

RESEARCH ARTICLE

## Systematics of genus *Garcinia* L. (Clusiaceae) in Sri Lanka: new insights from vegetative morphology

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**Abstract:** The genus *Garcinia* L. (Family: Clusiaceae) is represented by ten species in Sri Lanka including five endemic species. Systematics of the genus hitherto were primarily based on reproductive morphology. A recent study was carried out in order to evaluate the species limits of Sri Lankan *Garcinia* using anatomical characters. However, the field identification of *Garcinia* is challenging due to the presence of unisexual flowers, strict seasonality in flowering and fruiting, and failure to use anatomical characters.

The aim of this study was to investigate the relationships among the species and to construct an identification key based on the vegetative morphological characters. Specimens of all representative taxa were sampled from different geographical locations. A total of 97 specimens were collected and similarity and cladistic analyses were performed based on 47 vegetative morphological characters. The results suggest that the sampled species fall into two major groups characterized by leaf morphological features. Major groups can be further divided into subgroups by bark colour and tertiary venation. The overall results demonstrate within species variations of *G. hermonii* and *G. terpnophylla* highlighting the presence of different varieties. The dichotomous key constructed using conspicuous vegetative characters will be a valuable tool for identification of Sri Lankan *Garcinia* species in the field. The relationships found in this study provide insights to the phylogeny of the species. The results are congruent with those of the recent anatomical study and revealed the importance of combined multidisciplinary data analysis to elucidate more robust species relationships.

**Keywords:** Bootstrap, cladistic, *Garcinia*, phylogeny, strict consensus tree, vegetative morphology.

### INTRODUCTION

*Garcinia* L. (Clusiaceae) is a pantropically distributed angiosperm genus with high species richness in South

East Asia, and composed of 240 woody tree species<sup>1</sup>. *Garcinia* belongs to the tribe Garcinieae Choisy, in which the genera *Pentaphalangium* Warb., *Rheedia* L., and *Tripetalum* K. Schum. are included<sup>2</sup>, and has a close relationship with the genus *Allanblackia* Oliver, which is indigenous to Africa and not found in Sri Lanka.

In Sri Lanka the genus is represented by 10 species including five endemic species (*G. quaesita*, *G. zeylanica*, *G. hermonii*, *G. terpnophylla* and *G. thwaitesii*), three Indo-Sri Lankan species (*G. morella*, *G. echinocarpa* and *G. spicata*), one species cultivated for more than seventy five years (*G. mangostana*) and an introduced species (*G. xanthochymus*) which is now considered as a semi-naturalized species<sup>3</sup>. The relationships of the above ten *Garcinia* species and their delimitation has been a subject of controversy (Table 1). Trimen<sup>4</sup> recognized five species of *Garcinia* growing in Sri Lanka. Later, Alston<sup>5</sup> provided a supplement to Trimen's system<sup>4</sup>, incorporating the semi naturalized species *G. xanthochymus*. Kostermans<sup>3</sup> recognized nine species. However, during the revision of the 'Handbook to the Flora of Ceylon', ten species have been identified on the basis of morphological characters.

Except for *G. spicata*, the other nine species are distributed in the wet zone of Sri Lanka. Among those *G. echinocarpa* is confined to the montane and submontane regions<sup>3</sup>. *G. thwaitesii* and *G. zeylanica* are listed as globally endangered and *G. quaesita* is listed as globally vulnerable<sup>6</sup>. However, the Sri Lankan *Garcinia* species have not been assessed for conservation status in the latest IUCN national red data list (2007)<sup>6</sup>.

*Garcinia* species play a key role as subcanopy species in the lowland wet evergreen forests and submontane

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forests in Sri Lanka. The seedlings of these species are a major constituent of the undergrowth of the lowland wet zone forests. As a result *Garcinia* species can be successfully used in forest restoration programmes in the degraded lands of the wet zone of Sri Lanka. In addition they can be used in community forestry programmes and in programmes for buffer zone management of forest reserves, as these species have economical value<sup>7</sup>. Xanthones, a group of chemicals found in *Garcinia* species has important pharmacological effects<sup>8,9</sup>.

The phylogenetic relationships within the ten species of *Garcinia* studied are far from resolved. The substantial floral diversity of the *Garcinia* species is very important

in field identification<sup>2</sup>. Therefore, the identification keys for the Sri Lankan *Garcinia* species are mostly based on the flower and fruit morphology<sup>3</sup>.

The flowering and fruiting of *Garcinia* species are strictly seasonal (from April to June/ July<sup>10</sup>) and these species are dioecious. Therefore to identify the species both male and female plants should be sampled.

A comprehensive study has been recently carried out to assess the phylogenetic relationships of the genus using vegetative anatomy<sup>11</sup>, which resolved the species limits of individual species. The anatomical characters however are not very useful and practical in field identification of

**Table 1:** Comparison of selected taxonomic treatments of genus *Garcinia* in Sri Lanka

Trimen (1893) <sup>4</sup>	Alston (1931) <sup>5</sup>	Kostermans (1980) <sup>3</sup>
<i>G. cambogia</i> Desr.	<i>G. cambogia</i> Desr.	<i>G. quaesita</i> Pierre
<i>G. morella</i> (Gaertn.) Desr.	<i>G. morella</i> (Gaertn.) Desr.	<i>G. morella</i> (Gaertn.) Desr.
<i>G. echinocarpa</i> Thw.	<i>G. echinocarpa</i> Thw.	<i>G. echinocarpa</i> Thw.
<i>G. terpnophylla</i> (Thw.)	<i>G. terpnophylla</i> (Thw.)	<i>G. terpnophylla</i> (Thw.) Thw.
<i>G. spicata</i> (W. & A.) Hook f.	<i>G. spicata</i> (W. & A.) Hook f.	<i>G. spicata</i> (W. & A.) Hook f.
	<i>G. xanthochymus</i> Hook f.	<i>G. zeylanica</i> Roxb.
		<i>G. thwaitesii</i> Pierre
		<i>G. hermonii</i> Kosterm.
		<i>G. mangostana</i> L.
		<i>G. xanthochymus</i> Hook f.

**Table 2:** Species codes for specimens, the species represented as three letter codes (in capital letters) and the locations are coded as two letter codes (in simple letters). The locality number refers to the number on the map in Figure 1. (PW- Peak Wilderness Sanctuary)

Botanical name	Code	Botanical name	Code
<i>G. morella</i> (Gaertn.) Desr.	GMO	<i>G. zeylanica</i> Roxb.	GZE
<i>G. echinocarpa</i> Thw.	GEC	<i>G. quaesita</i> Pierre	GQU
<i>G. terpnophylla</i> (Thw.) Thw.	GTE	<i>G. thwaitesii</i> Pierre	GTH
<i>G. spicata</i> (W. & A.) Hook f.	GSP	<i>G. hermonii</i> Kosterm.	GHE
<i>C. rosea</i>	CRO		
Locality	Code	Locality	Code
1 Ritigala	ri	Diyadawa	di
2 Dehiattakandiya	da	Walankanda	wa
3 Gampaha	ga	Gilimale	gi
4 Bulathsinhala	bu	Kitulgala	ki
5 Kalugala	ka	Udawalawa	ud
6 Morapitiya	mo	Sinharaja/ Morningside	si
7 Haycock	ha	PW- Maliboda	mb
8 Kanneliya	kn	PW- Marathenna	mt
9 Kottawa	ko	PW- Moray Estate	me
10 Rumassala	ru	PW- Nallathanniya trail	na
11 Mulatiyana	mu	Dehiowita	de
12 Rammalekanda	ra	Ginigathhena	gh

these species because of the difficulty in taking transverse/longitudinal sections of the vegetative parts (stem, leaves) in the field and inconvenience of transportation and usage of necessary scientific instruments to and from the field. The taxonomic treatments for the Sri Lankan *Garcinia* species by different authors<sup>3-5</sup> and opportunistic field observations have shown that the species circumscriptions are still contradictory and ambiguous. Therefore, the field identification of these species is challenging and a comprehensive revision of species circumscriptions of the genus *Garcinia* will be an important contribution to the systematics of the Sri Lankan flora.

The main objective of this study was to carry out a comprehensive systematic survey of Sri Lankan *Garcinia* species using vegetative morphological features. The specific objectives were to i) Construct the relationships among the *Garcinia* species using similarity/ distance and phylogenetic analysis methods, ii) Elucidate the species circumscriptions of Sri Lankan *Garcinia* species based on vegetative morphological characters and iii) Construct species identification keys using vegetative morphological characters to identify *Garcinia* species in the field.



