

## Taxonomic Study of the subtribe *Andrographinae*, tribe *Ruellieae*, family *Acanthaceae* in Thailand

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### ABSTRACT

The *Andrographinae* is one of the famous subtribes in the Tribe *Ruellieae* (*Acanthaceae*). The most famous genera in this subtribe are *Andrographis* Wall ex Nees, *Graphandra* Imlay, *Gymnostachyum* Nees and *Phlogacanthus* Nees. Their large diversity was found in various tropical countries including Thailand. However, there is no revision of their diversity in Thailand for decades. Thus, this study aimed to collect and revise the species list for the Flora of Thailand. Data was collected from relevant previous documents, herbarium specimen supplied from 5 herbaria, and field survey across country during October 2009 to September 2010. Morphological characters including pollen morphology were determined and recorded. Twenty-three species of the *Andrographinae* were found in this study viz. *Andrographis* (2), *Graphandra* (1), *Gymnostachyum* (8), and *Phlogacanthus* (12). The differences of morphological characters among them were discussed and the checklist of the subtribe was provided.

**Keywords** : Subtribe *Andrographinae*, *Acanthaceae*

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## Introduction

The Family Acanthaceae is a large pantropical family of about 229 genera and 3,450 species in the world (Mabberley, 1997). Most of them are herbs or shrubs, including twining forms, some are spiny. There were about 40 genera and over 230 species in Thailand and the majority are confined all over the country (Hansen, 1985). At present, the studies on the family Acanthaceae are diagnosed through the details of corolla aestivation, palynological and phylogenetic data to serve the classification of this family. This family was treated by Scotland & Vollesen (2000), which was classified it into three subfamilies, i.e. Nelsonioideae, Thunbergioideae and Acanthoideae. The last one is the largest sub family which composts of two tribes, i.e. Acantheae and Ruellieae. The tribe Ruellieae was classified into 4 subtribes, i.e. Ruelliinae (48 genera), Andrographinae (8 genera), Justiciinae (103 genera) and Barleriinae (9 genera). All of them are famous for medicinal purposes, especially both subtribe Barleriinae and Andrographinae. The famous genera in the subtribe Andrographinae are as follows : Barleriinae (2 genera : *Barleria* L. and *Lepidagathis*

Willd.) and Andrographinae (2 genera : *Andrographis* Wall. ex Nees and *Phlogacanthus* Nees) (Hansen, 1985). The subtribe Andrographinae is an interesting acanthus group to be revised for this research, due to lacking the clear-cut taxonomic data and also they are well-known as the Thai medicinal plants used by traditional herbalist. Some well-known species used as medicinal purposes in minority groups of Thailand are *Phlogacanthus curviflorus* Nees and *P. pulcherimus* T. Anders. This study aim to collect, revise, do a check list to serve as an information to the Flora of Thailand and agricultural purposes by emphasizing on botanical literature, herbarium study, field survey, specimen collection, morphological study and to conduct a taxonomic treatment for this genus and enumerate the number of species and construct the keys to genera. As mentioned, this research is fruitful for the taxonomic works for the Flora of Thailand in the future.

## Materials and Methods

### 1. Taxonomic Study

Taxonomic study was carried out by diagnosing through details of corolla

aestivation, cystolith, retinacula, number of stamen and seed data followed Benoist (1936), Cramer (1998) and Scotland & Vollesen (2000). The literature and herbarium specimens of the subtribe Andrographinae were examined at Thai and foreign herbaria as follows: Bangkok Herbarium, Department of Agriculture (BK), Forest Herbarium, National Park, Wildlife and Plant Conservation Department (BKF), Queen Sirikit Botanic Garden Herbarium, The Botanical Organization, Ministry of Natural Resources and Environment (QBG), the Biological Department Herbarium, Chiang Mai University (CMU), Prince of Songkhla University Herbarium (PSU), Royal Botanic Gardens, Kew (K). Field surveys and specimen collections were conducted throughout the country by choosing the three represented conserved area from each region, such as north, north-eastern, eastern, south-western, eastern, central, south-eastern and peninsular Thailand. Measurement of vegetative and reproductive features of dried and spirit specimens collected by the author, and herbarium specimens deposited in the above Thai and foreign herbaria are

