OBSERVATIONS ON *PLEIONE FORMOSANA* HAYATA[1]

YOU-LONG CHIANG[2] and YUNG-REUI CHEN[3]

ABSTRACT

The form and structure of *Pleione formosana* Hayata are described in detail. Evidences are given to show that *Pleione Pricei* Rolfe and *Pleione formosana* Hayata form. *nivea* Fukuyama should be reduced to synonyms of *Pleione formosana* Hayata. As of date, *Pleione formosana* Hayata is the only known native species of *Pleione* in Taiwan.

INTRODUCTION

The Formosan orchid, *Pleione formosana* Hayata, known as Taiwan I-yeh-lan[4] (臺灣一葉蘭), meaning one-foliate orchid of Taiwan, has a characteristic 'single-leaf' and one to three conspicuous flowers which are probably the largest among all the known orchids indigenous to Taiwan (Figs. 1, 2). As an ornamental plant, this species is fast gaining attraction to orchid-lovers in Japan and Europe.

Fig. 1. A color photograph of *Pleione formosana* Hayata. Photographed on Feb. 30, 1967. Left, a plant with a 2-flowered scape. The fully opened flower is the first flower. Right, a white flower specimen (called form. *nivea* by Fukuyama).

(1) The authors thank Sister Julita Ruelo of Biology Department, Fu-Jen Catholic University, Taiwan and Dr. Charles E. DeVol of our department for correcting the English. A grant to the senior author from the Biological Research center, Academic Sinica, and from the National Council of the Long Range Planning for Science Development in the Republic of China is also acknowledged.

(2) (江有龍) Associate professor of Botany, National Taiwan University.
(3) (陳榮銳) Assistant in Botany, National Taiwan University.

(4) Another Chinese name is Ta-lun-chu-lu-lan (大輪朱露蘭) (Lin, 1953). Japanese name is Tairintokiso (Masamune, 1933).

This orchid was first collected by U. Mori[1] in April, 1909 in Taiwan and was described and named by B. Hayata in 1911. In 1914, Hayata made a short supplemental description of this plant, based on the material collected at Mt. A-li (阿里山) in March, 1914 by T. Ito and himself. R. A. Rolfe studied the plants which were grown from pseudobulbs (collected by R. W. Price in 1914 in Taiwan) in Kew, noting that the plant he studied was different from *P. formosana* in having a shorter scape, single flower, shorter bract and less number of keels on the lip, and thus gave it a name, *P. Pricei* Rolfe in 1917. In addition to *P. formosana*, this new species was listed by Hayata in 1919 in his book, Icones Plantarum Formosanarum, vol. 8. In 1932 N. Fukuyama gave a name, *P. formosana* Hayata form. *nivea*[2] to those *Pleione* growing in Hsin-chu (新竹), Taiwan, which had green pseudobulbs with white flowers. In 1933, *P. Pricei* was listed as a synonym of *Pleione formosana* by *G. Masamune*.

MATERIALS AND METHODS

Observations were made on plants grown from pseudobulbs which were collected on the rocky precipice facing southeast at about 1,500 m above sea level on Mt. Chia-li-hsien (加利仙山) on October 25, 1966 by F. S. Chang[3] (張福生). Mt. Chia-li-hsien (Fig. 18) is located on the boundary between Nan-chuang (南庄) and Tai-an (泰安), Miao-li, Taiwan. These pseudobulbs collected from the field vary in size, shape and color. Field collection of pseudobulbs of this plant showed four different groups: (1) purplish-red and large, (2) purplish-red, small and depressed-ovoid, (3) purplish-red, small and elongated and (4) green pseudobulbs. Ten healthy pseudobulbs were selected from each group and were planted on Nov. 25, 1966. The dimension, weight and color of the pseudobulbs were recorded. All pseudobulbs were planted in wet *Sphagnum* in flower pots, the upper half of the pseudobulb was exposed in the air. The pots were kept in a shaded vinyl house[4] in Taipei, where the intensity of light is about half that of outdoors. The pots were watered once or twice every day.

For anatomical studies, plant materials were fixed in FAA, dehydrated with a tertiary butanol series and embedded in paraffin. Serial sections were made at a thickness of 8 μ and stained with safranin and fast green.

(1) Based on herbarium specimen of Botany Department of National Taiwan University.
(2) Japanese name is Yuki-hitotuba-ran (Fukuyama, 1932).
(3) Specialist in the field collection of orchids; present address: 95, Hsi-tsun, Nan-chuang Hsiang, Miao-li Hsien, Taiwan.
(4) Located at 27, Lane 244, Section III, Roosvelt Road, Taipei, Taiwan. This house will be removed to erect a new building after May 1969.

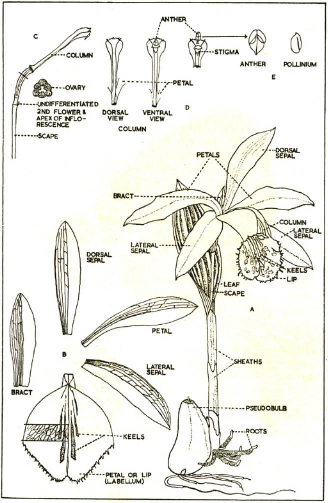

Fig. 2. *Pleione formosana* Hayata. Drawings were made on Feb. 23, 1964. A, an entire plant in flower with pseudobulb. ×0.75. B, a bract, sepals and petals. ×0.75. C, scape, ovary and column. ×0.75. D, dorsal and ventral view of column. ×0.75. E, anther and pollinium. Respectively ×2 and ×3.

OBSERVATIONS

1. Leaf.

A typical mature leaf of *Pleione formosana* is differentiated into leaf blade and petiole (Fig. 3A). The blade is oblanceolate. The apex of the blade is apiculate and its base is attenuate. The transition from blade to petiole is gradual indicating that the petiole is morphologically a continuous part of the blade. The lengths of blade and petiole are respectively 38 cm and 6 cm. An anatomical petiole, however, can be recognized at the base of the petiole very near the pseudobulb (Fig. 6E). This anatomical petiole is very short, usually not more than 1 cm in length. Morphologically, it is a sheath. Main veins stand in bold relief on the abaxial surface of the blade. They are parallel with each other, running from the base to the apex of the leaf. As in many other monocotyledons, these longitudinal bundles are laterally interconnected by many cross veins (Fig. 3C). The leaf is folded. Transections of the leaf show that the leaf is uneven or zigzag (Fig. 38). The width of the folded leaf at its widest portion is 9 cm. Its extended width, however, is 11 cm. The folding of the leaf blade contributes undoubtedly to the increase of photosynthetic area per unit space. The folding of the blade also may prevent the plant from being damaged by continuous sunshine, since a certain area (plane) of the folded surface is not illuminated continuously for a long time (Fig. 3E). The uneven surface of the leaf also effectively prevents the excessive loss of water vapor, since diffusion shells on the uneven surface overlap one another whereas those on the plain surface are separated (Fig. 3D).