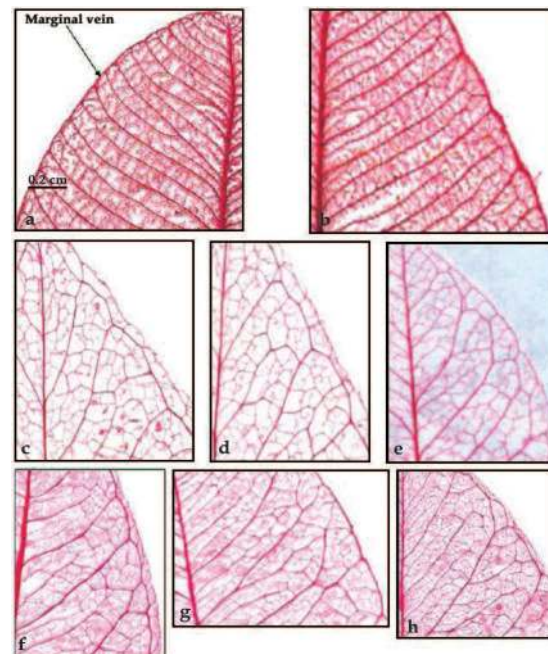
**Figure 1:** Sampling locations (numbered in red), of the present study. The location codes are in accordance with Table 2.

## METHODS AND MATERIALS

**Study species:** The indigenous *Garcinia* species of Sri Lanka, namely *G. morella* (Gaertn.) Desr., *G. echinocarpa* Thw., *G. terpnophylla* (Thw.) Thw., *G. spicata* (W. & A.) Hook f, *G. zeylanica* Roxb., *G. quaesita* Pierre, *G. thwaitesii* Pierre and *G. hermonii* Kosterm. were sampled and included in the present study. The two exotic *Garcinia* species, *G. mangostana* L. and *G. xanthochymus* Hook f. were not included due to wide variations in their morphology, which may interfere and cause imprecise relationships within the study group.

**Outgroup selection:** The closest Sri Lankan relative to the genus *Garcinia* is the genus *Clusia* (Clusiaceae)<sup>2</sup>. The genus *Clusia* is represented by only one species (*C. rosea*) in Sri Lanka; two specimens of *C. rosea* were sampled for this study from Ginigathena.

**Taxon sampling:** A total of 97 individual plants representing the 8 indigenous *Garcinia* species were sampled. Efforts were made to attain comprehensive species sampling, representing diverse geographical locations (Figure 1) of each species.



**Figure 2:** Venation patterns of Sri Lankan *Garcinia* species. a-*G. echinocarpa*, b-*G. hermonii*, c-*G. quaesita*, d-*G. zeylanica*, e-*G. morella*, f-*G. terpnophylla*, g-*G. spicata*, h-*G. thwaitesii*; Two major groups can be identified; leaves with loosely arranged secondary veins (c,d,e,f,g,h) and leaves with densely arranged secondary veins (a,b).

The individual plants were randomly selected from the populations depending on the population size. Mature twigs from sun-exposed portions of the lower crown were harvested from each tree. The sets of leaves for each of the 97 individuals were carefully examined and only intact leaves with no visible damage were selected for analysis, with a sample size of 50 leaves per individual.

The collected specimens were given a species code based on their species name and the location where the specimens were collected (Species codes and the location codes are given in Table 2). Voucher specimens were deposited in the National Herbarium, Peradeniya (Table 3).

*Character coding:* After careful observation of the sampled specimens, 47 vegetative morphological characters and the respective character states (Table 4), based on the available literature and observations, were selected for the purposes of cluster and cladistic analyses. Among the characters 13 were quantitative and others were qualitative characters. All data were converted into a data matrix.

*Leaf clearing:* The leaves were cleared for observing distinct veinlet arrangements of different species. The protocol followed for leaf clearing was adapted from that used by Radford *et. al.*<sup>12</sup>. The original method was modified as follows and used as the most suitable method for the specimens after several trials.

Leaves were placed in a fresh 7% NaOH (Sodium Hydroxide) solution, which was replaced with fresh solution daily for 10 d. Leaves were then washed well with water and bleached with 3% H<sub>2</sub>O<sub>2</sub> (Hydrogen Peroxide) for 12 h. Finally, leaves were thoroughly washed with water followed by 30% ethanol for 10 min, 50% ethanol for 10 min, 1% safranin in 50% ethanol for 2 min, 70% ethanol for 10 min, 95% ethanol for 10 min and absolute ethanol for 5 min. The leaves were then mounted on glass slides and the leaf venation observed (cleared leaves of the *Garcinia* species are illustrated in Figure 2).

*Cluster analysis:* Cluster analysis was performed with PAUP\* (Phylogenetic Analysis Using Parsimony) Version 4.0b10 for Macintosh (i book G4/Altivec). The relationships among the Sri Lankan *Garcinia* species were evaluated using two major clustering methods; Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Neighbour Joining (NJ) methods. UPGMA analysis was performed for the 97 in-group taxa with randomly initiated seed option. NJ analysis was carried out with randomly initiated seed option and mid point rooting option.

*Principal component analysis (PCA):* Principal component analysis was carried out using PC-ORD version 4.0, based on correlation matrices of characters. Only those axes corresponding to components with eigen values greater than 1.0 were extracted and plotted.

*Phylogenetic analysis:* Phylogenetic analysis was performed with PAUP\* Version 4.0b10 for Macintosh (i book G4/Altivec). The two *C. rosea* specimens collected from Ginigathhena were used as the out group.

Heuristic searches of 1000 random unordered taxon addition replicates with equal weight criterion were executed with TBR (Tree Bisection and Reconstruction) branch swapping and MULtrees (Multiple Parsimonious trees) in effect, keeping only 10 trees for each replicate in order to reduce time spent in swapping on suboptimal trees. The resulting trees were then used as starting trees to find as many trees of maximum parsimony as possible. Branches were collapsed if minimum length is zero. Finally, a cladogram and a phylogram were obtained. Strict consensus trees were constructed from all the most parsimonious trees obtained from individual and combined searches. Consistency Index (CI) and Retention Index (RI) were calculated. Bootstrap analysis (100 replicates, full heuristic search using random addition sequence and TBR branch swapping) was applied to each matrix as an internal support. All clades discovered in at least 50% of these replicates are reported.

*Preparation of key to the Sri Lankan Garcinia species:* Although the genus has comprehensive keys based on flower and fruit characters, field identification is challenging as a result of the strict seasonality of flowering and fruiting of these species. Therefore, a dichotomous key was constructed using conspicuous vegetative morphological characters.

## RESULTS

### Cluster analysis

Two different dendrograms were obtained from the cluster analysis using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Neighbour Joining (NJ) methods.

Both the UPGMA and NJ analyses distinctly delimited the eight Sri Lankan *Garcinia* species used in the present study. The relationships shown by the species based on the overall similarity are similar with both UPGMA and NJ methods except for the variation in the placement of *G. spicata*. Therefore, only the UPGMA dendrogram is illustrated in Figure 3.



