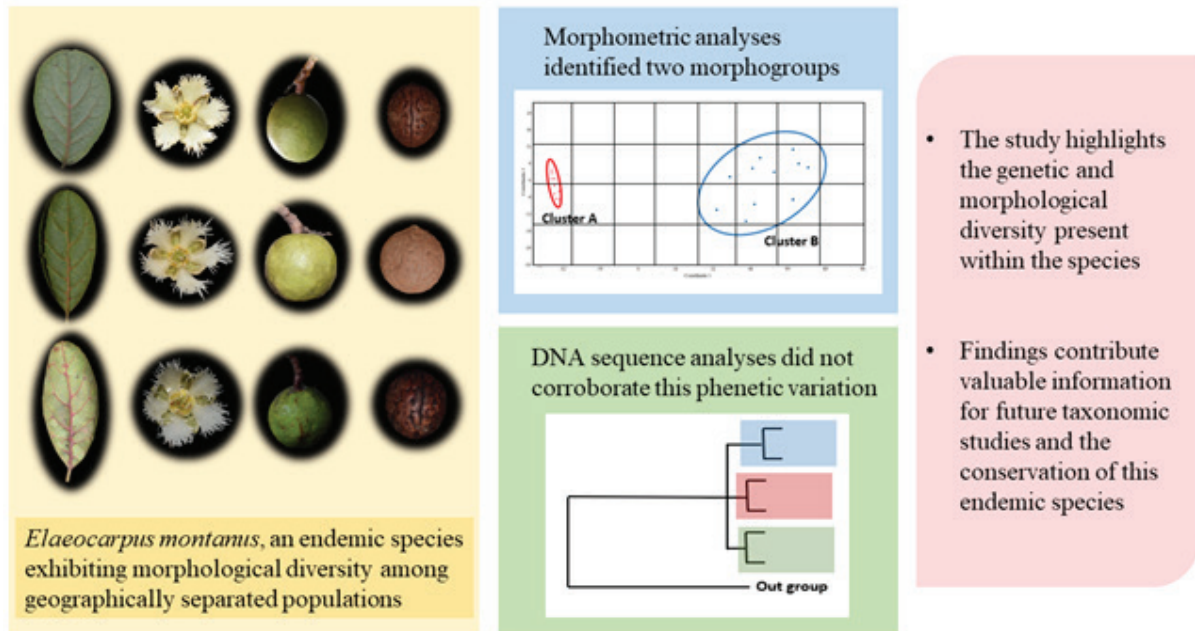


RESEARCH ARTICLE

Intraspecific Diversity in the *Elaeocarpus montanus* Thwaites (Elaeocarpaceae) Species Complex in Sri Lanka: Insights from an Integrative Morphological and Molecular Approach

S.I. Hapuarachchi, G.G.N.D. Silva, D.M.D. Yakandawala* and K. Yakandawala



Highlights

- *Elaeocarpus montanus* exhibits intraspecific diversity, initially suggested a species complex
- An integrative approach combining morphometrics and molecular phylogenetics was employed
- Morphometrics revealed two morphogroups, but DNA data did not support this differentiation
- Recording intraspecific genetic and morphological diversity is important
- Findings contribute for future taxonomic studies and conservation

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Intraspecific Diversity in the *Elaeocarpus montanus* Thwaites (Elaeocarpaceae) Species Complex in Sri Lanka: Insights from an Integrative Morphological and Molecular Approach

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Abstract: Documenting both morphological and genetic diversity in plant populations is a vital component of taxonomic research and plays a key role in biodiversity conservation. While many studies culminate in the recognition of new species or infraspecific taxa, instances where observed variation does not justify formal taxonomic recognition are often underreported. This study presents evidence of intraspecific diversity in *Elaeocarpus montanus* (Elaeocarpaceae), an endemic species in Sri Lanka, based on field observations that initially suggested a species complex. The study focuses on an integrative approach combining morphometric analysis and molecular phylogenetics. Morphometric results supported the existence of two distinct morphogroups; however, molecular data did not corroborate this division. The morphological variation corresponded to three geographically and attitudinally distinct populations; low, mid, and high elevations in the central mountains. In addition to reporting these findings, we provide an updated morphological description and distribution records with taxonomic clarification. These data contribute significantly to the future taxonomic studies and the conservation of this endemic species.

Keywords: Conservation biology; Elevation gradient; Integrative taxonomy; Morphometric analysis; Molecular analysis

INTRODUCTION

Sri Lanka harbors an exceptionally high proportion of endemic species across various taxa, including 28% of flowering plants, 85% of amphibians, 60% of reptiles, and 50% of freshwater fishes (Gunatilleke et al., 2017). However, this remarkable biodiversity is facing serious threats due to habitat loss, degradation, fragmentation, overexploitation of biological resources, pollution, and the rapid expansion of invasive alien species. The country's designation as part of a global biodiversity hotspot is attributed not only to its high levels of endemism but also to this growing pressure from anthropogenic activities. At present, Sri Lanka together with the Western Ghats of Southern India is recognized as one of the world's 34 biodiversity hotspots (Mittermeier et al., 2004). According to the National Red List 2020, 1,496 species which are nearly 50% of the country's 3,087 native angiosperms are classified as threatened (Ministry of Environment, 2020), underscoring the urgent need for focused conservation

initiatives.

Taxonomic studies provide the fundamental knowledge of biodiversity and play a crucial role in conservation (Kim & Byrne, 2006). Despite its importance, taxonomy remains an often-neglected field, yet effective biodiversity conservation cannot be achieved without proper species identification (Madola et al., 2022). Modern integrative taxonomic approaches, combining morphological, molecular and ecological data, can successfully resolve complex taxonomic problems and clarify species boundaries. Insufficient understanding of the quantity and distinctions among species can impede successful conservation efforts (Baba, 2013). Recent taxonomic studies focusing on the island's flora have provided valuable insights into resolving species limits (Yakandawala & Samarakoon, 2006; Guruge et al., 2017), clarifying past misidentifications (Guruge et al., 2016; Manawaduge et al., 2016), and describing new species (Manawaduge et al., 2018; Madola et al., 2021) all of which are critical contributions to conservation efforts. Therefore, in the absence of comprehensive taxonomic studies, species may become extinct before they are even recognized or documented. One such unresolved taxonomic complex in Sri Lanka is the genus *Elaeocarpus* (Elaeocarpaceae), for which a taxonomic revision has now been initiated (Silva et al., 2023).

The last revision of *Elaeocarpus* was carried out in 2001 by Zmarzty where the study concentrated on Sri Lankan and South Indian species belonging to the sect. *Elaeocarpus*. The study of Sri Lankan material was based on herbarium specimens collected during the revision by Meijer's in 1995. Presently in Sri Lanka, the genus is represented by ten species which are endemic to the island (Silva et al., 2023). The ongoing taxonomic study on the genus *Elaeocarpus* (Silva et al., 2021) has revealed distinct morphotypes among field-collected specimens from different locations within *E. montanus* Thwaites. Interestingly, these locations are also geographically separated by elevation: Horton Plains, Hakgala, and Pidurutalagala occur at high elevations (above 1,770 m a.s.l.), while the High Forest/Mathurata populations (1,700–1,760 m a.s.l.) occupy an intermediate

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elevation between the Loolkandura populations (1,500–1,526 m a.s.l.). All these *E. montanus* populations are confined to the upper montane zone and occur at distinct elevation ranges; high, mid, and low respectively within the zone.

These morphological variations (leaf shape, number of domatia on a leaf, leaf base angle average, petal division numbers etc.) raise the possibility of a species complex and highlight the need for further investigations. *Elaeocarpus montanus* is an endemic taxon confined to the upper montane zone from 1900–3000 m altitude (Meijer, 1995) and is recognized as a vulnerable species (VU category) in the most recent national red listing in 2020 (Ministry of Environment, 2020).

To clarify the taxonomic boundaries within the *E. montanus* species complex, this study re-evaluated the identified morphotypes using morphometric analyses combined with DNA sequence data from nuclear and chloroplast markers. We employed phylogenetic framework to test whether the observed morphological variation corresponds to distinct evolutionary lineages.

MATERIALS AND METHODS

Study sites and sampling

Twenty individuals from five populations, representing the morphotypes of *E. montanus*, identified during field visits were collected from different locations in the forest reserves of the wet zone, Sri Lanka between November 2019–December 2023. A GPS (Garmin Etrex 10, USA) was used to record the location. Each sample from a different location was given an acronym for their easy reference. Details of the collected specimens are given in Table 1, and the locations are illustrated on a map in Figure 1. In addition, vouchers of the samples were collected.