carried out by using a hand ruler or scale calibrated in cm and mm under the stereomicroscope for the characters of indumentum, leaves and flowers. Illustrations and photographs are provided for clarification. The herbarium specimens together with the voucher specimens are identified by taxonomic literature and the comparison with the type specimens. Preparation the keys to subtribe, genera and species based on the significant morphological characters are constructed to prove their taxonomic units. The study on the subtribe Andrographinae, family Acanthaceae in Thailand during October, 2009 to September, 2010.

## **2. Pollen morphology**

Selected pollen of some species were collected to be fixed in 3 % glutaraldehyde in phosphate buffer and 1% osmium tetroxide. (Hopwood and Milne, 1991). The SEM study was conducted by dropletting of pollen-ethanol sputter-coated with a platinum. Observation and measurements was conducted by using scanning electron microscopy (SEM).

## Results and Discussion

### 1. Taxonomic Study

The classification of Acanthaceae and taxonomic characters among this subtribe (4 genera) were listed in Table 1. Among this 4 genera, 23 taxa were enumerated in Thailand. Two taxa were belong to *Andrographis*, whereas had 1, 8 and 12 taxa were classified to the genera *Graphandra*, *Gymnostachyum* and *Phlogacanthus* respectively (Table 2).

#### **Andrographis Wall.**

Herbs or very small shrubs, erect or procumbent. Leaves opposite, simple, entire. Inflorescence: axillary and terminal racemes, lax and paniced, or dense, often 1-sided; bracts small; bracteoles 2 or absent. Calyx small, divided almost to the base into 5 sepals. Corolla small, obscurely or definitely bilabiate, with a short tubular base; lobes imbricated in the bud. Stamens 2, inderted near the mouth of the corolla tube; filaments ciliate or not; anthers included or exserted, bilocular, the cells equal or slightly unequal, parallel, muticous, bearded at the base; pollen “Daubenpollen” (Lindau) – grains ellipsoid, subglobose or obscurely trigonous, finely pitted, germ – pores 3 in

short and broad grooves. Ovary bilocular, 6-12 ovulate; disc fleshy; style minutely bifid at the apex. Capsule linear or oblong-elliptic, compressed at right angles to the septum. Seeds subglobose, glabrous, pitted. (Figure 1A)

There are about twenty species from India, and tropical Asia. Only two species are found in Thailand.

#### **Graphandra J.B. Imlay**

Small herb, procumbent. Leaves opposite, small, entire, sessile or subsessile. Inflorescence terminal and axillary; small simple bracteates spikes; flowers alternate; bracts opposite, more or less imbricated, larger than the calyx; bracteoles equaling or a little longer than the calyx. Calyx of 4 sepals free to the base. Corolla tube almost as long as the limb; limb bilabiate, the upper lip narrow, slightly concave, entire or subentire, the lower lip oblong, 3-lobed; lobes imbricated in the bud. Stamens 2, affixed to the corolla tube by a thin membrane, free in the mouth of the tube; anther cells 2 equal, parallel, oblong, minutely mucronate at the base, hairy but not bearded at the base; pollen “Daubenpollen” (Lindau) – almost globose, finely pitted, 3

**Table 1** Plant characters of 4 genera in the sub-tribe Andrographinae

Taxa	Habit	Inoescence					fruit/seed
		Inoescence type/bract	calyx	corolla	stamen	ovule	
<i>Andrographis</i> Wall. ex Nees	Annual herb	raceme/bract small	contracted	corolla sub-equally 5-lobed	2	6-12	linear/ seed attened/ 6-12 seeded
<i>Graphandra</i> J.B. Imlay	Annual herb	spike/bract larger than calyx	Small, calyx lobe 4, deeply lobed	corolla sub-equally 5-lobed	2, adnate to corolla tube	12	oblong/ 12- seeded
<i>Gymnostachyum</i> Nees	Annual herb	spike	small, calyx lobe 5,	bilabiate/ corolla tube long	2	6 ovules/ many ovules	cylindrical/8-20 seeded
<i>Phlogacanthus</i> Nees	Annual / perennial herb	panicle	Corolla lobe 5 shallowly lobed	corolla sub-equally 5-lobed, corolla tube ventricose, curved towards the throat/ straight	2	10-16	cylindrical/10- 16 seeded or many seeds

