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Assessment of the Morphometric Variation of Fruits and Phylogenetics of *Elaeocarpus ganitrus* (Woodenbegar) and *Elaeocarpus serratus* (Ceylon Olive)

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ABSTRACT

Purpose : *Elaeocarpus ganitrus and E. serratus are two significant tropical fruit tree species. E. ganitrus is also important for its endocarps that are popularly known as Rudraksha. However, these are underutilized species in Sri Lanka. Therefore, present study was conducted to assess the morphometric diversity of fruits and endocarps and the phylogenetic relationships of two species using DNA barcoding markers.*

Research Method : *The ripe fruits were collected from a set of trees of two species in Matale and Kandy districts. The fruit and endocarp size parameters were measured in two seasons and subjected to statistical analysis. The trnH-psbA and trnL-trnF sequences were obtained from the selected trees and subjected to phylogenetic analysis.*

Findings : *The fruit size traits were predominantly affected by the intraspecific variation than the environment. The length and diameter of the endocarps were seasonal independent and tree specific. Except colour, people preferred E. serratus fruits than E. ganitrus fruits. The markers, trnH-psbA and trnL-trnF unravelled the species delimits of E. ganitrus and E. serratus. The trnL-trnF based phylogeny positioned the trees of E. ganitrus in the respective clade. E. serratus trees were cladded with E. stipularis, E. tectorius, E. dongnaiensis and E. glaber.*

Research Limitations: *A limited sequence pop-set for Elaeocarpus spp. is available in literature for comparison. The meta-scale attempts are needed to resolve the phylogenetic relationships of Elaeocarpus germplasm.*

Originality / Value : *The revealed morphological and phylogenetic relationships of E. ganitrus and E. serratus lay the platform for germplasm conservation and crop improvement programs in Sri Lanka.*

Keywords: *Blue Weralu, Elaeocarpaceae, Indian bead tree, Indian olive, Nil Weralu, Rudraksha, Weralu*

INTRODUCTION

The members of the genus *Elaeocarpus* L. of the family Elaeocarpaceae are well-known for their delicious fruits in South Asia (Pant *et al.,* 2013). Typically, *Elaeocarpus serratus* L., (English: Ceylon Olive / Indian olive; Sinhala: *Weralu*; Tamil: *Veralikkai*) and *E. ganitrus* Roxb. Ex G. Don [Alias *E. spharicus*] (English: Woodenbegar / Indian Bead Tree; *Rudraksha*-Fruit; Sinhala: *Nil Weralu*; Tamil: Ruttiratcam) are preferred over the other species in the genus because *E. ganitrus* and *E. serratus* bear colourful fruits with edible pulp (Coode, 2004; Das, 1987; Rahman *et al.,* 2003). The higher preference for the fruits of *Elaeocarpus* spp. is attributed to the unique

taste and the rich medicinal values (Coode, 2004). Though *Elaeocarpus* is the most popular genus, the family Elaeocarpaceae contains a total of 12 genera and more than 550 known species (Christenhusz and Byng, 2016). Among them, more than 350 species belong to the genus *Elaeocarpus* L. and within this genus; majority

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of the species possess edible mesocarps (Coode, 2004). However, despite having numerous and well-known regional floras, there is an ample amount of ambiguity over the number of taxa within the genus (Baba, 2013; Coode, 1983; Coode, 2010; Zmarzty, 2001).

The tropical and sub-tropical regions provide the ideal habitats to almost all the genera of the family Elaeocarpaceae, however, some chose to grow in temperate countries (Baba, 2013). Moreover, the family Elaeocarpaceae is known to have a southern origin. Members of this family are more abundant in South America, Southeast Asia, Madagascar and Australia. Furthermore, no evidence is found to confirm the existence of this family in Africa, thereby supporting the theory of southern origin (Raven and Axelrod, 1974). *E. serratus* has a wide range of distribution extending from southern India to Nepal and Java, and said to be indigenous to India and Sri Lanka (https://npgsweb.ars-grin.gov). Moreover, *E. serratus* trees are cultivated mainly in Australia, Brazil, China, Ghana and, other Asian countries (Pushpakumara *et al.,* 2007). *E. ganitrus* species is mainly distributed in the South-east Asian countries such as India, Nepal, Myanmar (Singh *et al.,* 2016) and central areas of Sri Lanka.

E. ganitrus is a large evergreen tree bearing bright-deep blue coloured fruits upon maturation (Singh *et al.,* 2003; Kumar *et al.,* 2008). However, the blue colour is limited to the epidermis of the exocarp, and the inner edible mesocarp is usually bright green (Lee, 1991). The endocarp is a hard-lignified stone (i.e., endocarp) with strong electromagnetic properties (Singh *et al.,* 2016; Pant *et al.,* 2013). Moreover, the leaves are broad and simple and, they turn in to red near to senescence (Pant *et al.,* 2013). Particularly, *E. ganitrus* is not an ornamental tree; instead, it inhabits tropical evergreen forests (Singh *et al.,* 2003). Surprisingly, the unripen fruits are not edible however, ripen fruits have a sweet-sour taste and usually being eaten raw or as pickles (Coode, 2004). Furthermore, the endocarp is a famous curative agent in Ayurvedic medicine due to their electromagnetic properties and the Hindu people use endocarps as Rudraksha (i.e., tear drops of load Shiva) beads to make necklaces or rosaries, to wear as religious accessories (Coode,

2004; Coode, 2010). In general, leaf, seed and fruit pulp extracts of *E. ganitrus* are known to have significant applications in treating the patients with blood and heart-related diseases, brain and nerve disorders and, abnormalities associated with respiratory and digestive tract (Kumar *et al.,* 2008; Pant *et al.,* 2013; Singh *et al.,* 2003; Singh *et al.,* 2016). In Sri Lanka, *E. serratus* is an abundantly growing tree in the lower altitude forests in wet and intermediate zones (Dassanayake, 1980; Pushpakumara *et al.,* 2007). There is a tremendous amount of medicinally valuable constituents present in *E. serratus* (Mohanan and Sabu, 2002). The *E. serratus* fruits are consumed fresh at ripen stage and prepared as spiced and salted pickle in raw stage. Moreover, the leaf extracts of *E. serratus* contain anti-oxidative components.

However, *E. ganitrus* and *E. serratus* of Sri Lanka remain as under-utilized tree species. The morphological diversity of fruits and endocarps has not been assessed with respect to the individual tree variation and the environment to use in germplasm utilization and conservation. The phylogenetic relationships of species in family Elaeocarpaceae have been recently studied (Baba, 2013). However, *E. ganitrus* and *E. serratus* of Sri Lanka have not been studied for the species delimits and the phylogenetic relationships. Therefore, the present study was carried out to assess the fruit and endocarp morphological diversity and the phylogenetic relationships of *E. ganitrus* and *E. serratus* of Sri Lanka in relation to the sequences reported for the other species in Elaeocarpaceae.

