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RE-EVALUATION OF SPECIES LIMITS OF *HORTONIA* (MONIMIACEAE) BASED ON EMPIRICAL METHODS

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ABSTRACT

A study was carried out to re-evaluate the species limits of the endemic genus *Hortonia*. The latest revision in Flora of Ceylon recognizes three species of *Hortonia*. A recent chemical study claims to have found identical chemical compounds among the three species. Therefore during this study the three species were studied in greater detail to see if the three species possess distinct morphological features or they were identical as suggested by the chemical study. Specimens were collected from all different geographical locations within Sri Lanka where they occur. More emphasis was given to morphological characteristics of leaves and flowers that had not been previously studied. Different chemical methods were adopted to clear the veins and to study the venation patterns in detail. A total of 57 characteristics were coded. Species limits were determined by clustering methods and phylogenetic analysis. Both analyses support the recognition of three species of *Hortonia*. Additional stable morphological features were identified in defining the three species. *H. ovalifolia* and *H. floribunda* are resolved as a monophyletic group which is well supported. Based on the additional characters, a modified key for the identification of the three species is proposed. The results corroborate the recent revision of the genus.

Key words

Hortonia, Monimiaceae, morphological data, species limits

INTRODUCTION

Hortonia is a genus endemic to Sri Lanka, belonging to the family Monimiaceae Juss. The family Monimiaceae is thought to have originated in the Gondwanaland about 100-120 million years ago. The family has played an important role in discussions of the history of land masses (Laurasia/Gondwanaland) inhabited by their living and extinct members.

The family consists of about 28-34 genera and more than 450 species distributed in tropical and sub-tropical regions (Renner, 1998). In Sri Lanka the family Monimiaceae is represented only by the endemic genus *Hortonia*. The family includes mostly trees and shrubs and rarely climbers (Dassanayake, 1996).

Hortonia is a shrub or a small tree. The arrangement of leaves is opposite or opposite-decussate. Flowers are borne on inflorescences. The small, bisexual and regular flowers consist of many perianth segments arranged in many series; the outer sepaloid changing gradually inwards to 16-20 petaloid segments. The number of stamens varies from 4 -12 and they are shorter than the perianth segments. They are attached to the margin of the hypanthium in one or two series. Filaments are linear, somewhat short and bent outward at the apex with two large turbinate appendages (glands) at the base on the abaxial side. Eight to ten free, sessile carpels are attached to the centre of

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the hypanthium. Druplets are ovoid in shape, shortly stalked and with a hard endocarp (Dassanayake, 1996).

The latest revision of the Handbook to the Flora of Ceylon recognizes three species of *Hortonia viz. H. floribunda, H. angustifolia* and *H. ovalifolia. H angustifolia* is recorded in the moist lowlands, at elevations of about 700 m at edges of streams, in secondary wet forests and along road - sides. Mature trees reach a height of about 7m. Flowering occurs from July to November. *H. floribunda* occurs in the montane zone above 1300 m, in secondary montane forests, by streams, rooted in rocky eroded ground and flowering from December to January. *H. ovalifolia* is recorded in the Peak Wilderness area, about 1600 m and higher, by streams, rooted in rocky eroded ground and flowering from March to August.

Taxonomic history

The monotypic Sri Lankan genus is the sole perfect flower (bisexual) of the family and also has given rise to many controversial ideas regarding the interfamily relationships. In most of the major treatments because of this bisexual flower, a trait absent in the other members, the genus was treated as a separate basal subfamily (Perkins & Gilg, 1901; Money *et al.*, 1950; Philipson,1993). The situation is even further complicated as the species limits of the genus are also a point of debate. Arnott, in 1838 (Dassanayake, 1996 and reference therein) first validly published the species *Hortonia floribunda* which was recognized by Wight. Since then several taxa have been added and recognized at species and subspecies level. Circumscriptions in these major works have been very different. However, the most recent revision of the family Monimiaceae by Dassanayake (1996) recognizes three species of *Hortonia; H. floribunda* Wight ex. Arn, *H. ovalifolia* Wight and *H. angustifolia* (Thw.) Trimen. A summary of the different taxonomic treatments is given in Table 1.

Table 1

A summary of different taxonomic treatments. (Hooker and Thomas, 1855; Engler, 1901; Hooker, 1887; Dassanayake, 1996)

Wightex.Arn. 1838	Wight 1853	Hooker & Thomas 1855	Thwaites 1864	Trimen 1895	Dassanayake 1996
H.floribuna	*H. floribunda* *H. ovalifolia* *H.acuminata*	*H. floribunda* var. *acuminata* var. *ovalifolia*	*H.floribunda* var. a.*acuminata* b.*ovalifolia* c.*angustifolia*	*H. floribunda* var. *ovalifolia* *H.angustifolia* var.*angustifolia* var.*acuminata*	*H.floribunda* Wighex. Arn. *H.ovalifolia* Wight *H.angustifolia* (Thw.) Trimen

A recent study based on the phytochemistry of the genus *Hortonia* has once again raised the question of the species limits. The study has isolated many different compounds from the three species of *Hortonia,* two butanoloides, one tetracyclic sesquiterpene and two oxygenated butanoloides being the main bioactive compounds (Ratnayake *et al.*, 1998; Ratnayake *et al.*, 1999;

Ratnayake *et al.*, 2001). Based on the results the conclusion was that the three species of *Hortonia* had shown identical chemical composition. Therefore, it was suggested that as there were no significant phyto-chemical differences among the three species recognized by the revision (*H. floribunda, H. angustifolia* and *H. ovalifolia*) they belonged to the same species (Ratnayake *et al.*, 1998; Ratnayake *et al.*, 2001).

Further, a recent study on the phylogenetic affinities of the Monimiaceae based on cpDNA gene and spacer sequences also treated the genus as with one species. Money's statement (Money and Swamy, 1950), "*H. ovalifolia* and *H. angustifolia* Trimen in my view are identical with *H. floribunda* Wight ex. Arn" is quoted as the basis for this study (Renner, 1998).

The past classifications of *Hortonia*, are primarily based on a few leaf morphological characters, especially the leaf shape; leaves elliptic, rounded at base and apex being *H ovalifolia*, leaves lanceolate to narrowly ovate, major veins arched defining *H. floribunda* and leaves narrowly lanceolate to narrowly elliptic with major veins parallel to margin being *H.angustifolia.* As the leaf size and shape could show phenotypic variation and plants always do contain a percentage of odd ones, it makes it difficult to differentiate between the species only by considering the leaf shape.

