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# MULTIVARIATE MORPHOMETRIC ANALYSIS OF MANGOSTEEN (*GARCINIA MANGOSTANA* VAR. *MANGOSTANA*, CLUSIACEAE) AND ITS WILD RELATIVES

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Mangosteen (*Garcinia mangostana* var. *mangostana*) is a dioecious and agamospermous cultivated fruit tree. It has two recognised hypothetical wild ancestors, *Garcinia mangostana* var. *malaccensis* and *G. mangostana* var. *borneensis*, distributed in the lowland dipterocarp-dominated forests of Sumatra, the Malay Peninsula, and Borneo. The highly similar morphological characters between the cultivated and wild varieties have posed challenges in identification. Additionally, *Garcinia penangiana* is often mistaken for *G. mangostana* var. *malaccensis*, and *G. venulosa* is regarded as morphologically similar to *G. mangostana* var. *borneensis*. In the present study, we conducted morphometric analyses of *Garcinia mangostana* var. *mangostana*, *G. mangostana* var. *borneensis*, *G. mangostana* var. *malaccensis*, *G. penangiana* and *G. venulosa*. We assessed the efficacy of morphological characters in combination (vegetative–male flowers–female flowers) in distinguishing the taxa as recognised in the current taxonomy. In our morphometric analyses, we found that *Garcinia penangiana* and *G. venulosa* are well delimited and congruent with their current taxonomic designations. A combination of vegetative and male-flower characters provided the most definitive delimitation. We recovered the specific coherence of *Garcinia mangostana*, but the infraspecific delineations of *G. mangostana* var. *mangostana*, *G. mangostana* var. *borneensis* and *G. mangostana* var. *malaccensis* are not supported.

*Keywords*. Clustering analyses, cultivated tree, *Garcinia mangostana*, Southeast Asia, wild relatives.
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## Introduction

Clusiaceae is a pantropical family consisting of shrubs and trees represented in 27 genera and slightly more than 1000 species (Stevens, 2007). The genus *Garcinia* L. is pantropical and comprises close to 250 species (Stevens, 2001–). It is one of the most diverse tree genera in Asian tropical forests (Davies, 2005), and is taxonomically difficult (Sosef & Dauby, 2012). *Garcinia* is usually dioecious (Stevens, 2001–). Gynodioecy (Pangsuban *et al.*, 2007) and trioecy (Joseph & Murthy, 2015) are known in paleotropical species, and androdioecy

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(van den Berg, 1979), monoecy and andromonoecy (Leal *et al.*, 2013) in Neotropical species. However, the variants of breeding systems, especially described based on morphological observations of flowers, do not always reflect their true sexual functions. Some species are known to exhibit facultative apomixis and ploidy variants (Ha *et al.*, 1988; Soepadmo, 1989; Thomas, 1997).

*Garcinia mangostana* L. var. *mangostana*, a fruit-tree species producing mangosteen, is undoubtedly the most well-known taxon of the genus. Originating from the Malay Archipelago, its fruits have been available in markets for at least 600 years (Yao *et al.*, 2023). It has now been widely cultivated throughout the humid tropics (Murthy *et al.*, 2018) and is recognised as a major tropical fruit in the international market.

Because of the commercial importance of *Garcinia mangostana* var. *mangostana* as a widely cultivated tropical fruit tree, various recent studies have been carried out to investigate its genomes (Abu Bakar *et al.*, 2016; Wee *et al.*, 2022, 2023) and transcriptomes (Goh *et al.*, 2019; Matra *et al.*, 2019). Genetic sequences of *Garcinia mangostana* cultivars, namely manggis and mesta, have also been made available in the past two decades (Wee *et al.*, 2023). However, despite the economic importance of the species, taxonomic studies of the genus *Garcinia* have received relatively little attention. Although deep-morphology and breeding-system studies on cultivated mangosteen received attention very early on (Sprecher, 1919), studies focusing on morphological characters for taxonomic delimitation are limited (Kochummen & Whitmore, 1973; Nazre *et al.*, 2018).

*Garcinia mangostana* represents the type species of the genus. *Garcinia* sect. *Garcinia* was recently revised with 13 species being recognised (Nazre *et al.*, 2018). The morphological characters delineating the section are terminal inflorescences of simple cymes or solitary female flower in some species, male flowers with 4-lobed or 4-angled stamen bundles, and fruits with a smooth surface. The geographical distribution of sect. *Garcinia* spans across Eastern India, Bangladesh and Indochina, and throughout the Malesian region.

Nazre *et al.* (2018) recognised three varieties of *Garcinia mangostana*; *G. mangostana* var. *mangostana* represents the cultivated variety, whereas *G. mangostana* var. *borneensis* Nazre and *G. mangostana* var. *malaccensis* (Hook.f.) Nazre are wild varieties. Morphologically, the stamen bundles and the forms and surfaces of the persistent stigma plate on the fruit apex can be used to delimit the three varieties (Nazre *et al.*, 2018). A persistent smooth stigma-plate surface on the fruits is diagnostic for *Garcinia mangostana* var. *mangostana* and a corrugated plate for *G. mangostana* var. *malaccensis*, according to Nazre *et al.* (2018) and Whitmore (1973). However, Corner (1997) was sceptical of its efficacy as a diagnostic character. Hambali & Natawijaya (2016) observed that stigmas with smooth and corrugated surfaces could be found in both taxa, and that natural hybrids between male *Garcinia mangostana* var. *malaccensis* and female *G. mangostana* var. *mangostana* had been observed. It is not known how prevalent natural hybrids between *Garcinia mangostana* var. *mangostana* and *G. mangostana* var. *malaccensis* are, or how often they are represented in

herbarium collections. Therefore, there is a need to evaluate the morphological characters used in varietal delimitation of *Garcinia mangostana*.

There is clearly some confusion regarding species delimitation in the genus. *Garcinia venulosa* (Blanco) Choisy is recognised as morphologically similar to *G. mangostana* var. *borneensis* (Nazre *et al.*, 2018), and the fruit of *G. penangiana* Pierre is often confused with that of *G. malaccensis* var. *malaccensis* in herbaria. *Garcinia malaccensis*, now considered *G. mangostana* var. *malaccensis*, was entirely mistaken for *G. penangiana* by Kochummen (Kochummen, 1997), and partly so by Whitmore (1973), as inferred from information on determination slips by the authors on herbarium specimens.

Wild varieties of *Garcinia mangostana* and *G. penangiana* are sympatric in Sumatra, the Malay Peninsula and Borneo, whereas *G. venulosa* is confined to the Philippines (**Figure 1**). According to the Köppen–Geiger climate classification (Beck *et al.*, 2018), an equatorial climate prevails throughout Sumatra, the Malay Peninsula and Borneo, and a monsoon climate in the northwest of the Philippines, specifically northwest Luzon (part of the geographical distribution of *Garcinia venulosa*).

The dioecious nature of these taxa adds another layer of difficulty to their interpretation and delineation. *Garcinia mangostana* var. *mangostana*, a Linnean name most likely based on female material only (Linnaeus, 1753), contrasts with *G. penangiana* (Pierre, 1883), which was described based on male flower material only. By contrast, the protologues of *Garcinia mangostana* var. *malaccensis* (Anderson, 1874), *G. mangostana* var. *borneensis* (Nazre *et al.*, 2018) and *G. venulosa* (Blanco, 1837) include descriptions of both male and female flowers. Despite the acknowledged challenge in delimitating *Garcinia* taxa (Kochummen & Whitmore, 1973; Sosef & Dauby, 2012), no previous attempt has been made to address this problem using morphometric analyses.

Molecular phylogenetic approaches offer insights into relations among *Garcinia mangostana* varieties and between *G. mangostana* and other morphologically similar wild relatives, particularly *G. penangiana* (Yapwattanaphun *et al.*, 2004; Nazre, 2014). The results of a phylogenetic analysis based on ITS sequence data showed that *Garcinia mangostana* var. *malaccensis* forms a paraphyletic group with *G. mangostana* var. *mangostana* (Nazre, 2014). In the same study, *Garcinia mangostana* var. *borneensis* Nazre, represented by a single accession, was shown to be sister to the *mangostana–malaccensis* clade, and *G. penangiana* emerged as the sister group to the clade encompassing all *G. mangostana* varieties. *Garcinia venulosa* has not been included in any phylogenetic or morphological studies to date.