**Table 3:** Voucher information of the of 97 individuals of eight *Garcinia* species used in this study

Species	Code	Voucher No.	Collection	Deposited	Species	Code	Voucher No.	Collection	Deposited	Species	Code	Voucher No.	Collection	Deposited
<i>G. hermonii</i>	GHE_bu1	J 31	Bulathsinhala	PDA		GQU_ko2	J 61	Kottawa	PDA		GSP_ru2	J 94	Ritigala	PDA
	GHE_bu2	J 32	Bulathsinhala	Voucher not collected		GQU_mb1	J 62	Maliboda	PDA		GSP_ud1	J 95	Udawalawa	PDA
	GHE_gf1	J 33	Gilimale	PDA		GQU_mu1	J 63	Mulatiyana	PDA		GSP_ud2	J 96	Udawalawa	PDA
	GHE_gf2	J 34	Gilimale	PDA		GQU_wa1	J 64	Walankanda	PDA		GMO_bu1	J 97	Bulathsinhala	PDA
	GHE_ha1	J 35	Haycock	PDA		GQU_wa2	J 65	Walankanda	PDA		GMO_bu2	J 98	Bulathsinhala	PDA
	GHE_ka1	J 36	Katugala	PDA		GZE_bu1	J 66	Bulathsinhala	PDA		GMO_bu3	J 99	Bulathsinhala	PDA
	GHE_ka2	J 37	Katugala	PDA		GZE_bu2	J 67	Bulathsinhala	PDA		GMO_bu4	J 100	Bulathsinhala	PDA
	GHE_ki1	J 38	Kitulgala	PDA		GZE_de1	J 68	Dehiowita	PDA		GMO_mb1	J 101	Maliboda	PDA
	GHE_ki2	J 39	Kitulgala	PDA		GZE_ga1	J 69	Gampaha	PDA		GMO_mo1	J 102	Morapitiya	PDA
	GHE_kn1	J 40	Kanneliya	PDA		GZE_kn1	J 70	Kanneliya	PDA		GMO_mu1	J 103	Mulatiyana	PDA
	GHE_kn2	J 41	Kanneliya	PDA		GZE_mb1	J 71	Maliboda	PDA		GMO_mu2	J 104	Mulatiyana	PDA
	GHE_kn3	J 42	Kanneliya	PDA		GZE_mu1	J 72	Mulatiyana	PDA		GMO_ra1	J 105	Rammale Kanda	PDA
	GHE_ko1	J 43	Kottawa	PDA		GZE_ra1	J 73	Rammale Kanda	PDA		GTE_bu1	J 106	Bulathsinhala	PDA
	GHE_mb1	J 44	Maliboda	PDA		GZE_ru1	J 74	Rumassala	PDA		GTE_bu2	J 107	Bulathsinhala	PDA
	GHE_mb2	J 45	Maliboda	PDA		GZE_wa1	J 75	Walankanda	PDA		GTE_bu3	J 108	Bulathsinhala	PDA
	GHE_mo1	J 46	Morapitiya	PDA		GTH_bu1	J 76	Bulathsinhala	PDA		GTE_gf1	J 109	Gilimale	PDA
GHE_mo2	J 47	Morapitiya	PDA		GTH_bu2	J 77	Bulathsinhala	PDA		GTE_kn1	J 110	Kanneliya	PDA	
GHE_mo3	J 48	Morapitiya	Voucher not collected		GTH_bu3	J 78	Bulathsinhala	PDA		GTE_kn2	J 111	Kanneliya	PDA	
GHE_mo4	J 49	Morapitiya	Voucher not collected		GTH_bu4	J 79	Bulathsinhala	PDA		GTE_mb1	J 112	Maliboda	PDA	
<i>G. quaesita</i>	GHE_wa1	J 50	Walankanda	PDA		GTH_di1	J 80	Diyadawa	PDA		GTE_mb2	J 113	Maliboda	PDA
	GQU_bu1	J 51	Bulathsinhala	PDA		GTH_di2	J 81	Diyadawa	PDA		GTE_ru1	J 114	Rumassala	PDA
	GQU_bu2	J 52	Bulathsinhala	PDA		GTH_di3	J 82	Diyadawa	PDA		GTE_ru2	J 115	Rumassala	PDA
	GQU_bu3	J 53	Bulathsinhala	PDA		GTH_ha1	J 83	Haycock	PDA		GTE_ru3	J 116	Rumassala	PDA
	GQU_di1	J 54	Diyadawa	PDA		GTH_ka1	J 84	Kalugala	PDA		GTE_ru4	J 117	Rumassala	PDA
	GQU_ga1	J 55	Gampaha	PDA		GTH_ki1	J 85	Kitulgala	PDA		GEC_na1	J 118	Nallathanniya	PDA
	GQU_ka1	J 56	Kalugala	PDA		GTH_ki2	J 86	Kitulgala	PDA		GEC_si1	J 119	Sinharaja	PDA
	GQU_ka2	J 57	Kalugala	PDA		GTH_ko1	J 87	Kottawa	PDA		GEC_si2		Sinharaja	Voucher not collected
	GQU_ka3	J 58	Kalugala	PDA		GTH_ko2	J 88	Kottawa	PDA		GEC_si3		Sinharaja	Voucher not collected
	GQU_ka4	J 59	Kalugala	PDA		GTH_mb1	J 89	Maliboda	PDA		GEC_si4		Sinharaja	Voucher not collected
	GQU_ka5	J 60	Kalugala	PDA		GTH_mu1	J 90	Mulatiyana	PDA		GEC_ms1	J 120	Moray estate	PDA
GQU_ki1	J 61	Kitulgala	PDA		GTH_ra1	J 91	Rammale Kanda	PDA		GEC_ms2	J 121	Moray estate	PDA	
GQU_ki2	J 62	Kitulgala	PDA		GSP_da1	J 92	Dehiathakandiya	PDA		GTH_ko1	J 87	Kottawa	PDA	
GQU_ko1	J 63	Kottawa	PDA		GSP_ru1	J 93	Ritigala	PDA		GTH_ko2	J 88	Kottawa	PDA	

Sri Lankan *Garcinia* species cluster into two major groups in both analyses. One of these two major clusters includes *G. hermonii*, *G. spicata*, *G. thwaitesii* and *G. echinocarpa* (cluster 1) and the other group (cluster 2) includes *G. quaesita*, *G. morella*, *G. zeylanica* and *G. terpnophylla*.

Cluster 1 shows a further division into two subgroups. This division illustrates a slight variation in the two analyses due to the incongruence in the placement of *G. spicata*. The UPGMA analysis placed *G. spicata* in a common subgroup with *G. hermonii*, *G. thwaitesii* and *G. echinocarpa* from the second subgroup. On the contrary, the Neighbour Joining tree places *G. spicata* in the cluster in which *G. thwaitesii* and *G. echinocarpa* are included, while *G. hermonii* alone forms the other major subgroup.

Cluster 2 is divided into two subgroups (Figure 3). One subgroup includes only the individuals of *G. terpnophylla*. The other three species (*G. morella*, *G. quaesita* and *G. zeylanica*) of cluster 2 are comparatively similar in vegetative morphology, except for a few hardly identifiable vegetative morphological characters.

### Principal component analysis (PCA)

Component loading of each species along the component axes are given in Table 5. Species such as *G. spicata*, *G. hermonii*, *G. echinocarpa* and *G. thwaitesii* indicated a considerable contribution to the first principal component axis and *G. terpnophylla* shows its wide contribution for the second component axis.

On the other hand, the individuals of *G. morella* were heavily loaded along the third component axis. The fourth and fifth components loaded with *G. quaesita* and *G. zeylanica* could be considered as negligible component axes with the loading of variables along these components being insignificant. The resulting scatter plots revealed that there is a more or less clear grouping pattern of specimens and thus those characters considered in the study are important in the delimitation of the Sri Lankan *Garcinia* species (Figure 4).

The Principal Component Analysis illustrates that the Sri Lankan indigenous species clearly circumscribe the species limits, with few exceptions. *G. terpnophylla* and *G. hermonii* which show a division among the individuals supported by leaf characteristics. *G. spicata* shows a close relationship with the *G. hermonii* group. *G. echinocarpa* placed in a distant cluster is characterized by morphological characters such as the red coloured

petiole, stilt roots and convex lamina. Its distribution is confined to the lower montane and sub montane zones of Sri Lanka.

### Phylogenetic analysis

A comprehensive analysis was carried out to generate a cladogram with the maximum parsimony method using *C. rosea* as the out group species. One out of the 1198 most parsimonious trees (phylogram) is illustrated in Figure 5.

The most parsimonious trees have a length of 165 steps, CI of 0.3515 and a RI of 0.9254. The branch lengths are shown using tree number 1 of the 1198 trees (ACCTRAN optimization) (Figure 5).

The strict consensus tree of the 1198 most parsimonious trees of Sri Lankan *Garcinia* species is illustrated in Figure 6 including the clades with bootstrap support greater than 50. Monophyly of Sri Lankan *Garcinia* species is well supported (BP 100), and except for *G. hermonii* other Sri Lankan *Garcinia* species circumscribed into well-supported monophyletic clades.

