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# Two new natural hybrids in the genus Pleione (Orchidaceae) from China

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#### Abstract

Several species in the genus *Pleione* (Orchidaceae) have same or overlapping geographical distribution in China. In this study, two new natural hybrids, *Pleione* × *baoshanensis* and *Pleione* × *maoershanensis*, were described and illustrated. The parentage for these two hybrids was confirmed using molecular data from ITS of the nuclear ribosomal, *trnT-trnL* spacer and *trnL-trnF* region (*trnL* intron and *trnL-trnF* spacer) of the plastid DNA. *Pleione* × *baoshanensis* is intermediate between *P. albiflora* and *P. yunnanensis*, and characterized by its erose lamellae on the lip. Meanwhile, *Pleione* × *maoershanensis* is intermediate between *P. hookeriana* (*P. chunii*) and *P. pleionoides*, and characterized by its deep lacerate lamellae on the lip. For the individuals tested, molecular data suggest that *P. albiflora* is the maternal parent of *Pleione* × *baoshanensis*, and *P. hookeriana* (*P. chunii*) is the maternal parent of *Pleione* × *maoershanensis*. The history and taxonomic status of *P. chunii* is also discussed.

Keywords: Coelogyninae, lip callus, natural hybridization, Pleione phylogenetics, sympatric species

## Introduction

It is estimated that a quarter of plant species are involved in hybridization and potential introgression with other species (Mallet 2005). These natural hybridizations are recognized as an important source of evolutionary novelty (Arnold 1992; Soltis & Soltis 2009), and also, threats of extinction to rare species (Levin *et al.* 1996).

Typically, maintenance of species boundaries is the result of the combination of both prezygotic and postzygotic barriers (Rieseberg & Carney 1998). In the family Orchidaceae, prezygotic barriers, such as pollinator specificity, are usually considered predominant in the reproductive isolation of sympatric species (e.g. Banziger *et al.* 2008; Scopece *et al.* 2007), even though postzygotic barriers may have a comparable contribution in some cases (Cortis *et al.* 2009; Scopece *et al.* 2007; Scopece *et al.* 2008). However, despite these exquisite barriers that prevent invasion of the genome from one species to another, natural hybrids are quite common and have continuously been discovering in every main lineage of this family (e.g. Iamonico *et al.* 2011; Jakubska-Busse *et al.* 2017; Lee 2013; Liu & Chen 2007; Neto *et al.* 2012; Nielsen 2000).

The species of genus *Pleione* D. Don (1825: 36) distribute from central Himalayas eastwards to Taiwan Island and from central mainland China southwards to central Indo-China Peninsula. The highest species diversity was found in mountainous areas of southwest China, where several species have same or overlapping geographical distribution (Chen *et al.* 2009; Cribb & Butterfield 1999). Due to the weak interspecific incompatibility in this genus, hundreds of artificial hybrids have been made and therefore the chance of successful hybridization was supposed to be relatively high for sympatric species (Cribb & Butterfield 1999). Indeed, taking the relatively small size of the genus into account (~20 species), the number of natural hybrids in *Pleione* is quite substantial: five species have been suggested to be natural hybrid origin (Cribb & Butterfield 1999) and of which two has been conclusively confirmed their parentage by DNA evidence (Gravendeel *et al.* 2004).

Recently, several plants were collected with intermediate floral characteristics between co-occurring species in China. Based on a combination of morphology and molecular phylogeny, two new natural hybrids of *Pleione* were herein described and illustrated.

# Materials and methods

### Morphology observation

Characters of the leaves and flowers for two natural hybrids were described and measured on fresh specimens. The flowers of these two hybrids were compared with their respective putative parents, *Pleione albiflora* P. J. Cribb & C. Z. Tang (1983:117), *P. yunnanensis* (Rolfe 1903a: 23) Rolfe (1903b: 292), *P. hookeriana* (Lindl. 1854: 14) Rollisson (1875: 39) and *P. pleionoides* (Kraenzl. ex Diels 1901: 267) Braem & H. Mohr (1989: 124). The flower was focused more on the callus at the upper surface of the lip.

