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Cycas Taxa in Sri Lanka and their Morphological Characteristics of Taxonomic Significance

Asanka Mudannayake[1,2], Suneth S. Sooriyapathirana[3], Preminda Samaraweera[3] and Anoma Perera[2]*

[1]Postgraduate Institute of Science, University of Peradeniya, Sri Lanka.
[2]Department of Botany, Faculty of Science, University of Peradeniya, Sri Lanka.
[3]Department of Molecular Biology and Biotechnology, University of Peradeniya, Sri Lanka.
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ABSTRACT

Cycads are a primitive group of living seed plants which dignifies as an indispensable element in revealing evolutionary affinities of present day plants. However, cycads in Sri Lanka have received the least scientific attention so far. Aiming to identify the different cycad taxa present in Sri Lanka, to find out their vegetative and reproductive morphological features of taxonomic significance and to develop keys to identify these taxa, an island wide survey of Sri Lankan cycads was conducted. During the field survey, vegetative and reproductive morphological features (both qualitative and quantitative) of sixty *Cycas* plants were recorded. These plants were identified according to more recent, validly published circumscriptions of given taxa. Results revealed that *Cycas* species in Sri Lanka represent both subsection *Cycas* Greuter and subsection *Rumphiae* K. D. Hill of the section *Cycas.* This study further revealed the occurrence of *C. nathorstii* J. Schuster of the subsection *Cycas* in Sri Lanka. Accordingly, taxonomically useful morphological features in distinguishing different *Cycas* subsections were identified, and the taxonomic features of *C. nathorstii* were described. However, the individuals examined in both subsections showed wide but consistent variations, indicating the presence of more than a single species or several varieties in these subsections. Therefore, further investigations are needed to describe Sri Lankan *Cycas* species. Molecular characterization of Sri Lankan *Cycas* species would benefit in identifying different *Cycas* species of Sri Lanka and their intermediate forms, as well as in describing their biogeographic affinities and these are now being conducted at the University of Peradeniya, Sri Lanka.

Keywords: *Cycas nathorstii*, cycads, reproductive morphology, vegetative morphology.

INTRODUCTION

Cycads (Order: Cycadales) of Sri Lanka have received poor scientific attention over the past years though these represent an important link describing the biogeographic and evolutionary lineages of land plants. Cycads are a primitive group of living seed plants which have been in existence for more than 250 million years (Mamay, 1969). All cycad species are distributed in tropical, subtropical and warm temperate regions of both the northern and southern hemispheres. There are 331 validly-published species in this order belonging to 10 genera; *Bowenia*, *Ceratozamia*, *Cycas*, *Dioon*, *Encephalartos*, *Lepidozamia*, *Macrozamia*, *Microcycas*, *Stangeria*, and *Zamia* (Osborne *et al*., 2012). Of these, *Cycas* L. is the sole living cycad group in Asia (Lindstrom and Hill, 2007). Species of this genus belong to the unigeneric family Cycadaceae, and are considered as the basal lineage of all living cycads (Stevenson, 1992). About 100 species are reported for the genus *Cycas* worldwide, and around 40 species occur in Indo-Chinese region (Lindstrom and Hill, 2007). Of all Asian countries, the highest *Cycas* species richness of 24 species is reported from Vietnam followed by China and Thailand with 21 and 10 species respectively (Hill *et al*., 2003). However, most of the *Cycas* populations are declining due to uncontrolled human activities, and therefore, need to be conserved (IUCN 2015; Lindstrom and Hill, 2007; Mudannayake and Perera, 2014).

The genus *Cycas* has been examined by several researchers (de Laubenfels and Adema, 1998; Hill, 1994a & b, 1995a & b; Hill *et al.*, 2004; Jones, 1993; Lindstrom and Hill, 2002, 2007; Smitinand, 1971, 1972). For the ease of identification and description, this genus has been divided into several subsections (Hill, 1995b, 2008; Lindstrom *et al.,* 2008) despite that some researchers (de Laubenfels and Adema, 1998) disagree with divisions at subgeneric level. However, being a plant group which is difficult to identify to the species level at once, identification to subgeneric level would be helpful during field explorations. Six sections of this Genus have been recognized *viz*., *Cycas* Greuter, *Asiorientales* Schuster, *Indosinenses* Schuster, *Stangerioides* Smitinand (Hill, 1995a), *Wadeae* K. D. Hill and A. Lindstrom (Lindstrom *et al*., 2008) and *Panzhihuaenses* (D. Yue Wang) K. D. Hill (Hill, 2008). Among these, the section *Cycas* naturally

*Corresponding author's email: anomap@pdn.ac.lk

occurs in Sri Lanka (Lindstrom and Hill, 2002). This section is further divided into three subsections: *Cycas* Greuter (Lindstrom and Hill, 2007), *Endemicae* Schuster (Hill, 1994c, Lindstrom and Hill, 2007) and *Rumphiae* K. D. Hill (Hill, 1994a, Lindstrom and Hill, 2007). Of these, *Cycas* and *Rumphiae* subsections are known to occur in Sri Lanka (Lindstrom and Hill, 2002) but, the subsection *Endemicae* is restricted only to Australia and the New Guinea (Lindstrom and Hill, 2007).

