forests

MDPI

Article

Morphological Systematics of *Spathoglottis* Blume (Orchidaceae: Collabieae) in Peninsular Malaysia and Borneo

Farah Alia Nordin [1], Akmal Raffi [2], Rusea Go [3], Christina Seok Yien Yong [3], Kartini Saibeh [4] and Ahmad Sofiman Othman [1,*]

[1] School of Biological Sciences, Universiti Sains Malaysia, Gelugor 11800, Penang, Malaysia; farahalianordin@usm.my
[2] Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Kota Samarahan 94300, Sarawak, Malaysia; mrmakmal@unimas.my
[3] Department of Biology, Faculty of Science, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia; rusea@upm.edu.my (R.G.)
[4] Faculty of Tropical Forestry, Jalan UMS, Universiti Malaysia Sabah, Kota Kinabalu 88400, Sabah, Malaysia; k_saibeh@ums.edu.my
* Correspondence: sofiman@usm.my; Tel.: +60-46534019

Abstract: Seventy-two morphological characters and three ecological characteristics were measured to assess variation and phylogenetic relationships among twelve species and three infraspecific taxa of the genus *Spathoglottis* from Peninsular Malaysia and Borneo. The morphological analyses divided *Spathoglottis* into two main groups based on the colours of the flower: Purple-Flowered *Spathoglottis* and Yellow-Flowered *Spathoglottis*. Species within the two groupings were further classified based on the size of the plants (Large/Dwarf Purple *Spathoglottis* and Large/Dwarf Yellow *Spathoglottis*) and the shapes of the labellum (spathulate, bilobulate or narrow/thread–like). The selected morphological characters appeared to support the taxonomic boundaries between two mostly debated taxa in the genus, *S. aurea* and *S. microchilina*.

Keywords: Malesia; morphology; orchids; phylogeny; systematics; taxonomy; ecology

Citation: Nordin, F.A.; Raffi, A.; Go, R.; Seok Yien Yong, C.; Saibeh, K.; Othman, A.S. Morphological Systematics of *Spathoglottis* Blume (Orchidaceae: Collabieae) in Peninsular Malaysia and Borneo. *Forests* **2023**, *14*, 940. https://doi.org/10.3390/f14050940

Academic Editor: Jianquan Liu

Received: 21 March 2023
Revised: 27 April 2023
Accepted: 28 April 2023
Published: 3 May 2023

1. Introduction

Forty-nine species of the genus *Spathoglottis* Blume (subfamily Epidendroideae, tribe Collabieae) are recognized worldwide, and of these, forty-four species are predominantly Malesian [1] (Figure 1). There are eight species and three infraspecific taxa native to Peninsular Malaysia and Borneo, namely *Spathoglottis affinis* de Vriese, *S. aurea* Lindl., *S. confusa* J.J.Sm., *S. gracilis* Rolfe ex Hook.f., *S. hardingiana* C.S.P.Parish & Rchb.f., *S. kimballiana* Hook.f., *S. kimballiana* var. *angustifolia* Ames, *S. kimballiana* var. *kimballiana*, *S. microchilina* Kraenzl., *S. plicata* Blume and *S. plicata* var. *alba*.

In the western part of Malesia, early taxonomical works on the genus *Spathoglottis* have been carried out by several authors for Peninsular Malaysia [2–4], the Borneo Islands of Sabah and Sarawak [5–8] and from Java and Sumatra [9,10]. In spite of this, a comprehensive revision on *Spathoglottis* in Peninsular Malaysia and Borneo is almost unavailable, worsened by the small numbers of specimens deposited in the herbaria, some of which have been wrongly identified.

Moving northwards crossing the Peninsular Malaysia–Thailand political border, taxonomic treatments for the Indochinese *Spathoglottis* were made available from two prominent contributions [11,12]. Meanwhile, early taxonomic revision on this genus was first discussed for the Australian *Spathoglottis*, later followed by revisions for the Pacific Islands and the New Caledonian species [13–15]. Surprisingly, even though almost half of *Spathoglottis* species are concentrated in New Guinea, knowledge on the genus from this part of East Malesia is rather scarce. Similarly, *Spathoglottis* from central Malesia (Wallacea), which

includes the Philippines, Sulawesi, Lesser Sunda Islands and Maluku Islands, has been poorly studied.

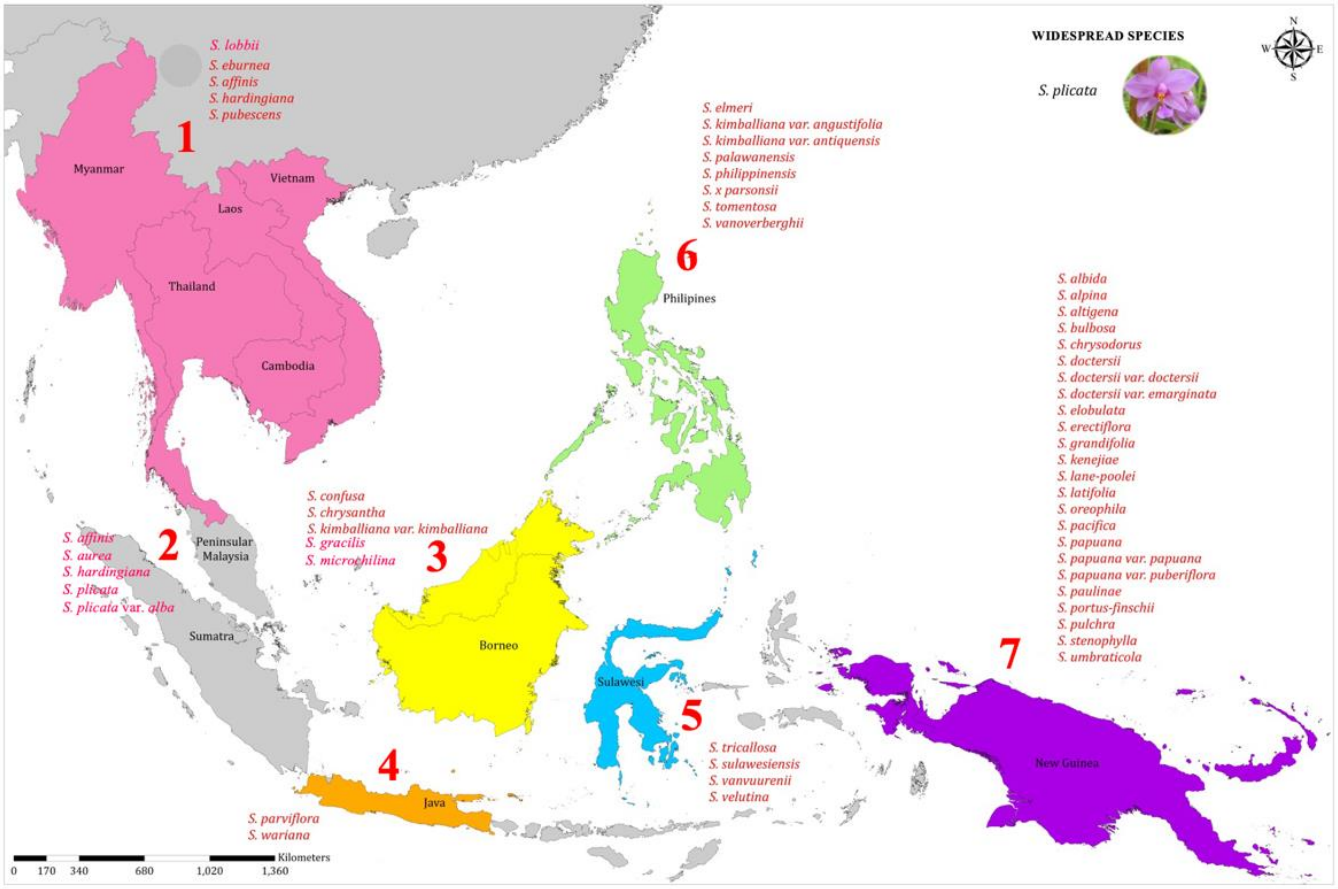


Figure 1. Distribution of *Spathoglottis* in Indo–China and throughout Malesia. Each numbering represents individual island grouping, with almost no cross-over in species occurrences: (1) Indo–China, (2) Peninsular Malaysia and Sumatra, (3) Borneo, (4) Java, (5) Sulawesi, (6) the Philippines and (7) New Guinea. *Spathoglottis plicata* is a widespread species throughout Malesia.

Species within the genus are morphologically still poorly defined. Classifications based on morphology have utilized both quantitative and qualitative characters for the informal groupings within *Spathoglottis* [3,4]. Nevertheless, this classification was made based on limited measurement of morphological characters. Species within *Spathoglottis* were first recognized by the colours of the flower, followed by shapes of the labellum (lip)/midlobe, size of the flowers, shapes of the sidelobes, and size of the sepals in proportion to the petals. However, a detailed study of the morphological variation, using analysis of many traits in resolving the taxonomic boundaries among species of the genus, has not previously been undertaken.

Meanwhile, analyses of independent gene regions coupled with combined dataset using molecular markers have proven that *Spathoglottis* is monophyletic. The phylogenetic relationships among species of *Spathoglottis* from Peninsular Malaysia and Borneo were successfully resolved, and species boundaries were successfully circumscribed [16,17]. At the genetic level, the split within *Spathoglottis* reflects an early differentiation of plant size, flower colours and flower size. Nonetheless, no attempt has ever been made to elucidate the phylogenetic patterns within the genus using a detailed study of the morphological variation. Thus, the present work aimed to explain species relationships between members of the genus *Spathoglottis* from Peninsular Malaysia and Borneo using morphological and ecological variation, and to resolve the taxonomic questions between the controversial narrow-lip *S. aurea* and *S. microchilina.*

2. Materials and Methods

2.1. Taxon Sampling

A total of 103 plant samples from 12 species and 3 infraspecific taxa of *Spathoglottis* were analysed in this present work as listed in Table 1. It was sufficient to use one outgroup only, namely *Tainia paucifolia* (Breda) J.J.Sm. from tribe Collabieae, as *Spathoglottis* is already known to be monophyletic. The plant samples were obtained from different localities throughout Peninsular Malaysia and Borneo (Sabah and Sarawak). Plant samples for the six *Spathoglottis* species from Thailand, Sumatra, Irian Jaya, Maluku Island and New Caledonia were provided by our collaborators. The samples were also included in this detailed study to examine discreet evolutionary relationships between members of the genus based on morphological character and ecological characteristic analyses.

Table 1. Species list, collection localities and herbarium voucher numbers of the *Spathoglottis* species and outgroup used in this present work.