The aims of this morphometric study were twofold: (i) to assess the efficacy of morphological characters used to delineate the morphologically highly similar taxa in *Garcinia*, and (ii) to determine the character combinations that best represent the resulting groupings and that align with the latest taxonomic delimitations. For this purpose, our sampling focused on the taxa mentioned in the above paragraph, known to be closely related to *Garcinia mangostana* var. *mangostana*, both genetically and morphologically.

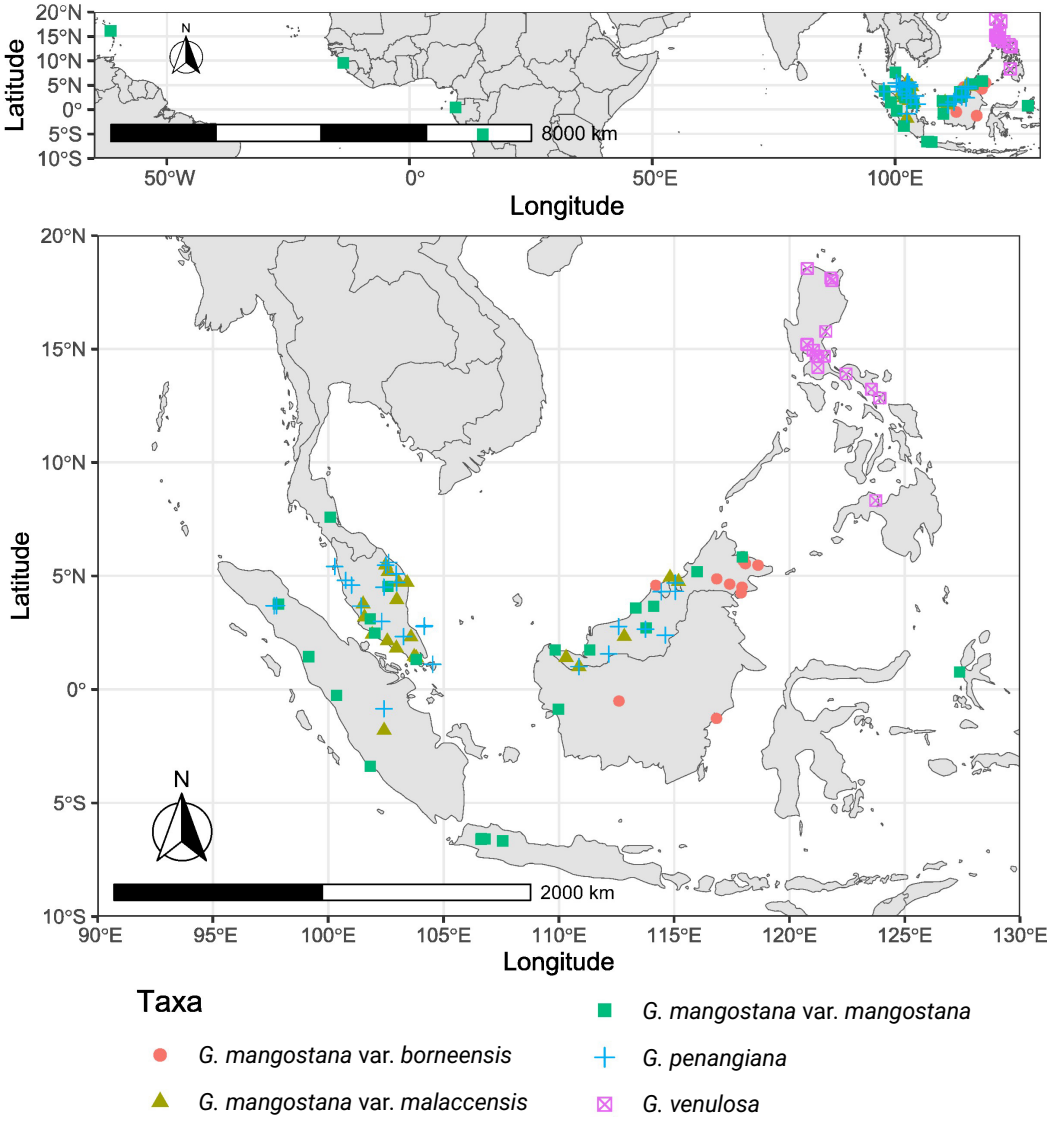


**Figure 1.** The geographical distribution of the *Garcinia* specimens examined and analysed.

## Materials and methods

### *Specimens and taxa included*

A total of 124 specimens representing *Garcinia mangostana* var. *mangostana* (*n* = 35), *G. mangostana* var. *borneensis* (*n* = 16), *G. mangostana* var. *malaccensis* (*n* = 23), *G. penangiana* (*n* = 30) and *G. venulosa* (*n* = 20) were included in the morphometric analyses.

The specimens examined and analysed included holdings in herbaria BO, K, KEP, KLU, L, MDI, MPU, P, SAN, SAR, SING, U, US and WAN (herbarium codes follow Thiers, continuously updated). To facilitate taxon identification, we used the identification key and description of Nazre *et al.* (2018), the identification lists in Nazre (2006), Nazre *et al.* (2018) and Nazre's specimen annotations, if available. Notably, these specimens collectively represent the entire geographical distribution of wild taxa (see **Figure 1**).

Character scoring was conducted using a specimen-based approach. Gatherings of the same collection were treated as duplicates. The full listing of the specimens is provided in Supplementary file 1. Assessments and measurements were made across duplicates, whenever they complemented missing or incomplete organs.

A collection of *Garcinia mangostana* var. *malaccensis*, *Maingay* 149, at K consisted of male flowers (accession no. 1643A, barcode K000380446), and at L, of female flowers and young fruit (QR code L.2416659). At K, Maingay's field numbers were partially replaced by herbarium accession numbers (Steenis-Kruseman, 1950). The replacement occurred before duplicates were distributed to other herbaria and although we were not able to confirm the source(s) of the multiple gatherings, we consider them to have originated from at least two different individuals. This distinction is based on our knowledge that the species is reported as dioecious, and we consistently observe male or female flowers on separate specimens. Consequently, these specimens are treated as two distinct collections.

Another notable collection is *Daud & Tachun* SFN36093 (KEP [barcode KEP239972], L [QR code L.2416581]), identified as a male-flowered representative of *Garcinia mangostana* var. *mangostana*. The label states "40' (feet) tall, flrs. yellow, fruit red"; however, no fruit material was found, only male flowers. Consequently, we have treated these specimens as duplicates of a single collection. For female material, we chose fruit rather than female flowers for analysis for two pragmatic reasons. First, petals of the examined taxa are caducous, and specimens with complete petals are rare. Second, the stigma plate, which persists in fruit, provides many key characters (Nazre *et al.*, 2018), is more developed, and can be better examined and coded in fruiting materials. Male material is represented by specimens bearing male flowers. In all the herbaria visited, only three male specimens of *Garcinia mangostana* var. *mangostana* were found. Details regarding the number of specimens analysed, along with information on taxa and specimen subsets, are summarised in **Table 1**.

### *Character and character state selection*

Because the examined taxa are genetically closely related and highly similar morphologically, selection of additional characters supplementary to key characters is challenging. A total of 38 characters are included in the present study (**Table 2**). The morphometric datasets assembled included qualitative and quantitative data and were organised in DELTA Editor (Dallwitz, 1980; Dallwitz *et al.*, 1999–). Beentje (2010) was followed for characters and character state descriptive terms.