The history of cycad studies in Sri Lanka dates back to 18th century with the first record of Sri Lankan cycads made by the distinguished scientist Carolus Linnaeus (1707– 1778) in his famous book, *Flora Zeylanica* (1747). According to Hill (1995), Linnaeus has cited treatments of *Cycas* from eight earlier works in the protologue of *Cycas circinalis* in 1753. He has treated these as a single element, *C. circinalis* L. (Lindstrom and Hill, 2002). About 100 years later, George Thwaites who served as the curator of the Royal Botanic Garden, Peradeniya from 1849 to 1880, has collected material of some Sri Lankan *Cycas* species and distributed these to different European herbaria through William Barbey-Boissier (1842-1914) (Lindstrom and Hill, 2002). However, there are no records of their usage in taxonomic research during that era. Henry Trimen (1898) has also included *C. circinalis* in the *Flora of Ceylon* but there are no evidences of his collections of Sri Lankan *Cycas* species. Later, in 1932, based on the specimen collected by Thwaites, Schuster has differentiated the Sri Lankan material as *C. nathorstii* J. Schuster, endorsing the type of *C. nathorstii* from Sri Lanka (*Thwaites 3689* in Schuster, 1932). According to Lindstrom and Hill (2002), Schuster's types were mainly in Berlin, Germany and may have been destroyed during the World War II. Later, de Laubenfels and Adema (1998) have again placed Sri Lankan material in the same taxon in *C. circinalis* in which the lectotype is from India. They have cited *C. nathorstii* J. Schuster as a synonym of *C. sphaerica* Roxb. but have not acknowledged *C. sphaerica* as a plant native to Sri Lanka.

In 2000, two *Cycas* species, *C. circinalis* L. emend J. Schust. and *C. rumphii* Miq. were recorded in the *Revised Handbook to the Flora of Ceylon*, with taxonomic descriptions of the two species (Wadhwa, 2000). However, soon after, in 2002, Lindstrom and Hill have confirmed the occurrence of *C. nathorstii* and *C. zeylanica* (J. Schuster) A. Lindstrom & K. D. Hill in Sri Lanka, mainly based on the specimens in various herbaria in the world and also through field explorations for the latter species. The specimen collected in 1866 by Thwaites (*no. 3689* in G) has been designated as the lectotype of *C. nathorstii* by Lindstrom and Hill (2002). In their description, they have cited that *Cycas nathorstii* (madu) which occurs in inland and upland forests in the northern parts of the island, usually in somewhat dry places, is an endemic plant to Sri Lanka while, *Cycas zeylanica* (maha madu) is restricted to the southern Sri Lanka and, the Andaman and Nicobar islands. Moreover, *C. circinalis* L. was considered as an endemic species to India (Hill, 1995). Accordingly, only *C. nathorstii* and *C. zeylanica* have been evaluated in the National Red List of Fauna and Flora of Sri Lanka in 2012 (National Red List, 2012) and, *C. circinalis* and *C. rumphii* in Sri Lanka remain unsubstantiated.

The past disagreements in taxonomic identification of Sri Lankan species may be partly due to the incomplete understanding of the Order Cycadales in that era and partly due to the unavailability of suitable Sri Lankan specimens for taxonomic research. *Cycas* species in Sri Lanka are not passably explored and hence the exact number of *Cycas* species of the country remains uncertain, urging the need for proper scientific exploration and description.

One of the major barriers for the conservation of *Cycas* species in Sri Lanka is the lack of information on the species identification and their distribution. Early records on the occurrence of different *Cycas* species are needed to be confirmed through extensive field explorations and taxonomic identification while investigating possible hybridization among species, if any. With this background, morphological, biochemical and molecular systematic studies of Sri Lankan *Cycas* species are being conducted at the Department of Botany, University of Peradeniya, Sri Lanka. However, the present paper is devoted only for the taxonomic identification of different *Cycas* taxa/species in Sri Lanka based on vegetative and reproductive morphological traits using available circumscriptions of the taxa, and to describe the characteristics of these taxa. A given morphological feature, especially the size of mature individuals or the magnitude of the vegetative and reproductive structures may vary from site to site which make the identification of individuals in the field rather difficult. Therefore, this study attempts to reveal taxonomically useful morphological features in distinguishing different *Cycas* taxa in Sri Lanka, to describe vegetative and reproductive morphology in *Cycas* species populations and, to develop keys for identification of Sri Lankan *Cycas* taxa. However, this paper does not intend to revise any accepted taxonomic circumscriptions of *Cycas* taxa.

MATERIALS AND METHODS

An island wide survey of Sri Lankan Cycads was carried out to find out different *Cycas* species present within the island. Forty nine morphological features (23 vegetative and 26 reproductive

characters given in Tables 1 and 2), both qualitative and quantitative, in mature individuals were recorded and species were identified based on the available taxonomic descriptions of the taxa (Lindstrom and Hill, 2002, 2007). These characters were measured on fully mature individuals and structures. Some of the parameters measured are generally accepted norms in describing plant morphology but some other parameters have been specifically defined for comparing different *Cycas* species, and such used parameters are explained in the Box 1 below.

Two samples *t* tests were performed using R commander 3.0.2 and R studio 0.98.501 statistical software to reveal significant differences of the examined quantitative morphological characters among different taxa. Taxonomic descriptions of the correctly identified *Cycas* species was prepared using important vegetative and reproductive features while the line diagrams were drawn to describe some of its morphological features.

