

AN EMPIRICAL STUDY ON THE TAXONOMY OF *CRINUM ZEYLANICUM* (L.) L. AND *CRINUM LATIFOLIUM* L. (AMARYLLIDACEAE) OCCURRING IN SRI LANKA

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ABSTRACT

Crinum latifolium L. and *C. zeylanicum* (L.) L. are two *Crinum* species native to Sri Lanka, but their species delimitation has been a point of debate since their establishment as separate species. During the recent revision of the Sri Lankan Amaryllidaceae, both species have been recognized. The separation of the two species is based on the leaf undulation and the size of the leaves. Field experiences suggest the occurrence of *Crinum* species with other distinct characters, raising the question of their species limits. Therefore, a detailed taxonomic study on species limits of *C. latifolium* and *C. zeylanicum* was carried out to solve the taxonomic ambiguity, based on empirical methods. Specimens were collected from all possible geographical locations. Morphological characteristics with distinct character states were studied at both macroscopic and microscopic level and coded into data matrices. Species limits were determined by phenetic and phylogenetic methods. The results clearly suggested the occurrence of two morphologically distinct groups supporting the recognition of *C. latifolium* L. and *C. zeylanicum* (L.) L. Furthermore, two morphologically distinct forms of *C. zeylanicum* were identified as occurring in Sri Lanka which had not been previously recorded. In view of the fact that the characters of these two types are stable and not dependent on the environment, formal taxonomic ranks could be offered.

INTRODUCTION

The genus *Crinum* is an 'eye-catching group' of tropical and subtropical Lilies distributed in almost every part of the world, mainly in Africa, Asia, Australia and America. Identification of *Crinum* is straightforward with its bulbous plants and lily-like flowers. The genus *Crinum* was first established by Linnaeus in 1737 (Hannibal and Williams, 1998). The name *Crinum* is derived from the Greek word 'Krinos', meaning trailing hair or comet-tail. It is said that one must witness a *Crinum* blossom with its long trailing petals to appreciate the significance of the name *Crinum* (Hannibal and Williams, 1998).

Though there are about 130 species of *Crinum* widely distributed in and around the tropical and sub-tropical regions of the world, only four are native to Sri Lanka; *Crinum asiaticum* L., *C. defixum* Ker-Gawl., *C. latifolium* L. and *C. zeylanicum* (L.) L. Local folks hardly distinguish *Crinum* species from other Lilies in other genera of the Amaryllidaceae, especially from *Pancratium* and *Hippeastrum* species. All of them are

known by same common name. In Sinhala, they are known as 'Goda Manel', 'Lunu Mal' or 'Tholabo', in Tamil, as 'Vichamunkil'. 'Sudharshana', 'Somawalli', 'Cakrangi' and 'Madhuparnika' are some of the Sanskrit names for *Crinum* species. Fig. 1 shows the different *Crinum* species and the other Amaryllid genera that occur in Sri Lanka.

Crinum species are widely used in indigenous medicine for many diseases; mainly leaf and bulb extracts are of medicinal importance. Leaf extract is used as a treatment for vomiting and for ear-aches. The bulbs are crushed and applied on to piles and abscesses to cause suppuration. In addition, the roasted bulbs are used as a rubefacient in rheumatism (Jayaweera, 1981).

Crinum plants are bulbous herbs with quite large globose to ovoid subterranean bulbs which often grow several inches in diameter with long necks up to 30 cm. Bulbs regularly propagate vegetatively and produce large clusters. Plants grow to a height of about 1m, depending on the species, and produce spirally arranged clumps of strap or

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linear shaped leaves and clusters of fragrant, red, pink, white or bicolor flowers borne terminally on an inflorescence axis about 1-1.5m long. Bulbs are often large, usually produced at the apex in to a longer or shorter neck. Leaves spirally arranged, sessile, linear, strap-shaped or lanceolate, with smooth or scabrous edges. Peduncle compressed, solid. Bracts 2, usually broad, and membranous. Bracteoles many, linear. Umbels with few or many large, subsessile or shortly pedicelled, white or reddish, regular to more less irregular, flowers. Perianth funnel or salver-shaped, with a long, straight or curved tube cylindrical or broadened at the apex, and linear, lanceolate or oblong, subequal, erect or spreading segments recurved at the apex and about as long as the tube. Stamens inserted at the throat of the perianth tube. Filaments long, free, filiform, spreading or declinate. Anthers medifixed, linear, often curved. Ovules usually few in each locule, biseriate, on thick, often projecting, axile placentae. Style long, filiform, more or less declinate. Stigma small, mostly capitate. Capsule subglobose or obovoid, with a membranous or leathery pericarp dehiscing irregularly. Seeds few, large, green, globose or irregularly compressed. Endosperm thick (Dassanayake, 2000).

The family Amaryllidaceae including the genus *Crinum*, evolved as semitropical marsh plants in East Africa (Hannibal and Williams, 1998). Some of the *Crinum* species moved from aquatic to savannah habitats and particularly to desert areas where unfavorable environmental stresses are frequent. These species exhibit various adaptations and modifications that evolved to conquer these environmental conditions successfully (Hannibal and Williams, 1998).

Taxonomic History of *Crinum*

Systematic revision of the Genus *Crinum* has been carried out from time to time by several scientists within the past few years in attempt to resolve the mix-ups in nomenclature that have occurred from the time of Linnaeus to the current decade. In the past, many *Crinum* species were described under other genera especially under *Amaryllis* and many species of other genera were described under *Crinum* (Hannibal and Williams, 1998). According to them, these problems were mostly due to inadequate research and due to misinterpretation or incorrect recognition of the material, as most of these characters of succulent

plants are lost during the preparation of herbarium specimens.

Linnaeus established the group *Crinum* in 1737 recognising four species, *Crinum latifolium*, *C. asiaticum*, *C. americanum* and *C. africanum* (Nordal, 1977). However, with time about 130 new species were added into the genus. Later on, *C. africanum* was found to belong to the genus *Agapanthus*. During 1781 when the younger Linnaeus was in England, he made a number of mistakes in the identification of *Crinum* species (Hannibal and Williams, 1998).

In 1806, Ker-Gawler, reviewing the available published plates and descriptions, lumped all the known broad-petalled forms in a group called *Amaryllis ornate* (*A ornate* K-G group). This name was also proposed by the junior Linnaeus in 1782 (Hannibal and Williams, 1998), as he thought the name *Amaryllis zeylanica* (*Zeylanica* = Ceylon) that was given by his father was too regional to recognise such a widely distributed species.