MATERIALS AND METHODS

Sample collection

The fruits were collected from *E. serratus* (ES) and *E. ganitrus* (EG) trees within *Matale* and Kandy Districts, Sri Lanka. Total of 30 trees were selected, 15 trees from each species (Figure 01). A total of 15 fully ripe fruits and five seeds were randomly collected from each tree for the morphological analysis. The fruit and seed morphological analyses were undertaken in 2018 and 2019 fruiting seasons (January-May).

The rainfall and temperature data of the fruit collecting districts are given in Figure 01.

Morphological data collection

The fruit and seed size parameters; weight, length and width, and colour in terms of Red-Green-Blue (RGB) were measured using a balance, Vernier calliper and by examining high-resolution photographs respectively. The leaf morphology was also qualitatively examined. All the morphological data collected were subjected to the normality testing followed by GLM procedure with main effects; species, season and tree ID, as well as their interactions. The significant mean differences were examined using LS-means and *pdiff* option in GLM procedure. All the statistical analytical procedures were performed using the Statistical Package SAS 9.4 (SAS Institute, NC, Cary, USA).

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Organoleptic assessment of ripe fruits

The ripe fruits of *E. serratus* and *E. ganitrus* (bulked samples harvested from studied groups of trees) were given to a group of 30 taste-panellists to observe, taste and rank the fruit samples for the preferred levels of five organoleptic parameters; colour, aroma, texture, sourness and overall taste. The tasters were requested to use a three-tier scoring system (1: low; 2: moderate; 3: high). The rank data generated by the taste panel were subjected to association analysis (taste parameter vs. species) using FREQ procedure in SAS.

DNA extraction

The freshly collected immature leaf samples were used to extract genomic DNA. The DNA extractions were carried out based on the CTAB method (Doyle, 1990) which was optimized separately for *E. ganitrus* and *E. serratus*. For *E. ganitrus*, original CTAB method was used and extracted DNA was purified using phenol:chloroform:isoamyl alcohol and precipitated using ice-cold isopropanol. For *E. serratus*, the original CTAB method was modified by adding poly-vinyl-pyrrolidone (PVP) (0.1 g in solid form) and β-mercaptoethanol (4% - 1.5 μl) to the initial grinding step. The DNA was purified using two consecutive purification steps of chloroform: isoamyl alcohol. The DNA precipitation was done using ice-cold isopropanol and 5M NaCl. The DNA pellets were washed with 70% ethanol and ammonium acetate, dissolved in TE buffer and stored at -20 °C.


Parameter		Kandy	Matale
Annual rainfall (mm)		2368.8	3070.4
Daily Temperature (°C)	Highest maximum	38	35
	Average maximum	29.4	31.1
	Lowest minimum	13.6	13.2
	Average minimum	29.4	20.1
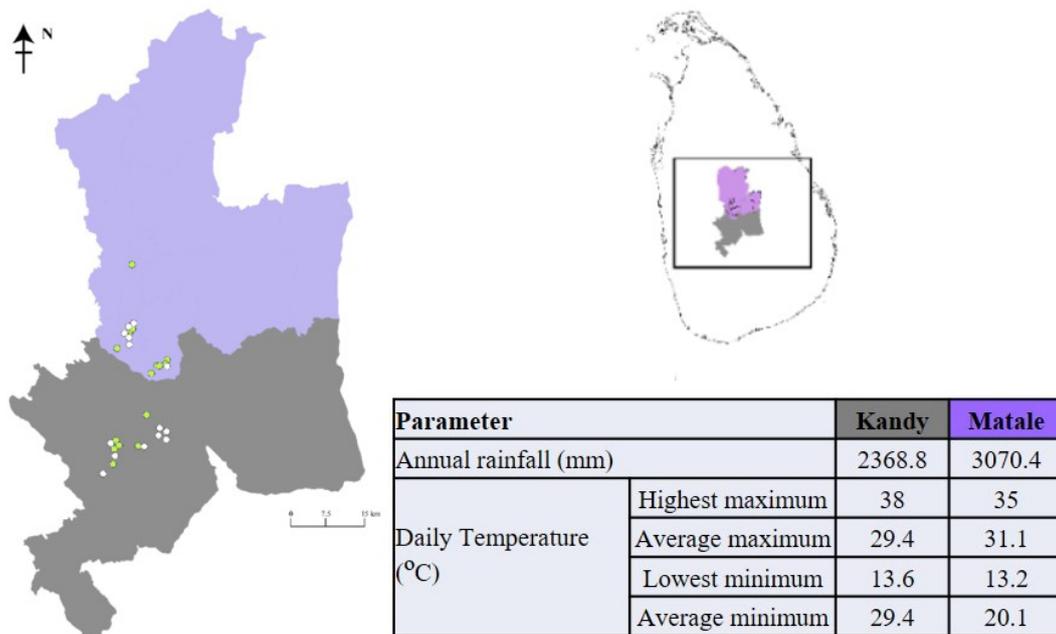
Figure 01: **A map showing sampling locations. Purple and grey regions represent *Matale* and Kandy Districts of Sri Lanka respectively. Green dots: *E. ganitrus* (Woodenbegar). White dots: *E. serratus* (Ceylon olive) The annual rainfall and temperature data for 2018 are given.**

PCR and DNA sequencing

The DNA samples were subjected to PCR using the standard DNA barcoding markers; *trnH-psbA* (Pf: 5' CGC GCA TGG TGG ATT CAC AAT CC 3'; Pr: 5' GTT ATG CAT GAA CGT AAT GCT C 3') (Sang *et al.,* 1997) and *trnL-trnF* (*tRNA-leu gene*) (Pf: 5' CGA AAT CGG TAG ACG CTA CG 3'; Pr: 5' GGG GAT AGA GGG ACTTGA AC 3') Taberlet *et al.,* 2007). The PCR solution mixtures (15μl) were prepared with 1× Go Taq® Green Master Mix (7.5 μl) (Promega Corporation, Madison, Wisconsin, USA), forward and reverse primers (10 ng/μl - 0.5 μl each), nuclease free water (5.5 μl), spermidine (1.34×10^{-4} mol/dm^3 - 3.5 μl), bovine serum albumin (BSA) (4% - 0.2 μl) and DNA template (1.0 μl - 60 ng/μl). All PCR work was carried out using a Takara thermal cycler (Otsu Shiga, Japan). The PCR cycles consisted of initial denaturation at 98 ºC for 45 sec for *trnH-psbA*, and 95 ºC for 5 min for *trnL-trnlF*, then 35 cycles of denaturation at 98 ºC for 10 sec for *trnH-psbA* and 95 ºC for 30 sec for *trnL-trnF*, 30 sec of primer annealing at 64 ºC for *trnH-psbA* and 50 ºC for *trnL-trnF*, extension at 72 ºC for 40 sec for *trnh-psbA*, and 2 min for *trnL-trnF*. The final extension was done at 72 ºC for 10 min for *trnH-psbA* and 4 min for *trnL-trnF* (Sang *et al.,* 1997; Taberlet *et al.,* 2007). The PCR products were visualized using 1% agarose gel electrophoresis and purified using Wizard SV gel® PCR clean-up system (Promega Corporation, Madison, Wisconsin, USA). The purified PCR products were subjected to DNA sequencing using an ABI 3500 Genetic Analyzer (Applied Biosystems, Cat. No. 4405186).