	Species	Collection Number/Voucher Number	Collection Locality	Altitude (m)
1.	*Spathoglottis affinis* de Vriese	FAN020/USMP12177	Ton Nga Chang, Songkhla, Thailand	100
2.	*Spathoglottis affinis* de Vriese	FAN023/USMP12178	Fang District, Chiang Mai, Thailand	1000
3.	*Spathoglottis affinis* de Vriese	FAN025/USMP12179	Thailand–Myanmar Border	1000
4.	*Spathoglottis affinis* de Vriese	FAN028/USMP12180	Padang Tok Sheikh, G. Jerai, Kedah	1131
5.	*Spathoglottis aurea* Lindl.	FAN006/USMP12181	G. Ledang, Johor	1200
6.	*Spathoglottis aurea* Lindl.	FAN008/USMP12182	G. Lari Tembakau, Pahang	1800
7.	*Spathoglottis aurea* Lindl.	FAN009/USMP12183	G. Ulu Kali, Pahang	1800
8.	*Spathoglottis aurea* Lindl.	FAN018/USMP12184	G. Jerai, Kedah	989
9.	*Spathoglottis aurea* Lindl.	FAN019/USMP12185	Taman Rimba, G. Jerai, Kedah	986
10.	*Spathoglottis aurea* Lindl.	FAN026/USMP12186	Taman Rimba, G. Jerai, Kedah	990
11.	*Spathoglottis aurea* Lindl.	FAN030/USMP12187	Fraser's Hill, Pahang	1300
12.	*Spathoglottis aurea* Lindl.	FAN031/USMP12188	Fraser's Hill, Pahang	1300
13.	*Spathoglottis aurea* Lindl.	FAN032/USMP12189	Fraser's Hill, Pahang	1292
14.	*Spathoglottis aurea* Lindl.	FAN035/USMP12190	Mile 49, Tanah Rata, Cameron Highlands, Pahang	1400
15.	*Spathoglottis aurea* Lindl.	FAN036/USMP12191	Mile 39, Tanah Rata, Cameron Highlands, Pahang	1410
16.	*Spathoglottis aurea* Lindl.	FAN037/USMP12192	G. Brinchang, Pahang	1800
17.	*Spathoglottis aurea* Lindl.	FAN038/USMP12193	G. Brinchang, Pahang	1600
18.	*Spathoglottis aurea* Lindl.	FAN039/USMP12194	G. Ulu Kali, Pahang	1800
19.	*Spathoglottis aurea* Lindl.	FAN040/USMP12195	G. Chin Chin, Pahang	1800
20.	*Spathoglottis aurea* Lindl.	FAN041/USMP12196	G. Brinchang, Pahang	1800
21.	*Spathoglottis aurea* Lindl.	FAN044/USMP12197	Padang Tok Sheikh, G. Jerai, Kedah	1200
22.	*Spathoglottis aurea* Lindl.	FAN052/USMP12198	G. Ulu Kali, Pahang	1800
23.	*Spathoglottis aurea* Lindl.	FAN053/USMP12199	G. Ulu Kali, Pahang	1800

Table 1. *Cont.*

	Species	Collection Number/Voucher Number	Collection Locality	Altitude (m)
24.	*Spathoglottis aurea* Lindl.	FAN054/USMP12200	Padang Tok Sheikh, G. Jerai, Kedah	1200
25.	*Spathoglottis aurea* Lindl.	FAN057/USMP12201	G. Bunga Buah, Selangor	1400
26.	*Spathoglottis aurea* Lindl.	FAN058/USMP12202	G. Bunga Buah, Selangor	1400
27.	*Spathoglottis aurea* Lindl.	FAN059/USMP12203	G. Bunga Buah, Selangor	1400
28.	*Spathoglottis aurea* Lindl.	FAN060/USMP12204	G. Bunga Buah, Selangor	1400
29.	*Spathoglottis aurea* Lindl.	FAN069/USMP12205	Tanah Rata, Cameron Highlands, Pahang	1419
30.	*Spathoglottis aurea* Lindl.	FAN070/USMP12206	Tanah Rata, Cameron Highlands, Pahang	1419
31.	*Spathoglottis aurea* Lindl.	FAN071/USMP12207	Huta Tinggi, Samarindo, Samosir, North Sumatra	1400
32.	*Spathoglottis aurea* Lindl.	FAN098/USMP12208	Tanah Rata, Cameron Highlands, Pahang	1419
33.	*Spathoglottis aurea* Lindl.	FAN099/USMP12209	Tanah Rata, Cameron Highlands, Pahang	1419
34.	*Spathoglottis eburnea* Gagnep.	FAN022/USMP12210	Fang District, Chiang Mai, Thailand	1000
35.	*Spathoglottis gracilis* Rolfe ex Hook.f.	FAN094/USMP12211 KIP1266f	Kg. Liposu, Ranau, Sabah	850
36.	*Spathoglottis hardingiana* C.S.P.Parish & Rchb.f.	FAN016/USMP12212	G. Baling, Kedah	450
37.	*Spathoglottis hardingiana* C.S.P.Parish & Rchb.f.	FAN056/USMP12213	G. Pong, Kenering, Perak	420
38.	*Spathoglottis hardingiana* C.S.P.Parish & Rchb.f.	FAN105/USMP12214 K20160013	Tg. Asan, Pulau Timun, Langkawi, Kedah	200
39.	*Spathoglottis kimballiana* Hook.f.	FAN085/USMP12215	Ranau, Sabah	1300
40.	*Spathoglottis kimballiana* var. *angustifolia* Ames	FAN076/USMP12216 THH13 6 99	Bidu Bidu FR, Telupid, Sabah	71
41.	*Spathoglottis kimballiana* var. *angustifolia* Ames	FAN077/USMP12217	Bidu Bidu FR, Telupid, Sabah	71
42.	*Spathoglottis kimballiana* var. *angustifolia* Ames	FAN104/USMP12218	Sungai Tongod, Telupid, Sabah	60
43.	*Spathoglottis kimballiana* var. *kimballiana*	FAN067/USMP12219	Mt. Kinabalu, Ranau, Sabah	1300
44.	*Spathoglottis kimballiana* var. *kimballiana*	FAN092/USMP12220	Kota Belud, Sabah	800
45.	*Spathoglottis kimballiana* var. *kimballiana*	FAN093/USMP12221	Pekan Nabalu, Ranau, Sabah	1400
46.	*Spathoglottis microchilina* Kraenzl.	FAN082/USMP12222	Bundu Tuhan View Trail, Kinabalu Park, Sabah	1601
47.	*Spathoglottis microchilina* Kraenzl.	FAN083/USMP12223	Kinabalu Park Research Centre, Sabah	1599
48.	*Spathoglottis microchilina* Kraenzl.	FAN084/USMP12224	Kinabalu Park, Sabah	850
49.	*Spathoglottis microchilina* Kraenzl.	FAN086/USMP12225	Mamut Copper Mine, Sabah	1324

Table 1. *Cont.*

	Species	Collection Number/Voucher Number	Collection Locality	Altitude (m)
50.	*Spathoglottis microchilina* Kraenzl.	FAN087/USMP12226	Mamut Copper Mine, Sabah	1485
51.	*Spathoglottis microchilina* Kraenzl.	FAN088/USMP12227	Mamut Copper Mine, Sabah	1372
52.	*Spathoglottis microchilina* Kraenzl.	FAN089/USMP12228	Mamut Copper Mine, Sabah	1405
53.	*Spathoglottis microchilina* Kraenzl.	FAN091/USMP12229	Sg. Lohan, Ranau, Sabah	1375
54.	*Spathoglottis microchilina* Kraenzl.	FAN095/USMP12230	Tambunan, Sabah	1000
55.	*Spathoglottis microchilina* Kraenzl.	FAN096/USMP12231	Ranau, Sabah	850
56.	Spathoglottis parviflora Kraenzl.	FAN061/USMP12232	Wamena, Irian Jaya	1679
57.	*Spathoglottis plicata* Blume	FAN001/USMP12233	Peak of G. Ledang, Johor	1000
58.	*Spathoglottis plicata* Blume	FAN002/USMP12234	Peak of G. Ledang, Johor	1100
59.	*Spathoglottis plicata* Blume	FAN003/USMP12235	Penang Hill, Penang	686
60.	*Spathoglottis plicata* Blume	FAN004/USMP12236	Penang Hill, Penang	735
61.	*Spathoglottis plicata* Blume	FAN005/USMP12237	Sungai Rui, Kedah	700
62.	*Spathoglottis plicata* Blume	FAN007/USMP12238	K. Kubu Bharu, Selangor	1392
63.	*Spathoglottis plicata* Blume	FAN010/USMP12239	G. Ulu Kali, Pahang	1800
64.	*Spathoglottis plicata* Blume	FAN011/USMP12240	G. Jerai, Kedah	980
65.	*Spathoglottis plicata* Blume	FAN012/USMP12241	G. Jerai, Kedah	1200
66.	*Spathoglottis plicata* Blume	FAN015/USMP12242	Gerik, Perak	1100
67.	*Spathoglottis plicata* Blume	FAN017/USMP12243	Baling, Kedah	650
68.	*Spathoglottis plicata* Blume	FAN021/USMP12244	Peak of G. Jerai, Kedah	1210
69.	*Spathoglottis plicata* Blume	FAN027/USMP12245	G. Jerai, Kedah	1000
70.	*Spathoglottis plicata* Blume	FAN029/USMP12246	G. Jerai, Kedah	1100
71.	*Spathoglottis plicata* Blume	FAN033/USMP12247	Raub, Pahang	1000
72.	*Spathoglottis plicata* Blume	FAN042/USMP12248	Taiping Hill, Perak	1200
73.	*Spathoglottis plicata* Blume	FAN043/USMP12249	G. Jerai, Kedah	926
74.	*Spathoglottis plicata* Blume	FAN046/USMP12250	Taman Negara Endau Rompin, Johor	78
75.	*Spathoglottis plicata* Blume	FAN047/USMP12251	Taman Negara Endau–Rompin, Johor	80
76.	*Spathoglottis plicata* Blume	FAN048/USMP12252	Kota Tinggi Waterfall, Johor	100
77.	*Spathoglottis plicata* Blume	FAN049/USMP12253	Ladang Lok Heng, Kota Tinggi, Johor	40
78.	*Spathoglottis plicata* Blume	FAN050/USMP12254	Jalan Mersing, Kahang, Johor	76
79.	*Spathoglottis plicata* Blume	FAN051/USMP12255	Peak of G. Ledang, Johor	1200
80.	*Spathoglottis plicata* Blume	FAN055/USMP12256	G. Jerai, Kedah	1200
81.	*Spathoglottis plicata* Blume	FAN063/USMP12257	Lata Chemerong, Terengganu	200