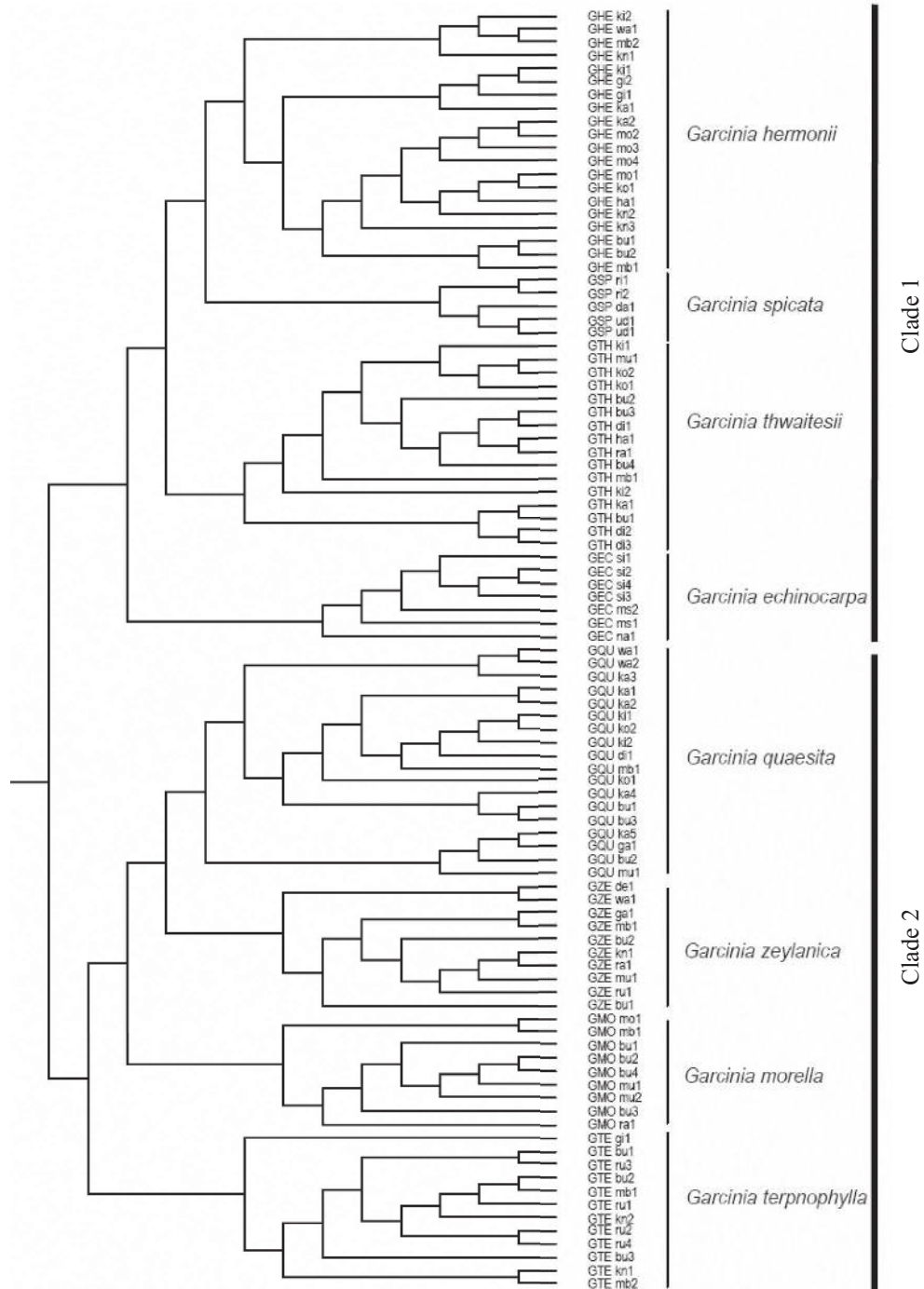
Sri Lankan *Garcinia* species form two major clades. The first clade consists of *G. hermonii*, *G. spicata*, *G. thwaitesii* and *G. echinocarpa* and these species possess coriaceous leaves (clade 1). The second clade which includes *G. quaesita*, *G. morella*, *G. zeylanica* and *G. terpnophylla* (clade 2) has non-coriaceous leaves (Figures 5 and 6).

*G. hermonii* in clade 1 shows a highly unresolved relationship forming a polytomy. Although it shows an unresolved relationship in the strict consensus tree, the phylogram obtained from the maximum parsimony method shows that it forms a distinct clade with eight out of the forty seven distinct morphological characters and the individuals are differentiated into two major groups, which is also supported by the PCA. The other three species, namely *G. spicata*, *G. thwaitesii* and *G. echinocarpa* form strong monophyletic clade inferred by the strict consensus tree. *G. spicata* is sister to *G. echinocarpa* and forms a strong monophyletic clade with bootstrap support of 92 (Figures 5 and 6). These three species show a distinct clade from *G. hermonii*, with thirteen character diversions in the phylogram.

Compared to the species of clade 1, the species belonging to clade 2 form strong relationships. *G. terpnophylla* forms a lonely but strong monophyletic subclade with bootstrap support of 83. In addition, it is sister to the other major subclade of clade 2 which comprises *G. quaesita*, *G. zeylanica* and *G. morella*.

The subclade consisting *G. quaesita*, *G. zeylanica* and *G. morella* is well supported with a bootstrap support of 91. The subclade containing these three species again forms a relationship dividing into two parts with bootstrap support of 71 and 79. *G. quaesita* and *G. morella* are

included in the part with 71 percent bootstrap support, being sister to each other, they strongly circumscribe with 95 and 92 percent of bootstrap values accordingly (Figure 6).



**Figure 3:** The UPGMA dendrogram of eight indigenous *Garcinia* species in Sri Lanka inferred by forty seven vegetative morphological characters. Major clusters and representative species are marked.

**Table 4:** The vegetative characters and character states used in the study

Code	Character	Character states				
		0	1	2	3	4
C1	Leaf length (LL)	0.0 > LL > 12.0cm	12.0 < LL ≤ 24.0 cm			
C2	Leaf width (LW)	LW ≤ 5.0cm	LW > 5.0cm			
C3	Petiole length (PL)	PL < 2.0cm	PL ≥ 2.0cm			
C4	Petiole diameter (PD)	PL < 0.2cm	PL ≥ 0.2cm			
C5	Leaf: length/ width (RI)	RI > 2.0	RI ≤ 2.0			
C6	Petiole: length/ width (Rp)	Rp ≤ 8.0	Rp > 8.0			
C7	Number of secondary veins (#V)	0 < #V < 25	25 < #V < 50	50 < #V < 75		
C8	Branchlet tip diameter (TD)	TD < 0.3	TD > 0.3			
C9	Groove length (GL)	GL < 0.3	GL > 0.3			
C10	Angle of leaf tip (T)	T < 50	T > 50			
C11	Angle of leaf base (B)	B < 100	B > 100			
C12	Leaf thickness (LT)	LT < 0.3	LT > 0.3			
C13	Angle in 1ry & 2ry veins (V)	0 < V < 45	45 < V < 90			
C14	Bark colour	Rusty black	Smooth brown			
C15	Live bark colour	Off white	Beefy red	Brown		
C16	Sap colour	Luminous Yellow	Brick red	White	Off white	
C17	branchlet TS shape	Cylindrical	Subcylindrical	Quadrangular		
C18	Leaf texture - Fresh leaf	Coriaceous	Non coriaceous			
C19	Leaf texture - Dried leaf	Coriaceous	Non coriaceous			
C20	Leaf shape	Elliptic	Ovate □	Oblong	Obovate	Oval
C21	Leaf bent down at tip region	Yes	No			
C22	shape of leaf apex	Acute	Cuspidate	Obtuse	Emarginate	
C23	shape of the leaf base	Attenuate	Rounded			
C24	Colour of the young leaf	Reddish brown	Light green	Dark green		
C25	Colour of the young leaf margin	Reddish brown	Green			
C26	Decurrent nature of leaf base	Present	Absent			
C27	Presence of a foveola	Present	Absent			
C28	Midrib nature/ upper surface	Flat	Raised	Impressed		
C29	Midrib nature/ lower surface	Flat	Raised	Impressed		
C30	Midrib nature at base	Flat	Raised			
C31	Colour of the midrib	Totally green	Reddish brown at base			
C32	Colour of the petiole	Totally green	Reddish brown			
C33	Colour of the young petiole	Totally green	Reddish brown			
C34	petiole nature	Stout	Slender			
C35	Groove in the branchlets	Present	Absent			
C36	Stilt roots	Present	Absent			
C37	Aerial roots	Present	Absent			
C38	Strangler roots	Present	Absent			
C39	Buttresses	Present	Absent			
C40	Secondary vein arrangement	Dense	Loose			
C41	Presence of inter marginal vein	Present	Absent			
C42	Pattern of tertiary veins	Reticulate	Parallel			
C43	Relationship of tertiary to mid vein	Parallel	Not parallel			
C44	Intermediate secondary vein nature	Reach margin	Not reach margin			
C45	Cup shape of the leaf	Present	Absent			
C46	Pubescence	Present	Absent			
C47	Mid vein invisible at the apex	Yes	No			



**Table 5:** Results of principal component analysis on forty seven vegetative morphological characters of ninety seven specimens of eight indigenous *Garcinia* species in Sri Lanka. Variables arranged in descending order according to loadings on first component. The heavily loaded components are shown in bold letters.

