & 2006). They found that the mixed camptodromous-brochidodromous secondary venation pattern is widespread in the family with mixed acrodromous-brochidodromous secondary venation patterns. While the acrodromous pattern is widespread in the subfamily Leptospermoideae (Klucking,1988).

Molecular markers have been discovered to be quite important in terms of molecular taxonomy. Grattapaglia *et al*. (2012) studied the genetic variation in natural and breeding populations in Myrtaceae by using different molecular markers as (microsatellite markers, random amplified polymorphism, amplified fragment length polymorphism, cpDNA, and finally restriction fragment length polymorphism).

A brand-new PCR approach called the SCoT method was introduced by Collard & Mackill (2009). This approach relies on the gene's start codon being translated. For both the forward and reverse primers, this marking uses a single primer. According to Xiong *et al*. (2010), the SCoT approach can be applied to a wide range of research areas since the SCoT primer can generate polymorphic dominant markers for functional gene regions. This marker has been widely used in the analysis and fingerprinting of several taxa *viz*. Rice (Collard & Mackill, 2009), *Arachis* (Xiong *et al*. 2010), *Citrus* (Han *et al*. 2011), *Vitis* (Zhang *et al*. 2011), Sugarcane and other plants (Wu *et al*. 2013). There is no information available on genomic DNA isolation, or the application of SCoT markers for molecular identification and genetic diversity assessment of Myrtaceae. The SCoT markers are simple, inexpensive, and reproducible.

The main objectives of the present work are to explore the contribution of the leaf morphological characters, lamina vein architecture and molecular data using SCoT markers in phenetic relationship and explanation of taxonomic treatment among the studied taxa.

Materials and methods

In the current study 26 cultivated species representing 15 genera belonging to the family Myrtaceae. Nine genera and 13 species belong to subfamily Leptospermoideae and six genera and 13 species belong to subfamily Myrtoideae were collected from Mazhar Botanic garden, Al-Baragil, Giza, Egypt (Table 1). The identification of taxa according to Bailey (1949) and Bailey and Bailey (1976). Synonyms were derived from Tropicos and IPNI. Voucher specimens were preserved at Botany Department Herbarium, Faculty of Science, Ain Shams University (Table 1).

1. Morphological investigation

A. Leaf macromorphological characters

Fresh leaves were used to describe the morphological features. Leaf macromorphological terminology was used by aid of Ellis *et al*. (2009).

B. Lamina vein architecture

The leaves were cleaned and immersed in a powerful home bleach (> 5% sodium hypochloride, > 5% sodium hydroxide, and water) till white. The bleach was then gently rinsed away with water. The leaves were stained with 1% safranin for two minutes. Terminology for leaf architecture according to Ellis *et al*. (2009). For numerical analysis the taxonomic treatment based on 85 morphological characters

Table 1. The studied taxa of Myrtaceae and their taxonomic postion according to (Niedenzu, 1893; Lughadha & Proenca1996).

Subfamily	Genera	Species
Myrtoideae	*Acca*	*A. sellowiana* (O.Berg) Burret
	Eugenia	*E. supraaxillaris* Spreng. Revise *E. uniflora* L.
	Myrtus	*M. communis* L.
	Pimenta	*P. dioica* (L.) Merr. *P. racemosa* (Mill.) J. W. Moore
	Psidium	*P. cattleyanum* Sabine *P. guajava* L.
	Syzygium	*S. antisepticum* (Blume) Merr. & L.M.Perry *S. jambos* (L.) Alston *S. malaccense* (L.) Merr. & L. M. Perry *S. paniculatum* Gaertn. *S. samarangense* (Blume) Merr. & L. M. Perry
Leptospermoideae	*Agonis*	*A. flexuosa* (Willd.) Sweet
	Backhousia	*B. citriodora* F. Muell.
	Callistemon	*C. rigidus* R. Br. *C. viminalis* (Sol. ex Gaertn.) G. Don
	Corymbia	*Co. ficifolia* (F.Muell.) K.D.Hill & L.A.S.Johnson
	Eucalyptus	*E. camaldulensis* Dehnh.
	Lophostemon	*L. confertus* (R.Br.) Peter G. Wilson & J. T. Waterh.
	Melaleuca	*M. armillaris* (Sol. ex Gaertn.) Sm. *M. ericifolia* Sm. *M. leucadendra* (L.) L. *M. linariifolia* Sm.
	Metrosideros	*M. excelsa* Gaertn.
	Xanthostemon	*X. fruticosus* Peter G.Wilson & Co

2. Start Codon Targeted (SCoT) Polymorphism marker analysis

DNA was extracted from about 50-100 mg of the fresh leaves of the 26 taxa, using DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). The genetic diversity of the studied 26 taxa was examined using eight SCoT primers (Table 2) selected based on their ability to produce clear reproducible banding pattern. SCoT primers were designed as previously described by Collard & Mackill (2009). The primers were dissolved in sterilized water to a final concentration of 100 µM and kept at -20°C. The polymerase chain reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM MgCl2, 0.2 mMdNTPs, 30 uM primer, 25-50 ng genomic DNA and one unit of Phusion® High-Fidelity DNA Polymerase (Espoo, Finland). PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 35 cycles after an initial denaturation cycle for three min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for one min, an annealing step at 55ºC for one min, and an elongation step at 72ºC for 1.5 min. The primer extension segment was extended to seven min, at 72ºC in the final cycle. PCR products were separated by electrophoresis (40min, 95v) through 1.5 % (m/v) agarose gel in 1XTBE buffer. Band sizes were visualized and determined based on (O'GeneRuler™1Kb) bp DNA ladder.

Table 2. Sequence of primers used in SCoT polymorphism analysis.

Primers name	Sequence
1	CAACAATGGCTACCACCA
2	CAACAATGGCTACCACCC
4	CAACAATGGCTACCACCT
5	CAACAATGGCTACCACGA
13	ACGACATGGCGACCATCG
14	ACGACATGGCGACCACGC
16	ACCATGGCTACCACCGAC
35	CATGGCTACCACCGGCCC

Data Analysis

The presence or absence of SCoT amplified bands was represented as (1) or (0). Only obvious distinguishable replicable bands were considered. The SCoT binary matrices were processed using the Bio-Rad diversity database software package and converted into similarity matrices according to coefficient (Dice, 1945; Sneath and Sokal, 1973). PAST4 software was used to perform the computation (Ryan et al. 1995).

Results and discussion

1. Morphological investigation

A. Leaf macromorphology

The leaf morphological data in the present study were summarized in (Table 3) and some specific leaf morphological characters were illustrated in plate I. All the studied taxa showed unifying feature of simple leaves with entire lamina margin. These results are compatible with Schmid (1980) and Wilson (2011).

Leaf arrangement: alternate in eight studied taxa (belonging to Leptospermoideae), while opposite in the remaining 17 taxa (belonging to Myrtoideae and Leptospermoideae). The observation is in accordance with Defaveri *et al.* (2011); Khan *et al.* (2016) and Oliveira *et al.* (2017) who indicated that the leaves of *Eugenia rotundifolia*, *Myrtus communis* and *Psidium* spp. are opposite. Leaf Attachment: sessile in seven of studied taxa (belonging to Leptospermoideae) and petiolate in the remaining 19 studied taxa. The presence of petiole in some taxa of Myrtaceae was mentioned by Oliveira *et al.* (2017).

Lamina shape: obovate in *Pimenta racemosa*, oblong in *Syzygium paniculatum* and *S. samarangense*, oblanceolate in *S. malaccense* and *Xanthostemon fruticosus*, linear in five taxa, lanceolate in five taxa, elliptic in five taxa and ovate in the remaining six studied taxa. In this respect, Oliveira *et al.* (2017) recorded the elliptic to obovate lamina shape in *Psidium* spp., while Khan *et al*. (2016) found the leaves of *Myrtus communis* ovate - lanceolate and this finding is consistent with the present results. Lamina base angle: Acute in 13 studied taxa while obtuse in the remaining 13 studied taxa. Defaveri *et al.* (2011) found that the lamina base angle in *Eugenia rotundifolia* is obtuse as in the present results. Lamina base shape: Concave in *Eugenia supraaxillaris*, decurrent in three taxa, rounded in three taxa, cuneate in seven taxa and convex in the remaining 12 studied taxa. Oliveira *et al.* (2017) stated that the lamina base shape of *Psidium* spp. varied between rounded and acute to cuneate, which in accordance with the present results. Lamina apex*:* Obtuse in four taxa acute in 11 taxa and acuminate in the rest 11 studied taxa. The present result is in accordance with that recorded by Khan *et al*. (2016) and Oliveira *et al.* (2017) who found that lamina apex acute in *Myrtus communis* and acute to acuminate in *Psidium* spp.

Table 3. Leaf macromorphological characters of the studied taxa of Myrtaceae. Abbreviations: (//) as previous.