Transections of petiole are V-shaped (Figs. 3B, 4A). As seen in a transverse section, vascular bundles are arranged in a line near the abaxial surface in the petiolar tissue (Fig. 4A). Nineteen vascular bundles are recognized in a mature petiole. They vary greatly in size, ranging from 78 μ to 667 μ in diameter. The largest bundle or midrib is located opposite to the notch of the petiole. Smaller bundles are located between larger bundles. All bundles of the petiole are collateral, the phloem is orientated toward the abaxial surface and the xylem toward the adaxial surface. As seen in a transverse section, the vascular bundle is elliptical in shape. Xylem is highly lignified. Phloem cells are very much smaller than those of xylem cells and are irregular in shape. They are parenchymatous. The vascular bundle is surrounded by sclerenchyma. Several layers of fibers are seen arching on the outside of the thick-walled cells, orientated toward the abaxial surface of the petiole. On the outside of the thick-walled cells and fibers, there is a layer of one-cell thick parenchymatous sheath (Fig. 5B). Although bundles vary in size, they are similar in structure and shape. The ground tissue of the petiole consists of isodiametric parenchymatous cells in which intercellular spaces are present (Fig. 5B). Stomata are found occurring on the abaxial surface of the petiole (Fig. 5B).

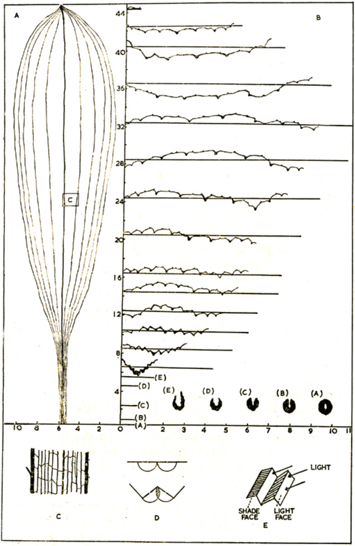

Fig. 3. Morphology of a mature leaf. A, surface view of a mature leaf showing blade, petiole and venation. ×0.4. B, transections of blade and petiole showing their folding and extended width. ×0.75. C, detail venation from small area of leaf depicted in Fig. 3A, showing cross anastomosis between the longitudinal bundles. ×23. D, diffusion shells on folded surface (upper) and plain surface (below). E, alternation of shade face and light face of folded blade.

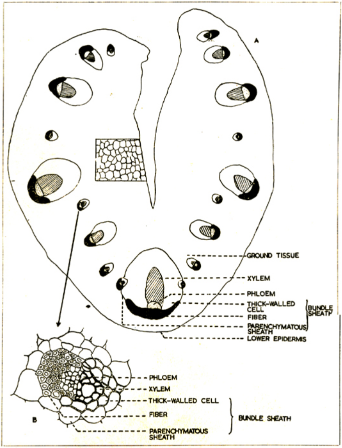

Fig. 4. Internal structure of petiole. A, transection of petiole, showing the arrangement of vascular bundles. ×34. B, detailed structure of a small bundle from the petiole. ×132.

The cells of the upper epidermis of the blade are larger than those of the lower epidermis (Fig. 5A). Stomata are present only on the abaxial surface of the blade. A significant characteristic of the leaf is the absence of palisade tissue, *i.e.*, the mesophyll is not differentiated into palisade and spongy parenchyma. The mesoyhyll is composed of rather irregular large parenchyma cells. (Fig. 5A).

Two-foliate plants were occasionally found in the cultures (Fig. 6C). Their

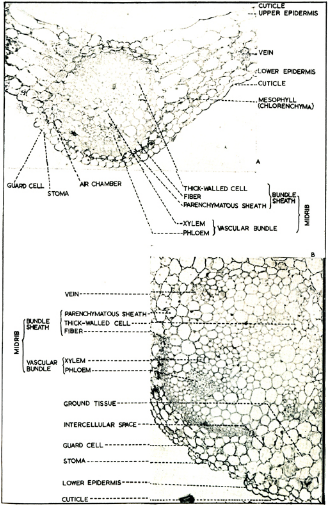

Fig. 5. Anatomy of mature leaf. A, transection of blade through midrib. ×88. B, transection of petiole through midrib. ×83.

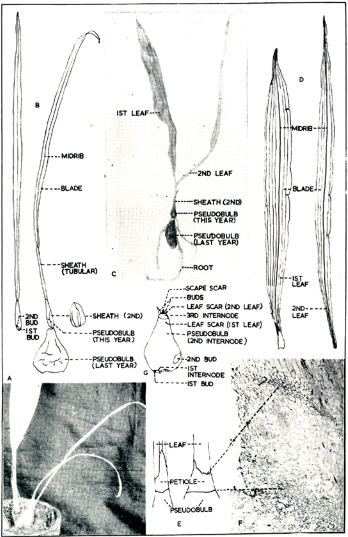

Fig. 6. Plantlets. A, photograph showing plantlets. ×0.4. B, a 1-foliate plantlet with a linear leaf. ×0.75. C, a 2-foliate plantlet. ×0.25. D, leaves of the 2-foliate plantlet depicted in Fig. 6C. E, petiole of 1st leaf. ×1.5. F, two abscission layers: one is between blade and petiole and the other is between petiole and pseudobulb. ×28. G, pseudobulb of the plantlet depicted in Fig. 6C, showing its external morphology. ×1.5.

leaves were lanceolate (Fig. 6D). The plantlet produced on the top of pseudobulbs has a linear leaf (Figs. 6A, 6B).

2. Pseudobulb (stem).

Pseudobulbs of *P. formosana* vary greatly in size and form. A typical pseudobulb is conical in shape (Fig. 7A). At a glance, it is glossy and without nodes. A careful observation, however, reveals that the pseudobulb consists of 3 nodes and 4 internodes. It looks like a nodeless or one-internode pseudobulb, because the 1st, 3rd and 4th internodes of the pseudobulb are very short and are hardly visible. The 2nd internode is enlarged and freshy and is called a pseudobulb (Fig. 6G) (Withner, 1959). The pseudobulb is tough and somewhat elastic. The following tissues are visible in pseudobulb: epidermis, chlorenchyma, storage tissue and vascular bundle (Figs. 7B, 7C). As seen in a transverse section (Fig. 7C), epidermal cells are tabular in shape and relatively small. They are arranged compactly in a one-celled layer. The cuticle occurs over the outer wall of the epidermal cells. No stomata are present. Directly under the epidermis, there is a layer of chlorenchyma cells. The storage tissue of pseudobulb is parenchymatous, consisting of larger cells and smaller cells. Many starch-containing chloroplasts are seen in the smaller cells but not in the larger cells. The larger cells of the pseudobulb may serve for water storage whereas the smaller cells are for food storage. The vascular bundle of the pseudobulb is similar to that of the leaf in structure but not in size. The bundle of the pseudobulb is smaller with less amount of both fibers and thick-walled tissue (Fig. 8). Starch-containing chloroplasts are present in every cell of the parenchymatous sheath but not in the tissues inside it.