Between 1800 and 1830, a number of *Crinum* species were collected from India, West Africa, the Caribbean, mostly from the British colonies, initiating a competition in naming these newly collected species. This led to many misinterpretations such as the broad petalled *Crinum* species like *C. zeylanicum* being included in the genus *Amaryllis* (*Amaryllis zeylanica*). Even during these times, forms with narrow petals like *C. asiaticum* were readily recognized as *Crinum* (Hannibal and Williams, 1998).

In the next few years classification and nomenclature of *Crinum* species was even more confusing due to different ideas in grouping them, sometimes the same *Crinum* species identified as different species by different authors and were given different names. Further, separate *Crinum* species were recognized as a single one. Therefore, attempts at identification and classification of *Crinum* progressed with increase in numbers of synonyms.

Studies on *Crinum* by Salisbury (1805), Hooker (1892) and Herbert (1820) aided in solving this confusion to some extent when they agreed that several *Amaryllis* species were not *Amaryllis* but *Crinum* (Hannibal and Williams, 1998).

In 1837, Herbert extracted some *Crinum* species from Ker-Gawler's *A. ornate* K-G group and included them under subdivision *Ornatae* (1837) and further identified *C. zeylanicum* as *C. ornatum* var. *zeylanicum*. This was an attempt to form a general grouping based upon the blossom and foliage characters. Even though this was step towards uniformity, it was far from achieving recognition as later authors like J.G. Baker, J. D. Hooker and I. Nordal were not in complete agreement with this grouping (Hannibal and Williams, 1998).

In 1888, the genus *Crinum* was divided into three subdivisions or subgenera by Baker, improving Herbert's classification (Nordal, 1977). His grouping was based upon the floral characters; shape and arrangement of the petals, where actinomorphic flowers with linear petals were placed in the subgenus *Stenaster*. Subgenus *Platyaster* contained plants with actinomorphic flowers and lorate petals while forms with broad elliptical petals in curved trumpet-shaped blossoms having bilateral symmetry were included in the subgenus *Codonocrinum*. According to Baker (1888), broad-petalled species like *Crinum latifolium* L. and *C. zeylanicum* (L.), L. fall into the subgenus *Codonocrinum* (Nordal, 1977). Further, Baker was careful to retain several older classical names to avoid the confusion set by both Ker-Gawler and Herbert, in their baseless classification of *Amaryllis ornata* K-G and *Crinum ornatum* var. *zeylanicum* Herb. and therefore, resurrected *C. latifolium* and *C. zeylanicum* to their original Linnaean state (Hannibal and Williams, 1998).

In 1894, J. D. Hooker in his treatment recombined *C. latifolium* and *C. zeylanicum* as a single species, *C. latifolium*. Hooker working on Flora of British India (1892) stated that he was unable to find any character whereby *C. zeylanicum* and *C. latifolium* are to be distinguished from each other. Further, he states that Baker's treatment also does not give any characters for their separation. However, Trimen in his Handbook to the Flora of Ceylon (1898), treated *C. zeylanicum* as a variety of *C. latifolium* as he was incapable of finding sufficient characters to discriminate between them.

In 1977-82, Ingar Nordal and her co-workers carried out a study to re-identify the African segment of *Crinum* series *Ornatae*; i.e. eight *Crinum* species including *C. zeylanicum* (L.) L.

(Nordal *et al.*, 1977). In their study, two different forms of *C. zeylanicum* were noted which were distinguished mainly by growth of the leaves and chromosome numbers. One restricted to forest ecosystems had intact leaf apices, less than 13 flowers on the inflorescence with little undulation on the leaves and a chromosome number $2n = 30$. The other *C. zeylanicum* form known as savannah ecotype was found in savannah – like ecosystems with strongly undulate leaf margins, more than 10 flowers, without entire leaf apices and with chromosome number $2n = 22$. She treated the two forms as ecotypes (Nordal, 1977). *Crinum latifolium* was not considered in her revision due to the fact that there was only one specimen in the Linnaean herbarium under *C. latifolium* (LINN415.1), in a very poor condition, appearing to her to belong to the *Stenaster* group, more or less near *C. asiaticum*. Further, the reference that was cited by Linnaeus under *C. latifolium* was difficult to interpret, as there was no connection between the description and the specimen (Nordal, 1977).

Further Nordal (1977) also noted that there was no material named as *A. zeylanicum* in the Linnaean herbarium but cited three other references, Van Royen (1740 p.36), Commelin's *Lilio narcissus zeylanicum* (1697 p. 73, Tab. 73) and Ehret's *Lilio narcissus africanus* (1748 Table 5, Fig. 2). From these three illustrations, Ehret's *Lilio narcissus africanus* was chosen as lectotype for *C. zeylanicum* (Nordal, 1977). Nevertheless, this idea has been rejected by Dassanayake (1981), and Wijnand (1983), who pointed out that Jan Moninckx's 1690 illustration of *Lilio narcissus zeylanicum*, which was republished by the Commelins in 1697, was Linnaeus's original isotype (Hannibal and Williams, 1998). Thus, this evidence disqualifies Nordal's suggestion that Ehret's *Lilio narcissus africanus* was a valid lectotype for *A. zeylanica* L. of 1753 (Hannibal and Williams, 1998).

However, Dassanayake (2000) during his treatment of the Sri Lankan Amaryllidaceae and *C. zeylanicum* and *C. latifolium* in particular, has considered the two as distinct species, based on the leaf characters undulation of the leaf margin and size of the leaves. Further, there is no mention of the occurrence of savannah and forest ecotypes of Nordal among the Sri Lankan *Crinums*.

Based on the present circumscription and the field experience, the occurrence of different character combinations in *Crinum* has aroused an interest to investigate if savannah and forest ecotypes do occur in Sri Lanka. Thus once again it raises the question of the taxonomic delimitation of *C. zeylanicum* (L.) L. and *C. latifolium* L. in Sri Lanka. Therefore, the present study is aimed at elucidating this taxonomic ambiguity using a large number of morphological characters, based on empirical methods.

MATERIALS AND METHODS

MATERIALS

All recorded locations for the occurrence of *C. latifolium* and *C. zeylanicum* together with other possible locations were visited periodically in order to collect plants with vegetative and reproductive parts. Specimens were collected from Ampara, Anuradhapura, Kandy, Kurunegala and Polonnaruwa districts (Fig. 2). All the collected specimens were identified and tagged separately with a different acronym, Z1-Z15 and L1-L17. Living specimens were also collected for further microscopical studies and to prepare voucher specimens. Collected bulbs were cultivated at the University of Peradeniya. Living material has also been examined at the Royal Botanical Garden, Peradeniya and cultivated material in the Department of Botany, University of Peradeniya.