Phylogenetic analysis

Initially, the two sets of sequences obtained for markers *trnH-psbA* and *trnL-trnF* were visualized in MEGA v7 (Kumar *et al.,* 2016). Then the initial and end buffers were trimmed, and the two sets of sequences were aligned separately using MEGA v7. To reconstruct the phylogeny of family Elaeocarpaceae, we adapted sequences reported in Baba, (2013) and other relatable reference sequences obtained from Basic Local Alignment Search Tool (BLAST) search of NCBI (Table 1). The alignment was carried out in MEGA v7 utilizing the Clustal W algorithm (Thompson *et al.,* 1994). Then the alignment was checked manually for further clarifications to remove unwanted gaps. The INDELS and SNPs were identified from the alignment. The Maximum Likelihood (ML) tree search was carried out in RAxML (Stamatakis, 2006) applying the rapid bootstrap algorithm (Stamatakis *et al.,* 2008). The DNA model was selected as GTRGAMMA and the analysis was carried out for 1000 iterations (Rodriguez *et al.,* 1990). By applying the bipartition option in RAxML, the bootstrap replicates were concluded into a single tree topology. Then the Bayesian analysis was carried out in MrBays (Huelsenbeck and Ronquist, 2001). A model selection was carried out using Corrected Akaike Information Criteria (AICc) (Cavanaugh, 1997), Bayesian Information Criteria (BI) and Decision Theory (DT). The best-suited parameters (HIK model- f (a): 0.34, f (c): 0.18, f (g): 0.17, f (t): 0.31, kappa: 2.45) were employed in the Bayesian tree search and ran four Markov Chain Monte Carlo (MCMC) chains for 50 million cycles. The 50% majority rule consensus tree was drawn for all the trees probed after the burn-in. All the analyses were carried out in CIPRES science gateway (Miller *et al.,* 2010). The phylogenetic trees were modified using FigTree v1.4.3 software (Rambaut, 2014) for better visualization.

The *E. ganitrus* trees are evergreen and grow up to 15-75 m in height. The trunk is prominent, and leaves and branches are nested as a crown in the top (Figure 2Ai). The *E. serratus* trees are also evergreen; however, medium sized (5-30 m) (Figure 2Bi) compared to *E. ganitrus* trees. In *E. serratus*, the tree branching could happen after 2-4 m of growth under natural conditions (Figure 2B). The fruiting is occurred in all the branches however, it is prominent in distal branches formed recently (Figures 2Aii and 2Bii).

Table 01: **The *Elaeocarpus* DNA sequences subjected to phylogenetic analysis**

Species	Common name	Voucher No.	Geographic area	GenBank accession		Reference
				trnL-trnF	*trnH-psbA*	
E. ganitrus	Woodenbegar	DMB123	Sri Lanka	MN128522	MN119517	
E. ganitrus		DMB124	Sri Lanka	MN128523	MN119518	
E. ganitrus		DMB125	Sri Lanka	MN128524	MN119519	This study
E. ganitrus		DMB126	Sri Lanka	MN128525	MN119520	
E. ganitrus		DMB127	Sri Lanka	MN128526	MN119521	
E. serratus	Ceylon olive	DMB128	Sri Lanka	MN128527	MN119522	
E. serratus		DMB129	Sri Lanka	MN128528	MN119523	
E. serratus		DMB130	Sri Lanka	MN128529	MN119524	This study
E. serratus		DMB131	Sri Lanka	MN128530	MN119525	
E. serratus		DMB132	Sri Lanka	MN128531	MN119526	
E. grandis		-	Australia	KC428500.1	-	
E. stipularis		-	Not available	AB920774.1	-	
E. tectorius		-	Not available	AB920775.1	-	
E. apiculatus		-	Not available	JN676060.1	-	
E. largiflorens		71	Australia	KC428501.1	-	NCBI
E. angustifolius		184418	Australia	DQ444689.1	-	
E. hainanensis		-	Not available	JN676061.1	-	
E. dentatus		630341	New Zealand	KF591277.1	-	
E. hookerianus		630369	New Zealand	KF591223.1	-	
E. blascoi		-	India	HM066938.1	-	
E. geminiflorus		710751	Malaysia	KJ675695.1	-	
E. ptilanthus		554	Papua New Guinea	KJ675716.1	-	
E. sphaericus		710753	India	KJ631299.1	-	
E. grandis		27569	Australia	KJ675700.1	-	
E. angustifolius		710750	India	KJ631298.1	-	
E. hylobroma		838	Australia	KJ675705.1	-	
E. rotundifolius		761	New Caledonia	KJ675718.1	-	
E. seringii		2852	New Caledonia	KJ675721.1	-	
E. speciosus		115	New Caledonia	KJ675725.1	-	
E. alaternoides		749	New Caledonia	KJ631296.1	-	
E. hortensis		2968	New Caledonia	KJ675704.1	-	
E. brachypodus		71	New Caledonia	KJ631303.1	-	
E. coumbouiensis		388	New Caledonia	KJ631307.1	-	
E. gordonii		300	New Caledonia	KJ675697.1	-	
E. gummatus		260	New Caledonia	KJ675701.1	-	
E. bullatus		2906	New Caledonia	KJ631304.1	-	
E. glaber		179	Java, Indonesia	KJ675696.1	-	
E. dongnaiensis		1118	Vietnam	KJ675690.1	-	
E. stipularis		10749	Java, Indonesia	KJ675727.1	-	
E. largiflorens		503	Australia	DQ444684.1	-	
Elaeocarpus sp.		397	Australia	KJ675724.1	-	Baba, 2013
E. elliffii		884	Australia	KJ675691.1	-	
E. thelmae		792	Australia	KJ675729.1	-	
E. grandiflorus		10751	Malaysia	KJ675699.1	-	
E. dentatus		NA	New Zealand	KJ675689.1	-	
E. ruminatus		446	Australia	KJ675719.1	-	
E. sedentarius		408	Australia	KJ675720.1	-	
E. holopetalus		605470	Australia	KJ675702.1	-	
Elaeocarpus sp.		2	Australia	DQ444676.1	-	
E. hookerianus		605721	New Zealand	DQ444686.1	-	
E. hookerianus		787	New Zealand	KJ675703.1	-	
E. stellaris		3531	Australia	KJ675726.1	-	
Elaeocarpus sp.		4312	Australia	KJ675723.1	-	
E. williamsianus		513	Australia	DQ444693.1	-	
E. bancroftii		502	Australia	DQ444685.1	-	
E. nouhuysii		533	Papua New Guinea	KJ675713.1	-	
E. reticulatus		605722	Australia	DQ444683.1	-	
E. eumundii		505	Australia	DQ444682.1	-	
E. eumundii		4459	Australia	KJ675692.1	-	
E. nouhuysii		530	Papua New Guinea	KJ675712.1	-	
E. ferruginiflorus		882	Australia	KJ675693.1	-	