These ambiguities and the identical chemical composition led us to re-evaluate the species limits of *Hortonia* using a large number of morphological characters and analyzing the data based on an empirical method and further, if three species exist, to identify additional stable morphological features to construct an identification key and to determine their phylogenetic relationships. Solving taxonomic ambiguities especially of endemic species like *Hortonia* is very important in order to plan out conservation and management.

MATERIALS AND METHODS

All three currently recognized taxa from different locations were used in the study. Each taxon with different character states was coded separately. Five individuals per taxa were coded. Voucher specimens were prepared from the collected specimens and deposited at the Herbarium of the Department of Botany, University of Peradeniya.

Morphological studies

Characters were selected by reviewing previous work and searching for variations that had not been previously analysed. (Dassanayake, 1996; Dassanayke, pers. com.).

Floral studies

Flowers were preserved in 70% alcohol and observed under the dissecting microscope. Different flower parts and peels of petals and sepals, were cleared and observed under the light microscope to study the diversity of the trichomes.

Leaf venation

The patterns made by the primary vein (mid-vein), secondary, tertiary and quaternary veins, were coded by clearing leaves and by observing their pattern. The protocol for leaf clearing was adopted from Dissanayake (1999). The original method was modified and several methods were tried out and the best was selected.

Method for leaf clearing: Leaves were placed in 5% sodium hydroxide (NaOH) solution which was repeatedly replaced with fresh solution. The cleared leaves were washed, bleached and taken through an alcohol series; 30% ethanol (10 min.), 50% ethanol (10 min.), 1% safranin in 50% alcohol (3-4 min.), 70% ethanol (10 min.), 95% ethanol (10 min.) and absolute alcohol (5 min.). Finally the leaves were mounted on glass slides with glycerin.

As leaf texture varied among the species, different concentrations of NaOH, temperatures and clearing times had to be adopted. Terminology for the leaf venation patterns was adopted from Dilcher (1974) and Yakandawala (2001).

A total of 59 Morphological characters was coded into discrete states Table 2. Some characters were coded as binary variables and most as multi-state due to extended variation.

Table 2

List of morphological characters together with their character states used in the study

(1) **Branchlet nature:** drooping = 0; slender = 1; stout = 2.
(2) **Petiole nature:** slender = 0; stout = 1.
(3) **Petiole shape:** cylindrical = 0; sub-cylindrical = 1.
(4) **Petiole channel:** absent = 0; present = 1.
(5) **Leaf arrangement:** opposite = 0; opposite-duccusete = 1; spiral = 2
(6) **Leaf base:** acute = 0; obtuse = 1.
(7) **Lamina shape:** linear-lanceolate = 0; lanceolate = 1; ovate = 2; narrow-ovate = 3.
(8) **Leaf lamina:** flat = 0; bent down at apex = 1.
(9) **Leaf apex:** obtuse = 0; acute = 1; acuminate = 2; obtuse+acute = 3
(10) **Leaf margin:** entire = 0; serrate = 1; slightly repand = 2.
(11) **Strongly revolved margin:** absent = 0; present = 1.
(12) **Leaf texture:** coriaceous = 0; other = 1.
(13) **Symmetrical leaf base:** symmetrical = 0; symmetrical and asymetrical = 1.
(14) **Semicraspedodromous venation:** absent = 0; present=1.
(15) **Prominently three-veined at base:** absent = 0; present = 1.
(16) **Primary vein size:** weak = 0; moderate = 1; stout = 2.
(17) **Primary vein channeled above:** absent = 0; present = 1.
(18) **Secondary veins more prominent above than at the base:** absent = 0; present = 1.
(19) **Angle of origin of secondary veins:** narrow = 0; moderate = 1; unequal = 2
{ unequal = 1/2 narrow/1/2 moderate}
(20) **Secondary veins parellel to the margin:** absent = 0; present = 1.
(21) **Variation in angle of divergence of secondary veins:** uniform = 0; not-uniform=1; uniform and not-uniform=2.
(22) **Secondary veins more acute on one side of the leaf than on the other side:** absent = 0; present = 1.
(23) **The lowest secondary pair more acute than the pairs above:** absent=0;present =1.
(24) **Behavior of loop forming branches:** acute = 0; right angles = 1.
(25) **Angle of origin of tertiary veins:** (acute-right angles) AR = 0; RR (right angles- right angles) = 1.
(26) **Tertiary veins approximately right angles to the primary vein:** absent = 0; present = 1.
(27) **Pattern of tertiary veins:** straight = 0; retroflexed = 1; convex = 2; straight and retroflexed = 3; straight and convex = 4.
(28) **Areole shape:** mostly quadrangular = 0; mostly polygonal = 1.
(29) **Peduncle nature:** mostly drooping = 0; slender or stout = 1.
(30) **Peduncle width:** narrow = 0; broad = 1.

(31) **Nearly sessile inflorescence:** very short = 0; long = 1.
(32) **Pedicel length:** less than 10mm=0; greater than 10mm= 1.
(33) **Pedicel colour:** reddish-brown = 0; yellowish green = 1.
(34) **A small appendage on the pedicel:** absent = 0; present = 1.
(35) **Duration of bracts:** caducous = 0; persistent = 1; sub-persistent = 2.
(36) **Bract shape:** linear lanceolate = 0; deltoid = 1; orbicular = 2.
(37) **Bract size:** small = 0; large = 1.
(38) **Bracteole:** absent = 0; present = 1.
(39) **Well-developed, cup-shaped, fleshy hypanthium:** absent = 0; present = 1.
(40) **Petals and sepals:** absent = 0; present = 1.
(41) **Sepal colour:** greenish = 0; reddish-brown = 1.
(42) **Sepal shape:** ovate = 0; deltoid = 1; rounded =2.
(43) **Two types of sepals:** absent = 0; present=1.
(44) **Sepal length:** short = 0; long = 1.
(45) **Sepaloides:** absent=0; present=1.
(46) **Tufts of trichomes on the margin of the sepal:** absent = 0; present = 1.
(47) **Tufts of trichomes on the surface of the sepal:** absent = 0; present = 1.
(48) **Inflorescence type:** raceme or panicle = 0; compound dichasium = 1; umbel = 2.
(49) **Diameter of the flower:** less than 15mm = 0; greater than 15mm = 1.
(50) **Number of petals:** less than 16 = 0; more than 16 = 1.
(51) **Petal colour:** greenish yellow to bright yellow = 0; orange yellow = 1.
(52) **Petal apex:** obtuse = 0; acute = 1.
(53) **Tufts of trichomes on the margin of the petal:** absent=0; present=1.
(54) **Stamen number:** (4-6) = 0; (8-10) = 1.
(55) **Filament length:** (1-1.5mm) = 0; (1.5-2 mm) = 1; (4-5mm) =2.
(56) **Broad, elliptic, spreading anthers:** absent = 0; present = 1.
(57) **Appendage color:** brownish = 0; yellowish = 1.
(58) **Appendage width:** (0.5mm) = 0; (1-2mm) = 1.
(59) **Appendage shape:** deltoid = 0; pyramidal = 1.