METHODS

Character coding: Data were obtained from randomly selected individuals from each population mainly focusing on individuals with blossoms. Both qualitative and quantitative characters were examined. Special attention was paid to characters with distinct variations and also other characters that had not been studied in detail before.

Data analysis: Both phenetic and phylogenetic approaches were adopted during species limit studies. During phenetic analysis, individuals are grouped according to total similarity while in cladistics monophyletic clades are recovered based on homologous characters and further if the trees are rooted the based on synapomorphies. Either of the methods of analysis has been adopted in various studies (Jacobseni, 1979; Khidir and Wright, 1982;

Khordhopani and Ingrouille, 1991; Baum and Donoghue, 1995; Somaratne and Herat, 2001, During the present study both methods have been utilised. More recently in addition to Cluster analysis, Principal component analysis (PCA) has also been used extensively for evaluating taxonomic data (Audrey *et al.*, 2005).

Phenetic Analyses: Characters and character states were coded into a data matrix. Multivariate methods of analysis, Cluster Analysis and Principal Component Analysis were carried out using the statistical packages MINITAB 13.2 and PC-ORD 4 version. Cluster analysis using MINITAB was performed under the Euclidian Distance Measures and Group Average Linkage methods options. Group Average Linkage methods and Euclidean (Pythagorean) distance measure options were employed under the PC-ORD 4. Resulting groups/clusters should indicate the phenetic similarity of the members in different populations and enable a user to identify different important characters in these phenetic groups.

Further, Principal Component Analysis (PCA) was conducted using PCORD to test if the *a priori* groups were recognizable and to evaluate the contribution of each character to the analysis (Poulsen and Nordal, 2005). Scatter plots and the first three eigen vectors were obtained.

Cladistic Analyses: Data were coded into a data matrix using the MacClade 3.04 (Maddison and Maddison, 1992) program. Phylogenetic trees were constructed using the PAUP* 4d55 for Macintosh program (Swofford, 1998). For all analyses heuristic searches were performed initially under the unordered and equal weighting criteria of Fitch parsimony (Fitch, 1971) with 500 replicates, random sequence additions, tree bisection-reconnection (TBR) branch swapping and MULPARS in effect, steepest descent on. Ten trees were held for each step. Strict consensus and 50% majority rule trees were obtained and branch lengths and tree scores were calculated using ACCTRAN (accelerated transformation optimisation). The initial trees found with equal (Fitch) weights were used as the basis for successive weighting.

Table 1. Qualitative and quantitative characters assessed for the morphometric analyses. The quantitative characters were measured in centimeters.

Character number	Description
Vegetative characters	
1.	Growth form / Habit of the plant
2.	Height of the plant
3.	Length of the pseudo-stem (neck)
4.	Colour of the leaves
5.	Leaf form
6.	Leaf development/ leaf apices
7.	Length of the leaves
8.	Keelation of leaves
9.	Nature of the midrib
10.	Woolly appearance
11.	Undulate leaf margin
12.	Amplitude of waves
13.	Subundulation
14.	Outgrowth frequency on leaf margin
Floral characters	
15.	Colour of the peduncle
16.	Flattening of the peduncle towards the apex
17.	Shape of the peduncle
18.	Peduncle length
19.	Number of bracts
20.	Colour of the bracts
21.	Texture of the bracts
22.	Persistence of bracts during anthesis
23.	Height of the umbel
24.	Number of flowers per inflorescence
25.	Colour of the bracteoles
26.	Colour of the pedicel
27.	Pedicel length
28.	Colour of the perianth tube
29.	Length of the perianth tube
30.	Shape of the buds
31.	Colour of the perianth segments
32.	Shape of the perianth segment
33.	Arrangement of perianth segments
34.	Colour of the style
35.	Colour of the stigma
36.	Shape of the stigma
37.	Colour of the filament
38.	Colour of the young anthers
39.	Fruit development



Figure 1. Native *Crinum* species of Sri Lanka, a – *C. asiaticum*, b - *C. defixum*, c and e - *C. latifolium*, and f - *C. zeylanicum* (Forest type).

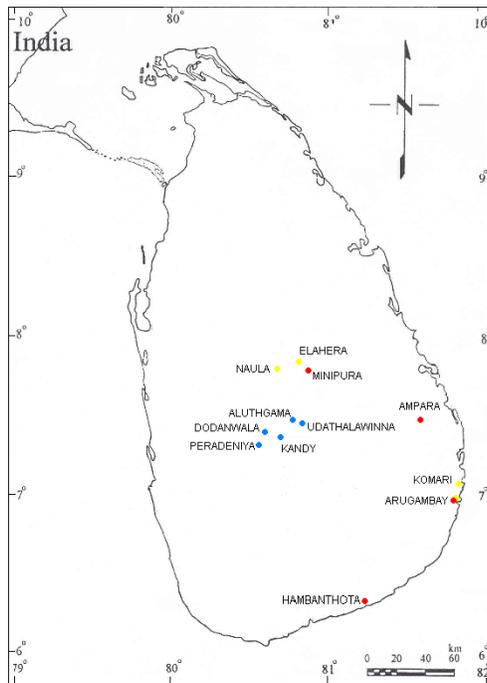


Figure 2. Distribution of the different populations of *Crinum* examined during the study.

Successive weighting was carried out using the Retention Index. Re-weighting was continued until the same length was obtained in two successive rounds. The tree was rooted under the mid point rooting option. Bootstrap analysis was carried out to evaluate the support for the groupings.

RESULTS

A total of 37 individuals was examined during the study but as certain data were missing in a few individuals, only 32 individuals were included for the final analysis. A total of 39 characters were coded. All characters were coded in the binary format. The list of characters and different character states used in the analyses are given in the Table 1.