Species	Common name	Voucher No.	Geographic area	GenBank accession		Reference
				trnL-trnF	*trnH-psbA*	
E. michaelii		310	Australia	KJ631310.1	-	
E. johnsonii		2122	Australia	KJ675706.1	-	
E. coorangooloo		695	Australia	KJ631308.1	-	
E. arnhemicus		341	Australia	KJ631300.1	-	
E. bifidus		649	Hawaii	KJ631302.1	-	Baba, 2013
E. kerstingianus		21433	Micronesia	KJ675707.1	-	
E. crenulatus		539	Papua New Guinea	KJ631309.1	-	
E. carolinae		4444	Australia	KJ631305.1	-	
E. foveolatus		856	Australia	KJ675694.1	-	
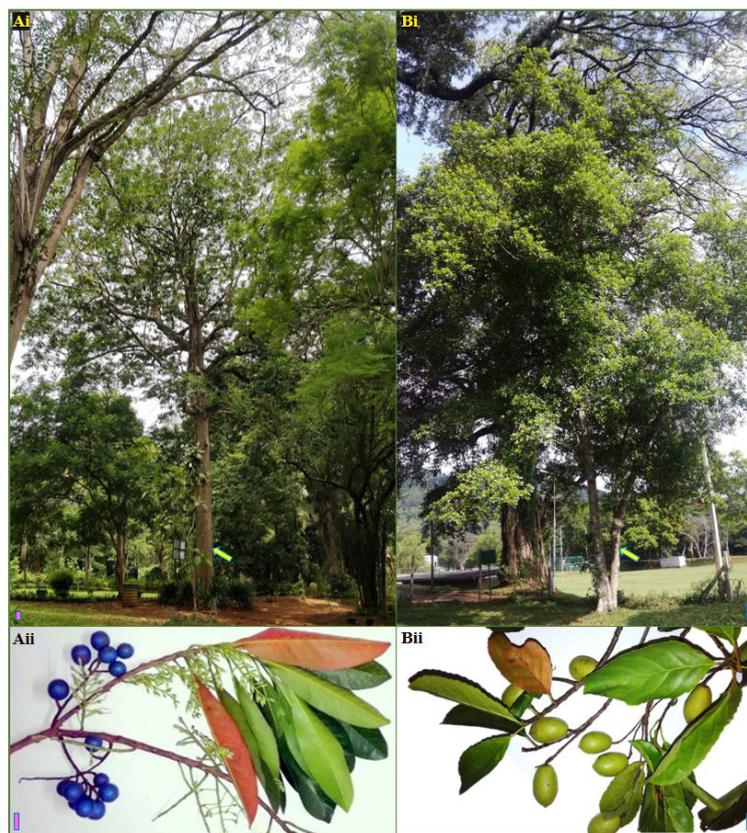
Figure 02: The fruit bearing trees and branches. A: *E. ganitrus* (Woodenbegar); B: *E. serratus* (Ceylon olive). i: trees; ii: branches. The scale bars represent 1m and 1cm in tree and branch figures respectively.

Morphological variation of fruits

E. ganitrus and *E. serratus* possess moderately glossy olive like fruits. *E. ganitrus* bears globular shaped fruits with bright ocean blue outer appearance. The blue coloration of the fruit skin of *E. ganitrus* can be slightly variable and the mean RGB values were found to be 15, 62 and 108. The *E. serratus* fruits are ovoid in shape and possess yellowish green skin and the mean RGB values reported were 135, 131 and 30 (Table 2). The reported colours represent the fruits at maturity (i.e. harvestable stage) and in younger stages fruits are relatively dark green in colour. The *E. ganitrus* fruits develop blue colour in the later stage (last three to four weeks) of fruit development.

The analysis of variance of the fruit size traits revealed that, the effect of seasonal variation was significant ($p < 0.05$). Within *E. ganitrus* and *E. serratus*, the fruit weight was determined by the seasonal effect and the individual tree; however, the effect of later was seven times higher (F values 10.9 vs 70.1) on deciding the fruit weight. The variation of the fruit length also affected significantly by the same trend of effects as in fruit weight; however, the interaction- effects were much more profound than those on fruit weight. The fruit diameter was not affected significantly by the seasonal variation and appeared to be a tree specific trait (F= 43.8; $p<0.05$). The mean values of fruit weight, length and diameter

were significantly different among two species (p≤0.05). The summary of the analysis of variation of fruit morphological variation is shown in Table 03. Table 04 displays the mean values of fruit size parameters for two seasons. It was apparent that *E. serratus* fruits were heavier, longer and broader than the *E. ganitrus* fruits (p < 0.05; Table 04).

The variation of the fruit weight, length and diameter are depicted in Figures 3A-3F. The seasonal effect was apparently much less than the effect of individual trees. The depicted variation of fruit size in Figure 3 is showcased with representative fruits taken from each tree in Figures 5Ai and 5Bi for *E. ganitrus* and *E. serratus* respectively. The longitudinal and cross sections of the fruits are shown in Figures 6Ai and 6Aii (*E. ganitrus*) and Figures 6Bi and 6Bii (*E. serratus*). The fruit size parameters were significantly and positively correlated with each other in which weight and length displayed the highest correlation (PCC= 0.71; p<0.05) (Table 05).

Table 02: **Qualitative morphological parameters of fruits, seeds and leaves of EG and ES**

Structure	Parameter	*E. ganitrus*	*E. serratus*
Fruit	Glossiness	Moderate	Moderate
	Color	Bright ocean blue	Yellowish green
	Mean RGB values of color	15, 62, 108	135, 131, 30
	Shape	Globular	Ovoid
Endocarp	Color	Brown to dark brown	Brown to dark brown
	Mean RGB values of color	127, 86, 71	134, 90, 71
	Shape	Spherical	Ovoid
	Surface texture	Rough	Rough
Leaf	Color	Dark green (adaxial)	Green (adaxial)
		Green (abaxial)	Light and green (abaxial)
	Mean RGB values of color	30, 45, 22 (adaxial)	65, 92, 61 (adaxial)
		83, 105, 45 (abaxial)	137, 157, 98 (abaxial)
	Shape	Oblong	Oval-obovate
	Margin	Serrulate	Shallowly serrate
	Surface texture	Glabrous	Glabrous
	Shape of the apex	Acute	Rounded-obtuse
	Shape of the base	Acute	Acute
	Venation pattern	Pinnate	Inter-marginal

Table 03: **F-values and their significance - analysis of variance for fruit and seed morphometric data**

Parameter	R^2	Season (S)	Species (sp)	Tree ID (T)	S × sp	S × T	sp × T	S × sp × T
Fruit								
Weight (g)	73.8%	10.9*	836.9*	70.1*	0.5ns	2.6*	34.9*	0.8ns
Length (cm)	86.6%	10.0*	4449.4*	31.3*	17.3*	8.3*	18.6*	8.1*
Diameter (cm)	51.5%	1.8ns	82.5*	43.8*	4.0*	3.3*	8.1*	2.3ns
Endocarp								
Weight (g)	52.8%	8.8*	2.8ns	9.5*	0.2ns	0.3ns	8.1ns	0.4ns
Length (cm)	91.8%	3.8ns	2098.2*	12.2*	2.5ns	1.3ns	26.2ns	1.8ns
Diameter (cm)	71.1%	2.8ns	125.5*	12.3*	0.6ns	1.9ns	18.0ns	0.9ns