RESULTS

A total of 435 individuals at different maturity stages were examined during field surveys and morphological traits were recorded in 181 mature individuals. Of these, 60 (48 female and 12 male) plants bore mature reproductive structures and only these were considered in identification of taxa in the present study. Results of the present study confirms the findings of Lindstrom and Hill (2002) that *Cycas* species in Sri Lanka represent both subsections of the Section *Cycas, i.e.,* the subsections *Cycas* and *Rumphiae and* the occurrence of *C. nathorstii* (Figs. 1 & 2) in Sri Lanka.

Morphological features of taxonomic significance in differentiating *Cycas* taxa

Several morphological features appeared to be helpful in distinguishing the subsection *Cycas* from that of *Rumphiae*. As Hill (1994a) stated, the presence of a layer of spongy endotests or a fibrous sarcotesta which are visible in a longitudinal section of a seed, is an important character in differentiating the two subsections. In the subsection *Cycas,* a fibrous sarcotesta exists but in the subsection *Rumphiae,* a spongy tissues are present in the endotesta. Thus, *Cycas* species in Sri Lanka too can be differentiated into subsections by this feature, but only the female individuals bearing seeds can only be identified using this feature. Therefore, the present study investigated other possible morphological traits in both male and female plants that can be used in distinguishing members of the subsection *Cycas* from those in the subsection *Rumphiae* in Sri Lanka and, are given in the Tables 1 and 2.

Some of the qualitative morphological characters, such as the shape of microsporophylls, megasprophylls and cataphylls appeared to be largely beneficial in differentiating the two subsections. Linear shaped cataphylls and upright apical spine of the microsporophylls in the individuals of the subsection *Rumphiae* can be used to distinguish these from the subsection *Cycas,* in which the apical spine of the microsporophylls is curved while the cataphylls are triangular in shape. Further, the narrowly ovoid pollen cone of the former group is different from ovoid-oblong pollen cones of the individuals in the subsection *Cycas* in Sri Lanka.

Box 1. Some of the specific morphological features used in describing *Cycas* species in the present study.

- Percentage spinescent (the length of the petiole with spines with respect to the total length of the petiole).
- Petiole circumference at the position of first leaflet.
- Petiole length + the lamina length as the full leaf length.
- Distance from one midrib to another of two adjacent leaflets at the central part of the leaf as a measure of leaflet spacing.
- Angle between median leaflets and rachis was considered as the insertion angle to rachis.
- Total length of the microsporophyll including the apical spine and width at the broadest point.
- Distance from the first ovule to the last ovule as the length of the fertile region.
- Distance from the last ovule to the apical spine as the length of the infertile region.
- Total length of the megasporophyll, *i.e*., the sum of lengths of fertile and infertile regions (the length from the base to the first ovule was not considered as it varies with the position of the megasporophyll on the tree).
- Total number of spines on both sides of the megasporophyll as the number of lateral spines.
- Colour of the sarcotesta as the seed colour.
- Presence of fibrous or spongy tissues/layers in the integument as seen in the transverse sections of seeds.