Determining the species limits

Literature on the species concept is enormous and several schools of thought exist regarding species limits. There are two major ones: according to one school of thought, species must first be determined outside a cladistic framework (Davis and Goldman, 1993) and the other, often termed the phylogenetic species concept, says that species can be defined only by cladistic analysis (Baum and Donoghue, 1995). Both approaches have been taken into account.

Out group selection

The genus *Neolitsea* (Lauraceae) was selected as the out-group based on the APG classification (APG, 1998). The data were coded by examining field-collected specimens and the literature.

Cluster analysis

For determining species limits out-side a cladistic frame work, clustering methods were used. The data were entered into an excel spreadsheet for cluster analysis (Table not given; the coding is similar to Table 3). Cluster analysis was carried out using UPGMA in SAS version 6.0.

Phylogenetic analysis

To determine species limits within a phylogenetic frame-work and their phylogenetic relationships, characters were coded taking into consideration the arguments on coding morphological features for phylogenetic analysis (Chappill, 1989; Stevens, 1991; Hawkins *et al.*, 1997; Kitching *et al.*, 1998). Data were coded using the MacClade program (Maddison and Maddison, 2000). The matrix is presented in Table 3. Two additional character codings for presence and absence were added in order to accommodate hierachical characters (peduncle present and absent; perianth present and absent). Of the 59 characters, 57 were parsimony-informative. Cladistic analysis was performed using P A P *Version 4.0b10 for Macintosh (Phylogenetic Analysis Using Parsimony, Swofford, 2000) to ensure the recovery of the most parsimonious tree or trees. Alternative tree topologies and resultant changes in tree lengths were explored using the MacClade program. For analysis branch and bound searches were performed initially under the unordered and equal weighting criteria of Fitch parsimony (Fitch, 1971) with 500 replicates, the 'MulTrees' option in effect. The number of trees retained is ten.

Strict consensus, 50% majority rule and Adams consensus tree were obtained and tree statistics were calculated. The initial trees found with equal (Fitch) weights were used as the basis for successive weighting. Successive weighting was carried out using the rescaled consistency index. Reweighting was continued until the same tree length was obtained in two successive rounds. Bootstrapping analyses was carried out to evaluate the robustness of the clades.

RESULTS

Cluster analysis

The dendrogram resulting from the cluster analysis is given in Figure 1. The cluster analysis identifies three major clusters each corresponding to *H. angustifolia, H. ovalifolia* and *H. floribunda.* The taxa separate into two groups, at a distance of 7.5, a group with *H. angustifolia* and the other with *H. ovalifolia* and *H. floribunda.* The later group divides at a distance of 6 into two separate taxa, *H. ovalifolia* and *H. floribunda.* The distance in the divisions within these groups is less than 2. There is no difference among HA1, HA2, HA3 and HA4, HA5 of *H. angustifolia,* HF1, HF2 and HF4, HF5 of *H. floribunda.* The 5 individuals of *H. ovalifolia* separated from each other at a distance of 1.5.

Phylogenetic analysis

Branch and bound search under the Fitch criterion yielded 10 most parsimonious trees (MPTs) of 84 steps. The strict consensus tree had a consistency index (CI) of 0.881 and rescaled consistency index (RI) of 0.962 (figure not shown). Successive weighting based on the rescaled consistency index of all 10 trees produced eight equally parsimonious trees of 73.95 steps, CI of 0.936 and RI of 0.980 (Fig. 2).