*P≤0.05; ns: nonsignificant (P>0.05)

Table 04:   **Variation of the size parameters of fruits and seeds**

Season	Species	Fruit			Seed		
		Weight (g)	Length (cm)	Diameter (cm)	Weight (g)	Length (cm)	Diameter (cm)
2018	EG	7.0[b]	2.2[b]	2.1[b]	2.0[a]	1.7[b]	1.5[a]
	ES	9.7[a]	3.2[a]	2.3[a]	2.1[a]	2.6[a]	1.4[b]
2019	EG	7.4[a]	2.2[b]	2.1[b]	2.1[a]	1.7[b]	1.6[a]
	ES	9.9[a]	3.1[a]	2.2[a]	2.2[a]	2.7[a]	1.4[b]

Means denoted by the same letters within columns are not significantly different at P<0.05. EG: E. ganitrus; ES: E. serratus
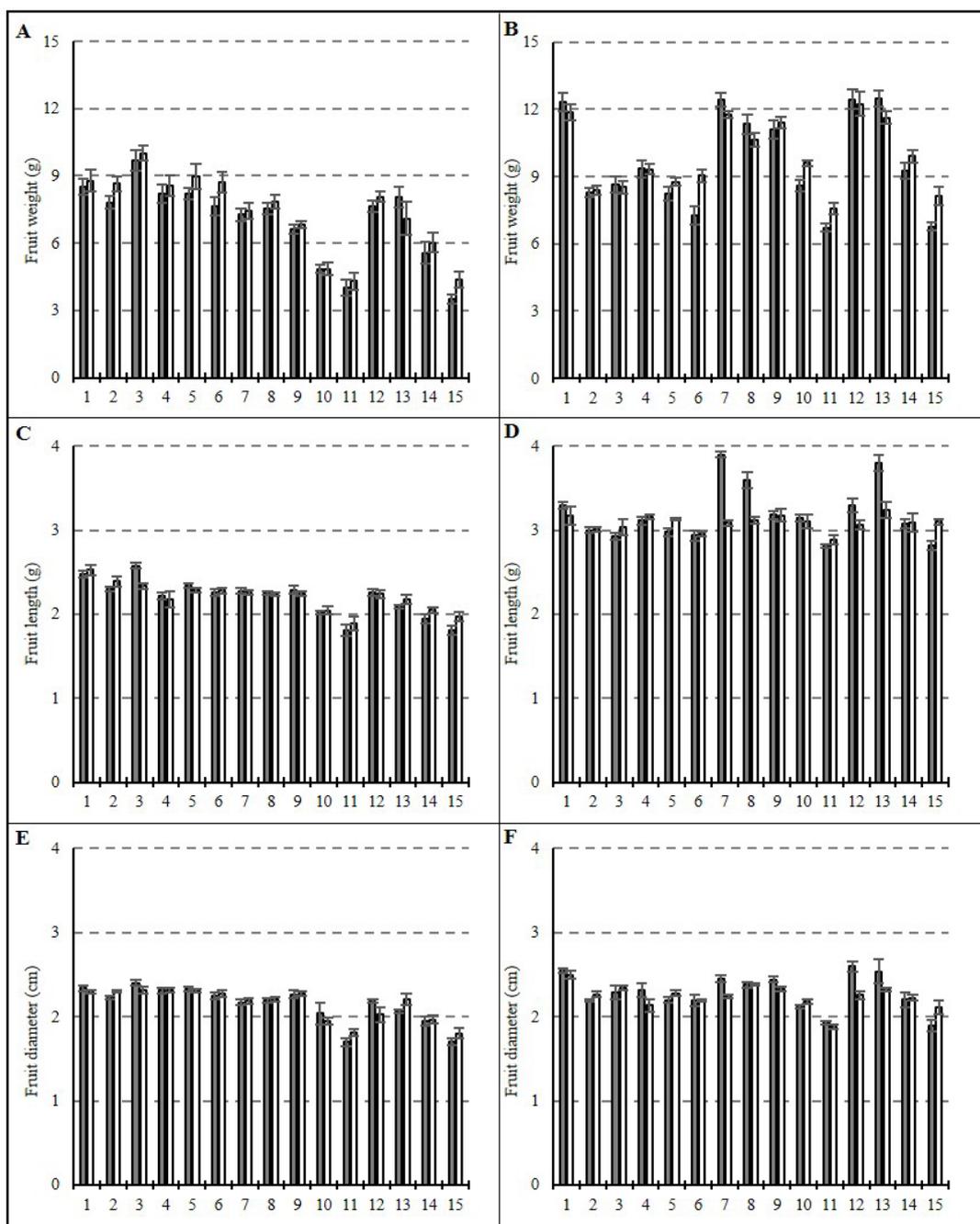
Figure 03:   **The variation fruit size parameters. A and B: Fruit weight (g); C and D: Fruit length (cm); E and F: Fruit Diameter. A, C and E: *E. ganitrus* (Woodenbegar); B, D and F: *E. serratus* (Ceylon olive). 1-15 are individual trees assessed for each species separately. The grey and white bars represent the mean variations in 2018 and 2019 fruiting seasons respectively. The error bars indicate the standard errors (±).**
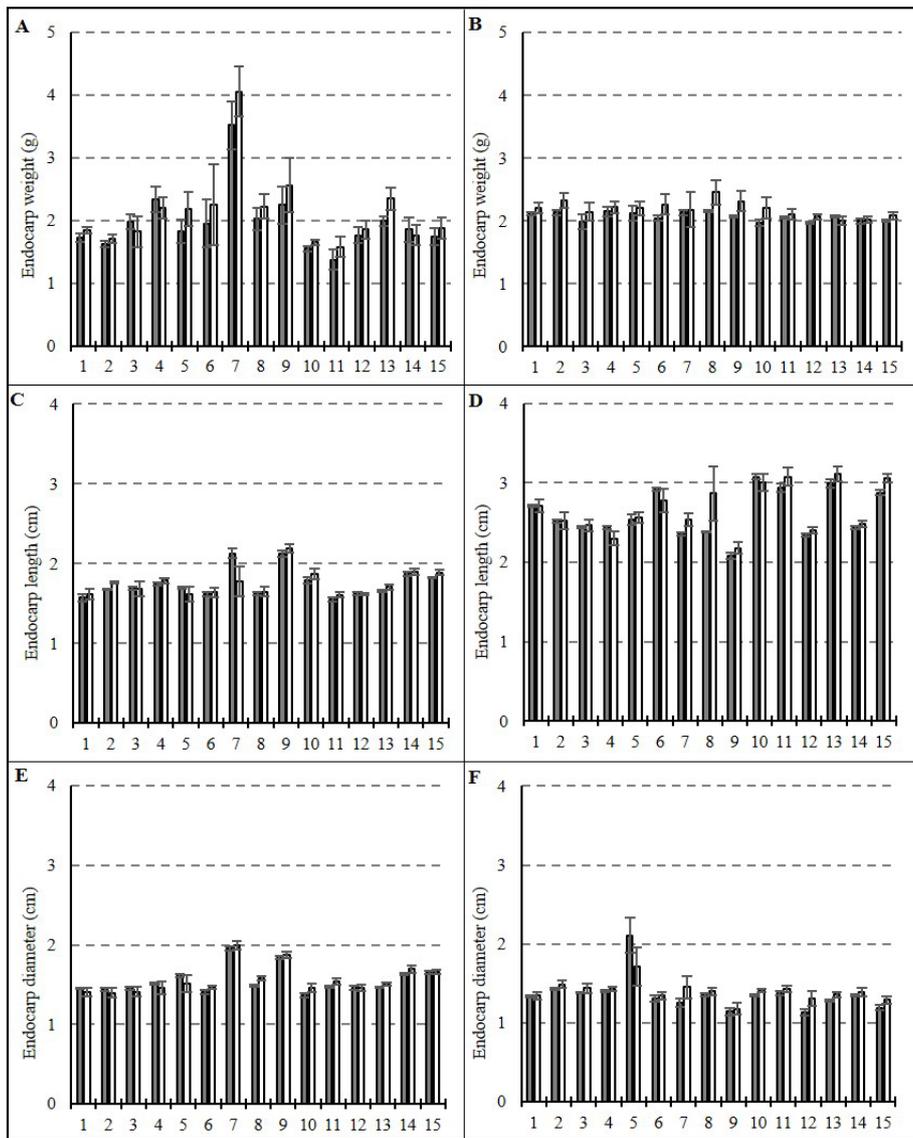
Figure 04: The variation seed size parameters. A and B: Seed weight (g); C and D: Seed length (cm); E and F: Seed diameter. A, C and E: *E. ganitrus* (Woodenbegar); B, D and F: *E. serratus* (Ceylon olive). 1-15 are individual trees assessed for each species separately. The grey and white bars represent the mean variations in 2018 and 2019 fruiting seasons respectively. The error bars indicate the standard errors (±).