Species	Origin	GenBank accession numbers			
		ITS	trnT-trnL	trnL	trnL-trnF
Pleione albiflora	China	AY101967	AF503655	AF503705	AF503680
P. aurita	China	AF461467	AF503660	AF503710	AF503685
P. bulbocodioides	China	AF461482	AF503657	AF503707	AF503682
P. formosana	unknown	AF461485	AF503661	AF503711	AF503686
P. forrestii	China	AF461478	AF503662	AF503712	AF503687
P. grandiflora	China	AF461477	AF503663	AF503713	AF503688
P. humilis	Nepal	AF461495	AF503666	AF503716	AF503691
P. limprichtii	China	AF461490	AF503667	AF503692	AF503717
P. maculata	unknown	AF461493	AF503668	AF503718	AF503693
P. pleionoides	China	AF461480	AF503670	AF503720	AF503695
P. praecox	unknown	AF461491	AF503669	AF503719	AF503694
P. saxicola	China	AF461492	AF503672	AF503722	AF503697
P. scopulorum	unknown	AF461471	AF503673	AF503723	AF503698
P. yunnanensis	China	AF461487	AF503675	AF503725	AF503700
Dendrochilum longifolium	New Guinea	AF281121	AF503678	AF503728	AF503703
Thunia alba	Nepal	AY008466	AF503679	AF503729	AF503704
P. albiflora Dali	Dali, Yunnan, China	MF775377	MF775393	-	MF775387
P. hookeriana Wenshan	Wenshan, Yunnan, China	MF775378	MF775391	-	MF775380
P. hookeriana Xing'an (P.	Xing'an, Guangxi, China	MF775379	MF775392	-	MF775381
chunii)					
P. pleionoides Chishui	Chishui, Guizhou, China	MF775374	MF775388	-	MF775384
P. pleionoides Yingjing	Yingjing, Sichuan, China	MF775375	MF775389	-	MF775385
P. yunnanensis Kunming	Kunming, Yunnan, China	MF775376	MF775394	-	MF775386
$P. \times baoshanensis$	Baoshan, Yunnan, China	MF775372	MF775390	-	MF775382
$P. \times$ maoershanensis	Xing'an, Guangxi, China	MF775373	MF775395	-	MF775383

TABLE S1. The origin of materials and GenBank accession numbers of the sequences used in the present study

Boldface: Data generated in this study.

DNA extraction and PCR amplification

Total genomic DNA of the two hybrids and their putative parents was extracted from fresh leaves using a plant DNA extraction kit (REDExtract-N-Amp, Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer's protocol. Primer pairs ITS4/ITS5 (White *et al.* 1990),a/b and c/f (Taberlet *et al.* 1991) were employed for amplification and sequencing of nuclear ribosome internal transcribed spacers (ITS), chloroplast regions between the *trnT* and *trnF* genes (*trnT-trnF*), respectively. Protocols for PCR and sequencing were according to Gravendeel *et al.* (2004). The amplified products were sequenced using an ABI 3730XL sequencer.

Trait	Pleione yunnanensis	$P. \times baoshanensis$	P. albiflora
Peduncle length	7–20 cm	8–10 cm	3–7 cm
Bract length	20–30 cm, shorter than ovary	30–36 mm, as long as ovary or slightly exceeding the ovary	20–35 mm, longer than ovary
		sugnity exceeding the ovary	
Flower colour	purplish, pink, or sometimes white	light pink	white, rarely with pale mauve lines on sepals and petals
Lip shape	obovate, apical margin erose- lacerate	ovate, apical margin lacerate	broadly ovate, apical margin lacerate
T in colour	winds with moments and are and	light might with held with to move he	antite anith hald animage to
Lip colour	spots	central stripes and spots	brownish yellow central stripes
Callus on lip	3–5 entire lamellae	4 erose lamellae	4–6 rows of long papillae
Column length	18–23 mm	28–30 mm	33–40 mm

**TABLE 1.** A comparison of *Pleione* × *baoshanensis* and its putative parents

TABLE 2. A comparison of Pl	eione × maoershanensis	and its putative parents
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Trait	Pleione hookeriana	$P. \times maoershanensis$	P. pleionoides
Peduncle length	5–10cm	2.5–5 cm	2–8 cm
Bract length	10–25mm, longer than ovary	18–28 mm, longer than ovary	25–39 cm, longer than ovary
Flower colour	pale pink or pale purple	bright rose	bright rose-purplish
Lip shape (when flattened)	oblate or subcordate	subrhombic, obovate or suborbicular	subrhombic to obovate
Lip colour	pale pink or pale purple, with purple spots at apical 1/3	bright rose, with crimson spots at apical 1/3	bright rose, with crimson spots or areas at apical 1/3
Callus on lip	5–9 long papillae	4-6 deeply lacerate lamellae	2 or 4 denticulate lamellae
Column length	15–30mm	30–40 mm	35–45 mm