The resulting dendrogram from the cluster analysis is given in Fig. 3. Major clusters were given a 'letter' for easy reference during the discussion. The studied individuals of the different populations grouped into two major clusters at a 14.28 % similarity level. Cluster A includes the individuals Z1- Z15, while the cluster B groups individuals L1- L17 (L 5 and L9 have been left out of the analysis due to missing data in floral features). As the separation of the clusters occurred

very early, it indicates a number of dissimilarities between the two groups. The character combinations of each individual group of a given cluster were analysed in detail with the available literature (Nordal, 1977; Dassanayake, 2000), and the characters of the cluster A corresponded to *C. zeylanicum* L. whereas the characters of the other cluster B agreed with *C. latifolium* L. Further the cluster A containing individuals Z1- Z15 further divided into two sub clusters C and D at about the 78 % similarity level. The cluster C includes the individuals; Z 1, 4 - 7, 9 and 15 and the cluster D comprises individuals Z 2, 3, 8 and 10 - 14 . Character combinations of these two subgroups could be explained on the basis of characters described for the two ecotypes savannah and forest recognised by Nordal (1977) during a study on African *Crinum*.

The simple scatter plot resulting from the PCA is given in Fig. 4. Based on the habit three distinct clusters can be identified. The resulting eigen values, percentage of total variance explained by each axis and cumulative percentage along the first three axes of the analysis are given in Table 2. The first three axes account for 93.264% of the total variation. The first component explains 80.423% of

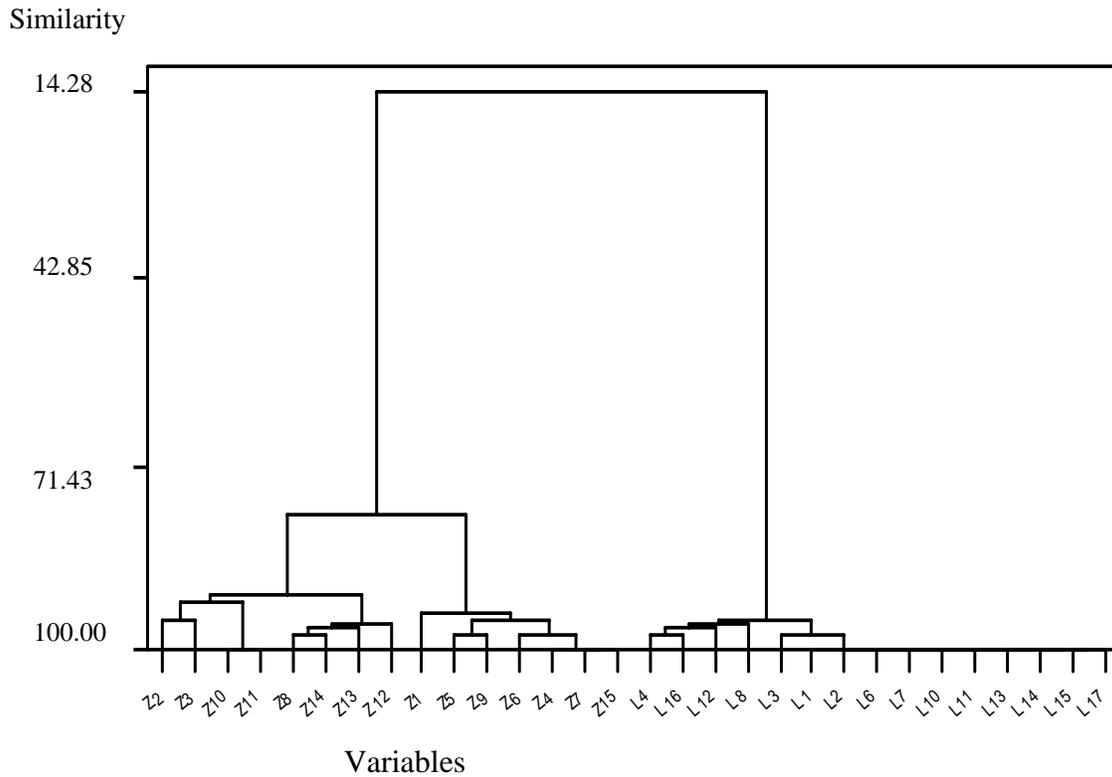


Figure 3. Dendrogram obtained from Cluster Analysis with Minitab 13.2, clustering method with Euclidean Distance Measure with Group Average Linkage method. Z1-Z15 and L1-L17 indicates the numbers allocated to each specimen.

Table 2. Results of PCA on 39 variables of 32 individuals of *Crinum* species. Eigen values, percentage of total variance explained by each axis and cumulative percentage along the first three axes.

AXIS	Eigen value	Percentage of variance explained	Cumulative percentage of variance explained
1	31.365	80.423	80.423
2	3.834	9.830	90.254
3	1.174	3.011	93.264

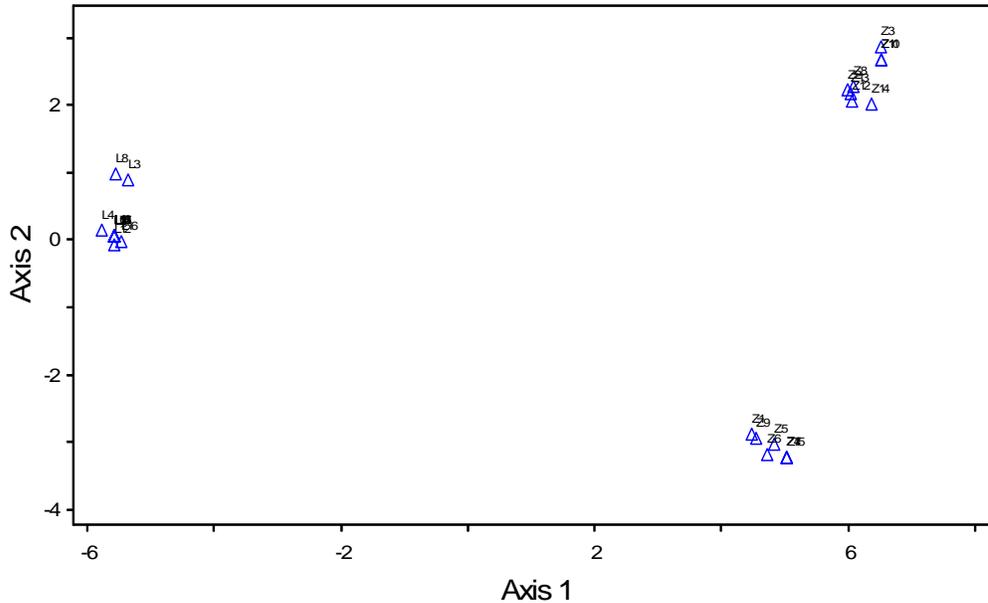


Figure 4. Simple scatter plot resulting from the Principal Component Analysis ordination diagrams based on measurement of 32 individuals from different geographical locations. Z1-Z15 and L1-L17 indicate the numbers allocated to each specimen.