Characters / Taxa	Leaf		Lamina		
	Arrangement	Attachment	Shape	Base shape /base angle	Apex
Acca sellowiana	Opposite	Petiolate	Elliptic	Convex /Acute	Obtuse
Agonis flexuosa	Alternate	Sessile	Lanceolate	Cuneate / Acute	Acuminate
Backhousia citriodora	Opposite	Petiolate	Elliptic	//	//
Callistemon rigidus	Alternate	Sessile	Linear	//	//
C. viminalis	//	//	//	//	//
Corymbia ficifolia	Opposite	Petiolate	Ovate	Rounded / Obtuse	//
Eucalyptus camaldulensis	Alternate	//	Lanceolate	Decurrent / Acute	Acute
Eugenia supraaxillaris	Opposite	//	Ovate	Concave / Obtuse	//
E. uniflora	//	//	//	Rounded / Obtuse	Acuminate
Lophostemon confertus	Alternate	//	//	Convex / Acute	Acute
Melaleuca armillaris	Opposite	Sessile	Linear	Cuneate / Acute	//
M. ericifolia	//	//	//	//	//
M. leucadendra	Alternate	Petiolate	Lanceolate	Decurrent / Acute	//
M. linariifolia	//	Sessile	Linear	Cuneate / Acute	Acuminate
Metrosideros excelsa	//	Petiolate	Ovate	Convex / Obtuse	Obtuse
Myrtus communis	Opposite	//	//	//	Acute
Pimenta dioica	//	//	Elliptic	//	Obtuse
P. racemose	//	//	Obovate	//	//
Psidium cattleyanum	//	//	Elliptic	//	Acuminate
P. guajava	//	//	//	Rounded / Obtuse	Acute
Syzygium antisepticum	//	//	Lanceolate	Convex / Obtuse	Acuminate
S. jambos	//	//	//	//	Acute
S. malaccense	//	//	Ob-lanceolate	//	Acuminate
S. paniculatum	//	//	oblong	Convex / Acute	//
S. samarangense	//	//	//	Convex / Obtuse	Acute
Xanthostemon fruticosus	Alternate	Sessile	Ob-lanceolate	Decurrent / Acute	//

B. Lamina vein architecture

A summary of leaf morphological characters and Lamina vein architecture is presented in (Table 4) and some of the most specific structures were illustrated in (Plates I-III). The study of leaf architecture reveled the following; Primary vein: category: Palmate flabellate in *Melaleuca ericifolia* and *M. leucadendra*, palmate basal acrodromous in *Callistemon rigidus, C. viminalis, M. armillaris* & *M. linariifolia* and pinnate in the remaining 20 studied taxa. Cardoso & Sajo (2004 & 2006) and Oliveira *et al*. (2017) recorded pinnate venation as the common type in Myrtaceae which is consistent with the present study. The most apparent variation is the number of basal veins *viz*. one basal vein in the 19 studied taxa, many in *M. ericifolia*, five in *Agonis flexuosa* and *M. leucadendra*, three in *C. rigidus, C. viminalis, M. armillaris* & *M. linariifolia* (Leptospermoideae).

Although simple brochidodromous is a common major secondary vein framework as recoded in 12 studied taxa, another pattern included Cladodromous in *M. leucadendra*, basal eucamptodromous in *Ag. flexuosa* and *M. ericifolia*, eucamptodromous becoming brochidodromous in *Lophostemon confertus, Metrosideros excelsa* and *Psidium guajava*, craspedodromous in *Backhousia citriodora, C. rigidus, C. viminalis, Corymbia ficifolia, M. armillaris, M. linariifolia, Pimenta dioica* & *Pi. racemosa* were also recorded. The data in the present study are in accordance with Costa *et al*. (1995); Farmacopeia Brasileira (2002) and Cardoso & Sajo (2004 & 2006) who mentioned that brochidodromous is the most common venation pattern in the genera of this family. Also, Oliveira *et al*. (2017) identified major secondary vein framework for some *Psidium* species as camptodromous–brochidodromous mixed venation pattern, then the brochidodromous pattern. Although Klucking (1988) supposed the acrodromous pattern as dominant for Myrtaceae.

Irregular spacing recorded in all the 26 studied taxa. This result support the finding of Oliveira *et al*. (2017) who recorded the irregular major secondary spacing in *Psidium*. Abruptly increasing proximally angle recorded in *Metrosideros excelsa*, ill-defined in *M. ericifolia*, uniform in *Acca sellowiana* and *Syzygium jambos*, smoothly increasing proximally in five taxa, smoothly decreasing proximally in six taxa and inconsistent in the remaining 11 studied taxa. Absence of Perimarginal vein is recoded in 16 studied taxa while marginal recorded in *Ag. flexuosa* and *Corymbia ficifolia*, fimbrial in *M. leucadendra, Pi. dioica* and *Xanthostemon fruticosus*, intramarginal in *Backhousia citriodora, E. camaldulensis, M. communis, P. racemosa & S. antisepticum*. In this respect Cardos & Sajo (2004 & 2006) and Oliveira *et al*. (2017) found that the absence of perimarginal vein in Myrtaceae as common character and this finding support our results. Intersecondaries wanting in seven taxa and present in the remaining 19 taxa. Oliveira *et al*. (2017) recorded the presence of intersecondaries in *Psidium* which agree with the present study.

In the present study reticulate irregular tertiary vein fabric was observed frequently (in 15 studied taxa) then mixed alternate opposite percurrent in two taxa, admedially ramified in in two taxa, transversely ramified in two taxa, Reticulate

composite admedially in *Acca sellowiana*, freely ramified in *M. leucadendra* or wanting in three taxa, although Cardos & Sajo (2006) claimed that the ramified admedially pattern is the common tertiary vein fabric pattern. Exterior course was mostly looped in 15 studied taxa, variable in *Me. excelsa*, terminating at margin in *Eucalyptus camaldulensis* or absent in seven taxa.

Quaternary vein fabric was frequently reticulate irregular in 14 studied taxa, freely ramified in eight taxa or absent four taxa while quinary vein fabric reticulate irregular in two taxa, freely ramified in ten taxa and absent in the rest 14 taxa. Areolation poorly developed in *Ps. cattleyanum* and *S. malaccense*, lacking in eight taxa, moderate developed in eight taxa well developed in the remaining eight taxa. Despite Oliveira *et al*. (2017) stated that the areoles well developed in *Psidium*. Unbranched freely ending veinlets recorded in *B. citriodora*, dichotomizing in *Me. excelsa* and *S. jambos*, absent in nine taxa and branched in the rest 14 taxa. Our results are in accordance with Oliveira *et al*. (2017) who recorded the presence of branched freely ending veinlets in *Psidium*. The marginal ultimate venation incomplete in *Me. excelsa*, absent in ten taxa and looped in the rest 15 taxa. In this respect Cardos & Sajo (2006) and Oliveira *et al*. (2017) identified the looped marginal ultimate venation pattern as common type in Myrtaceae, then absent and incomplete loop types and this finding is consistent with present study.

Foliar Oil glands (Table 4, Plate II & III)

Oil glands originally appeared in juvenile leaves (on the 3rd node) and continued to develop as the leaf expanded, while density per unit area dropped. The number of glands in a leaf peaked just before full leaf development. Oil glands present in all studied taxa except *Corymbia ficifolia* and *Melaleuca linariifolia*. This finding is consistent with Brooker & Nicolle (2013) who also recorded the absence of visible oil glands in *Co. ficifolia*. Moreover, they utilized this character to identify *Co. ficifolia* from other related taxa.

Irregular shape oil gland recorded in *Callistemon viminalis, Metrosideros excelsa, Psidium cattleyanum, P. guajava* & *Xanthostemon fruticosus* while rounded in the rest 19 taxa. The conspicuousness of oil gland was inconspicuous in 11 taxa and conspicuous in 13 taxa. This result is in accordance with Hambali *et al.* (2017) who mentioned the presence of inconspicuous oil glands in some *Syzygium* taxa. Frequency of oil gland rare (1≥ 5) in *S. antisepticum* and *X. fruticosus*, few (6≥20) in three taxa, abundant (more than 50) in seven taxa while moderate (21≥50) in the rest 12 taxa. In this respect Goodger *et al.* (2018) mentioned the presence of numerous oil glands in *Eucalyptus*, this in accordance with the present results. Also, Defaveri *et al.* (2011) recorded the presence of numerous oil glands on the leaf surface of *E. rotundifolia* and considered this character as key character to differentiate it from related taxa. Two gland types in leaves recorded; one is golden-brown in *E. uniflora*, *Pimenta dioica, Pi. racemose, Syzygium antisepticum, S. malaccense, S. paniculatum, S. samarangense* and the second is translucent appearance in the rest 19 studied taxa.

In conclusion, the characters of oil glands including their absence, presence, shape, conspicuousness, color and frequency can be used to identify some Myrtaceae, and this finding is supported by Brooker & Nicolle (2013).

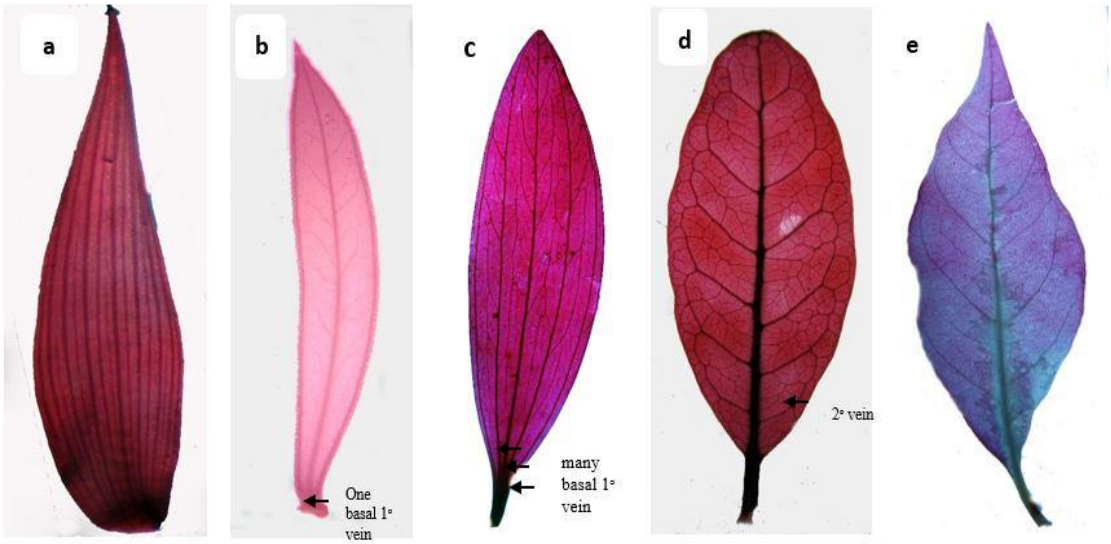

Plate I. Major categories of lamina vein architecture (1° vein category, number of basal veins, major 2° framework) **a.** Palmate flabellate 1° vein, many basal veins, basal eucamptodromous 2° vein (*Melaleuca ericifolia*); **b.** Palmate basal acrodromous 1° vein, three basal veins, craspedodromous 2° vein (*Callistemon rigidus*); **c.** Palmate flabellate 1° vein, five basal veins, cladodromous 2° vein (*Melaleuca leucadendra*); **d.** Pinnate 1° vein, one basal vein, simple brochidodromous 2° vein (*Acca sellowiana*); **e.** Pinnate 1° vein, one basal vein, eucamptodromous becoming brochidodromous 2° vein (*Lophostemon confertus*).