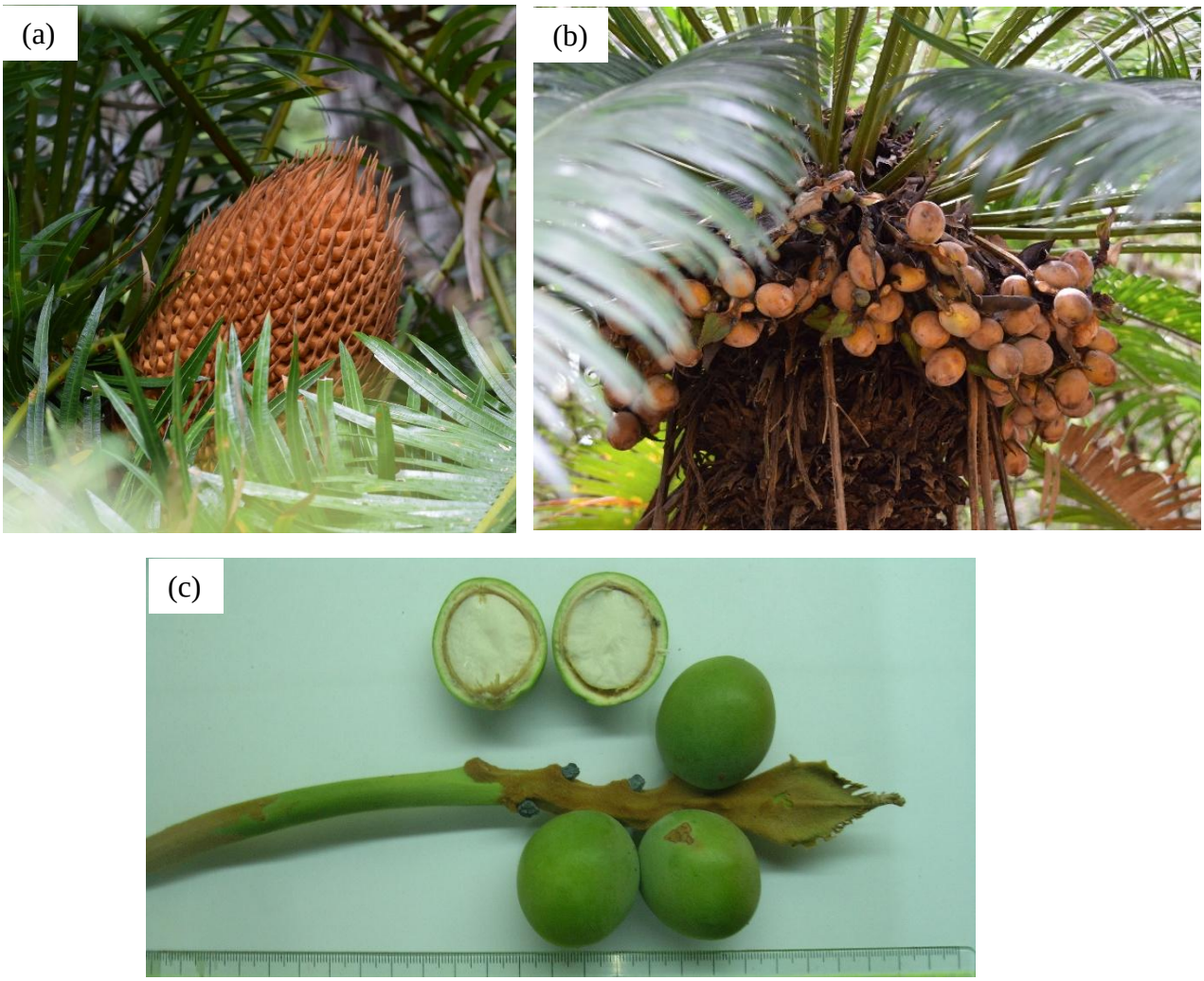

Figure 1. *Cycas nathorstii* (a) male cone (immature) (b) brownish orange mature seeds in a plant at Kudapathassa of Wilpaththu National Park (c) Megasporophyll with seeds and a longitudinal section of a seed

Of the 18 quantitative characters examined on reproductive structures, several (length:width ratio of microsporophylls, maximum width of the megasporophyll, the lengths of the fertile and infertile regions of megasporophylls, number of lateral spins on megasporophylls and the length of the apical spine) showed statistically significant differences between the two subsections but the ranges of values of these traits were overlapping between the two groups (Table 1). However, the total length of megasporophylls, the length:width ratio of the megasporphyll and length and width of seeds were significantly larger in the members of *Rumphiae* subsection than those in the subsection *Cycas* (*t* test $p<0.05$) and the ranges of values of these traits were not overlapping between the two groups. Therefore, these can be considered as prominent features in distinguishing the two taxa. The width of leaflets is the only morphological trait examined on vegetative structures which showed a significant differences (*t* test: $p<0.05$; Table 2) between the individuals of the two subsections without any overlapping between the two groups. In addition, the average values for the % spinescent, total leaf length, length of petiole, petiole circumference, leaflet spacing, length of leaflets, width of leaflets, insertion angle to rachis and the cataphyll length, were significantly larger while the number of leaflets were significantly smaller in individuals of the subsection *Rumphiae* than those in the subsection *Cycas* (*t* test: $p<0.05$). However, these should be used with caution as there can be individuals in species populations bearing extremes of these characters resulting in overlapping limits among different taxa. Further, these limits of the taxa have to be revised upon the correct identification of all the *Cycas* species in Sri Lanka and then, these characters can be used in species identification with better confidence.

Table 1. Comparison of morphology of reproductive structures in individuals of *Cycas* subsections in Sri Lanka. The numerical means of measured quantitative parameters are given with the range (within brackets) and the probability (*p*) value of *t* tests.

		Morphological trait	Subsection		*p* value
			Cycas	***Rumphiae***	
Microsporophyll	1.	Microsporophyll length (cm)	5.61 (4-8)	6.95 (6.6-7.3)	0.0770
	2.	Microsporophyll maximum width (cm)	1.89 (1.4-2.4)	1.75 (1.6-1.8)	0.3386
	3.	Microsporophyll length:width ratio	2.99 (1.25-3.81)	3.97 (3.8-4.1)	0.0027
	4.	Apical spine length (cm)	2.50 (1.2-3.6)	2.45 (2.3-2.6)	0.8783
	5.	Apical spine curved or upright	Curved	Upright	
	6.	Pollen cone shape	Ovoid-oblong	Narrowly ovoid	
	7.	Pollen cone colour	Brown	Pale fawn	
Megasporophyll	8.	Megasporophyll total length (cm)	12.73 (10.4-17)	20.96 (18-24)	<0.001
	9.	Megasporophyll maximum width (cm)	3.13 (2.5-4)	2.61 (2.1-3.2)	0.0147
	10.	Megasporophyll length:width ratio	4.14 (2.5-5.7)	8.13 (6.25-10)	<0.001
	11.	Fertile region length (cm)	6.04 (4.1-12)	8.75 (7-12)	0.0223
	12.	Number of ovules	6 (5-8)	5 (4-6)	0.0064
	13.	Infertile region length (cm)	6.89 (5-9.5)	12.21 (9-16)	0.0004
	14.	Infertile region margin	Dentate	Entire to dentate	
	15.	Number of lateral spines	42 (28-68)	14 (6-28)	0.0003
	16.	Length of lateral spine (mm)	5.67 (2-10)	4.37	0.4588
	17.	Width of lateral spine (mm)	0.73 (0.5-2.5)	0.875	0.6222
	18.	Apical spine length (cm)	1.67 (0.8-3.2)	6.31 (1-10.5)	0.0118
	19.	Fertile to infertile ratio	1.25 (0.42-1.73)	1.50 (0.8-2.28)	0.3355
	20.	Seed length (cm)	4.11 (3.1-4.8)	6.17 (5.7-6.8)	0.0040
	21.	Seed width (cm)	3.32 (2.8-4)	5.37 (5-6.2)	0.0086
	22.	Seed length:width ratio	1.24 (1.03-1.41)	1.15 (1.07-1.41)	0.0767
	23.	Seed colour	Yellow/Orange brown	Orange brown	
	24.	Seed shape	Subglobose	Ovoid	
	25.	Fibrous layer in the sarcotesta	Present	Absent	
	26.	Spongy endocarp layer	Absent	Present	

Table 2. Comparison of morphological features of vegetative structures in individuals of *Cycas* subsections in Sri Lanka. The numerical means of measured quantitative parameters are given with the range (within brackets) and the probability (*p*) value of *t* tests.

