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Field Level Identification of *Cinnamomum* Species in Sri Lanka Using a Morphological Index

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ABSTRACT

Cinnamomum zeylanicum Blume is a commercially important species cultivated in Sri Lanka and traded as Ceylon cinnamon or true cinnamon. In addition, seven endemic wild species of the genus *Cinnamomum* have been reported in Sri Lanka. The literature on wild relatives of *C. dubium*, *C. capparucoronde*, *C. citriodorum*, *C. litseaefolium*, *C. ovalifolium*, *C. rivulorum* and *C. sinharajaense* is limited. Therefore, proper field level identification and differentiation of both wild and cultivated species are critical for the conservation and utilization of such species. Since the cinnamon is a cross-pollinated species, intra-species diversity is also a possibility. Our work focused on inter- and intra-species diversity of *Cinnamomum* leaf morphology to develop a "Leaf Morphological Index" for field-level identification of reported species. Forty accessions, representing a minimum of two from each species, collected from natural and cultivated habitats, were assessed with 12 morphological characters. The highest within-species variation was observed in *C. zeylanicum*, followed by *C. dubium*. Of the morphological characters, five-leaf traits, leaf shape, apex, base, venation, and size significantly contributed to the main principle components. Therefore, those traits were used for developing a leaf morphological index. The morphological index could distinguish all the species at the field level.

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INTRODUCTION

The genus *Cinnamomum* Schaeff, belongs to the family Lauraceae, and consists of about 250 species distributed in Asia, Australia, and the Pacific Islands. Of them, *Cinnamomum zeylanicum* Blume (syn. *Cinnamomum verum* J. Presl), *C. cassia* Blume and *C. camphora* Seib are commercially cultivated around the world. Other than *C. zeylanicum,* seven endemic wild species of the genus *Cinnamomum* have been reported in Sri Lanka (Kostermans, 1995). *C. dubium* Nees. is a widely distributed species whereas *C. capparu-coronde* Blume., *C. citriodorum* Thw., *C. litseaefolium* Thw., *C. ovalifolium* Wight., *C. rivulorum* Kosterm. and *C. sinharajaense* Kosterm. are found in limited habitats. *C. ovalifolium* and *C. sinharajaense* are only naturally grown in isolated habitats and do not exist in cultivation or other parts of the country (Kostermans, 1995). *C. litseaefolium* and *C. rivulorum* are categorized as endangered species in Sri Lanka while all other wild species are categorized as vulnerable species (MOE, 2012).

The demand for true cinnamon has an increasing trend worldwide as a high price spice crop due to ethnobotanical uses and recent scientific evidence on its medicinal and pharmacological properties. Several *in vitro* and *in vivo* evidences suggest that *C. zeylanicum* has anti-microbial, anti-parasitic, anti-oxidant and free radical scavenging properties and ability to lower blood glucose, serum cholesterol and blood pressure, suggesting beneficial cardiovascular effects (Shan *et al*., 2007; Singhe *et al.,* 2007; Elumalai *et al*., 2011; Ranasinghe *et al.,* 2013). Similarly, several other wild relatives of cinnamon have also gained worldwide attention recently.

The *Cinnamomum* flower has naturally evolved for cross-pollination, having "protogyneous-dichorgamy" breeding system, which may result in intraspecies genetic diversity (Krishnamoorthy *et al*., 1992). Therefore, field-level identification and differentiation of both wild and cultivated *Cinnamomum* species are challenging. Liyanage (2010) and Liyanage and Senanayake (2010) studied the distribution ecology of wild *Cinnamomum* in Sri Lanka. Abeysinghe and colleagues assessed the genetic relatedness of cultivated and wild cinnamon in Sri Lanka using cpDNA (Abeysinghe *et al.,* 2009). Abeysignhe *et al.* (2014) also conducted a preliminary study on the identification of *Cinnamomum* species using randomly amplified polymorphic DNA (RAPD). Morphological differences among a few wild relatives and cultivated *C. zeylanicum* species were assessed (Ariyaratne *et al.,* 2018). A study also suggested a possible correlation of leaf morphological traits with bark yield, oil yield, and oil quality (Wijesinghe *et al*., 2004). A key has been developed by Kostermans (1995) using the persistent nature of the perianth segment in the fruit cup, panicle length, leaf characters, and smell of various plant parts, although the use of this key is not pragmatic as panicle and flowers are not available throughout the year under field conditions. Kumarathilake (2012) developed a key for the identification of Sri Lankan *Cinnamomum* species based only on morphological characteristics including characteristics of immature bark. Recently, Azad and colleagues reported that some leaf morphological traits are age and environment independent (Azad *et al*., 2016). Consequently, the objective of the present study was to develop a morphological index and a practical botanical key for field-level identification of different *Cinnamomum* species present in Sri Lanka. The study was also extended to identify the within-species diversity of *C. zeylanicum* with special emphasis on recently released *Sri Gemunu* and *Sri Wijaya* varieties by the Department of Export Agriculture (DEA), Sri Lanka.