Table 4. Lamina vein architecture of the studied taxa of Myrtaceae. Abbreviations: (+); present, (-); absent, (//); as previous.

Characters / Taxa	Primary vein farm work	No. of basal veins	Secondary vein					Tertiary vein		Quaternary fabric	Quinary fabric
			Major 2° frame work	Major 2° angle	Major 2° Attachment	Peri marginal veins	Inter 2° veins	Intercostal 3° vein fabric	Exterior 3° course		
Acca sellowiana	Pinnate	1	Simple brochido-Dromous	Uniform	Excurrent	-	+	Reticulate composite admedially	Looped	Reticulate irregular	Freely ramified
Agonis flexuosa	//	5	Basal eucampto-Dromous	Smoothly increase proximally	//	Marginal	+	Admedially ramified	-	Freely ramified	-
Backhousia citriodora	//	1	Craspedo-Dromous	Inconsistent	//	Intra-marginal	+	Reticulate irregular	Looped	Reticulate irregular	Freely ramified
Callistemon rigidus	Palmate basal acro-dromous	3	//	//	//	-	+	Transversely ramified	-	Freely ramified	-
C. viminalis	//	//	//	//	//	//	+	//	Looped	//	//
Corymbia ficifolia	Pinnate	1	//	Smoothly increase proximally	//	Marginal	+	Reticulate irregular	-	Reticulate irregular	Freely ramified
Eucalyptus camaldulensis	//	//	Simple brochido-Dromous	Smoothly decrease proximally	//	Intra-marginal	+	//	Termina-ting at the margin.	//	//

Characters / Taxa	Primary vein farm work	No. of basal veins	Secondary vein					Tertiary vein		Quaternary fabric	Quinary fabric
			Major 2° frame work	Major 2° angle	Major 2° Attachment	Peri marginal veins	Inter 2° veins	Intercostal 3° vein fabric	Exterior 3° course		
Eugenia supraaxillaris	Pinnate	1	Simple brochido-Dromous	Smoothly increase proximally	//	-	+	Reticulate irregular	Looped	Reticulate irregular	-
E. uniflora	//	//	//	Inconsistent	Proximal secondaries decurrent	//	+	//	//	//	//
Lophostemon confertus	//	//	Eucampto-dromous becoming brochido-dromous.	Smoothly increase proximally	//	//	+	//	//	//	Freely ramified
Melaleuca armillaris	Palmate basal acro-dromous	3	Craspedo-dromous.	Inconsistent	Decurrent	//	+	-	-	-	-
M. ericifolia	Palmate flabellate	Many	Basal eucampto-dromous	Ill-defined							
M. leucadendra	//	5	Clado-dromous	Inconsistent	Excurrent	-	+	Freely ramified	-	-	-
M. linariifolia	Palmate basal acro-dromous	3	Craspedo-dromous	//	Ill-defined						

Characters / Taxa	Primary vein farm work	No. of basal veins	Secondary vein					Tertiary vein		Quaternary fabric	Quinary fabric
			Major 2° frame work	Major 2° angle	Major 2° Attachment	Peri marginal veins	Inter 2° veins	Intercostal 3° vein fabric	Exterior 3° course		
Metrosideros excelsa	Pinnate	1	Eucampto-dromous becoming brochido-dromous	Abruptly increase proximally	Proximal secondaries decurrent	-	+	Reticulate irregular	Variable	Freely ramified	-
Myrtus communis	//	//	Simple brochido-dromous	Smoothly decrease proximally	Excurrent	Intra-marginal	+	Reticulate irregular	Looped	//	-
Pimenta dioica	//	//	Craspedo-dromous	//	Proximal secondaries decurrent	Fimbrial	-	//	//	Reticulate irregular	Reticulate irregular
P. racemose	//	//	//	//	Excurrent	Intra-marginal	-	//	//	//	//
Psidium cattleyanum	//	//	Simple brochido-dromous	//	Proximal secondaries decurrent	-	-	//	//	Freely ramified	-
P. guajava	//	//	Eucampto-dromous becoming brochido-dromous.	//	//	-	+	Mix. Alter. opposite percurrent	//	Reticulate irregular	Freely ramified
Syzygium antisepticum	//	//	Simple brochido-dromous	Smoothly increase proximally	Excurrent	Intra-marginal	-	Excurrent	//	//	//

Characters / Taxa	Primary vein farm work	No. of basal veins	Secondary vein					Tertiary vein		Quaternary fabric	Quinary fabric
			Major 2° frame work	Major 2° angle	Major 2° Attachment	Peri marginal veins	Inter 2° veins	Intercostal 3° vein fabric	Exterior 3° course		
S. jambos	Pinnate	1	Simple brochido-dromous	Uniform	Excurrent	-	+	Excurrent	Looped	Reticulate irregular	Freely ramified
S. malaccense	//	//	//	Inconsistent	Decurrent	-	+	Mix. Alter. opposite percurrent	//	Freely ramified	-
S. paniculatum	//	//	//	//	//	-	+	Admedially ramified	//	//	-
S. samarangense	//	//	//	//	Proximal secondaries decurrent	-	-	Reticulate irregular	//	Reticulate irregular	Freely ramified
Xanthostemon fruticosus	//	//	//	//	Excurrent	Fimbrial	+	//	//	//	//

Table 4 Cont. Lamina vein architecture of the studied taxa of Myrtaceae. Abbreviations: (+); present, (-); absent, (//); as previous.

Characters / Taxa	Areolation	Freely ending veinlet	Marginal ultimate venation	Oil gland		
				Shape	Conspicuousness/color	Frequency
Acca sellowiana	Moderate	Branched	Looped	Rounded	Conspicuous/ translucent	Moderate
Agonis flexuosa	Lacking	//	-	//	//	Abundant
Backhousia citriodora	Well developed	Unbranched	Looped	//	//	Moderate
Callistemon rigidus	Lacking	Branched	-	//	//	Few
C. viminalis	//	//	Looped	Irregular	Inconspicuous/ translucent	Moderate
Corymbia ficifolia	Well developed	//	-	-		
Eucalyptus camaldulensis	//	//	-	Rounded	Conspicuous/ translucent	Moderate
Eugenia supraaxillaris	Moderate	//	Looped	//	Inconspicuous/ translucent	//
E. uniflora	//	//	//	//	Conspicuous/ golden-brown	Abundant
Lophostemon confertus	Well developed	//	//	//	// translucent	Moderate
Melaleuca armillaris	Lacing	-	-	//	Inconspicuous/ translucent	//
M. ericifolia	//	-	-	//	Conspicuous/ translucent	//
M. leucadendra	//	-	-	//	Inconspicuous/ translucent	Abundant
M. linariifolia	//	-	-	-		
Metrosideros excelsa	Well-developed	Dichotomizing	Incomplete loops	Irregular	Inconspicuous/ translucent	Abundant
Myrtus communis	Moderate	Branched	Looped	Rounded	Conspicuous / translucent	Moderate
Pimenta dioica	Well developed	-	-	//	// / golden-brown	Abundant
P. racemose	//	-	Looped	//	Inconspicuous/ golden-brown	Moderate
Characters	Areolation			Oil gland		

Taxa		Freely ending veinlet	Marginal ultimate venation	Shape	Conspicuousness/color	Frequency
Psidium cattleyanum	Poorly developed	Branched	//	Irregular	// Translucent	//
P. guajava	Moderate	//	//	//	Inconspicuous/ translucent	Abundant
Syzygium antisepticum	//	//	//	Rounded	Conspicuous/ golden-brown	Rare
S. jambos	//	Dichotomizing	//	//	// / translucent	Moderate
S. malaccense	Poorly developed	Branched	//	//	Inconspicuous/ golden-brown	Few
S. paniculatum	Lacking	//	//	//	// / golden-brown	//
S. samarangense	Moderate	//	//	//	Conspicuous/ golden-brown	Moderate
Xanthostemon fruticosus	Well developed	//	-	Irregular	Inconspicuous/ translucent	Rare

