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Pinellia hunanensis (Araceae), a new species supported by morphometric analysis and DNA barcoding

YU-JING LIU[1], STEVEN G. NEWMASTER[2*], XIAN-JIN WU[3], YUE LIU[1], SUBRAMANYAM RAGUPATHY[2], TIMOTHY MOTLEY[1,4] & CHUN-LIN LONG[1,3,5*]

[1]*College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, China. E-mail: jing_yuzhouniao@126.com; lziqilziqi@yahoo.com.cn*

[2]*Centre for Biodiversity Genomics and Biodiversity Institute of Ontario Herbarium, University of Guelph, Guelph, Ontario, Canada. *Corresponding author, E-mail: snewmast@uoguelph.ca; ragu@uoguelph.ca*

[3]*Key Laboratory of Hunan Province for Study and Utilization of Ethnic Medicinal Plant Resources, Huaihua University, Hunan 418000, China. E-mail:hhuxianjin@163.com*

[4]*Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23529-0266, USA. E-mail:tmotley@odu.edu*

[5]*Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China. *Corresponding author, E-mail: long@mail.kib.ac.cn, chunlinlong@hotmail.com*

Abstract

Pinellia hunanensis, a new species from China, is described and illustrated. A key for the identification of all *Pinellia* species in China, Korea and Japan is included. A detrended correspondence analysis identified 6 groups of taxa including the new species. From the 20 samples, analyzing 38 morphological characters. A discriminant function analysis was used to rigorously test the classification of specimens provided in the cluster analysis. DNA barcoding provided phylogenetic support using NJ and Bayesian methods to distinguish all six taxa including the putative new species. This study provides preliminary evidence of morphometric variation within and among species of *Pinellia*, which allows further development of hypothesis concerning species boundaries. Discussions concerning medicinal product substitution within the genus *Pinellia* are presented in the context of conservation initiatives of species in China.

Introduction

The genus *Pinellia*, established by Tenore (1839) in honor of Giovanni V. Pinelli (1535–1601), belongs to the subfamily Aroideae in the family Araceae. *Pinellia* is a small genus with only nine species, distributed through China, Korea and Japan (Mayo *et al*. 1997, Zhu *et al*. 2007). China has the highest species diversity of *Pinellia* species, with eight species (Bogner & Li 2010, Li 1979, P'ei 1935, Zhu *et al*. 2007). One species, *P. tripartita* (Blume) Schott (1856: 5) is limited to Japan and Hong Kong (Ohwi 1984).

Among eight known *Pinellia* species occurring in China, only one species, *P. ternata* (Thunb.) Ten. *ex* Breitenbach (1879: 687) is widely distributed in the whole distribution range of the genus extending from China to Korea, southern and central Japan. Within China, the genus is absent from the North to Northwest (not present in Inner Mongolia, Qinghai, Xinjiang and Xizang) and confined to the East and Southeast. It has its greatest diversity in Eastern China (Figure 1). All species in the genus grow in humid environment. Most species like warm but not hot environment.

Our recent botanical expeditions in Zhongfang County, Hunan, Central China, were conducted in May and middle July from 2009 to 2011. Specimens of a *Pinellia* species had been collected. The morphological characteristics suggested our *Pinellia* specimen was probably an undescribed species. After examining all *Pinellia* specimens at PE and KUN, and studying all literatures on *Pinellia*, we confirmed it represented a new species.

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FIGURE 1. Sketch map of East Asia, indicating distribution of *Pinellia*.

This paper describes and illustrates *Pinellia hunanensis* C. L. Long & X. J. Wu, a new species from western Hunan, Central China. Morphological traits were compared for all nine *Pinellia* species in the world using morphometric analyses. A molecular classification is provided using DNA barcodes for the six most closely related taxa. A list of representative specimens examined and a morphological key to all 10 species (including the newly described one) occurring in the world are provided.

Materials and methods

Plant materials:—We gathered 24 plant samples from 5 species, namely, *P. peltata* P'ei (1935: 1), *P. polyphylla* Hu (1984: 713), *P. cordata* Brown (1903: 173), *P. fujianensis* Li & Zhu (2007: 512), *P. ternata* and *sp. nov.* (i.e. *Pinellia hunanensis*). *Pinellia hunanensis* was collected from 5 populations along cliffs in forested valleys in Zhongfang County, Huaihua City, Hunan Province, Central China, during flowering (May-June) and fruiting (July-August). Tubers from all individuals are cultivated at Minzu University of China (MUC), and voucher specimens and deposited at MUC and KUN (Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences) for further observation. Other species (*P. peltata*, *P. polyphylla*, *P. cordata*, *P. fujianensis*, and *P. ternata*) were selected to study their phylogenic relations because they are similar morphologically or biogeographically. Species sampled in this study with their source localities, and herbarium voucher number are listed in Table 1.