Table 3. A comparison of characters that serve to distinguish between *C. latifolium* and *C. zeylanicum*.

Character		<i>C. latifolium</i>	<i>C. zeylanicum</i>
Leaf	Form	Flaccid	Robust
	Margin	Slightly scabrous and undulate	Strongly scabrous and undulate
	Blade	Slightly keeled	Strongly keeled
	Woolly appearance when torn	Less	Dense
Peduncle	Colour	Prominent purplish	Totally green
	Shape	Oval	Trigonous
Bract	Colour	Purple coloured	White with pink tinge
	Texture	Papery, translucent	Fleshy
	Persistence	Early withered	Erect, persist
Perianth	Tube colour	Green with purple tinge	Greenish white
	Segment-Shape	More or less symmetric, lanceolate	Asymmetric, broadly lanceolate
	Curvature	More reflex	Prominent at the upper part,
	Stigma	Dark purplish	Pink
Filaments		White with purple colour distally	White without pink colour distally
	Anthers	Yellowish with purple median streak	Pale yellowish lack purple median streak
Fruit development	Lack fruit development	Present	

Table 4. A comparison of characters between the two groups of *C. zeylanicum*

Character	Forest type	Savannah type
Leaf orientation	Erect	Prostrate
Distinct neck (above ground)	Present	Absent
Leaf apices	Intact	Withered

of total variation and has a high positive loading for colour of the leaves, texture of the leaves, colour of the peduncle, flattening of the peduncle towards apex, shape of the peduncle, persistence of bract, colour of the bracteoles, perianth tube-colour, perianth segments-colour, perianth segments-shape, colour of the stigma, and colour of the filament contrasted with the strongly negative loading for several of the remaining variables. The second component explains 9.830 %, and third component gives 3.011 %.

Heuristic search under the Fitch criterion yielded 3 most parsimonious trees (MPTs) of 53 steps, CI = 0.736 (Consistency Index) and RI = 0.97 (Retention Index). The resulting strict consensus tree and majority rule trees were obtained (figures not shown). Successive weighting based on RI resulted in 3 MPTs of 48.76 steps, CI = 0.767 and RI = 0.975. The strict and majority rule consensus trees were obtained, and the resulting strict consensus tree is given in Fig. 5. A phylogram is given in Fig. 6. Two major monophyletic clades could be identified. Clade E, including Z1 - Z15, and Clade F, consisting of L1 - 16 (L 5 and L 9 have been left out due to missing data). Within the monophyletic Clade E, two other monophyletic groups could be identified, Clades G and H. The individuals in Clade F correspond to the characters of *C. latifolium* L. while the individuals of Clade E correspond to the characters of *C. zeylanicum* L. and the monophyletic groups within, G and H, corresponding once again to the characters described for the two ecotypes savannah and forest types identified by Nordal (1977) during a study on African *Crinum*. The Clade F corresponding the *C. latifolium* is strongly

supported by the bootstrap analysis (100% support). Clade G that corresponds to forest type is also strongly supported by the bootstrap analysis (95% support). However the Clade E is not supported by the bootstrap analysis. This clade corresponds to the cluster C in the cluster analysis.

DISCUSSION

The results of the two analyses corroborate each other where both analyses recognise the occurrence of two morphologically distinct species of *Crinum*, *C. latifolium* and *C. zeylanicum*. Further, both analyses recover two subgroups as occurring within *C. zeylanicum*. The specimen Z1, which is grouped under *C. zeylanicum* forest type groups well during the cluster analysis while it appears as an outlier in the cladistic analysis. This could be due to the fact that this particular specimen was collected from a home garden and due to that fact vegetative features such as plant height, peduncle length etc. would have showed a variation from the rest.

A comparative morphology of the two main clusters/clades and subclusters is given in Tables 3 and 4. Detailed rationalization of each character follows.

Habit Characters

The habit of each identified *Crinum* group is very distinct (Figure 1, 7 and 8). Vertical habit with erect leaves is shown by both *C. latifolium* and *C. zeylanicum* forest type.

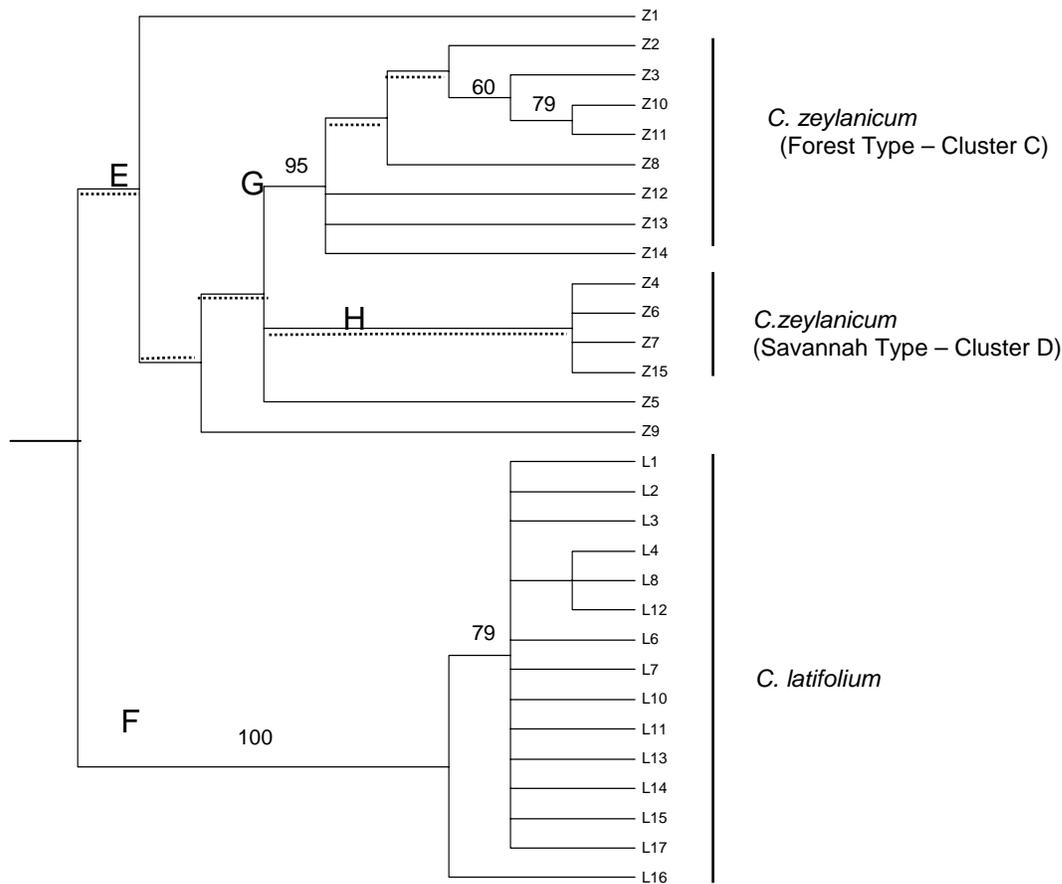


Figure 5. Strict consensus tree of the 3 MPTs recovered during the successive weighting of the morphological data. Length = 55.49 steps, CI = 0.674 and RI = 0.96. The bootstrap support values are shown above the branches. The branches that collapsed during bootstrap analysis are indicated by dotted lines. Z1-Z15 and L1-L17 indicates the numbers allocated to each specimen.