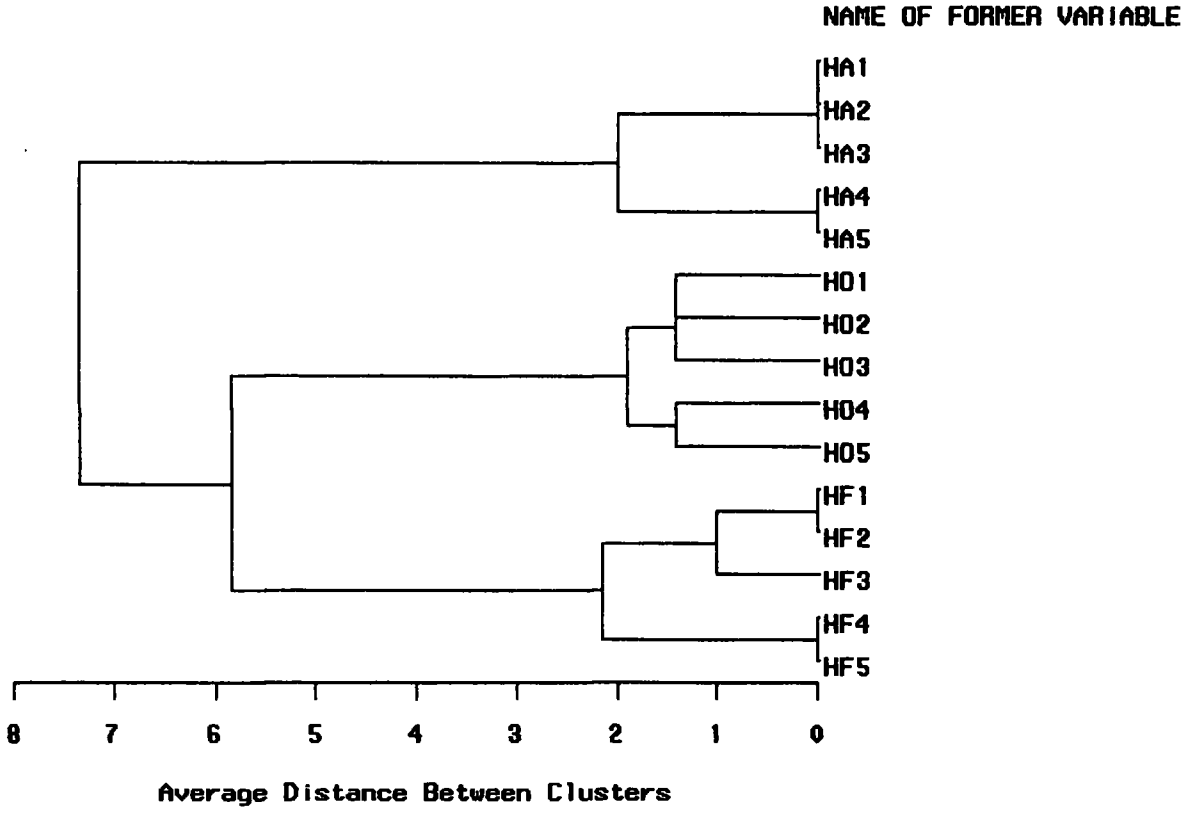

Figure 1. The resulting dendogram of the cluster analysis

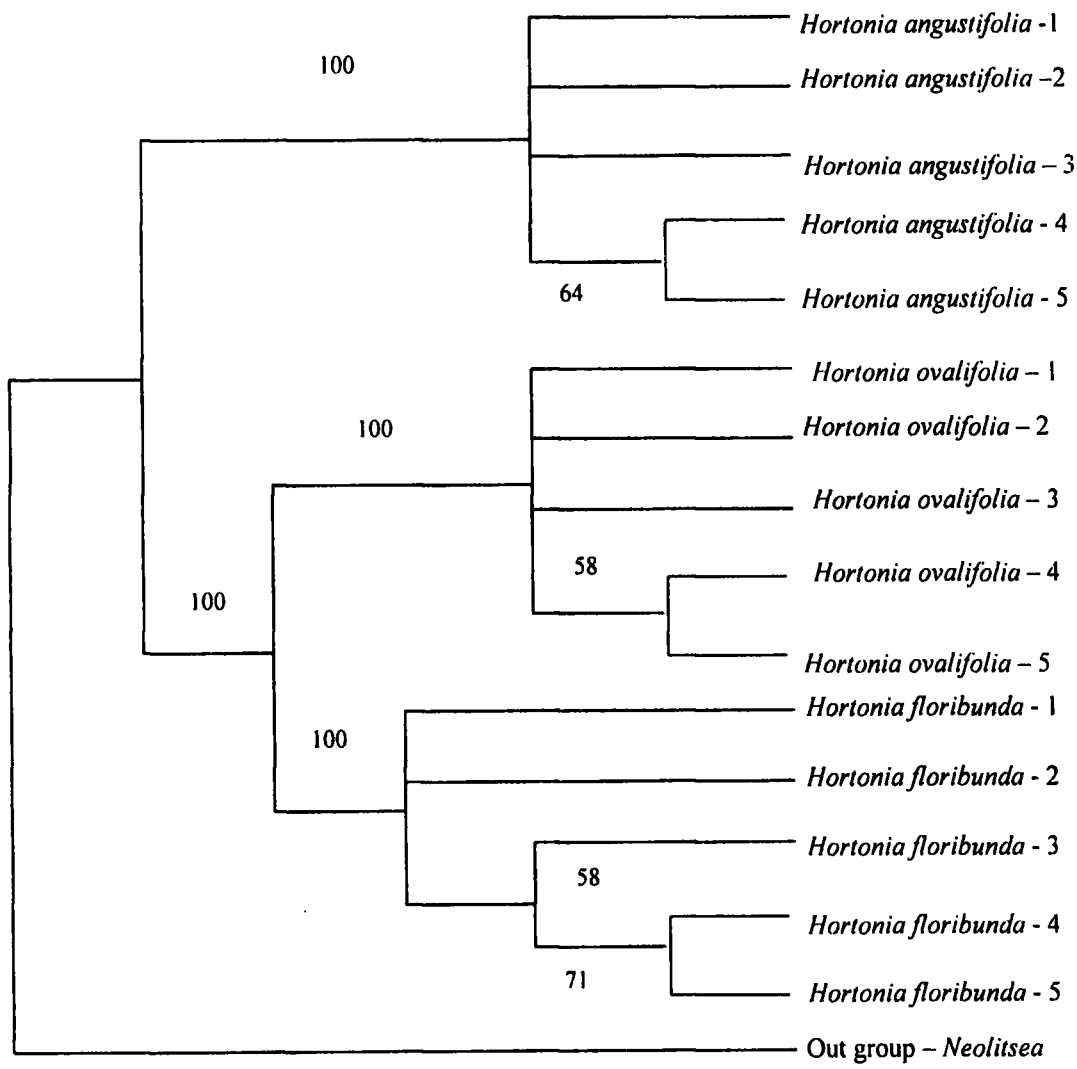

Figure 2. Strict consensus tree of the eight most parsimonious trees recovered during successive weighting based on RI Length = 73.95 steps, CI of 0.980. The bootstrap support values are shown above the branches.