Morphometric analysis

The field collected samples were studied in detail in the laboratory. Morphological data were obtained from two to three randomly selected individuals from each population. During the sample collection, emphasis was placed on capturing the maximum morphological diversity. Measurements were taken using a ruler or a stereomicroscope (10446322, 2X WD Leica), and the values were average. Morphometric analysis was carried out using ordination and clustering methods to assess patterns of morphological variation. The statistical program PAST (version 4.03; Hammer et al., 2001) was used to perform Hierarchical Cluster Analysis (CA) and Principal Coordinate Analysis (PCoA). Quantitative characters were represented by their mean values, calculated from three randomly selected measurements per individual for leaf, floral, and fruit dimensions. Uninformative characters, those exhibiting no observable variation, were excluded from the dataset and the data was normalized. The morphological data matrix consisted of 29 quantitative and 12 qualitative morphological characters. The final set of characters employed in the morphometric analyses, along with their respective character states, are listed in Table 2. To explore initial phenetic groupings of specimens based on morphological similarity, Cluster analysis (CA) was applied

to the complete data matrix. The analysis was conducted using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), with Euclidean distance (cophenetic correlation value = 0.9946) employed to calculate similarity measures. Ordination analysis was performed using Euclidean distance (transformation exponent C = 2) to generate a distance matrix for Principal Coordinate Analysis (PCoA). Unlike Principal Component Analysis (PCA), which can be distorted by binary characters, PCoA is based on distance between individuals, making it more suitable for datasets comprising mixed character types (Cupido, 2003).

Molecular analysis

Six individuals, two from each of the three morphologically distinct populations encountered across the three elevation ranges during the field work, were selected for the molecular analysis.

Genomic DNA extractions from 0.5 g of fresh leaf samples were carried out using a modified CTAB (Cetyltrimethylammonium bromide) method (Doyle & Doyle, 1990). The standard CTAB extraction protocol was modified by incorporating a sorbitol extraction buffer (pH 8), polyvinylpyrrolidone (PVP), and sodium acetate. The sorbitol extraction buffer [composed of 100.0 mM Tris–HCl (pH 8.0), 0.35 M sorbitol, 5.0 mM EDTA (pH 8.0), and 1% (w/v) PVP (molecular weight 40,000; PVP-40)] was prepared in advance, and β -mercaptoethanol was added just prior to use (Inglis et al., 2018). Extracted DNA was tested using Agarose gel electrophoresis to confirm the presence of DNA and stored in -20°C .

PCR amplification

Amplification of DNA was achieved through Polymerase Chain Reaction (PCR) performed in an Eppendorf Mastercycler thermal cycler (Eppendorf, Hamburg, Germany) for two intergenic regions (*trnL-trnF*, *trnH-psbA*), one protein-coding region (*matK*, partial sequence) and nuclear ribosomal locus (nrITS). The details of the primer sequences are given in Table 3. The PCR solution mixtures (40 μl) were prepared with 20 μl of 2X Green PCR Master Mix (Promega Corporation, Madison, Wisconsin, USA), 0.4 μl (0.1 mM) of each forward and reverse primers, 0.4 μl of GoTaq® Flexi DNA polymerase (Promega, USA), 2 μl of MgCl_2 (25 mM) (Promega, USA), 12.8 μl of nuclease free water (Promega, USA), 2 μl of bovine serum albumin (BSA) (0.01 $\mu\text{g}/\mu\text{l}$) and 2 μl of DNA template (10 ng/ μL). The PCR products were quantified on a 1% agarose gel using Low DNA Mass Ladder (Promega, USA). The amplified PCR products were sequenced at the Genetech, Colombo 08, Sri Lanka. The details of the PCR profiles are given in Table 4.

Phylogenetic analysis

All obtained sequences were edited for ambiguities using BioEdit version 7.2 (Hall, 1999) and aligned with ClustalW (Thompson et al., 1994) using BioEdit (Hall, 1999), with minor manual adjustments. *Elaeocarpus angustifolius* was used as the outgroup (Baba, 2013) and the respective sequences (Table 5) were retrieved from the NCBI GenBank database (<https://www.ncbi.nlm.nih.gov/>).

Table 1: Details of the collection sites of the *Elaeocarpus montanus* samples employed in the study.

Acronym	Location and District	Elevation (m a.s.l.*)	Elevation range within the upper montane zone
HP01	Horton plains National Park, Nuwara Eliya	2126	Above 1770 m a.s.l. High elevation
HP02	Horton plains National Park, Nuwara Eliya	2130	
HP12	Horton plains forest, Nuwara Eliya	2139	
HP14	Horton plains National Park, Nuwara Eliya	2115	
HP36 [#]	Horton plains National Park, Nuwara Eliya	2169	
HP21 [#]	Thotupala kanda, Nuwara Eliya	1874	
HP10	Thotupala kanda, Nuwara Eliya	2025	
PT04	Piduruthalagala Forest, Nuwara Eliya	2149	
PT05	Piduruthalagala Forest, Nuwara Eliya	2149	
HK02	Hakgala Strict Nature Reserve, Nuwara Eliya	1787	
HK03	Hakgala Strict Nature Reserve, Nuwara Eliya	1803	1700-1760 m a.s.l. Mid elevation
HK04	Hakgala Strict Nature Reserve, Nuwara Eliya	1824	
HK10	Hakgala Strict Nature Reserve, Nuwara Eliya	1770	
MR01 [#]	High Forest, Mathurata, Nuwara Eliya	1760	
MR02	High Forest, Mathurata, Nuwara Eliya	1739	
MR03	High Forest, Mathurata, Nuwara Eliya	1744	1500-1526 m a.s.l. Low elevation
MR07	High Forest, Mathurata, Nuwara Eliya	1767	
MR08 [#]	High Forest, Mathurata, Nuwara Eliya	1702	
LK03 [#]	Loolkandura, Kandy	1526	
LK12 [#]	Loolkandura, Kandy	1509	

Note: The individuals used in molecular study are indicated with a #.

*m a.s.l. = meters above sea level

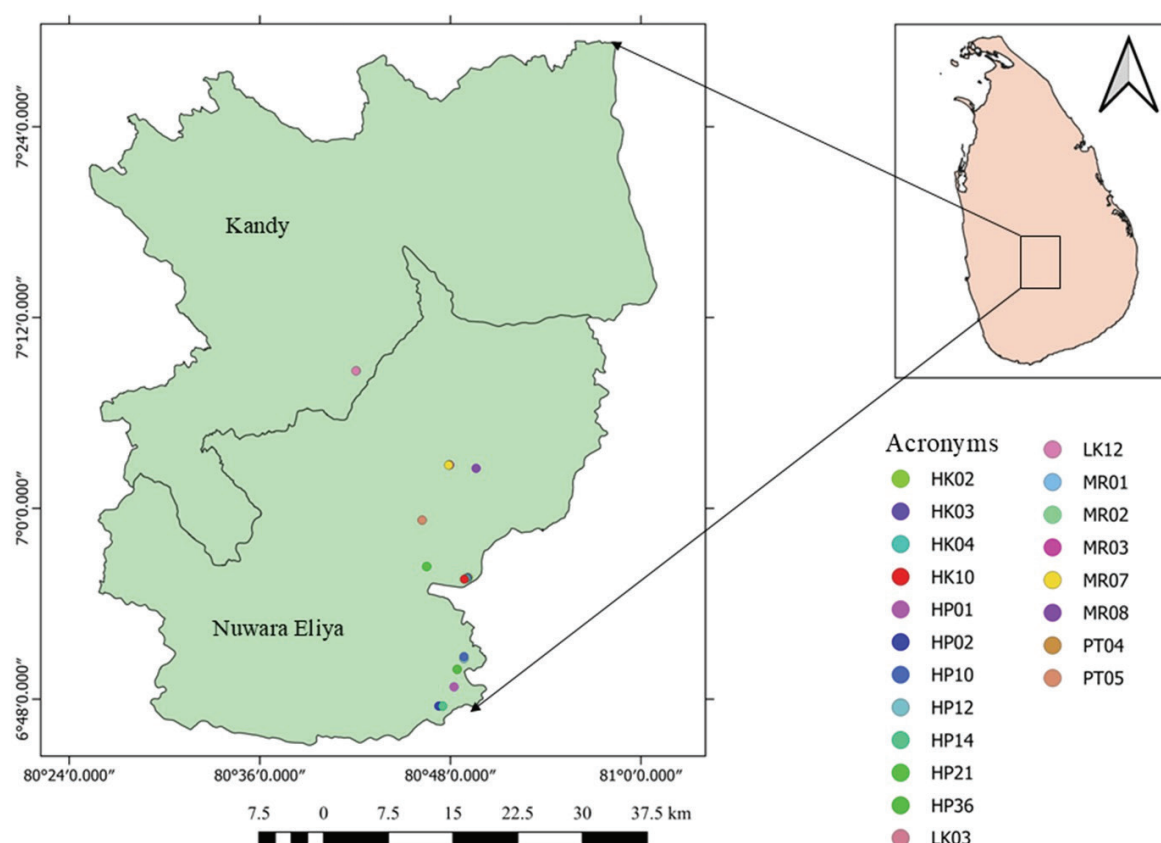


Figure 1: *Elaeocarpus montanus* sampling sites in the Kandy and Nuwara Eliya districts of Sri Lanka. Colored dots represent different sampling locations, labeled with site-specific acronyms. The inset map shows the location of the study area within Sri Lanka.