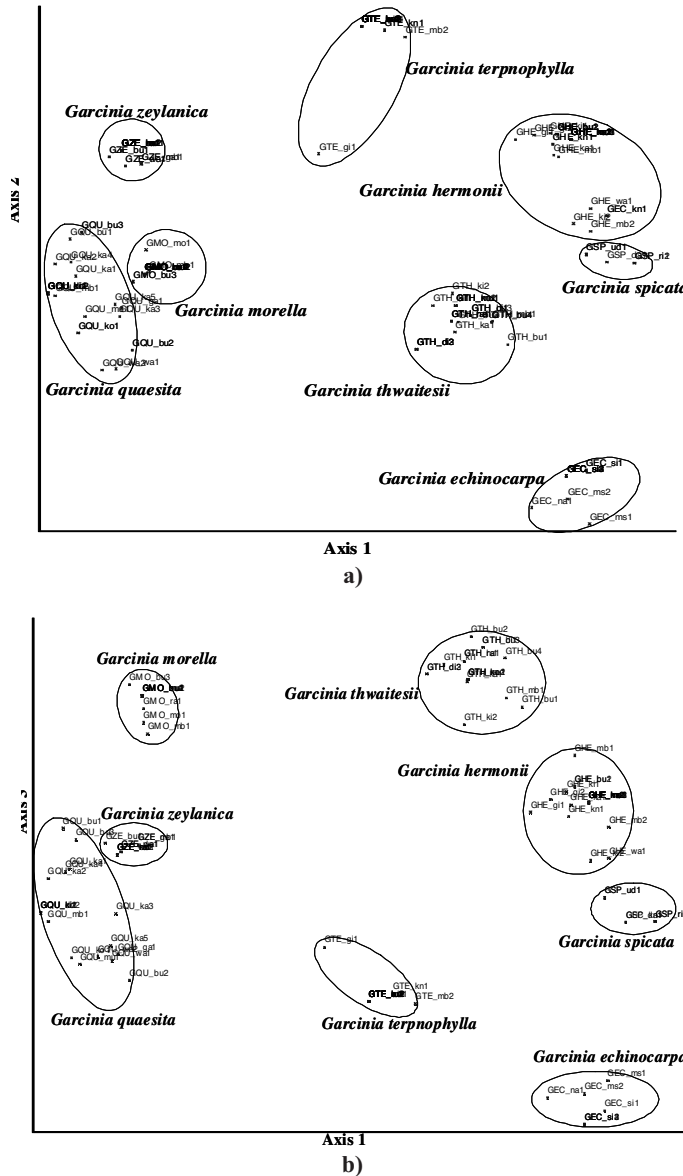
Species	Axis (Component)						Species	Axis (Component)					
	1	2	3	4	5	6		1	2	3	4	5	6
GSP_ri1	<b>5.4042</b>	-0.5489	-1.2430	1.0358	-0.2955	2.6689	GTE_kn1	0.6427	<b>4.1213</b>	-2.5492	3.2527	0.0199	-0.8107
GSP_ri2	<b>5.4042</b>	-0.5489	-1.2430	1.0358	-0.2955	2.6689	GTE_bu1	0.1941	<b>4.2190</b>	-2.6950	3.3567	0.2028	-0.7877
GEC_kn1	<b>4.8677</b>	0.3878	-1.2702	-2.4567	1.4041	0.5119	GTE_bu2	0.1941	<b>4.2190</b>	-2.6950	3.3567	0.2028	-0.7877
GSP_da1	<b>4.8649</b>	-0.5218	-1.2664	1.4116	-0.1121	2.6734	GTE_bu3	0.1941	<b>4.2190</b>	-2.6950	3.3567	0.2028	-0.7877
GHE_mb2	<b>4.5632</b>	0.0823	0.4430	-3.2360	1.7031	-0.4479	GTE_ru1	0.1941	<b>4.2190</b>	-2.6950	3.3567	0.2028	-0.7877
GHE_wa1	<b>4.5552</b>	0.5512	-0.1140	-3.5840	0.8216	0.0954	GTE_ru2	0.1941	<b>4.2190</b>	-2.6950	3.3567	0.2028	-0.7877
GEC_ms1	<b>4.5418</b>	-5.7815	-4.1061	0.9163	1.7329	-0.0201	GTE_kn2	0.1941	<b>4.2190</b>	-2.6950	3.3567	0.2028	-0.7877
GSP_ud1	<b>4.4820</b>	-0.3632	-0.8313	1.6264	-0.3932	2.6215	GTE_ru3	0.1941	<b>4.2190</b>	-2.6950	3.3567	0.2028	-0.7877
GSP_ud1	<b>4.4820</b>	-0.3632	-0.8313	1.6264	-0.3932	2.6215	GTE_ru4	0.1941	<b>4.2190</b>	-2.6950	3.3567	0.2028	-0.7877
GEC_si1	<b>4.4700</b>	-4.7154	-4.6487	-0.5567	1.2928	-0.2589	GTE_mb1	0.1941	<b>4.2190</b>	-2.6950	3.3567	0.2028	-0.7877
GHE_ki2	<b>4.2315</b>	0.2338	-0.1538	-3.2712	1.4827	0.0794	GTE_gi1	-0.6117	<b>1.6503</b>	-1.7167	2.1176	-1.6946	-1.4017
GHE_ka2	<b>4.1870</b>	1.9372	0.8886	-3.0228	-0.2178	-0.5107	GMO_mb1	-3.8295	-0.7338	<b>2.1137</b>	1.5391	3.3454	-1.0862
GHE_mo1	<b>4.1870</b>	1.9372	0.8886	-3.0228	-0.2178	-0.5107	GMO_ra1	-3.8934	-0.7703	<b>2.5691</b>	0.8208	3.9430	-1.0191
GHE_mo2	<b>4.1870</b>	1.9372	0.8886	-3.0228	-0.2178	-0.5107	GMO_mo1	-3.9022	-0.2855	<b>2.3328</b>	1.5239	2.5370	-1.2247
GHE_mo3	<b>4.1870</b>	1.9372	0.8886	-3.0228	-0.2178	-0.5107	GMO_bu1	-3.9254	-0.7886	<b>2.7968</b>	0.4617	4.2417	-0.9856
GHE_mo4	<b>4.1870</b>	1.9372	0.8886	-3.0228	-0.2178	-0.5107	GMO_bu2	-3.9254	-0.7886	<b>2.7968</b>	0.4617	4.2417	-0.9856
GHE_ha1	<b>4.1870</b>	1.9372	0.8886	-3.0228	-0.2178	-0.5107	GMO_bu4	-3.9254	-0.7886	<b>2.7968</b>	0.4617	4.2417	-0.9856
GHE_kn2	<b>4.1870</b>	1.9372	0.8886	-3.0228	-0.2178	-0.5107	GMO_mu1	-3.9254	-0.7886	<b>2.7968</b>	0.4617	4.2417	-0.9856
GHE_ka1	<b>4.1870</b>	1.9372	0.8886	-3.0228	-0.2178	-0.5107	GMO_mu2	-3.9254	-0.7886	<b>2.7968</b>	0.4617	4.2417	-0.9856
GEC_ms2	<b>4.1189</b>	-5.2889	-4.3389	0.2572	2.2772	-0.6905	GZE_ga1	-3.9984	1.4534	<b>0.1245</b>	-1.0856	0.5904	2.8129
GEC_si2	<b>4.1109</b>	-4.8199	-4.8960	-0.0909	1.3957	-0.1473	GZE_mb1	-3.9984	1.4534	<b>0.1245</b>	-1.0856	0.5904	2.8129
GEC_si3	<b>4.1109</b>	-4.8199	-4.8960	-0.0909	1.3957	-0.1473	GMO_bu3	-4.1536	-0.9147	<b>2.9983</b>	0.4817	4.2685	-0.2735
GEC_si4	<b>4.1109</b>	-4.8199	-4.8960	-0.0909	1.3957	-0.1473	GQU_bu2	-4.1565	-2.3085	-2.2998	-0.2857	-2.3554	1.1061
GHE_mb1	<b>3.9398</b>	1.5740	1.7358	-2.5316	0.4763	-1.0886	GZE_de1	-4.2948	1.4136	0.0068	-1.5262	-0.0399	2.3083
GHE_bu1	<b>3.9317</b>	2.0429	1.1787	-2.8797	-0.4053	-0.5454	GZE_wa1	-4.2948	1.4136	0.0068	-1.5262	-0.0399	2.3083
GHE_bu2	<b>3.9317</b>	2.0429	1.1787	-2.8797	-0.4053	-0.5454	GQU_ga1	-4.3631	-1.4413	-1.8390	-0.5332	-2.1947	-0.3077
GHE_ka1	<b>3.8634</b>	1.6198	0.8488	-2.7101	0.4433	-0.5267	GZE_bu2	-4.3734	1.7000	-0.0491	-0.8704	0.8671	2.4536
GHE_kn1	<b>3.8279</b>	1.8326	0.6413	-2.5570	-0.1149	-0.3991	GZE_kn1	-4.3734	1.7000	-0.0491	-0.8704	0.8671	2.4536
GHE_ki1	<b>3.7907</b>	2.0681	1.0679	-2.7253	-0.3652	-0.6652	GZE_mu1	-4.3734	1.7000	-0.0491	-0.8704	0.8671	2.4536
GHE_gi2	<b>3.4942</b>	2.0283	0.9502	-3.1659	-0.9955	-1.1698	GZE_ra1	-4.3734	1.7000	-0.0491	-0.8704	0.8671	2.4536
GEC_na1	<b>3.4386</b>	-5.4592	-4.4255	0.3250	2.5366	-0.3871	GZE_ru1	-4.3734	1.7000	-0.0491	-0.8704	0.8671	2.4536
GHE_gi1	<b>3.1350</b>	1.9237	0.7030	-2.7000	-0.8926	-1.0582	GQU_ka3	-4.4050	-1.6304	-1.1144	-1.0579	-1.6100	0.2423
GTH_bu1	<b>2.9798</b>	-2.1978	2.5935	1.8043	-1.2020	0.3837	GQU_wa1	-4.4704	-2.6689	-1.9570	-1.1874	-1.0711	-0.1048
GTH_mb1	<b>2.6889</b>	-1.7208	2.7636	2.1445	-1.4035	-0.1512	GQU_ka5	-4.4908	-1.3884	-1.6940	-0.4616	-2.2884	-0.3250
GTH_bu4	<b>2.6721</b>	-1.7316	3.4852	1.4842	-1.4521	-0.1084	GZE_bu1	-4.6016	1.5739	0.1524	-0.8504	0.8939	3.1657
GTH_bu3	<b>2.2757</b>	-1.6007	3.6646	1.7818	-1.5995	-0.2629	GQU_wa2	-4.7350	-2.6940	-1.8898	-1.2844	-0.8005	0.1422
GTH_di1	<b>2.2757</b>	-1.6007	3.6646	1.7818	-1.5995	-0.2629	GQU_mu1	-5.0576	-1.6152	-2.0195	-0.3281	-0.9327	-0.2988
GTH_bu2	<b>2.0475</b>	-1.7268	3.8660	1.8018	-1.5727	0.4492	GQU_bu3	-5.1281	0.0559	0.2132	-0.7352	-0.8991	-0.0957
GTH_mu1	<b>1.9985</b>	-1.4060	3.0911	2.8538	-1.7311	0.0498	GQU_ko1	-5.2013	-1.9533	-1.9051	-1.3850	-2.2406	-0.8325
GTH_ko1	<b>1.9985</b>	-1.4060	3.0911	2.8538	-1.7311	0.0498	GQU_ka1	-5.2506	-0.8159	-0.3007	-1.6114	-2.1539	-0.7066
GTH_ko2	<b>1.9985</b>	-1.4060	3.0911	2.8538	-1.7311	0.0498	GQU_ka4	-5.3291	-0.5295	-0.3566	-0.9555	-1.2469	-0.5613
GTH_ka1	<b>1.9735</b>	-1.9281	3.0526	2.0941	-0.9322	0.3164	GQU_bu1	-5.3563	-0.0703	0.4146	-0.7152	-0.8723	0.6164
GTH_ki2	<b>1.9224</b>	-1.1662	2.2857	3.5464	-1.3597	0.0596	GQU_ka2	-5.6256	-0.5692	-0.4743	-1.3961	-1.8772	-1.0659
GTH_ha1	<b>1.9166</b>	1.7052	3.4173	2.2476	-1.4966	-0.1512	GQU_mb1	-5.6361	-1.2123	-1.2428	-1.6124	-2.0531	-1.5342
GTH_ra1	<b>1.9166</b>	-1.7052	3.4173	2.2476	-1.4966	-0.1512	GQU_ki1	-5.7638	-1.1594	-1.0977	-1.5408	-2.1468	-1.5515
GTH_ki1	<b>1.5549</b>	-1.3982	3.3678	2.5059	-1.2274	-0.2358	GQU_ki2	-5.7638	-1.1594	-1.0977	-1.5408	-2.1468	-1.5515
GTH_di2	<b>1.2585</b>	-2.2746	3.1948	2.2012	-1.0894	0.5499	GQU_di1	-5.7638	-1.1594	-1.0977	-1.5408	-2.1468	-1.5515
GTH_di3	<b>1.2585</b>	-2.2746	3.1948	2.2012	-1.0894	0.5499	GQU_ko2	-5.7638	-1.1594	-1.0977	-1.5408	-2.1468	-1.5515
GTE_mb2	<b>1.0391</b>	<b>3.9904</b>	-2.7286	2.9552	0.1673	-0.6562							