TABLE 1. Specimens of *Pinellia* used in this study.

Herbarium Voucher #	Species	Locality	Origin
Yujing Liu, P1(KUN)	*P. polyphylla*	KIB, China	W
Yujing Liu, P2(KUN)	*P. polyphylla*	KIB, China	C
Yujing Liu, P3(KUN)	*P. polyphylla*	KIB, China	C
Yujing Liu, P4(KUN)	*P. peltata*	KIB, China	W
Yujing Liu, P10(KUN)	*P. peltata*	KIB, China	C
Yujing Liu, P6(KUN)	*P. peltata*	KIB, China	C
Yujing Liu, P21(KUN)	*P. fujianensis*	KIB, China	C
Yujing Liu, P68(KUN)	*P. fujianensis*	KIB, China	W
Yujing Liu, P90(KUN)	*P. fujianensis*	KIB, China	W
Yujing Liu, P91(KUN)	*P. fujianensis*	KIB, China	C
YujingLiu, P108(KUN)	*P. fujianensis*	KIB, China	C
Yujing Liu, P5(KUN)	*P. ternata*	KIB, China	W
Yujing Liu, P38(KUN)	*P. ternata*	KIB, China	W
Yujing Liu, P39(KUN)	*P. ternata*	KIB, China	W
Yujing Liu, P40(KUN)	*P. ternata*	KIB, China	W
Yujing Liu, P41(KUN)	*P. ternata*	KIB, China	W
Yujing Liu, P1(MUC)	*P. hunanensis*	Hunan, China	W
Yujing Liu, P43(MUC)	*P. hunanensis*	Hunan, China	W
Yujing Liu, P44(MUC)	*P. hunanensis*	Hunan, China	W
Yujing Liu, P45(MUC)	*P. hunanensis*	Hunan, China	W
Yujing Liu, P46(MUC)	*P. hunanensis*	Hunan, China	W
Yujing Liu, P67(KUN)	*P. cordata*	KIB, China	W
Yujing Liu, P68(KUN)	*P. cordata*	KIB, China	C
YujingLiu, P107(KUN)	*P. cordata*	KIB, China	C

KIB: Kunming Institute of Botany, MUC: Minzu University of China, W: wild, C: cultivated.

Morphometric analyses:—38 morphological variables (Table 2) were measured and recorded from 20 specimens noted above. A matrix of 18 specimens and 38 morphological characters were used in a multivariate analysis. Canonical ordination was used to detect groups of specimens and to estimate the contribution of each variable to the ordination. Unimodal, indirect ordination Detrended Correspondence Analysis (DCA) was used to explore variation in species scores in this study. A cluster analysis was used to classify the specimens, as it is better in representing distances among similar specimens, whereas DCA is better in representing distances among groups of specimens (Sneath & Sokal 1973). Cluster analysis was performed with NTSYS (Rohlf & Corti 2000). A distance matrix was generated using an arithmetic average (UPGMA) clustering algorithm and standardized data based on average taxonomic distance subjected to the unweighted pair-group method. A discriminant function analysis (Base 1999) was used to rigorously test the classification of specimens provided in the cluster analysis. The object of DFA is to predict multivariate responses that best discriminate subjects among different groups (Ramsey & Schafer 2012). A total of 38 morphological characters for each of the 20 specimens were used as input for a DFA. The 20 specimens used as input for a DFA were each coded as belonging to one group as designated a priori groups which 1) determined if the classification was accurate, 2) provided discriminant functions for the classification of the taxa and, 3) indicated if there are important morphological characters for each of the canonical discriminate functions.

TABLE 2. Diagnostic characters for separating *Pinellia ternata* from *Pinellia hunanensis*.

Characters	*Pinellia hunanensis*	*Pinellia ternata*
Subterranean part	tuber	tuber
Tuber shape	subglobose-globose	subglobose-globose
Tuber diameter (cm)	1–2.5	2–3
Leaf number	1	1–5
Petiole length (cm)	10–30	10–40
Petiole color	green/red/purple	green/red/purple
Bulbil location	petiole	petiole and blade
Leaf shape	trifoliate	trifoliate
Leaf blade length (cm)	8–30	10–20
Leaf blade width (cm)	<10	<10
Vein number	5–9	6–9
Peduncle	longer than petiole	same/greater than petiole
Spathe constriction	straight-slightly constricted	slightly constricted
Spathe length (cm)	5–7	5–7
Tube (shape)	subglobose-cylindrical	cylindrical
Limb (shape)	variable	elliptic/oblong
Limb size (cm)	4–5.5×1.5–3	3–51.2–3
Spathe shape	erect-slightly incurved	erect-slightly incurved
Spadix length (cm)	8–15	10–20
Female zone (cm)	1.5–2	2–3
Pistil length (mm)	2–2.2	2–2.2
Ovary (shape)	ellipsoid-ovoid/oblong	ovoid/oblong
Ovary diameter (mm)	1.3–1.5	0.8–1.2
Style	absent	attenuate
Stigma (shape)	disciform-hemispheric	disciform
Stigma diameter (mm)	0.16–0.2	0.2
Sterile zone length (mm)	1–1.5	3–4
Male zone (cm)	>10	4–10
Thecae	ellipsoid-elongate	elongate
Appendix (shape)	erect-outcurved	erect
Appendix (color)	green/yellow/violet	green/violet
Appendix length (cm)	7.8–15	7.8–10
Berries (color)	yellow green/white	yellow green/white
Berries (shape)	ovoid-oblong	oviod
Berry diameter (mm)	3–4	2–3
Seed diameter (mm)	1.6–3	0.5–1.5
Flowering time	April–July	May–July
Fruiting time	May–September	May–September