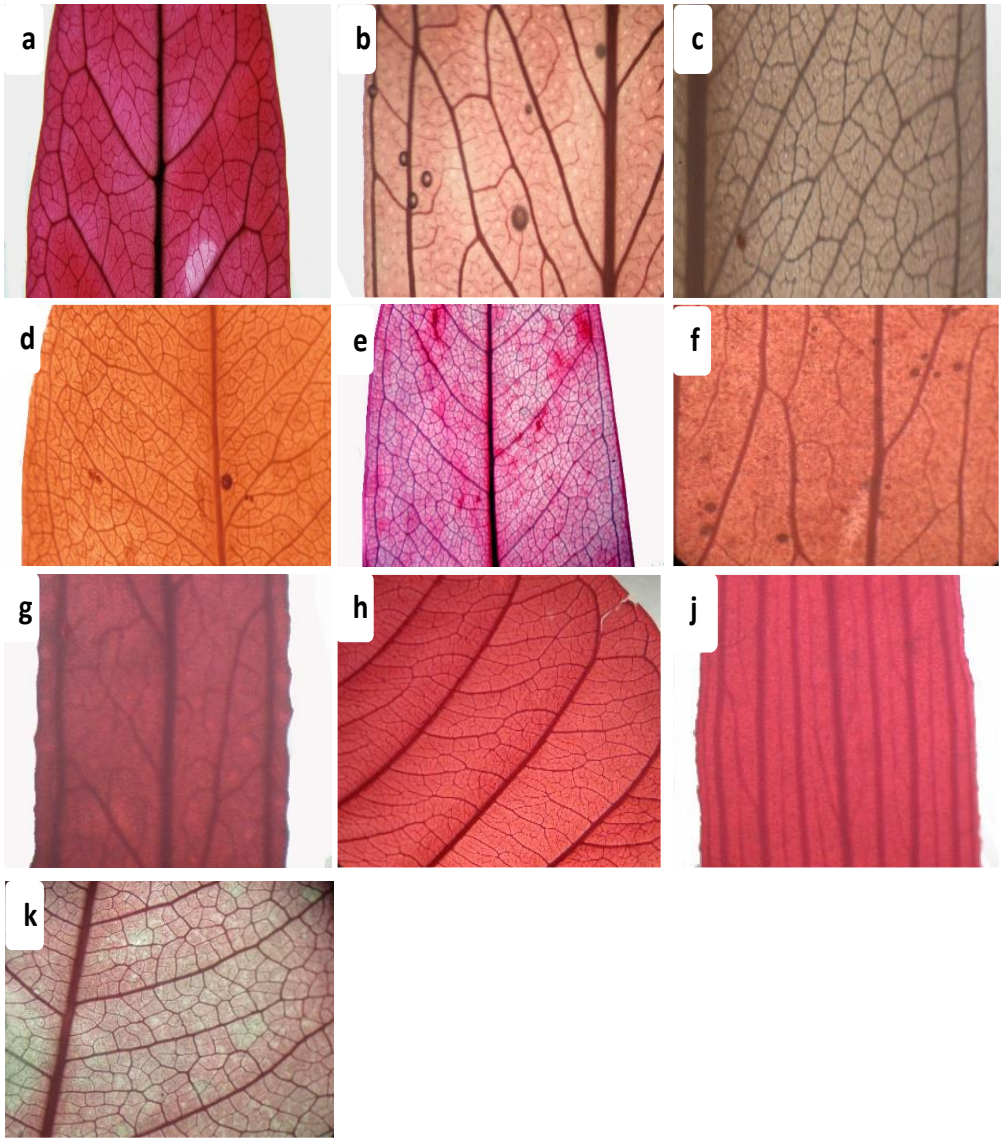

Plate II. Major categories of lamina vein architecture (perimarginal vein, exterior 3^{o} course, marginal ultimate venation, 3^{o} vein fabric, 4^{o} vein fabric, 5^{o} vein fabric) **a.** *Acca sellowiana*, **b.** *Agonis flexuosa*, **c**. *Eucalyptus camaldulensis*, **d.** *Metrosideros excelsa*e. *Xanthostemon fruticosus*, **f**. *Melaleuca leucadendra*, **g.** *Callistemon rigidus*, **h**. *Psidium guajava***, j.** *Melaleuca armillaris*, **k**. *Backhousia citriodora*.

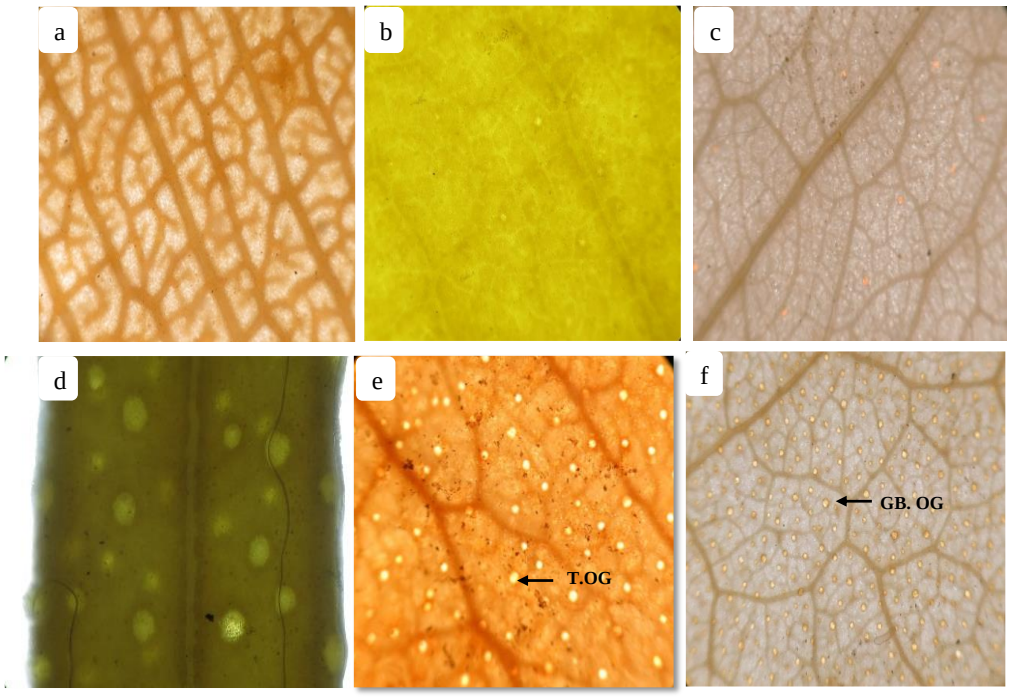

Plate III. Oil glands (shape, conspicoiusness, frequency). **a.** Oil glands absent (*Corymbia ficifolia*); **b.** Irregular, inconspicuous, rare oil glands (*Xanthostemon fruticosus*); **c**. Rounded, conspicuous, rare oil glands and golden brown (*Syzygium antisepticum)*; **d.** Rounded, conspicuous, few translucent oil glands (*Callistemon rigidus*); **e.** Rounded, conspicuous ,and moderate translucent oil glands (*Myrtus communis*); **f.** Rounded, conspicuous, abundant and golden brown oil glands (*Eugenia uniflora*), T. OG; Translucent oil glands, GB.OG; golden brown oil glands

2. Molecular study

The total number of amplified fragments was 125; 119 of them were polymorphic bands (95%) and the other six bands were monomorphic.The amplification products resulted from eight SCoT primers are presented in (Table **5)** in terms of percentage of PCR products for the studied taxa.

Species specific markers based on SCoT analysis

The specific markers produced by eight SCoT primers were 19 out of 125 total amplified bands as shown in (Table 6, Plate IV- Plate VII). Only one of them scored for absence of the band (negative band) while the other 18 markers scored for the presence of unique band (positive marker).The largest number of SCoT specific markers (5 markers) were scored by *Callistemon viminalis*, (3

markers) recorded by *Corymbia ficifolia* and *Melaleuca leucadendra*, (2 markers) scored by *Xanthostemon fruticosus*. While, the lowest number of specific markers (one) was scored for *Eucalyptus camaldulensis, Eugenia uniflora, M. linariifolia, Pimenta dioica, Syzygium malaccense* and *S. paniculatum*.

On the other hand, the largest number of specific markers was generated by primer SCoT35 with six markers, followed by primer SCoT1 (four markers), primer SCoT16 (three markers), primers SCoT13 and SCoT14 (two markers for each), while primers SCoT2 and SCoT5 scored only by one marker for each. Primer SCoT4 didn't reveal any specific markers.In this work, the SCoT markers could distinguish among Myrtaceae taxa. This finding is in agreement with those find by Han *et al.* (2011), Zhang *et al.* (2011) and Shahlaei *et al.* (2014).

Table 5. Total number of bands, monomorphic, polymorphic bands and percentage of polymorphism as obtained by SCoT markers among 26 studied taxa.