Table 1. *Cont.*

	Species	Collection Number/Voucher Number	Collection Locality	Altitude (m)
82.	*Spathoglottis plicata* Blume	FAN064/USMP12258	Lata Chemerong, Terengganu	70
83.	*Spathoglottis plicata* Blume	FAN065/USMP12259	Lata Chemerong, Terengganu	110
84.	*Spathoglottis plicata* Blume	FAN066/USMP12260	Lata Chemerong, Terengganu	70
85.	*Spathoglottis plicata* Blume	FAN072/USMP12261	Tasik Kenyir, Terengganu	30
86.	*Spathoglottis plicata* Blume	FAN073/USMP12262	Mile 49, Tamparuli to Ranau Road, Sabah	839
87.	*Spathoglottis plicata* Blume	FAN074/USMP12263	Jalan Kaung to Ranau, Kota Belud, Sabah	880
88.	*Spathoglottis plicata* Blume	FAN075/USMP12264	Kg. Labong–Labong, Kota Belud, Sabah	800
89.	*Spathoglottis plicata* Blume	FAN078/USMP12265	Sg. Keripir, 45 miles to Tambunan, Sabah	750
90.	*Spathoglottis plicata* Blume	FAN079/USMP12266	Tambunan Road, Sabah	757
91.	*Spathoglottis plicata* Blume	FAN080/USMP12267	Tambunan Road, Sabah	757
92.	*Spathoglottis plicata* Blume	FAN081/USMP12268	Tambunan Road, Sabah	468
93.	*Spathoglottis plicata* Blume	FAN090/USMP12269	Mamut Copper Mine, Sabah	1405
94.	*Spathoglottis plicata* Blume	FAN097/USMP12270	Hulu Telom, Pahang	708
95.	*Spathoglottis plicata* Blume	FAN100/USMP12271	Semengoh Nature Reserve, Sarawak	46
96.	*Spathoglottis plicata* Blume	FAN101/USMP12272	Semengoh Nature Reserve, Sarawak	50
97.	*Spathoglottis plicata* Blume	FAN102/USMP12273	Bario, Sarawak	1065
98.	*Spathoglottis plicata* Blume	FAN103/USMP12274	Long Baleh, Sarawak	944
99.	*Spathoglottis plicata* var. *alba*	FAN034/USMP12275	Lata Tembakah, Terengganu	36
100.	*Spathoglottis plicata* var. *alba*	FAN045/USMP12276	Lata Tembakah, Terengganu	36
101.	*Spathoglottis pubescens* Lindl.	FAN068/USMP12277	Fang District, Chiang Mai, Thailand	800
102.	*Spathoglottis unguiculata* (Labill.) Rchb.f.	FAN024/USMP12278	Isle of Pines, New Caledonia	200
103.	*Spathoglottis vanvuurenii* J.J.Sm.	FAN062/USMP12279	Seram, Maluku Island	834
104.	*Tainia paucifolia* (Breda) J.J.Sm.	FAN597/USMP12280	Taman Rimba Kenong, Pahang	20 m

The species list, localities and voucher information for all *Spathoglottis* species and the outgroup used are listed in Table 1. All voucher specimens were deposited in the Herbarium, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia (USMP) [18].

2.2. Species Identification and Enumeration

The *Spathoglottis* species were identified using the morphological characters described and identification keys prepared by preceding authors [2–11]. The scientific names adopted here are those accepted by the latest Kew's Plants of the World Online, accessed via the web [1].

2.3. Morphological Character and Ecological Characteristic Analyses

Observations on the vegetative and reproductive macromorphological characters were made in the field. The geographical, ecological and geological attributes were also documented. For detailed micromorphological characters, specimens were examined under a stereo microscope, particularly on the parts of the flower.

In this study, morphological revision of *Spathoglottis* was also conducted using ordinary practices of herbarium taxonomy. The morphological characters were thoroughly examined and compiled from various literature searches [2–15] and herbarium specimen holdings of the Kew Royal Botanic Gardens Herbarium (K), Leiden University Herbarium (L), Singapore Botanic Gardens Herbarium (SING), Australian National Herbarium (CANB), Forest Research Institute Malaysia Herbarium (KEP), University of Malaya Herbarium (KLU), Universiti Kebangsaan Malaysia Herbarium (UKMB), Universiti Putra Malaysia Herbarium (UPM), Sarawak Forest Department Herbarium and Sabah Park (Mount Kinabalu) Herbarium. Collectively, more than 200 individuals of *Spathoglottis* have been examined in the wild. Each species is represented by at least three different flowering individuals, except for *S. eburnea*, *S. gracilis*, *S. pubescens*, *S. unguiculata* and *S. vanvuurenii*. This is due to the small population size (*S. gracilis*, for instance, is rather rare) and long dormancy period (for *S. eburnea* and *S. pubescens*, measurements were taken from flowering individuals of the whole population). From the analyses, 72 morphological characters and 3 ecological characteristics were measured quantitatively and qualitatively. These characters were gathered from the plant samples collected and all literature sources available, and by examining approximately 400 herbarium voucher specimens. Each of the characters are numerically coded as shown in Table 2.

Table 2. Morphological characters and ecological characteristics measured in this present work. The characters were measured quantitatively and qualitatively. Each of the character states are represented by a numerical code.

Character		Character States
Vegetative Characters		
1.	Plant size	0 = dwarf (≤ 30 cm tall), 1 = large (≥ 30 cm up to 2 m tall)
2.	Root shape	0 = thick, 1 = filiforme
3.	Pseudobulb shape	0 = ovoid, 1 = conical, 2 = flattened
4.	Colour of pseudobulb	0 = green, 1 = purple, 2 = white
5.	Fibres enclosing pseudo. bulb	0 = presence, 1 = absence
6.	Stem appearance	0 = distinct, 1 = indistinct
7.	Stem sheath	0 = presence, 1 = absence
8.	Stem colouration	0 = greenish, 1 = tinged with purple
9.	Number of leaves per pseu dobulb	0 = 1, 1 = 2–3, 2 = 4, 3 = > 4
10.	Leaf sheath attachment	0 = sheath clasping the stem, 1 = petiolated
11.	Leaf vernation	0 = plicate with strong midrib, 1 = non-plicate
12.	Leaf texture	0 = thin, 1 = coriaceous (tough), 2 = velvety
13.	Leaf ornamentation	0 = glabrous, 1 = pubescent
14.	Leaf margin	0 = entire, 1 = crisp
15.	Leaf shape	0 = linear (grass-like), 1 = linear–lanceolate, 2 = lanceolate, 3 = broadly elliptic
16.	Leaf apex	0 = acute, 1 = acuminate
17.	Leaf colouration	0 = greenish throughout, 1 = greenish-grey, 2 = green tinged with purple

Table 2. *Cont.*

Character	Character States
18. Leaf width	0 = grass-like (≤ 1.0 cm wide), 1 = narrow (c. 1.1–2.0 cm wide), 2 = medium (2.1–5.0 cm wide), 3 = very broad (>5.1 cm wide)
19. Leaf length	0 = short (≤15.0 cm long), 1 = medium (15.1–50.0 cm long), 2 = long (50.1–100 cm long), 3 = very long (>100.1 cm long)
Reproductive Characters	
20. Inflorescence habit	0 = shorter than plant, 1 = longer than plant
21. Inflorescence colouration	0 = green, 1 = purple
22. Inflorescence ornamentation	0 = glabrous, 1 = covered with fine hairs
23. Sterile bract shape	0 = elliptic, 1 = obovate
24. Floral bract senescence	0 = deciduous, 1 = persistent
25. Floral bract shape	0 = obovate, 1 = elliptic, 2 = triangular with wavy margin
26. Floral bract attachment	0 = free, 1 = clasping the peduncle
27. Floral bract colour	0 = green, 1 = purple
28. Floral bract texture	0 = hard, 1 = soft
29. Pedicel ornamentation	0 = glabrous, 1 = covered in fine hairs
30. Ovary swelling	0 = upon flower senescence, 1 = flower still in bud stage
31. Number of flowers per rachis	0 = 2–3 (<5 flowers), 1 = many up to 20 flowers
32. Flower development	0 = determinate (terminal flower mature first), 1 = indeterminate (flower at the bottom mature first), 2 = all bloom at once
33. Flower colour	0 = white, 1 = yellow, 2 = pink, 3 = mauve to deep purple
34. Flower size (when open)	0 = small (c. 3.0 cm in dimension), 1 = medium (c. 3.1–5.0 cm in dimension), 2 = large (>5.1 cm in dimension)
35. Shades of yellow	0 = creamy yellow, 1 = pale yellow, 2 = golden yellow, 3 = other shades (not yellow)
36. Flower resupination	0 = presence (resupinate), 1 = absence (non resupinate)
37. Flower pollination strategy	0 = insect pollinated, 1 = dimorphic cleistogamy, 2 = geitonogamy
38. Fruit set percentage	0 = 50% set into fruit, 1 = almost 100% set into fruit
39. Sepal and petal architecture	0 = spreading, 1 = drooping, 2 = turned backwards, 3 = remained closed
40. Sepal and petal size	0 = different in size, 1 = almost equal in width
41. Sepal shape	0 = ovate, 1 = elliptic, 2 = narrow–elliptic
42. Sepal texture	0 = thin, 1 = tough
43. Sepal colouration	0 = flush red or brown on the outer part, 1 = no colouration, 2 = streaks with red in the inner part
44. Sepal ornamentation	0 = hairy at base, 1 = hairy on the entire outer part, 2 = glabrous
45. Sepal apex	0 = obtuse, 1 = tapering towards end, 2 = thickened, 3 = mucronate
46. Lateral sepal arrangement	0 = free, 1 = concave, 2 = curved backwards
47. Sepal carination	0 = presence, 1 = absence
48. Petal shape	0 = obtuse, 1 = elliptic, 2 = oblong–elliptic
49. Labellum/lip shape	0 = narrow, 1 = spathulate, 2 = obcordate, 3 = oblong–obovate, 4 = fiddle-shaped, 5 = square
50. Lip size	0 = length half of the sepals, 1 = length as long as sepals
51. Lip margin	0 = incurved, 1 = entire, 2 = wavy