Sequences alignment and phylogenetic analyses

The sequences generated in this study were deposited in GenBank as MF775372–MF775395 and each accession number and origin of materials were shown in Table S1. To understand the relationships among two putative hybrids and their putative parents, a total of 48 ITS and *trnT-trnL*, *trnL* and *trnL-trnF* sequences representing 12 species of *Pleione* were retrieved and combined in our analyses (Table S1). Sequences were aligned using Clustal X v.1.83 (Thompson *et al.* 1997) with manual adjustment in BioEdit (Hall 1999). Sequence lengths of the ITS, *trnT-trnL*, and *trnL-trnF* matrices are 672bp, 554bp and 965 bp, respectively. The sequences of *trnT-trnL* and *trnL-trnF* were concatenated with Phyutility 2.2 (Smith & Dunn 2008) to carry out a combined analysis. *Dendrochilum longifolium* and *Thunia alba* were included as outgroups, based on the methods of Gravendeel *et al.* (2004). Sequences of *P*.

*hookeriana* in the GenBank were not used because the individuals were without a specific geographical origin and will create phylogenetic incongruence (Gravendeel *et al.* 2004).

Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). ML analyses were performed using RAxML-VI-HPC (Stamatakis 2006) with the substitution model GTR+G and 1000 rapid bootstrap searches (BS). The BI analysis was carried out using MrBayes version 3.2.1 (Ronquist & Huelsenbeck 2003). The optimal substitution model HKY+G for nDNA and GTR+I for cpDNA dataset were selected by MrModeltest v2.2 according to Akaike Information Criterion (AIC) (Nylander 2004). All parameters in the ML analyses were used as the default settings, and statistical supports were obtained with rapid nonparametric bootstrapping with 1000 replicates. The BI analysis was performed by running four chains of 10 million generations and using the STOPRUL command by setting a convergence value of 0.01 and ESS values>200. Trees were sampled every 100 generations, and statistic supports were obtained after discarding the first 10% trees as burn-in.

### Results

The comparison of flowers of two hybrids and their respective putative parents were shown in Fig. 1 and Fig. 2. The lip color, lip shape and callus character indicate the intermediate state of these two hybrids between their putative parents (Fig. 1 and Fig. 2). More flower traits of these two hybrids and their putative parents were compared in Table 1 and Table 2.

For the 25 accessions included in our phylogenetic analyses, ML tree showed a very similar topology to the Bayesian tree and there was no strongly supported incongruence. *Pleione* × *maoershanensis* is closely related to two individuals of *P. pleionoides* as indicated by the tree inferred from ITS sequences (Fig 3). In contrast to the ITS phylogeny, sample of *P.* × *maoershanensis* and *P. hookeriana* (collected from Guangxi, China) clustered together according to the plastid sequences, suggesting that the latter one might have donated the plastid genome (Fig 4). *P.* × *baoshanensis* was found to be clustered in a clade consists of two specimens of *P. yunnanensis* as indicated by ITS sequences (Fig 3), while plastid sequences place *P.* × *baoshanensis*, *P. albiflora*, *P. coronaria* and *P. humilis* together (Fig 4). The latter two species spread restricted in the middle Himalayas, where is very far from the known distribution of *P.* × *baoshanensis* (Baoshan, west Yunnan). Thus, by combining the morphological evidence with molecular data, *P. albiflora* is the maternal parent of *P.* × *baoshanensis*.

#### Discussion

The hybrid origin of *Pleione* × *maoershanensis* and *P.* × *baoshanensis* can be easily recognized by their flower morphology. Both of these two hybrids have one parent with callus consisting of papillae and the other parent with callus consisting of lamellae (see Fig. 1 and Fig. 2). The earlier described hybrids *P.* × *confusa* and *P.* × *kohlsii*, are of this type. The discovering of *P.* × *confusa* and *P.* × *kohlsii* was the result of introduction of wild plants into cultivation, and hybrid individuals were usually found mixed with one of the parent (Cribb & Butterfield 1999). Similarly, *P.* × *maoershanensis* and *P.* × *baoshanensis* share similar habitat with their respective parents. This confirms the great potential of hybridization between sympatric species in this genus. Most of earlier described hybrids were proved to be F<sub>1</sub> generation (Gravendeel *et al.* 2004), and further research will help to understand the hybridization process or reticulate evolution in this taxa.