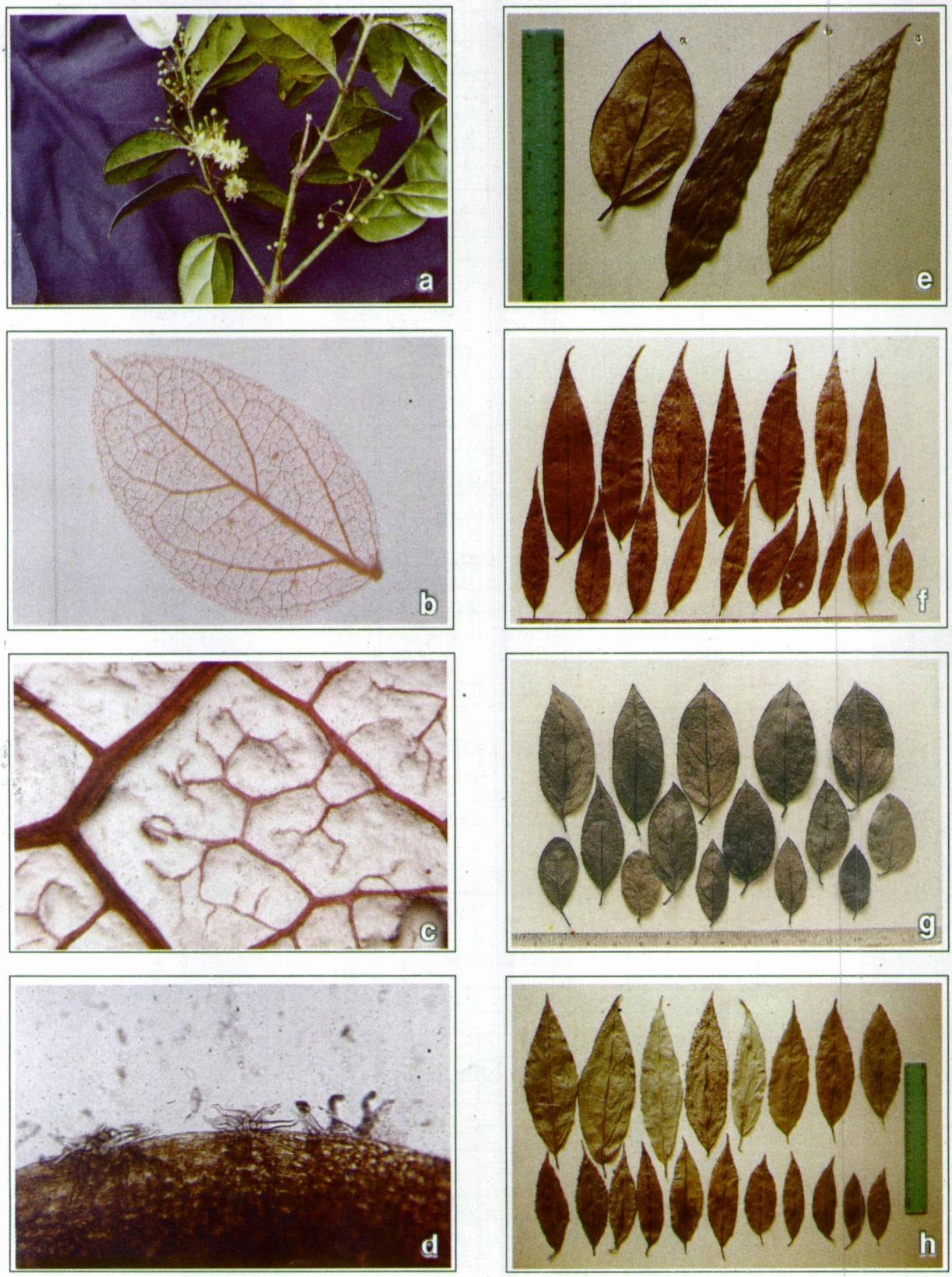

Figure 3. a. Inflorescence of *H. ovalifolia*; b. cleared leaf of *H. ovalifolia;* c. well developed areoles; d. tufts of trichomes on petals of *H. angustifolia* ; e .comparison of leaves of the three species; f. *H. angustifolia*; g. *H. ovalifolia*; h. *H. floribunda*

Table 3

Data Matrix for the Cladistic analysis HA=*H.angustifolia* HO=*H.ovalifolia*
HF=*H.floribunda*, LA= Out Group (Lauraceae)

	Character 1	Character 2	Character 3	Character 4	Character 5	Character 6	Character 7	Character 8	Character 9	Character 10	Character 11	Character 12	Character 13	Character 14	Character 15	Character 16	Character 17	Character 18	Character 19	Character 20	Character 21	Character 22	Character 23	Character 24	Character 25	Character 26	Character 27	Character 28	Character 29	Character 30	Character 31	Character 32	Character 33	Character 34	Character 35	Character 36	Character 37	Character 38	Character 39	Character 40	Character 41	Character 42	Character 43	Character 44	Character 45	Character 46	Character 47	Character 48	Character 49	Character 50	Character 51	Character 52	Character 53	Character 54	Character 55	Character 56	Character 57	Character 58	Character 59
HA1	0	0	0	1	0	1	0	1	2	0	0	1	1	0	1	0	1	1	0	1	1	0	0	1	0	1	3	0	0	0	0	0	1	0	1	0	1	1	0	1	0	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0
HA2	0	0	0	1	0	1	0	1	2	0	0	1	1	0	1	0	1	1	0	1	1	0	0	1	0	1	3	0	0	0	0	0	1	0	1	0	1	1	0	1	0	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0
HA3	0	0	0	1	0	1	0	1	2	0	0	1	1	0	1	0	1	1	0	1	1	0	0	1	0	1	3	0	0	0	0	0	1	0	1	0	1	1	0	1	0	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0
HA4	0	0	0	1	0	1	0	1	2	2	0	1	1	0	1	0	1	1	0	1	1	0	0	1	0	1	3	0	0	0	0	0	1	0	1	0	1	1	0	1	0	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0
HA5	0	0	0	1	0	1	0	1	2	2	0	1	1	0	1	0	1	1	0	1	1	0	0	1	0	1	3	0	0	0	0	0	1	0	1	0	1	1	0	1	0	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0
HO1	1	1	1	1	1	0	2	0	0	0	1	0	0	0	0	1	1	0	1	0	1	1	0	0	0	0	2	1	1	1	0	1	0	0	0	1	0	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	0	1	0
HO2	2	1	1	1	1	0	2	0	0	0	1	0	0	0	0	1	1	0	2	0	1	1	0	0	0	0	2	1	1	1	0	1	0	0	0	1	0	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	0	1	0
HO3	1	1	1	1	1	0	2	0	1	0	1	0	0	0	0	1	1	0	2	0	1	1	0	0	0	0	2	1	1	1	0	1	0	0	0	1	0	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	0	1	0
HO4	2	1	1	1	1	0	3	0	0	0	1	0	0	0	0	1	1	0	1	0	1	1	0	0	0	0	2	1	1	1	0	1	0	0	0	1	0	0	1	1	1	2	1	1	1	1	1	1	1	0	1	1	1	1	0	1	0
HO5	2	1	1	1	1	0	2	0	0	0	1	0	0	0	0	1	1	0	1	0	1	1	0	0	1	0	2	1	1	1	0	1	0	0	0	1	0	0	1	1	1	2	1	1	1	1	1	1	1	0	1	1	1	1	0	1	0
HF1	1	1	1	0	1	1	1	0	2	1	0	0	0	1	0	2	0	0	1	0	2	0	1	0	1	0	4	1	1	1	1	1	1	1	0	1	0	0	0	1	0	0	1	0	0	1	0	1	1	0	0	1	1	1	0	1	1
HF2	1	1	1	0	1	1	1	0	2	1	0	0	0	1	0	2	0	0	1	0	2	0	1	0	1	0	4	1	1	1	1	1	1	1	0	1	0	0	0	1	0	0	1	0	0	1	0	1	1	0	0	1	1	1	1	1	1
HF3	1	1	1	0	1	1	1	0	1	1	0	0	0	1	0	2	0	0	1	0	2	0	1	0	1	0	4	1	1	1	1	1	1	1	0	1	0	0	0	1	0	0	1	0	0	1	0	1	1	0	0	1	1	1	1	1	1
HF4	1	1	1	0	1	1	3	0	1	1	0	0	0	1	0	2	0	0	1	0	2	0	1	0	1	0	4	1	1	1	1	1	1	1	0	1	0	0	0	1	0	0	1	0	0	1	0	1	1	0	0	1	1	1	1	1	1
HF5	1	1	1	0	1	1	3	0	1	1	0	0	0	1	0	2	0	0	1	0	2	0	1	0	1	0	4	1	1	1	1	1	1	1	0	1	0	0	0	1	0	0	1	0	0	1	0	1	1	0	0	1	1	1	1	1	1
LR	1	0	1	1	2	0	1	1	2	0	0	0	0	0	1	1	0	1	1	1	1	0	0		1	1	5	0	1	1	1	1	0	0	2	2	0	0	0	0			0					2			0				0				

