

A New Hybrid Species of *Pulsatilla* (Ranunculaceae): *P. x yanbianensis* H.Z. Lv

Hui-Zi Lv, Soonku So¹ and Muyeol Kim^{1*}

College of Pharmacy, Yanbian University, 1829 Juzi Road, Yanji City, Jilin Province, China

¹Dep. of Biological Sciences, Chonbuk National University, Jeonju 561-756, Korea

(Received 28 November 2011; Revised 10 December 2011; Accepted 15 December 2011)

할미꽃속의 신잡종: 연변할미꽃(*Pulsatilla x yanbianensis* H.Z. Lv)

여혜자 · 소순구¹ · 김무열^{1*}

중국 연변대학교 약학원, ¹전북대학교 자연과학대학 생명과학과

ABSTRACT: In this paper, a new hybrid species, *Pulsatilla x yanbianensis* H.Z. Lv, is named and described from the Yanbian area of Jilin Province in China. Molecular data confirm that this taxon is a result of natural hybridization between *P. dahurica* and *P. cernua*. As intermediate and mosaic phenotypes are expected for an interspecific hybrid, the leaves of *P. x yanbianensis* are fully expanded at the anthesis, resembling one of the parental species, *P. dahurica*. However, the hybrid taxon is distinct from *P. dahurica* by having reddish violet sepals and sterile fruits. *Pulsatilla x yanbianensis* also resembles the other parental species, *P. cernua*, in that it has dark red sepals and styles. The new hybrid, however, can be distinguished from *P. cernua* because it has leaf margins with short hairs and fully expanded leaves at the anthesis.

Keywords: *Pulsatilla x yanbianensis*, *Pulsatilla cernua*, *Pulsatilla dahurica*

적 요: 중국 연변지역에서 할미꽃속의 신잡종인 연변할미꽃(*Pulsatilla x yanbianensis* H.Z. Lv)을 새로이 발견하여 기재하였다. 연변할미꽃은 개화시 잎이 완전히 벌어져 분홍할미꽃과 유사하나, 꽃받침이 적자색이고 과실이 불임인 점에서 뚜렷이 구별된다. 연변할미꽃은 꽃받침과 화주가 흑적색인 가는할미꽃과 유사하나, 잎의 엽연에 짧은 털이 있고 개화시 잎이 완전히 벌어진 점에서 뚜렷하게 차이가 난다.

주요어: 연변할미꽃, 가는할미꽃, 분홍할미꽃

The genus *Pulsatilla* Miller consists of approximately 33 species and is distributed primarily in Asia, Europe and North America (Tamura, 1993). Eleven species of *Pulsatilla* are distributed in China (Wang and Bartholomew, 2001). Of 11 species, three are reported to occur in Jilin Province (Wang, 2000): *P. cernua* (Thunb.) Berchtold & Presl, *P. dahurica* (Fisch. ex DC.) Sprengel, and *P. chinensis* (Bunge) Regel. Two species, *P. dahurica* and *P. cernua*, occur widely in Yanbian area of Jilin Province, while *P. chinensis* is rather rare and sporadically distributed.

During field work in 2007, a putative hybrid species from

Antu region in Yanbian area of Jilin Province, China was discovered. In this paper, we named this new hybrid taxon, *Pulsatilla x yanbianensis* H.Z. Lv., and described several morphological features relative to its putative parental species, *P. dahurica* and *P. cernua*. The new hybrid species shows several intermediate and mosaic phenotypes of the two parental species, but it can be distinguished based on sepal color (i.e., reddish violet), leaf margin (i.e., short hairs), fruit fertility (i.e., sterile achenes), etc. We found several hybrid populations in Yanbian area, such as Antu, Tumen, Hunchun, Longjing, Dunhua, etc. The dichotomous key based on morphological traits was provided and paternal and maternal parents based on molecular data were discussed briefly.