Figure 05: The variation of the fruits and seeds. A: *E. ganitrus* (Woodenbegar); B: *E. serratus* (Ceylon olive). i: Representative fruit from each of the 15 trees; ii: Representative seed from each of the 15 trees. Scale bars represent 1 cm.
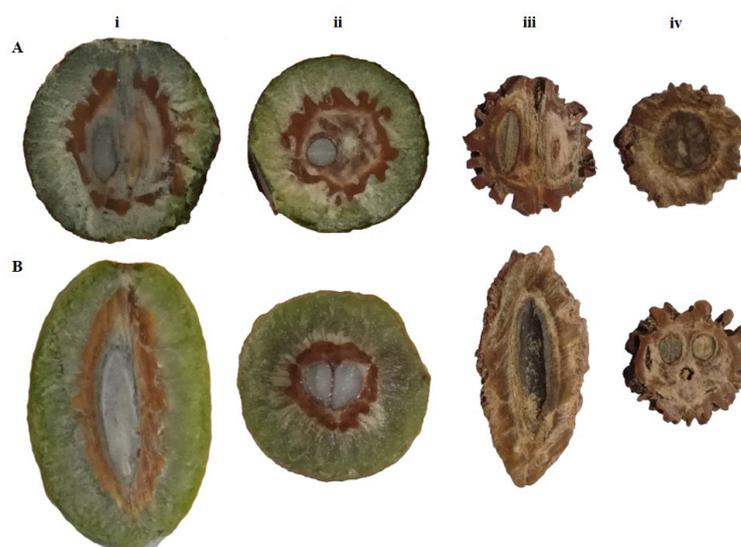
Figure 06: Sectional views of fruits and seeds. A: *E. ganitrus* (Woodenbegar); B: *E. serratus* (Ceylon olive). i and iii: Longitudinal sections; ii and iv: Cross sections. Scale bar represents 1 cm.
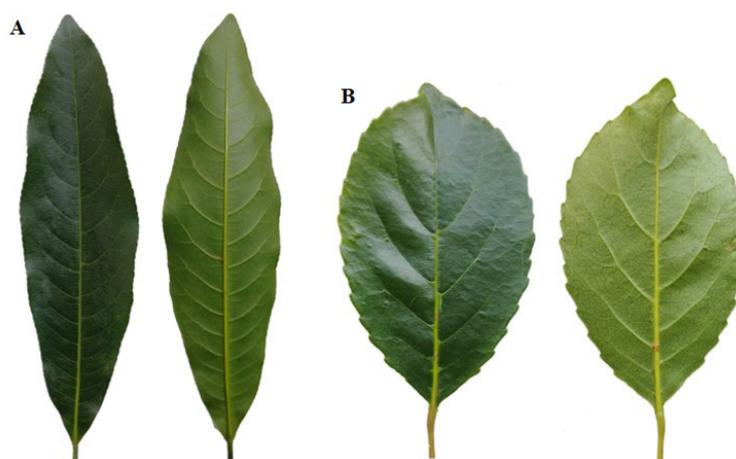

Figure 07: The appearance of leaves. A: *E. ganitrus* (Woodenbagar); B: *E. serratus* (Ceylon olive). Adaxial side (Left); Abaxial side (Right). Scale bar represents 1 cm.

Table 05: Pearson Correlation coefficients among fruit size parameters

	Weight (g)	Length (cm)
Length (cm)	0.71*	
Diameter (cm)	0.63*	0.45*

$*P \leq 0.05$; ns: nonsignificant ($P > 0.05$)

Morphological variation of endocarps

The external colours of the endocarps of two species were not significantly varying and a variation of brown to dark brown coloration was observed. The mean RGB values were also not different in providing perceivable colour differences. The seeds are lighter in colour (creamish in early stages) and turn brown in maturity. The intense brownness was observed after processing endocarps for measurements or practically to make bead-necklaces. The colours reported in the present study represent the mature endocarps. The spherical and ovoid shapes could be observed for the endocarps of *E. ganitrus* and *E. serratus* respectively (Table 2).

The analysis of the variance of weight, length and diameter of endocarps revealed that the seasonal effect was only significant in determining the

weight (F-value = 8.8; $p<0.05$). The length and diameter of endocarps were not significantly affected by season ($p>0.05$). The individual tree (i.e. Tree ID) variation for weight, length and diameter were significantly profound ($p<0.05$). The endocarp weights of two species were not significantly different whereas length and diameter reported significant differences between the two species. The summary of the analysis of variance for endocarp-size traits are given in Table 03. It is also important to note that the interaction terms of the main effects did not have a significantly different effect on the size of the endocarps. Table 04 summarizes the mean differences of endocarp weight, length and diameter. Although the weight of the endocarps of both the species are similar (2.0 – 2.2 g), the endocarps of *E. ganitrus* were significantly shorter and broader compared to that of *E. serratus* ($p<0.05$; Table 4). The mean variations of the endocarps of the individual trees are depicted in Figures 4A and 4B (weight), Figures 4C and 4D (length) and Figures 4E and 4F (diameter). The variation of endocarp size given in Figure 4 is shown Figure 5Aii (*E. ganitrus*) and Figure 5Bii (*E. serratus*) by means of representative endocarp selected from each assessed tree. The longitudinal and cross sections of fruits and endocarps of *E. ganitrus* and *E. serratus* are displayed in Figures 6Aiii, 6Aiv, 6Biii and 6Biv respectively. The size traits

of the endocarps were not strongly correlated where only diameter was separately correlated with weight and length ($p<0.05$). The endocarp length and diameter were negatively correlated showing an inverse relationship between the two traits (PCC = -0.24; $p<0.05$) (Table 06).