DNA barcoding:—We used two plastid regions (*mat*K and *rbc*L) as recommended by the CBOL Plant Working Group (Hollingsworth *et al*. 2009). Total DNA was isolated from silica gel-dried leaves. Genomic DNA was isolated using Plant Genomic kit (Tiangen Co., Ltd, China). The DNA products were then visualized by electrophoresis on an SYBR Green I stained 0.6% agarose gel with 1.5 kb DNA ladder (Ding Guo Co., Ltd, China) to evaluate the quality. Amplification of DNA regions was performed using PCR. DNA was amplified by PCR performed in 25μL volumes containing 10×buffer (2.5μL), dNTP (0.375μL), each primer (10mM, 0.5μL) (Synthesized by BGI. Co.), Mg^{2+} (1.5μL), five units of Taq polymerase (Fermentas, 0.3μL), temple (0.75μL) and ddH_2O (18.575μL). The resultant PCR products were separated by electrophoresis through 2.0% (w/v) agarose gel in TAE buffer at 120V for ~30min, stained with SYBR Green I, transilluminated under ultraviolet light and then photographed in Quantity One software. If multiple bands were detected, an additional electrophoresis was performed to excise and analyze them separately (Table 3). The length of PCR products were evaluated by comparison with a molecular weight 100bp marker. Amplified products were sequenced in both directions with the primers used for amplification to ensure high accuracy of data scoring. We believe that a high percentage of bidirectional reads will be critical for a successful plant barcoding system, given the generally low amount of variation that separates many plant species, and the increased danger of mis-assignment due to sequencing error that can be anticipated with incomplete bidirectional reads (Fazekas *et al*. 2008, Kress & Erickson 2007). Purifying and sequencing were completed by Invitrogen Co., Ltd. We submitted nucleotide sequences of 6 species in our study to GenBank (Table 4). The bidirectional sequences were first manually adjusted in Chromos (Version 1.62) and assembled in DNAMAN (Version 6) and then aligned using ClustalX program (Version 1.83). Two phylogenetic analyses were utilized; neighbor-joining (NJ) and Bayesian inference. NJ tree method was used to exhibit the molecular identification results and test the monophyly of species. We entered sequences into MEGA4.0 for construction of the NJ phylogenetic trees. Bootstrap (1000 replications) analysis was performed to estimate the confidence of the topology of the consensus tree (Ren *et al*. 2010), pairwise K2P (Kimura 2-parameter) distances for *mat*K and *rbc*L were calculated in MEGA to evaluate intraspecific and interspecific divergence among species of *Pinellia*. The data were then analyzed using Bayesian inference, with a best-fit model (HKY) selected model of sequence evolution for the two genes (i.e., *mat*K and *rbc*L). A binary model (Lset coding=variable) was applied to the coded gaps. Bayesian runs were performed with MrBayes (version 3.1), using one cold and three heated Markov chain Monte Carlo (MCMC) chains run for 5×10^6 cycles, sampling trees every 100 generations, and with a default temperature parameter value of 0.2. Bayesian runs were started from independent random starting trees and repeated at least twice (Cusimano *et al*. 2011, Mansion *et al*. 2008).

TABLE 3. PCR primers and profiles.

Region	Name of primer	Primer sequence 5'-3'	Reference
matK	390f	CGATCTATTCATTCAATATTTC	Cuénoud *et al*. 2002
	1326r	TCTAGCACACGAAAGTCGAAGT	Cuénoud *et al*. 2002
trnH-psbA	PA-r	GTTATGCATGAACGTAATGCTC	Sang *et al*. 1997
	TH-f	CGCGCATGGTGGATTCACAATCC	Tate 2002
rbcL	R-F	ATGTCACCACAAACAGAAACT	Terachi *et al*. 1987
	R-R	TCGCATGTACCTGCAGTAGC	Fay *et al.* 1997

Region	PCR profile					
	Initial Denaturation temp./time	Denaturation temp./time	Annealing temp./time	Extension temp./time	Final extension temp./time	No.of cycles
matK	94°/4min	94°/1min	48°/30s	72°/1min	72°/7min	35
trnH-psbA	94°/5min	94°/1min	55°/1min	72°/1.5min	72°/7min	35
rbcL	95°/2min	94°/1min	55°/30s	72°/1min	72°/7min	34

TABLE 4. PCR product size, variation and accession number of studied samples. A hyphen (-) indicates that sequencing of the PCR product failed.