MATERIALS AND METHODS

Sample collection

Field surveys were carried out in seven agro-ecological regions to capture both within and between species diversity. A total of 40 samples including 30 wild and 10 cultivated plants were included in the analysis as given in Table 1. While some of the collections sites were new, others were selected based on the previous eco-geographic map developed by Liyanage (2010) and Liyanage *et al.* (2010). Cultivated *C. zeylanicum* samples were

Table 1: *Cinnamomum* species used in the study and their sampling locations.

Species	Location	GPS coordinates	Remarks	
			Location	Origin
C. capparu-coronde	NCRTC, *Thihagoda*	E-6.0263 N-80.5616	PL	Cu.
	NCRTC, *Thihagoda*	E-6.0258 N-80.5616	PL	Cu.
	NCRTC, *Thihagoda*	E-6.0249 N-80.5616	PL	Cu.
	MRS, *Delpitiya*	E-7.1346 N-80.5851	PL	Cu.
C. citriodorum	NCRTC, *Thihagoda*	E-6.0225 N-80.5623	PL	Cu.
	Balangoda-Norwood Road	E-6.7548 N-80.7204	PL	NG
	Watawala	E-6.5431 N-80.4344	NL	NG
C. Dubium	NCRTC, *Thihagoda*	E-6.0285 N-80.5610	PL	Cu.
	NCRTC, *Thihagoda*	E-6.0279 N-80.5610	PL	Cu.
	NCRTC, *Thihagoda*	E-6.0270 N-80.5610	PL	Cu.
	MRS, *Delpitiya*	E-7.1334 N-80.5856	PL	Cu.
	Sinharaja MAB	E-6.4108 N-80.5088	NL	NG
	Sinharaja MAB	E-6.4267 N-80.4146	NL	NG
	Balangoda-Norwood Road	E-6.7537 N-80.7200	NL	NG
	Balangoda-Norwood Road	E-6.7454 N-80.7440	NL	NG
	Balangoda-Norwood Road	E-6.7548 N-80.7224	NL	NG
C. litseaefolium	BGH, *Hakgala*	E-6.9683 N-80.7700	PL	Cu.
	NCRTC, *Thihagoda*	E-6.0297 N-80.5600	PL	Cu.
	MRS, *Delpitiya*	E-7.1325 N-80.5879	PL	Cu.
C. ovalifolium	HPNP, *Ohiya*	E-6.8035 N-80.8031	NL	NG
	HPNP, *Ohiya*	E-6.8025 N-80.8027	NL	NG
	HPNP, *Ohiya*	E-6.7948 N-80.8060	NL	NG
	BGH, *Hakgala*	E-6.9696 N-80.7700	PL	Cu.
C. rivulorum	NCRTC, *Thihagoda*	E-6.0222 N-80.5624	PL	Cu.
	NCRTC, *Thihagoda*	E-6.0228 N-80.5624	PL	Cu.
	NCRTC, *Thihagoda*	E-6.0231 N-80.5624	PL	Cu.
C. sinharajaense	NCRTC, *Thihagoda*	E-6.0241 N-80.5619	PL	Cu.
	NCRTC, *Thihagoda*	E-6.0239 N-80.5619	PL	Cu.
	NCRTC, *Thihagoda*	E-6.0235 N-80.5619	PL	Cu.
	Sinharaja MAB	E-6.4352 N-80.4197	NL	NG
C. zeylanicum (*wild*)	GONP, *Bibila*	E-7.1905 N-81.3745	NL	NG
	GONP, *Bibila*	E-7.1885 N-81.3732	NL	NG
	GONP, *Bibila*	E-7.1892 N-81.3711	NL	NG
	Balangoda-Norwood Road	E-6.7567 N-80.7315	NL	NG
C. zeylanicum (*Sri wijaya)*	NCRTC, *Thihagoda*		PL	Cu.
	MRS, *Delpitiya*		PL	Cu.
			PL	Cu.
C. zeylanicum (*Sri gamumu*)	NCRTC, *Thihagoda*		PL	Cu.
	MRS, *Delpitiya*		PL	Cu.
	Nillambe		PL	Cu.