The central part of pseudobulb is light grass-green in color. The section of about 0.5 mm thick from this portion is slightly grass green. The thinner sections (below 0.5 mm), however, are colorless. Under the microscope, no grass-green bodies were observed in this region, instead, many colorless or transparent small spherical bodies (about 4 μ in diameter) are seen in cells, especially in the bundle sheath (Fig. 8). They are not found in the large water storage cells. They are chloroplasts, which appear colorless, owing to their minute size and low chlorophyll content. By placing a drop of iodine potassium iodide solution, the entire chloroplast becomes bright blue and finally almost black, indicating that it contains starch. The outer portion (near surface) of pseudobulb is grass-green, and the cells contain many grass-green chloroplasts. The pseudobulb has no aeration system, *i.e.*, no air spaces and no stomata, and the outer tangential walls of the epidermis are cutinized (Figs. 7A, 7B, 7C), and furthermore, the roots are functionless after leaf fall. Evidently, the chloroplasts in the pseudobulb are not for vigorous photosynthesis. Since it has been known that the material present in chloroplasts is able to absorb CO_2 (Willstätter and Stoll, 1918 cited in Koriba, 1960), the chloroplast in the pseudobulb of this orchid may function to remove CO_2 accumulated in respira-

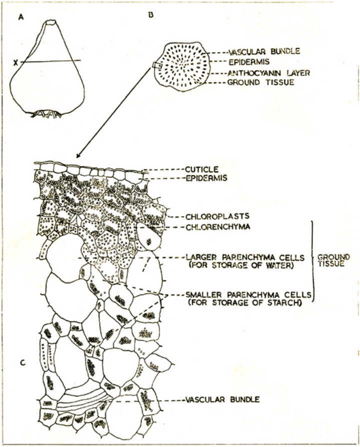

Fig. 7. Anatomy of pseudobulb. A, pseudobulb from the field. ×0.75. B, transection of the pseudobulb indicated in Fig. 7A showing arrangement of vascular bundles and anthocyanin layer. ×0.75. C, transection of the pseudobulb near the epidermis. ×65.

tion. The chloroplasts in the pseudobulb presumably carry out a low efficient photosynthesis, without gas exchange, which may be termed as 'anaerobic' photosynthesis. Another function of the chloroplast in the pseudobulb is the storage of food. It is evident that starch present in chloroplasts is not the assimilated starch, but is reserve starch which is the product of the photosynthetic activity of the

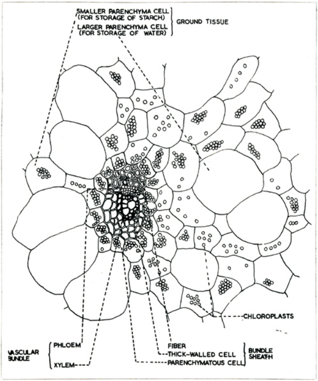

Fig. 8. Vascular bundle and its surrounding tissues from the central portion of pseudobulb, showing internal structure of pseudobulb and distribution of starch-containing chloroplasts. ×132.

leaf. The evidence for this is the dense distribution of starch in cells surrounding vascular bundles (Fig. 8).

Chow Cheng[1] (周鎮) obtained pseudobulbs from Mt. A-li, in autumn, 1967, and pointed out that they were much smaller than those collected in other areas. He also said that the leaves produced by them were also smaller. He, however, has not seen any in flower.

(1) Former chairman of editorial committee of Taiwan Orchid Society

3. Buds.

Several buds (typically four) were always found occurring on each pseudobulb that was harvested about time of defoliation (Fig. 9A). Two of them, *i.e.*, the 1st and 2nd buds, were located near the base of the pseudobulb. The 2nd bud is always larger than the 1st bud. Sometimes the 2nd bud is found very close to the tip of the pseudobulb, especially on elongated pseudobulbs. The internal structure of the 2nd bud is shown in Fig. 9. The bud at this stage of development consists of several scales and a rudimentary inflorescence. Scale 1 turned brown in color.

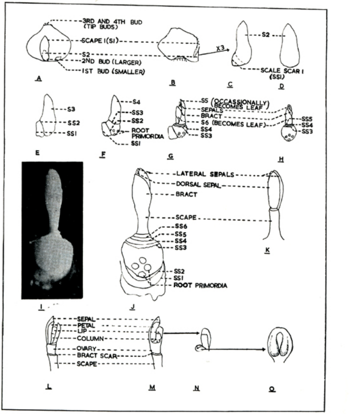

Fig. 9. Structure of a floral bud, showing various parts and their arrangement. This bud was harvested on Sept. 8, 1967 in vinyl house in Taipei. A, ×0.75; B, ×0.75; C, ×2.3; D, ×2.3; E, ×2.3; F, ×2.3; G, ×2.3; I, ×7.5; J, ×7.5; K, ×7.5; L, ×7.5; M, ×7.5; N, ×9; O, ×45.

Scales 2 to 6 remained light grass-green in color, indicating that they were still alive. Scales 2 to 5 grew as sheaths, whereas scale 6 as a leaf. Scale 5 occasionally developed into a leaf. All scales except 5 and 6 formed an envelope. Scale 1 is an elongated envelope. Two internodes, *i.e.*, the 1st and 2nd, are recognized: one is located between scale 1 and scale 2, and the other between scale 2 and 3. Several root primordia were seen on the lower side of the second internode. In a bud observed, 4 root primordia were seen. They were 1 mm in diameter and 0.3 mm in length. Since scales 3 to 6 occur very close to each other, no distinct internodes can be recognized. There is a young flower inside scale 6. The following rudimentary floral parts are recognized: 1 scape, 1 bract, 1 dorsal sepal, 2 lateral sepals, 2 lateral petals, 1 lip, 1 column and 1 ovary. The anther is still not differentiated, but 2 small protuberances could be recognized. It is important to note that the floral buds were formed before defoliation, indicating that the presence of a leaf is essential for floral bud formation in this orchid. As well as in the 2nd bud, a rudimentary flower was also found in the 1st or the smaller bud. This indicates that flowers may occur simultancously in the 1st and 2nd buds of the same pseudobulb. As described later, two-scaped pseudobulbs actually exist in nature. The arrangement of various rudimentary floral parts could be seen more clearly in transverse sectiong (Fig. 10A). As seen in these transections the scape is roughly round; 6 or 7 provascular strands are scattered in the scape. Each of these provascular strands is traceable to each floral part. A second flower primordium is found occurring on the ovary a short distance above the point of the bract or on the base of the ovary, proving that the inflorescence of this orchid is a spike. Floral parts are arranged in counter-clockwise direction (Fig. 10A).

Not all buds on the pseudobulb are floral. The buds produced on the tip of the pseudobulb, *i.e.*, the 3rd and 4th buds are always vegetative. The possibility of the 1st bud developing into a flower is very small. Only the 2nd bud has a high probability of becoming a flower. In a vegetative bud (Figs. 11A, 11B, 11C), a delicate, dome-shaped apical meristem will appear after the removal of 6 bud scales.