Primer	Total no. of bands	Monomorphic bands	Polymorphic bands	% of polymorphism
SCoT 1	14	0	14	100
SCoT 2	24	1	23	96
SCoT 4	15	1	14	93
SCoT 5	16	0	16	100
SCoT 13	16	1	15	94
SCoT 14	10	1	9	90
SCoT 16	13	2	11	85
SCoT 35	17	0	17	100
Total	125	6	119	
Average	15.6	0.75	19.9	

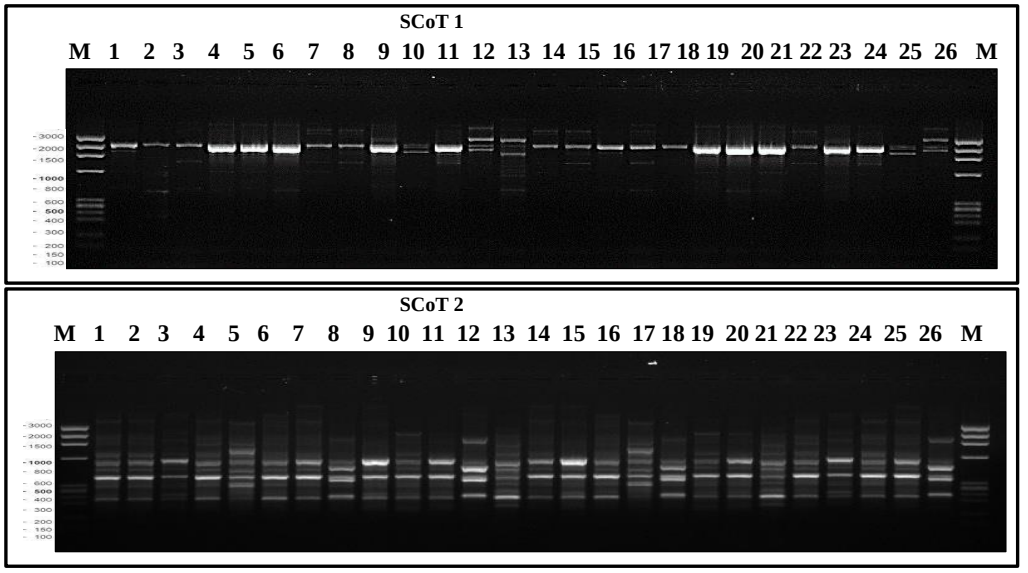

Plate IV. SCoT profile of the studied taxa of Myrtaceae by primers SCoT1 and SCoT2, **M** refers to 1Kb (O'GeneRuler™1Kb) DNA Ladder marker, numbers from 1 to 26 refer to studied taxa.

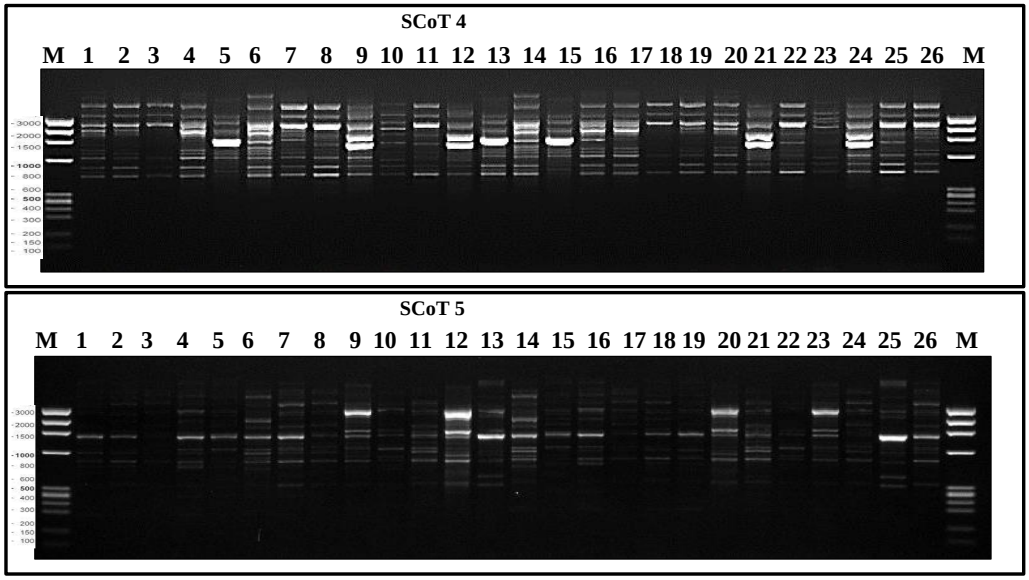

Plate V. SCoT profile of the studied taxa of Myrtaceae by primers SCoT4 and SCoT5, **M** refers to 1Kb (O'GeneRuler™1Kb) DNA Ladder marker, numbers from 1 to 26 refer to studied taxa

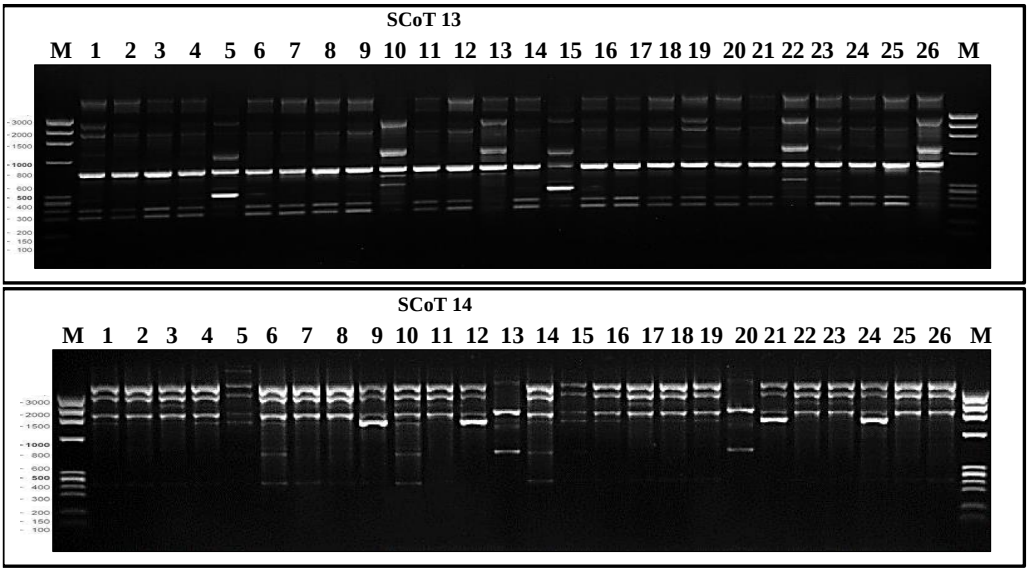

Plate VI. SCoT profile of the studied taxa of Myrtaceae by primers SCoT13 and SCoT14, **M** refers to 1Kb (O'GeneRuler™1Kb) DNA Ladder marker, numbers from 1 to 26 refer to studied taxa

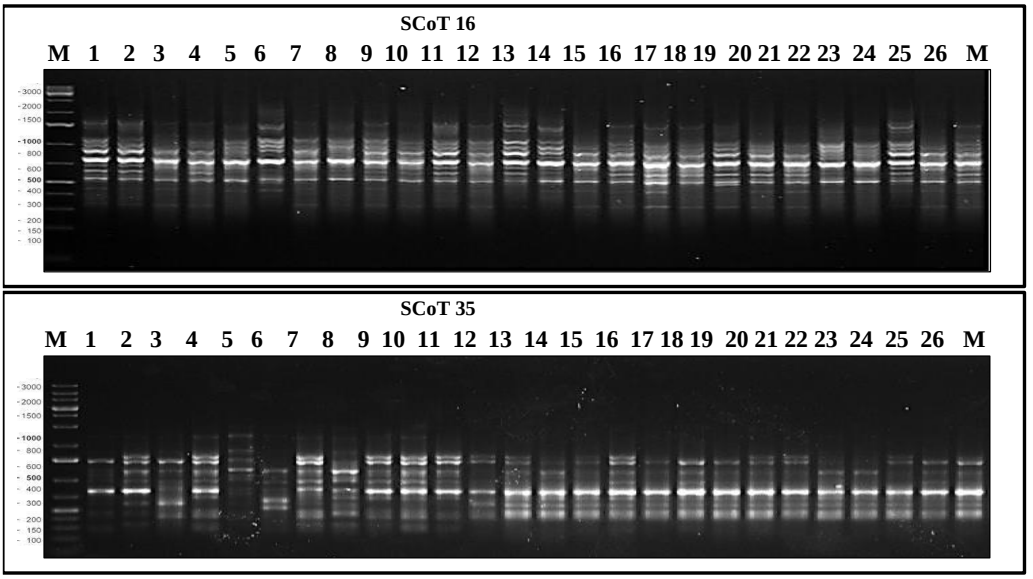

Plate VII: SCoT profile of the studied taxa of Myrtaceae by primers SCoT16 and SCoT35, **M** refers to 1Kb (O'GeneRuler™1Kb) DNA Ladder marker, numbers from 1 to 26 refer to studied taxa

Table 6. Specific markers and number of amplified fragments of studied taxa based on SCoT analysis. Abbreviations: (AB) amplified band, (SM) spesific marker.

Primer / Taxa	SCoT 1		SCoT 2		SCoT 4		SCoT 5		SCoT 13		SCoT 14		SCoT 16		SCoT 35		T S M
	AB	SM	AB	SM	AB	SM	AB	SM	AB	SM	AB	SM	AB	SM	AB	SM	
Acca sellowiana	1	0	9	0	9	0	2	0	7	0	4	0	7	0	3	0	0
Agonis flexuosa	2	0	9	0	7	0	3	0	6	0	3	0	6	0	7	0	0
Backhousia citriodora	5	0	6	0	5	0	3	0	6	0	4	0	6	0	5	0	0
Callistemon rigidus	4	0	9	0	8	0	5	0	6	0	4	0	8	0	8	0	0
C. viminalis	2	0	9	0	5	0	2	0	8	0	5	1	6	0	6	4	5
Corymbia ficifolia	3	0	10	0	10	0	7	0	7	0	5	0	8	1	5	2	3
Eucalyptus camaldulensis	5	0	10	0	7	0	7	0	6	0	3	0	7	1	6	0	1
Eugenia supraaxillaris	3	0	5	0	7	0	5	0	6	0	3	0	5	0	9	0	0
E. uniflora	3	1	4	0	9	0	6	0	7	0	4	0	7	0	6	0	1
Lophostemon confertus	2	0	9	0	9	0	3	0	7	0	7	0	5	0	8	0	0
Melaleuca armillaris	4	0	5	0	8	0	7	0	7	0	4	0	6	0	6	0	0
M. ericifolia	4	0	5	0	8	0	7	0	7	0	4	0	7	0	6	0	0
M. leucadendra	7	3	10	0	9	0	7	0	9	0	4	0	9	0	7	0	3
M. linariifolia	5	0	8	0	10	0	10	1	6	0	6	0	7	0	5	0	1

