

IDENTIFICATION AND CHARACTERIZATION OF LONG- STALKED *PAPHIOPEDILUM INSIGNE* (WALL. EX LINDL.) PFITZER FROM SIKKIM HIMALAYA, INDIA

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Abstract

Splendid slipper orchid *Paphiopedilum insigne* (Wall. ex Lindl.) Pfitzer is a species of ornamental significance both in domestic and international trade. The present study was undertaken to characterize *Paphiopedilum insigne* specimens for various morphological and floral characters and to investigate genetic variability and relationships amongst *P. insigne* specimens and the type species for the purposes of further breeding.

Introduction

PAPHIOPEDILUM INSIGNE (Wall. ex Lindl.) Pfitzer (Orchidaceae), also called as *Splendid slipper orchid*, is a highly valued pot plant. The genus *Paphiopedilum* belongs to subtribe Paphiopedilinae and comprises about 70 species; these are mostly found in the SouthEast Asia which includes NorthEast India (Meghalaya), China (NorthWest Yunnan), Thailand (Do Chiang Dao), Bangladesh (Sylhet), Myanmar, Philippines, Indonesia, New Guinea, and the Solomon Islands (Rankou and Kumar, 2015). The species is taxonomically very complex and *P. insigne* is the type species for the genus *Paphiopedilum*. Rao (2006) reported that there are about 60 species of *Paphiopedilum* in the world, of which 08 occur in India; 07 in NorthEastern India and 01 in peninsular India. It is a terrestrial orchid that grows on dolomite limestone outcrops near waterfalls, in shade (Pradhan, 1976). It is valued for its hardiness and attractive long-lasting flowers which last upto 60-90 days on the plant (Rao, 2006). Commercially, *P. insigne* is in high demand and used as an ornamental plant in domestic and international trade. The species has been used extensively for the development of new hybrids. *P. insigne* has been used 35 times as the female parent and 49 times as the male parent to develop primary hybrids. Two hundred and eighty-nine first generation hybrids have been produced using this species and the species has contributed to the genome of 19,130 hybrids. In recent years, habitat degradation and over-exploitation by ruthless collection for horticultural purposes in regional and international trade, has lead to the significant reduction of its wild populations (Rankou and Kumar, 2015).

The type specimen of *P. insigne* has been described as; terrestrial herb with 2.0-4.0 cm long stem enveloped by equitant leaf-bases; *leaves* 5-6, ligulate, linear-lanceolate, acute, unequally bilobed at apex, leathery, up to 35 cm long and 3.5 cm wide, dark pale green above and spotted purple at base on underside. Usually solitary flowered *inflorescence* (13.5-30.0 cm long) arising between the apical leaves; *peduncle* up to 25 cm long, green with very short purple-pubescent. *Ovary* pedicellate, up to 5.3-8.0 cm long, erect, and pale green in colour with dark purple hair. *Floral bract* elliptic or oblong-elliptic, 4.5-5.3 cm long, green spotted, purplish at base. *Floral bracts* are 4.0-5.3 cm long, convolute, glabrous, pale-green, with dark green veins. *Flowers* 8-11 cm across, spreading unequally, pale green colour with veins stubbed with dark-purple spots, margin undulating. *Dorsal sepal* ovate to orbicular-ovate, 5.7-6.7 cm long and 3.8-4.0 cm wide, pale green with dark purple spots. *Lateral sepal* oblong, 4.6-5.5 cm long and 1.4-1.5 cm wide, pale green, and spotted dark purple. *Petals* yellow-brown, 5.0-6.3 cm long and 1.0-1.8 cm wide, slightly incurved, spatulate, margin undulating, veins purple with dark purple spots, spreading. *Lip* 2.4-5.0 cm long, pale green with purple-brown. *Column* yellow, 1.9-2.0 cm long. *Staminode* obcordate, 1.5 cm long and 1.0 cm wide, yellow with purple hair on the surface and with a central raised boss (Lucksom, 2007; Pradhan, 2006). Previously, the species within the genus *Paphiopedilum* were reported to exhibit remarkable diversity and vary in their morphological and floral characters (Zhang *et al.*, 2016). The present study was undertaken to characterize *Paphiopedilum insigne* specimens for various morphological and floral characters and to investigate genetic variability and relationships amongst *P. insigne* specimens and the type species for the purposes of further breeding.