Table 2: Characters and the character states employed in the morphometric analysis

	Characters	Character states	Character type
1	Grouping of leaves at the apex of the stem	Compact = 0, Lax = 1	Binary
2	Leaf length (cm)	-	Continuous
3	Leaf width (cm)	-	Continuous
4	Leaf shape	Widely elliptic = 1, Obovate = 2	Binary
5	Petiole length (cm)	-	Continuous
6	Petiole hair density	Low = 0, High = 1	Binary
7	Leaf apex angle	-	Continuous
8	Leaf base angle	-	Continuous
9	Number of main vein pairs	-	Count
10	Angle of the secondary veins	-	Continuous
11	Leaf adaxial surface	Glabrous = 0, Pubescent = 1	Binary
12	Leaf lower surface vein colour	Pink = 0, Redish-brown = 1	Binary
13	Leaf domatia size (mm)	-	Continuous
14	Leaf domatia colour	Pink = 0, Redish-brown = 1	Binary
15	Number of domatia on the leaf	-	Count
16	Inflorescence length (cm)	-	Continuous
17	Number of florets	-	Count
18	Pedicel colour	Pink = 0, Rusty-red = 1	Binary
19	Pedicel length (mm)	-	Continuous
20	Pedicel width (mm)	-	Continuous
21	Sepal colour (outer)	Greenish brown = 0, Rusty-red = 1	Binary
22	Sepal hair density	Low = 0, High = 1	Binary
23	Sepal length (mm)	-	Continuous
24	Sepal width (mm)	-	Continuous
25	Petal division numbers	-	Count
26	Floret diameter (cm)	-	Continuous
27	Petal length (mm)	-	Continuous
28	Petal width (mm)	-	Continuous
29	Petal division length (mm)	-	Continuous
30	No of stamens	-	Count
31	Disk height (mm)	-	Continuous
32	Ovary length (mm)	-	Continuous
33	Fruit length (cm)	-	Continuous
34	Fruit width (cm)	-	Continuous
35	Fruit Shape	Globular = 0, Widely elliptic = 1	Binary
36	Fruit texture	Smooth = 0, Hairy = 1	Binary
37	Drupe flesh thickness (mm)	-	Continuous
38	Seed length (cm)	-	Continuous
39	Seed width (cm)	-	Continuous
40	Stone wall thickness (mm)	-	Continuous
41	Seed base	Round = 0, Acute = 1	Binary

Table 3: Details of the primers used to amplify different DNA regions

Region	Primer	DNA sequence	Reference
<i>matK</i>	3F – KIM	CGT ACA GTA CTT TTG TGT TTA CGA G	(Baba, 2013)
	1R – KIM	ACC CAG TCC ATC TGG AAA TCT TGG TTC	
<i>trnL-trnF</i>	5' <i>tab f</i>	ATT TGA ACT GGT GAC ACG AG	(Taberlet et al., 1991)
	3' <i>tab c</i>	GCA AAT CGG TAG ACG CTA CG	
<i>trnH-psbA</i>	5' <i>psbA3_f</i>	GTT ATG CAT GAA CGT AAT GCT C	(Sang et al., 1997)
	3' <i>trnHf_05</i>	CGC GCA TGG TGG ATT CAC AAT CC	
nrITS	5' ITS5	GGA AGT AAA AGT CGT AAC AAG G	(White et al., 1990)
	3' ITS4	TCC TCC GCT TATT GAT ATG C	

Table 4: PCR profiles employed in the study (Silva et al., 2025)

Region	PCR Profile					
	Initial denaturation temp./ time	Denaturation temp./ time	Annealing temp./ time	Extension temp./ time	Final extension temp./ time	No. of cycles
<i>matK</i>	95 °C/ 2 min	95 °C/ 30 sec	56.8 °C/1 min	72 °C/ 1 min	72 °C/ 2 min	35
<i>trnL-trnF</i>	95 °C/ 2 min	95 °C/ 30 sec	55 °C/ 30 sec	72 °C/ 1 min	72 °C/ 2 min	35
<i>trnH-psbA</i>	94 °C/ 3 min	94 °C/ 30 sec	56.6°C/ 30 sec	72 °C/ 1 min	72 °C/ 7 min	35
nrITS	95 °C/ 2 min	95 °C/ 30 sec	52 °C/30sec	72 °C/ 1 min	72 °C/ 7min	35

Table 5: Details of the GenBank accession numbers of sequences of the outgroup

Region	GenBank accession number
<i>matK</i>	KR530755
<i>trnL-trnF</i>	KJ631297
<i>trnH-psbA</i>	KY565484
nrITS	KJ675645

Phylogenetic analyses were subsequently conducted using Bayesian Inference (BI). Bayesian phylogenetic analysis was performed using MrBayes version 3.2.1 (Ronquist & Huelsenbeck, 2003). The best-fitting nucleotide substitution model for each DNA region was determined using jModelTest (version 2.1.10) (Darriba et al., 2012) based on model selection criteria outlined by Posada and Buckley (2004). HKY+GAMMA was applied to *matK* and nrITS regions whereas F81+F+GAMMA model was applied to *trnH-psbA* and *trnL-trnF* regions. The Markov Chain Monte Carlo (MCMC) algorithm was run for 10,000,000 generations, with two independent runs of four chains each (one cold and three heated). Trees were sampled every 1,000 generations and Tracer version 1.5 (Rambaut & Drummond, 2009) was used to estimate burn-in and check for convergence. The trees that fell under the 25% burn-in were discarded, while the remaining trees were utilized to build a Bayesian consensus tree.

Clade support was considered significant for posterior probability values ≥ 0.95 (Larget & Simon, 1999). The resulting phylogenetic tree was visualized using FigTree version 1.4.4 (Rambaut, 2018).

Prior to performing the combined analyses, phylogenetic trees generated from individual regions were compared to assess incongruence. All individual datasets resulted in

unresolved trees, except for the *trnH-psbA* region, which yielded a slightly resolved tree. As combining datasets typically provide stronger phylogenetic signals, we conducted two separate combined analyses: one using only the three chloroplast regions, and another incorporating all regions, including nrITS.

RESULTS

Morphometric analysis

The dendrogram that resulted from the CA identified two distinct morphogroups separating at a distance value of 92 (cophenetic correlation value = 0.9946) each corresponding to Horton plains, Hakgala and Piduruthalagala populations (Cluster A) and the Lookandura and High Forest/Mathurata populations (Cluster B) (Figure 2).

Similarly, the PCoA plot of the first and second coordinates also resolved two discrete clusters (Figure 3) each of which precisely matched one of the clusters shown by the UPGMA dendrogram (Figure 2). The first four (principal) eigenvalues recovered from the PCoA (366.74, 273.02, 175.88 and 99.826) accounted for 99.60% of the total variance (98.134%, 0.73057%, 0.47064% and 0.26712% respectively).

According to the analysis, average leaf base angle, number of domatia on a leaf, angle of the secondary veins, leaf apex angle, number of main vein pairs, leaf shape and petal division numbers were the most contributing characters to the divisions (Table 6).

Molecular analysis

The results of the sequence alignments included nrITS (516 bp), *matK* (756 bp), *trnL-trnF* (867 bp), *trnH-psbA* (490 bp), and a concatenated matrix of chloroplast regions

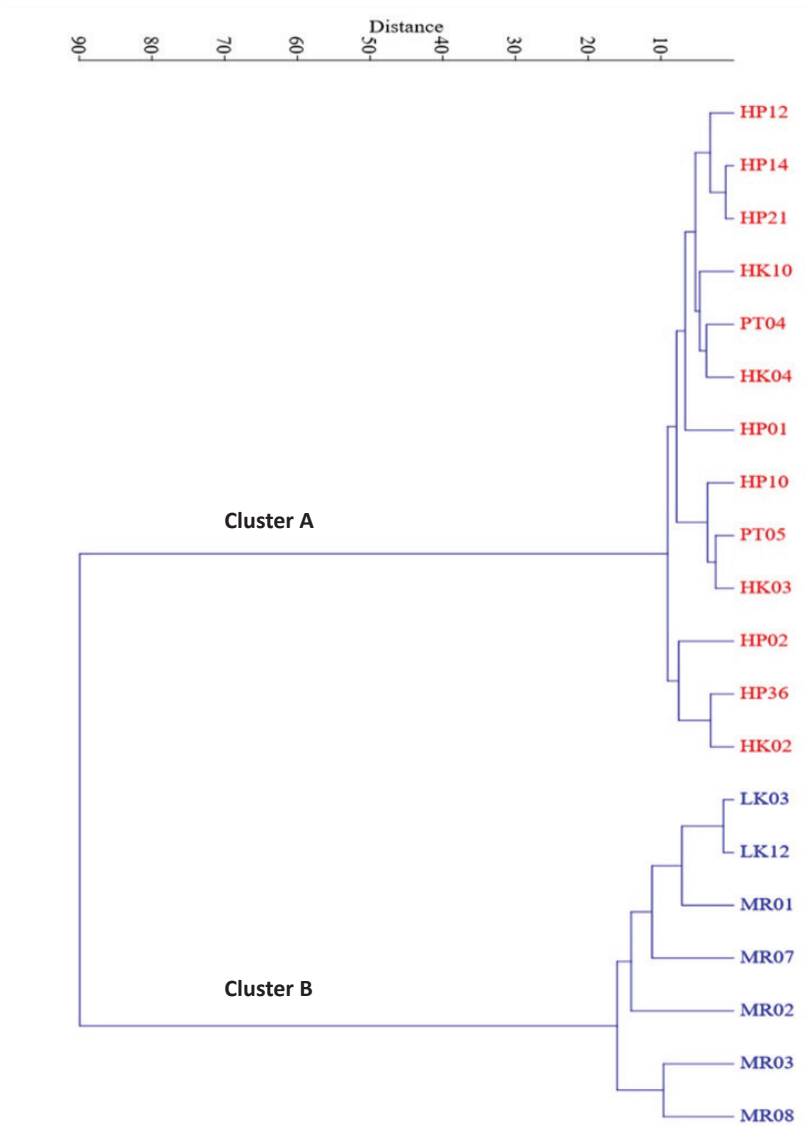


Figure 2: The dendrogram resulted from the cluster analysis with two distinct morphogroups (Cluster A & Cluster B) of *Elaeocarpus montanus* identified from the morphological characters employed in the study