	Morphological trait	Subsection		*p* value
		Cycas	***Rumphiae***	
1.	% spinescent	76.67 (60-100)	95.00 (80-100)	0.0014
2.	Petiole length (cm)	45.83 (32-69)	74.58 (45-112)	0.0020
3.	Petiole circumference (cm)	3.10 (2-5)	5.53 (4-7)	<0.001
4.	Petiole length:circumference ratio	15.47 (10.67-23.6)	13.43 (7.29-16.83)	0.2628
5.	Leaf lamina length (cm)	145.42 (89-186)	158.25 (106-190)	0.3358
6.	Full leaf length (cm)	191.25 (140-278)	232.83 (157-292)	0.0363
7.	Number of leaflets	201 (156-266)	131 (98-184)	<0.001
8.	Leaflet spacing (cm)	1.55 (0.8-2)	3.23 (1.5-4)	0.0004
9.	Leaflet length (cm)	22.17 (16-31)	32.55 (27.3-37.5)	<0.001
10.	Leaflet width (cm)	0.99 (0.9-1)	1.57 (1.2-1.8)	<0.001
11.	Leaflet length:width ratio	22.39 (16-27)	20.97 (17.2-25)	0.2933
12.	Midrib above	Raised	Raised	
13.	Midrib below	Raised - Flat	Flat	
14.	Leaflet margin	Entire	Entire	
15.	Leaflet apex	Acuminate	Acuminate	
16.	Insertion angle to rachis	51.67 (45-80)	72.92 (45-80)	0.0002
17.	Cataphyll shape	Triangular	Linear	
18.	Cataphyll length (cm)	6.67 (6-9.5)	12.77 (7-17)	<0.001
19.	Cataphyll-persistent or shedding	Persistent	Persistent	
20.	Leaflet colour – mature	Dark green	Dark green	
21.	young leaves – colour	Light green/Bluish green	Light green	
22.	Tomentum	Present	Present	
23.	Tomentum colour	Brown	Golden brown	

Moreover, the shape of cataphylls, upright/curved nature of the apical spine of microsporophylls, shape of pollen cone are important qualitative features in differentiating individuals of the two subsections.

The present study revealed that some samples collected from individuals of the subsection *Cycas* show clear similarities to *C. nathorstii*. Further, it is easy to differentiate *C. nathorstii* from those in the subsection *Rumphiae* by their external morphology. The length of the leaflets in the former is significantly shorter than in the latter (*t* test: p=0.00004). Also, the midrib beneath the leaflets is raised in *C. nathorstii* but it is flat in *C. rumphii* complex. The number of lateral spines is significantly higher while the lengths of the fertile and infertile regions of megasporophylls are significantly lower in *C. nathorstii* than in

C. rumphii complex in Sri Lanka (t test: number of lateral spines, p=0.009; the length of the fertile region, p=0.0029 and the length of the infertile region, p= 0.0002).

Cycas zeylanica (J.Schust.) A.Lindstr. & K.D.Hill is reported as a native species occurring in southern coastal regions of Sri Lanka (Lindstrom and Hill, 2002), growing in littoral forests near the sea in sandy soil and are often found on stabilized dunes (Lindstrom and Hill, 2002; IUCN Red List, 2015). During our field explorations, a few individuals that suspected to be *C. zeylanica* were found but these were not bearing reproductive structures at that time. Therefore, the morphological features of the species has not adequately being assessed and therefore, the species is not being described in this paper. Further, the individuals examined in the subsection *Rumphiae* showed a wide variations indicating the presence of several species or varieties.

Keys to the identification of subsections of *Cycas*

With an intension of easy identification of Sri Lankan *Cycas* taxa during field explorations, keys have been prepared including all remarkable characteristic features of taxonomic significance in vegetative and reproductive structures of both male and female plants.

Order Cycadales, Section *Cycas* in Sri Lanka

1 Width of leaflets 0.9-1 cm; cataphylls triangular; apical spine of the microsporophyll curved; ovoid-oblong pollen cone; megasporophyll 10-17 cm long, megasporophyll length:width ratio 2.5-5.7 cm, number of lateral spines 28-68; subglobose seeds, not very large (3.1-4.8 cm x 2.8-4 cm) with a fibrous sarcotesta, no spongy tissues in the endotesta.

Subsection: *Cycas*

1 Width of leaflets 1.2-1.8 cm; cataphylls linear in shape; apical spine of the microsporophyll upright; narrowly ovoid pollen cone; megasprophyll 18-24 cm long, megasporophyll length:width ratio 6.25-10 cm, number of lateral spines 6-28; Ovoid seeds, larger (5.7-6.8 cm x 5-6.2 cm) with spongy tissues in the endotesta, no fibrous sarcotesta.