Material and Methods

A total of eight *P. insigne* collections (NOAC-2362, 2353, 2348, 2350, 2356, 2347, 2365, 2366) maintained at the germplasm conservation unit, ICAR-NRC for Orchids, were studied for morphological and floral characters during the flowering season (October-December) of 2020-2021. These genotypes were collected from farmer's field near Pakyong, Sikkim (1,120 m amsl). Each plant was observed for different characters including number, length (cm), breadth (cm), shape, apex, variegation, upper surface colour, lower surface colour and margin of leaves, inflorescence

length, peduncle length (cm), pedicel length (cm), length and width of dorsal synsepals, petals, lip, and column. Further, the colour pattern of the flowers was observed. The descriptive statistical analysis for quantitative characters was carried out using Microsoft Excel, 2010 and agglomerative hierarchical cluster analysis was performed using 'factoextra' package of R Software.

Results and Discussion

In the present study, the morphological variations observed in the eight *P. insigne* collections were compared with that of the type specimen (Table 1). An

Table 1. Morphological variations observed amongst the eight *Paphiopedilum insigne* collections and the type species.

Characters		<i>P. insigne</i> specimens		<i>P. insigne</i> type species		P-Values
		Mean±SD	Range	Mean±SD	Range	
Leaf	number	4.88±0.83	4.0-6.0	3.5±0.71	3.0-4.0	0.066
	length (cm)	30.05±6.4	20-39.4	23.1±5.8	19.0-27.2	0.202
	breadth (cm)	2.29±0.31	1.8-2.9	2.9±0.71	2.4-3.4	0.077
Inflorescence	length (cm)	33.36±3.15	27.5-36.5	21.65±0.92	21.0-22.3	0.001 **
Peduncle	length (cm)	28.24±2.87	23.0-31.0	17.0±1.41	16.0-18.0	0.0008 ***
Pedicel	length (cm)	5.13±0.35	4.5-5.5	4.65±0.49	4.3-5.0	0.140
Dorsal sepal	length (cm)	5.15±0.13	5.0-5.4	5.2±0.42	4.9-5.5	0.752
	breadth (cm)	3.78±0.16	3.6-4.0	3.9±0.57	3.5-4.3	0.542
Synsepal	length (cm)	4.29±0.25	4-4.8	4.05±0.49	3.7-4.4	0.330
	breadth (cm)	2.5±0.16	2.3-2.8	2.5±0.28	2.3-2.7	1
Petal	length (cm)	5.16±0.17	5-5.4	4.55±0.35	4.3-4.8	0.004 **
	breadth (cm)	1.68±0.1	1.5-1.8	1.6±0.14	1.5-1.7	0.409
Lip	length (cm)	5.01±0.2	4.8-5.4	4.4±0.28	4.2-4.6	0.005 **
	breadth (cm)	3.09±0.15	2.8-3.2	2.75±0.07	2.7-2.8	0.015 *
Column	length (cm)	1.44±0.05	1.4-1.5	1.4±0.14	1.3-1.5	0.514
	breadth (cm)	1.3±0.08	1.2-1.4	1.3±0.14	1.2-1.4	1

Significance. codes: 0 '****' 0.001 '***' 0.01 '**' 0.05

Agglomerative hierarchical cluster analysis was performed on all the traits which resulted in two clusters with all the 08 collections in one group and two type

specimens in another group. This indicates the presence of diversity between the two groups (Fig. 3). Cluster I is the largest cluster having 8 collections and

Table 2. Variations observed amongst qualitative characters of eight *Paphiopedilum insigne* collections and the type species.