Table 2. *Cont.*

Character	Character States
52. Midlobe of lip	0 = thread-like, 1 = almost rounded, 2 = square, 3 = bilobulate
53. Width of midlobe	0 = mesochile narrow towards the end (≤4.0 mm wide), 1 = mesochile broad (c. 4.1–15 mm wide)
54. Lip apex	0 = dilate, 1 = pointed, 2 = truncate
55. Lip ornamentation	0 = keels or raised ridges presence, 1 = keels or raised ridges absence
56. Lip colouration	0 = spotted red or purple, 1 = splashed with yellow/white at the base
57. Sidelobe shape	0 = triangular, 1 = square, 2 = oblong, 3 = falcate, 4 = auriculate
58. Sidelobe colouration	0 = flushed with purple or crimson, 1 = splashed in yellow at base, 2 = spotted red or crimson or purple
59. Sidelobe ornamentation	0 = sparsely hairy at base, 1 = glabrous
60. Sidelobe length	0 = half as the width, 1 = almost double as wide
61. Sidelobe width	0 = half as the length, 1 = almost similar to the length
62. Lip claw	0 = presence, 1 = absence
63. Position of lip claw	0 = on one plane, 1 = bending at an acute angle to the left
64. Auricles (teeth) of lip	0 = distinct, 1 = indistinct, 2 = absence
65. Auricles shape	0 = small appendages, 1 = triangular
66. Callus shape	0 = oblong, 1 = cuneate, 2 = obcuneate, 3 = falcate, 4 = rounded
67. Callus ornamentation	0 = sparsely hairy at base, 1 = hairy entirely, 2 = hairy on top surface
68. Callus colouration	0 = spotted red or purple, 1 = yellow
69. Callus architecture	0 = united at base, 1 = risen individually
70. Column architecture	0 = arched, 1 = slender, 2 = curved
71. Column size	0 = shorter than the lip, 1 = as long as the lip
72. Column cap shape	0 = almost tubular, 1 = broadly winged
Ecological Characteristics	
73. Habit	0 = terrestrial, 1 = lithophytic
74. Ecological niches	0 = montane forest above 900 m, 1 = lowland to hill forest (0–700 m asl), 2 = riverside
75. Geology	0 = multisubstrates, 1 = limestone/quartzite, 2 = granite, 3 = ultramafic

2.4. Character Matrix and Phylogenetic Analysis

To build a phylogenetic tree based on morphological characters, the first step was to group the species by their similarities (shared characters) in order to recognize what characters they share and what characters are different. The information was then organized into a character matrix, which is a chart of characters that is important in categorizing the species and the names of the species. A phylogenetic analysis using the Maximum Parsimony method was carried out using data from the matrix in an attempt to ascertain whether these characters were phylogenetically significant, and to elucidate the relationships between members of *Spathoglottis* based on their morphological variation. The character matrix and phylogenetic tree were generated using Mesquite version 3.70 [19].

The distribution of character states among the 12 species and 3 infraspecific taxa of *Spathoglottis* measured in this study is presented in Table 3 below:

Table 3. Distribution of character states measured from the 12 species and 3 infraspecific taxa of *Spathoglottis*. A '?' symbol denotes a missing or not applicable character.

Species	Characters and Character States																				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
S. affinis	0	1	2	0	1	1	0	1	1	0	1	0	0	0	0	1	0	0	0	0	1
S. aurea	1	0	0	0	0	0	0	1	1	0	0	1	0	1	2	0	2	3	3	1	1
S. eburnea	0	1	2	2	1	1	1	0	1	0	0	0	0	1	1	0	0	2	1	1	1
S. gracilis	1	1	1	1	0	0	0	1	0	1	0	1	0	1	2	0	0	3	1	0	0
S. hardingiana	0	1	0	0	1	1	1	0	1	1	1	2	1	0	1	1	1	2	2	0	1
S. kimballiana	1	1	0	0	1	0	0	0	2	0	0	1	0	1	2	0	0	3	3	1	0
S. kimballiana var. *angustifolia*	1	1	0	0	1	1	0	0	1	0	1	0	0	0	0	1	0	0	1	1	0
S. kimballiana var. *kimballiana*	1	1	0	0	1	1	0	0	1	0	1	0	0	0	0	1	0	1	1	1	0
S. microchilina	1	0	0	0	0	0	0	0	1	0	0	1	0	1	2	0	0	3	3	1	0
S. parviflora	1	1	0	0	1	0	0	1	3	0	0	1	0	1	2	0	0	3	3	1	0
S. plicata	1	0	0	0	1	0	0	0	3	0	0	1	0	1	2	0	0	3	3	1	0
S. plicata var. *alba*	1	0	0	0	1	0	0	0	3	0	0	1	0	1	2	0	0	3	3	1	0
S. pubescens	0	1	2	2	1	1	1	0	1	1	1	0	0	0	0	0	0	0	0	1	0
S. unguiculata	1	1	0	0	1	0	0	0	2	0	0	1	0	1	2	1	0	3	3	0	0
S. vanvuurenii	1	0	0	0	1	0	0	0	3	0	0	1	0	1	2	0	0	3	3	1	0
Tainia paucifolia	1	0	1	1	1	0	1	0	0	1	0	0	0	0	3	0	0	3	2	1	1
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
S. affinis	1	0	0	1	0	1	1	1	0	0	1	1	0	2	0	0	0	0	1	0	0
S. aurea	0	1	1	2	1	1	0	0	1	1	1	1	1	2	0	1	1	3	1	0	1
S. eburnea	1	0	0	1	0	1	1	1	0	0	1	1	0	0	0	0	0	0	1	0	0
S. gracilis	0	1	1	0	1	1	0	0	0	1	1	1	2	2	0	0	0	1	0	0	0
S. hardingiana	1	0	0	1	0	1	1	1	0	0	1	3	0	3	1	0	0	2	0	1	0
S. kimballiana	0	1	1	0	0	0	0	0	0	1	1	1	2	2	0	0	0	0	0	0	1
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
S. kimballiana var. *angustifolia*	0	1	1	0	0	0	0	0	0	1	1	1	2	2	0	0	0	1	0	0	1
S. kimballiana var. *kimballiana*	0	1	1	0	0	0	0	0	0	1	1	1	1	2	0	0	0	0	0	0	1
S. microchilina	0	1	1	0	1	0	0	0	1	1	1	1	1	1	0	1	1	3	0	0	1
S. parviflora	0	1	1	1	0	1	1	1	0	1	1	3	2	3	0	2	0	0	0	0	0
S. plicata	0	1	1	1	0	1	1	1	1	1	1	3	1	3	0	2	1	0	0	0	0
S. plicata var. *alba*	0	1	1	1	0	0	1	1	0	1	1	0	0	3	0	0	0	0	1	1	0
S. pubescens	1	0	0	1	0	1	1	1	0	0	1	1	0	3	0	0	0	0	1	1	0
S. unguiculata	0	1	1	1	0	1	1	1	0	1	1	3	0	3	0	0	0	0	0	0	1
S. vanvuurenii	0	1	1	1	0	1	1	1	0	1	1	2	1	3	0	0	1	0	0	0	0
Tainia paucifolia	0	0	1	1	0	1	1	1	1	1	2	3	1	3	0	0	?	0	0	2	1

Table 3. *Cont.*

Species	Characters and Character States																				
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**
	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63
S. affinis	2	0	0	0	1	0	1	0	1	3	1	0	1	0	2	2	0	0	0	0	0
S. aurea	0	0	2	0	0	0	0	0	1	0	0	1	0	0	3	0	0	0	0	1	0
S. eburnea	0	1	0	0	1	0	5	0	1	1	1	0	0	0	1	2	0	0	0	1	0
S. gracilis	0	0	0	0	1	0	1	1	1	3	1	0	0	0	1	2	0	1	0	0	1
S. hardingiana	0	2	1	0	1	1	0	0	1	0	0	1	1	0	0	2	1	0	0	1	0
S. kimballiana	0	0	0	0	1	0	1	1	1	3	1	0	0	0	4	2	0	1	1	0	0
S. kimballiana var. *angustifolia*	1	0	0	0	0	0	4	1	1	1	1	2	0	0	4	2	0	1	1	1	0
S. kimballiana var. *kimballiana*	1	0	0	0	1	0	1	1	0	3	1	0	0	0	2	2	0	0	0	0	0
S. microchilina	1	0	0	0	1	0	0	0	1	0	0	1	0	0	2	0	0	0	0	1	0
S. parviflora	1	0	0	0	1	0	3	0	1	1	1	0	0	1	3	1	0	0	0	1	0
S. plicata	1	0	0	0	0	0	1	0	1	3	1	0	0	0	1	2	0	0	0	0	0
S. plicata var. *alba*	1	0	1	0	1	0	1	0	1	3	1	0	1	1	1	1	0	0	0	0	0
S. pubescens	1	1	1	1	1	0	2	0	1	2	1	0	0	0	2	2	0	0	0	1	0
S. unguiculata	1	2	0	0	0	0	2	0	0	1	1	0	1	1	2	1	0	0	0	1	0
S. vanvuurenii	1	0	0	0	1	0	1	0	1	3	1	0	0	0	2	2	0	0	0	1	0
Tainia paucifolia	1	2	3	2	0	2	2	1	2	1	1	2	0	0	2	0	1	0	0	1	0
	64	65	66	67	68	69	70	71	72	73	74	75									
S. affinis	0	1	0	2	0	1	2	1	1	1	0	1									
S. aurea	0	1	1	0	0	0	0	1	1	0	0	2									
S. eburnea	0	1	3	0	1	1	2	1	1	1	0	1									
S. gracilis	0	1	0	0	0	1	2	1	1	0	0	3									
S. hardingiana	1	0	4	0	0	1	1	1	0	1	1	1									
S. kimballiana	0	1	0	0	0	1	2	1	1	0	0	3									
S. kimballiana var. *angustifolia*	0	1	0	0	0	0	2	1	1	0	2	3									
S. kimballiana var. *kimballiana*	0	1	0	0	0	0	2	1	1	0	0	3									
S. microchilina	0	1	3	0	0	1	0	1	1	0	0	3									
S. parviflora	1	0	4	0	1	1	2	1	1	0	0	0									
S. plicata	0	1	2	2	0	0	2	1	1	0	1	0									
S. plicata var. *alba*	0	1	2	2	1	0	2	1	1	0	2	0									
S. pubescens	1	0	2	0	1	1	2	1	1	1	1	1									
S. unguiculata	1	0	4	1	1	1	2	0	1	0	1	0									
S. vanvuurenii	0	1	0	0	0	1	2	1	1	0	0	0									
Tainia paucifolia	2	?	?	?	?	?	0	1	1	0	2	0									

3. Results

Morphological characters and ecological characteristics appeared to support the informal groupings in *Spathoglottis*. Based on the morphological variation analyses, the

relationships among species of *Spathoglottis* in Peninsular Malaysia and Borneo are represented in a well-resolved phylogenetic tree.

3.1. Phylogeny Based on Morphological and Ecological Variation Analyses

The early groupings of *Spathoglottis* are suggested to be driven by the different colours of the flowers, followed by the plant size, and species are further distinguished by the shapes of the labellum/lip (Figure 2). Two main groups based on the colours of the flowers are recognized for this separation; namely Purple-Flowered *Spathoglottis* (Group G1) and Yellow-Flowered *Spathoglottis* (Group G2). A chart on the different tons of purple and yellow representing each species of *Spathoglottis* was prepared, as shown in Figure 3. It was observed that the character for purple colour flower was gained twice in *Spathoglottis*, as seen in the lilac *S. hardingiana* embedded within the Yellow-Flowered *Spathoglottis* group.