Note: *NL*=New location, *PL*=Previously known location, *Cu*=Cultivated plants, and *NG*=Naturally grown plants.

collected from the following locations: The National Cinnamon Research and Training Centre (NCRTC), Palolpitiya, Thihagoda, Matara; Mid Country Research Station (MRS), Delpitiya; Sub Research Stations of Wariyagala, and Nillambe, and Hakgala Botanical Gardens. Samples of naturally grown populations were collected from Sinharaja Man and Biosphere (MAB) Reserve, Gal Oya National Park (GONP), Bibila, Horton Plains National Park (HPNP) and Balangoda-Norwood Road. Voucher specimens were prepared and identified using specimens at the National Herbarium at the Royal Botanical Gardens, Peradeniya, Sri Lanka.

Selection of characters for the development of morphological index

As the initial step, both wild and the cultivated species were evaluated on ten identified leaf morphological characters and two bark organoleptic characters. The leaf traits included leaf apex, base, shape, size, venation, margin, texture, arrangement, leaf colour and flush colour. The fragrance and taste of bark were included as bark traits. All the studied characters were recorded following the cinnamon descriptor (TURIS 2013 project, 2016). The 10th leaf from the tip of the branch with no symptoms of pest and disease attack was selected for assessing leaf colour, texture, margin, and venation. The leaf and flush colour were recorded using the Royal Horticultural Society colour chart (RHS, 2015).

Principle component analysis (PCA) was performed on 12 characters to select the most appropriate traits for the total variance, and such characters were used to develop leaf morphological index. According to the PCA, the first PC (PC1) accounted for 63% of the total variance. Leaf venation pattern, leaf size, leaf base and leaf apex positively correlated with the PC1. The second PC (PC2) described 17% of the total variation and the leaf shape, colour, and bark fragrance contributed to the PC2 (Table 2). In order to develop the morphological index, age and environment independent leaf morphological characters such as shape, apex, and base (Azad *et al.*, 2015), and venation pattern and leaf size were used.

Table 2: Total Variance explained based on the Principal Component Analysis.

Component	Initial Eigen Values		
	Total	% Variance	Cumulative %
1	5.06	63	63
2	1.39	17	80
3	0.77	9	90
4	0.61	9	100

Development of leaf morphological index

Thirty matured leaves were selected from each plant and leaf shape, apex, base, and venation pattern along with size were observed. This was done for each species on four different characters (Table 3). Selected observations were grouped into four separate measurement scales by giving the lowest value to *C. ovalifolium* and the highest value to *C. dubium*. The values were assigned considering their morphological relativity previously described by Saumyasiri *et al.* (2006). Accordingly, *C. ovalifolium* and *C. dubium* are the most distinctly related (Saumyasiri *et al.,* 2006) whilst other species are located in-between (Table 3). As such, four different morphological index-scoring scales were developed in this study for selected leaf characters (Table 3). The "Ideal Morphological Index Value" *(IMIV)* was introduced for each species by adding Morphological Index Score *(MIS)* values of the apex, base, shape and venation (Table 3). The leaf size was incorporated with the leaf shape (Figure 1 D). Both leaf apex and base were evaluated on a 1 to 10 score scale while leaf shape and the venation were evaluated on a 1 to 20 score scale (Table 3 and Figure 1).

Table 3: Ideal morphological index values for genus *Cinnamomum* in Sri Lanka.

Spp.	Typical observations				Morphological Index Score (MIS)				*IMIV*
	Apex	Shape	Base	Venation	Apex	Shape	Base	Venation	
C1	Obtuse	Oval	Acute	A	1	3	1	2	7
C2	Acute	Ovate	Subacute	A	3	3	3	2	11
C3	Acuminate	Narrowly elliptic	Subacute	B	5	3	3	5	16
C4	Acute	Lanceolate	Subscute	C	3	8	3	7	21
C5	Acute	Lanceolate	Obtuse	C	3	8	8	7	26
C6	Acute	Ovate	Rounded	d/c	3	12	10	9	34
C7	Acute	Oval	Obtuse	d/h	3	14	8	14	39
C8	Narrowly acuminate	Elliptic	Obtuse	e/h	7	16	8	16	47
C9	Short acuminate	Ovate-lanceolate	Rounded	F	6	20	10	18	54
C10	Long acuminate	Ovate-lanceolate	Rounded	G	10	20	10	20	60