Qualitative Characters	Pattern	
	Collections (08)	Type Species (02)
Spot pattern on dorsal sepal and synsepal of the flower	Random	Linear
Spot colour on dorsal sepal and synsepal of the flower	Light brown	Dark Brown
Spot appearance	Blotched	Spotted
Spot pattern on side petals	Linear	Absent

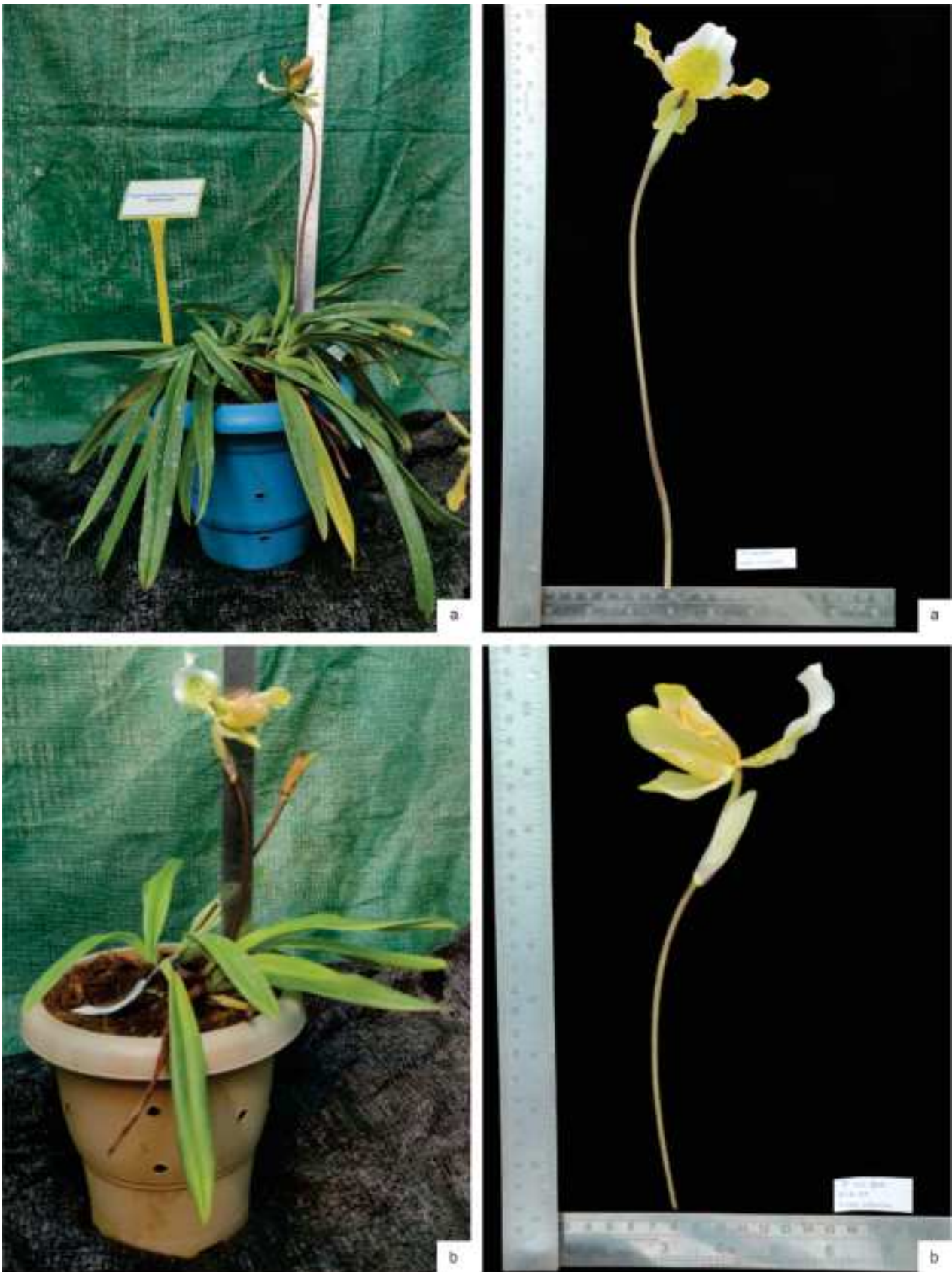


Fig. 1. *Paphiopedilum insigne* inflorescence and peduncle length of a, Specimen; b, Type species.

its Intra-Cluster distance I is 5.81 and 8.15 for Cluster II. Inter-Cluster distance (which denotes the how diverse

the groups are) is 10.15 (Table 3). The Cluster means for all the quantitative traits are given (Table 5). The



Fig. 2. *Paphiopedilum insigne* floral morphology and colour variation of a, Specimen b, Type species.

cluster mean of inflorescence length and leaf length is highest in Cluster I indicating thereby that these traits

Table 3. Inter- and Intra- cluster distances.

	Cluster I	Cluster II
Cluster I	5.81	10.15
Cluster II	10.15	8.15

have higher contribution towards cluster formation than the rest of the traits and lowest cluster mean was recorded for column traits.

Table 4. Cluster size and collections distributed amongst the clusters.

Cluster name	Cluster size	Collection number (NOAC)
Cluster I	8	2362, 2353, 2348, 2350, 2356, 2347, 2365, 2366
Cluster II	2	Type Sps 1, Type Sps 2

The species *P. insigne* has been previously described by various researchers (Lucksom, 2007; Pradhan, 2006; Yonzone, 2017) who reported the inflorescence length in the range of 13.5-30.0 cm (Lucksom, 2007; Pradhan, 1976; Yonzone, 2017) and peduncle length in the range of 8.0-25.0 cm (Lucksom, 2007; WFO, 2021; Yonzone, 2017). During the present