Table 4

Characters that showed variation

Character	*H. angustifolia*	*H. ovalifolia*	*H. floribunda*
leaf shape	Linear-lanceolate	Ovate-shaped leaves	Lanceolate-shaped eaves
2 types of bracts smaller & larger	Present	Absent	Absent
Duration	Persistent	Caducous	Caducous
Leaf base	Acute leaf base	Obtuse leaf base	Acute leaf base
Leaf margin	Entire margin.	Strongly revolved margin	Entire and some leaves with serrate margins
Flower and inflorescence	Narrow, drooping peduncles & small flowers	Slender or stout peduncles and larger flowers.	Slender or stout peduncles and larger flowers.
Inflorescence type	Flowers born on racemes or panicles	Flowers in simple or compound dichasia	Flowers born on racemes or panicles
Sepals	Sepals of only one type	Two types of sepal	Sepals of only one type
Venation type	Only brochidodromous venation present	Only brochidodromous venation present	Brochidodromous and Semicraspedodromous venation in serrate leaves
Trichomes on petals	Tufts of trichomes on the margin of petals	Trichomes are absent from the margin of the petals	Trichomes are absent from the margin of the petals
Leaf arrangement	Oppositely arranged leaves	Opposite-decussate leaf arrangement	Opposite decussate leaf arrangement
Flower	Yellow colored small flowers	Light orange-yellow colored, bigger flowers	Yellow colored medium sized flowers
Petiole	Cylindrical petiole and channeled above	Sub-cylindrical petiole and channeled above	Sub-cylindrical petiole and not channeled
Primary vein	Channeled primary vein	Primary vein channeled	Primary vein not channeled
Angle of divergence of secondary	Angle of divergence of secondary veins not uniform from base to apex	Angle of divergence of secondary veins not uniform from base to apex	Angle of divergence of secondary veins, uniform or lowest pair more acute than the pairs above
Secondary veins	Secondary veins run parallel to the leaf margin	Secondary veins run parallel to the leaf margin	Secondary veins run parallel to the leaf margin
Hypanthium	Hypanthium is not cup shaped or fleshy	Well developed, cup-shaped, fleshy hypanthium	Hypanthium is not cup shaped or fleshy
Inflorescence axis	Long inflorescence axis	Long inflorescence axis	Short inflorescence axis
Appendage on the pedicel	No appendage on the pedicel	No appendages on the pedicel	Presence of pair of bract-like appendages on the pedicel
Trichomes on sepals	Trichomes are present on the surface of the sepals	Absence of trichomes from the surface of sepals	Trichomes are present on the surface of the sepals
Bracts	Presence of two types of bracts	Presence of only one type of bract	Presence of only one type of bract