Note: C1=*C. ovalifolium*, C2=*C. litseaefolium*, C3=*C. citriodorum*, C4=*C. capparu-coronde*, C5=*C. zeylanicum* (wild), C6=*C. zeylanicum (Sri Wijaya)*, C7=*C. zeylanicum (Sri Gemunu),* C8=*C. sinharajaense*, C9=*C. rivulorum*, C10=*C. dubium. IMIV:* Ideal morphological index values for different species

Assessment of leaf morphological index values for genus *Cinnamomum* in Sri Lanka

All the collected species were evaluated based on the developed morphological index (Figure 1) to address inter- and intra-species diversity. The *MIS* values were recorded for each species and the average morphological index score *(AMIS)* and standard deviations were calculated (Table 4). By adding all four *AMIS* together, an average morphological index value *(AMIV*) was introduced with standard deviations. Based on *AMIV's* standard deviations, morphological index value range *(MIVR)* was introduced for each species to address their morphological diversities (Table 4).

Statistical analysis

The technical replication depended on the availability of sampling locations. As such, *C. ovalifolium*, *C. rivulorum,* and *C. citriodorum* consisted of three replicates while *C. capparu-coronde, C. sinharajaense* and *C. litseaefolium* had four replicates. Further, *C. zeylanicum* and *C. dubium* consisted of 9 replicates each. The principal component analysis (PCA) was performed including 12 observed characters to identify major components responsible for the total variation. Minitab (version 18) software package was used for statistical analysis.

Development of botanical key for field level identification of *Cinnamomum* species

A botanical key was developed for field-level identification of *Cinnamomum* species by modifying Kostermans (1995), Kumarathilake (2012), and Ariyarathne *et al.* (2018). Because of the limited availability of flowers and immature leaves throughout the year and difficulty in identifying the correct maturity stage, more attention was given to the leaf venation pattern and its association than to other characters.