**Table 1.** Number of *Garcinia* specimens analysed, with details of taxa and specimen subsets

| Taxon | Specimen subset[a] | | |
|---|---|---|---|
| | VG | FR | MF |
| *G. mangostana* var. *mangostana* | 35 | 30 | 3 |
| *G. mangostana* var. *borneensis* | 16 | 11 | 5 |
| *G. mangostana* var. *malaccensis* | 23 | 11 | 11 |
| *G. penangiana* | 30 | 15 | 13 |
| *G. venulosa* | 20 | 12 | 6 |
| Total | 124 | 79 | 38 |

[a] Specimen subset: VG, vegetative; FR, vegetative and fruit; MF, vegetative and male flower.

For qualitative data, 'character states' were predominantly treated as 'conventional multistate factorial data', and 'unconventional coding methods', as defined by Hawkins (2000), included (i) composite coding, (ii) logically related coding, (iii) positional coding, and (iv) mixed coding. Because 13 of the 38 characters had three or more character states, multistate coding was preferred over absent-or-present coding, to avoid unnecessary expansion of the dataset. Quantitative data, namely measurements and counts, were treated as numerical and integer data types, respectively. ImageJ (Schneider *et al.*, 2012) was used for measurements of secondary vein angles and for the counting of number of secondary veins forming loops. Each mean value in the datasets represents the mean calculated from three measurements.

All characters (18) used in the identification keys of Nazre *et al.* (2018) for the selected taxa were included (here termed 'key characters'). Additionally, 20 'additional characters' were specifically identified and included in the present study.

The selection of appropriate diagnostic characters for use in delineating taxa is fundamental to taxonomy (Borkent, 2021), and we approached this task with an open perspective. Additional characters incorporated in this study were not previously employed as diagnostic characters. The rationale behind selecting these additional characters was twofold. First, it was observed that some of these characters were potentially informative in morphometric analyses during the early stages of specimen examination and assessment. The nine additional characters included on this basis were characters 5, 12, 15, 16, 22, 23 and 35–37 (see **Table 2**). Second, an additional 11 characters (characters 3, 4, 6–11, 24, 27, 28; see **Table 2**) were included to supplement the key characters and to assess whether they enhance taxa delineation. There was no *a priori* expectation regarding how these characters would affect the results.

Most characters and their character states are self-explanatory; however, some require further explanation. Characters 8, 9, 16 and 28 (see **Table 2**) represent the ratios of two ratios (i.e. means) instead of direct measurements. The 'density of secondary vein pairs forming loops at intramarginal vein' was calculated by dividing the 'mean count

**Table 2.** Characters and character states examined and analysed[a]

| No. | Character (unit) | Character states (no. of states) | Organ[b] | Data type[c] | Cluster[d] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | VG-key | VG-add | MF-key | MF-add | FR-key | FR-add |
| 1 | **Twig form** | slender/stout (2) | TW | F | • | • | • | • | • | • |
| 2 | **Petiole base 'ligule-like' appendage conspicuity** | conspicuous/inconspicuous (2) | LF | F | • | • | • | • | • | • |
| 3 | Petiole length (cm), mean | | LF | N | | • | | • | | • |
| 4 | Lamina shape | ovate/elliptic/narrowly elliptic/elliptic-oblong/elliptic-ovate/broadly elliptic/obovate (7) | LF | F | | • | | • | | • |
| 5 | Lamina texture | chartaceous/coriaceous (2) | LF | F | | • | | • | | • |
| 6 | Lamina length (cm), mean | | LF | N | | • | | • | | • |
| 7 | Lamina width (cm), mean | | LF | N | | • | | • | | • |
| 8 | Lamina length-to-width ratio, mean | | LF | N | | • | | • | | • |
| 9 | Lamina-to-petiole length ratio, mean | | LF | N | | • | | • | | • |
| 10 | Lamina base | cuneate/acute/obtuse (1) | LF | F | | • | | • | | • |
| 11 | Lamina apex shape | caudate/acuminate/acute/obtuse (4) | LF | F | | • | | • | | • |
| 12 | Lamina apex form | drip tip bend downwards/flat (2) | LF | F | | • | | • | | • |
| 13 | **Midrib upper surface cross-section shape (observed at the middle length)** | keel shape/convex/square (3) | LF | F | • | • | • | • | • | • |
| 14 | **Secondary veins general form** | brochidodromous (looping near margin) (1) | LF | F | • | • | • | • | • | • |
| 15 | Secondary veins angle (°) (angle between secondary veins and midrib), mean | | LF | N | | • | | • | | • |
| 16 | Density of secondary vein pairs forming loops at intramarginal vein | | LF | N | | • | | • | | • |
| 17 | **Intramarginal veins observed on lamina lower surface** | Single/predominantly single, in some double circa mid-length of the lamina/double (3) | LF | F | • | • | • | • | • | • |
| 18 | **Glandular line form** | predominantly long wavy lines, occasionally with shorter ones/a mix of long wavy lines and short lines (2) | LF | F | • | • | • | • | • | • |
| 19 | **Glandular line conspicuity** | weakly to moderately raised on lamina lower surface/strongly raised on lamina lower surface (2) | LF | F | • | • | • | • | • | • |
| 20 | **Glandular line colour** | greenish or brownish grey/dark grey or black (2) | LF | F | • | • | • | • | • | • |
| 21 | **Glandular line orientation** (angle between glandular line and midrib) | 10–55° running from midrib towards margin/almost parallel (180°) to the midrib and margin (2) | LF | F | • | • | • | • | • | • |
| 22 | Male flower count, maximum | | MF | I | | | | • | | |
| 23 | Male flower pedicel form | slender/stout (2) | MF | F | | | | • | | |
| 24 | Male flower petal shape | ovate/oblong-obovate/oblong/obovate (4) | MF | F | | | | • | | |

Table 2 (*continued*).

| No. | Character (unit) | Character states (no. of states) | Organ[b] | Data type[c] | Cluster[d] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | VG-key | VG-add | MF-key | MF-add | FR-key | FR-add |
| 25 | **Stamens bundle shape** | in a single mass, lateral view obovoid in outline, top view circular, top convex/in a single mass, lateral view obovoid in outline, top view circular, top flat/in a single mass, lateral view broadly ovate in outline, top view circular, top pointed/in a single mass, lateral view broadly ovate in outline, top view subtly four-angled, top pointed/in a single mass, lateral view cylindrical in outline, top view circular, top pointed/in a single mass, lateral view cylindrical in outline, top view subtly four-angled, top pointed (6) | MF | F | | | • | • | | |
| 26 | **Stamen bundle length (mm)** | | MF | N | | | • | • | | |
| 27 | Stamen bundle width | | MF | N | | | | • | | |
| 28 | Stamen bundle length-to-width ratio | | MF | N | | | | • | | |
| 29 | **Pistillode presence** | present/absent | MF | F | | | • | • | | |
| 30 | **Pistillode form** | fungiliform, with gap/capitate-clavate, no gap/convex, no gap (3) | MF | F | | | • | • | | |
| 31 | **Pistillode length (mm)** | | MF | N | | | • | • | | |
| 32 | **Fruit shape** | globose/lopsidedly globose/globbose with a distinct stipe/ovoid/ellipsoid/oblate (6) | FR | F | | | | | • | • |
| 33 | **Persistent stigma attachment** | on a stipe/on attenuated apex/on truncate/in depressed apex (4) | FR | F | | | | | • | • |
| 34 | **Persistent stigma form** | a disc-shaped protuberance/2–4 clusters (adjoined or separated) of tubercles/a polygonal protuberance (3) | FR | F | | | | | • | • |
| 35 | Persistent stigma lobes/clusters count, maximum | | FR | I | | | | | | • |
| 36 | Persistent stigma plate best-fit outline | circular/oval/square or rhomboid/triangle (4) | FR | F | | | | | | • |
| 37 | Persistent stigma lobes or clusters dissection | in clusters, adjoined or separated/dissected more than half of the stigma plate radius/dissected less than half of the stigma plate radius (3) | FR | F | | | | | | • |
| 38 | **Persistent stigma surface** | papillate/rugose/smooth (3) | FR | F | | | | | • | • |
| | | No. of characters included | | | 9 | 21 | 14 | 31 | 13 | 28 |