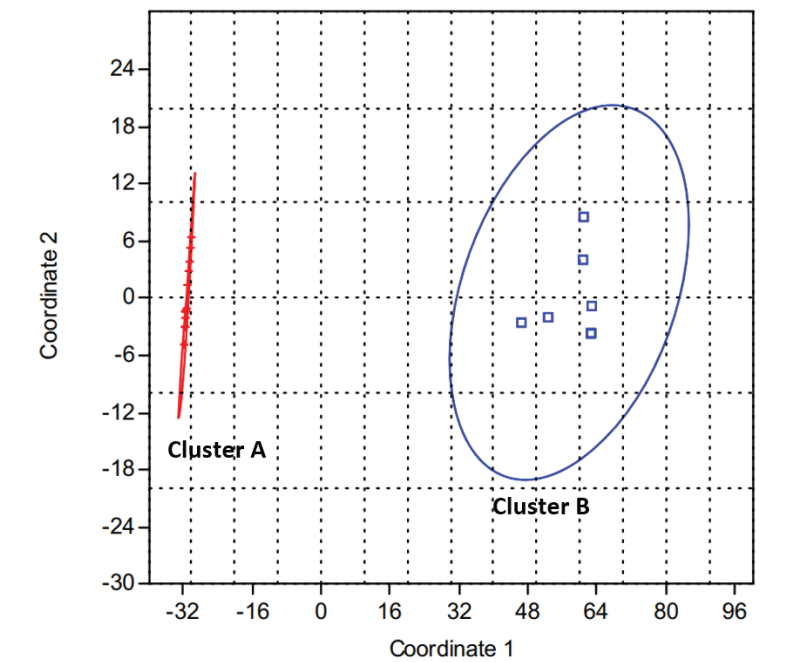


Figure 3: The scatter plot resulting from Principal Coordinate Analysis (PCoA) shows two distinct morpho-groups identified through cluster analysis of *Elaeocarpus montanus*, based on the morphological characters used in the study.

Table 6: The key morphological characters most contributing to the distinction between the two morpho-groups identified in the morphometric analysis

Character	Cluster A (Morphogroup corresponding to Horton plains, Hakgala, and Piduruthalagala populations)	Cluster B (Morphogroup corresponding to Loolkandura and High Forest/Mathurata populations)
Leaf base angle (°)	160 ⁰	67-83 ⁰
Number of domatia on a leaf	28-36	18-26
Angle of the secondary veins (°)	57-65 ⁰	43-57 ⁰
Leaf apex angle average (°)	143-153 ⁰	140-147 ⁰
Number of main vein pairs (average)	8	5-7
Leaf shape	Widely elliptic	Obovate
Petal division numbers	19-22	20-24

totaling 2,113 bp, while the complete dataset comprised 2,629 bp. The phylogenetic tress that resulted from individual data sets, nrITS, *matK* and *trnL-trnF* are not presented. The trees of the individual *trnH-psbA* region analysis and combined analysis of all regions, including nrITS are given in Figures 4 and 5 respectively.

All independent analyses, except *trnH-psbA* region, resulted in phylogenetic trees that were unresolved. The phylogenetic tree of the *trnH-psbA* region (Figures 4) supported all *E. montanus* accessions as monophyletic with a strong support, a posterior probability (PP) of 1. Within this *E. montanus* clade, the three-field observed morphologically distinct populations were separated as clades but unresolved, each receiving strong support (between PP 0.97-1).

Both analyses, one using the three chloroplast regions and the other using all regions (Figures 5), resolved *E. montanus* as monophyletic with a PP of 1. In both analyses, the internal structure of the clade was unresolved: the

three morphologically distinct populations observed in the field were separated into three clades, while the two morphogroups identified through morphometric analysis were not supported as monophyletic.

DISCUSSION

Understanding and resolving intraspecific variation is essential for accurately assessing and conserving biodiversity. Although three morphogroups were visually observed among the geographically separated populations confined to different elevation ranges, the morphometric analysis, both Cluster Analysis and Principal Coordinates Analysis, did not support this observation, instead identified only two morphogroups.

Populations from Horton Plains, Hakgala, and Piduruthalagala formed one morphogroup (Cluster A), while those from Loolkandura and High Forest/Mathurata formed the second morphogroup (Cluster B) (Figure 2).

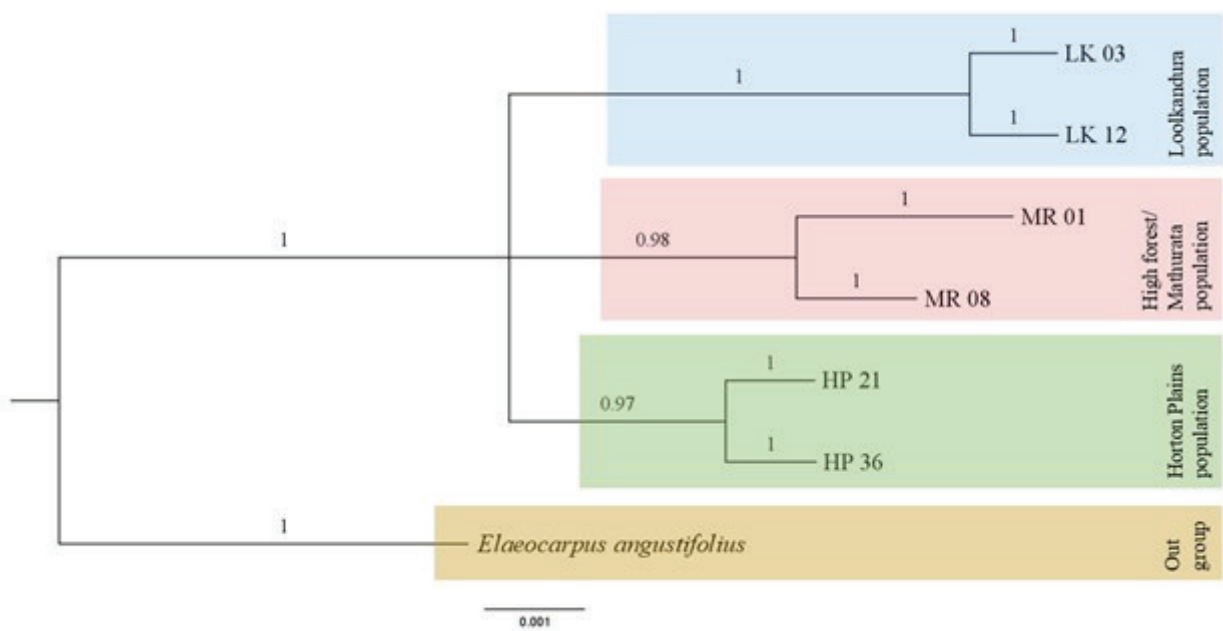


Figure 4: Phylogenetic tree recovered from BI analysis based on the individual *trnH-psbA* sequence data. The posterior probability values are indicated on the branches.

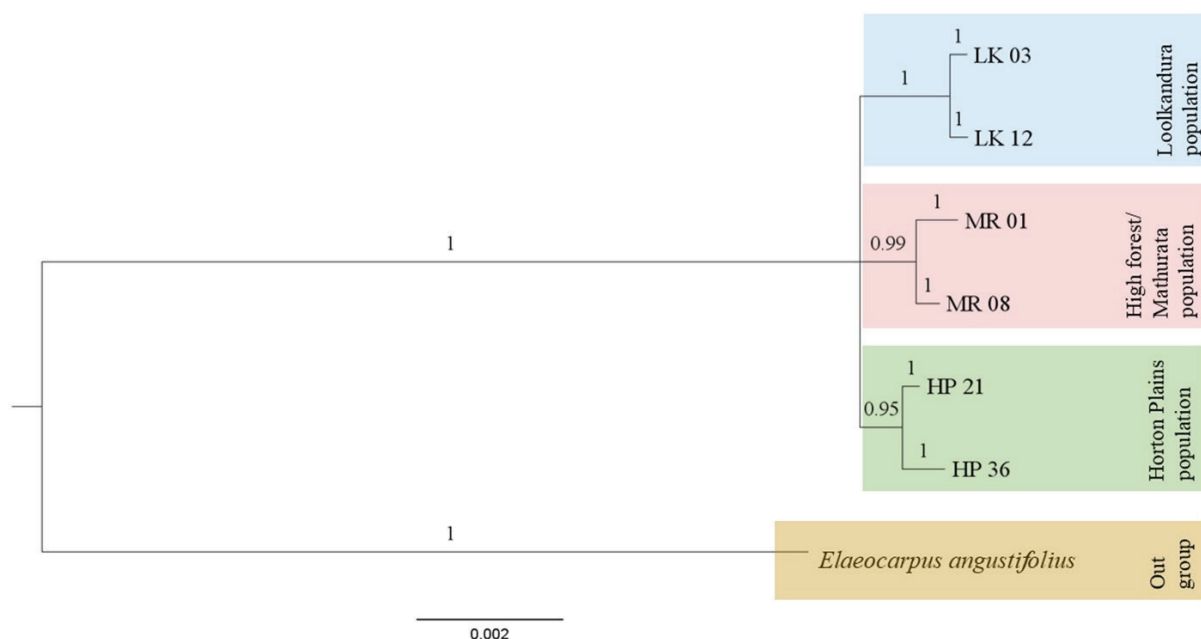


Figure 5: Phylogenetic tree recovered from BI analysis based on the concatenated data from all DNA regions (nrITS, *matK*, *trnH-psbA*, and *trnL-trnF*). The posterior probability values are indicated on the branches.