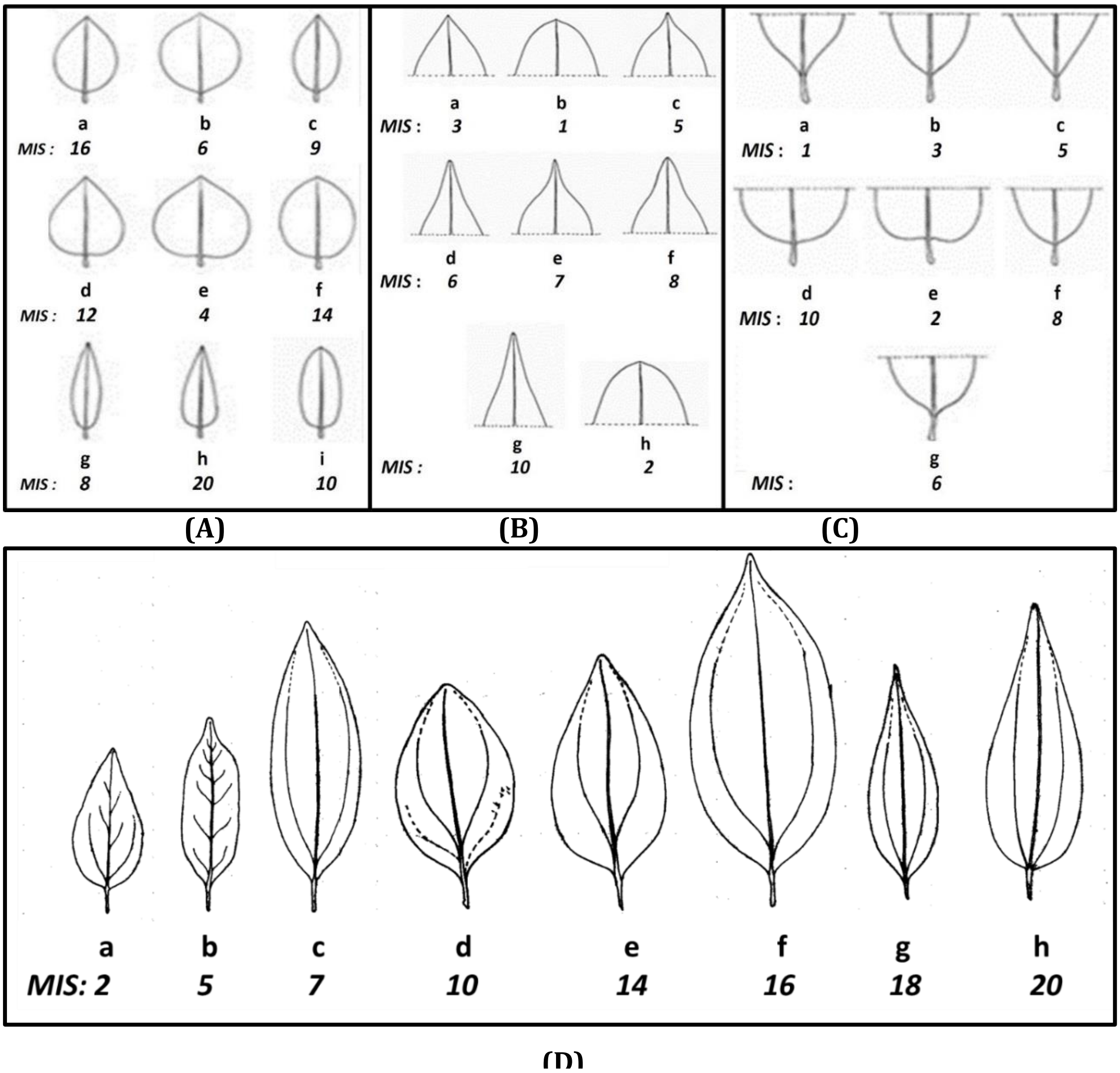

Figure 1: Leaf morphological characters and their index scores for genus *Cinnamomum* in Sri Lanka.

Notes: Leaf shape (A) (a- Elliptic, b- Broadly elliptic, c- narrowly elliptic, d-ovate, e- broadly ovate, f- oval, g- lanceolate, h- ovate-lanceolate, i- oblong-lanceolate); Leaf Apex (B) (a- Acute, b- Obtuse, c- Acuminate, d- Blunt, e- Long acuminate, f- Narrowly acuminate, g- Acuminate with broad acumen, h- Short acuminate); Leaf Base (C) (a- Acute, b- Subacute, c- Cuneate, d- Rounded, e- Subcordate, f- Obtuse, g Obtuse) and leaf venation (D) (a- *C. ovalifolium*, *and C. litseaefolium*, b- *C. citriodorum*, c- *C. capparu-coronde*, d/e- *C. zeylanicum*, f- *C. sinharajaense* , g- *C. rivulorum*, h- *C. dubium*). *MIS*: Morphological index score; *MIS* leaf shape: for leaves with leaf length less than 10 cm, *MIS* should be divided by 4

RESULTS AND DISCUSSION

Leaf morphological index

Average morphological index values *(AMIV)* (Table 4) are useful in evaluating both inter and intraspecies morphological diversity. For example, mean separation values separated *C. zeylanicum*, *C. sinharajaense, C. citriodorum* and *C. capparu-coronde* each other, while *C. ovalifolium*, *C. litseaefolium* and *C. rivulorum, C. dubium* showed some overlapping. Nevertheless, the species could be differentiated in the field using morphological index value range *(MIVR)* together with specific morphological characters. Based on *AMIV*, the highest within-species variation was observed in *C. zeylanicum* followed by *C. dubium.* The lowest within species diversity was observed in *C. citriodorum*.

Table 4: Calculated morphological index values for genus *Cinnamomum* in Sri Lanka.

Species	*AMIS*				*AMIV*	*MIVR*
	Apex	Base	Shape	Venation		
C. ovalifolium	1.7±0.9	3.3±0.9	2.1±0.6	2.0±0.0	9.1±2.1	7 - 11
C. litseaefolium	2.3±1.2	3.3±0.5	4.7±2.9	2.0±0.0	12.3±3.5	11 - 15
C. citriodorum	4.7±0.6	3.7±1.2	4.3±0.3	5.0±0.0	17.7±1.6	16 - 19
C. capparu-coronde	3.5±0.4	9.0±0.8	3.8±1.6	7.0±0.0	23.3±2.2	20 - 25
C. zeylanicum (wild)	3.7±0.5	9.1±2.1	8.7±0.2	7.0±0.0	28.4±2.5	26 - 30
C. zeylanicum (cult.)	4.1±1.1	13.2±2.8	8.9±1.2	9.1±2.5	36.1±5.7	31 - 41
C. sinharajaense	6.4±0.9	15.8±1.7	7.9±0.2	15.9±0.9	45.9±3.2	42 - 48
C. rivulorum	7.9±0.9	18.3±1.6	8.9±0.6	19.3±0.8	54.4±2.6	50 - 55
C. dubium	9.1±1.2	18.4±3.1	9.2±0.7	19.8±0.3	56.4±4.3	55 - 60