Thus, they have a distinctive height of about 40-150 cm. On the other hand *C. zeylanicum* savannah type appears very short and has leaves that spread parallel to the ground.

Less support is given for delimitation by bulb characters of *C. latifolium* and *C. zeylanicum*. However, it seems that *Crinum latifolium* has comparatively smaller bulbs than *Crinum zeylanicum*. This may be due to the reason that *C. latifolium* prefers to grow in the wet zone where additional water storage is not required. The size difference also depends on the growth stage of *C. zeylanicum* as it propagates from seeds rather than

vegetatively. Thus, smaller plants with smaller bulbs can be found.

A distinct neck-like region between the bulb and leafy area (Fig. 8) could be identified in *C. latifolium* and *C. zeylanicum* forest type where they produce extended necks above the ground level. In contrast, *C. zeylanicum* savannah type produces leaves from the ground level. This character is useful in the field identification of the *C. zeylanicum* savannah type, but less important in herbarium specimen identification and delimitation of *C. zeylanicum* and *C. latifolium* as all species have below ground extended neck region.

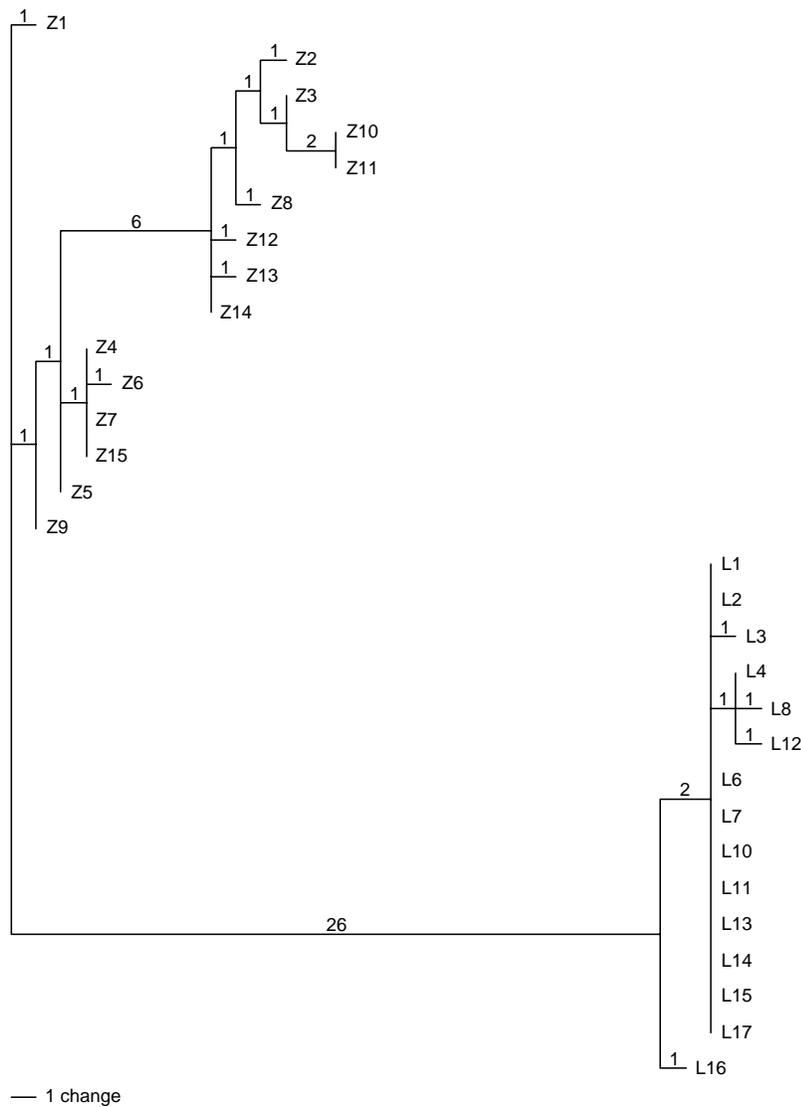


Figure 6. One of the MPTs resulting from the successive weighting of the strict consensus tree of the morphological data. The figures above the branches are the Fitch lengths. Z1-Z15 and L1-L17 indicate the numbers allocated to each specimen.

Foliar Characters

All the specimens showed spirally arranged leaves. But two distinct leaf development patterns can be observed. Leaves of *C. latifolium* are bright green in colour, have intact apices throughout the growing season and are produced in greater numbers (Fig. 1). *Crinum zeylanicum* forest type also shows the same pattern (Fig. 1). But *C. zeylanicum* savannah type shows a different leaf

development pattern. The leaves die back completely or partially in harsh environmental conditions and develop again in the next growing season or when conditions are suitable. When they resume growth, some of the partly dead, older leaves continue to develop again and those leaves lack leaf-apices. Thus, these plants predominantly have leaves without intact apices (Fig. 7).



Figure 7. Habit of the *C. zeylanicum*, savannah type, a – natural habitat and b – in cultivation, Woolly nature of leaves, c – *C. latifolium* and d – *C. zeylanicum*, Leaf margins, e - *C. latifolium* and f – *C. zeylanicum*.



Figure 8. Habit of the three different groups of *Crinum* identified, a - *C. zeylanicum* forest type. Note the vertically orientation robust leaves; b - *C. zeylanicum* savannah type. Note the robust leaves spread parallel to the ground, c - *C. latifolium*. Note the drooping nature of the leaves, d - *C. zeylanicum* forest type, note the distinct neck region.