investigation, we observed that the length of inflorescence and peduncle in the range of 27.5-36.5 cm and 23.0-31.0 cm, respectively amongst the eight *P. insigne* collections (Table 1; Fig. 1). A MANOVA test was conducted on quantitative traits to see the significance ($p < 0.05$) amongst the different traits between collections and type species (Table 1; p-Values). The peduncle and inflorescence length was found to be

Table 5. Cluster means for various traits.

Characters	Cluster means		
	Cluster I	Cluster II	
Leaf	number	4.87	3.5
	length (cm)	30.05	23.1
	breadth (cm)	2.28	2.9
Inflorescence	length (cm)	33.36	21.65
Peduncle	length (cm)	28.23	17
Pedicel	length (cm)	5.12	4.65
	length (cm)	5.15	5.2
Dorsal sepal	length (cm)	5.15	5.2
	breadth (cm)	3.77	3.9
Synsepal	length (cm)	4.28	4.05
	breadth (cm)	2.5	2.5
Petals	length (cm)	5.16	4.55
	breadth (cm)	1.67	1.6
Lip	length (cm)	5.01	4.4
	breadth (cm)	3.08	2.75
Column	length (cm)	1.43	1.4
	breadth (cm)	1.3	1.3

significantly higher than that of type specimen. This long inflorescence is an important character for breeding potted plants of *P. insigne*. In addition, there is a significant variation for traits such as petal length, lip length, and lip breadth. Variations observed in several qualitative characters such as spot pattern of dorsal sepal, synsepal, side petals, and colour of the spots between collections and type species (Table 2; Fig. 2) can be exploited for developing potted plant varieties of *P. insigne*. The morphological variations observed in the floral traits such as inflorescence and peduncle length amongst the *P. insigne* specimens would be of immense value to breeders for utilizing in crossing programmes to develop