**Dichotomous key to the Sri Lankan *Garcinia* species**

The dichotomous key prepared using distinct morphological characters that can be easily identified in the field is illustrated in Figure 7.

The initial categorization of the species is easy with the character of coriaceousness or non-coriaceousness of leaf texture. *G. echinocarpa*, *G. hermonii*, *G. spicata* and *G. thwaitesii* are included in the category of the species

with coriaceous leaves. *G. echinocarpa* and *G. hermonii* can be separated from the rest of the species of this group based on the arrangement of the secondary veins. Both these species have a densely arranged (0.2-0.8 cm between two secondary veins) secondary venation and they can be distinctly identified by their petiole colour. The other two species *G. spicata* and *G. thwaitesii* possess loosely arranged secondary veins (> 0.8 cm between two secondary veins) and they are characterized by the of the leaf tip nature (Figure 7).



**Figure 4:** a) PCA scatter diagram produced by plotting the first PC against the second for vegetative morphological characters of the Sri Lankan *Garcinia* species; b) PCA scatter diagram produced by plotting the first PC against the third for vegetative morphological characters of the Sri Lankan *Garcinia* species; species codes are as follows. GZE- *G. zeylanica*, GQU- *G. quaesita*, GMO- *G. morella*, GTE- *G. terpnophylla*, GTH- *G. thwaitesii*, GHE- *G. hermonii*, GEC- *G. echinocarpa* GSP- *G. spicata*

*Garcinia morella*, *G. quaesita*, *G. zeylanica* and *G. terpnophylla* possess non-coriaceous leaves. *G. terpnophylla* can be distinctly identified among the species of this group by the presence of an intermarginal vein. The other three species *G. morella*, *G. quaesita* and *G. zeylanica* are very similar to each other. *G. morella* and *G. zeylanica* have brilliant yellow coloured latex, which separates them from *G. quaesita*. *G. morella* can be differentiated by the presence of foveola in the leaf petiole. In addition it possesses very thick leaves and reddish coloured juvenile leaves. On the other hand *G. zeylanica* is characterized by its blood red coloured live bark. *G. quaesita* possesses a reddish brown coloured petiole and can be easily characterized by the orangish yellow (brick red) coloured latex (Figure 8).

## DISCUSSION

The phylogeny of genus *Garcinia* is researched extensively all over the world, due to the paraphyletic nature of the genus according to the present circumscription. However the present study reveals that the Sri Lankan *Garcinia* species form a strong monophyletic clade. The species circumscriptions are also supported by a number of distinct morphological characters.

When the results of the similarity methods are compared, Sri Lankan *Garcinia* species cluster into two major groups in both the NJ and UPGMA analyses. This separation is inferred by the leaf texture and the leaf size of the species. The major cluster which includes *G. hermonii*, *G. spicata*, *G. thwaitesii* and *G. echinocarpa* have coriaceous leaves (cluster 1), while the other group has non-coriaceous small leaves compared to the species of cluster 1 (Figure 3).

As the results illustrate the species in cluster 1 show a further division into two subgroups (Figure 3). The similarity relationship inferred by the analyses is robustly supported by many morphological features including leaf size, leaf shape, leaf tip and leaf base characteristics as well as the entire venation pattern.

Cluster 2 is divided into two subgroups (Figure 3). One major group includes only the individuals of *G. terpnophylla*. Although *G. terpnophylla* and the other species in cluster 2 are very similar in leaf characters such as the leaf size and texture, they are dissimilar from the related species in other major morphological characters showing more similarity to the species in cluster 1 such as white coloured sap, densely arranged secondary veins, which are nearly at right angles to the midvein, and oblong leaf shape.

*G. terpnophylla* not only possesses features common to the species of cluster 1 but is also characterized by distinct morphological characters such as presence of an intermarginal vein and a canopy tree with buttresses. These characters may have given *G. terpnophylla* a unique position in the UPGMA and analyses.

Even though *G. terpnophylla* has non-coriaceous leaf texture in common with the species in cluster 2, it has varying levels of thickness (coriaceous nature). One specimen collected from Gilimale (GTE gi1) is highly coriaceous similar to the species in cluster 1 and the specimens collected from Maliboda trail to the Peak Wilderness Sanctuary (GTE mb2) and Kanneliya Forest Reserve (GTE knl) are similar to species of cluster 1 in leaf length. Their leaves have intermediate leaf thickness compared to the related species of the same cluster and those of cluster 1 (Figure 3). However, the other common characters among the individuals of *G. terpnophylla* place them in a single major group of cluster 2 (Figure 3).