Although distinctive features, such as larger seeds and reddish-tinted sepals were observed in the Highforest/Mathurata populations, these individuals were still grouped with those from Lookkandura in the analyses. However, two individuals (MR 03 and MR 08) were separated from the rest of the Mathurata and Lookkandura samples, indicating that they are morphologically distinct. This suggests that, when considered alongside all characters across all populations, the observed distinctive traits are insufficient on their own to warrant a separate grouping.

Considering the results of the molecular analyses of the independent analyses of four DNA regions, only *trnH-psbA* region supported *E. montanus* as a clade as well as internal grouping for the accessions representing different populations. During the concatenated analysis of all regions, the same topology was exhibited (Figures 5), with no support for the two Clusters (morphogroups) recognized by the morphometric analysis.

Even though the molecular sequence analysis was failed to support the phenetic variations recognized through morphometric analysis of *E. montanus* populations in Sri Lanka, the results indicate genetic diversity among populations. Furthermore, tree-building algorithms may not fully capture these differences, which could be better analyzed and visualized through population-level approaches. The resulting phylogenies from the nuclear and chloroplast datasets were not congruent, indicating possible differences in the evolutionary histories reflected by these genomic regions. Among the chloroplast markers used, only the *trnH-psbA* region produced a well-resolved tree, suggesting that the other chloroplast regions lacked sufficient phylogenetic signal or were influenced by factors such as incomplete lineage sorting or recent divergence. The study documents the morphological variation among populations confined to different elevation ranges and their genetic diversity. Although the results do not support the

recognition of taxa below the species level, they underscore the importance of conserving these populations, particularly in light of ongoing rapid deforestation. Furthermore, in most populations, no regeneration was observed, emphasizing the urgent need to initiate restoration efforts aimed at conserving their genetic diversity. An updated morphological description with the new data together with coloured plates (Figures 6, 7 and 8) are given below.

Morphological description

Medium sized tree 6-10 m. Twig tips lined with sparse to medium-dense, short, wavy, spreading or semi-spreading hairs. Terminal bud with hairs, usually reddish brown. Leaves loosely grouped towards twig tips or scattered. Petioles 10-20 mm, brownish red, with sparse to medium-dense, very short hairs, without pegs or teeth at apex. Leaves chartaceous to coriaceous, 6-9 x 5-7 cm, widely elliptic to obovate, apex rounded, never acuminate, apex angle 143-153°, base very broadly obtuse to rounded, base angle 67-160°; Blade glabrous above, beneath with sparse to medium-dense, short, spreading, brown hairs; mid-vein and main veins impressed above, raised and very prominent beneath, main veins 5-8 pairs at 50-65° to mid-vein (wider angles towards leaf-base), breaking up one quarter to halfway inside margin; domatia regularly present along mid-vein and in vein-branch forks, conspicuous; leaf margin revolute or not, weakly serrate or occasionally obviously serrate throughout the leaf-length. Domatia 18-36, cup-shaped, 2-3.5 mm, pink to reddish-brown. Inflorescence scattered behind leaves, rachises 5-9 cm long, Flowers 15-22 per inflorescence. Bracts early caducous, oblanceolate or ovate-elliptic, curved, 1.5-1.8 mm long, toothed, each with a conspicuous blackish-mucronate tip. Flower-buds ovoid, apex acute, rusty red. Pedicel pink to rusty red, 4-6 x 0.5-1 mm. Sepals persistent after petals and stamens, 3-5 mm long, indumentum dense on both surfaces, hairs wavy, semi-spreading or spreading, inner rusty red, outer

greenish-brown to rusty red. Petals creamy white, obovate to broadly obovate, 5.5–6 x 3–5 mm, with 19–24 apical divisions up to 4 mm long, indumentum dense throughout on both sides including divisions and margins, hairs fairly long, wavy. Stamens 20–25, falling before sepals or petals or both, filament straight or slightly sigmoid, 0.7–1.5 mm long, anther 1–1.7 mm long, outer and inner anther-teeth of about the same both teeth with or without short setae. Disk yellow, with fused lobes, 1–1.5 mm tall, with dense, short, spreading, rusty brown hairs. Ovary placed above the disk globular, 1.5–2 mm long, style 1.2–2 mm long. Fruit round, 2.3–3.1 cm diameter, apex obtuse to rounded, base rounded, skin smooth to slightly undulate, greenish red. Pedicels 7–8 mm long in fruit. Drupe flesh 3–4 mm thick. Stone round, 2.2–2.6 cm in diameter, surface finely and irregularly channelled and pitted, channels short, shallow, sutures well-defined, stone wall 4–5 mm thick, round apex, round to acute base.

Ecology: A species of the upper montane zone, from 1500–3000 m altitude.

Phenology: Flowering January–July, September; fruiting June–December.

Specimens examined

CENTRAL PROVINCE: Nuwara Eliya District, Moon Plains, 2000 m a.s.l., 27 November 1971, frs, *Balakrishnan et al.* 1135 (PDA, barcode PDA00118187!, US, MO); Kandapola FR., 19 June 1972, frs, *Maxwell & Jayasuriya* 872 (PDA, barcode PDA00118175!, US, MO); 1/3 up the climb to Horton Plains, 1950 m a.s.l., 7 October 1967, *Comanor* 461 (PDA, barcode PDA00119049!, US); Trail behind Farr Inn, 2130 m a.s.l., 23 April 1961, fls, *Hladik* 737 (PDA, barcode PDA00118176!, US); Hakgala jungle, 1500 m a.s.l., 18 May 1971, fls, *Kostermans* 24184 (PDA, barcode PDA00118185!, US); Nuwara Eliya, September 1851 and April 1856, *Thwaites* C.P. 2524, p.p. (PDA, barcode PDA00003404!); Horton Plains, border of secondary montane forest beside Pattipola Road, 2200 m a.s.l. (fr), 26 January 1977, *Cramer* 4812 (PDA, barcode PDA0119145!, K, US); West of Hewaheta, 80°44'E 07°06'N, forest above Hope Estate Upper Division, 1875 m a.s.l., fl., 23 August 1984, *Jayasuriya et al.* 2861 (PDA, barcode PDA00119054!); Horton Plains, Word's End Lookout, 2350 m a.s.l., fl., 19 April 1973, *Stone* 11261 (PDA, barcode PDA00118182!, US). High forest, Mathurata, 1760 m a.s.l., 15 June 2020, *N. Silva, D. Yakandawala & K. Yakandawala* MR01 (PDA!); High forest, Mathurata, 1702 m a.s.l., 15 June 2020, *N. Silva, D. Yakandawala & K. Yakandawala* MR08 (PDA!); Thotupola kanda, 1874 m a.s.l., 04 February 2022, *N. Silva, D. Yakandawala & K. Yakandawala* HP21 (PDA!); Horton plains National Park, 2169 m a.s.l., 04 February 2022, *N. Silva, D. Yakandawala & K. Yakandawala* HP36 (PDA!); Kandy District, Loolkandura, 1526 m a.s.l., 03 November 2019, *N. Silva, D. Yakandawala & K. Yakandawala* LK03 (PDA!).

Notes on taxonomic updates

The identities of the following specimens cited by Meijer (1995) during the under 'specimens examined'

in *E. montanus* have since been revised. PDA00112222 (CENTRAL PROVINCE: Kandy District, Hunnagiriya, 1000 m altitude, 6 June 1971, frs, *Kostermans* 24418) is now recognized as *E. hedyosmus* (Zmarzty, 2001). PDA00119143 (UVA PROVINCE: Badulla District, secondary montane forest, 2100 m altitude, 24 September 1974, fls, *Waas* 856) and PDA00119146 (Debedde, Namunukula, 1000 m altitude, 9 June 1971, *Kostermans* 24457) are now recognized as *E. taprobanicus* (Zmarzty, 2001). PDA00003399 (SABARAGAMUWA PROVINCE: Ratnapura District, Dumbanagala Hill, Rangala, September 1851 & April 1888, *Trimen s.n.*) that was recognized as *E. taprobanicus* by Zmarzty (2001) was subsequently recognized as *E. mahamayensis* (Silva et al., 2023). PDA00118186 (Adam's Peak, above Carney Road, 1700 m altitude, 21 December 1975, fls, *Bernardi* 16097) is supposed to be a new species (Silva et al., unpublished).