Primer / Taxa	SCoT 1		SCoT 2		SCoT 4		SCoT 5		SCoT 13		SCoT 14		SCoT 16		SCoT 35		T S M
	AB	SM	AB	SM	AB	SM	AB	SM	AB	SM	AB	SM	AB	SM	AB	SM	
Metrosideros excelsa	3	0	4	0	5	0	3	0	6	0	4	0	5	0	6	0	0
Myrtus communis	2	0	6	0	9	0	7	0	7	0	4	0	7	0	7	0	0
Pimenta dioica	5	0	9	0	8	0	3	0	6	0	5	1	8	0	5	0	1
Pi. Racemose	1	0	5	0	5	0	3	0	6	0	3	0	6	0	5	0	0
Psidium cattleyanum	4	0	8	0	7	0	3	0	7	0	4	0	8	0	5	0	0
Ps.guajava	3	0	6	0	9	0	5	0	6	0	4	0	5	0	6	0	0
Syzygium antisepticum	4	0	10	0	7	0	7	0	5	0	4	0	6	0	5	0	0
S. jambos	3	0	9	0	8	0	2	0	8	0	4	0	3	0	4	0	0
S. malaccense	2	0	10	1	6	0	6	0	7	0	4	0	3	0	5	0	1
S. paniculatum	2	0	9	0	9	0	6	0	7	0	4	0	9	1	5	0	1
S. samarangense	2	0	10	0	8	0	8	0	8	0	3	0	5	0	5	0	0
Xanthostemon fruticosus	4	0	5	0	8	0	7	0	14	2	3	0	7	0	5	0	2
Total polymorphic bands	14	4	24	1	15	0	16	1	16	2	10	2	13	3	17	6	19 125

Numerical analysis based on macromorphological characters

In the present study, the taxonomic treatment based on 85 morphological characters including leaf macromorphology and lamina vein architecture characters were scored as binary codes (0 & 1) to clarify the taxonomic relationships among the studied taxa.

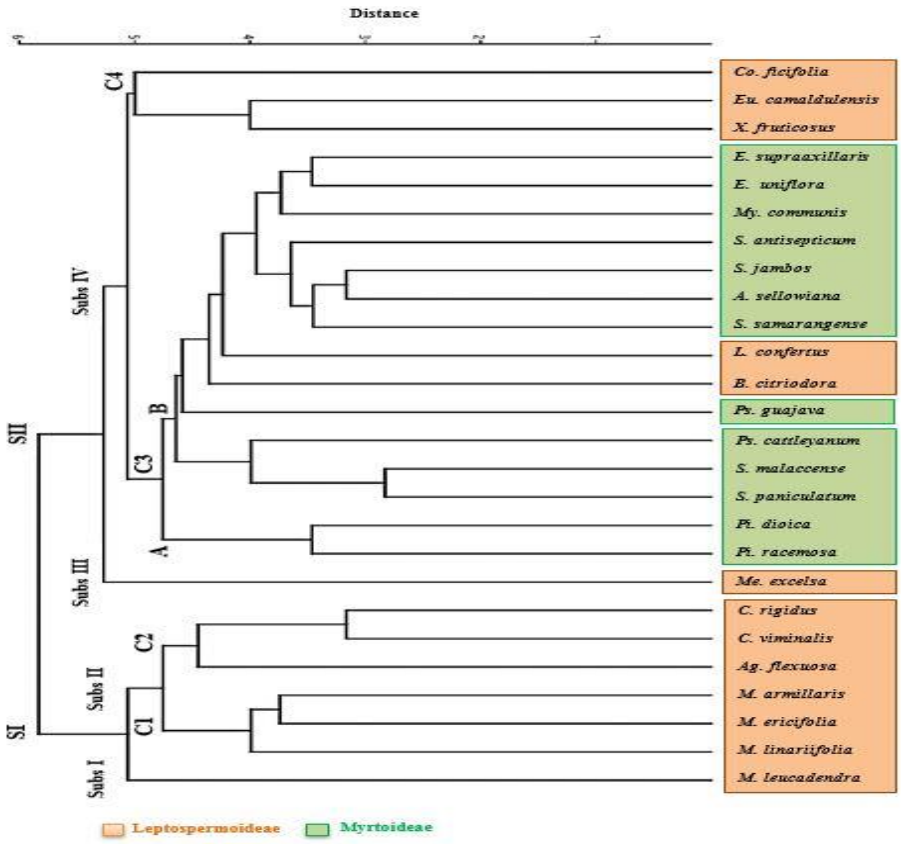

Fig.1. Dendrogram indicating the relationships among the studied taxa of Myrtaceae based on numerical analysis of macromorphological characters

From the obtained dendrogram (Fig. 1), the taxa under investigation separated under two main series (SI & SII). SI comprises of seven studied species belonging to subfamily Leptospermoideae (with capsular fruit) *viz. Melaleuca leucadendra, M. linariifolia, M. ericifolia, M. armillaris, Agonis flexuosa, Callistemon rigidus, C. viminalis.* In Series I, taxa grouped together based on leaves with three to numerous basal veins. SII comprises the rest 19 studied species, all 13 Myrtoideae taxa (with berry fruit) *viz. Acca sellowiana, Eugenia supraaxillaris, E. uniflora, Myrtus communis, Pimenta dioica, Pi. racemosa, Psidium cattleyanum, Ps. guajava, Syzygium antisepticum , S. jambos, S. malaccense, S.*

paniculatum, S. samarangense in addition to six Leptospermoideae taxa *viz. Backhousia citriodora, Corymbia ficifolia, Eucalyptus camaldulensis Lophostemon confertus, Metrosideros excelsa, Xanthostemon fruticosus.* Taxa in SII grouped together owing to sharing of pinnate venation with one basal vein.
SI subdivided into two subseries (Subs I & Subs II). Subs I, includes only *Melaleuca leucadendra* due to Cladodromous framework of major secondary vein and freely ramified tertiary vein fabric. Subs II, divided into two clusters (C1&C2). C1 included *M. linariifolia, M. ericifolia, M. armillaris* owing to linear lamina shape, acute cuneate apex, lacking of areolation, absence of marginal ultimate veinlet. C2 included *Agonis flexuosa, Callistemon rigidus, C. viminalis* owing to presence of rounded conspicuous oil glands, sharing of alternate leaves, absence of 5^{o} vein fabric, lacking of areolation and branched of freely ending veinlet. Also, the dendrogram exhibits clustering of genus *Callistemon* with *Melaleuca*. This is in accordance with (Ladiges et al. 1999 and Brown et al. 2001) who stated that, there is argument regarding the generic limits of the two genera.
SII subdivided into two subseries (Subs III & Subs IV). Subs III *Metrosideros excelsa* (Leptospermoideae) separated away from the remaining taxa through unique morphological characters *viz.* abruptly increase proximal of major secondary angle, variable exterior tertiary course and dichotomizing freely ending veinlets. Subs IV divided into two clusters (C3 & C4). C3 divided into two groups (A & B). Groups A&B includes all Myrtoideae taxa with Backhousia *citriodora and Lophostemon confertus* (Leptospermoideae) capsular fruit and each of them is separated as a single entity. Numerous characteristics of leaf macromorphology and lamina vein architecture of the berry genera are shared with *Backhousia* and *Lophostemon.* C4 included *Corymbia ficifolia, Eucalyptus camaldulensis, Xanthostemon fruticosus* (Leptospermoideae) due to sharing of excurrent major secondary attachment, preimarginal veins and well-developed areolation.
Regarding the results of the above dendrogram, the separation of the studied species into series I & II is in accordance to some extent with Niedenzu (1893) who stated that Myrtaceae divided into two subfamilies Myrtoideae and Leptospermoideae based on fruit type. The deviation from Niedenzu system may be due to significant variation between Myrtoideae and Leptospermoideae in other different criteria.

Numerical analysis based on molecular characters

The resulted cluster analysis based on SCoT markers (125 molecular bands) was represented as dendrogram (Fig. 2). The dendrogram clarifies that *Melaleuca leucadendra* basally separated away as a distinct entity. This was confirmed by several authors who clarify that the tribe Melaleuceae includes a variety of myrtaceous species with bottlebrush inflorescence, such as *Agonis* (Bentham & Hooker, 1867), *Callistemon* (Craven,2006). Johnson & Briggs (1984) stated that

Melaleuceae have never been fully characterized morphologically, and being an informal group based on the sharing of capsular fruit, sessile flowers, and narrow leaves. Edwards *et al*. (2010) strongly supported the non-monophyly *Melaleuca* with the genera in their study, and separated *M. leucadendra* (broad leaf) in one separate clade using cpDNA sequence.