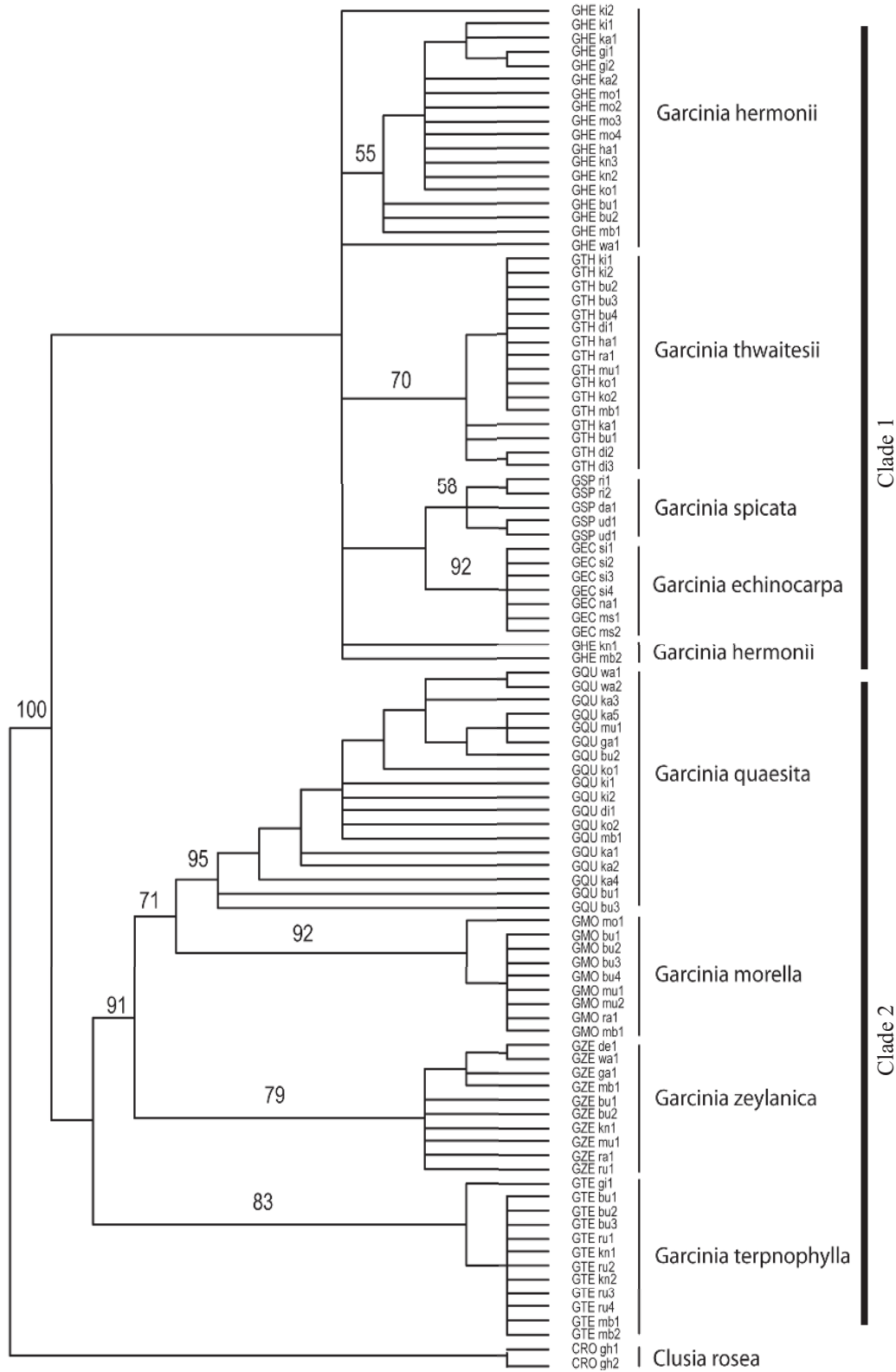
The other three species of cluster 2 are similar in vegetative morphology, except for a few specimens with hardly identifiable vegetative morphological characters. Trimén 1893<sup>4</sup> delimited these three species into two species; *G. morella* and *G. cambogia*. Both *G. quaesita* and *G. zeylanica* were included in *G. cambogia*, and *G. zeylanica* was considered as one variety (*G. cambogia* var. *papilla*).

A similar relationship was observed during the current study. These three species form one subgroup of cluster 2. *G. quaesita* and *G. zeylanica* show a very close relationship. The red coloured young leaf and the presence of a foveola in the petiole are characteristic features of *G. morella*. *G. morella* has thicker leaves than other two species. These characters may cause *G. morella* to be separated from the other two species.

Other than these unique characters, *G. morella* shares some characteristics in common with *G. quaesita* and *G. zeylanica* of the same subgroup. Both *G. zeylanica* and *G. morella* possess bright yellow coloured latex, while *G. quaesita* has bright orange coloured latex. The colour of the live bark of *G. morella* and *G. quaesita* is light brown and *G. zeylanica* has a blood red coloured live bark. Almost all the leaf lamina characteristics of the three species are similar.

The phylogenetic analysis shows an unresolved polytomy in *G. hermonii*. The vegetative morphology points to the presence of two types of *G. hermonii* in the field. It is interesting to note that both types coexist in the same ecological niche. Therefore, this morphological variation is not imposed by environmental factors. The





**Figure 6:** The strict consensus tree of the 1198 most parsimonious trees of Sri Lankan *Garcinia* species obtained from the phylogenetic analysis using 97 ingroup and 2 outgroup taxa inferred by forty seven vegetative morphological characters. The most parsimonious trees have a length of 165 steps, CI of 0.3515 and a RI of 0.9254. The clades with bootstrap support greater than 50 are also included



KEY TO THE SRI LANKAN <i>GARCINIA</i> SPECIES (LEAF ARCHITECTURE)	
1. Coriaceous leaves	
2. Leaves with densely arranged veins	
4. Petiole reddish brown .....	<i>G. echinocarpa</i>
4. Petiole green.....	<i>G. hermonii</i>
2. Leaves with loosely arranged veins	
5. Leaves with emarginated leaf tip.....	<i>G. spicata</i>
5. Leaves with acuminate leaf tip.....	<i>G. thwaitesii</i>
1. Non- coriaceous leaves	
6. Leaves without intermarginal vein	
7. Petiole with foveola.....	<i>G. morella</i>
7. Petiole without foveola	
8. Basal region of mid vein red coloured .....	<i>G. quaesita</i>
8. Basal region of mid vein green coloured.....	<i>G. zeylanica</i>
6. Leaves with strong intermarginal vein.....	<i>G. terpnophylla</i>

Figure 7: The dichotomous key prepared for the *Garcinia* species in Sri Lanka using leaf characteristics

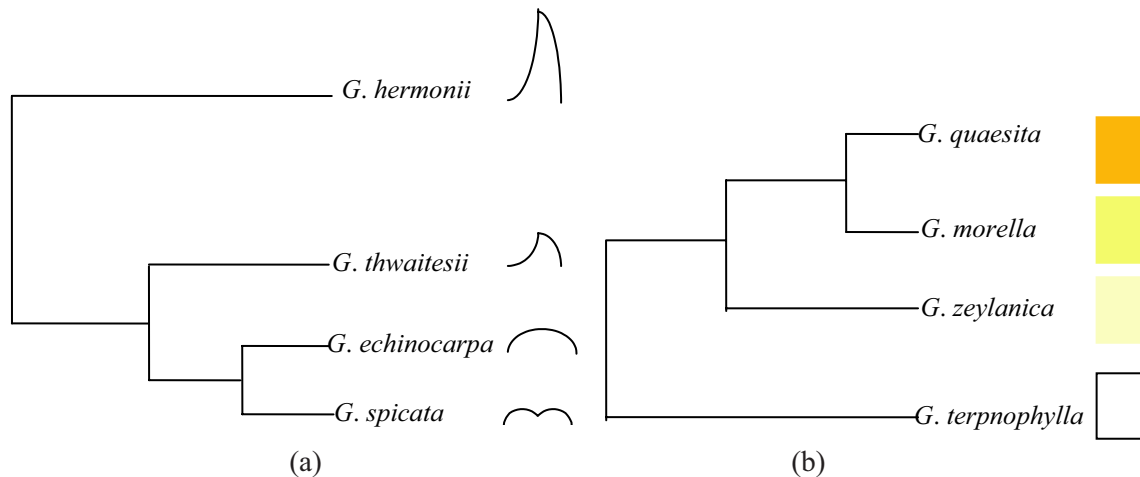


Figure 8: a) The leaf tip shapes of *G. hermonii*, *G. thwaitesii*, *G. echinocarpa* and *G. spicata* and their relationship appeared in the phylogenetic analysis. b) The latex colour variation in *G. quaesita*, *G. morella*, *G. zeylanica* and *G. terpnophylla*.

individuals with the similar vegetative morphology form a monophyletic clade in the strict consensus tree, while the positions of the individuals that diverged from the common situation are still unresolved. The PCA also showed such a division within the species. This suggests the presence of two distinct varieties of *G. hermonii*. The divergence could have occurred in the near past and the varietal characters are not strong enough to consider

them as one clade.