Specimen	*matK*, length (bp)	Accession number	*rbcL* length (bp)	Accession number	*trnH-psbA length (bp)*	Accession number
P. polyphylla	812	JF828125	758	JF828106	543	JF828145
P. polyphylla	806	JF828126	738	JF828107	-	-
P. polyphylla	804	JF828127	718	JF828108	-	-
P. peltata	822	JX123072	685	JX123069	759	JX123085
P. peltata	810	JF828128	719	JF828109	-	-
P. peltata	809	JX123089	719	JX123083	-	-
P. fujianensis	808	JX123084	717	JX123075	-	-
P. fujianensis	816	JX123062	716	JX123071	-	-
P. fujianensis	810	JF828129	719	JF828110	-(320bp?	JX123082
P. fujianensis	813	JX123063	719	JX123081	-(183bp)	-
P. fujianensis	815	JX123078	714	JX123077	586	JX123080
P. ternata	806	JX123079	707	JX123064	640	JF828148
P. ternata	781	JF828130	716	JX123070	547	JF828147
P. ternata	785	JX123073	741	JX123066	554	JF828146
P. ternata	806	JX123086	744	JX123074	672	JX129927
P. ternata	746	JX123065	721	JX123068	504	JX123090
P. hunanensis	-	-	721	JX123067	-	-
P. hunanensis	743	JX123088	734	JX123076	640	JX123087

Results and discussion

The morphometric analysis revealed considerable variation among the 5 known taxa and the new species. A discriminant function analysis (DFA) used 38 quantitative characters to classify heterogeneity in 20 specimens into what is currently considered 5 known taxa of *Pinellia* and the new species (*Pinellia hunanensis*): *P. peltata*, *P. polyphylla*, *P. cordata*, *P. fujianensis*, *P. ternata*. The canonical correlation from the discriminant functions is the ratio of the between groups sums of squares to the total sums of squares. Thus, the first discriminant function is responsible for 61.4% of the between group differences (variability in the discriminant scores). The second function is responsible for the remaining 38.6% of the between group variance. Wilk's Lambda was used to test the hypothesis that there are no difference in variance ($p<0.001$) between the groups of taxa which represent different species (Base 1999). There were significant differences (p0.008) for first two canonical functions. 100% of the groups (representing 5 known and the new species) were correctly classified using the DFA into 6 distinct groups of taxa including *Pinellia hunanensis.*

The ordination analyses utilized DCA in the separation of 6 taxa of *Pinellia* (including *Pinellia hunanensis*) from the 20 specimens that were analyzed. This provided a measure of the important morphological variables in the classification. A DCA was used to classify the 20 specimens into distinct groups representing 5 known and one new species. High eigenvalues for the X-axis (0.349) and the Y-axis (0.222) indicated that the gradient axes were of considerable length and justified the use of DCA. The X-axis (axis 1) is strongly correlated with 16 characters; these include Bulbil location, Vein number, Peduncle, Spathe constriction, Tube (shape), Spathe shape, Female zone (cm), Ovary diameter (mm), Style, Stigma (shape), Stigma diameter (mm), Sterile zone length (mm), Male zone (cm), Appendix (shape), Berry diameter (mm), Seed diameter (mm) (Table 3, Fig. 4). The Y-axis (axis 2) is strongly correlated with 11 characters; Subterranean part, Tuber shape, Bulbil location, Tube (shape), Limb size (cm), Pistil length (mm), Ovary (shape), Style, Stigma (shape), Thecae, Flowering time (Table 3, Fig. 4).

TABLE 5. DCA analysis of 38 quantitative variables (taxonomic characters) for 24 specimens (classification of 6 *Pinellia* species). Pearson correlations indicate the characters significant to the classification (** = p value < 0.01, * = p value < 0.05).