•, character included in the character subset.
[a] Characters in bold are 'key characters' used by Nazre *et al.* (2018); characters not in bold are additional characters used in the present study.
[b] Organ: FR, fruit; LF, leaf; MF, male flower; TW, twig.
[c] All qualitative characters are treated as factorial (F) data, quantitative characters include numerical (N) and integer (I) data.
[d] Character subsets: VG-key, vegetative key characters; VG-add, vegetative additional characters; MF-key, vegetative and male flower characters; MF-add, vegetative and male flower additional characters; FR-key, vegetative and fruit characters; FR-add, vegetative and fruit additional characters.

of secondary vein pairs that form loops at intramarginal vein' by 'mean lamina length'. This approach was adopted to mitigate bias caused by plasticity in leaf sizes (Chitwood *et al.*, 2021). Measurements of secondary vein angle and glandular line angle were taken between the midrib and the secondary vein and between the midrib and the glandular line, respectively. Although three measurements were taken for the secondary veins of each specimen, measurements of the glandular line angle were coded as 'glandular line orientation' (character 21) and treated as factorial data. Because specimens with fruits at various maturity stages were included to obtain a larger sample size for statistical analyses, characters directly linked to maturity, such as fruit length and width, were deliberately avoided.

In our analyses, the dataset was organised into six subsets (see **Table 2**) based on criteria pertaining to specimens (samples) and characters (variables). All 124 specimens were designated as 'vegetative' (VG) because they included only vegetative characters. Owing to the dioecious nature of the taxa examined, specimens were divided into specimen subsets based on the reproductive organs. The female specimen subset, termed 'vegetative and fruit' (FR), comprised 73 specimens, and the male specimen subset, 'vegetative and male flower' (MF), comprised 38 specimens. Both the FR and the MF subsets include vegetative characters, plus fruit materials and male flower materials, respectively. Each of the three specimen subsets were assigned two character subsets: 'key characters' (key) only and 'additional characters' (add). Thus, the six subsets are denoted as follows: 'VG-key', 'VG-add', 'FR-key', 'FR-add', 'MF-key' and 'MF-add'. 'Key characters' character subsets consisted of key characters only, whereas 'additional characters' included both key and additional characters.

### *Algorithms in principal coordinates analysis, ascendent hierarchical classification and CH index*

Principal coordinates analysis (PCoA) and ascendent hierarchical classification (AHC) were employed to evaluate the morphological characters used in species delimitation; these methodologies have demonstrated success in prior studies (Pierre *et al.*, 2014; Morel *et al.*, 2021). Analyses were performed using R version 4.1.1 (R Core Team, 2021) on RStudio (Posit team, 2022). Three R packages were utilised, namely 'ade4' (Dray & Dufour, 2007), 'vegan' (Oksanen *et al.*, 2022), and 'cluster' (Maechler *et al.*, 2022). R functions were used to enhance the dendrogram plot, namely 'fColorLeaf.R', 'fLabelNoeud.R' and 'EnvelopingEllipse.R' (Le Moguédec, 2020). The morphometric datasets were analysed using morphological characters *per se*, without considering the specimen's taxonomic identification. Thus, no *a priori* assumptions were made regarding the formation of clusters, thereby ensuring an unbiased approach.

The algorithms employed in the analyses were modelled after Morel *et al.* (2021) and Pierre *et al.* (2014). Given that the dataset comprised both quantitative and qualitative

variables, Gower's (1971) coefficient of dissimilarity was applied due to its ability to analyse heterogenous variables simultaneously. Subsequently, the dissimilarity matrix was converted into Euclidean distance through square-root transformation.

A PCoA was conducted to obtain an overview of the grouping. In the PCoA, axes are ranked in descending order based on their total inertia, calculated as eigenvalues. The cumulative total of the six axes with highest eigenvalues was then calculated. The results of PCoA are presented in scatter plots, using the two axes with the highest eigenvalues.

An AHC was then applied for clustering analysis. The same transformed dissimilarity matrix for PCoA was employed in AHC. To construct the dendrogram, the aggregation criterion Ward distance (Ward, 1963) was utilised for clustering. The best partition on the datasets was assessed using Caliński–Harabasz (CH) index (Caliński & Harabasz, 1974), based on the same distance matrix used to construct AHC dendrograms *a posteriori*. Between 2 and 10 partitions were tested for each data subset. The CH index indicates the number of partitions that represents the optimal clusters for the examined dataset (Legendre & Legendre, 1998).

## Results

Our early observations during examination of the specimens for character assessments and measurements were that typical *Garcinia penangiana* has chartaceous lamina texture (character 5), a relatively wide angle between secondary veins and midrib (character 15), and a relatively high density of secondary vein pairs forming loops at the intramarginal vein (character 16). Typical *Garcinia mangostana* var. *mangostana* displays prominent lamina apex form (character 12) that bend downwards. In male flowers, only *Garcinia malaccensis* and *G. penangiana* have more than 5 flowers in a simple cyme (character 22), and the pedicels of *G. penangiana* are slender (character 23).

It is crucial to emphasise that the representations of clustering in each six data subsets correspond in their PCoA and AHC plots. This means that the identity of the datapoints and their taxon in PCoA plots can be cross-referenced to the specimens in AHC dendrograms. In the plots, three groups, denoted as clusters in PCoA and assemblages in AHC, were determined and labelled as A, B1 and B2. These labels are used consistently across **Figures** 2, 3, 4 and 5 as well as **Supplementary files** 2, 3 and 4, and the datapoints encircled in the dashed-line ellipses (**Figure** 2) correspond to the specimens at the ends of the nodes (**Figure** 3, 4 and 5; **Supplementary files** 2, 3 and 4) with the same labels; these ellipses and nodes are colour-coded. Individual samples are colour-coded according to their current taxon name. In CH tests, four out of six (67%) showed partitioning into three clusters best representing the respective datasets, whereas only one showed partitioning into 2 and 10 as the best scenario (Table 3).

**Table 3.** Caliński–Harabasz indices of six data subsets tested with 2–10 partitions[a]

| Cluster[b] | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| VG-key | 382.8 | 445.1 | 440.6 | 427.2 | 418.6 | 436.3 | 441.7 | 449.7 | **474.1** |
| VG-add | 209.0 | **209.9** | 173.5 | 155.0 | 132.9 | 118.6 | 109.3 | 103.2 | 97.3 |
| FR-key | **212.7** | 203.6 | 175.3 | 148.8 | 136.2 | 130.3 | 126.1 | 126.4 | 126.6 |
| FR-add | 121.9 | **150.0** | 125.8 | 104.6 | 90.7 | 80.6 | 72.8 | 67.3 | 62.8 |
| MF-key | 123.2 | **127.7** | 120.8 | 113.7 | 112.3 | 107.0 | 106.4 | 106.2 | 108.0 |
| MF-add | 66.5 | **79.2** | 63.4 | 54.8 | 49.3 | 45.6 | 42.8 | 40.9 | 39.5 |

[a] The highest index value for each subset is in bold font.
[b] Character subsets: VG-key, vegetative key characters; VG-add, vegetative additional characters; FR-key, vegetative and fruit characters; FR-add, vegetative and fruit additional characters; MF-key, vegetative and male flower characters; MF-add, vegetative and male flower additional characters.

## *Principal coordinates analysis*

Eigenvalues presented as a percentage of variance show that the distance matrix can be effectively summarised using the first six axes, accounting for 86.98–95.16% of the total inertia (**Table 4**). To illustrate the results of the PCoA, we plotted the first two axes (see **Figure 2**). Cluster A, which uniquely represents *Garcinia penangiana*, is clearly delineated from other clusters across all character subsets. Ellipses of clusters B1 and B2 overlap in **Figure 2A** and **B**. These two clusters include specimens of *Garcinia mangostana* varieties and *G. venulosa*. Three distinct clusters were observed in FR-add (see **Figure 2D**). However, cluster B2 in FR-add (see **Figure 2D**) comprises *Garcinia venulosa* and all *G. mangostana* varieties. By contrast, the clustering results of MF-add (see **Figure 2F**) best represent the current taxonomic delimitation of the five taxa included. It shows unique clusters for *Garcinia penangiana* (A) and *G. venulosa* (B1), whereas all three *G. mangostana* varieties uniquely grouped within B2.