Subsection: *Rumphiae*

Descriptions of species based on vegetative and reproductive morphology of species populations

Both vegetative and reproductive features of the population of *C. nathorstii* recorded during field explorations are given in the descriptions below. Although the authors have not seen the types, the circumscriptions by Hill (1995b) and Lindstrom and Hill (2002) have mainly been followed.

***C. nathorstii* J. Schuster**

Vernacular: Madu (S)

Perennial trees, Stems arborescent, sometimes forked, bark thick and corky with persistent cataphylls and leaf bases. Petiole glabrous, 32-56 cm long with 60-100% of spinescent. Mature leaves dark green, semi glossy, 108-186 cm long, flat (not keeled) in section with 156-246 leaflets. Light brown tomentum shedding as leaf mature. Median leaflets simple, lanceolate, 16-24 cm long and 0.9-1 cm wide, 1.3-2.0 cm spacing on rachis; entire margin with acuminate apex. Midrib raised above and slightly raised below. Cataphylls narrowly triangular and 6-7.2 cm long. Male cone brown and ovoid to conical shape when immature, more elongated when mature. Microsporophyll lamina firm; ovoid to deltoid but narrows gradually; 4-5.5 cm long and 1.4-1.9 cm wide; apical spine prominent, curved with 1.2-3.6 cm in length. Megasporophyll possesses orange tomentum; fertile region 4.1-6.5cm long; infertile region 5.4-7.0 cm long and 2.5-3.4 cm wide; ovules 5-7, glabrous; infertile region shortly dentate with 34-68 lateral spines, 2-5 mm long and 1 mm wide at base. Seeds subglobose, 3.1-4.8 cm long, 3.1-3.4 cm wide; sarcotesta orange brown, fibrous; spongy endotesta absent.

Notes on the subsection *Cycas* in Sri Lanka

Individuals of the subsection *Cycas* examined showed a wide variation in their morphological features (Tables 1 and 2). As a result, the ranges of some quantitative traits were broader. However, some of the collected material looks similar to the circumscriptions of *C. nathorstii*. Wide and distinct variations of examined morphological parameters within this subsection indicates the presence of different taxa within this subsection, at species or variety levels, or possible hybridization among different taxa. Therefore, it is necessary to study these further for clear clarification and identification of *Cycas* taxa in this subdivision. However, the morphological features of Sri Lankan samples do not match with *C. circinalis* (Personal communications with Dr A. J. Lindstrom, the Nong Nooch Tropical Gardens, Sattahip, Thailand) and therefore, there is a high possibility that *C. circinalis* does not naturally grow in Sri Lanka.