4. Root.

The root (Fig. 2) is about 1.5 mm in diameter. Roots are devoid of secondary growth and thus they remain slender. They are densely covered with root hairs. Root hairs occur 2–3 mm behind the root tip when the root is in contact with a solid substrate. Roots and root hairs are white and the root tip is yellow in color. The roots turn green when they are exposed to light.

5. Flower.

The flower of *Pleione formosana* is large and showy (Figs. 1, 2A). It consists of an axis (*i.e.*, ovary and column) and lateral appendages (*i.e.*, bract, sepal and petal) (Fig. 2). It is bisexual and zygomorphic. Perianth is epigyous, consisting of 3 sepals and 3 petals in two whorls. 3 sepals are petaloid and also free from

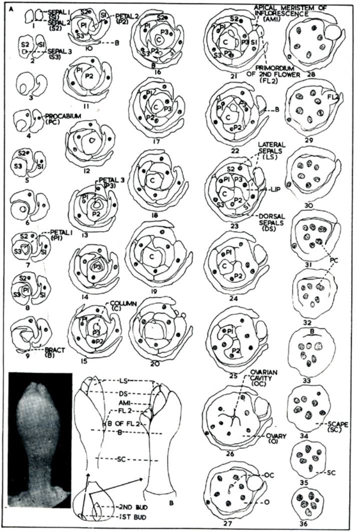

Fig. 10. Structure of rudimentary inflorescence. A, transections of a rudimentary inflorescence in the 2nd bud depicted in Fig. 10B at various levels, showing various floral parts and their arrangement of 1st flower, primordium of 2nd flower and apical meristem of the inflorescence. The 1st bud born on the same pseudobulb was also floral (Fig. 10B). These buds were fixed on Sept. 5, 1967. The pseudobulb was produced in a vinyl house in Taipei. 1, 2, 3,⋯⋯⋯⋯36 are respectively at the following numbers of microns from the tip of the 1st flower: 8, 96, 104, 114, 184, 192, 200, 216, 224, 256, 336, 352, 384, 400, 448, 528, 536, 576, 704, 712, 736, 752, 784, 792, 800, 808, 968, 984, 992, 1040, 1080, 1104, 1128, 1176, 1432, 1744. All ×45. B, photograph and drawings of rudimentary inflorescence. ×23.

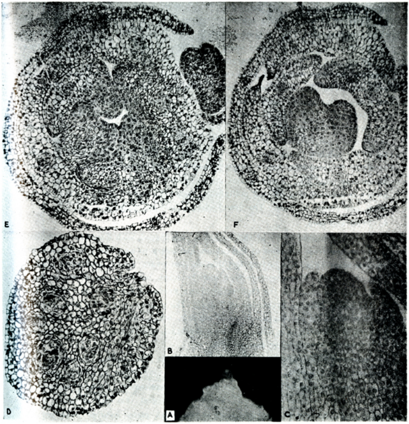

Fig. 11. Anatomy of vegetative and floral bud. A-C, vegetative bud. A, shoot apex of a vegetative bud. ×20. B, longitudinal section of a vegetative bud. ×70. C, enlarged view of Fig. 11B. ×325. D-F, transections of floral bud. All ×140. D, transection through the scape of a rudimentary inflorescence. This section corresponds to Fig. 10A-(34). E, transection of a rudimentary inflorescence near the point of floral parts and ovary. This section corresponds to Fig. 10A-(23). F, transection of a rudimentary flower. This section corresponds to Fig. 10A-(19).

one another. One of 3 petals, labellum or lip, is larger and highly modified as to shape and structure. Two lateral petals and the lip are free from each other. The lip, however, is united to the column at its base (Fig. 2D). The front margin of the lip is fimbrate. The lip is adorned with 2 to 4 keels (Fig. 2B). The keels are yellow in color· Various brown spots of irregular shape occur on adaxial surface of the lip. As in the leaf, the parallel veins are laterally interconnected by small veins in the bract, sepal and petal except in the lip where veins are more or less reticulate (Fig. 2B). Since sepals and petals are succulent and vascular bundles are very fine, veins are invisible when the flower is living. The ovary of this orchid is 3-carpelled. It is unilocular with 3 parietal placentae (Fig. 2C). In describing the flower of this orchid, Rolfe (1917) mistook the ovary for a pedicel. The ovary is not erect, but curved slightly downward. The stigma occurs laterally near the tip of the column, facing downward. Unlike ovary and scape, the column is colorless or white. *P. formosana* has a single terminal anther, and thus is a member of Monandrae (Fig. 2D). Pollen grains are agglutinated into pollinia (Fig. 2E). The anther is 2-celled (Fig. 2E). 2 pollinia are found in each cell. They are firm and lobed, and yellow in color. The flower of *P. formosana* is odorless.

6. Fruit and seed.

The unfertillized ovary of this orchid is club-shaped, with 6 sunken longitudinal lines running from the joint of the column and ovary to near the joint of the ovary and the scape (Fig. 2C). After fertilization, the ovary swells and gives rise to fruit (Fig. 12A). The immature fruit and the scape below it are green in color. Just before its maturity, the fruit turns brown gradually from its tip. This color change began in early September in Taipei. The mature fruit is 7 mm in diameter and 2 or more cm in length. It is a capsule, which splits along 6 longitudinal grooves at maturity (Fig. 12C). As with the bract, sepals, petals and column, the fruit does not abscise after maturity.

Some herbarium specimens of *P. formosana* have fruits, indicating that fruitification occurs in the field. In the present study in a vinyl house in Taipei, the fruit was produced when the flower was artificially cross-pollinated. Artificial self-pollination, however, resulted in no fruit. These facts suggest that hybridization occurs in the field. As will be described later in this work many hybrids were actually recognized.

The seed consists of an undifferentiated embryo and a flimsy, membranous seed coat (Fig. 12F). Endosperm is absent. The embryo is spherical and is 30 μ in diameter. The embryo does not vary very much in size and shape. The seed coat, however, varies greatly in shape and size, owing to its elasticity and thinness. The shape of seed is, therefore, polymorphic (Fig. 12E). A seed coat is composed approximately of 150 thin-walled cells. Seeds are minute and very abundant.

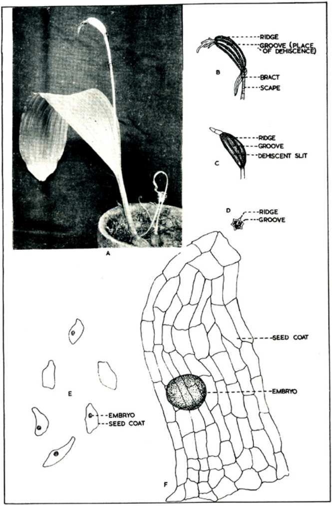

Fig. 12. Fruit and seed. A, an entire plant with a fruit. This fruit was harvested at maturity in vinyl house in Taipei on September 27, 1967. ×0.53. B-C, gross structure of fruit showing its dehiscence. In Fig. 12C, bract, sepals and petals were removed. D, transection of fruit. ×0.75. E, seeds showing variation in shape, note embryos are absent in some seeds. ×45. F, seeds showing transparent seed coat enclosing a spherical undifferentiated embryo (stippled). ×450.