The Guangxi type of one of the parents *P. hookeriana* were treated today as a separate species [*P. chunii* Tso (1933: 148)] by some authors (Chen *et al.* 2009; Cribb & Butterfield 2001; Hareesh *et al.* 2017). When it comes to the name "*Pleione chunii*", a long story has to be told. The botanist Ching Lieh Tso described this species in honor of his teacher, Prof. Woon Youg Chun. The type specimen was from cultivated plants said originated from Lokchong (Lechang), northern Guangdong (Tso 1933). Dry specimen of another species, *P. aurita*, which Phillip Cribb described based on the specimen in Yunnan (Cribb & Pfennig 1988), is so similar to *P. chunii* to urge Phillip Cribb to synonymize *P. aurita* with the older name *P. chunii* (Cribb & Butterfield 1999; Cribb & Chen 1994). In the late 90s and early 2000s, more Chinese species, including the spectacular *P. aurita*, were introduced into Europe to meet horticultural demand, which gave a chance to *P. aurita* to regain its name and to confirm the alliance of *P. chunii* to *P. hookeriana* (Chen *et al.* 2009; Cribb & Butterfield 2001; Torelli 2001).



**FIGURE 1.** Flower morphology of *Pleione* × *baoshanensis* and putative parents. A. Flower shape; B. Lip flattened. A1. P. yunnanensis; A2. P. × *baoshanensis*; A3. P. *albiflora*. B1. Lip of P. yunnanensis; B2. Lip of P. × *baoshanensis*; B3. Lip of P. *albiflora*.



**FIGURE 2.** Flower morphology of *Pleione* × *maoershanensis* and putative parents. A. Flower shape; B. Lip flattened. A1. *P. hookeriana*; A2. *P.* × *maoershanensis*; A3. *P. pleionoides*. B1. Lip of *P. hookeriana*; B2-B5. Lips of *P.* × *maoershanensis*; B6. Lip of *P. pleionoides*.



**FIGURE 3.** Phylogenetic tree inferred from ITS sequences using Bayesian inference. Bayesian posterior probability (PP,  $\ge 0.90$ ) and bootstrap values ( $\ge 50$ ) derived from ML analyses (ML-BS) are showed above branches in a follow of PP/ML-BS. The new hybrids were shown in bold.



**FIGURE 4.** Phylogenetic tree inferred from *trnT-trnL* and *trnL-trnF* sequences using Bayesian inference. Bayesian posterior probability (PP $\ge$  0.90) and bootstrap values ( $\ge$  50) derived from ML analyses (ML-BS) are showed above branches in a follow of PP/ML-BS. The new hybrids were shown in bold.

In fact, Kleinhans tried to find real *P. chunii* in April 2005, but no living plants was rediscovered in its type locality, except another *Pleione* species *P. formosana* Hayata (1911: 326) (Kleinhans 2005). Besides the holotype, only one out of hundreds of *Pleione* specimens was labeled as "*Pleione chunii* Tso" in the herbarium of South China

Botanical Garden (IBSC). After a careful examination of this one (*L. Teng 4119*, IBSC), we found the pressed plant displayed on the sheet is quite different from the holotype of *P. chunii* (*N. K. Chun 43047*, IBSC), while the flower size and lip callus characteristics fall into the range of *P. formosana*. Another attempt was made by us to find real *P. chunii* and another suspectable species, *P. microphylla* S. C. Chen & Z. H. Tsi (2000: 182) in Guangdong in April, 2017. However, unexceptionally, only plants that were typical *P. formosana* were found growing in the mountains in the southern part (Nankun Shan, Guangzhou and Huizhou border) and the northern part (Nanling National Nature Reserve, Ruyuan County) of Guangdong, although these plants were given a name of *P. bulbocodioides* in literatures (Tang *et al.* 2011; Wu 2006) and specimens (e.g. *H. Z. Tian 387* and *A. Q. Hu 23*, IBSC).



FIGURE 5. Flower of an individual from Maoer Shan, Northern Guangxi (A) and the holotype of Pleione chunii Tso (B).



**FIGURE 6.** *Pleione* × *baoshanensis*. A. Plant with flower; B. Flower—side view; C. Flower, different parts in separation; D. Mature leaf. Bar=2cm.



**FIGURE 7.** *Pleione*  $\times$  *maoershanensis.* A. Plant with flower in the natural habitat; B. Flower—side view; C. Flower, different parts in separation; D. Mature leaf. Bar=2cm.