*AMIV*s are mean ± SD; *AMIS*= Average morphological index score; *AMIV*= Average morphological index values for each species; *AMIV = AMSV* (Apex) + *AMSV* (Base) + *AMSV* (Shape) + *AMSV* (Venation); *MIVR*= Morphological index value range for species identification.

A closer relationship was reported among *C. ovalifolium*, *C. litseaefolium* and *C. rivulorum, C. dubium* (Saumyasiri *et al.,* 2006; Saumyasiri *et al.,* 2007). This study also provided closer *AMIV*s in those species (Table 4) indicating *AMIV* also provides an idea about relationships among species. Interestingly, *C. capparu-coronde* and *C. zeylanicum* (wild) have close *AMIV*s, as such similar leaf shape, apex, and base (Figure 1(A)(B)(C)). Nevertheless, these two species can be identified with unique volatiles and leaf types (Kostermans, 1995). While *C. capparu-coronde* leaf has a strong clove-like volatiles *C. zeylanicum* has unique cinnamon volatiles (Kostermans, 1995). Further, *C. zeylanicum* comprises of several leaf types; twisted, upward, or downward curled leaves while *C. capparu-coronde*, in general, has straight leaves.

The index developed in the current study could separate individuals of two varieties released by the DEA, Sri Lanka; variety *Sri Gemunu* has *MIVs* closer to 40 whilst variety *Sri Wijaya* has *MIVs* around 30. Hence, this index may also be useful in the identification of popular varieties among the other cultivated germplasm. For example, *MIV*s beyond 40 and less than 30 will not represent DEA recommended varieties.

Leaf morphological indexes have previously been developed and used in the identification of (i) both wild and cultivated species of japonica rice (Qian *et al.,* 2000), (ii) natural variation, and the genetic control of the leaf expansion process of poplar (Hirokazu, 2002) and, (iii) the relationship between the morphological index and net photosynthesis by individual leaves of *Quercus rubra* seedlings (Hanson *et al*., 1986). Therefore, further studies on the association of morphological traits and yield parameters, biochemical properties, and other quality parameters are encouraged as such findings would be useful for the growers to assess the planting materials and the quality of harvest at the field level.

Botanical key for field level identification of *Cinnamomum* Species

The leaf venation pattern can also be used to differentiate species. In both *C. rivulorum* and *C. dubium*, two basal veins start at the leaf stalk junction together with the midrib and runs towards the apex (Figure 1 (D) g &

h). In other species, two basal veins start slightly above the base (Figure 1 (D)). Further, *C. dubium* can be separated from *C. rivulorum,* based on the arrangement of the basal veins where the basal veins of *C. dubium* run closer and parallel to the midrib especially at the mid-point (Figure 1 (D) h).