*Author for correspondence: mykim@jbnu.ac.kr

Materials and Methods

Living materials of the newly discovered hybrid *Pulsatilla x yanbianensis* and its two putative parental species, *P. dahurica* and *P. cernua*, were collected from Yanbian area in Jilin Province (Table 1). The voucher specimens were deposited in the Herbarium of Chonbuk National University (JNU). Eighteen vegetative and reproductive characteristics were measured and relationships among these taxa were assessed (Table 2).

Results and Discussion

1. Morphological study

Eighteen morphological characteristics of *Pulsatilla x*

yanbianensis H.Z. Lv and its putative parental species were compared in Table 2 and described as follows:

Leaves: *Pulsatilla x yanbianensis* H.Z. Lv and its putative parents have basal leaves only and leaf blades are pinnately divided (Figs. 1-3). *Pulsatilla dahurica* and *P. cernua* have glabrous and long haired leaf margins, respectively, and the putative hybrid *P. x yanbianensis* has an intermediate phenotype (i.e., leaf margins with short hairs). The leaves of *P. dahurica* and *P. x yanbianensis* are fully expanded at anthesis, while those of *P. cernua* are not fully expanded.

Flowers: *Pulsatilla x yanbianensis* shares several floral characteristics with *P. dahurica* and *P. cernua*, including stamen

Table 1. Collection data of *Pulsatilla x yanbianensis* and its putative parental species.

Taxa	Localities
<i>P. dahurica</i> (Fisch. ex DC.) Sprengel 분홍할미꽃	Tumen, Jilin Prov., China. May 14, 2009. <i>H. Lv 5101</i> .
<i>P. x yanbianensis</i> H.Z. Lv 연변할미꽃	Antu, Jilin Prov., China. May 14, 2009. <i>H. Lv 5105</i> .
<i>P. cernua</i> (Thunb.) Berchtold & Presl 가는 할미꽃	Xiaoyingxiang, Jilin Prov., China. Apr. 26, 2009. <i>H. Lv 5108</i> .

Table 2. Qualitative and quantitative characteristics of *Pulsatilla x yanbianensis* and its putative parental species. Measurements were based on 15 individuals for each taxon.

	Characters	<i>P. dahurica</i>	<i>P. x yanbianensis</i>	<i>P. cernua</i>
Leaf	Leaf at anthesis	fully expanded	fully expanded	not fully expanded
	Blade margin	glabrous	short hairs	long hairs
	Petiole length*	9.4 ± 1.6	12.5 ± 3.7	11.7 ± 4.3
	Terminal leaflet lobe length**	3.9 ± 0.6	4.6 ± 0.8	4.3 ± 2.3
	Terminal leaflet lobe width	0.8 ± 0.3	1.1 ± 0.4	1.0 ± 0.3
	Terminal leaflet petiole length	3.1 ± 1.1	3.3 ± 0.4	3.1 ± 1.1
Flower	Flowering time	May	May	April
	Scape length	23.7 ± 3.5	25.9 ± 3.4	19.7 ± 6.2
	Involucral tube length	1.7 ± 0.2	1.4 ± 0.3	1.4 ± 0.3
	Involucral lobe length	3.0 ± 0.4	3.5 ± 0.5	3.2 ± 0.4
	Pedicel length	8.4 ± 3.2	9.0 ± 4.9	11.6 ± 3.3
	Sepal color	pale violet	reddish violet	dark red
	Sepal length	2.2 ± 0.3	2.4 ± 0.3	2.6 ± 0.3
	Sepal width	1.2 ± 0.2	1.2 ± 0.3	1.0 ± 0.2
	Stamen length	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.2
	Pistil length	1.8 ± 0.3	1.9 ± 0.2	2.2 ± 0.3
Style color	pale violet	reddish violet	dark red	
Fruit	Fertility	fertile	sterile	fertile

*unit is cm

**mean ± SD



Fig. 1. Photographs of *Pulsatilla x yanbianensis* H.Z. Lv at type locality. A. adult plants; B. flower. Scale bars 5 cm and 1 cm in A and B, respectively.