Since no living plant was rediscovered in its type locality or nearby region, we found the plants in northern Guangxi (a neighboring province of Guangdong) perfectly fit with the holotype of P. chunii (see Fig 5). The materials collected from these areas were usually identified as P. hookeriana by Chinese botanists (e.g. G. Z. Li 12077, IBK; Huaping Nature Reserve Expedition H0051, PE), as well as the isotype of P. chunii that from Guangdong (N. K. Chun 43047, PE). Phylogeny of *Pleione* indicates that *P. hookeriana* is polyphyletic, and the authors suggested the different phylogenetic position of Chinese accession and Indian accession of P. hookeriana could be caused by three processes: hybridization, introgression and/or incomplete lineage sorting (Gravendeel et al. 2004). However, the material Gravendeel et al. used was without a specific locality, and after the examination of specimens and photos of living plants from Guangxi, Yunnan and Tibet, we found even within China the Guangxi origin of P. hookeriana shows some differences with those of Yunnan and Tibet, which seemingly supports dividing these entities into two species (P. hookeriana and P. chunii). For example, a two-year observation on plants cultivated in Kunning after collected from southeastern Yunnan (Wenshan County) and northern Guangxi (Maoer Shan, Xing'an County) unraveled the differences in pseudobulb shape, flower size and flowering time. No stolon was observed in both populations but the Guangxi plants had larger pseudobulbs, larger flowers and earlier flowering time in April while the Yunnan plants had slim pseudobulbs, smaller flowers and a flowering time a month later (see also Hareesh et al. 2017). Flora of China distinguishes *P. hookeriana* and *P. chunii* by the row number of papillae on the lip. The former has a callus on the lip composed of seven rows of papillae while the latter's composed of six (Chen et al. 2009). We therefore investigated 20 plants of the Maoer Shan population (Guangxi) and found that the row number of papillae varies from 5 to 9, similar to other species described in this genus (see Chen et al. 2009). To date, all have left to distinguish P. hookeriana and P. chunii is plant size, which seems not enough for P. chunii to be regarded as a separate species, given the fact that twice the normal sized *P. hookeriana* has been collected in Nepal, where is far from the region of *P. chunii* (see  $p_{sy}$  Cribb & Butterfield 1999).



**FIGURE 8.** Pleione  $\times$  baoshanensis (A–B) and Pleione  $\times$  maoershanensis (C–D). Pleione  $\times$  baoshanensis, A. Habit; B. Lip. Pleione  $\times$  maoershanensis, C. Habit; D. Lip. Drawn by Jiao Qin.

To sum up, morphologically *P. chunii* seems to deserves a variety of *P. hookeriana*, however, it's too early to synonymize *P. chunii* with *P. hookeriana* for the lack of extensive sampling and sufficient molecular evidence. Thus, we tend to follow Prof. X. Q. Chen (S. C. Chen) and Prof. K. Y. Lang's treatments by temporarily labeling plants from Guangxi and Guangdong as *P. hookeriana* (*G. Z. Li 12077*, IBK; *Huaping Nature Reserve Expedition H0051*, PE; *N.* 

*K. Chun 43047*, PE). Multi-discipline study on extensive sampling is needed to elucidate the relationship of plants continuously distributed from Nepal and India in the west and Guangdong Province, China in the east, and to clarify the taxonomic position of *P. chunii*.

# Taxonomy

*Pleione* × *baoshanensis* W. Zhang & S. B. Zhang *hybr. nov.* [*P. albiflora* P. J. Cribb & C. Z. Tang × *P. yunnanensis* (Rolfe) Rolfe.] Fig. 1 A2, B2; Fig. 6 and Fig. 8 A, B

Intermediate between *P. albiflora* and *P. yunnanensis*. It differs from *P. albiflora* by the longer peduncle, light pink flower (not white), and erose lamellae (not long papillae) on the lip. It also differs from *P. yunnanensis* by the floral bract that is as long as ovary or slightly longer than ovary (not shorter than ovary) and erose (not entire) lamellae on the lip.

**Type:**—CHINA. Yunnan: Baoshan City, Longyang District, Yangliu Township, 2500 m, December 2014 (flowered in cultivation at Kunming Institute of Botany in April, 2015) , *W. Zhang & S. B Zhang ZW002* (holotype: KUN!).