CONCLUSION

The morphological diversity observed in the field was supported by morphometric analyses, which identified two distinct clusters supporting two morphogroups within *E. montanus*. However, molecular data analyses did not corroborate this phenetic variation, and thus, the study does not support the recognition of taxa below the species level. Nonetheless, the study highlights the genetic and morphological diversity present within the species and updates the existing morphological description and distribution. Although morphometrics identified two morphogroups, the lack of genetic support suggests they do not warrant recognition as separate species, providing taxonomic clarity.

Additionally, the findings contribute valuable information for future taxonomic studies and the conservation of this endemic species by identifying new localities and providing accurate data relevant to initiatives such as the national red listing.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION

Conceptualization, Design of the study: **D.M.D.Y., K.Y.**; fieldwork and field data collection: **G.G.N.D.S., D.M.D.Y.,**

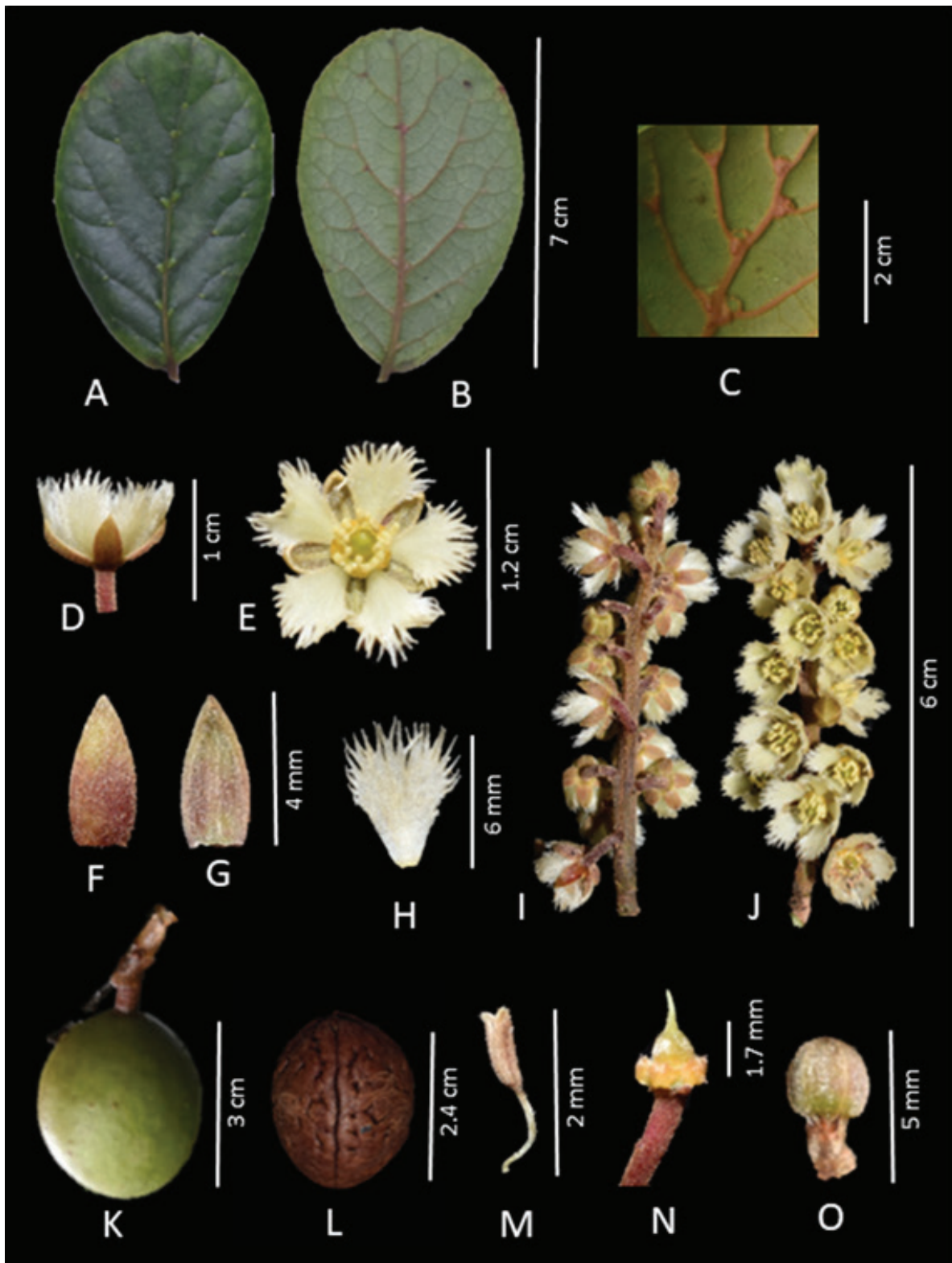


Figure 6: (A): Adaxial and (B): Abaxial surface of leaf. (C): leaf domatia. (D): Side view of a floret. (E): Birds eye view of a floret. (F) and (G): Sepals. (H): Petal. (I) and (J): Inflorescence. (K): Fruit. (L): Seed. (M): Stamen. (N): Disk and ovary. (O): Flower bud of Horton Plains population

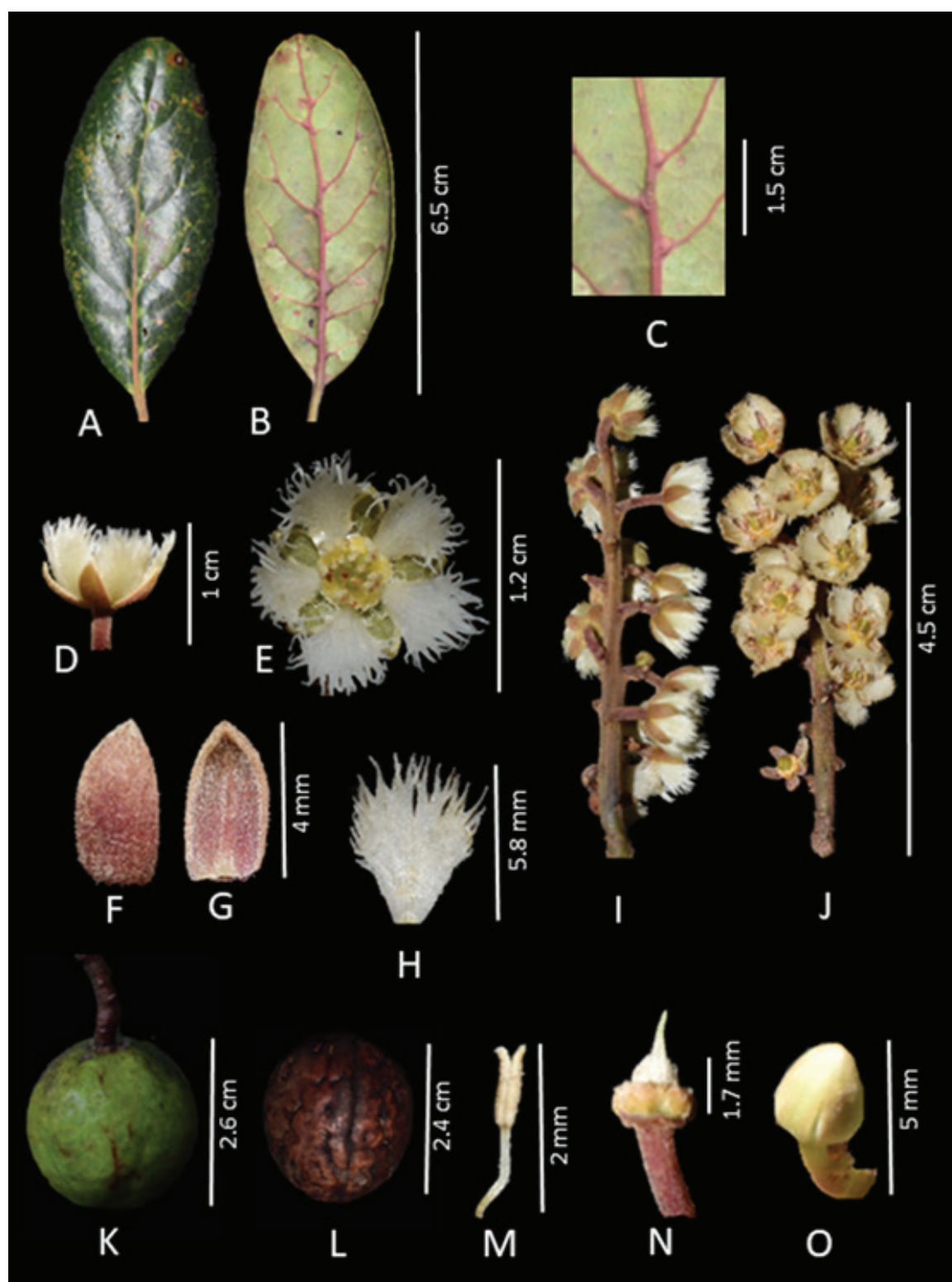


Figure 7: (A): Adaxial and (B): Abaxial surface of leaf. (C): leaf domatia. (D): Side view of a floret. (E): Birds eye view of a floret. (F): and (G): Sepals. (H): Petal. (I) and (J) Inflorescence. (K): Fruit. (L): Seed. (M): Stamen. (N): Disk and ovary. (O): Flower bud of Lookandura population