Fig. 2. *Pulsatilla x yanbianensis* H.Z. Lv A. compound leaf; B. flower; C. sepal; D. stamen; E. pistil. Scale bars = 1 cm.

shape, anther color, pistil shape, etc. The new hybrid, however, has reddish violet sepals and thus can be distinguished from *P. cermua* with dark red sepals and *P. dahurica* with pale violet sepals.

Fruits: *Pulsatilla x yanbianensis* and its putative parents have a fruit type of achenes. *Pulsatilla x yanbianensis* does not produce fertile achenes perhaps due to interspecific sterility, while *P. dahurica* and *P. cermua* produce fertile achenes.

In summary, *P. x yanbianensis* shows an intermediate or a mosaic of patterns found in two purported parental species, *P. dahurica* and *P. cermua*. Some intermediate characteristics between the two parental species include leaf margins and sepal and style

colors. However, a combination of vegetative and reproductive characteristics, including reddish violet sepals, short haired leaf margins, and sterile achenes, can be used to distinguish from its parental species.

2. Taxonomic study

Pulsatilla x yanbianensis H.Z. Lv, hybrid nov. Fig. 1, 2, 3
Korean name: **Yeonbyeon-Halmikkuch 연변할미꽃**

Herba perennis. Rhizoma erectus. Folia basalis; petiolus 8.8 (12.5) 16.2 cm longus, pilosus; lamina pinnatidivisis, 6–8 cm longa, 5–7 cm lata. abaxialis margo ad brevis trichomata. Flos Maius florens, rubellus purpura. Scapus 22.5 (25.9) 29.3 cm.



Fig. 3. Holotype of *Pulsatilla x yanbianensis* H.Z. Lv

Involucris tubus 1.1 (1.4) 1.7 cm, lobi 3.0 (3.5) 4.0 cm. Pedicelli 4.1 (9.0) 13.9 cm. Sepalum 5–6, rubellus purpura; 2.1 (2.4) 2.7 cm longi, 0.9 (1.2) 1.5 cm lati. Petalum absens. Stamina numerosus. anthera aureus. Pistili numerosus; styli linearis, rubellus purpura.

Fructus sterilis achenia.

Perennial herbs. Rhizomes erect. Leaves basal; petiole 8.8 (12.5) 16.2 cm in length, with pilose hairs; leaf blade pinnately

divided, 6–8 × 5–7 cm, adaxial surface glabrous, abaxial surface margin with short hairs. Flowering May, reddish violet. Scape 22.5 (25.9) 29.3 cm. Involucral tube 1.1 (1.4) 1.7 cm in length, lobes 3.0 (3.5) 4.0 cm in length. Pedicels 4.1 (9.0) 13.9 cm. Sepals 5–6, reddish violet, 2.1 (2.4) 2.7 cm in length, 0.9 (1.2) 1.5 cm in width. Petals absent. Stamens numerous; anthers yellow. Pistils numerous, with long pilose hairs; style linear, reddish violet. Fruit achenes, sterile.

Type Locality: Liangjiang, Antu, Jilin Province, China. alt. 448 m, 43°05'N, 128°30.7'E.

Holotype: *H. Lv 5105*. May 14, 2009. Herbarium of Chonbuk National University (JNU). Liangjiang, Antu, Jilin Province, China.

Isotype: Liangjiang, Antu. May 14, 2009. *H. Lv 5110* (JNU).

Paratypes: Xidong, Yanji. May 7, 2007. *H. Lv 4990* (YBU); Taiyan, Yanji. May 8, 2008. *H. Lv 5002* (YBU); Limin, Yanji. May 8, 2008. *H. Lv 5005* (YBU); Weizigou, Tumen. May 22, 2008. *H. Lv 5220* (YBU); Changxing, Antu. May 10, 2009. *H. Lv 5312* (YBU); Biyan, Longjing. May 13, 2009. *H. Lv 5314* (YBU); Weizigou, Tumen. May 16, 2009. *H. Lv 5318* (YBU); Limin, Yanji. May 21, 2009. *H. Lv 5320* (YBU); Weizigou, Tumen. May 22, 2010. *H. Lv 5402* (YBU); Longjia, Tumen. May 28, 2010. *H. Lv 5409* (YBU); Liangbing, Antu. May 27, 2011. *H. Lv 5503* (YBU).