Herbs, lithophytic. Pseudobulb green or dark olive-green, conic-ovoid,  $2-2.5 \times 1-2$  cm, 1-leaved. Leaf not developing at flowering, lanceolate to narrowly elliptic when mature,  $15-25 \times 3-4$  cm, apex acuminate. Inflorescence erect; peduncle 8–10 cm, prolonged when fruit; floral bract 30–36 mm long, as long as ovary or slightly exceeding the ovary. Flower solitary, rarely two, light pink. Dorsal sepal lanceolate, 42–48 mm long, apex acute; lateral sepals 40–46 mm long, slightly wider than dorsal sepal. Petals oblanceolate, 45–50 mm long, narrower than dorsal sepal, apex acute; lip ovate, 40–48 × 25–32 mm, apical margin lacerate or denticulate; disk with 4 erose lamellae. Column 28–30 mm.

**Phenology:**—Flowering from April to May.

Etymology:-Named after the City of Baoshan where this taxon was discovered.

**Distribution and habitat**:—Known only from the type locality. This hybrid grows on humus-covered rocks along streams, mixed with one of its parents *P. albiflora* at an elevation of 2400–2600 m, where the other parent *P. yunnanensis* and an autumn flowering species *P. praecox* can be found within an area of ten square meter. This hybrid has also been raised artificially by Paul Cumbleton and registered at Royal Horticulture Society as "*Pleione* Siamang" in 2009.

*Pleione* × *maoershanensis* W. Zhang & S. B. Zhang *hybr. nov.* [*P. pleionoides* (Kraenzlin) Braem & H. Mohr ×*P. hookeriana* (Lindley) Rollisson (*Pleione chunii* C. L. Tso)]. Fig. 2 A2, B2–B5; Fig. 7 and Fig. 8 C, D

 $Pleione \times maoershanensis$  is a hybrid intermediate between *P. pleionoides* and *P. hookeriana*. It differs from the former by the lacerate lamellae (not denticulate) on the lip. It also differs from *P. hookeriana* by the brighter colour of flower and the deeply lacerate lamellae (not papillae or hair) on the lip.

**Type:**—CHINA. Guangxi: Xing'an County, Maoer Shan, Huilong Temple, 1600m, April 11, 2016, *W. Zhang & Y. Yang ZW003* (holotype: KUN!).

Herbs, lithophytic. Pseudobulb ovoid to conic,  $1.5-2.5 \times 1-2$  cm in diam, 1-leaved. Leaf immature at flowering, mature leaf elliptic-lanceolate,  $10-20 \times 3-4$  cm, papery, apex acute. Inflorescence erect or suberect; peduncle 2.5–5 cm; floral bract 18–28 mm, exceeding ovary. Flowers solitary, bright rose. Dorsal sepal narrowly elliptic, 40–55 mm long, apex acute; lateral sepals slightly oblique, 35–50 mm long, slightly wider than dorsal sepal, apex acute. Petals oblanceolate, 35–60 mm long, narrower than dorsal sepal, apex acute; lip subrhombic, obovate or suborbicular when flattened, 35–50  $\times$  30–49 mm, apical margin denticulate; disk with 4–6 deeply lacerate lamellae. Column 30–40 mm.

Phenology:—Flowering from late March to May.

Etymology:--Named after the highest mountain in South China (Maoer Shan), where this natural hybrid was discovered.

**Distribution and habitat**: Known only from the type locality. This hybrid grows on humus-covered rocks in forests or cliffs at forest margins, mixed with *P. hookeriana* at an elevation of 1300–1600 m. The hybrid was found only on rocks while *P. hookeriana* grows both on rocks and moss-covered trunks or branches of *Lithocarpus hancei* (Bentham) Rehder in a *Tsuga/Rhododelldron* forest from 1300 to 2000 m. The other parent, *P. pleionoides*, was not seen during our brief exploration of Maoer Shan. However, we collected samples of that species from Yinzhulaoshan Nature Reserve in Ziyuan County, a place less than 50 km away from Maoer Shan. The variation of lip morphology of *P. × maoershanensis* implies backcross between the hybrid and the parents (see Fig 2). This hybrid has also been raised artificially by Paul Cumbleton and registered as "*Pleione* Potto" (*P. chunii × P. pleionoides*) in 2009 at Royal Horticulture Society.

#### Acknowledgement

We thank Mr. Dapeng Yu, Dr. Binjie Ge (Shanghai Chenshan Botanical Garden), Dr. Guangda Tang (South China Agricultural University), Prof. Yechun Xu (Guangdong Academy of Agricultural Sciences) and Mr. Xuewei Fu (Kunming Institute of Botany) for their help in field investigation, and Ms. Lingling Zhang (South China Botanical Garden) for her help in specimen examination. This study was supported by the National Natural Science Foundation of China (31670342, 31370362), the National Key Project of the Ministry of Science and Technology of China (2015BAD10B03), and the Science and Technology Research Program of Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences (2016-006).