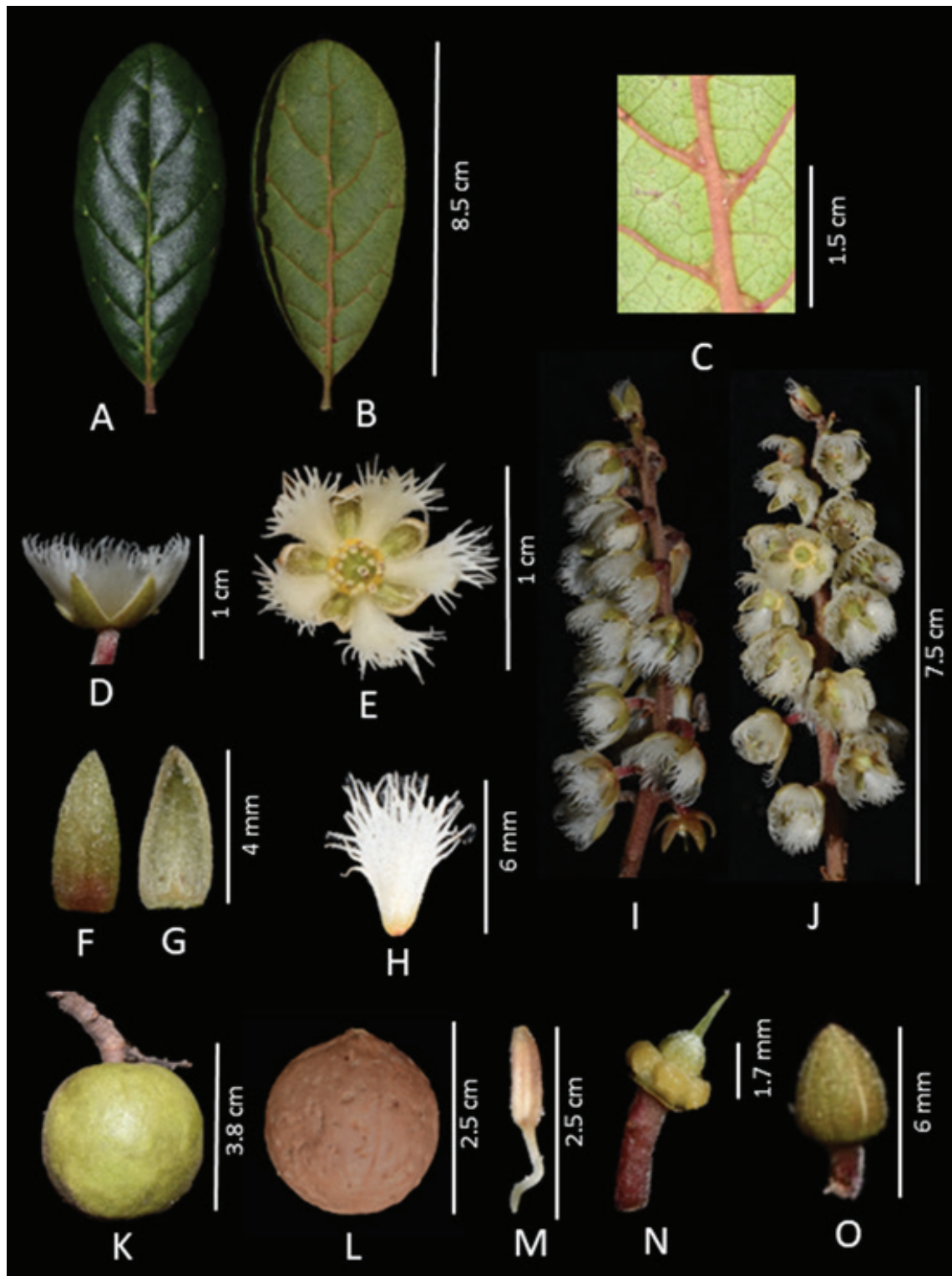


Figure 8: (A): Adaxial and (B): Abaxial surface of leaf. (C): leaf domatia. (D): Side view of a floret. (E): Birds eye view of a floret. (F): and (G): Sepals. (H): Petal. (I) and (J) Inflorescence. (K): Fruit. (L): Seed. (M): Stamen. (N): Disk and ovary. (O): Flower bud of High Forest/Mathurata population

K.Y.; Laboratory studies and analysis: **S.I.H., G.G.N.D.S.;** data interpretation preparation of the manuscript: **S.I.H., D.M.D.Y., G.G.N.D.S.** All authors contributed to the development of the final manuscript and approved its submission.

REFERENCES

- Baba, Y. (2013). *Evolution, systematics and taxonomy of Elaeocarpus (Elaeocarpaceae) in Australasia* (Doctoral dissertation, James Cook University). doi.org/10.25903/2sh7-ym93
- Cupido, C.N. (2003). Systematic studies in the genus *Merciera* (Campanulaceae): A re-assessment of species boundaries. *Adansonia*, **25**(1), 33–44.
- Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and high-performance computing. *Nature methods*, **9**(8), 772. https://doi.org/10.1038/nmeth.2109
- Doyle, J. J., & Doyle, J. L. (1990) Isolation of plant DNA from fresh tissue. *Focus* **12**(1), 13–15.
- Gunatilleke, N., Pethiyagoda, R., & Gunatilleke, S. (2017). Biodiversity of Sri Lanka. *Journal of the National Science Foundation of Sri Lanka*, **36**(0), 25–62. https://doi.org/10.4038/jnsfsr.v36i0.8047
- Guruge, D. S. K., Yakandawala, D., & Yakandawala, K. (2016). Confirming the identity of newly recorded *Nymphaea rubra* Roxb. Ex Andrews discerning from *Nymphaea pubescens* Willd. using morphometrics and molecular sequence analyses. *Bangladesh J. Plant Taxon.*, **23**(2), 107–117. https://doi.org/10.3329/bjpt.v23i2.30819
- Guruge, S., Yakandawala, D., & Yakandawala, K. (2017). A taxonomic synopsis of *Nymphaea nouchali* Burm. f. And infraspecific taxa. *Journal of the National Science Foundation of Sri Lanka*, **45**(3), 307. https://doi.org/10.4038/jnsfsr.v45i3.8194
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic acids symposium series*. **41**(41), 95–98.
- Hammer, Ø., Harper, D.A., & Ryan, P.D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia electronica*, **4**(1), 1–9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm.
- Inglis, P. W., Pappas, M. de C. R., Resende, L. V., & Grattapaglia, D. (2018). Fast and inexpensive protocols for consistent extraction of high-quality DNA and RNA from challenging plant and fungal samples for high-throughput SNP genotyping and sequencing applications. *PLOS ONE*, **13**(10), e0206085. https://doi.org/10.1371/journal.pone.0206085
- Kim, K. C., & Byrne, L. B. (2006). Biodiversity loss and the taxonomic bottleneck: emerging biodiversity science. *Ecological Research*, **21**(6), 794–810. https://doi.org/10.1007/s11284-006-0035-7
- Larget, B., & Simon, D. L. (1999). Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution*, **16**(6), 750–759. https://doi.org/10.1093/oxfordjournals.molbev.a026160
- Madola, I., Yakandawala, D., & Yakandawala, K. (2022) A new *Lagenandra* species with two varieties from the Wet Zone of Sri Lanka. *Phytotaxa* **567**(2), 110–126. https://doi.org/10.11646/phytotaxa.567.2.1
- Madola, I., Yakandawala, D., Yakandawala, K., & Karunaratne, S. (2021). Two new species of *Lagenandra* (Araceae) from the Wet Zone of Sri Lanka. *Phytotaxa*, **522**(3), 187–199. https://doi.org/10.11646/phytotaxa.522.3.2
- Manawaduge, C. G., Yakandawala, D., & Les, D. H. (2016). A reappraisal of leaf morphology in *Aponogeton natans* (Aponogetonaceae). *Phytotaxa*, **265**(1), 95. https://doi.org/10.11646/phytotaxa.265.1.12
- Manawaduge, C.G., Yakandawala, D., & Les, D.H. (2018). Morphometric analysis reveals a new species of *Aponogeton* (Aponogetonaceae) in Sri Lanka (Erratum). *Phytotaxa*, **575**(3), 243–262. https://doi.org/10.11646/phytotaxa.367.1.13
- Meijer, W. (1995). Elaeocarpaceae. In M. D. Dassanayake, F. R. Fosberg, & W. D. Clayton (Eds.), *Revised handbook to the flora of Ceylon* (Vol. 9, pp. 80–90). Amerind Publishing Co. Pvt. Ltd.
- Ministry of Environment (2020). *The National Red List 2020 of Sri Lanka; Conservation Status of the Fauna and Flora*. Ministry of Environment, Colombo, Sri Lanka, pp.254.
- Mittermeier, R.A., Robles, G., Hoffman, M., Pilgrim, J., Brooks, T., Mittermeier, C.G., Lamoreux, J., & da Fonseca, G.A.B. (Ed.). (2004) *Hotspots revisited: Earth's biologically richest and most threatened terrestrial ecoregions*. CEMEX, Mexico DF.
- National Center for Biotechnology Information. (2022, December 8). GenBank. U.S. National Library of Medicine. Retrieved June 20, 2024, from https://www.ncbi.nlm.nih.gov/genbank/
- Posada, D., & Buckley, T. R. (2004). Model Selection and Model Averaging in Phylogenetics: Advantages of Akaike Information Criterion and Bayesian Approaches Over Likelihood Ratio Tests. *Systematic Biology*, **53**(5), 793–808. https://doi.org/10.1080/10635150490522304
- Rambaut, A. & Drummond, A.J. (2009) *Tracer v1.5*. Available from: http://beast.bio.ed.ac.uk/Tracer (accessed 20 October 2024)
- Rambaut, A. (2018) *FigTree-Tree Figure Drawing Tool Version v. 1.4. 4*. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh.
- Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**(12), 1572–1574. https://doi.org/10.1093/bioinformatics/btg180
- Sang, T., Crawford, D. J., & Stuessy, T. F. (1997). Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany*, **84**(8), 1120–1136. https://doi.org/10.2307/2446155
- Silva, G.G.N.D., Yakandawala, D.M.D., & Yakandawala, K. (2021). Morphometric analysis Genus *Elaeocarpus* L. (Elaeocarpaceae) in Sri Lanka. Proceedings of The Peradeniya University International Research Sessions, Pp.134.