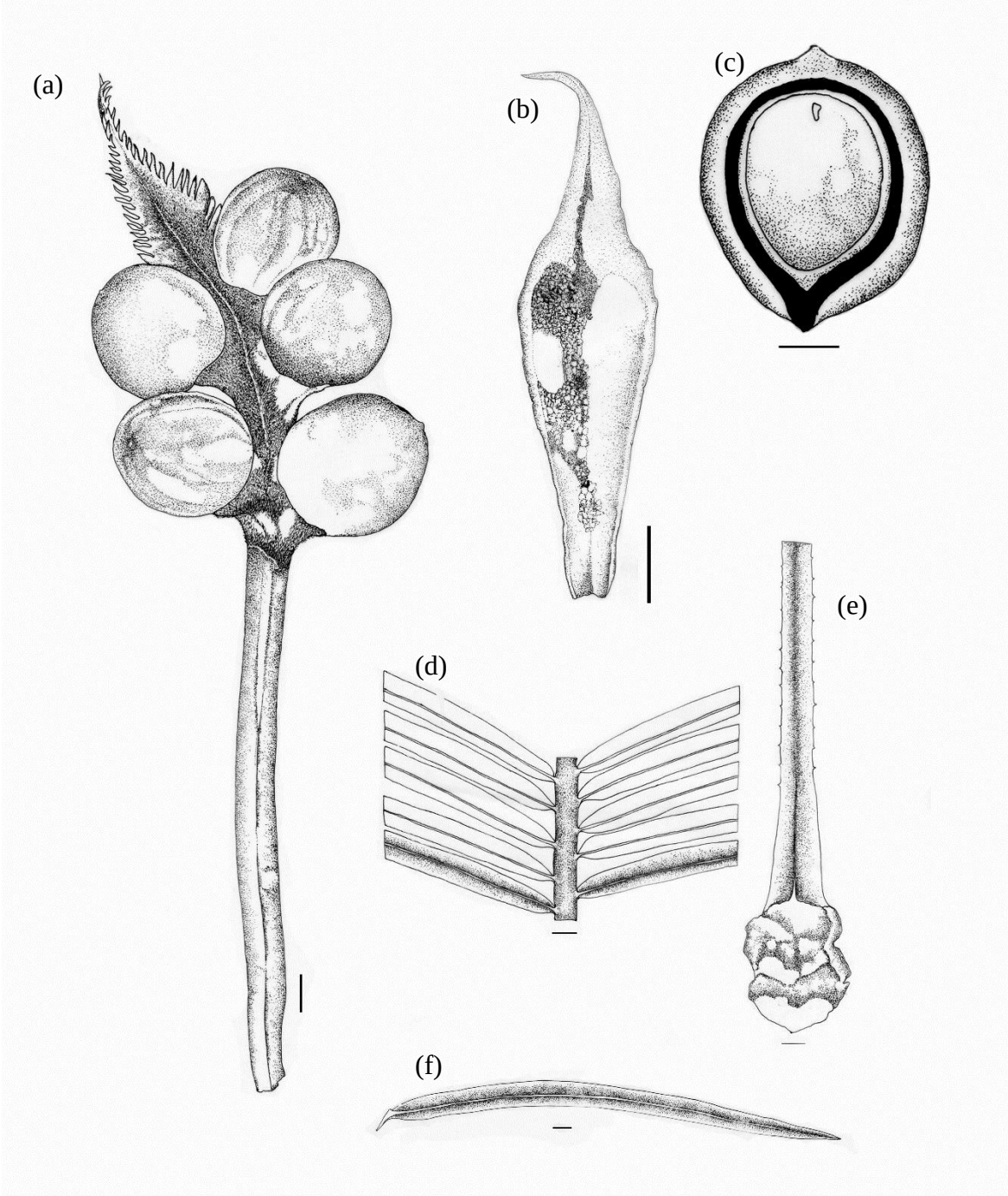

Figure 2. *Cycas nathorstii* (a) megasporophyll from a middle whorl, (b) a microsporophyll, (c) Longitudinal section of a seed, (d) median leaflets showing the insertion angle to rachis (e) petiole and (f) a leaflet [scale bar = 1 cm]

In the past, there were some instances which reported *C. circinalis* in Sri Lanka (Linnaeus, 1753, Trimen 1898 and Wadhwa, 2000). Hill (1995b) has clearly pointed out that it was designated as the single constituent species when Linnaeus established the genus *Cycas* and therefore, the name of *C. circinalis* has often been used in the past in an aggregate sense. Of the eight references cited by Linnaeus (1753) in the protologue of *C. circinalis*, only two have now been typified as *C. circinalis* (Lindstrome and Hill, 2007). Later, some of the specimens collected from Sri Lanka, which formerly identified as *C. circinalis* have been treated as *C. nathorstii* by Schulster, and the specimen collectd by *Thawaites (no.* 3689) was considered as the isosyntype of *Cycas nathorstii* (Lindstrom and Hill, 2002). However, de Laubenfels and Adema (1998) have continuously considered Sri Lankan *Cycas*

species as *C. circinalis* and, they cited *C. nathorstii* under the synonym of *C. spherical,* which is not acknowledged as a native Sri Lankan species. When preparing the Revised Handbook to the Flora of Ceylon, Wadhwa (2000) has also reported the presence of *C. circinalis* in Sri Lanka but has not acknowledged the occurance of *C. nathorstii* in Sri Lanka. Once Lindstrom and Hill (2002) cited the occurrence of *C. nathorstii* as a species endemic to Sri Lanka, while acknowledging *C. circinalis* as an endemic species in India which restricted to Western Ghats, Kerala, Karnataka, Tamil Nadu and the Rajasthan (Lindstrom and Hill, 2002), the occurrence of *C. circinalis* in Sri Lanka was treated as a misidentification and therefore, largely neglected. The specimens used by Wadhwa (2000) for preparing the section Cycadaceae for the Revised Handbook to the Flora of Ceylon are available at the Kew Gardens herbarium, but of these, duplicates of *Jayasuriya 1278* from the ridges of Na-Ulpotha of the Ritigala Strict Nature Reserve and *Hepper & G. de Silva 4723* from Bibile are available in the PDA herbarium at present. These have not probably been seen by Wadhwa as no annotations were made on the herbarium sheets. Of these, the former has been labeled as *Cycas* cf. *circinalis* while the latter has been identified only to the genus level by collectors, urging the necessity of further identification. Wadhwa (2000) has identified these two in the Kew Gardens as *C. circinalis* while Lindstrom and Hill (2002) differentiated these as *C. nathorstii*. However, the corresponding author of this article (AP) noted that the duplicates of these in the PDA herbarium are not in a good condition to come to a strong conclusion. In addition, there were three other specimens in PDA which have not been included in the Revised Handbook to the Flora of Ceylon. Among these, the specimen by *F. N. Hepper & G. de Silva 4723* from Bibile has not been identified to the species level. Two other specimens, *i.e*., *F.R. Fosberg & M.-H. Sachet 53148* collected from 2 miles east of Bibile and *Sohmer & D.B. Sumithrarachchi 10,711* collected from Timbiriyawila were female plants with megasporophylls and were labelled as *C. circinalis* but the taxonomic identification of these specimens remained questionable. Lindstrome and Hill (2002) recorded that the duplicate of the former (*F.R. Fosberg & M.-H. Sachet 53148*) in the Kew Gardens and New York Herbaria as *C. nathorstii* while AP noted that the latter specimen (*Sohmer & D.B. Sumithrarachchi 10,711*) signifies more towards *C. nathorstii*. However, the other specimens observed by Lindstrom and Hill (2002) are not available in the PDA herbarium.

Notes on the subsection *Rumphiae* in Sri Lanka

About 11 species are reported worldwide under the subsection *Rumphiae* in the Genus *Cycas* (Lindstrom and Hill, 2002) but the taxonomy of this group is difficult and unclear (Hill 1994). All species in the *C. rumphii* complex have been erroneously cited as *C. circinalis* at some stage (Hill 1994; Lindstrom and Hill, 2002). *Cycas rumphii* Miq. has been separated from *C. circinalis* L. by Miquel in 1839 (Lindstrom and Hill, 2007).