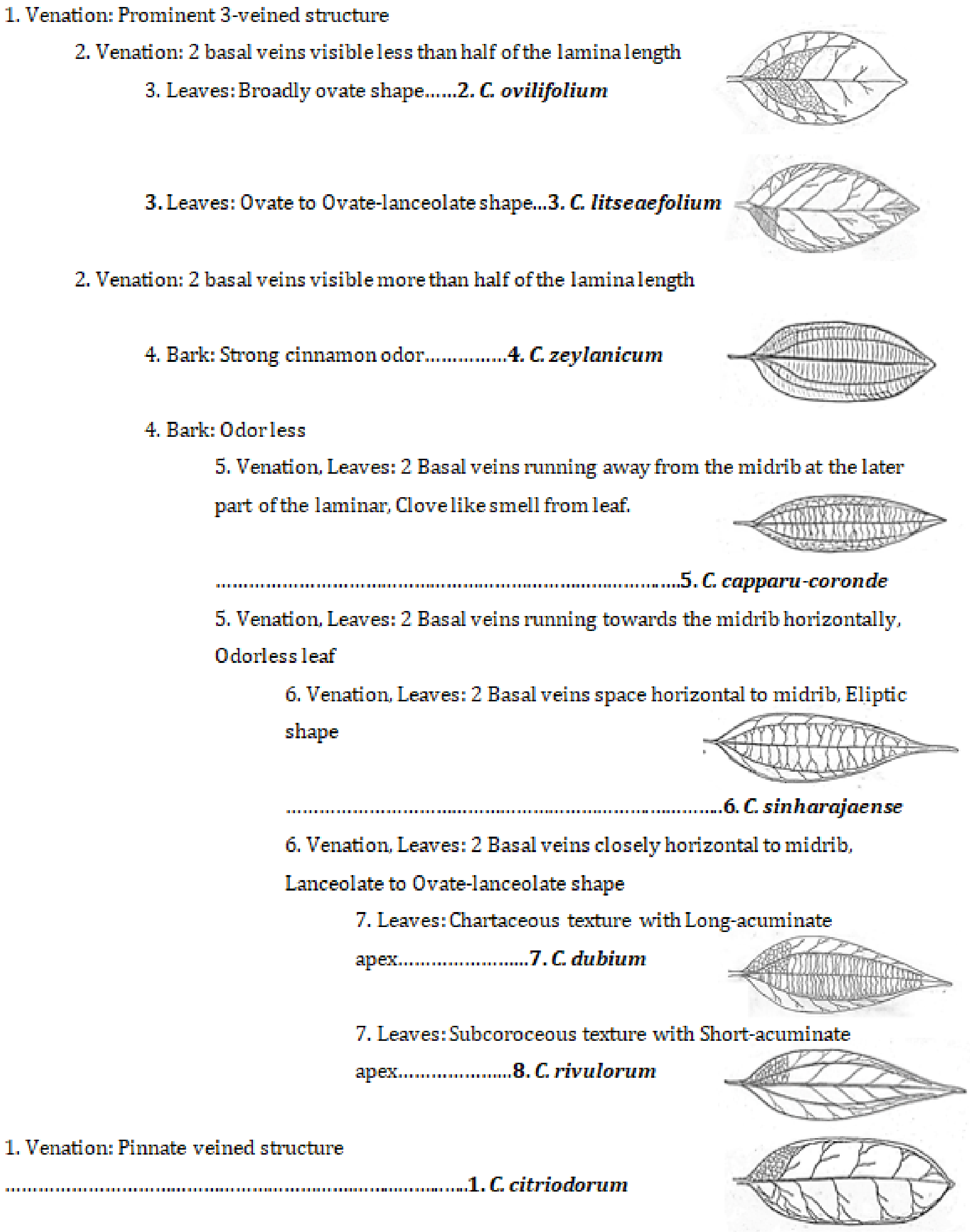

Figure 2: Revised key for identification of *Cinnamomum* species in the field level.

Besides, leaf texture and apex are also useful in the differentiation of *C. rivulorum* and *C. dubium.* For example, *C. rivulorum* has sub coriaceous texture and gradually acuminated apex while *C. dubium* has a chartaceous texture and long acuminated apex (Sritharan, 1984; Kostermans, 1995). *C. citriodorum* consists of a clear pinnate venation pattern (Figure 1 (D) b) with strong citronella like fragrance, distinguishing it from the other species (Sritharan, 1984; Kostermans, 1995). Both *C. litseaefolium* and *C. ovalifolium* have a pinnate venation pattern with two short basal veins. Further, *C. litseaefolium* has twisted leaves with an upward curled leaf margin while *C. ovalifolium* has straight leaves with downward curled leaf margin. *C. sinharajaense* differs from others due to its large leaf size, elliptic leaf shape, and the prominent reticulated secondary venation pattern. As such, a botanical key was developed (Figure 2) including unique field-level observations by modifying the key proposed by Kostermans (1995) giving major attention to the leaf venation.

This will be useful in the identification of species especially in the farmer's field and/or natural habitats without floral characters and chemical constituents as suggested by Kostermans (1995) and Ariyarathne *et al.* (2018). Further, the proposed key is developed without using characters of immature leaves (colour, thickness, and texture) as suggested by Kumarathilake (2012).

CONCLUSIONS

The leaf morphological index and the modified botanical key developed in this study are useful tools for accurate filed level identification of *Cinnamomum* species in Sri Lanka. Besides, the index can also be used to identify cultivated varieties such as *Sri Wijaya*, *Sri Gemunu* from other species.

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