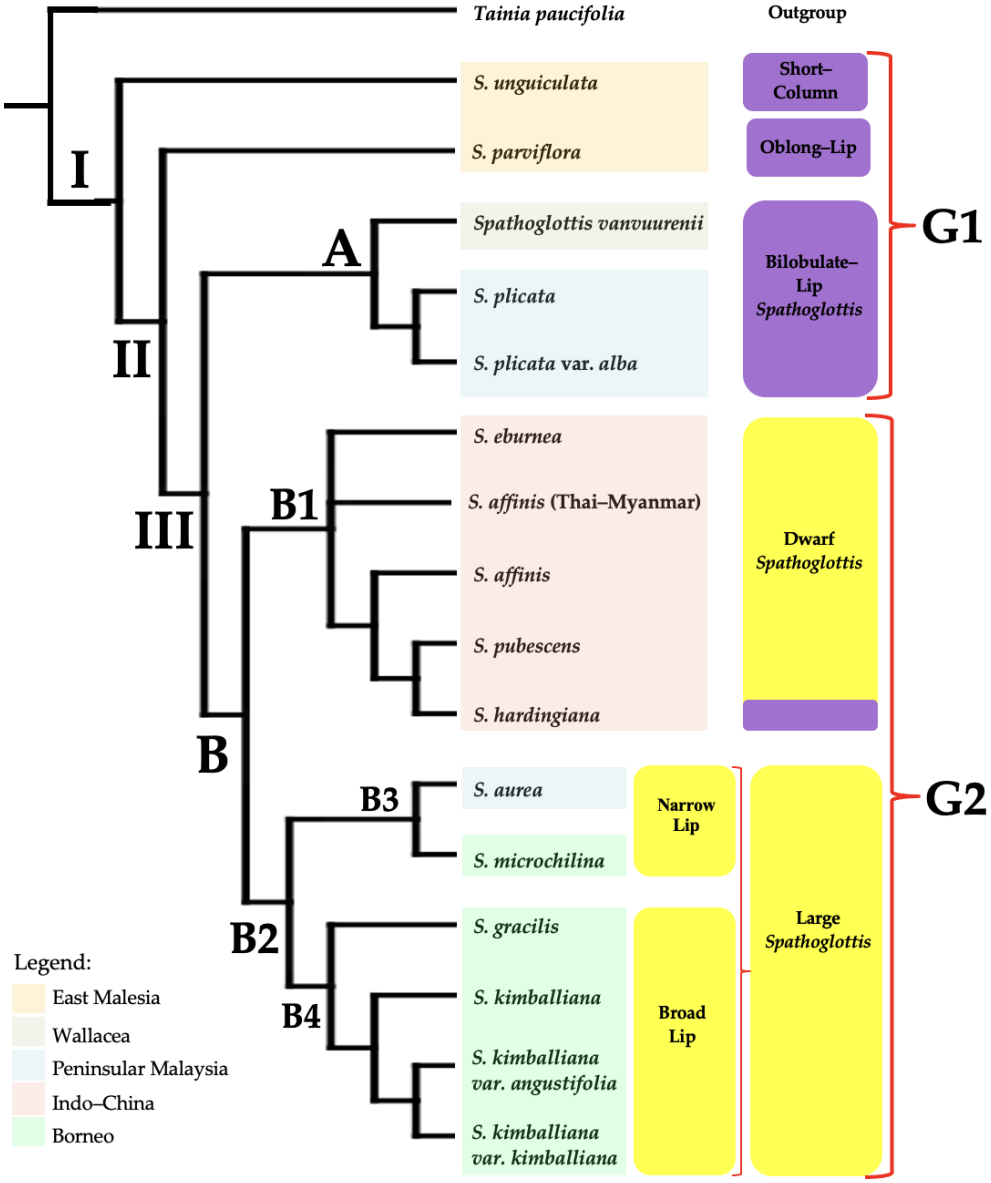
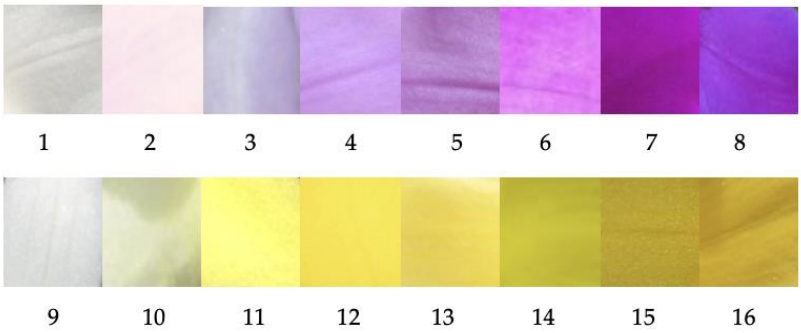


Figure 2. A majority rule consensus of 100 equally parsimonious trees of *Spathoglottis* species based on morphological and ecological sequence data. Tree length = 251; CI = 0.51; RI = 0.54. Groups G1 and G2 denote the Purple-Flowered *Spathoglottis* and Yellow-Flowered *Spathoglottis*, respectively. Numbers at nodes represent the informal groupings in *Spathoglottis* from Peninsular Malaysia, Borneo, Thailand, Sumatra, Irian Jaya, Maluku Island and New Caledonia.



Reference:

1 *Spathoglottis plicata* var. *alba*
2 *Spathoglottis vanvuurenii*
3 *Spathoglottis hardingiana*
4 *Spathoglottis plicata*
5 *Spathoglottis plicata*
6 *Spathoglottis parviflora*
7 *Spathoglottis unguiculata*
8 *Spathoglottis plicata*
9 *Spathoglottis pubescens*
10 *Spathoglottis eburnea*
11 *Spathoglottis affinis*
12 *Spathoglottis kimballiana* var. *kimballiana; S. kimballiana* var. *angustifolia*
13 *Spathoglottis microchilina*
14 *Spathoglottis kimballiana*
15 *Spathoglottis affinis*
16 *Spathoglottis aurea; S. gracilis*

Figure 3. A chart on the different shades of purple and yellow representing each species of *Spathoglottis*. The chart was prepared from the correct colours of *Spathoglottis* flowers, taken in natural light.

From the phylogenetic tree, it can be seen that Clade I consists of *S. unguiculata*, a purple-flowered *Spathoglottis* from the island of New Caledonia which was found to be a sister to all *Spathoglottis* species. The densely hairy callus and short column are characteristics unique to this species, and appeared nowhere else in any species of the genus (Figure 4).

Meanwhile in Clade II, *S. parviflora* from Irian Jaya appeared to be a sister to the rest of the purple-flowered *Spathoglottis*. The lip of *S. parviflora* is spathulate or almost boat-shaped, and thus is very distinctive from the broad/bilobulate lip of *S. plicata*, *S. plicata* var. *alba* and *S. vanvuurenii* from Seram (Figure 5). Generally, all members of these two clades share many similarities in vegetative habit, size and characters of the flowers. However, the clades are separated by the shapes of the lip and the sidelobes of the flower; *S. parviflora* has a spathulate lip, and the sidelobes are falcate, in contrast to the bilobulate lip in *S. plicata*, *S. plicata* var. *alba* and *S. vanvuurenii* (Figure 6).

Clade III contains all the purple- and yellow-flowered *Spathoglottis* in Peninsular Malaysia and Borneo and is monophyletic. This clade is further divided into Subclade III-A (Purple-Flowered *Spathoglottis* group) and Subclade III-B (Yellow-Flowered *Spathoglottis* group) according to their respective flower colours. The Purple-Flowered *Spathoglottis* group comprises the purple-flowered species, namely *S. vanvuurenii*, *S. plicata* and its white form *S. plicata* var. *alba*. Species within this group are held together by shared vegetative characters: medium-to-large-sized plants with four to five plicate leaves, having broad and coriaceous leaves, production of persistence floral bracts; and reproductive characters: flowers in the shades of pink to mauve to deep purple, thin-textured flowers, free-spreading sepals and petals, and broad midlobe with the presence of a narrow claw. However, vegetatively, it is difficult to differentiate *S. vanvuurenii* from *S. plicata* in the absence of the flowers. They are almost similar in habit, but *S. vanvuurenii* is less robust and has fewer leaves than *S. plicata*, and the inflorescence is less dense with only a few pinkish flowers blooming at one time. The shape and architecture of the sidelobes and calli (plural of callus) on the lip are different in *S. vanvuurenii* and *S. plicata*. The two species are thus separable based on the oblong vs. square sidelobes, and cuneate vs. obcuneate calli in *S. vanvuurenii* and *S. plicata*, respectively. The calli in *S. plicata* are also united at the base, in contrast to the calli of *S. vanvuurenii* which rise individually.