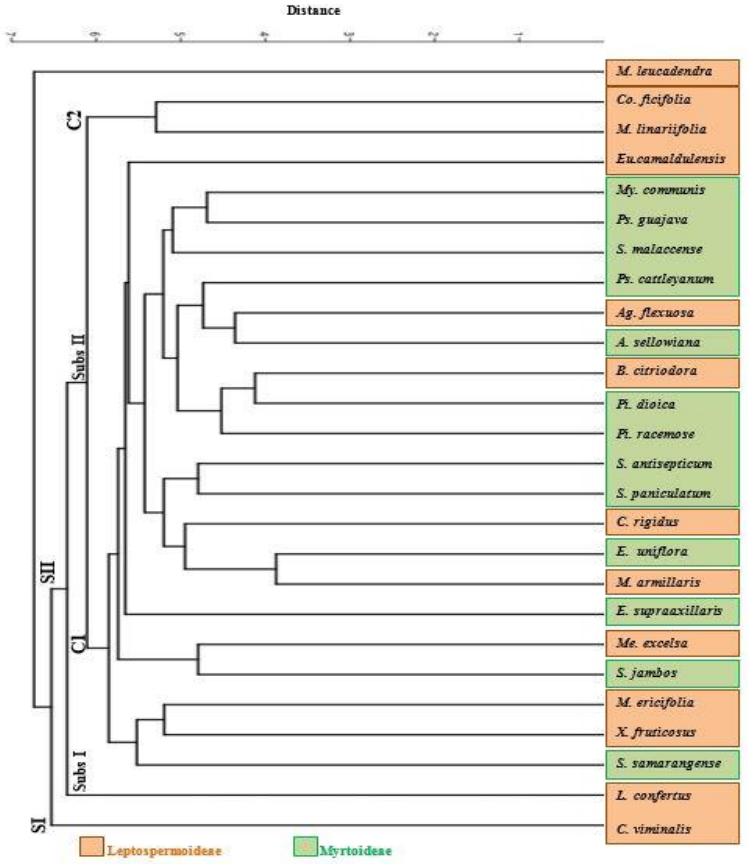

Fig.2.

Dendrogram of the studied taxa of Myrtaceae based on numerical analysis of molecular characters

The remaining taxa under investigation divided into two main series (SI & SII). SI included *Callistemon viminalis*. The segregation of *C. viminalis* as a distict identity due to the largest number of Scot specific markers (five positive markers; one marker scored for Scot 14 and four markers scored for Scot35). SII comprises all the remaining taxa Myrtoideae and Leptospermoideae. SII subdivided into two subseries (I, II), two clusters(C1&C2). The dendrogram showed that the studied taxa of subfamily Myrtoideae (with berry fruits) are scattered with the capsular fruited Leptospermoideae taxa (with capsule fruits). This result is in

harmony with that of Wilson (2011) who stated that the classification of the family Leptospermoideae and Myrtoideae was invalid.

Numerical analysis based on combined characters

The combination of the obtained character states of leaf macromorphology, lamina vein architecture and molecular analysis of the studied taxa contributes to construct a combined dendrogram (Fig.3). The resulted dendrogram elucidates that *Melaleuca leucadendra* basally separated in a distinct identity. The rest taxa under investigation divided into two main series (SI & SII). SI contains five species Leptospermoideae *viz. M. ericifolia, M. linariifolia, M. armillarias, Callistemon rigidus, C. viminalis.* SII includes all Myrtoideae taxa with seven Leptospermoideae taxa *viz. Corymbia ficifolia, Eucalyptus camaldulensis, Agonis flexuosa, Backhousia citriodora, Lophostemon confertus, Metrosideros excelsa, Xanthostemon fruticosus*. SI subdivided into two subseries (Subs I& Subs II). SII subdivided into two subseries (Subs III& Subs IV), two clusters and two groups.
Amazingly, *Corymbia* and *Eucalyptus* didn't cluster together although Wilson (2011) allocated them together in tribe Eucalypteae. *Callistemon* and *Melaleuca* are grouped as supported by (Edwards *el al*. 2010) and confirmed with morphological characters. *Backhousia* grouped with fleshy fruited taxa in clusters C2 while Wilson (2011) placed it in tribe Backhousieae and the fleshy fruited taxa in tribe Myrteae.

The combined data resulted dendrogram grouped all the 13-studied berry fruited Myrtoideae with seven capsular fruited Leptospermoideae, while the remaining five capsular fruited taxa grouped together. This dendrogram agrees with the morphological- based dendrogram. Both of them recommend the maintenance of two subfamilies based on fruit type (Hickey & King. 1988; Judd et al. 1999 and Tantawy, 2004), despite both dendrograms showed deviation from the original of Niedenzu (1893).

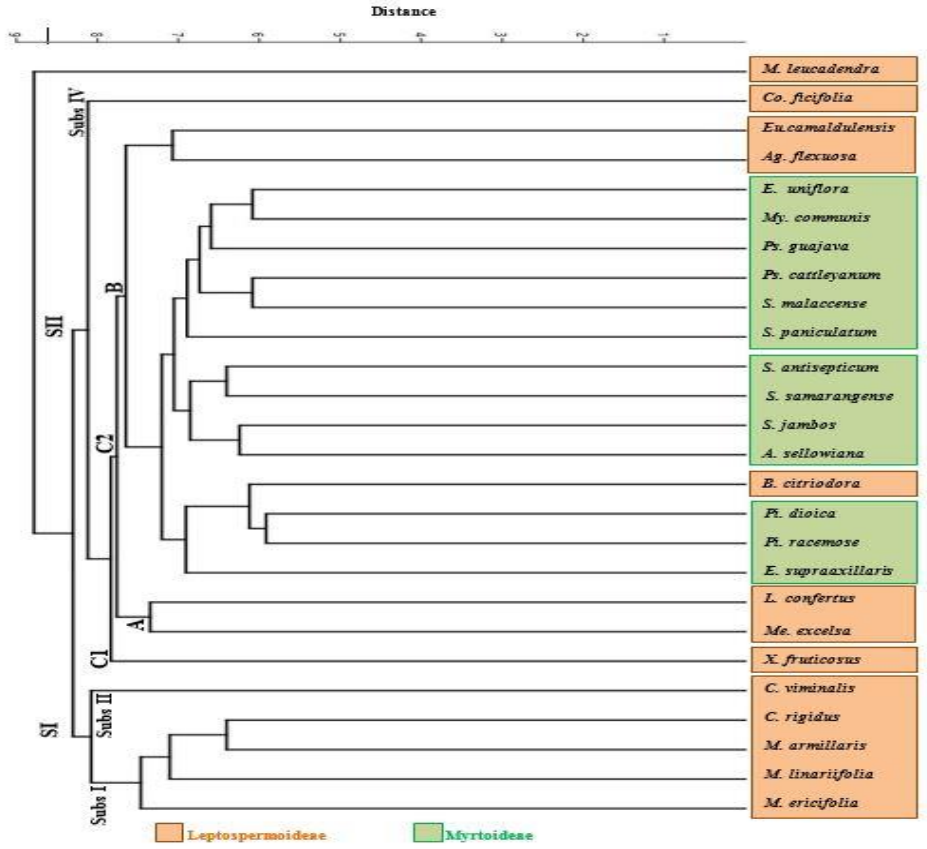

Fig.3. Dendrogram indicating the relationships among the studied taxa of Myrtaceae based on numerical analysis of combined characters.

Conclusion

The current study recommended more assignment must be performed on berry and capsular fruited Myrtaceae taxa to classify in one subfamily Myrtoideae or two separate subfamilies Myrtoideae and Leptospermoideae, Additionally, the molecular data obtained from the present study did not always was in congruent with the result of traditional taxonomy. So according to the present work and the opinion which suggests that key clade connections within the Myrtaceae cannot be resolved only by morphological data (Wilson et al. 2001& 2005; Biffin et al. 2007), further studies based on more taxonomic criteria and molecular markers should be performed to have a clear insight.

References

Bailey L.H. and Bailey E.Z. (1976) *A Concise Dictionary of Plants Cultivated in the U.S. and Canada. Hortus Third* 'Revised by staff of the L.H. Bailey Hortium'. The Macmillan Publishing Company, New York, 450 pp.

Bailey L.H. (1949) *Manual of Cultivated Plants*. The Macmillan Company, New York, pp.431-450.

Bentham G. and Hooker J.D. (1867) *Genera Plantarum* 1, pars III. Lovell Reeve & Co., Londini, London, pp. 721–1040.

Biffin E. Lucas E.J. Craven L.A. Costa I.R. Harrington M.G. & Crisp M.D. (2010) Evolution of exceptional species richness among lineages of flesh fruited Myrtaceae. *Annals of Botany* 106: 79-93.

Brooker M.I.H. and Nicolle D. (2013) Atlas of leaf venation and oil gland patterns in the *Eucalyptus*. CSIRO Publishing, Collingwood, pp.5-15.

Byrne M., Marquez-Garcia M.I., Uren T., Smith D.S. and Moran G.F. (1996) Conservation and genetic diversity of microsatellite loci in the genus *Eucalyptus*. *Australian Journal of Botany* 44: 331–341.

Cardos C.M.V. and Sajo M.G. (2004) Vascularizac¸ ão foliar e a identificac¸ ão de espéciesde *Eugenia* L. (Myrtaceae) da bacia hidrográfica do Rio Tibagi, PR. *Revista Brasileira de Botânica* 27(1): 47-54.

Cardos C.M.V. and Sajo M.G. (2006) Nervac¸ ão foliar em espécies brasileiras de Myrtaceae Adans. *Acta Botanica Brasilica* 20: 657–669.

Christenhusz M.J.M. and Byng J.W. (2016) The number of known plants species in the world and its annual increase. *Phytotaxa* 261(3): 201-217.

Collard B.C.Y. and Mackill D.J. (2009) Start Codon Targeted (SCoT) polymorphism: A simple novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology* 27: 86–93.

Costa C.G. Machado R.D. and Fontenelle J.B. (1995) Sistema vascular em folhas de *Eugenia* L. (Myrtaceae). *Bradea* 6(42): 345-356.

Craven L.A. (2006) New combinations in *Melaleuca* for Australian species of *Callistemon* (Myrtaceae). *Novon* 16(4): 468–475. DOI: 10.3417/1055-3177(2006)16[468:NCIMFA]2.0.CO;2

Cronquist A. (1981) *An Integrated System of Classification of Flowering Plants*. Columbia University Press, New York, 1262 pp.