The details of the morphological appearance of the leaves of *E. ganitrus* and *E. serratus* are indicated in Table 2. In general, the leaves of *E. ganitrus* are larger and darker. Two representative leaves at fully grown stage of the two species are given in Figure 7A (*E. ganitrus*) and 7B (*E. serratus*).

Variation in organoleptic preference on fruits

All the organoleptic parameters of the ripe fruits got significant associations with the species ($p<0.05$). The colour of the fruits of *E. ganitrus* fruits were highly preferred (as ranked by 70.0 % of respondents) than *E. serratus* fruits (33.3 %). However, the higher ratings were received by *E. serratus* fruits for all the other organoleptic parameters. The highest significant association was reported for aroma (Chi-square=26.9; $p<0.05$). The summary of the association analysis for organoleptic properties are given in Table 07.

Table 06:   **Pearson Correlation coefficients among seed size parameters**

	Weight (g)	Length (cm)
Length (cm)	0.01^{ns}	
Diameter (cm)	0.26*	-0.24*

$P\leq0.05$; ns: nonsignificant ($P>0.05$)

Table 07:   **Association analysis of the organoleptic parameters of fruits**

Association	Species	Percentage respondents			
		Low (1)	Moderate (2)	High (3)	Chi-square value
Color *vs.* species	*E. ganitrus*	6.7	23.3	70.0	12.1*
	E. serratus	0.0	66.7	33.3	
Aroma *vs.* species	*E. ganitrus*	76.7	20.0	3.3	26.9*
	E. serratus	16.7	23.3	60.0	
Texture *vs.* species	*E. ganitrus*	36.7	46.7	16.7	14.1*
	E. serratus	3.3	43.3	53.3	
Sourness *vs.* species	*E. ganitrus*	46.7	40.0	13.3	11.5*
	E. serratus	10.0	50.0	40.0	
Overall taste *vs.* species	*E. ganitrus*	46.7	33.3	20.0	13.3
	E. serratus	10.3	27.6	62.1	

$P\leq0.05$

Phylogenetic analysis

There are no nucleotide sequence differences of two loci within each species for the trees assessed. However, there is a considerable variation present between two species. In the *trnH-psbA* marker region, there are five SNPs and two INDELS present; a nine-base deletion in *E. serratus* (49-57 bp) and a six-base deletion in *E. ganitrus* (102-107 bp). In the *trnL-trnF* marker region, there are four SNPs two INDELs present; one-base deletion in *E. serratus* (773 bp) and a 17-base deletion in *E. ganitrus* (815-831 bp) (Figure 08). Almost similar topologies were obtained for both ML and Bayesian trees drawn for *trnL-trnF* marker region. However, the Bayesian tree was more resolved than the ML tree thus shown in the present study (Figure 09). According to the Bayesian tree, separate clusters are formed by *E. ganitrus* and *E. serratus*. A separate clade is formed by the assessed trees of *E. serratus* accessions and other five *Elaeocarpus* spp. (*E. stipularis, E. tectorius, E. dongnaiensis, E. glaber*) while the assessed trees of *E. ganitrus* are assembled with nine *Elaeocarpus* spp. (*E. grandis, E. angustifolius, E. ptilanthus, E. sphaericus, E. hylobroma, E. geminiflorus, E. largiflorens* subsp.) forming a separate clade (Figure 09).
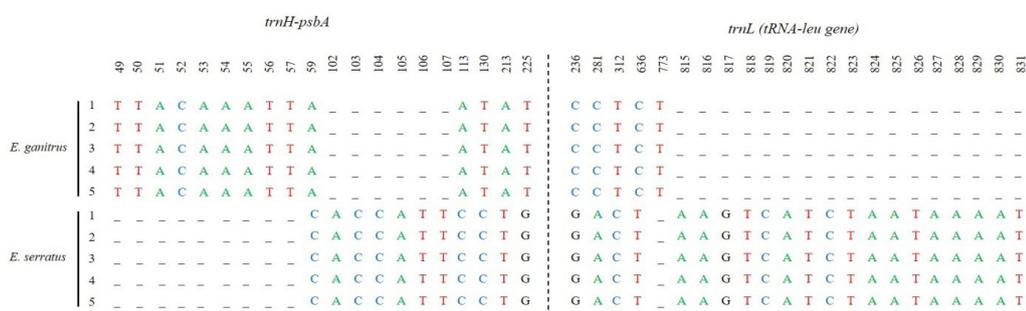

Figure 08:   The SNP and INDEL profile *E. ganitrus* and *E. serratus* at the polymorphic sites of *trnH-psbA* and *trnL-trnF.* Names of the markers and the positions of the SNPs and INDELS are given above the sequences. 1, 2, 3, 4 and 5 indicate five different trees for each species.
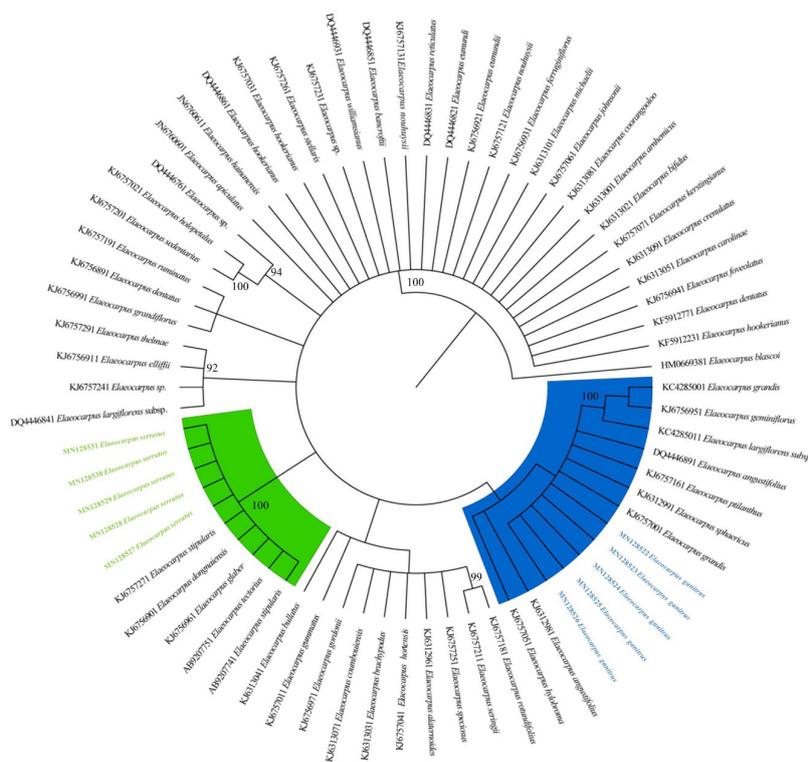

Figure 09:   The rooted Bayesian tree constructed using sequences obtained for *trnL-trnF* (*tRNA-leu gene*) marker region. *E. serratus* (green) and *E. ganitrus* (blue) species included into two separate clades. The posterior probability values (>80) are shown at the respective clades.