The two Indo-Sri Lankan species *G. spicata* and *G. echinocarpa* show a close affinity to each other, while forming a monophyletic clade with endemic *G. thwaitesii* and *G. hermonii*. This may be an indication of the evolution of an endemic species. The relationships of these species are illustrated in relation to the nature

of their leaf tips in Figure 8 a. Except for *G. terpnophylla*, the other species of clade 2 (Figure 5) show affinities with each other. *G. quaesita*, *G. morella* and *G. zeylanica* are very difficult to distinguish in the field without flowering specimens. However, the varying colours of their latex can be used in field identification (Figure 8 b).

Individuals of *G. quaesita* are highly variable in vegetative morphology. Polymorphism in leaf shape plays a major role in variability within the species.

Kostermans<sup>3</sup> identified two distinct varieties of *G. terpnophylla*; var. *acuminata* found in the lowland wet zone and var. *terpnophylla* found in the montane zone. The montane variety has not been observed in the wild for nearly a century and is not sampled in the present study. All *G. terpnophylla* specimens in the present study were collected from the lowland wet zone. A within-species divergence is observed in *G. terpnophylla* like in *G. hermonii*.

The vegetative anatomical study<sup>11</sup> of the genus *Garcinia* has also resulted in a similar relationship to that from clustering methods (Figure 5). The indigenous *Garcinia* species form two major clusters as evidenced from anatomical studies. The two clusters are composed of the same species as in the present study. However, the placement of *G. morella* in the preceding anatomical study shows a wide deviation and is grouped with the cluster 1 species.

The same anatomical study illustrates that *G. hermonii* forms one distinct cluster as inferred by the NJ tree. The other three species (*G. thwaitesii*, *G. spicata* and *G. echinocarpa*) form another cluster together with *G. morella*. The other major cluster includes *G. quaesita*, *G. zeylanica* and *G. terpnophylla*, and *G. terpnophylla* forms one subgroup with a close relationship between *G. quaesita* and *G. zeylanica* as in the present study.

### Conservation status of Sri Lankan *Garcinia* species

The history of the field of taxonomy has shown that poorly resolved taxonomies have a negative impact on conservation assessment and lack of systematic knowledge of threatened taxa prevents effective conservation<sup>11</sup>. Thus, systematic studies impose an enormous influence on conservation programmes.

Comprehensive revision on the conservation status of the *Garcinia* species appears to be vital. *Garcinia* species have not been assessed in detail for their distribution and conservation since the National Conservation Review (NCR)<sup>12</sup>. The NCR<sup>12</sup> was done as a rapid biodiversity

survey to identify the localities with high biodiversity and to use in conservation strategies, and did not focus on the species composition due to several limitations.

Observations made during the current study revealed that the *Garcinia* species were absent even from the recorded NCR locations, while some locations recorded higher *Garcinia* species richness than was recorded in the NCR database.

Most of the herbarium specimens of lowland wet zone *Garcinia* species deposited in the National Herbarium have been collected from Morapitiya. During the current study, only a few individuals of three *Garcinia* species were sampled from Kalugala and Morapitiya areas, which are now highly disturbed due to encroachments. Our observations reveal that the diversity of *Garcinia* species has considerably declined in these locations.

The national IUCN red data list has not considered *Garcinia* species for the latest revision<sup>7</sup> mainly due to financial, time and resource constraints and the poor knowledge on the distribution of the species in Sri Lanka. Even where information is available the data is limited. Inadequate herbarium collections of plant species were a constraint to confirmation of the occurrence of species. Thus, the conservation status of these species is also uncertain.

The observations made during the present study revealed that *G. terpnophylla* is a highly threatened species in Sri Lanka and one of the varieties has not been recorded in the wild for nearly a century while the lowland wet zone variety is confined to small patches in the wet zone.

Wathurana, a fresh water swamp forest in Bulathsinhala, which is privately owned and comprises about 12 ha in land area, recorded the highest diversity of *Garcinia* species. All possible wet lowland species were sampled from the site and the species showed an even distribution. The individuals possess adventitious roots regardless of the species which may be an adaptation to the water logged, swampy environment. The Peak Wilderness Sanctuary is another location that recorded high species diversity of *Garcinia*. Being a large area and showing a wide variation in environmental, ecological and geographical factors, it recorded seven out of eight indigenous *Garcinia* species (explicitly *G. spicata*, which is the dry zone species).

### CONCLUSION

Vegetative morphology can be successfully used to resolve species circumscriptions and identification

of Sri Lankan *Garcinia* species. The present study reveals that Sri Lankan *Garcinia* forms two major groups within the genus; species with coriaceous leaves and species with non-coriaceous leaves.

The species can be distinctly identified by certain vegetative morphological characters. *G. hermonii* and *G. echinocarpa* have densely arranged secondary veins and the two species can be differentiated by the colour of the petiole and the shape of the leaf tip. *G. spicata* and *G. thwaitesii* can be easily distinguished from the venation characteristics. The veins of *G. thwaitesii* can be felt by hand.

*G. quaesita*, *G. zeylanica* and *G. morella* are very similar in vegetative morphology, and are commonly known as *Goraka*. The sap colour of *G. quaesita* trees is the best vegetative character for identifying them in the field. *G. morella* and *G. zeylanica* can be distinguished by the colour of live bark. *G. zeylanica* possess a dark reddish brown coloured live bark. *G. terpnophylla* have the unique character of an intermarginal vein and they are canopy trees of lowland wet evergreen forests. The mature individuals possess buttresses.

The study has revealed that *Garcinia* species diversity is declining in certain areas. Therefore, accurate and broad systematic studies of the genus will be an asset for the conservation perspectives of these species in Sri Lanka.

The genus *Garcinia* of order Malpighiales is one of the taxa with poorly resolved phylogenetic relationships. The molecular markers for the genus are now being developed, and those markers can be used to carry out DNA based studies for the Sri Lankan *Garcinia* species.

The results of the current study are congruent with those of the previous study which was based on vegetative anatomy<sup>9</sup>. Therefore combined multidisciplinary analysis of anatomy, vegetative and reproductive morphology as well as chemotaxonomy is necessary to study the more robust phylogeny of this group which could be used for studies of phytogeography and evolutionary radiation of the species.

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### References

1. Angiosperm phylogeny. <http://www.mobot.org/MOBOT/research/APweb>. Accessed on 21 April 2008.
2. Gustafsson M.H.G. & Bittrich V. & Stevens P.F. (2002). Phylogeny of Clusiaceae based on rbcL sequences. *International Journal of Plant Sciences* **163**(6):1045-1054.
3. Kostermans A.J.G.H. (1980). In: *A Revised Handbook to the Flora of Ceylon*. vol 1 (Eds. Dassanayake M.D. & Fosberg F.R.) pp. 72-89. Amerind Publishing Company (Pvt) Ltd, New Delhi **1**:72-89.
4. Trimen H. (1893). *A Handbook to the Flora of Ceylon*, iii, pp. xvi+327. Bishen Singh Mahendrapal Singh, New Connaught Place, Dehra Dun, India.
5. Alston A.G.H. (1931). *Supplement to Trimen's Handbook to the Flora of Ceylon, Part VI*, p. 101. Dalau & Co. Ltd., London.
6. International Union for conservation of Nature, Sri Lanka and the Ministry of Environment and Natural Resources (2007). The 2007. Red List of Threatened Fauna and Flora of Sri Lanka, Colombo.
7. Ashton M.S., Gunatilleke C.V.S., Singhakumara B.M.P. & Gunatilleke I.A.U.N. (2001). Restoration pathway for main forests in South West Sri Lanka: a review of concepts and models. *Forest Ecology and Management* **154**(3): 409-430.
8. Chomnawang M.T., Surassmo S., Nukoolkarn V.S. & Gritsanapan W. (2005). Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. *Journal of Ethnopharmacology* **101**(1-3): 330-333.
9. Jiang D.J. (2004). Pharmacological effects of xanthenes as cardiovascular protective agents. *Cardiovascular Drug Reviews* **22**(2):91-102.
10. Jayaweera D.M.A. (1981). *Medicinal Plants- Indigenous and Exotic-Used in Ceylon- Part III*, pp. 66-71. National Science Council of Sri Lanka. 47/5, Maitland Place, Colombo 07.
11. Pathirana P.S.K. & Herat T.R. (2004). Comparative vegetative anatomical study of the genus *Garcinia* L. (Clusiaceae/ Guttiferae) in Sri Lanka. *Ceylon Journal of Science (Biological Sciences)* **32**: 39-66.
12. Radford D.G., Carplo C.B. & Santos G.A. (1974). Coconut varieties in the Philippines. *Proceedings of the Fifth Annual Meeting of the Crop Science Society of the Philippines*, May Naga City.
13. Bacon C.D. & Baily C.D. (2006). Taxonomy and Conservation: A Case study from *Chamaedorea allernans*. *Annals of Botany* **98**(4):755-763.
14. International Union for conservation of Nature (1997). *Designing an optimum protected areas system for Sri Lanka's natural forests*, Vol. 1 and 2, pp. X+ 299. Forest Department. Ministry of Forestry and Environment, Battaramulla.