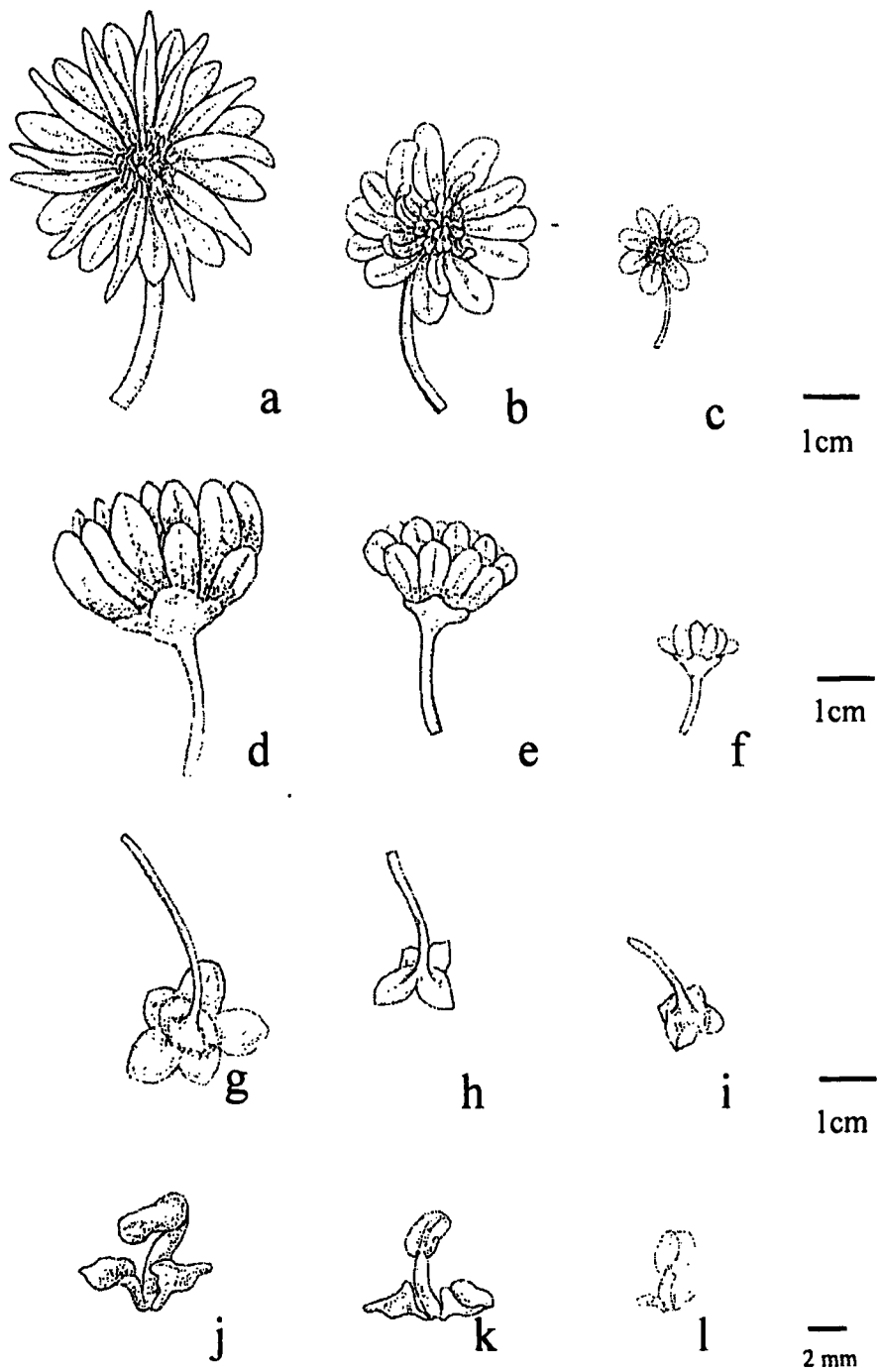

Figure 4. Differences among the flowers of the three taxa *H. ovalifolia* (a), *H. floribunda* (b), *H. angustifolia* (c). Bud of *H. ovalifolia* (d), *H. loribunda* (e), *H. angustifolia* (f). Hypanthium of *H. ovalifolia* (g), *H. floribunda* (h),*H. angustifolia* (i). Appendage of *H. ovalifolia* (j), *H. floribunda* (k), *H. angustifolia* (l).

1.Branchlets mostly drooping, leaves linear-lanceolate, acuminate apex, flowers small, petals with tufts of trichomes on the margin..... .....*H. angustifolia*
1.Branchlets slender or stout, leaves lanceolate, ovate or narrowly ovate.....2
1.Leaves ovate, base obtuse, margin entire and strongly revolved, petiole channeled above, flowers large, orange yellow with a well developed, cup-shaped , fleshy hypanthium.....*H. ovalifolia*
1.Leaves lanceolate to narrowly ovate, base acute, petiole not channeled above, flowers small greenish yellow with small hypanthium..... .....*H. floribunda*

Figure 5. Modified key for the identification of the three species. Features underlined are newly added characters.

DISCUSSION

This study has identified a number of features that are common to the three species and several characters with distinctly different states. A number of characters could be identified as generic level characters that group them in one genus. Some of these characters have not been listed previously and they include angle of origin of secondary veins which becomes more obtuse towards the apex of the leaf than near the base, incomplete marginal ultimate venation (freely ending veinlets adjacent to the margin), randomly arranged areoles, no veinlets inside areoles and brochidodromous venation (secondaries joined together in a series of prominent arches without terminating at the margin) (Fig. 3). Even though the leaves of *H. floribunda* with serrate margins showed semicraspededromous venation, all the leaves of the three species with entire margins showed brochidodromous venation.

The morphological features that show a variation in character state among different taxa are strong enough to recognize the three species of *Hortonia*; the acuminate leaf apex, small flowers (Table 4) and petals with tufts of trichomes on the margin distinguished *H. angustifolia* while obtuse leaf base, entire and strongly revolved leaf margin, channeled petiole and large orange yellow flowers with a well developed, cup-shaped, fleshy hypanthium (Fig. 3) represent the main features of *H. ovalifolia,* and acute leaf base, unchanneled petiole, medium sized greenish yellow flowers with small hypanthium characterized *H. floribunda* (Fig. 4). Serrate margins were observed only in *H. floribunda* but previous studies record the presence of serrate margins in *H. angustifolia* as well (Ratnayake, per. com.). However, *H. ovalifolia* does not posses leaves with serrate margins.

The difference in the morphological data is strongly supported by results of both cluster and phylogenetic analyses. During the cluster analysis divisions at species level are very low and the individuals of the three species separate at an acceptable distance into three separate clusters. Similarly, during the phylogenetic analysis the genus is recovered as monophyletic with no support. The individuals of the three taxa appear in three separate clades with good support (100% bootstrap) on the strict consensus tree.

Considering the phylogenetic relationships, *H. ovalifolia* and *H. floribunda* are more closely related to each other than to *H. angustifolia. H. angustifolia* is sister to the other two taxa. The clade bearing *H. ovalifolia* and *H. floribunda* is well supported by 100% bootstrapping. The reason for not

recovering the genus as a monophyletic group may be due to the fact that the data was always coded with aim of looking for differences helping in recognizing the three species rather than for similarities.

Even though the chemical studies suggest that the three species are closely similar, because the three species of *Hortonia* (*H. angustifolia, H. ovalifolia* and *H. floribunda*) show the same number of spots with identical R_f values on TLC plates and contain identical bioactive compounds and protein bands, the present study strongly supports the recognition of three distinct species of *Hortonia* and identifies several additional stable morphological features as diagnostic features of the three species. The reason why the three species of *Hortonia* possess identical biologically active compounds could be that they have been retaining the primitive chemical composition of their ancestor while evolving as three different species with distinct morphological features.