	DCA1		DCA2	
Characters	**Pearson Correlation**	**Sig. (2-tailed)**	**Pearson Correlation**	**Sig. (2-tailed)**
Subterranean part	-0.346	0.135	-0.707**	0
Tuber shape	0.185	0.436	-0.534*	0.015
Tuber diameter (cm)	0.154	0.726	0.183	0.274
Leaf number	0.385	0.082	0.217	0.583
Petiole length (cm)	-0.091	0.704	0.285	0.224
Petiole color	0.43	0.059	0.031	0.896
Bulbil location	-0.505*	0.023	0.566**	0.009
Leaf shape	0.392	0.087	0.342	0.14
Leaf blade length (cm)	-0.28	0.232	0.1	0.676
Leaf blade width (cm)	-0.269	0.251	0.286	0.221
Vein number	-0.592**	0.006	0.164	0.488
Peduncle	0.928**	0	-0.114	0.633
Spathe constriction	-0.867**	0	0.186	0.432
Spathe length (cm)	0.185	0.464	-0.022	0.931
Tube (shape)	0.614**	0.004	0.519*	0.019
Limb (shape)	0.371	0.411	0.427	0.239
Limb size (cm)	0.615	0.058	-0.684*	0.029
Spathe shape	0.686**	0.001	-0.096	0.687
Spadix length (cm)	0.142	0.551	-0.436	0.055
Female zone (cm)	0.797**	0	-0.263	0.263
Pistil length (mm)	-0.336	0.148	0.825**	0
Ovary (shape)	0.305	0.191	0.755**	0
Ovary diameter (mm)	0.62**	0.004	0.261	0.266
Style	0.669**	0.001	0.483*	0.031
Stigma (shape)	0.451*	0.046	-0.715**	0
Stigma diameter (mm)	-0.778**	0	-0.233	0.323
Sterile zone length (mm)	-0.768**	0	-0.365	0.114
Male zone (cm)	0.475*	0.034	0.027	0.91
Thecae	0.005	0.983	0.64**	0.002
Appendix (shape)	-0.559*	0.01	-0.124	0.601
Appendix (color)	0.138	0.561	0.38	0.099
Appendix length (cm)	-0.035	0.883	0.197	0.406
Berries (color)	0.393	0.087	-0.182	0.443
Berries (shape)	0.347	0.134	0.079	0.739
Berry diameter (mm)	0.795**	0	0.029	0.904
Seed diameter (mm)	0.88**	0	-0.128	0.59
Flowering time in wild	0.255	0.277	0.56*	0.01
Fruiting time in wild	-0.054	0.821	0.389	0.09

The DCA ordination of the first two canonical functions identifies a distinct cluster of five specimens that represent the new species, *Pinellia hunanensis*, in the proximity of the respective allied species (Fig. 4). Intraspecific variation among the samples of the new species is within the natural range of variation for the 6 species of *Pinellia* in the ordination (Fig. 4). This study provides preliminary evidence of morphometric variation within and among species of *Pinellia*, which allows further development of hypotheses concerning species limits.

DNA barcoding provides additional support for *Pinellia hunanensis* with clear differentiation among the six species of *Pinellia*. Interspecific sequence variation identified considerable barcode variation among all of the samples. The K2P interspecific distances for *mat*K among the five known species of *Pinellia* was 0.022; *Pinellia hunanensis* had a K2P interspecific distance of 0.026 with the closest morphological species, *P. ternata*. The K2P interspecific distance for *rbc*L among the five known species of *Pinellia* was 0.008; *Pinellia hunanensis* had a K2P interspecific distance of 0.016 with that of *P. ternata*. The intraspecific K2P distance was <0.001 for both *mat*K and *rbc*L within all species including *Pinellia hunanensis*.

A neighbor-joining tree and Bayesian analysis of the DNA barcodes provides strong support for *Pinellia hunanensis*. The Barcode of Life Data System (BOLD) identifies clusters of barcodes using a neighbor-joining (NJ) tree that makes use of an average Kimura-2-parameter model (Kimura 1980). In our analysis, the support from the *rbc*L NJ tree is congruent with that of the *mat*K NJ tree. The combined data sets produce a single resolved (100%) tree that strongly supports recognition of *Pinellia hunanensis* (Fig. 5) (Cabrera *et al*. 2008; Mansion *et al*. 2008; Newmaster & Ragupathy 2010). This tree revealed barcode clusters for the six *Pinellia* species and identified considerable interspecific variation between *P. hunanensis* and *P. ternata*. The new species clearly formed one clade (100% bootstrap support) (Fig. 5), with the morphologically most similar species, *P. ternata* placed in a different clade; although morphologically similar, these two taxa are not sister species. *P. hunanensis* appears to be more closely related to *P. fujianensis* than it is to *P. ternata* as it has considerable divergence from *P. ternata*, *P. polyphylla* and *P. peltata*.