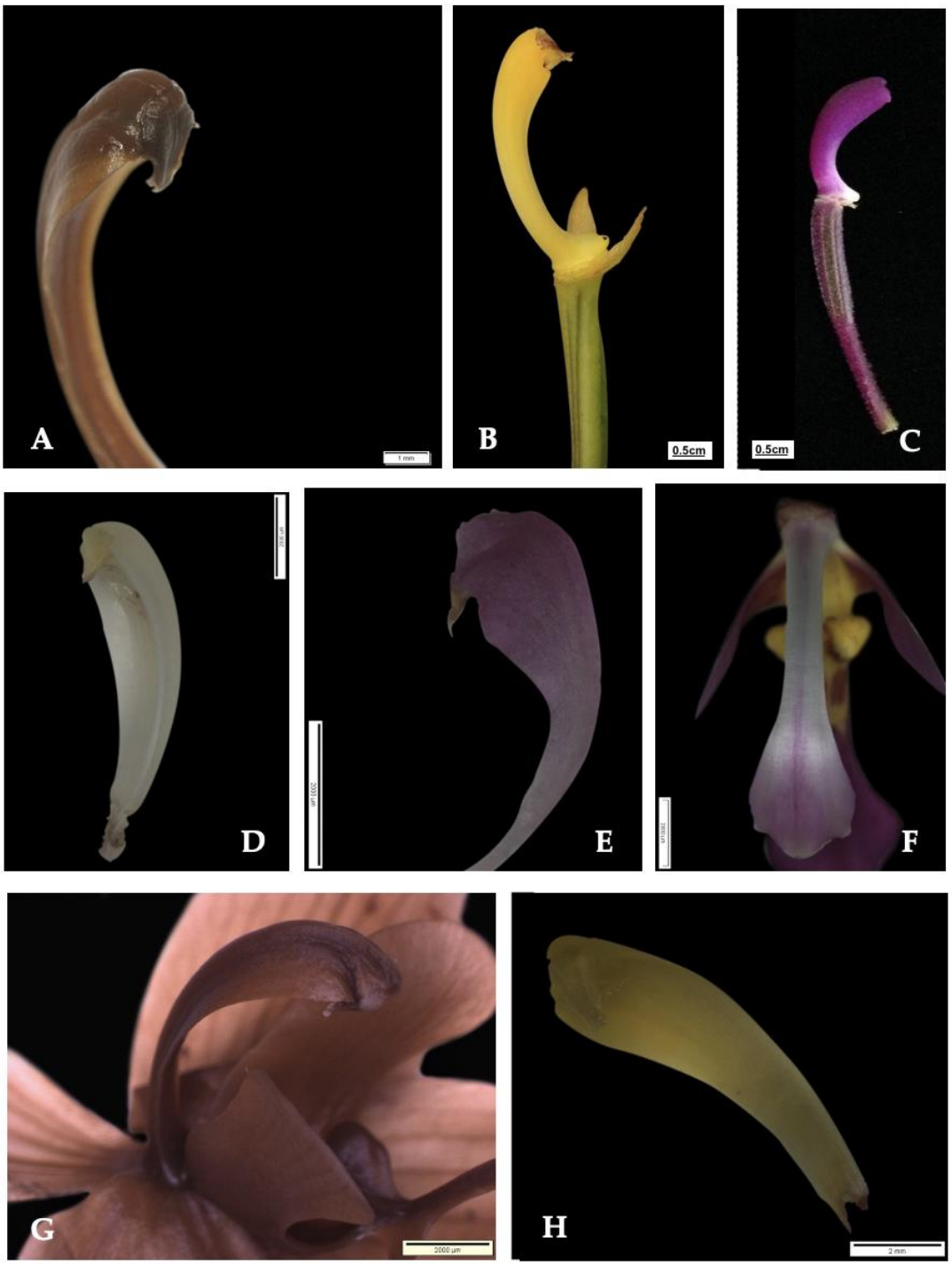


Figure 4. Column (Characters 70–72), side view of (**A**) *S. microchilina* (scale bar 1 mm, 4× magnification); (**B**) *S. aurea* (scale bar 0.5 cm, 4× magnification); (**C**) *S. unguiculata* (scale bar 0.5 cm, 4× magnification); (**D**) *S. pubescens* (scale bar 2000 µm, 4× magnification); (**E**) *S. hardingiana* (scale bar 2000 µm, 4× magnification); (**F**) *S. plicata* (scale bar 2000 µm, 4× magnification); and dorsal view of (**G**,**H**) *S. affinis* (scale bar 2 mm, 4× magnification). Photos: Farah Alia Nordin.

Meanwhile, the Yellow-Flowered *Spathoglottis* group is further divided into two separate groupings based on the size of the plants. Subclade III-B1 (Dwarf Yellow *Spathoglottis* group) houses all the dwarf-sized yellow *Spathoglottis* from Indo–China, whereas Subclade III-B2 (Large Yellow *Spathoglottis* group) comprises the large-sized species from Peninsular Malaysia and Borneo. These morphological variation analyses have shown that the

Yellow-Flowered *Spathoglottis* group is monophyletic. Independently, the Large Yellow *Spathoglottis* group is also monophyletic. However, the Dwarf Yellow *Spathoglottis* group appears to be paraphyletic. This is due to the placement of *S. hardingiana*, a dwarf species with a purple flower that nests within the clade, rather than forming a distinct clade of its own.

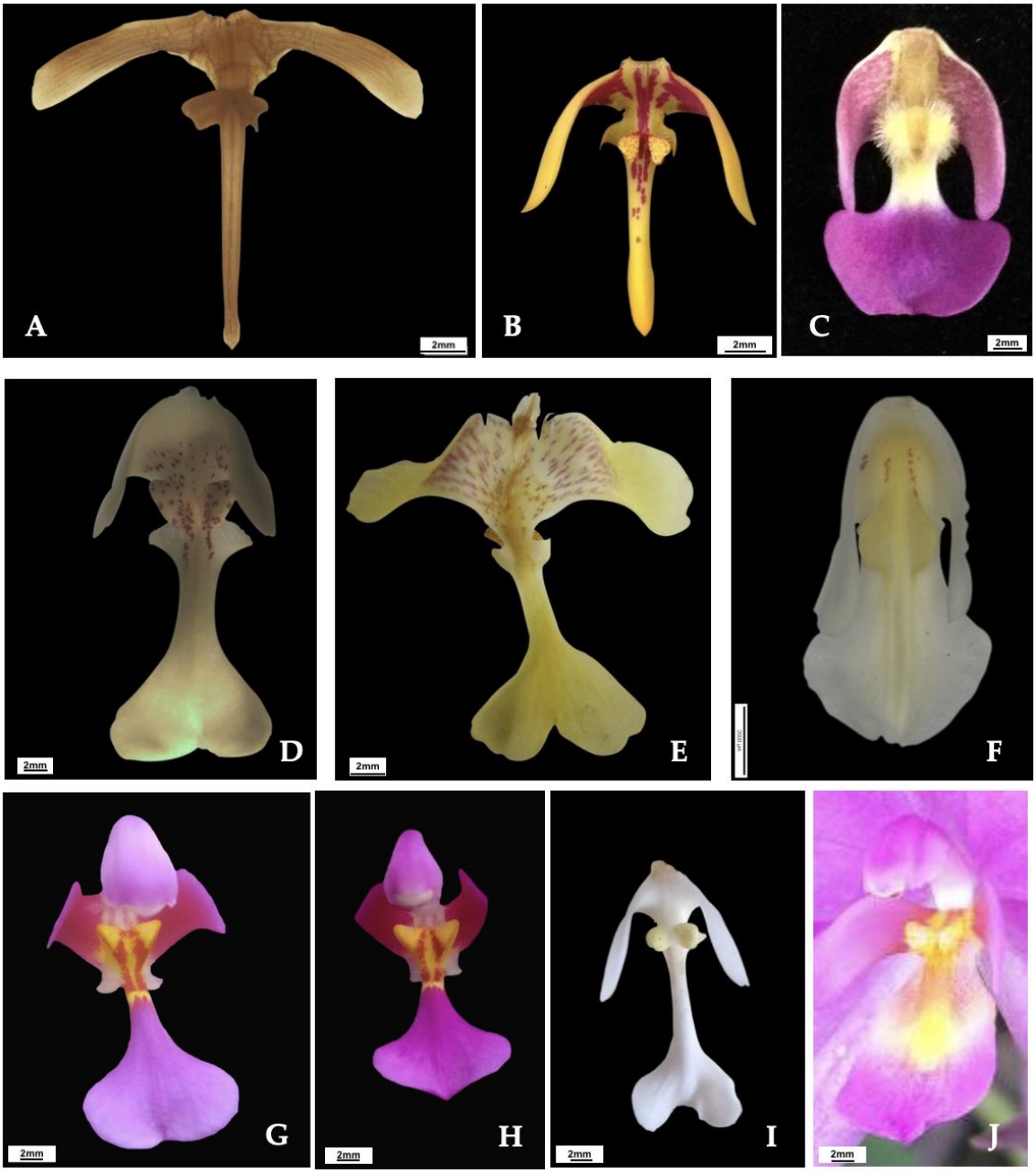


Figure 5. Labellum/lip (Characters 49–56) of (**A**) *S. microchilina*; (**B**) *S. aurea*; (**C**) *S. unguiculata*; (**D**,**E**) *S. affinis*; (**F**) *S. eburnea*; (**G**,**H**) *S. plicata*; (**I**) *S. plicata* var. *alba*; and (**J**) *S. parviflora.* Scale bar 2 mm, 4× magnification. Photos: Farah Alia Nordin.

The four species, namely *S. eburnea*, *S. affinis*, *S. hardingiana* and *S. pubescens*, are distinctly dwarf in habit. A flattened pseudobulb of irregular shape is unique to members in the Dwarf Yellow *Spathoglottis* group, and a white colour pseudobulb was only observed in *S. eburnea* and *S. pubescens*. The group also shows diverse similarities of characters, such as the petioles and thin leaf, the elliptic sterile bracts that are deciduous, purplish

inflorescence and finely hairy throughout, bearing less than five flowers per inflorescence of which only one blooms at a time, having sepals and petals almost equal in width, and predominantly, all are lithophytic species that dwell on rocky substrates.

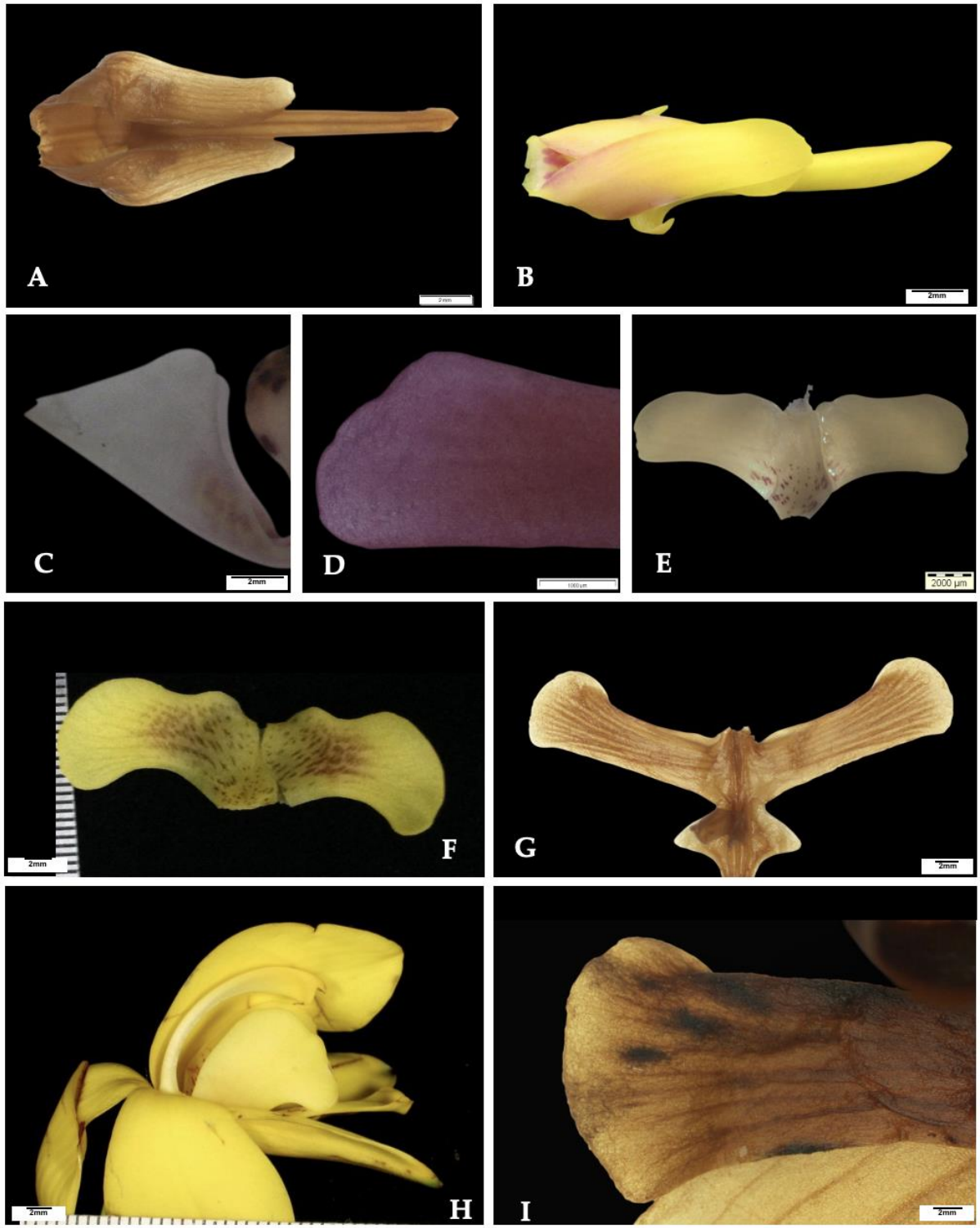


Figure 6. Sidelobes (Characters 57–61) of (**A**) *S. microchilina*; (**B**) *S. aurea*; (**C**) *S. hardingiana*; (**D**) *S. plicata*; (**E**,**F**) *S. affinis*; (**G**) *S. kimballiana* var. *kimballiana*; (**H**) *S. kimballiana* var. *angustifolia*; and (**I**) *S. gracilis.* Scale bar 2 mm, 4× magnification. Photos: Farah Alia Nordin.