#### References

- Arnold, M.L. (1992) Natural hybridization as an evolutionary process. *Annual Review of Ecology and Systematics* 23: 237–261. https://doi.org/10.1146/annurev.es.23.110192.001321
- Banziger, H., Sun, H.Q. & Luo, Y.B. (2008) Pollination of wild lady slipper orchids *Cypripedium yunnanense* and *C. flavum* (Orchidaceae) in south-west China: why are there no hybrids? *Botanical Journal of the Linnean Society* 156: 51–64. http://doi.org/10.1111/j.1095-8339.2007.00755.x.
- Braem, G.J. & Mohr, H. (1989) *Pleione pinkepankii* and *Pleione pleionoides*, two new taxa of Asiatic terrestrial orchids. *Orchis* 1989: 121–126.
- Chen, S.C. & Tsi, Z.H. (2000) *Pleione microphylla*, a new orchid from southern Guangdong, China. *Acta Phytotaxonomica Sinica* 38: 182–184.
- Chen, X., Cribb, P.J. & Gale, S.W. (2009) *Pleione* D. Don. *In:* Wu, Z.Y., Raven, P.H. & Hong, D.Y. (Eds.) *Flora of China*. Missouri Botanical Garden Press, St.Louis, pp. 325–333.
- Cortis, P., Vereecken, N.J., Schiestl, F.P., Lumaga, M.R.B., Scrugli, A. & Cozzolino, S. (2009) Pollinator convergence and the nature of species' boundaries in sympatric Sardinian *Ophrys* (Orchidaceae). *Annals of Botany* 104: 497–506. https://doi.org/10.1093/aob/mcn219
- Cribb, P. & Butterfield, I. (1999) The Genus Pleione (Second edition). Richmond: Royal Botanic Gardens, Kew.
- Cribb, P. & Butterfield, I. (2001) A synopsis of the genus Pleione. Orchid Digest 65: 163-187.
- Cribb, P. & Chen, S. (1994) Pleione chunii, an earlier name for P. aurita. The Orchid Review 102: 276-277.
- Cribb, P. & Pfennig, H. (1988) Pleione aurita-a new species from China. Die Orchidee 39: 111-114.
- Cribb, P.J. & Tang, C.Z. (1983) The genus Pleione. In: Curtis's Botanical Magazine. London, pp. 117.
- Don, D. (1825) Prodromus florae Nepalensis. London.
- Gravendeel, B., Eurlings, M.C.M., van den Berg, C. & Cribb, P.J. (2004) Phylogeny of *Pleione* (Orchidaceae) and parentage analysis of its wild hybrids based on plastid and nuclear ribosomal ITS sequences and morphological data. *Systematic Botany* 29: 50–63. https://doi.org/10.1600/036364404772973988
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *In: Nucleic acids symposium series*. [London]: Information Retrieval Ltd., pp. 95–98.
- Hareesh, V.S., Kumar, P. & Sabu, M. (2017) *Pleione arunachalensis* (Orchidaceae: Epidendroideae: Arethuseae: Coelogyninae), a new species from North-East India. *Phytotaxa* 291: 294–298.

https://doi.org/10.11646/phytotaxa.291.4.7

Iamonico, D., Forbicioni, L. & Frangini, G. (2011) A new hybrid in the genus *Ophrys* (Orchidaceae) from the Elba Island (central Italy). *Annales Botanici Fennici* 48: 435–438.

http://doi.org/10.5735/085.048.0508

- Jakubska-Busse, A., Zolubak, E. & Lobas, Z. (2017) *Epipactis* × *subtilis* (Orchidaceae), a new hybrid between E. albensis and E. purpurata. *Annales Botanici Fennici* 54: 139–144.
  - https://doi.org/10.5735/085.054.0322

- Kraenzlin, F.W.L. (1901) Orchidaceae. *In*: Diels, L. Die flora von Central-China: nach der vorhandenen litteratur und neu mitgeteiltem original-materiale. *Botanische Jahrbücher fur Systematik, Pflanzengeschichte und Pflanzengeographie* 29: 263–272.
- Lee, Y.I. (2013) *Calanthe* × *hsinchuensis* (Orchidaceae), a new natural hybrid from Taiwan. *Botanical Studies* 54: 5. https://doi.org/10.1186/1999-3110-54-5
- Levin, D.A., Francisco-Ortega, J. & Jansen, R.K. (1996) Hybridization and the extinction of rare plant species. *Conservation Biology* 10: 10–16.