germ – pores in 3 very small grooves. Ovary bilocular, 12 – ovulate; style minutely bifid at the apex. Capsule oblong, scarcely flattened, not stalked, 12 – seeded from the base. Seeds flattened, smooth, almost glabrous. (Figure 1B) Only one species is listed in Thailand

### **Gymnostachyum Nees**

Herbs or under shrubs, erect, often sub-acauline. Leaves cauline or almost radical, opposite, equal, entire or

subentire. Inflorescence terminal and axillary, simple, branched or paniced; flowers. Flowers opposite or alternate, solitary, clustered or cymose, sessile or subsessile, forming simple spikes or terminal lax panicles, sometimes unilateral; bracts and 2 bracteoles very call. Calyx 5- lobed almost to the base; sepals subequal, the posterior sometimes smaller than the others. Corolla tube slender straight or slightly curved, narrow at the base then ridening slightly

**Table 2** List of taxa in the subtribe Andrographinae, family Acanthaceae in Thailand.

Taxa	Specimen Investigation					Document					
	BK	BKF	CMU	PSU	K	1	2	3	4	5	6
<i>Andrographis laxiora</i> Lindau	x	x	x	x	x			x		x	x
<i>A. paniculata</i> Nees	x	x		x	x		x	x		x	x
<i>Graphandra procumbens</i> Imlay	x				x			x			
<i>Gymnostachyum canescens</i> T.Anderson	x	x			x		x	x			
<i>Gymnostachyum coriaceum</i> Imlay	x	x			x	x		x		x	
<i>Gymnostachyum gracile</i> Bremek.	x	x			x			x			
<i>Gymnostachyum hirtum</i> Ridl.	x	x			x			x		x	
<i>Gymnostachyum leptostachyum</i> Nees.	x	x			x		x	x	x		
<i>Gymnostachyum signatum</i> (Benoist) Imlay	x	x			x			x			
<i>Gymnostachyum trilobum</i> Ridl.	x	x			x		x	x			
<i>Gymnostachyum venustum</i> T. Anderson	x	x			x			x			
<i>Phlogacanthus asperulus</i> Nees	x	x			x			x			
<i>Phlogacanthus colaniae</i> Bebiust & Benoist					x						
<i>Phlogacanthus curviorus</i> Nees.	x	x			x		x	x	x		x
<i>Phlogacanthus murtonii</i> Craib	x	x			x			x			
<i>Phlogacanthus paniculatus</i> (T.Anderson) Imlay	x				x			x			
<i>Phlogacanthus pauciorus</i> Imlay	x	x	x		x			x			
<i>Phlogacanthus pedunculatus</i> Imlay	x	x			x		x	x	x		
<i>Phlogacanthus pulcherrimus</i> T. Anderson	x	x	x		x			x			
<i>Phlogacanthus racemosus</i> Bremek.	x				x			x	x		
<i>Phlogacanthus rectorius</i> Imlay	x				x	x		x			
<i>Phlogacanthus thysiorus</i> Nees.			x	x	x			x			x
<i>Phlogacanthus vitellinus</i> T. Anderson	x	x			x			x	x		

Remarks: BK = Bangkok Herbarium, Thailand BKF = The Forest Herbarium, Thailand CMU = Chiang Mai University Herbarium, Thailand; PSU = Prince of Songkla University Herbarium, Thailand; K = Botanic Gardens, Kew Herbarium, UK (1) = Clarke (1885); (2) = Benoist (1935); (3) = Imlay (1939); (4) = Bremekamp (1965); (5) = Cramer (1998); (6) = The Forest Herbarium (2001)

upwards; limb spreading, bilabiate; upper lip shortly 2-lobed or emarginated; lower lip shortly 3-lobed, the lobes imbricated in the bud. Stamens 2, inserted below the middle of the corolla tube; anthers included or sub-exsert, bilocular; loculi oblong, muticous or very shortly

mucronata at the base; pollen “Daubenpollen” (Lindau) – grains ellipsoid and finely pitted, “Daubenpollen” (Lindau) – grains ellipsoid and finely pitted, with 3 short and broad grooves pointed at both ends and Uned with 2 rows of minute papillae, containing the 3 germ – pores.