Occasionally seeds without embryos are found. The seeds do not germinate in the laboratory when they are supplied only with water.

7. Flowering.

In the field, defoliation occurs before late October (Fig. 13). The harvest of pseudobulbs begins about this time every year. The harvest time lasts for about one and a half months. In the field, the blooming period starts in the beginning of April and lasts to the middle of May. From the cultures used in the present study, the first flower opened on January 11, 1967 and the last flower withered on March 23, 1967. The blooming period lasted about two and a half months. The blooming period in Taipei came two months earlier than in the field. The blooming period of an individual flower varies from 6 to 14 days. Generally, the blooming period of the flower produced in January and February was longer than that of the flowers produced in March (Fig. 14). As is true in some other plants, the winter flowers are more durable than the spring flowers. As can be seen in Fig. 14, most flowers produced by large pseudobulbs opened before Feb. 16, while most flowers produced by small pseudobulbs opened after Feb. 16. In other words, the flowers which opened earlier were produced on large pseudobulbs. In the 2-scaped pseudobulb, the flower bud borne on the 2nd scape opened several days (*e.g.*, 9 days) earlier than the flower bud borne on the 1st scape (Fig. 14). In one of the 2-flowered scapes, the 1st flower opened 5 days carlier than the 2nd flower (Fig. 14).

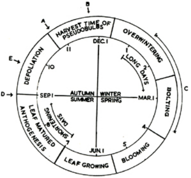

Fig. 13. The life history of *Pleione formosana* Hayata growing at the altitude of 1,500 m on Mt. Chia-li-hsien in Taiwan. A-E indicate the growth of the same plant in a vinyl house in Taipei. A, the time at which the pseudobulbs were harvested from the mountain for culture; B, the time of planting; C, blooming period; D, the time at which the well-developed floral bud was recognized; E, fruit matured.

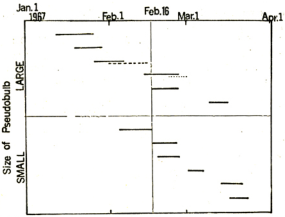

Fig. 14. Relation between the size of pseudobulbs and blooming period. Solid lines indicate the blooming period (from opening to withering) of individual solitary flower born on the 2nd scape. Broken lines indicate the blooming period of the 2nd flower on the same scape. Dotted lines indicate the blooming period of the solitary flower born on the 1st scape produced on the same pseudobulb.

The pseudobulbs collected from the field were able to produce daughter pseudobulbs in an uncontrolled vinyl house. These daughter pseudobulbs were able to bear flowers. Some of them were planted on Sept. 4, 1967. The first flower produced

Table 1. Morphological characteristics of an individual parent plant and of its daughter plant.

	Parent plant (from the field)	Daughter plant (from a vinyl house in Taipei)
Weight of pseudobulb (gm)	21.77	14.02
H/D ratio of pseudobulb	1.16	0.81
Color of pseudobulb	purplish red	purplish red
Date planted	Nov. 25, 1967	Sept. 4, 1967
Sheath number	4	3
Scape length (cm)	9.4	12
Bract length (cm)	3.9	3.5
Ovary length (cm)	1.5	1.9
Sepal length (cm)	5.1	4.9
Petal length (cm)	5.1	4.9
Keel number and length	2L2S1VS	2L1S
Flowering period	1967-1-18~28	1968-1-5~12

by them opened on Jan. 5 and withered on Jan. 12, 1968 (Table 1). Both 1st and 2nd bud of the pseudobulb harvested in Taipei may be floral (Fig. 10B).

8. Flowering rate of pseudobulbs.

Not all pseudobulbs produced flowers. As is shown in Tables 2 and 3, it was found that pseudobulbs flowered regardless of their size, shape and color except that those small purplish-red pseuddobulbs of the elongated type bore no flowers at all. Six out of ten of the large purplish-red pseudobulbs produced flowers. Six

Table 2. Weight and H/D ratio* of purplish red pseudobulb which flowered and not flowered.

10 large pseudobulbs (above 10 gm)				10 depressed and 10 elongated small pseudobulbs (under 10 gm)					
Flowered		Not flowered		Flowered		Not flowered			
Weight (gm)	H/D	Weight (gm)	H/D	Weight (gm)	H/D	Weight (gm)	H/D	Weight (gm)	H/D
21.8	1.1	22.8	0.7	6.2	0.6	5.4	0.7	7.2	1.9
18.7**	1.1	16.0	1.2	6.0	0.7	5.3	0.8	7.1	1.5
15.4	1.5	16.0	1.2	5.7	0.6	4.9	0.8	7.0	1.9
15.3***	1.2	14.9	1.1	5.7	0.8	4.7	0.6	6.8	1.7
15.2	1.1			5.3	0.7			6.6	1.5
14.3				4.6	0.7			6.5	1.5
								6.5	1.4
								6.0	1.4
								6.0	1.4
								4.5	1.2
Mean 16.8	1.2	17.4	1.1	5.8	0.7	5.1	0.7	6.4	1.5

* H/D ratio=Height/diameter ratio of pseudobulb.
** This pseudobulb produced two 1-flowered scapes.
*** This pseudobulb produced one 2-flowered scape.

Table 3. Weight and H/D ratio* of green pseudobulb which flowered and not flowered.

Flowered		Not flowered	
Weight (gm)	H/D*	Weight (gm)	H/D*
10.0	1.3	7.6	1.2
7.7	1.2	7.5	1.1
5.0	1.2	5.0	1.0
6.5	0.9	4.8	1.0
6.8	0.7	5.4	0.8
Mean 7.2	1.1	6.1	1.0

* H/D ratio=Height/diameter ratio of pseudobulb.

out of ten of the small purplish-red pseudobulbs of the depressed type produced flowers. Five out of ten of the green pseudobulbs produced flowers.

9. The cause of flowering.

Since the pseudobulbs of *Pleione formosana* lives overwinter in the mountains where it is usually below 0°C and then flowers in next spring (Fig. 13), one may suggest that a low temperature is required for flowering as in biennials. This suggestion, however, is not true for this orchid. The buds on the pseudobulbs harvested on September 25, 1967 from the mountain were examined under the dissecting microscope and were found that they were floral, having rudimentary flower buds with well differentiated young sepals, petals and a recognizable column. The buds produced by those pseudobulbs cultured in a vinyl house in Taipei were harvested on September 1, 1967 and were also examined. They were also found floral, having well developed rudimentary flower buds. These observations show that the anthogenesis of *Pleione formosana* does not require a low temperature of winter, because flower buds are found before winter. The next question is: "then what is the cause of anthogenesis?" One possibility is the influence of day length. Since the leaves mature during period of shortening days (from July 1 in Taiwan) and since anthogenesis occurs also during these days, this orchid must be a short-day plant if the day length is a limiting factor (Fig. 13).