The Large Yellow *Spathoglottis* group is observed as a well-resolved clade. The group consists of species with large yellow flowers (about 5.0–8.0 cm cross), the sepals and petals of which are thick-textured; the pedicel holding the flower is glabrous as compared to the hairy one in the Dwarf Yellow *Spathoglottis* group and the rest of the purple lower *Spathoglottis*; and the floral bracts are coriaceous, persistent and ovate to triangular in shape. Species within the Large Yellow *Spathoglottis* group are further separated into two distinct groupings based on the shapes of the lip. Subclade III-B3 (Narrow-Lip *Spathoglottis*) holds together the two species with a narrow lip, namely *S. aurea* and *S. microchilina*, whereas Subclade III-B4 (Broad-Lip *Spathoglottis*) consists of all *Spathoglottis* species from Borneo with a broad lip.

In the Narrow-Lip *Spathoglottis* group, *S. aurea* and *S. microchilina* formed a distinct grouping, predominantly characterized by a narrow and pointed lip which is almost thread-like, in contrast to the broad, spathulate midlobe of *S. gracilis*, *S. kimballiana* and its two infraspecific taxa. Additionally, this group is separated from the broad-lip *Spathoglottis* by the number of leaves, which is only two to three per plant, sometimes tinged with purple (*S. aurea*), the arched column, and also the high percentage of fruit sets in each individual plant. Autogamy or self-pollination is also unique to this group, and swelling of the ovary is commonly observed even before the flower bud is open.

Together, *S. gracilis*, *S. kimballiana*, *S. kimballiana* var. *angustifolia* and *S. kimballiana* var. *kimballiana* form the Broad-Lip *Spathoglottis* group. This group consists of species that show a mixture of various vegetative characters, from the one-leaf *S. gracilis* to the four-leaf *S. kimballiana*, to the grass-like leaves of *S. kimballiana* var. *kimballiana* and *S. kimballiana* var. *angustifolia*. Despite of the differences, members of this group are all ultramafic species and are held together by many similarities in their floral characters. A pair of broad and long auriculate sidelobes is a feature special to this group, along with flowers spotted with crimson on the reverse of the sepals and the distinctive curved column.

3.2. Delimiting Species Using Discreet Morphological Examination

Over the years, the taxonomic status between *S. aurea* and *S. microchilina* has been repeatedly questioned. The two narrow-lip, yellow-flowered *Spathoglottis* inhabit montane forests at 1300–2000 m asl and scarcely survive anywhere lower than 1000 m. They were separated as two different species predominantly based on the width of the lip/midlobe, which is wider in *S. aurea* (2.5–4.0 mm) as compared to the thread-like instances in *S. microchilina* (c. 1.5 mm). *Spathoglottis microchilina* was also reported to be autogamous, which is in contrast to the insect-pollinated *S. aurea*. However, this generalization is partly agreed, because self-pollination was also observed to be rather common in *S. aurea.* The flower of *S. aurea* is also relatively larger (c. 5.0–7.0 cm cross) and golden yellow as compared to the medium-sized (c. 4.0 cm cross) and pale yellow flower of *S. microchilina* (Figure 7).

Both *S. aurea* and *S. microchilina* share many similarities in their vegetative habits, such as size of plants, shapes of leaves and length of inflorescence. However, individuals in wild populations of *S. aurea* show great phenotypic variations across habitat, thus leading many authors to suggest *S. microchilina* as one of the highly variable forms of *S. aurea*. Nevertheless, the two species are well separated geographically, as *S. aurea* is rather common in the mountains of Peninsular Malaysia, while *S. microchilina* is confined to the ultramafic forests in Borneo [17].

Discreet morphological examination of the flower characters of *S. aurea* and *S. microchilina* has successfully resolved the taxonomic confusion between these two species. *Spathoglottis aurea* is distinguished from *S. microchilina* (*S. aurea* vs. *S microchilina*.) by (1) having a wider lip (c. 2.5–4.0 mm vs. <1.5 mm), (2) a triangular vs. obovate floral bract, (3) golden yellow flower vs. pale yellow flower, (4) sepals and petals almost equal in size vs. relatively larger petals, (5) falcate sidelobe vs. oblong sidelobe, (6) cuneate callus vs. falcate callus and (7) callus united at base vs. callus risen individually (Figures 4–6).

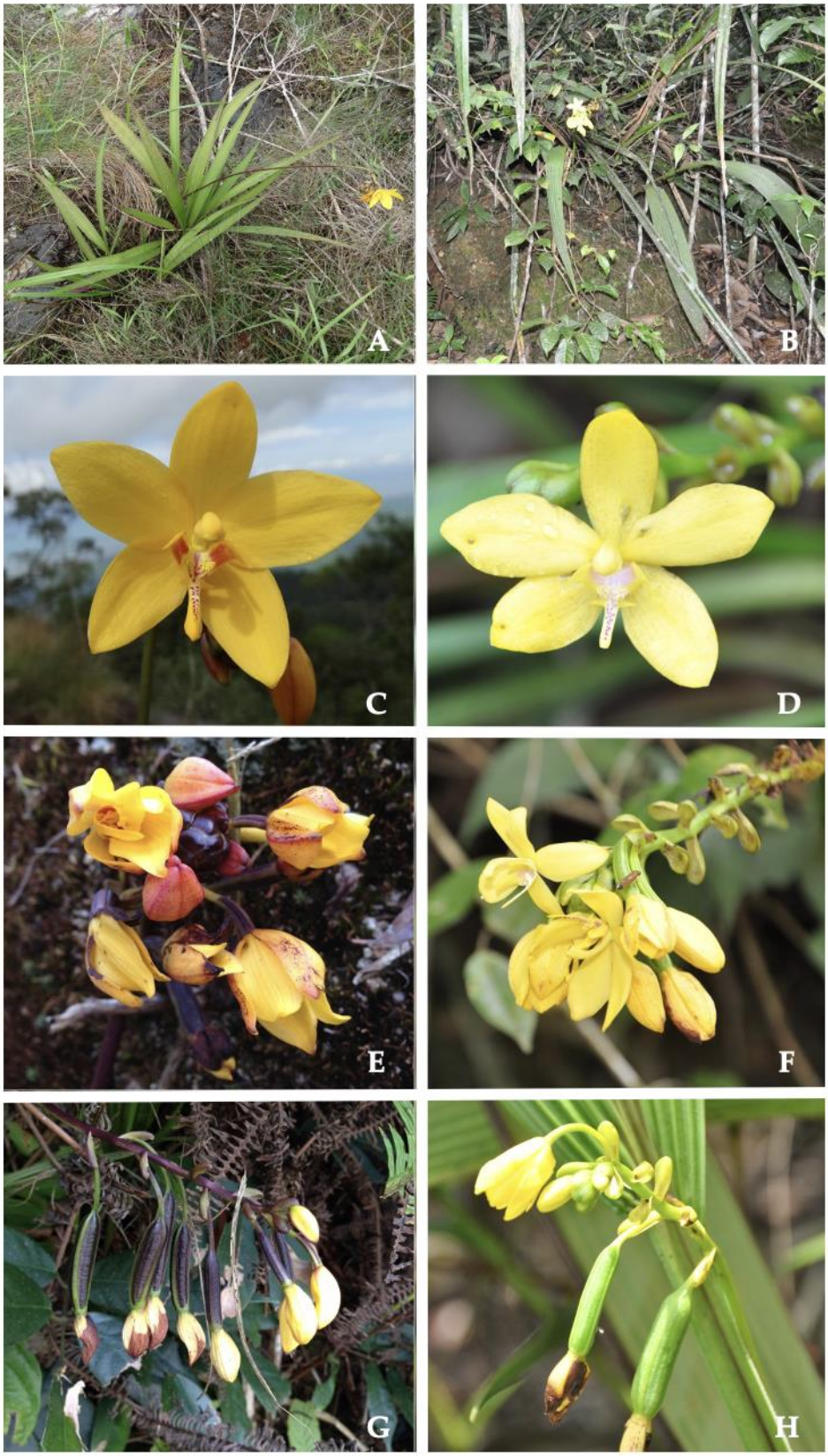


Figure 7. *Spathoglottis aurea* (**A**) habit; (**C**) flower; (**F**) inflorescence and (**G**) self-pollinated flowers and fruits, and *S. microchilina* (**B**) habit; (**D**) flower; (**E**) inflorescence; (**H**) self-pollinated flowers and fruits. Photos: Farah Alia Nordin.

4. Discussion

4.1. Morphological Variation and Phylogenetic Relationships between Species of Spathoglottis

Morphology may show prominent signals in elucidating phylogenetic relationships and phenotypic character evolution as discussed in various morphological systematics of Orchidaceae [20–22]. Morphological and ecological variation analyses in this present work have successfully resolved the evolutionary relationships between the eight species and three infraspecific taxa of *Spathoglottis* from Peninsular Malaysia and Borneo. Additionally, species boundaries within the genus have been successfully circumscribed.

Plant size, flower colours and shapes of labellum are synapomorphic characters that support monophyly in *Spathoglottis*.