Every bilocular, 8 – 30 – ovulate; disc conspicuous, rather fleshy; style minutely bifid at the apex. Capsule linear, terete or cotusely 4 – sided, seed – bearing from the base, each valve 3 – grooved seeds many, obliquely ovate, flattened, covered with hygroscopic hairs; ejaculatura hard curved. (Figure 1C-1F) There are about

forty species from tropical Asia and Malaysia. Nine species are enumerated from Thailand.

### Phlogacanthus Nees

Broad; midrib conspicuous above, prominent below; lateral nerves 6-8 pairs, conspicuous above, prominent below;



**Figure 1** Plant forms in some species of *Andrographis*, *Graphandra* and *Gymnostachyum* (A) *Andrographis laxiflora*, (B) *Graphandra procumbens* (C) *Gymnostachyum leptostachyum*, (D) *G. signatum* (E) *G. trilobum* (F): *G. venustum*

margin subentire, glabrous; petiole 1-1.5 cm long, glabrous. Inflorescence a narrow terminal panicle, 2-6 cm long, 1-2 cm broad; upper flowers solitary, opposite, the lower flowers in small shortly peduncled cymes; pedicels ca 3 mm long or less; peduncles and pedicels shortly pubescent; bracts at the base of the pedicel, lanceolate, acuminate, shortly pubescent, 3-4 mm long; bracteoles 1.5 mm long, linear, shortly pubescent, affixed about the middle of pedicel. Calyx shortly

pubescent outside, 4.5-5 mm long; sepals lanceolate, acuminate, united at the base for ca 1.25 mm, shortly pubescent inside towards the apex, minutely ciliate, 3-nerved. Corolla pink with yellow patch on lower lip (ex Kerr), or red, densely shortly pubescent outside, many hairs glandular, 0.8-1.2 cm long; tube narrow at the base, then suddenly curved and inflated, glabrous inside except for an incomplete ring of short hairs near the throat; limb shortly obscurely bilabiate; lobes 3-5 mm



**Figure 2** Plant forms in some species of *Phlogacanthus* (A) *Phlogacanthus curviflorus*, (B) *P. pauciflorus*, (C) *P. pedunculatus*, (D) *P. pulcherrimus*, (E) *P. rectiflorus*, (F.) *P. vitellinus*



long, ovate or ovate-oblong, acute, puberulous inside towards the margin. Stamens 2 fertile and 2 minute rudiments inserted 5 mm from the base of the corolla; filaments 6mm. long, curved, shortly hairy at the base; anthers exserted, oblong 2.5 mm long, glandular at the back. Ovary glabrous. 8-ovulate; style 9 mm long, glabrous. Capsule 1.7 cm long, glabrous, 8-seeded from the base. (Figure 2) There are about twenty species from tropical India, and Indo-China. Twelve species are found in Thailand.

## **2. Distribution of subtribe Andrographinae, family Acanthaceae in Thailand**

The field survey of 4 genera were reported with distribution map in 8 floristic regions of Thailand, North, North-eastern, Eastern, South-western, Eastern, Central, South-eastern and peninsular Thailand. The results showed that both of *Andrographis laxiflora* (Blume) Lindau and *A. paniculata* (Burm.) Wall ex Nees were found in North, South-east and Central. *A. laxiflora* was found in East and South – West (Figure 3A). *Gymnostachyum canescens* (Nees) T. Anders. was found in North and South-west. *G. coriaceum*