It is important to consider this new rare species for conservation of biological diversity given that it is easily confused with a common species widely used as a traditional Chinese medicine of economic and cultural importance adopted by the Chinese pharmacopoeia in 2010 (The Editorial Committee of Pharmacopoeia of the People's Republic of China 2010). The processed tuber of *P. ternata*, known as *Ban Xia*, is one of the most important herbs in Chinese medicine to reduce lung congestion, vomiting, morning sickness, cough, influenza, pain, swelling (inflammation) and as a birth control (The Editorial Committee of Pharmacopoeia of the People's Republic of China 2010). The unprocessed tuber is only used externally in traditional Chinese medicine. In recent years, it has become very popular in both Japan and China to use *P. ternata* as an adjuvant therapy in treating bronchus, chronic hepatitis, breast cancer and diabetes. Product substitution is a serious problem within *Pinellia* herbal products as species of lower potency such as, *P. fujianensis, P. polyphylla, P. peltata* and *P. cordata* are often used as substitutes for the desirable *P. ternata* (Wei & Peng 2003). This is because the identification of *P. ternata* products and those from morphologically similar species are impossible to differentiate when they are young, as there are no inflorescences. Over harvesting for medicinal use of rare species such as *P. hunanensis* is possible since it would be very difficult to differentiate even mature plants of *P. hunanensis* from that of *P. ternata.* It would be desirable to have a molecular tool such as DNA barcoding to differentiate species of *Pinellia* used commercial herbal products to support highly quality products and the conservation of rare species.

Key to the species of *Pinellia* (modified from Zhu *et al.*, 2007)

1. Leaf blade entire 2
— Leaf blade compound, trifoliolate or pedate 6
2. Leaf blade not peltate 3
— Leaf blade peltate, ovate or oblong ..... *P. peltata*
3. Petiole lacking bulbils 4

— Petiole or base of leaf blade bearing bulbils 5
4. Leaf blade deltoid-ovate or broadly ovate, base deeply cordate, 6–33×4–22cm ..... *P. polyphylla*
— Leaf blade ovate or oblong, base obtuse or shallowly cordate, 5–19 × 1.5–6 cm ..... *P. integrifolia* Brown (1889: t. 1875)
5. Tuber globose; leaf blade sagittate-oblong, cordate-ovate, base deeply cordate; bulbils present at the base of the petiole and at the base of the leaf blade ..... *P. cordata*
— Rhizome cylindrical; leaf blade widely sagittate; bulbils at the base of the petiole ..... *P. fujianensis*
6. Leaf trifoliolate or pedate with 5 leaflets 7
— Leaf blade always pedate, leaflets 6–11; bulbils absent ..... *P. pedatisecta* Schott (1857: 341)
7. Leaf blade only deeply tripartite, anterior lobe broadly ovate or ovate-oblong, sessile; bulbils absent..... *P. tripartita*
— Leaf blade trisect, sometimes pedate with only 5 leaflets, leaflets oblong or lanceolate 8
8. Petiole lacking bulbils, bulbils emerging only from the tuber; lateral leaflets usually bifid ..... *P. yaoluopingensis* Liu & Guo (1986: 223)
— Bulbils present at petiole below middle, or both at lower part of petiole and at the base of the leaf blade 9
9. Inflorescence with peduncle longer than petioles, 25–35 cm long ..... *P. ternata*
— Inflorescence with peduncle much longer than petioles, 45–55 cm long ..... *P. hunanensis*

Pinellia hunanensis C. L. Long & X. J. Wu, *sp. nov.* (Figs. 2, 3)

Pinellia hunanensis differs from *Pinellia ternata* (Thunb.) Ten. ex Breitenb. in having very tall inflorescence, and long appendix.

Type:—CHINA. Hunan Province: Zhongfang County, Tongwan, cliff in moist forest in the valley, elev. 650 m, 6 July 2009, *Long Chun-lin 113* (holotype: KUN!, isotype MUC!).

Perennial aroid herb, 20 cm tall. Tuber globose, 1.5–3 cm in diameter. With one leaf, 18–22 cm long, with a green, unspotted petiole and nearly absent sheath. Bulbil at lower portion of petiole, 0.8–1.1 cm in diam. Leaf blades trisect or pentasect, leaflets with obvious petioles; central leaflet lanceolate, 16–20 cm long, 4–5.5 cm wide, upper side green, lower side silver-white, margin crisp; primary lateral veins of the leaf blade pinnate, forming a submarginal collective vein, 1 distinct marginal vein present, higher order venation reticulate; two lateral leaflets smaller, oblique at base. Young leaves simply cordate, 5–6 cm long, 3–4 cm wide. Inflorescence solitary, appearing with leaves, 45–55 cm long; peduncle green, much taller than petiole. Spathe persistent, 5–7.5 cm long, 0.4–0.7 cm in diameter, slightly constricted between tube and blade; tube 2–2.5 cm long, 0.4–0.5 cm in diameter, convolute, ellipsoid, almost closed inside by a transverse septum; limb of spathe oblong, fornicate, green, 3.5–5.5 cm long, much longer than tube. Spadix 12–15 cm long, longer than spathe, female zone 2–3 cm long, adnate to spathe, separated from the male zone by the spathe septum, and by the short, free, naked portion of spadix axis (0.9–1.4 cm long); male zone 1–1.5 com long, cylindric. Appendix smooth, erect or slight curve, elongate-linear, long-exserted from spathe, 8–12 cm long. Flowers unisexual, perigone absent. Male flowers 2-androus, stamens united congenitally in pairs, short, laterally compressed; anthers sessile, connective slender, thecae ellipsoid, 2-celled, dehiscing by apical slit; pollen extruded in amorphous mass, inaperturate, spherical, small, white. Female flowers with ovary ovoid-oblong, 1-locular; ovule 1, orthotropous, 2–2.5 mm long, 1.2–1.5 mm in diam., funicle very short; placentation basal, stylar region attenuate, stigma small, hemispheric.