Flowering: May

Distribution: Yanbian area, Jilin Province, China

Etymology: The specific epithet is derived from type locality name, Yanbian where Antu is located.

Habitats: Yanbian area has deciduous broad-leaved forest comprising *Quercus mongolica*, *Ulmus macrophylla*, *Acer ginnala* etc. The natural habitats of *Pulsatilla x yanbianensis* H.Z. Lv were discovered at sunny hillside in the Yanbian area. This new hybrid occurs on grassy slopes under shrub forests dominated by *Acer ginnala*, *Syringa reticulata* var. *manshurica*, *Corylus heterophylla*. The hybrid species also occurs with other herbaceous species dominated by *Potentilla chinensis*, *Polygonatum humile*, and *Iris nertschinskia*.

Remark: This new hybrid was firstly discovered at Antu in Yanbian area, China. This species is distinct from congeneric sympatric species *P. dahurica* and *P. cernua*. Thus, the authors described this species as a new taxon, *Pulsatilla x yanbianensis* H.Z. Lv.

Key to *Pulsatilla x yanbianensis* and its related taxa.

1. Leaf margin hairs present; sepals dark red or reddish violet.
2. Leaf margin with long hairs; leaves not fully expanded

at anthesis; sepals dark red; achenes fertile

..... *P. cernua* 가는할미꽃

2. Leaf margin with short hairs; leaves fully expanded at anthesis; sepals reddish violet; achenes sterile

..... *P. x yanbianensis* 연변할미꽃

1. Leaf margin hairs absent; sepals pale violet

..... *P. dahurica* 분홍할미꽃

This new hybrid, *Pulsatilla x yanbianensis* H.Z. Lv shows a mosaic patterns of vegetative and floral characteristics between two putative parental species, *P. dahurica* and *P. cernua*. However, this hybrid is distinct from *P. dahurica* which has pale violet sepals and glabrous leaf margins. Also, it is different from *P. cernua* which has dark red sepals and leaf margins with long hairs. Therefore, the authors treated this taxon as a new hybrid species of the genus *Pulsatilla*.

Molecular data provided some insights into the hybrid origin of *Pulsatilla x yanbianensis*. For example, RAPD analysis confirmed that *P. x yanbianensis* H.Z. Lv is a result of natural hybridization between *P. dahurica* and *P. cernua* (Jin et al., 2010). In addition, nrDNA ITS and plastid *psbA-trnH* intergenic spacer sequences suggested that *P. dahurica* and *P. cernua* contributed as a maternal and paternal parent, respectively (S. So et al., unpubl. data).

Acknowledgement

We are deeply indebted to professor Seung-Chul Kim of Sungkyunkwan University for his taxonomical review and Mr. Dogeun Lee for his kindly help.

Literature Cited

- Jin, Y.-Z., D.-C. Jin and H.-Z. Lu. 2010. RAPD analysis of *Pulsatilla* in Yanbian, Jilin Province. *Jiangsu Agricultural Sciences* 3: 24-25.
- Tamura, M. 1993. Ranunculaceae. In *The Families and Genera of Vascular Plants: Flowering Plants-Dicotyledons*. Vol. 2. Kubitski, K., J. G. Rohwer, and V. Bittrich (eds.). Springer-Verlag, Berlin. Pp. 563-583.
- Wang, J. 2000. *Pulsatilla* Miller. In *Higher Plants of China*. Vol. 3. Fu, L., T. Chen, L. Lang, and T. Hong (eds.). Qingdao Pub., Qingdao, China. Pp. 501-505.
- Wang, W-C and Bruce Bartholomew. 2001. *Pulsatilla* Miller. In *Flora of China*. Vol. 6. Wu, Z.-Y. and P. H. Raven (eds.). Science Press, Beijing and Missouri Botanical Garden Press, St. Louis, USA. Pp. 329-333.