Further, the lengths of the leaves show a distinct variation. *Crinum latifolium* and *C. zeylanicum* forest type produce leaves longer than 40 cm. while *C. zeylanicum* savannah type produces shorter leaves. Even though the savannah type possesses withered apices due to unfavourable weather conditions, still intact leaves are shorter than 40 cm. Therefore, this character also can be used to recognize the savannah type rather than as a feature to distinguish *C. zeylanicum* from *C. latifolium*.

Leaf colour can be used in the delimitation of *C. latifolium* and *C. zeylanicum* as they show distinct colour variation. *Crinum latifolium* possesses bright dark green coloured leaves. But *C. zeylanicum* shows another colour pattern as they produce dull green coloured leaves (glaucous) or yellowish green coloured leaves.

The leaf of *C. latifolium* is always flaccid. Therefore, though erect they droop down beyond the basal half due to their flaccidity. But in contrast leaves of *C. zeylanicum* are robust and the leaf cluster spread either erect or parallel to the ground. *Crinum zeylanicum* savannah type has its leaf cluster parallel to the ground whereas the forest type has erect leaves and only distal ends of the outer leaves may droop (Fig. 1, 7 and 8).

A considerable difference can be noted in the morphology of the leaf blade. A deep and sharp longitudinal adaxial alley with a abaxial thickening of a paler colour, resembling a 'mid rib', makes the leaves keeled. Most *Crinum* species of the studied group produce keeled leaves. Compared to the other *Crinum* species (*C. asiaticum*, *C. defixum*) all studied *Crinum* species have a visible midrib-like region with variable prominence. The leaves of *C. latifolium* and *C. zeylanicum* forest type often have a distinctly marked 'mid rib'. But savannah type has only a faintly marked 'midrib'.

Both forest and savannah types of *C. zeylanicum* produce strongly keeled leaves. Due to the extensive keel development, leaves even become folded along the midrib. *Crinum latifolium* produces slightly keeled leaves or leaves without a keel and others produce leaves with keel developed strongly (Fig. 9).

Considering the leaf width, *C. latifolium* produces somewhat narrow leaves. But as leaf

widths of a few *C. zeylanicum* (savannah type) overlapped with *C. latifolium*, this feature was not used in the analysis. But generally it could be considered that *C. latifolium* produces long narrow leaves.

The leaf margin is strongly scabrous in both savannah and forest types of *C. zeylanicum* and slightly scabrous to smooth in *C. latifolium* (Fig. 7). In addition, undulated leaf margins are always present in *C. zeylanicum*. They have strongly and deeply undulated leaf margins with more than three waves per foot of the leaf while *C. latifolium* lacks undulation or are only, slightly undulated with shallow waves. In addition, most *C. zeylanicum* specimens show subundulation due to extensive undulation. However, *C. latifolium* never shows subundulation. This character has been used in the species key to delimit the two species (Dassanayake, 2000).

In all *C. zeylanicum* examined dense white woolly fibres vascular appear when a leaf is torn apart. However, *C. latifolium* shows less woolly appearance (Fig. 7).

Most of the leaf characters that were described above are valuable field characters in distinguishing between *C. latifolium* and *C. zeylanicum*. Nevertheless, they are less significant in herbarium identification as most characters like leaf colour, undulation and keel disappear during herbarium preparation. However, subundulation and woolly fibres can still be noticed even in the herbarium specimens.

Floral Characters

Focussing on floral features, several characters could be identified for delimiting the species. Inflorescences are borne in the axil of older leaves; peduncles are solid and slightly compressed towards the apex. There is a distinct variation in the peduncle colour and shape where all *C. zeylanicum* species produce pale green coloured trigonous slightly compressed peduncles while *C. latifolium* possesses dark green coloured peduncles with a purplish tinge or completely purple peduncles, strongly compressed towards the end and oval shaped in transverse section (Fig. 10). Both species have white scales on the peduncle, which disappear gradually. These characters are also strong and stable in field determination of the two species but are of less importance in herbarium identification.

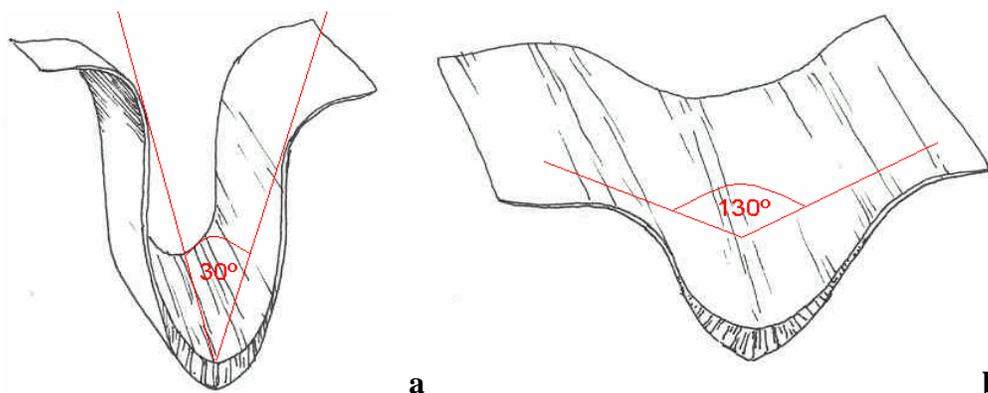


Figure 9. Keelation in leaves, a - Strongly keeled, b - Slightly keeled.

Furthermore, there is a variation in the length of the savannah type, which produces relatively short peduncles about 20-55 cm long and they rarely exceed 60 cm. But *C. latifolium* differs by producing longer peduncles more than 1 m long, while *C. zeylanicum* forest type produces long peduncles of about 50-80 cm but not as long as *C. latifolium*. Another feature associated with the length of the peduncle is twisting. Due to the length of the peduncles, they become twisted towards the top. Most *C. latifolium* species possess about 1-3 twists. However, in general, *C. zeylanicum* has relatively shorter peduncles, they lack twisting or if present twist only once. Since the width of peduncles measured at half-length overlapp among each group, peduncle width has less value in the delimitation of the species.

Involucres with two spathe segments (bracts) that envelope the buds were observed except in a few members of *C. latifolium* species that had only one bract. Generally, the two bracts are arranged contortedly and split regularly along the margins. Before opening of the involucre, the segments are fleshy, robust and thick in all species. However, once the spathe valves open they show differences. In *C. latifolium* they wither very early, even before anthesis, appearing dry and papery. However, in *C. zeylanicum* both savannah and forest types they stay erect enveloping the perianth tubes, maintaining their fleshy texture even after anthesis (Figure 10). Due to this persistent involucrum segments, perianth tubes of the florets in *C. zeylanicum* are curved downwards only from the upper part while in the case of *C. latifolium*, the

perianth tubes are more reflexed. Considerable variation in the length and width of the bracts could be observed, certain measurements overlapped between the two species, therefore these features were not considered as contributing to species delimitation.