Defaveri A.C.A., Arruda R.C.O. and Sato A. (2011) Leaf anatomy and morphology of *Eugenia rotundifolia* applied to the authentication of the "abajurú" commercially sold. *Brazilian Journal of Pharmacognosy* 21(3): 373-381.

Edwards R.D., Craven L.A., Crisp M.D. and Cook L.G. (2010) *Melaleuca* revisited: cpDNA and morphological data confirm that *Melaleuca* L. (Myrtaceae) is not monophyletic. *Taxon* 59:744–754.

Ellis B., Daly D.C., Hickey L.J., Johnson K.R., Mitchell J.D., Wilf P. and Wing S.L. (2009) *Manual of Leaf Architecture*. Cornell University Press, Ithaca, https://hdl.handle.net/10088/93918

Farmacopeia Brasileira, 2002. *Monografia Goiabeira*, 4th ed. parte 2. fasc. 4, Atheneu, São Paulo.

Goodger J.Q., Samiddhi S., Nicolle D. and Woodrow I.E. (2018) Differential metabolic specialization of foliar oil glands in *Eucalyptus brevistylis* Brooker (Myrtaceae), *Tree Physiology*, Volume 38, Issue 10, October 2018, pp. 1451-1460, https://doi.org/10.1093/treephys/tpy077

Grattapaglia D., Vaillancourt R.E., Shepherd M., Thumma B.R., Foley W., Külheim C., Potts B. M. and Myburg A.A. (2012) Progress in Myrtaceae genetics and genomics: *Eucalyptus* as the pivotal genus. *Tree Genetics & Genomes* 8: 463–508.

Hambali G.G., Sunarti S. and Low Y.W. (2017) *Syzygium jiewhoei* (Myrtaceae), a new endemic tree from Western New Guinea, Indonesia. *Gardens' Bulletin Singapore* 69(2): 201–210.

Han G.H., Su Q., Wang W.S., Jia Z.G., Hong Q.B. and Liang G.L. (2011) Establishment and application of SCoT molecular marker system for citrus. *Acta Horticulturae Sinica* 38(7): 1243-1250.

Heywood V.H. (1993). *Flowering plants of the world* (Rev.). Oxford University Press, New York, pp. 239-240.

Hickey M. and King C. (1988) 100 *Families of Flowering Plants*. Cambridge University Press, Cambridge, UK, pp.130–133.

Hora F.B. (1978) Myrtaceae. In: V.H. Heywood (ed.), *Flowering Plants of the World*: Oxford University Press, London, pp. 161-162.

IPNI (2023). International Plant Name Index, https://www.ipni.org/ The Royal Botanic Gardens, Kew, Harvard University Herbaria & Libraries and Australian National Herbarium.

Johnson L.A.S. and Briggs B.G. (1984) Myrtales and Myrtaceae - a phylogenetic analysis. *Annals of the Missouri Botanical Garden* 71: 700-756.

Judd W. S. Campbell C. S. Kellogg E.A. and Stevens P.F. (1999) *Plant systematics, a phylogenetic approach*. Sinauer Associates, Sunderland, Massachusetts, 464 pp.

Khan D. Zaki M. J. and Khan A. (2016) Leaf Architecture, Ornamentation and Estimation of Lamina Area in *Myrtus communis* L. (Myrtaceae). *International Journal of Biology and Biotechnology* 13(4): 537-550.

Klucking E.P. (1988) *Leaf venation patterns*: Myrtaceae, vol. 3, J. Cramer, Stuttgart, 279 pp.

Keszei A., Brubaker C. L., Carter R., Köllner T., Degenhardt J. and Foley W. J. (2010) Functional and evolutionary relationships between terpene synthases from Australian Myrtaceae. *Phytochemistry* 71: 844-852.

Lambert J. B., Donnelly E. W., Heckenbach E. A., Johnson C. L., Kozminski, M. A., Wu Y. and Santiago-Blay J. A. (2013) Molecular classification of natural exudates in rosids. *Phytochemistry* 94: 171-183.

Larano A.A.P. and Buot Jr.I.E. (2010) Leaf Architecture of selected species of Malvaceae *sensu* APG and its Taxonomic Significance, *Philippines Journal of Systematic Biology* 4: 54-211

Ash A., Ellis, B., Hickey L., Johnson, K., Wilf, P. and Wing, S. (1999). *Manual of Leaf Architecture: Morphological description and categorization of dicotyledonous and net-veined monocotyledonous angiosperms.* Smithsonian Institution, Washington DC, pp. 26-45. DOI:10.13140/2.1.3674.5282.

Ladiges P.Y., McFadden G.I., Middleton N., Orlovich D.A., Treloar N. and Udovicic F. (1999). Phylogeny of *Melaleuca, Callistemon*, and related genera of the Beaufortia Suballiance (Myrtaceae) Based on 5S and ITS-1 Spacer Regions of nrDNA. *Cladistics* 15(2):151-172. doi: 10.1111/j.1096-0031.1999.tb00257.x. PMID: 34902912.

Lucas E.J. (2007). Myrtaceae. In: Heywood V.H., Brummitt R.K., Culham A. and Seberg O. (Eds.) *Flowering Plant Families of the World*. Royal Botanic Gardens, Kew, UK, pp. 225–226.

Lughadha E. N. and Proenca C. **(1996)** A Survey of the Reproductive Biology of the Myrtoideae (Myrtaceae). *Annals of the Missouri Botanical Garden* 83(4):480–503.

Marsh K. J., Kulheim C., Blomberg S. P., Thornhill A. H., Miller J. T., Wallis I. R., Nicolle D., Salminen J.-P. and Foley W. J. (2017) Genus-wide variation in foliar polyphenolics in eucalypts. *Phytochemistry* 144: 197-207. doi: 10.1016/j.phytochem.2017.09.014. Epub 2017 Sep 25. PMID: 28957714.

Niedenzu F. (1893) Myrtaceae. In: Engler A, Prantl K. (Eds.) Die Natürlichen Pflanzenfamilien 3(7). Leipzig, Verlag von Wilhelm Engelmann, pp. 57-105.

Oliveiraa E.F., Bezerraa D.G., Santosa M.L., Rezendeb M.H. and Paulaa J.A.M. (2017) Leaf morphology and venation of *Psidium* species from the Brazilian Savanna. *Brazilian Journal of Pharmacognosy* 27: 407–413.

Ryan P.D., Harper D.A.T. and Whalley J.S. (1995) *PALSTAT, Statistics for palaeontologists.* Chapman & Hall, London.

Schmid R. (1980) Comparative anatomy and morphology of *Psiloxylon* and *Heteropyxis*, and the subfamilial and tribal classification of Myrtaceae. *Taxon* 29: 559-595.

Shahlaei A. Torabi S. and Khosroshahli M. (2014) Efficiacy of SCoT and ISSR marekers in assesment of tomato (*Lycopersicum esculentum* Mill.) *Genetic diversity* 5(2): 14-22.

Stevens P. F. (2001 onwards). Angiosperm Phylogeny Website. Version 14, July 2017 [and more or less continuously updated since]." will do. http://www.mobot.org/MOBOT/research/APweb/.

Tantawy M.E. (2004) Morpho-anatomical Study on Certain Taxa of Myrtaceae. *Asian Journal of Plant Science* 3:274-285.

TROPICOS. Nomenclature Database of the Missouri Botanical Gardens. http://www.tropicos.org/

Wagner W.L., Herbst D.R. and Sohmer S.H. (1990) *Manual of the Flowering Plants of Hawaii.* University of Hawaii Press and Bishop Museum Press, Honolulu, Hawaii, 1952 pp.

Westbrook J., Kitajima K. and Wright S. J. (2009) Patterns of silica and fiber accumulation in the leaves of 400 neotropical woody species: Physical defense in a phylogenetic context. *Botany and Mycology. Snowbird, Utah, July* 25-29, 214 pp.

Wilson P. G. (2011) Myrtaceae**.** In: Kubitzki K. (Ed.). The Families and Genera of Vascular Plants, Volume 10. Flowering Plants: Eudicots - Sapindales, Cucurbitales, Myrtaceae. Springer Verlag, Heidelberg, pp. 212–271.

Wilson P.G. O'Brien M.M. Heslewood M.M. and Quinn C.J. (2005) Relationships within Myrtaceae *sensu lato* based on a matK phylogeny. *Plant Systematics and Evolution* 251: 3–19.

Wilson P.G. O'Brien M.M. Gadek P.A. and Quinn C.J. (2001) Myrtaceae Revisited: A reassessment of Infrafamilial Groups. *American Journal of Botany* 88 (11): 2013–2025.

Wu J.M., Y.R. Li, L.T. Yang, Fang F.X., Song H.Z., Tang H.Q., Wang M. and Weng M.L. (2013): cDNA-SCoT: a novel rapid method for analysis of gene differential expression in sugarcane and other plants. *Australian Journal of crop science* 7(5): 659-664.

Xiong F.Q., Jiang J., Zhong R.C., Han Z.Q., He L.Q., Li Z., Zhuang W.J. and Tang R.H. (2010) Application of SCoT molecular marker in genus *Arachis*. *Acta Agronomica Sinica* 36(12): 2055-2061.

Zhang J.Y., Guo D.L., Gong Y., Liu C.H., Li M. and Zhang G.H. (2011) Optimization of start codon targeted polymorphism PCR (SCoT-PCR) system in *Vitis vinifera*. *Journal of Fruit Science* 28(2): 209-214.0