**Table 4.** Summary of eigenvalues in percentage of variance calculated in principal coordinates analysis

| | Cluster[a] | | | | | |
|---|---|---|---|---|---|---|
| eigenvalue | VG-key | VG-add | FR-key | FR-add | MF-key | MF-add |
| Axis 1 | 79.07 | 73.13 | 78.05 | 70.23 | 79.39 | 70.79 |
| Axis 2 | 16.09 | 14.71 | 12.58 | 17.03 | 13.53 | 16.19 |
| Sum of axes 1 and 2 | **95.16** | **87.83** | **90.63** | **87.26** | **92.92** | **86.98** |
| Axis 3 | 3.08 | 3.60 | 3.22 | 2.50 | 3.33 | 4.11 |
| Axis 4 | 1.11 | 2.05 | 2.37 | 2.07 | 1.91 | 2.21 |
| Axis 5 | 0.35 | 1.59 | 1.13 | 1.54 | 0.61 | 1.87 |
| Axis 6 | 0.24 | 1.19 | 0.81 | 1.36 | 0.47 | 1.19 |
| Total | **99.94** | **96.26** | **98.16** | **94.73** | **99.24** | **96.36** |

[a] Character subsets: VG-key, vegetative key characters; VG-add, vegetative additional characters; FR-key, vegetative and fruit characters; FR-add, vegetative and fruit additional characters; MF-key, vegetative and male flower characters; MF-add, vegetative and male flower additional characters.

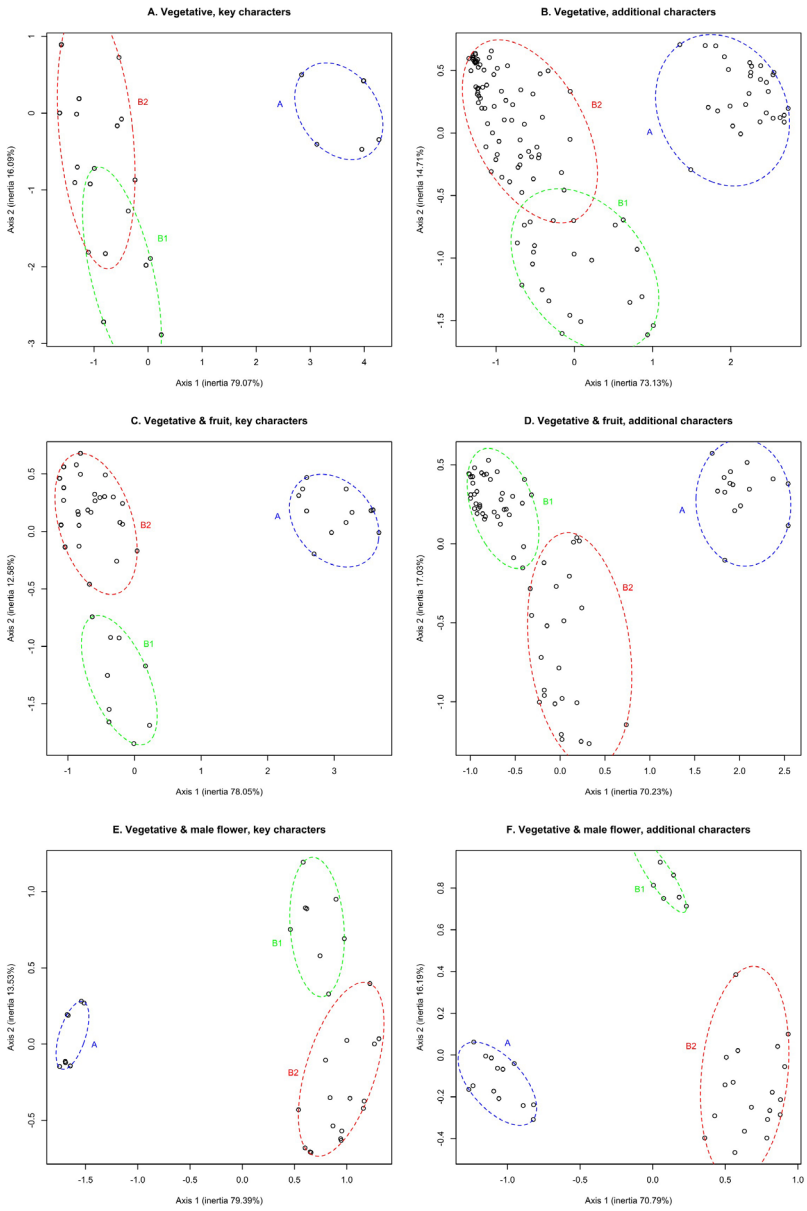


**Figure 2.** Scatter plots showing the results of principal coordinates analysis (PCoA) for six data subsets: A, vegetative, key characters; B, vegetative, additional characters; C, vegetative and fruit, key characters; D, vegetative and fruit, additional characters; E, vegetative and male flower, key characters; F, vegetative and male flower, additional characters. Each empty circle represents a single specimen. Data points in clusters A, B1 and B2 correspond to the specimens in the clades of the same name in **Figures** 3, 4 and 5 and **Supplementary files** 2, 3 and 4. Overlapping points in the PCoA plots are due to specimens sharing identical positions.

### *Ascendent hierarchical classification*

The dendrograms depict the results of the AHC based on analysis using the six data subsets: VG-key (Figure 3), VG-add (Supplementary file 2), FR-key (Figure 4), FR-add (Supplementary file 3), MF-key (Supplementary file 4) and MF-add (Figure 5). These dendrograms were generated utilising the same distant matrices as those plotted in the PCoA for each data subset, thereby providing additional details on the specimen and taxonomic identity. Compared with the circumscription in the PCoA, they provide more detailed resolution with which to determine recognisable unique taxonomic subassemblage(s) within assemblages A, B1 and B2. The undifferentiated specimens in AHC, best exemplified in Figure 3, assemblage A, in which 30 specimens ended in only five leaves, are represented by the five overlapping datapoints in the PCoA (see Figure 2**A**).

Assemblage A consistently contains only individuals of *Garcinia penangiana* across all datasets, in both the dendrograms and the PCoA results. In VG-key (see Figure 3) and VG-add (Supplementary file 2), assemblages B1 and B2 consist of individuals of three *Garcinia mangostana* varieties and *G. venulosa*, without forming any clear-cut group that corresponds to the current taxonomic delineation. Nevertheless, the introduction of additional characters improved the partition of taxa, as observed in assemblage B1, to which all *Garcinia venulosa* are confined (Supplementary file 2).

For FR-key (see Figure 4), assemblage B1 consists of *Garcinia venulosa* uniquely, and this distinction is supported by the PCoA results (see Figure 2**C**). However, the CH index results support the partition of two instead of three assemblages (Table 3). In FR-add, AHC recovered a subassemblage within assemblage B2 (Supplementary file 3), consisting of *Garcinia venulosa* uniquely. However, the CH index results more strongly support three assemblages instead of four. By contrast, in MF-add, a unique assemblage of *Garcinia venulosa* is observed as assemblage B1 (see Figure 5), and this assemblage is supported in the PCoA and CH index results.

Throughout the analyses of all six data subsets (see **Figures** 3, 4 and 5, and **Supplementary files** 2, 3 and 4), we did not recover any assemblage that clearly delineates the three varieties of *Garcinia mangostana*. Individuals of *Garcinia mangostana* var. *mangostana* and *G. mangostana* var. *malaccensis* are especially unresolved. In one exception, we recovered a subassemblage in MF-add within assemblage B2 (see Figure 5) that uniquely consists of *Garcinia mangostana* var. *borneensis* individuals, but this assemblage lacks support in the PCoA and CH index results.