Paratype:—CHINA. Hunan Province: Zhongfang Xian, Tongwan, on cliff within a moist forest in the valley, 670 m, 24 July 2009, *Long 179* (KUN!). In the same valley on June 25 2011 at different altitudes: 580 m, *Long 829* (KUN!); 640 m, *834* (KUN!); 650 m, *836* (KUN!).

Phenology:—*Pinellia hunanensis* starts to grow leaves in late April or early May, flowers in June-July, and fruits from mid-July to August. After August, the whole plant will get withered.

Etymology:—Named after Hunan Province where it was firstly discovered.

Habitat and distribution:—The new species was only found in Zhongfang County, western Hunan, Central China. It is restricted to wet cliffs in moist forests and is morphologically similar to *Pinellia ternata*, which does not grow on cliffs. There are subtle diagnostic characters that can be used to distinguish *Pinellia hunanensis* from *P. ternata*, such as the presence of only one leaf; a tall inflorescences up to 55 cm long,

which is 2–3 times longer than petiole; long spadix axis; erect and very long appendix; three leaflets with a crisp, lanceolate margin; one large, central leaflet with two small lateral leaflets that are oblique at base (Fig. 3, Table 2).

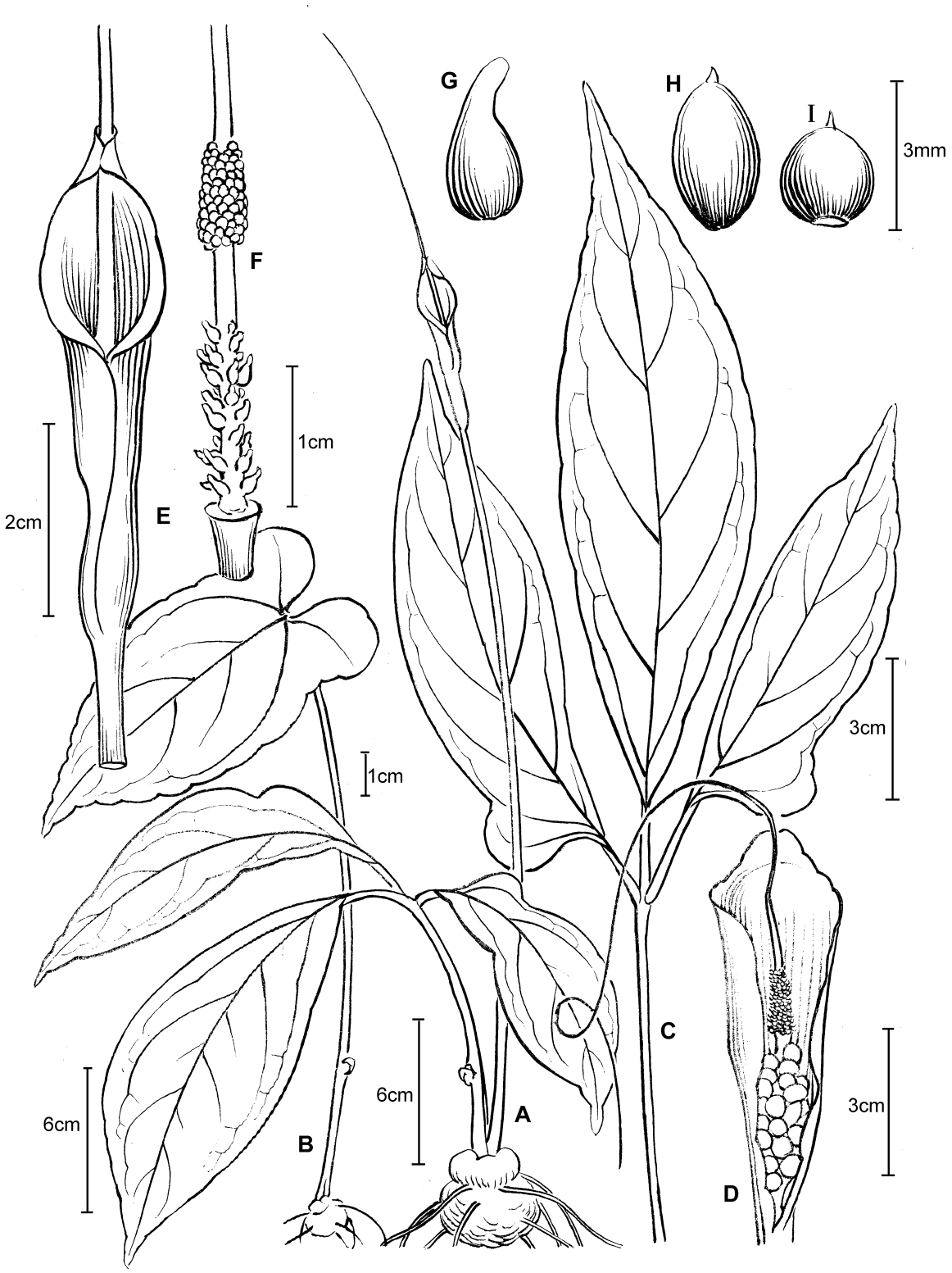

FIGURE 2. *Pinellia hunanensis*—A: habit; B: juvenile plant; C: leaf; D: inflorescence; E: spadix; F: infructescence; G: pistil; H: fruit; I: seed (Drawn after the holotype by Yitao Liu).

FIGURE 3. Habitats of *Pinellia hunanensis* (Photographed by Chunlin Long).

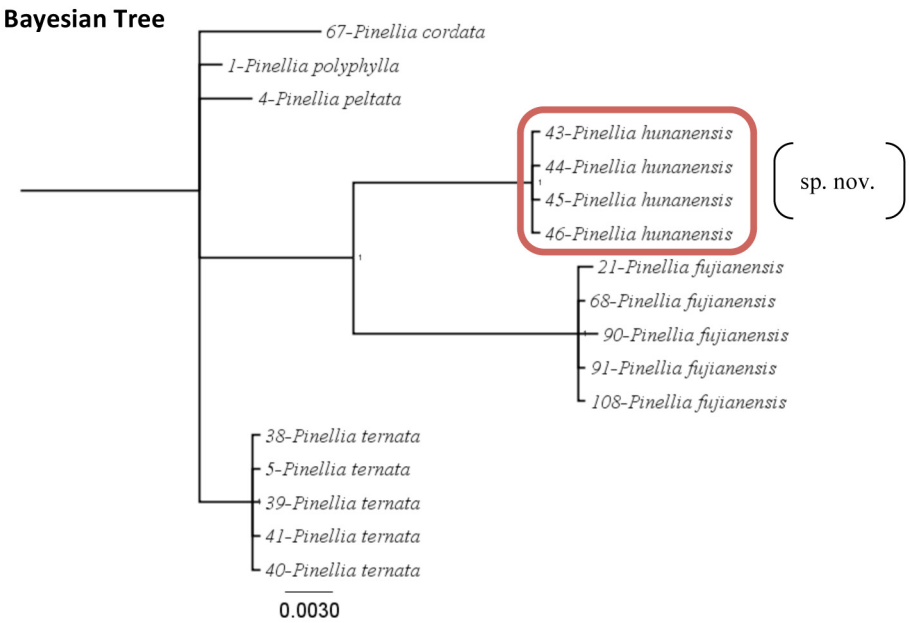

FIGURE 4. A classification tree of combined *rbc*L and *mat*K data using neighbor-joining (NJ) and Bayesian of phylogenetic methods.

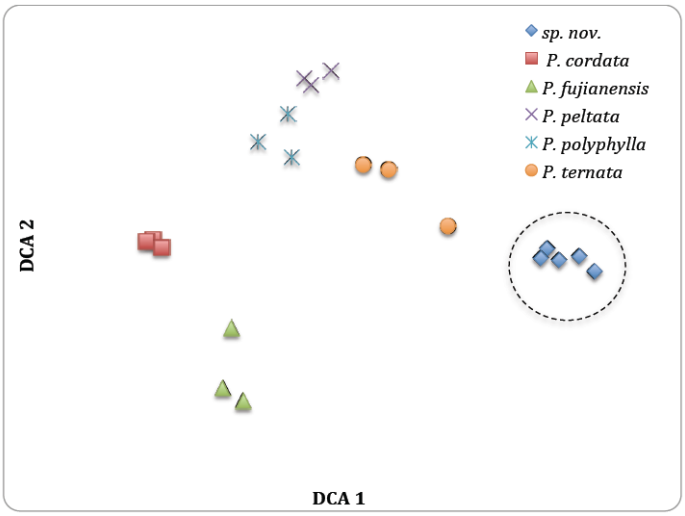

FIGURE 5. Scatter plot of the first two axes from a detrended correspondence analysis (DCA) for 38 quantitative morphological variables (taxonomic characters) of 24 specimens (classification of 6 *Pinellia* species). The new species *Pinellia hunanensis* is circled, including its respective intraspecific variation.

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