Bracteoles of *C. latifolium* are translucent and papery, thus withering early, whereas bracteoles of *C. zeylanicum* are fleshy and persistent for a relatively long period of time and white in colour. Both types contain a pinkish tinge distally. Although the bract and bracteole characters contribute little in herbarium identification, they serve as marked features in the field delimitation of the two species.

Considering the floral buds, the buds of both are of equal widths, but the buds of *C. latifolium* are longer and slightly more slender (Figure 10), while the buds of *C. zeylanicum* are somewhat stout.

The number of flowers per inflorescence varies within the species and between species. Mostly *C. latifolium* produces more than 10 flowers per inflorescence but *C. zeylanicum* produces less than 10 (Figure 1).

Height/size of the umbel can be used for the delimitation of the two species to some extent as *C. latifolium* has a larger umbels, with the height more than 20 cm. Whereas *C. zeylanicum* has smaller umbels with a height less than 20 cm (14 – 20 cm).



Figure 10. Peduncle colour, a - *C. latifolium*. b - *C. zeylanicum*, Bracts, c - withered, papery bract of *C. latifolium*. Note the withered bracteoles, purplish tinge of pedicel and perianth tube. D - Umbel of *C. zeylanicum* with pinkish, fleshy bracts and perianth segments curved only from the upper part of the segment. Buds, e – *C. latifolium* - slender appearance of the bud, F - *C. zeylanicum* - stout appearance of the bud.

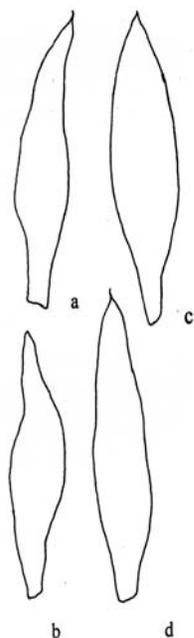


Figure 11. Shape and size of the perianth segments, a - outer segment of *C. zeylanicum*, b - inner segment of *C. zeylanicum*, c - outer segment of *C. latifolium*, d - inner segment of *C. latifolium*

Crinum latifolium produces pedicels with a length between 2- 3 cm, while *C. zeylanicum* has somewhat shorter pedicels where the length is between 1.2-1.9 cm. As the difference is not significant, it makes no contribution to the species differentiation of the studied *Crinums*. Nevertheless, the colours of the pedicel and perianth tube are varying. Pedicels of *C. zeylanicum* are completely pale green in colour and the tube is greenish white, sometimes with slight purplish tinge (Fig. 10). *Crinum latifolium* has green coloured pedicels and greenish white tube with strong purplish tinge at pedicel and perianth tube. Besides the colour, perianth tubes of *C. latifolium* are longer than those of *C. zeylanicum*.

Colour differences of the flowers can be used in the delimitation of the two species to a certain extent. *Crinum zeylanicum* has prominent pink streaks varying from pale to dark. In *C. latifolium*, mostly the streaks are paler and are purple coloured (Fig. 1 and 2).

The perianth parts are united forming a basal bell shaped tube. The free distal segments are reflexed. In *C. latifolium* the tube is relatively short and the upper segments are more reflexed than the lower, making the flower strongly zygomorphic. In *C. zeylanicum*, the perianth tube is relatively longer and all the segments are about equally reflexed, resulting in a funnel-shaped perianth. In both species the perianth segments are acuminate. In *C. zeylanicum* their apices are asymmetric, while in *C. latifolium* they are symmetrical (Fig. 1 and 11).

The style, which is greenish white at the base, changes distally to purple or red and ends in a purple, more or less trigonous stigma, in *C. latifolium*. In *C. zeylanicum* the tip is pink and the stigma is globose.

Length and curvature of the style and filament vary with the maturity of the flower. The filaments are white in colour. In *C. latifolium* the distal end is slightly pinkish to purple in the young filament but with maturity the colour disappears.

The young anther is 0.7 to 3.0 cm long and fleshy. With maturity it dries and shrinks to about 0.5 cm, turns black and becomes curved and sometimes twisted. The young anther in *C. latifolium* is sometimes yellowish with a median purplish streak. *C. zeylanicum* has cream colour anthers.

Considering the above described characters, many features can be identified to separate the two species; *C. latifolium* and *C. zeylanicum*. Further, the two forms of *C. zeylanicum* can be distinguished with confidence using foliar features, but it is difficult to delimit the two forms by floral characters. Even though Nordal (1977) indicates a difference in the number of flowers per inflorescence, where the forest type possesses less than 13 flowers in the inflorescence and the savannah type more than 10 flowers, this feature has not been consistent for the observed individuals. Further she also considers the chromosome number as a good feature, the forest ecotype with a chromosome number of 30 (2n) and chromosome number 22 (2n) for the savannah ecotype. An attempt to count the chromosome numbers during this study was up to now inconclusive due to various reasons. Other Nordal (1977) characters such as intact leaf apices and little

undulation of the leaves for the forest ecotype and leaves without entire apices and strongly undulate leaf margins defining the savannah type have been well supported. She regarded the two forms as ecotypes adapted to different water supply conditions without giving them a formal taxonomic rank (Nordal, 1977). During the present study, the plants have been grown together in the gardens of the Department of Botany, University of Peradeniya, for over two years and the habits of the forest and savannah types have been maintained by both retaining their identity, specially the habit and leaf characters. This confirms the fact that these characters are not phenotypic variations but a genetically stable feature. Therefore formal taxonomic ranks could be offered for the two types. Thus, the study reveals and confirms the occurrence of two forms/types of *C. zeylanicum* in Sri Lanka.

CONCLUSION

The results of the present study provide an answer, based on empirical methods, for the long-standing uncertainty of the species delimitation of *Crinum latifolium* and *Crinum zeylanicum* by identifying several stable morphological features aiding in their identification as two distinct species.

Furthermore, two morphologically distinct forms of *Crinum zeylanicum* were identified as occurring in Sri Lanka which has not been recorded before. Therefore, the study corroborates the finding of Nordal (1977) on African *Crinum* species that identifies two ecotypes of *C. zeylanicum*, forest and savannah ecotypes. In view of the fact that characters of these ecotypes are stable and do not appear to be dependent on environmental factors, formal taxonomic ranks could be offered.

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