The application of parsimony to the morphological matrix in this work reproduced the well-supported topology, and is in accordance with the early revision efforts on the classification of *Spathoglottis* prior to the advent of molecular phylogenetics. This genus is classified into two main groups (Purple-Flowered *Spathoglottis* and Yellow-Flowered *Spathoglottis*) based on the colours of the flower (Character 33), size of the plants (Character 1) and shapes of the labellum/lip (Character 49). The other equally important morphological characters and ecological characteristics used to identify and delimit species within *Spathoglottis* are: leaf shape and vernation (Character 11, 15), size of flower (Character 34), size and shape of midlobe (Character 52, 53), callus architecture (Character 69), column architecture (Character 70), pollination strategies (Character 37), ecological niches (Character 74) and geological history of the species (Character 75).

4.2. Spathoglottis aurea and S. microchilina as Two Distinguished Species

Based on the morphological variation analyses, *S. aurea* and *S. microchilina* should be accepted as two separate taxa, of which *S. aurea* (native to Peninsular Malaysia) has wider geographical occurrences, while *S. microchilina* is known only in Borneo.

The narrow lip possessed by both *S. aurea* and *S. microchilina* is suggested to appear due to a floral divergence driven by adaptation to specific pollinators in particular habitat. The much-reduced lip of *S. aurea* and *S. microchilina*, the underdeveloped rostellum, and the swollen ovary with developed pollen tubes during the floral bud stage are evidence of a self-pollination strategy. In this study, each of the individuals of *S. microchilina* from Borneo was observed to be completely cleistogamous or occasionally geitonogamous. The flower does not open fully, and fruit set percentage is very high; a sign of a successful self-pollination strategy. Likewise, similar observation was noticed among individuals of *S. aurea* from different populations in Peninsular Malaysia. A complete cleistogamous form of *S. aurea* is usually stunted or smaller in size, in comparison to the geitonogamous or insect-pollinated *S. aurea* which are vegetatively and reproductively larger in size.

4.3. Phylogenetic Relationships Based on Morphological Data and Molecular Data

Comparison-wise, the morphological tree obtained in this study appeared incongruent to the molecular trees published in separate studies [17]. The comparisons are discussed as in Table 4 below:

However, both in morphology and molecular trees, the Large Yellow-Flowered *Spathoglottis* have been proven to be monophyletic. In addition, the taxonomic status of the two profoundly confused narrow-lip *Spathoglottis*, *S. aurea* and *S. microchilina*, is now resolved and they should be considered as two separate taxa.

This phylogenetic incongruence between trees built from morphological data versus molecular data might be due to several reasons, such as homoplasious characters resulting from convergent evolution, and morphological variation across character states inflicted by environmental variation impacting on plastic morphological traits [20].

Table 4. Phylogenetic relationships between species of Spathoglottis compared from morphological data and molecular data.

	Relationships from Morphological Data	Relationships from Molecular Data
1.	Species were first separated according to the colour of the flowers.	Groupings of *Spathoglottis* were first made according to plant size.
2.	The Dwarf *Spathoglottis* is nested within the Yellow-Flowered *Spathoglottis* group.	The Dwarf *Spathoglottis* formed a separate group from the Large *Spathoglottis* group.
3.	The Large Yellow-Flowered *Spathoglottis* group is well resolved, and species are grouped according to the shapes of labellum.	Species are arranged based on their geographical distribution, ecological niches and flower size.
4.	*Spathoglottis kimballiana* is well placed within the Large Yellow-Flowered *Spathoglottis* group with other *Spathoglottis* species from Borneo.	*Spathoglottis kimballiana* is nested within the Large Purple *Spathoglottis* group.

5. Conclusions

Morphological characters and ecological characteristics have been proven to show a strong taxonomic signal in elucidating evolutionary relationships within *Spathoglottis*. Plant size, flower colours and shapes of labellum are synapomorphic characters that support the monophyly of the groups within *Spathoglottis*. However, it was proven that similar morphological characters were not necessarily inherited, but could be independently derived.

Author Contributions: Data curation, F.A.N. and A.S.O.; formal analysis and investigation, F.A.N., C.S.Y.Y., A.S.O. and K.S.; methodology, F.A.N., A.S.O., A.R., R.G. and C.S.Y.Y.; resources, F.A.N., A.S.O., K.S., A.R. and R.G.; supervision, A.S.O. and K.S.; validation, A.S.O.; writing—original draft, F.A.N.; writing—review and editing, F.A.N. and A.S.O. All authors have read and agreed to the published version of the manuscript.

Funding: The study was funded by the USM Short Term Grant of assignment No. 304/PBIOLOGI/6315549 awarded to the corresponding author and the first author. All funders provided financial supports for the present work but did not contribute any additional role in in the research design, data collection and analysis and preparation of the manuscript.

Data Availability Statement: Data presented in this article are available on request from the corresponding authors.

Acknowledgments: The authors would like to express their deepest gratitude to the administrative and field personnel of School of Biological Sciences, Universiti Sains Malaysia; Faculty of Resource Science and Technology, Universiti Malaysia Sarawak; Department of Biology, Universiti Putra Malaysia; and Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah for the facilities and assistance provided during the study conducted. Thank you to the Forestry Department of Peninsular Malaysia, Forestry Department of Sarawak, Forestry Department of Sabah, Sabah Biodiversity Centre and Kinabalu Park for the research permits granted. Many thanks to Richard Chung (FRIM), Ong Poh Teck (FRIM), Yong Kien Thai (KLU) and Nik Norhazrina Nik Mohd Kamil (UKMB) for the assistance provided during visits to the herbaria. Special thanks to our local and overseas collaborators; Anuar, Lawrence and C. K. Lim of Suriana Botanic Conservation Gardens.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. POWO, Plants of the World Online (Facilitated by the Royal Botanic Gardens, Kew). Available online: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:325895- (accessed on 20 January 2023).
2. Ridley, H.N.; Hutchinson, J. *The Flora of the Malay Peninsula Vol. 4*; A. Asher & Co.: Utrecht, Holland; L. Reeve & Co.: London, UK, 1924.
3. Holttum, R.E. *A Revised Flora of Malaya: Orchids of Malaya Vol. 1*, 3rd ed.; Government Printing Office: Singapore, 1964.
4. Seidenfaden, G.; Wood, J.J. *The Orchids of Peninsular Malaysia and Singapore: A Revision of R.E. Holttum: Orchids of Malaya*; Olsen and Olsen in association with The Kew Royal Botanic Gardens, and Singapore Botanic Gardens: Fredensborg, Denmark, 1992.
5. Chan, C.L.; Lamb, A.; Shim, P.S.; Wood, J.J. *Orchids of Borneo Vol. 1 Introduction and a Selection of Species*; The Sabah Society in Association with the Bentham—Moxon Trust, England: Kota Kinabalu, Malaysia, 1994.

6. Wood, J.J. *Orchids of Borneo Vol. 3: Dendrobium, Dendrochilum and Others*; The Sabah Society in Association with the Bentham Moxon Trust, England: Kota Kinabalu, Malaysia, 1997.
7. Wood, J.J. *Orchids of Borneo Vol. 4*; The Sabah Society in Association with the Bentham—Moxon Trust, England: Kota Kinabalu, Malaysia, 2003.
8. Beaman, T.E.; Wood, J.J.; Beaman, R.S.; Beaman, J.H. *Orchids of Sarawak*; Natural History Publications in Association with the Royal Botanic Gardens, Kew: Kota Kinabalu, Malaysia, 2001.
9. Comber, J.B. *Orchids of Java*; The Royal Botanic Garden's Kew, Bentham Moxon Trust: London, UK, 1999.
10. Comber, J.B. *Orchids of Sumatra*; Natural History Publications in Association with the Royal Botanic Gardens, Kew and Botanic Gardens, Singapore: Kota Kinabalu, Malaysia, 2001.
11. Seidenfaden, G.; Smitinand, T. *The Orchids of Thailand: A Preliminary List*; The Siam Society: Bangkok, Thailand, 1959.
12. Schuiteman, A.; de Vogel, E.F. *Orchid Genera of Thailand, Laos, Cambodia and Vietnam*; Nationaal Herbarium Nederland: Leiden, The Netherlands, 2000.
13. Dockrill, A. Australian Indigenous Orchids Vol. 1. In *Spathoglottis (Orchidaceae) in Australia and the Pacific Islands*; Cribb, P.J., Tang, C.Z., Eds.; Springer: Berlin/Heidelberg, Germany, 1969; Volume 36, pp. 721–729.
14. Cribb, P.J.; Tang, C.Z. *Spathoglottis* (Orchidaceae) in Australia and the Pacific Islands. *Kew Bull.* **1981**, *36*, 721–729. [CrossRef]
15. Hallê, N. Flore de la Nouvelle Calêdonie et Dêpendances and Orchidacées. In *Spathoglottis (Orchidaceae) in Australia and the Pacific Islands*; Cribb, P.J., Tang, C.Z., Eds.; Springer: Berlin/Heidelberg, Germany, 1977; Volume 36, pp. 721–729.
16. Ginibun, F.C.; Saad, M.R.M.; Hong, T.L.; Othman, R.Y.; Khalid, N.; Bhassu, S. Chloroplast DNA barcoding of *Spathoglottis* species for genetic conservation. *Acta Holtic.* **2010**, *878*, 453–459. [CrossRef]
17. Nordin, F.A.; Saibeh, K.; Go, R.; Mangsor, K.N.A.; Othman, A.S. Molecular Phylogenetics of the Orchid Genus *Spathoglottis* (*Orchidaceae*: *Collabieae*) in Peninsular Malaysia and Borneo. *Forests* **2022**, *13*, 2079. [CrossRef]
18. Thiers, B. Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff. Available online: https://sweetgumnybg.org/science/ih (accessed on 14 March 2023).
19. Maddison, W.P.; Maddison, D.R. Mesquite: A Modular System for Evolutionary Analysis. Version 3.70. Available online: http://mesquiteproject.org (accessed on 10 December 2022).
20. Bateman, R.M.; Murphy, A.R.M.; Hollingsworth, P.M.; Hart, M.L.; Denholm, I.; Rudall, P.J. Molecular and Morphological Phylogenetics of the Digitate–Tubered Clade within Subtribe *Orchidinae* s.s. (*Orchidaceae*: *Orchideae*). *Kew Bull.* **2018**, *73*, 54. [CrossRef]
21. Venhuis, C.; Venhuis, P.; Oostermeijer, J.G.B.; van Tienderen, P.H. Morphological Systematics of *Serapias* L. (*Orchidaceae*) in Southwest Europe. *Plant Syst. Evol.* **2007**, *265*, 165–177. [CrossRef]
22. Devey, D.S.; Bateman, R.M.; Fay, M.F.; Hawkins, J.A. Friends or Relatives? Phylogenetics and Species Delimitation in the Controversial European Orchid Genus *Ophrys*. *Ann. Bot.* **2008**, *101*, 385–402. [CrossRef] [PubMed]