10. Number of flowers on the scape.

The number of flowers on the scape has been used as a taxonomic characteristic by R. A. Rolfe (1917). He recognized the plant with a 1-flowered scape as *P. Pricei* and the one with a 2- or 3-flowered scape as *P. formosana*. In the present study, it has been observed that a large pseudobulb is likely to produce a 2-flowered scape (Fig. 1 left) or a two 1-flowered scape on a common pseudobulb. As is shown in Table 2, one out of ten of the large purplish-red pseudobulbs produced a 2-flowered scape and one out of the large purplish-red pseudobulbs produced two 1-flowered scapes on a common pseudobulb. The small pseudobulb, on the other hand, always produced a 1-flowered scape. The presence of a 2nd flower primordium and the apical meristem of the inflorescence on a rudimentary inflorescence (Fig. 10A), the presence of a small undifferentiated bud a the joint of ovary and scape (Fig. 2C) and the actual occurrence of 2-flowered scapes (Fig. 1, left) are the evidences that the flower of *P. formosana* has a tendency to be a spike although most flowers produced in Taipei are solitary.

11. Keels on the lip.

R. A. Rolfe (1917) used the keel number as a characteristic to classify this orchid. He said that *P. Pricei* Rolfe had two keels whereas *P. formosana* Hayata had 4 keels on the lip. As shown in Table 4, four types of lips as regard to their keel number and length have been recognized in the present cultures. Most flowers, *i. e.*, 15 out of 17, have 2 long and 2 short keels (2L2S), the *formosana* type.

Table 4. The number of different color and keel characteristics, produced by purplish red and green pseudobulbs.*

Number and length of keels		Flower color			Total
		Pink	Pinkish white	White	
Pricei type	2L2T (or 2L)	1(1)	—	—	1(1)
	2L2S	7(1)	(1)	(1)	7(3)
formosana type	2L2S1VS	—	—	(1)	(1)
	2L2S1VSM	4	—	—	4
Total		12(2)	(1)	(2)	12(5)

*L=long keel; T=trace of keel; S=short keel; VS=very short keel; VSM=very short keel on mid-rib near the tip of the lip.
The number in ( ) is the number of flowers produced by green pseudobulbs; the number not in ( ) is the number of flowers produced by purplish red pseudobulbs.

Only a small proportion of plants, *i. e.*, 2 out of 17, have 2 long and or 2 trace keels (2L2T or 2L), or the *Pricei* type. A reduction of the number of keels was observed in the present study. In Taipei a 5-keeled parent plant propagated vegetatively gave rise to a daughter pseudobulb which had 3 keels (Table 1).

12. Relation between size of flower and of pseculobulb.

As shown in Table 5, flowers produced by smaller pseudobulbs were smaller than those flowers produced by larger ones. The size of the flower (represented by sepal length), however, varies extremely little as against a high variation of pseudobulbs in weight or size. The length of bracts also varies very little. The mean length of sepal of small pseudobulbs is 4.8 cm, that is, 0.2 cm or 4% shorter than that of large pseudobulbs. The mean length of bract of small pseudobulbs, on the other hand, is 3.2 cm, that is, 0.4 cm or 11% shorter than that of large pseudobulbs. In other words, the length of bract is much more variable than that of sepal. In fact, bract is more vegetative in nature than sepal. The pseudobulb is a vegetative organ. The 2nd flower produced on the same scape is much smaller than the 1st flower Table 5). The flower produced on the 1st scape was smaller than the flower produced on the 2nd scape of the same pseudobulb (Table 5). The length of scape and the weight of pseudobulb are positively correlated (Fig. 16). The heavier pseudobulb has a longer scape.

13. Formation of daughter pseudobulbs.

Reproduction occured vegetatively by forming daughter pseudobulbs on the parent or last year's pseudobulb (Fig. 6). The bud on the parent pseudobulb may develop and bear a leaf, a new pseudobulb is then formed below it. Typically, four pseudobulbs are produced on a parent pseudobulb. Two of them, from 1st and 2nd bud, may grow into about the same size as that of their parent pseudobulb. The rest of them, from 3rd and 4th bud, formed on the top of the pseudobublbs are always smaller and much elongated (Fig. 6).

Table 5. Relation between the size of flower (represented by the length of lateral sepal and of bract) and the weight of pseudobulb*

Weight of pseudobulb (gm)	Bract length (cm)		Sepal length (cm)	
	Flower on 2nd scape	2nd flower	Flower on 2nd scape	2nd flower
Large pseudobulb				
21.8	3.9	—	5.1	—
18.7	3.1	2.5**	5.0	4.6**
15.4	3.0	—	4.6	—
15.3	?	?	4.8	3.7***
15.2	3.4	—	4.8	—
14.3	4.5	—	5.4	—
Mean 16.8	3.6	2.5	5.0	4.2
Small pseudobulb				
6.2	3.3	—	5.0	—
6.0	3.3	—	4.8	—
5.7	3.0	—	5.0	—
5.7	3.2	—	4.6	—
5.3	3.0	—	4.7	—
4.6	?	—	4.7	—
Mean 5.6	3.2	—	4.8	—

* All pseudobulbs were purplish red. All flowers except the flower denoted by *** were solitary on the 2nd scape. Measurement were made on the day of flowering.
** The solitary flower on the 1st scape.
*** The 2nd flower of the 2nd scape.

Table 6. H/D ratio and weight of parent pseudobulb and of daughter pseudobulb (from 2nd bud) produced vegetatively in a vinyl house in Taipei*.

Color, size and form of parent pseudobulb	H/D ratio of pseudobulb		Weight of pseudobulb (gm)	
	Parent	Daughter	Parent	Daughter
Green	1.10	0.87	6.61±1.57	5.82±2.54
Purplish red, large	1.20	0.79	17.01±2.87	12.01±4.05
Purplish red, small and depressed	0.75	0.84	5.37±0.16	5.62±1.49
Purplish red, small and elongated	1.58	0.98	6.40±0.75	7.18±2.10
Mean	1.16	0.87	8.85	7.66

* Average of ten pseudobulbs.

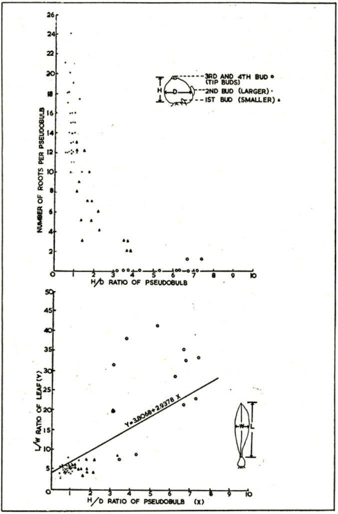

Fig. 15. Correlations between the number of root and the form of pseudobulbs (upper), and between the form of leaf and of pseudobulb (below).

Table 6 shows that larger pseudobulbs from the mountains produced larger pseudobulbs in Taipei, whereas those with smaller parent pseudobulbs produced smaller ones. The H/D ratios of daughter pseudobulbs tend to be a similar value whereas those of parent pseudobulbs have a higher deviation Table 6. The results show that the formation of pseudobulbs.