A modification of the present key to the species published in the Revised Handbook to the Flora of Ceylon was constructed with additional characters. New features are underlined (Fig. 5). Regarding the characters used in the key (Dassanayake, 1996), the leaf shapes could be considered as a more or less stable feature for the three species. 95% of the leaves of a given tree fitted well into the described leaf shape (Fig. 3).

The set of morphological data coded in the present study could be supplemented with other data sets such as chemical and molecular biological data in order to gain more support for the species limits and to test the monophyly of the genus.

Finally the present study confirms the presence of three species of *Hortonia*: *H. floribunda, H. angustifolia* and *H. ovalifolia* in Sri Lanka. This corroborates with the recent revision of the genus.

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REFERENCES

Angiosperm Phylogeny Group.(APG) (1998). An ordinal classification of the families of flowering plants. *Annals of Missouri Botanical Gardens* **85**: 531-553.

Baum, D.A. and Donoghue, M.J. (1995). Choosing among alternative "phylogenetic" species concepts. *Systematic Botany* **20**(4): 560-573.

Chappill, J.A. (1989). Quantitative characters in phylogenetic analysis. *Cladistics* **5**: 217-234.

Dassanayake, M. D. (1996). Monimiaceae. In *A Revised Hand book to the Flora of Ceylon*, Vol. **X.**, M.D.Dassanayake and W. D. Clayton, (eds.), Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi. Pp. 284 -284.

Davis, J.I. and D.H. Goldman (1993). Isozyme variation and species delimitation among diploid populations of the *Puccinellia nuttalliana* complex (Poaceae): Character fixation and the discovery of phylogenetic species. *Taxon* **42**: 585-599.

Dilcher, D.L. (1974). Approaches to the identification of angiosperm leaf remains. *The Botanical Review*. Department of Plant Sciences, Indiana University, Bloomington, Indiana **40**: 1-84.

Dissanayake, D.M.D. (1999). Phylogenetic research of the family Chrysobalanaceae. Ph. D. Thesis. Centre of Plant Diversity and Systematics, Department of Botany, University of Reading, UK.

Engler, A. (1901). *Das Pflanzenriech, Monimiaceae. Leipzig, Verlag von Wihelm Engelmann.* Pp.14-16.

Fitch, W.M. (1971). Toward defining the course of evolution: minimum change for a specified tree topology. *Systematic Zoology* **20**: 406-16.

Hawkins, J.A., Hughes, C.E. and Scotland, R.W. (1997). Primary Homology Assessment, Characters and Character states. *Cladistics* **13**: 275-283.

Hooker, J.D. (1887). *Flora of British India.* Vol. V. L Reeve and Co., London. Pp. 115.

Hooker, J. D. and Thomson, T. (1855). *Flora Indica.* A Systematic Account of the Plants of British India. W. Pamplin, London. Pp. 166-167.

Kitching, I.J., Forey, P.L., Humphries, C.J. and Williams, D.M. (1998). *Cladistics*: *The theory and practice of parsimony analysis.* The Systematics Association. publication No.11. Oxford University Press.

Maddison , W.P. and Maddison. D.R. (2000). *MacClade 4: Analysis of Phylogeny and Character Evolution*, Sinauer Associates, Inc. Sunderland, Massachusetts, USA.

Money, L.L., Bailey, I.W. and Swamy, B.L.G. (1950). The morphology and relationships of the Monimiaceae. *Journal of the Arnold Arboretum* **31**: 372-404.

Perkins, J. and Gilg, E. (1901). *Monimiaceae.in Das Pflanzenreich.* A. Engler (ed), W. Engelmann, Leipzig. Pp. 1-122.

Philipson, W.R. (1993). *Monimiaceae. The families and Genera of Vascular Plants.* Vol. II. K.Kubitzki, J.G. Rohwer and V. Bittrich (eds.), Springer-Verlag, Berlin. Pp. 426-437.

Ratnayake, R., Karunaratne, V., Kumar, V., Bandara, B. M. R., Wijesundara, S., Dharmasena, S., Weerasooriya, A. and Dassanayake, M. D. (1998). A Phytochemical approach to the classification of genus *Hortonia* in Sri Lanka. *Proceedings of the 54th Annual Sessions, Sri Lanka Association for the Advancement of Science. Part I (Abstracts).*, December, 1998, Sri Jayawardenapura .Pp. 291-292.

Ratnayake, R., Karunaratne, V., Kumar, V. and Bandara, B. M. R. (1999). Two new oxygenated lactones from the genus *Hortonia. Proceedings of the 55th Annual Sessions, Sri Lanka Association for the Advancement of Science, Part I (Abstracts)*, 1999. p. 232.

Ratnayake, R., Karunaratne, V., Bandara, B.M.R. and Kumar, V. (2001). Two new lactones with mosquito larvicidal activity from three *Hortonia* species. *Proceedings of the 57th Annual Session, Sri Lanka Association for the Advancement of Science, Part I (Abstracts)*. December, 2001,Moratuwa. Pp. 376-377

Renner S.S. (1998). Phylogenetic affinities of Monimiaceae based on cpDNA gene and spacer sequences. *Perspectives in Plant Ecology, Evolution and Systematics* **1/1**: 61-77.

Stevens, P.F. (1991). Character states, Morphological variation, and phylogenetic analysis: A review. *Systematic Botany* **16** (3): 553-583.

Swofford D.L. (2000). *PAUP*: Phylogenetic analysis using parsimony (and other methods)* Version 4.0b10 for Macintosh. Computer Software, Sinauer Associates, Sunderland.

Thwaites, G.H.K. (1864). *Enumeratio Plantarum Zeylaniae: an Enumeration of Ceylon Plants.* Dalau & Co. London. Pp. 11-12.

Trimen, H. (1885). *Systematic Catalogue of the Flowering Plants and Ferns indigenous to or growing wild in Ceylon.* Government Printers, Colombo.

Trimen, H. (1895). *A Hand book to the Flora of Ceylon,* Vol.111. Dalau and Co., London Pp.436-437.

Wight, R. (1853). *Icones Plantarum Indiae Orientalis.* Madras. Vol. VI. Pl. 1997-1998.

Yakandawala, D.M.D. (2001). *Teaching material for Plant Taxnomy I – Basic Terminology.* Department of Botany, University of Peradeniya.