DISCUSSION

E. serratus fruits are very popular in Sri Lanka. *E. serratus* trees are predominantly found in wet and intermediate zones of the country; however, home garden trees are scattered all over the country. *E. ganitrus* trees are mainly found in wet zone and especially in districts *Matale* and Kandy. Therefore, in the present study we selected a set of trees of the two species from same area (Figure 2) in order to perform a comparative morphological analysis of fruits and endocarps.

The colour of *E. ganitrus* fruits is caused by the iridosomes present under the outer cell walls of epidermis. The iridosome reflect the blue light and that is why *E. ganitrus* fruits are blue in appearance (Lee, 1991). In the present study, we observed that, at complete maturity (ripe stage), fruits of different trees show a variation indicating complex genotype × environment interaction causing the blue coloration of fruits (Figure 5Ai). The large size of *E. ganitrus* trees and presence of apical crown of branches at great heights did not permit us to collect fruits to study the gradual development of iridosomes, colour and within plant variations. The fruits of *E. serratus* do not contain iridosomes and remain in lightish or olive green in skin colour (Figure 5Bi). The endocarps of *E. ganitrus* are culturally significant and hence economically important. We observed that endocarp size traits are not significantly affected by the environment but there is a significant within species variation observed (Table 3 and 4; Figure 4). In making wooden-bead chains for religious matters; the size, shape and the contour-pattern of the endocarp surface are important. It is evident from the present analysis that the tree to tree variation of endocarp morphology (Figure 4 and 5ii), permits the selection of a specific tree for specific necklace types for particular devotees and the religious occasion or observance. The endocarps of *E. serratus* have no religious value probably because of their elongated shape causing less applicability to string as beads and the shallow contours of the endocarps make them less attractive compared to the more prominently patterned endocarps of *E. ganitrus* (Figure 6).

Although seasonal effect is significant for fruit size traits and the weight of endocarps, the tree-to-tree (i.e., intraspecific) variation is the highest for all the size traits (Table 3), indicating the heterogeneous nature and the presence of major effect quantitative trait loci (QTLs) causing such a high variation. It is also interesting to note that length and diameter are negatively correlated probably implying that during the cause of fruit development, certain degree ($\sim$ 25 % according to the present study) of length to diameter ratio is maintained retaining the identity of fruit geometry to keep the dispersal agents like birds attracted or an avenue of co-evolution with them. If there are specialized dispersal agents (Lee, 1991 reported fruit-eating pigeons, small mammals and Cassowary for *E. ganitrus* citing Crome, 1976; Frith *et al.,* 1976; Stocker and Irvine, 1983; Pratt and Stiles, 1985), the genetic determination of specific endocarp-geometry is important for the survival of the species.

The organoleptic analysis revealed that the tasters prefer the blue colored appearance of the *E. ganitrus* fruits than the olive-green colored *E. serratus* fruits. However, for all the other taste parameters, *E. serratus* fruits were more preferred (Table 7). The blue look of *E. ganitrus* fruits is gorgeous and everyone prefers this uniqueness; however, the reason for high preference on the other parameters due to the fact that *E. serratus* is more common in Sri Lanka as a fruit. The people are so used to the '*weralu*' taste, aroma, texture and sourness. Many people do not even know the existence of 'blue *weralu*' within the country and it is a relatively less known fruit type for the consumption which may have caused the lower preference-ratings. Those who see the *E. ganitrus* fruits for the first time in Sri Lanka, always think that they are 'toys' made from play-clay. The less awareness of *E. gantrus* makes it a more under-utilized crop than *E. serratus*.

According to the sequence polymorphism obtained from the *trnH-psbA* and *trnL-trnF* (*tRNA-leu gene*) marker regions, it was evident that there is no intraspecific genetic variation present within the species (Figure 8). According to the clustering pattern in Bayesian tree, *E. ganitrus* and *E. serratus* form two separate clusters. Furthermore, within *E. serratus* cluster, there is no significant interspecific variation (Figure 9). There is a detectable variation within

the *E. ganitrus* cluster. According to Baba, (2013), *E. stipularis*, *E. dongnaiensis* and *E. glaber* form the Asian clade, and according to the obtained results, *E. stipularis*, *E. tectorius*, *E. dongnaiensis*, *E. glaber* and all five *E. serratus* accessions form a monophyletic clade (pp= 100). Though *E. angustifolius* and *E. sphaericus* are generally used as synonyms for *E. ganitrus*; *E. angustifolius* and *E. sphaericus* are not completely homogenous (Baba, 2013). However, according to out results, *E. grandis*, *E. angustifolius*, *E. ptilanthus*, *E. sphaericus*, *E. hylobroma*, *E. geminiflorus*, *E. largiflorens* subsp. and all five *E. ganitrus* accessions form a separate cluster (pp= 69). According to the phylogenetic analysis of *E. ganitrus* and *E. serratus*, the interspecific genetic variation is more prominent, and the intraspecific variation is not apparent implying that the genetic variation is independent from the selection pressures exerted by environment.

CONCLUSIONS

The morphometric analysis of fruits and endocarps of *E. ganitrus* and *E. serratus* revealed that the size traits are predominantly affected by the individual tree (i.e. intraspecific variation) than the environmental effect. Although fruit size and endocarp weight parameters are affected by the season ($p<0.05$), the length and diameter of the endocarps are seasonal independent and tree specific. This tree specificity enables the devotees to harvest characteristic beads from specific trees to string diverse necklaces for various religious functions and devotees. Except colour, people prefer *E. serratus* fruits than *E. ganitrus* fruits. The *trnH-psbA* and *trnL-trnF* barcoding markers clearly point out the species difference among *E. ganitrus* and *E. serratus*. The sequence pop set available in Baba (2013) for *trnL-trnF* (only pop set available for *Elaeocarpus* spp. to date) positioned the trees of *E. ganitrus* assessed in the present study in the respective clade and *E. serratus* in a clade with *E. stipularis*, *E. tectorius*, *E. dongnaiensis* and *E. glaber*. Although present study set the species delimits for two studied species, worldwide attempts are needed to fully resolve the molecular phylogenetic relationships of *E. ganitrus*, *E. serratus* and other worldwide *Elaeocarpus* spp.

Data Availability Statement

The nucleotide dataset generated during and/or analysed during the current study are available in the GenBank, and https://www.ncbi.nlm.nih.gov/nuccore (*trnH-psbA*: MN119517-MN119526; *trnL-trnF*: MN128522-MN128531).

The other datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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