14. Correlations among root. leaf and pseudobulb.

As can be seen in Fig. 15 (below), there is a correlation between form of leaf and pseudobulb. It shows that the narrower leaves occur on the more elongated pseudobulbs. A statistical test concluded that this correlation is fairly reliable ($p=0.01$). Fig. 15 (below) also shows the relationships between size of bud and form of leaf, and between size of bud and form of pseudobulb. The larger bud results in the wider leaf and the thicker pseudobulb below it. Correlations between root number and pseudobulb form, and between root number and bud size are shown in Fig. 15 (upper). This figure shows that more roots occur on the larger bud that gives rise to a thicker pseudobulb.

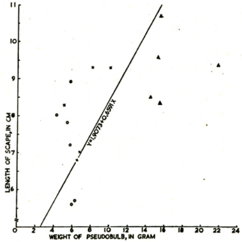

Fig. 16. Correlation between the weight of pseudobulb and the length of scape. ▲ denotes the scape produced by purplish red, large pseudobulb; × denotes the scape produced by green pseudobulb; o denotes the scape produced by purplish red, small, depressed-ovoid pseudobulb.

15. Identification of purplish red pigments in pseudobulb.

Extraction of pigments.

Purplish-red pigments were extracted from the pigment-containing tissue of fresh pseudobulbs with 1% hydrochloric methanol for 2 days in a dark room.

Color test on fresh tissue and crude extract of pseudobulb.

Several pieces of the pigment-containing tissue of fresh pseudobulbs were separated carefully from chlorenchyma and other tissues by a razor blade and were placed in a small Petri-dish containing concentrated aqueous ammonia. The purplish-red color of the tissue and the red color of the crude extract turned blue immediately upon contact with the fumes of ammonia, indicating that the pigments were anthocyanins (Geissman, 1955).

Separation of anthocyanins by paper chromatography (Endo, 1954).

The crude extract was chromatographed by the one-dimensional ascending method at 25±3°C. n–Butanol–36% HCl-water (5:1:4) was used as a solvent, but it produced a poor result. Toyo No. 50 filter paper (26×3 cm) was used through out this study. The results are summarized in Table 7. Six round to oval-shaped spots in purplish-red and pink color were clearly recognized on the chromatogram. The relative amounts of these anthocyanins contained in the pseudobulb were estimated from the size the spots as follows: b<c<d<a<f<e. The extract in 1% hydrochloric methanol was stored in a refrigerator at 5°C in the dark for 2 months and was also chromatographed. The same result was obtained, indicating that the pigments were stable in the dark at a low temperature.

Table 7. Rf values, colors and relative amounts of anthocyanins found in the pseudobulb of *Pleione formosana* chromatographed with n–butanol–36% HCl-water (5:1:4).

Anthocyanins	Rf values	Colors	Relative amounts
f	0.31	purplish red	5
e	0.23	purplish red	6
d	0.17	purplish red	3
c	0.13	purplish red	2
b	0.09	purplish red	1
a	0.04	pink	4

Observation of the absorption spectrum in the spectroscope.

A study of the absorption spectrum of the crude extract and of the pigment-containing tissue may provide some hints to understand the physiological and ecological functions rather than the biochemical nature of anthocyanins presented in the pseudobulb of this orchid. For this purpose and also for the identification of pigments, a spectroscope was used. The crude extract (in 1% hydrochloric methanol) of the pigment-containing tissue of pseudobulbs was placed in a glass cell and observed in the spectroscope. As known from the paper chromatographic analysis described earlier, this crude extract is a mixture of six anthocyanins with very little impurities. A dark band appears in the green region at about 550 mμ when the solution is thin. As the solution increases in thickness, another band in the blue to purple range of the spectrum, between 400 and 500 mμ is apparent. This light absorbing property of the extract is in agreement with that of anthocyanins (Geissman, 1955). The absorption spectrum of the purplish-red tissue of pseudobulb also shows a similar result, but the blue to purple wavelengths of the spectrum are absorbed less than that in the solution.

16. Occurrence of anthocyanins.

As seen in Table 8, purplish-red pseudobulbs always bore purplish red flowers and produced purplish-red daughter pseudobulbs. Green pseudobulbs, on the other hand, showed an irregular phenomenon. Some of them had white flowers and the daughter pseudobulbs had anthocyanins. Some others bore purplish-red flowers, but their daughter pseudobulbs were green. Some flowers showed an intermediate type, producing white flowers with a trace of anthocyanin color. Anthocyanin color was always found in the outer bud of daughter pseudobulbs, regardless of the presence or absence of anthocyanins in their parent pseudobulbs. As can be seen in Table 8, although the occurrence of anthocyanins is genetically controlled, the influences of other unknown factors are great.

It is a significant fact that the green pseudobulbs occur very rarely, whereas anthocyanin-containing pseudobulbs occur abundantly in the mountains, indicating

Table 8. The occurrence of anthocyanins in flowers, daughter pseudobulbs and buds of daughter pseudobulbs*.

	Parent pseudobulb	Flower (from bud of parent pseudobulb)	Daughter pseudobulb	Bud (on daughter pseudobulb)
Green pseudobulbs	–	–	±	+
	–	–	+	+
	–	±	+	++
	–	+	–	+
	–	+	–	++
	–	**	++	++
Purplish red pseudobulbs	+++	+++	+++	+++

* – denotes the absence of anthocyanins, ± denotes the presence of a trace of anthocyanins, + denotes the presence of anthocyanins in low concentration, ++ denotes the presence of anthocyanins in median concentration, +++ denotes the presence of anthocyanins in high concentration.

** Failed to flower.

the high adaptability of anthocyanin-containing pseudobulbs in the field. It may be suggested that anthocyanins of pseudobulbs protect the pseudobulbs against the harmful effects of ultraviolet rays and cold temperature at high altitudes and also against harmful organisms.

17. Geographical location.

The localities of *P. formosana* in Taiwan known from the literature, herbarium specimens (in NTU herbarium) and specialists are summarized in Table 9. These localities are also illustrated on the contour-line map of Taiwan (Fig. 17). As can be seen from these data, *P. formosana* has been collected in the mountains at various altitudes ranging from 500 m to 2,500 m. Most of them were collected from the northern Taiwan. But there is no definite evidence that it is not found in southern Taiwan. It was proved in this investigation that this orchid could be propagated vegetatively in an uncontrolled vinyl house in Taipei where winter temperature is very much higher than its localities on the mountains. The daughter pseudobulbs produced in Taipei was able to flower in Taipei. These data indicate that this orchid is presumably distributed also in the mountains in southern part of the island. Possibly these orchids have thrived previously at low altitudes in areas which are now under cultivation. Low temperature and low altitude do not seem to be the limiting factors for the growth of this orchid. So far *P. formosana* has only been reported from Taiwan.

Table 9. Localities of *Pleione formosana* in Taiwan.