Figure 6 shows the glandular lines on the lamina of *Garcinia mangostana* var. *mangostana*, *G. mangostana* var. *borneensis*, *G. mangostana* var. *malaccensis*, *G. penangiana* and *G. venulosa*. Figure 7 shows the persistent stigma plate surface of varieties of *Garcinia mangostana*.

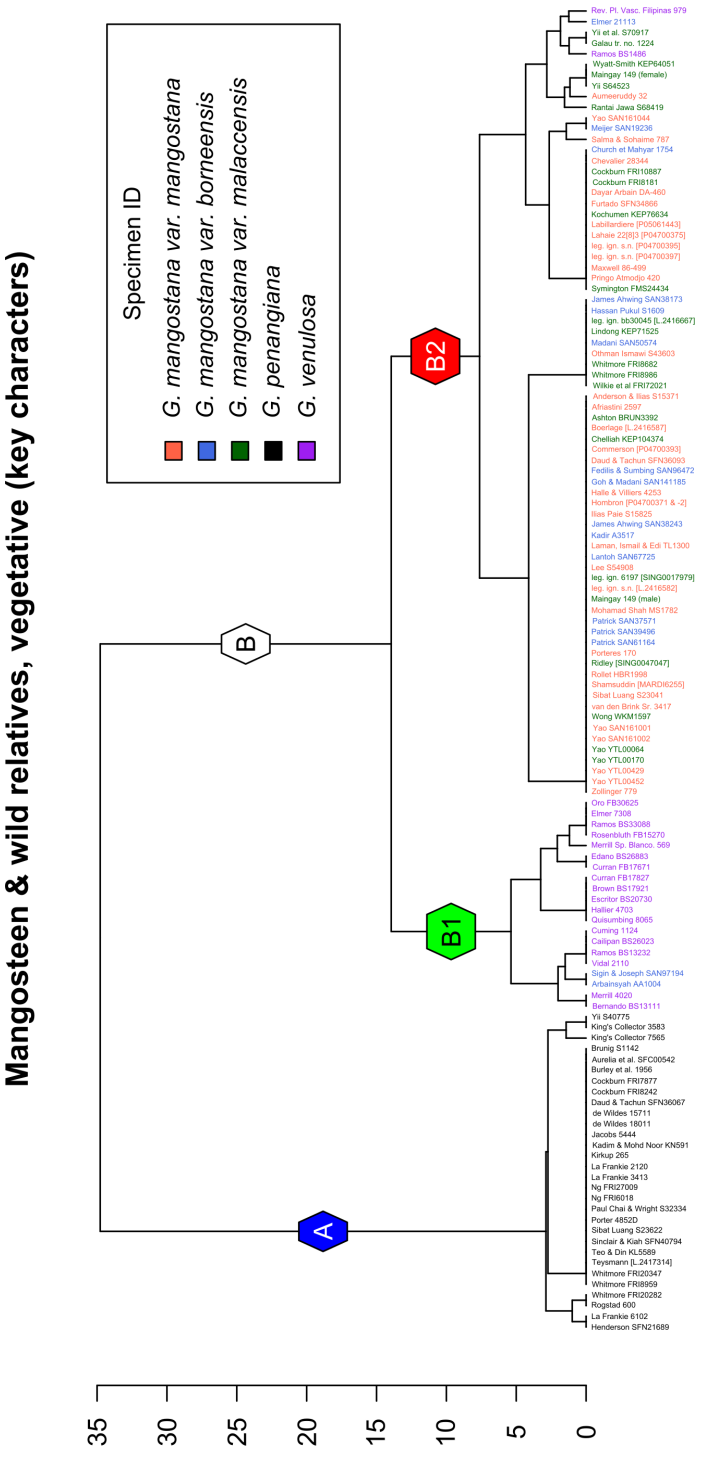


**Figure 3.** Ascendent hierarchical classification dendrograms for the vegetative key characters specimen subset.

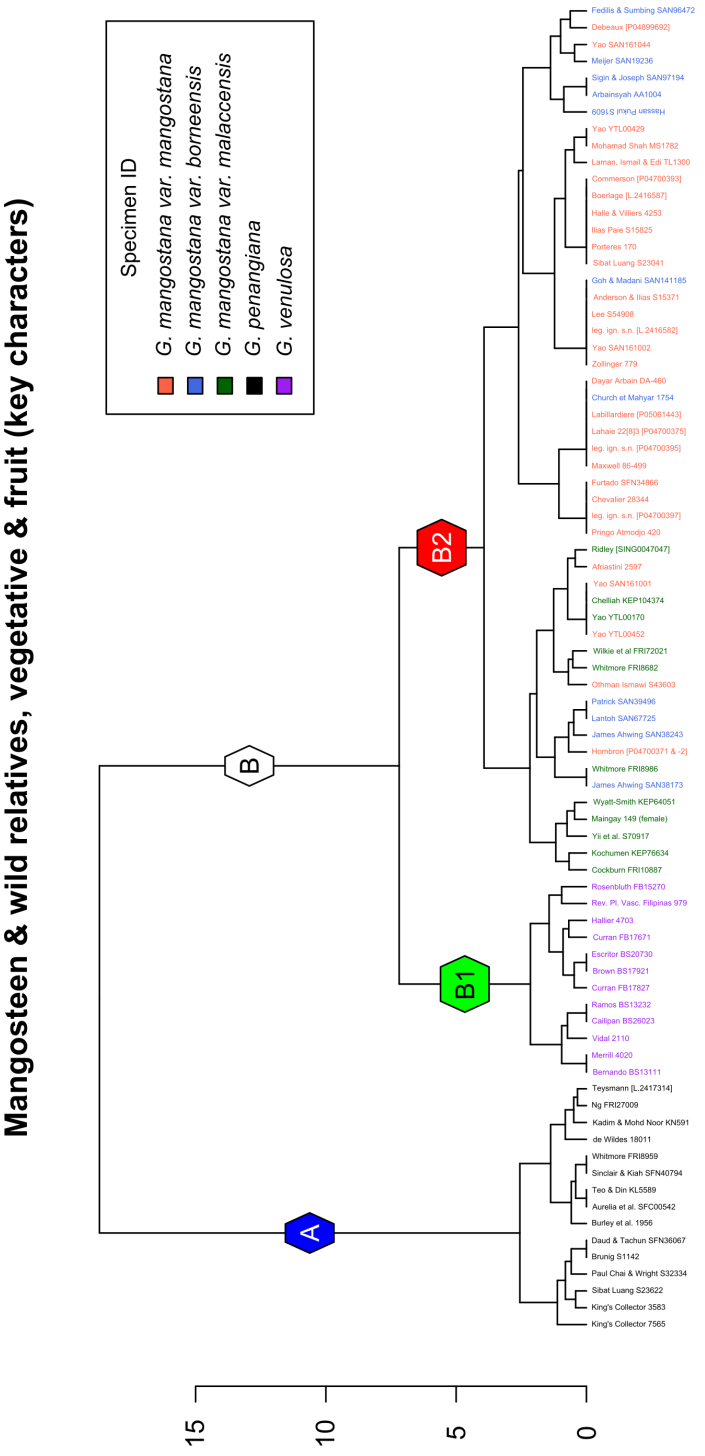


**Figure 4.** Ascendent hierarchical classification dendrograms for the vegetative and fruit key characters specimen subset.