The individuals examined in the subsection *Rumphiae* in Sri Lanka showed a wide but consistent variation and need further investigations to identify and describe these at species level. Some of the morphological traits considered in the Tables 1 and 2 show drastic variations resulting a wider ranges of examined quantitative characters. It is obvious that there could be more than one species or many intermediate forms in this group too. Consequently, the overall distribution of the different species is not clear. Therefore, thorough field explorations and molecular characterization of the group are required before determining the limits of *Cycas* species in this subsection and these investigations are currently being carried out at the Department of Botany, University of Peradeniya, Sri Lanka.

Two species of the *C. rumphii* complex have been reported in Sri Lanka so far; *viz. C. rumphii* Miq. (Wadhwa, 2000) and *Cycas zeylanica* (Lindstrom and Hill 2002). Of these, *C. zeylanica* is listed as a Globally Vulnerable species due to the overall decline in the population size (30-50% reduction in the last 50 years alone). Populations in the wet zone of Sri Lanka have been categorized as reduced and Extinct in the Wild, but the species is reported as still relatively abundant in the Indian parts of its range, *i.e*., on the Andaman and Nicobar islands (Lindstrom and Hill, 2002; IUCN, 2015). During our field explorations, we were able to locate a few individuals of the species but no natural populations were noticed. Further, no male plants of *C. zeylanica* were found so far. However, the occurrence of *C. rumphii* in Sri Lanka has not been confirmed so far by other scientists.

Identification of *Cycas* taxa based on morphological traits

Identification of *Cycas* species based on morphological features observed in herbarium specimens may be a difficult task. In general, the morphological traits may vary from region to region or from one country to another, the age of the plant or the position of the sample (plant part) on the plant. For instance, in the subsection *Cycas*, the megasporophylls in the lower whorls contain a broader infertile region with more regular lateral teeth whereas those in the upper whorls are less broad with irregular lateral teeth and a long apical spine. Therefore, we propose to develop norms describing the morphological traits of *Cycas* species. It is very helpful if details of the maturity stage and the position of the micropsporophyll within the pollen cone or the megasporophyll within the tree (lower or upper whorl) are provided on herbarium

specimens. Further, it is worth to consider as many traits as possible in the identification of *Cycas* taxa and, include many traits when preparing identification keys of *Cycas* taxa. To avoid confusions, it is much easier and more practical if the examining individuals are first identified to subsection levels and then proceeded with species level identification.

We have further observed that most of the collectors tend to collect immature reproductive structures of *Cycas* species in preparing herbarium specimens, especially the megasporophylls with ovules, as those with seeds are bulky and crooked. Some of the significant morphological traits may not have fully developed in these immature plant parts. All these have made the identification and description of *Cycas* species using herbarium specimens, a more dubious task. Recent advancements of molecular phylogenetic studies would help in correct identification of *Cycas* species however, these may not be a solution for quick recognition of species during field explorations. Therefore, the morphological traits that help in identifying *Cycas* species have to be investigated further.

Concluding remarks

Cycas species in Sri Lanka represent both subsections *Cycas* and *Rumphiae* of the section *Cycas*. However, the present paper only confirms the *C. nathorstii* in the subsection *Cycas* as a native endemic to the country. Although, *C. zeylanica* is reported from Sri Lanka (Lindstrom and Hill, 2002), it is not considered in the present paper due to insufficient amount of collected material during our field explorations. Moreover, further taxonomic research are required in confirming or identifying the occurrence of other *Cycas* species in Sri Lanka.

It appears that most of the taxonomic work on Sri Lankan *Cycas* taxa were based on a few herbarium material collected in the past (*e.g.* George Thwaites) which are located in different herbaria in the world. No detailed field explorations have been conducted to represent the species diversity of the country and no taxonomic studies have been conducted to elucidate the species limits of Sri Lankan *Cycas* species. Therefore, all *Cycas* taxa of Sri Lanka may not have been acknowledged in the past for taxonomic revisions. The inherent difficulties in identifying different *Cycas* species using morphological features and the dubious nature of collected specimens in early times might also responsible for that.

Taxonomically useful morphological features in distinguishing different *Cycas* subsections in Sri Lanka and in identifying *C. nathorstii* were revealed from the current study. Shape of cataphylls, upright/curved nature of the apical spine of microsporophylls, shape of pollen cone, length of megasporophylls, length:width ratio of the megasporophyll, length and width of seeds and, width of leaflets can be considered as important traits of taxonomic significance which allows to distinguish the individuals of the subsection *Rumphiae* from the subsection *Cycas* in Sri Lanka. Several other quantitative features show significant differences among the taxa but ranges (based on maximum or minimum values) of these characters may sometimes overlap among the taxa examined. Therefore, these have to be considered carefully with other suitable characters in identifying *Cycas* taxa. Moreover, the limits of the taxa have to be revised upon the correct identification of *Cycas* species in Sri Lanka and, subsequently, these traits can be useful in taxonomic identification of *Cycas* species in the country. Different Sri Lankan *Cycas* species share many common features and therefore, it is always advised to differentiate the two subsections of the genus first, before attempting to identify the species straight away. Further, molecular characterization of Sri Lankan cycads is required to identify all the *Cycas* species and their intermediate forms in Sri Lanka, and to describe their biogeographic affinities. These studies are currently being conducted at the Department of Botany, University of Peradeniya, Sri Lanka.

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