Localities	Altitudes (m)	Collecters	Notes or literatures
Mt. Yang-mei, Nan-chuang, Miao-li (楊梅山), (南庄), (苗栗)	800	F. S. Chang*	Based on personal communication to the authors; pseudobulbs collected from these localities are for market
Mt. Lu-chang-ta, Nan-chuang, Miao-li (鹿場大山), (南庄), (苗栗)	1,500	F. S. Chang	
Mt. Chia-li-hsien, Nan-chuang, Miao-li (加利仙山), (南庄), (苗栗)	1,500	F. S. Chang	
Mt. Kuai-mu, Wu-feng, Hsin-chu (檜木山), (五峯), (新竹)	1,800–2,000	F. S. Chang	
Hyahōsha, Igiris ( ), ( )	2,000	U. Mori (1909)	Literature 8
Mt. A-li, Chia-i (阿里山), (嘉義)	2,000	B. Hayata *et al* (1914)	Literature 9
Mt. Tai-ping, I-lan (太平山), (宜蘭)	?	S. Suzuki (1928, 1929)	Specimens in NTU** herbarium
Kianraha, Taipei ( ), (臺北)	?	S. Suzuki (1930)	Specimen in NTU herbarium
Mt. Ta-tuen, Taipei (大屯山), (臺北)	?	S. Sasaki (1931)	Specimen in NTU herbarium
Mt. Syakaroo-taizan, Hsin-chu ( ), (新竹)	2,000	N. Fukuyama (1932)	Literature 3
Tai-lu-ko, Hua-lien (太魯閣), (花蓮)	1,800 2,500	I. M. Liu*et al* (1956)	Specimen in NTU herbarium

* See footnote on p. 2 (3).

** NTU=National Taiwan University, Taipei.

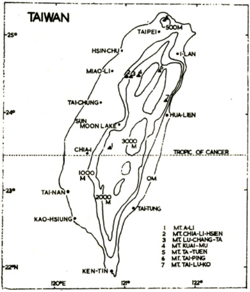

Fig. 17. Localities of *Pleione formosana* Hayata in Taiwan. Black triangulars denote the localities of this orchid formerly collected by botanists and specialists.

DISCUSSION

Though Taiwan does not have a large territory (36,000 Km^2), there are a considerable number of species of orchids. According to Masamune (1954), 372 species of orchids have been listed out of about 4,000 species of vascular plants from Taiwan, in other words, there is one orchid plant for about ten vascular plants in Taiwan. A considerable volume of pseudobulbs of *Pleione formosana* was exported at about US $4.00 per kilogram to Japan, U. S. A., England and other European countries last year (1967), whereas *Phalaenopsis amabilis* Blume var. *aphrodite* Ames (蝴蝶蘭), one of the famous precious orchids in Taiwan, is threatened with extinction from its native home in Taiwan. The wild orchid is an important part of the economic resources of any country. It deserves protection. Little has been published concerning the native orchids of Taiwan since World War II.

Pleione formosana is not usually an epiphyte on trees. Most of them grow on the precipitous rocks. Little wonder this native species is being destroyed by the cutting of forests, and building of highways. On the other hand, a single pseudobulb gives rise to four new pseudobulbs each year. Two of them develop almost the same size as that of their parent pseudobulb. The extinction of this plant hardly can happen except for the excessive collection of this species. Though this plant seems to be protected naturally, government protection from abusive collectors is needed, as well. In a study concerning the survival and reproduction of wild orchids in Rhode Island, Stuckey (1967) had been worried about the extinction of rare native American orchids.

In 1932 Fukuyama gave a name, *Pleione formosana* Hayata form *nivea* to those plants which had green pseudobulbs with white flowers. Since the flowers as well as daughter pseudobulbs produced by the green pseudobulbs were not limited to white color, since some of them were pink, and others were intermediate or pinkish-white in color, a variety name based on the color of flowers is not justified, and the establishment of *P. formosana* Hayata form. *nivea* Fukuyama or of other new name for these plants will result in nothing but confusion.

LITERATURE CITED

(1) BENTHAM, G. and J. D. HOOKER. 1883. Genera Plantarum. L. Reeve and Co., Covent Garden.

(2) ENDO, T. 1954. Biochemical and genetical investigation of flower color in *Viola tricolor* L. I. Inter-relationships of pigment constituents occurring in ten varieties. Jap. Jour. Bot. 14: 187-193.

(3) FUKUYAMA, N. 1932. Neue Orchideen von Formosa. Trans. Nat. Hist. Soc. Formosa 22: 413-416.

(4) FUKUYAMA, N. 1934. Orchidacearum Formosanarum Prodromus—Taxonomical stucy of Formosan orchidaceous plants with special reference to floral characters. Taihoku Imp. Univ. Thesis.

(5) FUKUYAMA, N. 1936. Orchidaceae. *In* Short flora of Formosa (Masamune, G. ed.): 282-302. The Editorial Department of Kudoa, Taihoku.

(6) Geissman, T. A. 1955. Anthocyanins, chalcones, aurones, flavones and related water-soluble plant pigments. *In* Modern methods of plant analysis, vol 3 (Paech, K. and M. V. Tracey ed.) 450-498. Springer-Verlag, Berlin.

(7) HAYATA, B. 1911. Materials for a flora of Formosa—Supplementary notes to the Enumeration Plantarum Formosanum and Flora Montana Formosae (based on a Study of the Collections of the Botanical Survey of the Government of Formosa, principally made at the Herbarium of the Royal Botanic Garden, Kew). Jour. College Sci. Imp. Univ. Tokyo, XXX. Art. 1: 326-327.

(8) HAYATA, B. 1914. Icones Plantarum Formosanarum. 4: 64-64.

(9) HAYATA, B. 1919. Plantarum Formosanarum 8: 132-132.

(10) KORIBA, H. 1960. Physiology and ecology of plants (植物生理生態). Yokendo Co., Tokyo.

(11) KUDO, Y. 1934. List of cultivated plants in the Department of Forestry, report no. 17. Department of Forestry, Government Research Institute, Taihoku. Taiwan.

(12) LIN, C. C. EDITOR, 1953. A preliminary draft of topography of Taiwan, The Historical Research Commission of Taiwan Province, vol. 1(2): 47-48. (臺灣省通志稿, 卷一 (第二册), 臺灣省文獻委員會編).

(13) MASAMUNE, G. 1933. A list of orchidaceous plants indigenous to Formosa. Tropical Horticulture 3 (1-4): 22-28.

(14) MASAMUNE, G. 1954. A list of vascular plants of Taiwan. (Mimeographed).

(15) ROLFE, R. A. 1917. *Pleione Pricei* Rolfe. Bot. Mag. 143: t. 8729.

(16) SCHLECHTER, R. 1919. Orchideologiae Sino-Japonicae Prodromus. Verlag des Repertoriums, Berlin.

(17) STUCKEY, I. H. 1967. Environmental factors and the growth of native orchids. Am. J. Bot. 54: 232–241.

(18) WITHNER, G. 1959. The orchids. The Ronald Press Co., New York.