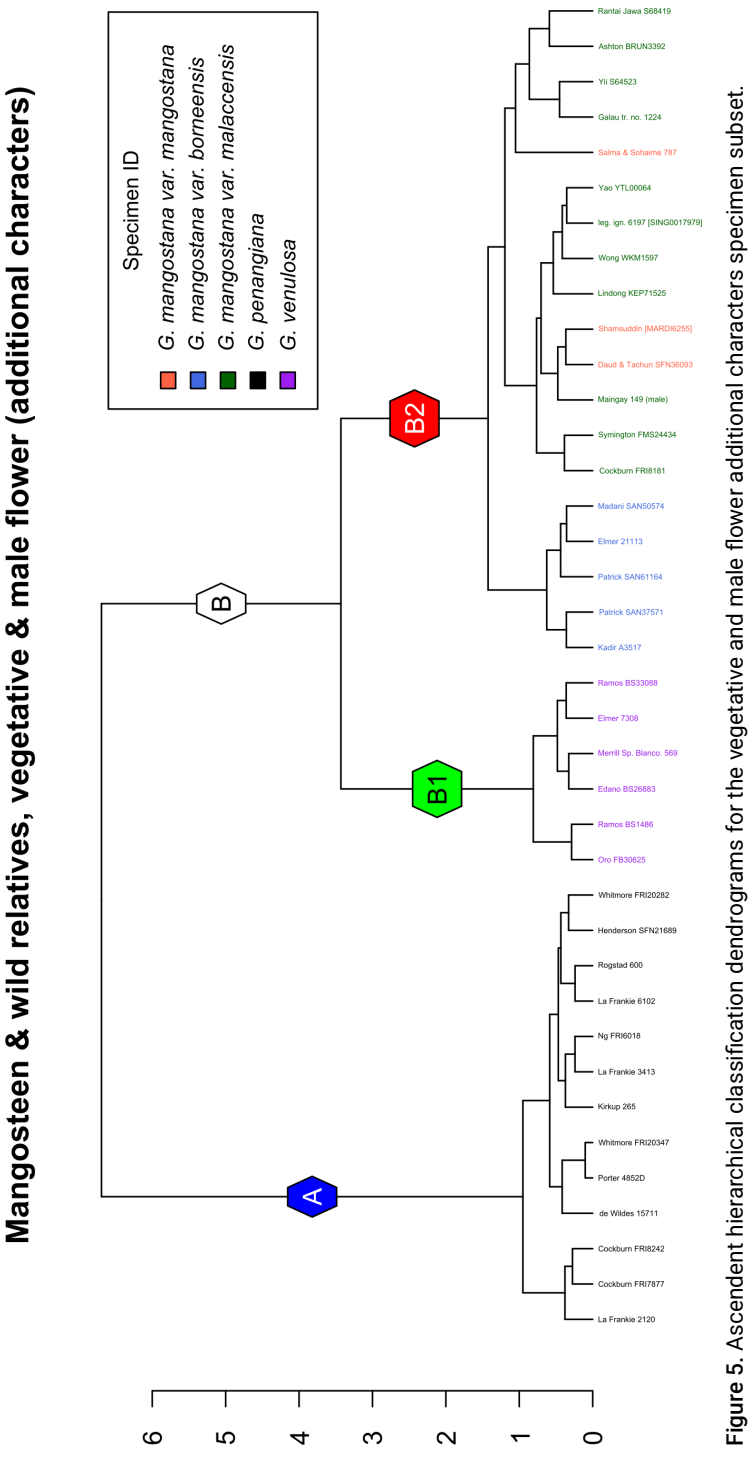


**Figure 5.** Ascendent hierarchical classification dendrograms for the vegetative and male flower additional characters specimen subset.

## Discussion

We assessed the efficacy of morphological characters for use in delineating the varieties of *Garcinia mangostana* and the closely related taxa *G. penangiana* and *G. venulosa*. Additionally, we investigated the character combinations that best represent the current taxonomic delimitation and explored the effect of including additional characters in these combinations. Table 5 summarizes the combined effect of additional characters in *Garcinia*, based on the results of PCoA, AHC and CH analyses.

**Table 5.** The combined effect of additional characters in the genus *Garcinia*, based on the results of principal coordinates analysis, ascendent hierarchical classification, and Caliński–Harabasz analyses

| Delineation | Vegetative | Fruit | Male flower |
|---|---|---|---|
| Species | Positive | Negative | Positive |
| *G. mangostana* varieties | Neutral | Neutral | Positive |

### *Efficacy of morphological characters in defining the closely related taxa*

Generally, vegetative characters alone are not effective for distinguishing the taxa investigated; the exception is *Garcinia penangiana*. Across all six data subsets analysed, *Garcinia penangiana* consistently formed a cluster clearly delineated from the other taxa. Our findings highlight that this species is a well-defined taxon, based on the key characters used in Nazre *et al.* (2018). On close scrutiny, we identified specific characters that delineate *Garcinia penangiana* from the other taxa. These diagnostic characters and character states include: (i) the presence of single intramarginal veins, as observed on the lower surface of the lamina; (ii) a dark grey or black glandular line; and (iii) a glandular line form consisting of a mix of long wavy lines and short lines.

The misapplication of *Garcinia malaccensis* (a synonym of *G. mangostana* var. *malaccensis*) to *G. penangiana* by Kochummen (1997) and Whitmore (1973) indicates that their taxonomic species concept of *G. malaccensis* was too broad. *Garcinia venulosa* is indistinguishable from *G. mangostana* var. *borneensis*, using solely vegetative characters. However, characters used in the male flower additional data subset can uniquely delineate *Garcinia venulosa*. We also observed a trend that when fruit characters datasets (both key and additional data subsets) are used, the taxon is distinguishable from *Garcinia mangostana* varieties. On examination of which individual characters delineate *Garcinia venulosa* from other taxa, we identified one character: glandular line orientation, which is almost parallel (180°) to the midrib and margin. By contrast, in other species, glandular line orientation ranges between 10 and 55°, running from the midrib towards the margin. In short, our results suggest that morphological characters could be used to satisfactorily delineate both *Garcinia penangiana* and *G. venulosa* from *G. mangostana*.

Individuals of *Garcinia mangostana* generally formed an inseparable cluster, with the exception of *G. mangostana* var. *borneensis*, which formed a unique taxon assemblage

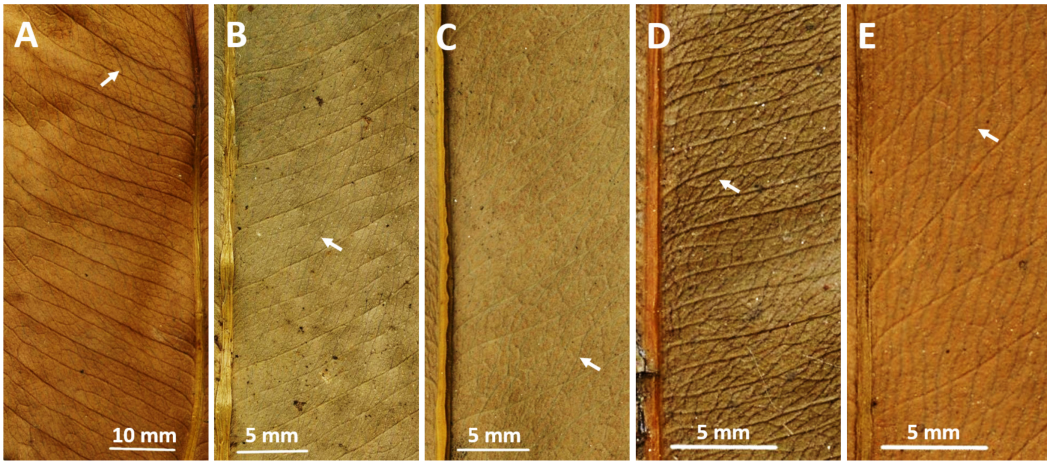


**Figure 6.** Glandular lines on the lamina: A, *Garcinia mangostana* var. *mangostana*; B, *G. mangostana* var. *borneensis*; C, *G. mangostana* var. *malaccensis*; D, *G. penangiana*; E, *G. venulosa*. All photographs: T. L. Yao.