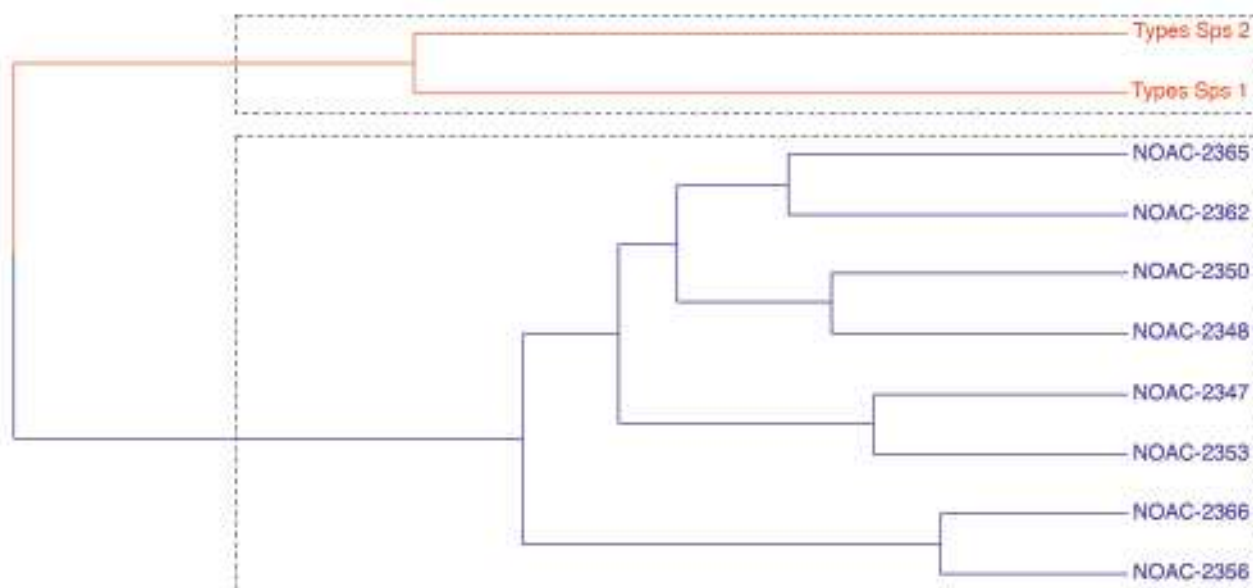


Fig. 3. Hierarchical cluster dendrogram indicating the distribution of individual plants into different clusters.

improved cultivars. A brief description of the *P. insigne* variant is given below:

Plant terrestrial with *stem* enveloped by leaf base; *leaves* 4-6, linear, forked leaf apex, 20.0-39.4 cm long, and 1.8-2.9 cm wide, green; *inflorescence* one-flowered, 27.5-36.5 cm long; *peduncle* 23.0-31.0 cm long, green, purple, pubescent; *flower* 7.5-12.1 cm across; *floral bract* elliptic, obtuse, pale green; *dorsal sepal* 5.0-5.4 cm long and 3.6-4.0 cm wide, pale yellow green, horse-shoe shaped, white margin, linear veins stubbed with large purple spots (0.3-0.8 × 0.2-0.5 cm size) on inner surface (more number of spots compared to type species); *synsepal* elliptic, obtuse 4.0-4.8 cm long and 2.3-2.8 cm wide, pale green, purple spots; petals 5.0-5.4 cm long, 1.5-1.8 cm wide, incurved slightly, obtuse, purple hairs externally, inside glabrous, glossy, base with few purple hairs, veins purple with spots spreading at the base. *Lip* 4.8-5.4 cm long and 2.8-3.2 cm, wide pale green, dark purple veins, small incurved, truncated below staminode. *Column* 1.4-1.5 cm long and 1.2-1.4 cm wide, having a bigger central raised boss.

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