Imlay was found in north-west. *G. decurrens* Stapf and *G. hirtum* Ridl. were found in South-west (Figure 2B). *G. leptostachyum* Nees were found in North-east and South-east. *G. signatum* (Benoist) Imlay and *G. trilobatum* Ridl. were found in South-east and peninsular. *G. venustum* (Wall.) T. Anders. was found in South-west (Figure 3C). *Phlogacanthus asperulus* was found in north. *P. colaniae* Benoist was found in South-east. *P. curviflorus* (Wall.) Nees was found in North and South-west. *P. murtonii* Craib was found in South-east region. (Figure 3D). *P. paniculatus* (T. Anders.) J.B. Imlay was found in North and North-east. *P. pauciflorus* J.B. Imlay was found in North. *P. pedunculatus* J.B. Imlay was found in North and North-west. *P. pulcherrimus* T. Anders. was found in East and peninsular (Figure 3E). *P. racemosus* Brem. was found in North. *P. rectiflorus* J.B. Imlay and *P. thyrsoiflorus* Nees were found in peninsular. *P. vitellinus* T. Anders. was found in South-east (Figure 3F). *Graphandra procumbens* J.B. Imlay was found as only one species at Tha Uthen District, Nakhon Phanom Province in North-east.



*Andrograhis* Wall.



*Gymnostachyum* Nees (1)



*Gymnostachyum* Nees (2)



*Phlogacanthus* Nees (1)



*Phlogacanthus* Nees (2)



*Phlogacanthus* Nees (3)

**Figure 3** Distribution maps of subtribe Andrograpinae in Thailand (A) *Andrograhis* Wall. (B) *Gymnostachyum* Nees 1. (C) *Gymnostachyum* Nees 2 (D) *Phlogacanthus* Nees 1 (E) *Phlogacanthus* Nees 2 (F) *Phlogacanthus* Nees 3

## 2. Pollen Morphology

The pollen types within subtribe Andrographinae has the evidence for the discovery of taxa. As in the study of Scotland and Vollesen (2000) it may be that pollen morphology is the most accurately utilized and informative at fairly low taxonomic levels, and best used in studies of individual genera and relationships between small groups of genera. The preliminary results showed that the shape and ornamentation were recognized the differences among genera.

The pollen types from the selected species of 3 genera (*Andrographis*, *Gymnostachyum* and *Phlogacanthus*) are investigated (Figure 4) as follows:

*Andrographis laxiflora* : tricolporate, distinct aperture margins (Figure 4A).

*Gymnostachyum leptostachyum*: tricolporate, open reticulate tectum with scattered granules (Figure 4B).

*G. signatum*: tricolporate, open reticulate tectum (Figure 4C).

*G. trilobum*: tricolporate, open reticulate tectum (Figure 4D).

*G. venustum*: tricolporate, open reticulate tectum (Figure 4E).

*Phlogacanthus curviflorus*:

tricolporate, distinct aperture margins (Figure 4F).

*P. pauciflorus*: tricolporate, distinct aperture margins (Figure 4G).

*P. pedunculatus*: tricolporate, distinct aperture margins (Figure 4H).

*P. pulcherrimus*: tricolporate, distinct aperture margins (Figure 4I).

*P. rectiflorus*: tricolporate, distinct aperture margins (Figure 4J).