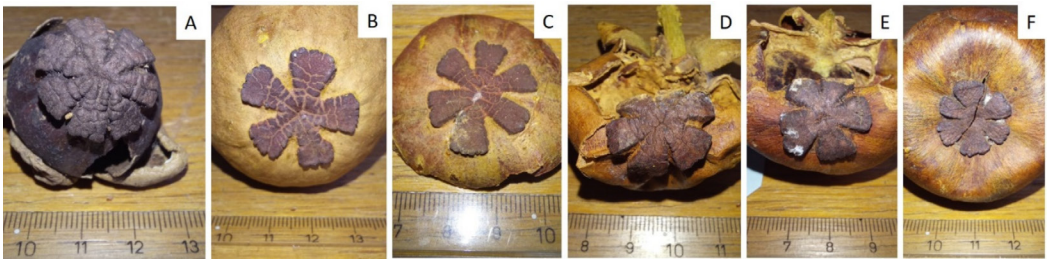


**Figure 7.** The persistent stigma plate surface of *Garcinia mangostana* varieties: A, YTL00170, var. *malaccensis*, Johor, Peninsular Malaysia; B, YTL00452, var. *mangostana*, Wanayasa, West Java; C, YTL00429, var. *mangostana*, Leuwiliang, West Java; D, *Yao* SAN161001, var. *mangostana*, Sabah, Malaysia; E, *Yao* SAN161002, var. *mangostana*, Sabah, Malaysia; F, *Yao* SAN161044, var. *mangostana*, Sabah, Malaysia. All deposited in MPU. Photographs: T. L. Yao.

based on the male flower additional characters data subset. Delimitation between *Garcinia mangostana* var. *mangostana* and *G. mangostana* var. *malaccensis* is not readily observed. The key character of the surface of the persistent stigma plate on fruit apex, 'smooth vs rugose', was used to delimitate the two varieties (Nazre *et al.*, 2018). However, we observed a continuum in this character state, leading to challenges in taxa delineation if using only vegetative and fruit characters. The stigma plate surface is especially variable in the mangosteen cultigens from Java. This observation is not novel; based on their examination of numerous samples since early 1996, Hambali & Natawijaya (2016) found the presence of smooth and corrugated surface stigma in both *Garcinia mangostana* var. *mangostana* and

*G. mangostana* var. *malaccensis*. These character states of the stigma plate surface are also observed for fresh fruits.

The morphometric analysis did not identify any character that would allow delineation of *Garcinia mangostana* varieties. We confirmed that the pistillode is always present in male flowers of *Garcinia mangostana* var. *mangostana* and always absent in *G. mangostana* var. *borneensis*, but both character states are applicable to *G. mangostana* var. *malaccensis* (Nazre *et al.*, 2018). Additionally, we did not find disjunctive measurements in stamen bundle length among the three varieties (Nazre *et al.*, 2018).

Findings from genetic studies appear to confirm our observations. The entire chloroplast genomes of *Garcinia mangostana* var. *mangostana* and *G. mangostana* var. *malaccensis* were found to be almost identical except for two indels and the presence of one single-nucleotide polymorphism (Wee *et al.*, 2023). The distance matrix of ITS sequences ($n$ = 18) of *Garcinia mangostana* var. *mangostana* ($n$ = 10) and *G. mangostana* var. *malaccensis* ($n$ = 8) ranges merely from 0.16% to 0.33% (Nazre, 2014). Crossing experiments between male *Garcinia mangostana* var. *malaccensis* and female *G. mangostana* var. *mangostana* (Hambali & Natawijaya, 2016) confirmed that gene flow between these two varieties can occur. Ploidy variants also exist in the taxa we studied: tetraploidy and aneuploidy in *Garcinia mangostana* var. *mangostana* (Matra *et al.*, 2016; Midin *et al.*, 2018) and diploidy in *G. mangostana* var. *malaccensis* (Hambali & Natawijaya, 2016; Midin *et al.*, 2018). The existence of these variants may influence morphological plasticity (Vichiato *et al.*, 2014). Considering the potential for interbreeding between these two varieties (Hambali & Natawijaya, 2016; Nazre, 2014), their failure to form distinguishably delineated assemblages congruent with the current taxonomic varieties in AHC analyses is not surprising.

Our findings support the inclusion of *Garcinia mangostana* var. *borneensis* and *G. mangostana* var. *malaccensis* within *G. mangostana*. However, the recognition of varietal rank is not supported, and construction of an identification key based on morphological characters is not achievable. Harlan & de Wet (1971) emphasised the difficulties in the circumscribing and naming of cultivated plants. They proposed the use of subspecies rank for cultivated and close wild relatives that form a ‘primary gene pool’. This idea may be applicable to well-studied crops and their wild relatives whose population genetics have been clarified, a situation not yet realised in mangosteen. In modern taxonomy, the rank of subspecies is more commonly used to delineate taxa with geographically disjunctive populations (Pipoly, 1987). *Garcinia mangostana* var. *borneensis* is confined to eastern Borneo whereas *G. mangostana* var. *malaccensis* is confined to Sumatra, the Malay Peninsula and western Borneo. However, there is no consensus among taxonomists on the differentiation between subspecies and varieties (Hamilton & Reichard, 1992). We have refrained from proposing taxonomic changes until deeper knowledge of the population genetics of both the cultivated and the wild compartments is available.

### *Assessment based on character-combination data subsets*

In the vegetative characters datasets, the improvement of clustering with the use of additional characters is exemplified by the results for the CH indices. The use of additional characters resulted in recognition of two clusters with the highest score, which could better explain the clustering of all *Garcinia penangiana* specimens in one assemblage and other taxa in another assemblage. This contrasts with recognising 10 clusters, or even more considering the increasing trend, if only key characters are used.

The male flower characters dataset best reflects the current taxonomic circumscription among taxa, and the use of additional characters improves the clustering topology. *Garcinia venulosa* is distinguished from *G. mangostana* varieties, and this is supported in the results for PCoA and CH index score. Additionally, we observed a unique taxonomic subassemblage formed by *Garcinia mangostana* var. *borneensis* within assemblage B2. The formation of a unique assemblage by a taxon among the *Garcinia mangostana* varieties is observed only in the male flower additional characters data subset. However, this subassemblage is not supported in the PCoA clustering or CH index score results.

The inclusion of additional characters in the fruit characters dataset has a negative effect on distinguishing *Garcinia venulosa* from *G. mangostana* varieties (**Supplementary file 3**), whereas key characters of fruit *per se* can be used to distinguish *G. penangiana* and *G. venulosa* from *G. mangostana* varieties. Clearly, there were mixed effects of the inclusion of additional characters. The additional characters recognised in this study, especially those with positive effect in delineating the taxa, should be considered in future taxonomic studies of *Garcinia*.

## Conclusions

The results of morphometric analyses showed that *Garcinia penangiana* is essentially delineated by vegetative characters, whereas *G. venulosa* is delineated from *G. mangostana* varieties by a combination of vegetative and male flower characters. Generally, all *Garcinia mangostana* varieties formed a single mixed assemblage. Our findings confirmed the coherence of *Garcinia mangostana* as a taxonomic species. However, our findings do not support the designation of infraspecific taxa, because we did not find apomorphies. This conclusion is consistent with Corner's (1997) opinion that varieties *mangostana* and *malaccensis* cannot be distinguished, although whether a varietal rank would appropriate to var. *malaccensis* was not discussed. Hambali & Natawijaya (2016) viewed var. *malaccensis* as a diploid form of the tetraploid var. *mangostana*, and they favoured the recognition of var. *malaccensis* at specific rank, a view that is not supported in our morphometric analyses. A deeper investigation of the delimitation of *Garcinia mangostana* varieties, using nuclear markers in a population genetics framework, would help clarify the taxonomic delimitation of these varieties.

We acknowledge the limitations of using morphometric analysis in studies of dioecious plants. The species concept in dioecious plants typically involves delineation utilising both female and male characters. However, using a specimen-based approach, we could not assess all the species-delineating characters in a single integrated dataset without the results being adversely affected by excessive missing data, as discussed by Pierre *et al.* (2014).

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## Supplementary material

Supplementary material is available from the *Edinburgh Journal of Botany* online portal.

**Supplementary file 1.** List of specimens included in the present study. In specimens without a collection number, barcodes or QR codes are provided.
**Supplementary file 2.** Ascendent hierarchical classification dendrograms of the vegetative additional characters specimen subset.
**Supplementary file 3.** Ascendent hierarchical classification dendrograms of the vegetative and fruit additional characters specimen subset.
**Supplementary file 4.** Ascendent hierarchical classification dendrograms of vegetative and male flower key characters specimen subset.

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