Kleinhans, G. (2005) Pleiones in Canton. In: The Pleione review. pp. 38-48.

https://doi.org/10.1046/j.1523-1739.1996.10010010.x

Lindley, J. (1854) Folia Orchidacea: An enumeration of the known species of orchids. London.

- Liu, Z.J. & Chen, S.C. (2007) *Paphiopedilum* × *yingjiangense*, a new hybrid of Orchidaceae from China. *Acta Botanica Yunnanica* 29: 289–290.
- Mallet, J. (2005) Hybridization as an invasion of the genome. *Trends in Ecology and Evolution* 20: 229–237. https://doi.org/10.1016/j.tree.2005.02.010
- Neto, V.P.C., Motte, D. & Dubuisson, J.Y. (2012) *Cattleya* × *itabapoanaensis* (Orchidaceae), a new natural hybrid from Rio Janeiro State (Brazil). *Phytotaxa* 50: 64–68.

https://doi.org/10.11646/phytotaxa.50.1.6

- Nielsen, L.R. (2000) Natural hybridization between Vanilla claviculata (W. Wright) Sw. and V. barbellata Rchb.f. (Orchidaceae): genetic, morphological, and pollination experimental data. Botanical Journal of the Linnean Society 133: 285–302. https://doi.org/10.1111/j.1095-8339.2000.tb01547.x
- Nylander, J.A.A. (2004) MrModeltest. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.
- Rieseberg, L.H. & Carney, S.E. (1998) Plant hybridization. New Phytologist 140: 599-624.

https://doi.org/10.1046/j.1469-8137.1998.00315.x

Rolfe, R.A. (1903a) The genus Pleione. The Orchid Review 130: 292.

Rolfe, R.A. (1903b) Orchidaceae: Epidendreae *In*: Enumeration of all the plants known from China Proper, Formosa, Hainan, Corea, the Luchu Archipelago, and the Island of Hongkong, together with their distribution and synonymy. Part—XIV. *Journal of the Linnean Society of London, Botany* 36: 1–72.

http://doi.org/10.1111/j.1095-8339.1903.tb02550.x

Rollisson, W. (1875) General Catalogue of Stove, Greenhouse, Hardy, and Bedding Plants by William Rollisson & Sons. Pollet, London.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

https://doi.org/10.1093/bioinformatics/btg180

Scopece, G., Musacchio, A., Widmer, A. & Cozzolino, S. (2007) Patterns of reproductive isolation in Mediterranean deceptive orchids. *Evolution* 61: 2623–2642.

https://doi.org/10.1111/j.1558-5646.2007.00231.x

Scopece, G., Widmer, A. & Cozzolino, S. (2008) Evolution of postzygotic reproductive isolation in a guild of deceptive orchids. *American Naturalist* 171: 315–326.

https://doi.org/10.1086/527501

Smith, S.A. & Dunn, C.W. (2008) Phyutility: a phyloinformatics tool for trees, alignments and molecular data. *Bioinformatics* 24: 715–716.

https://doi.org/10.1093/bioinformatics/btm619

- Soltis, P.S. & Soltis, D.E. (2009) The role of hybridization in plant speciation. *Annual Review of Plant Biology* 60: 561–588. https://doi.org/10.1146/annurev.arplant.043008.092039
- Stamatakis, A. (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22, 2688–2690.

https://doi.org/10.1093/bioinformatics/btl446

- Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109. https://doi.org/10.1007/BF00037152
- Tang, G., Zhuang, X.Y, Qiu, J.L., Ma, H.Y., Zheng, M.X., Xu, Z.C. & Hou, B.Q. (2011) Two new recorded genera in Guangzhou and their geographic significance. *Subtropical Plant Science* 2011 (4): 69–70. http://doi.org/10.3969/j.issn.1009-7791.2011.04.017
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997) The CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876–4882. https://doi.org/10.1093/nar/25.24.4876
- Torelli, G. (2001) *Pleione aurita*: a different species from *Pleione chunii*. *In: National Pleione report, incorporating hardy orchids*. pp. 15–19.

Tso, C.L. (1933) Notes on the orchid flora of Kwangdung. Sunyatsenia 1: 131-156.

White, T.J., Bruns, T., Lee, S. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*. Academic Press, San Diego, pp. 315–322.

https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wu, T. (2006) Flora of Guangdong (Vol. 7). Guangzhou: Guangdong Science and Technology Press.