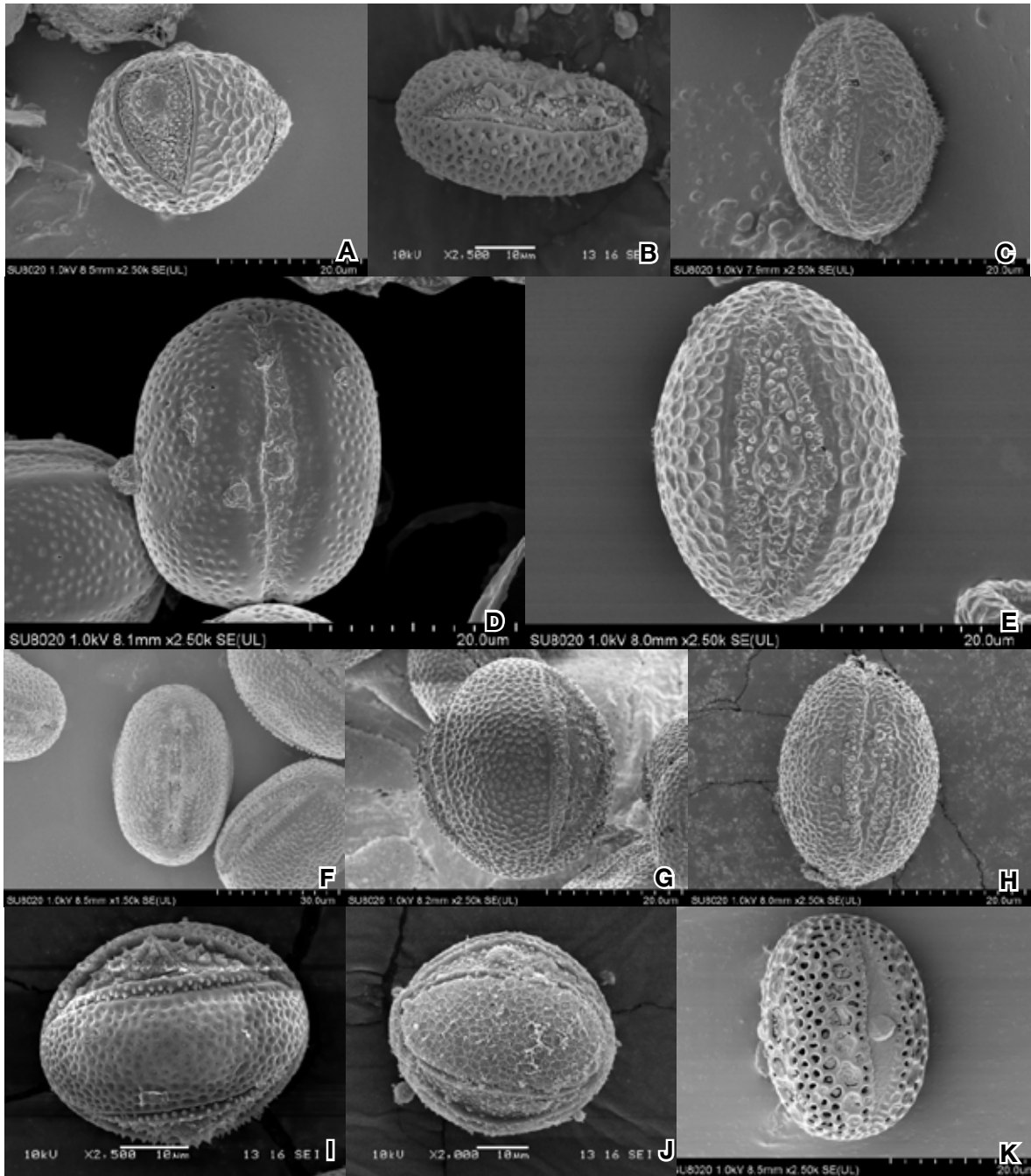
*P. vitellinus*: tricolporate, distinct aperture margins (Figure 4K).

## Conclusions

The overview characters of the subtribe Andrographinae comprise corolla imbricate or ascending cochlear aestivation; stamen 2; ovules 6 – many, capsule extending to the base, bearing 6 ovules – many; pollen tricolporate with distinct aperture margin.

The differences in morphological characters among them were the ones that bear the corolla sub-equally 5-lobed; sepal 4 or 5 (*Andrographis*, *Grapahndra*, *Phlogacanthus*) and the others with the corolla bilabiate; sepal 5 (*Gymnostachyum*).

The number of the subtribe Andrographinae were enumerated into 23 species, *Andrographis* (2), *Grapahndra* (1),



**Figure 4** Pollen types, (A) *Andrographis laxiflora*; (B) *Gymnostachyum leptostachyum*; (C) *G. signatum*; (D) *G. trilobatum*; (E) *G. venustum*; (F) *Phlogacanthus curviflorus* (G) *P. pauciflorus*; (H) *P. pedunculatus*; (I) *P. pulcherrimus*; (J) *P. rectiflorus*; (K) *P. vitellinus*

*Gymnostachyum* (8), and *Phlogacanthus* (12)

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