- Silva, N., Dasanayaka, S., Yakandawala, D., & Yakandawala, K. (2025). Taxonomic resolution of the *Elaeocarpus hedyosmus* Zmarzty (Elaeocarpaceae) species complex in Sri Lanka using an integrative approach. *Phytotaxa*, **706**(2), 101–128. <https://doi.org/10.11646/phytotaxa.706.2.1>
- Silva, N., Yakandawala, D., & Yakandawala, K. (2023). *Elaeocarpus mahamayensis* (Elaeocarpaceae), a new endemic species from the Knuckles Mountain Range in Sri Lanka. *Phytotaxa* **616**(1), 1–26. <https://doi.org/10.11646/phytotaxa.616.1.1>
- Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, **17**(5), 1105–1109. <https://doi.org/10.1007/bf00037152>
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research*, **22**(22), 4673–4680. <https://doi.org/10.1093/nar/22.22>.
- White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis (Ed.), *PCR protocols a guide to methods and applications* (pp. 315–322). Academic Press.
- Yakandawala, D.M.D., & Samarakoon, T.M. (2006). An empirical study on the taxonomy of *Crinum zeylanicum* (L.) L. and *Crinum latifolium* L. (Amaryllidaceae) occurring in Sri Lanka. *Cey. J. Sci. (Biol. Sci.)* **35**(1), 53–72.
- Zmarzty, S. (2001). Revision of *Elaeocarpus* (Elaeocarpaceae) section *Elaeocarpus* in Southern India and Sri Lanka. *Kew Bulletin*, 405–447.

Appendix 1: Morphological characters employed in the morphometric analysis

Characters																				
Acronym	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
HP01	1	7.5	6.3	1	1.8	1	147	160	8	63	1	1	3.5	1	35	5.2	17	1	5.0	1.0
HP02	1	6.2	6.2	1	1.3	1	145	160	8	60	1	1	2.8	1	29	6.0	15	1	6.0	1.0
LK03	0	6.5	6.7	2	1.5	0	145	67	5	57	0	1	2.5	1	22	4.5	16	1	4.3	1.0
HP12	1	7.5	6.3	1	1.8	1	143	160	8	63	1	1	3.5	1	35	5.2	17	1	5.0	1.0
HP14	1	7.8	5.0	1	2.0	1	145	160	8	62	1	1	2.8	1	36	5.2	18	1	4.3	1.0
MR01	0	7.8	4.2	2	1.6	0	145	67	7	53	0	1	3.0	1	24	6.2	18	1	4.0	1.0
MR02	1	6.5	4.1	2	1.4	0	147	70	5	43	0	1	2.3	1	22	5.5	16	0	4.0	1.0
MR03	1	8.8	6.1	2	1.9	0	143	83	6	57	0	1	2.7	1	26	8.5	22	0	4.0	1.0
MR07	1	8.3	4.2	2	1.8	0	140	70	5	53	0	0	3.3	0	18	7.5	18	1	4.7	0.7
MR08	1	8.3	5.1	2	1.4	0	143	77	6	57	0	0	2.3	0	24	6.5	18	1	4.0	0.7
LK12	0	6.7	5.8	2	1.5	0	145	67	5	57	0	1	2.5	1	22	4.5	16	1	4.3	1.0
HP21	1	7.8	5.3	1	2.0	1	145	160	8	62	1	1	2.8	1	36	5.2	18	1	4.3	1.0
HP10	1	7.7	6.3	1	1.8	1	150	160	8	63	1	1	3.5	1	35	5.2	17	1	5.0	1.0
HP36	1	7.7	6.3	1	1.8	1	145	160	8	57	1	1	3.0	1	28	5.3	19	1	5.0	1.0
HK10	1	7.7	6.3	1	1.8	1	147	160	8	63	1	1	3.5	1	32	5.6	18	1	5.0	1.0
PT04	1	7.5	6.3	1	1.8	1	143	160	8	60	1	1	2.8	1	31	5.4	19	1	5.0	1.0
PT05	1	7.7	6.3	1	1.8	1	153	160	8	63	1	1	3.5	1	33	5.1	18	1	5.0	1.0
HK02	1	7.5	6.3	1	1.8	1	147	160	8	57	1	1	3.0	1	29	5.2	18	1	5.0	1.0
HK03	1	7.7	6.3	1	1.8	1	152	160	8	65	1	1	3.5	1	34	5.3	18	1	5.0	1.0
HK04	1	7.5	6.3	1	1.8	1	145	160	8	60	1	1	3.5	1	33	4.7	17	1	5.0	1.0

Appendix 1: Continued

Characters																					
Acronym	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
HP01	1	0	4.0	1.7	20	1.1	5.8	4.5	4.2	20	1.0	1.7	3.0	2.5	1	1	3.0	2.4	2.1	4.0	0
HP02	1	0	4.0	1.7	20	1.1	5.8	4.3	4.3	20	1.0	1.7	2.8	2.4	1	1	3.0	2.4	2.1	5.0	0
LK03	1	0	4.0	1.7	20	1.2	5.8	4.5	4.2	25	1.0	1.7	2.6	2.4	0	1	3.5	2.4	2.1	4.7	0
HP12	1	0	4.8	1.5	21	1.2	6.0	4.5	4.2	25	1.0	1.7	3.0	2.5	1	1	3.0	2.4	2.3	4.0	0
HP14	1	0	4.5	1.8	21	1.1	6.0	4.5	4.0	25	1.0	1.7	2.8	2.6	1	1	2.8	2.2	2.2	4.0	0
MR01	1	0	4.0	1.8	23	0.9	5.7	3.3	3.8	25	1.2	1.5	3.1	2.7	1	1	4.3	2.5	2.5	4.3	1
MR02	0	0	4.0	1.8	23	0.9	5.5	3.3	3.8	25	1.5	1.7	3.0	2.7	1	1	4.3	2.5	2.5	4.0	1
MR03	0	0	4.0	1.5	23	0.9	5.2	3.7	3.7	25	1.0	1.5	3.1	2.8	1	1	4.3	2.5	2.5	4.3	0
MR07	1	0	4.3	1.8	24	1.0	6.0	3.3	3.7	20	1.0	1.7	3.0	2.8	1	0	4.3	2.5	2.5	4.0	0
MR08	1	0	4.5	2.0	23	1.0	5.8	3.5	3.7	20	1.0	1.7	2.8	2.6	1	0	3.7	2.5	2.5	4.3	0
LK12	1	0	4.0	1.7	20	1.2	5.8	4.5	4.2	25	1.0	1.7	2.6	2.4	1	1	3.5	2.4	2.1	4.3	0
HP21	1	1	4.5	1.8	21	1.1	6.0	4.5	4.0	25	1.0	1.7	2.8	2.6	1	1	2.5	2.2	2.2	4.0	0
HP10	1	1	4.8	1.5	21	1.2	6.0	4.5	4.2	25	1.0	1.7	2.9	2.5	1	1	3.0	2.4	2.3	4.3	0
HP36	1	1	4.8	1.5	22	1.2	6.0	4.5	4.2	25	1.0	1.7	2.9	2.5	1	1	3.0	2.4	2.3	4.3	0
HK10	1	1	4.8	1.5	20	1.2	6.0	4.5	4.2	25	1.0	1.7	2.9	2.5	1	1	3.0	2.4	2.3	4.0	0
PT04	1	1	4.8	1.5	20	1.2	6.0	4.5	4.2	25	1.0	1.7	2.8	2.5	1	1	3.0	2.4	2.3	4.3	0
PT05	1	1	4.8	1.5	20	1.2	6.0	4.5	4.2	25	1.0	1.7	2.9	2.5	1	1	3.0	2.4	2.3	4.0	0
HK02	1	1	4.8	1.5	20	1.2	6.0	4.5	4.2	25	1.0	1.7	2.8	2.5	1	1	3.0	2.4	2.3	4.3	0
HK03	1	1	4.8	1.5	20	1.2	6.0	4.5	4.2	25	1.0	1.7	2.9	2.5	1	1	3.0	2.4	2.3	4.3	0
HK04	1	1	4.8	1.5	19	1.2	6.0	4.5	4.2	25	1.0	1.7	2.9	2.5	1	1	3.0	2.4	2.3	4.0	0