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Full Length Research Paper

Morphological diversity of wild medicinal *Paris* L. from China and Vietnam

Jin-Yu Zhang[1, 2], Ji Zhang[2], Wei-Ze Yang[2], Yuan-Zhong Wang[2], Hong Yu[1] and Hang Jin[2]*

[1]Yunnan Herbal Laboratory, Institute of Herb Biotic Resources, Yunnan University, Kunming 650091, China.
[2]Institute of Medicinal Plants, Yunnan Academy of Agricultural Sciences, Kunming 650223, China.

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***Paris* L. (Trilliaceae) is a temperate genus of about 24 perennial herbaceous species distributed from Europe to Eastern Asia. *Paris* is notable in China for its medicinal value. An investigation was conducted to determine the variations of 27 morphological characters of 196 accessions from 8 populations of medicinal *Paris* from China and Vietnam. Variations of qualitative and quantitative characters showed high diversity in medicinal *Paris*. The first four principal components in the principal component analysis explained 59.36% of the total variation among 8 medicinal *Paris* taxon. The clustering of the accessions based on morphological similarity reflected their geographic origin. Overall, the results would be provided for the conservation and sustainable utilization of this medicine resource.**

Key words: Morphological characterization, genus *Paris* resource, wild medicinal plant, conservation.

INTRODUCTION

Paris L. (Trilliaceae) is a temperate genus of about 24 species of perennial herbs distributed from Europe to eastern Asia (Heywood, 1980; Li, 1986, 1998; Jacquemyn et al., 2008). According to the DNA sequence data analysis, *Paris* is monophyletic (Ji et al., 2006). Most species are restricted to East Asia and the Yunnan-Guizhou Plateau in China as the diversity centre of this genus (Li, 1998). The rhizome of *Paris* plant is a famous Chinese traditional medicine named “Chonglou” which is widely used to treat traumatic injuries, snake bite, abscess, parotitis and mastitis (Tang, 2003; Huang et al., 2005; Yang et al., 2011). Steroidal saponins are the major active compounds (Jacquemyn et al., 2008; Liao, 2009; Ma, 2009).

Natural and man-made disturbances are the major factors responsible in the loss of *Paris* (He et al., 2007a). Wild population of this genus, such as *Paris polyphylla* var. yunnanensis, have greatly declined and become fragmentized (He et al., 2007b). For a successful species variability play a vital role because long-term survival and evolution of species depend on the maintenance of sufficient genetic variability within and/or among populations to accommodate new selection pressures brought about by environmental changes and artificial selection (Barrett and Kohn, 1991; Rodríguez et al., 2011; Arslanoglu et al., 2011). Asudi et al. (2010) reported that high diversity of some morphological characterization of *Carica papaya L.* points to ample possibilities of obtaining desirable trait combinations in specific cultivars.

Previous studies on *Paris* species were focused on genetic structure, taxonomic relationship and DNA barcoding (Zhang et al., 2004; Weng, 2006; Zhang, 2006; Weng et al., 2008; Zhao et al., 2010; Zhu et al., 2010). In order to preserve the natural resources and to retain a stable and renewable source of the important medicinal *Paris*, more research should be dedicated to understanding the morphological diversity of this genus. However, only the study by Li et al. (2010) reported the morphological variations of *P. polyphylla* var. *yunnanensis*. They found that P. polyphylla var. yunnansensis had plenty of phenotypic diversity for its breeding. The aim of this study was to describe the variation in morphological characters of some medicinal *Paris* from China and Vietnam.

*Corresponding author. E-mail: hongyu@ynu.edu.cn, jinhang2009@126.com.

Table 1. Sample's origin.

Name	Population	Location	Altitude (m)	Habitat
P. polyphylla var. *yunnanensis*	P1	Kunming, Yunnan, China	2100	Evergreen broad-leaved forest
	P2	Yuxi, Yunnan, China	2500	Forest of *Pinus yunnanensis*
P. polyphylla var. *chinensis*	P3	Baise, Guangxi, China	1200	Subtropical evergreen broad-leaf forest
P. vietnamensis	P4	Puer, Yunnan, China	1500	Subtropical evergreen broad-leaf forest
P. cronquistii var *cronquistii*	P5	Cao Bang, Vietnam	800 - 1000	Rainforest
P. xichouensis	P6	Cao Bang, Vietnam	800 - 1000	Rainforest
P. caobangensis	P7	Cao Bang, Vietnam	800 - 1000	Rainforest
P. forrestii	P8	Nujiang, Yunnan, China	1600	Evergreen broad-leaved forest

Table 2. Quantitative characters considered in the medicinal *Paris.*

Character	Abbreviation	Measurement
Plant height	PH	cm
Stem diameter	SD	mm
Leaf stalk length	LSL	cm
Leaf length	LL	cm
Leaf width	LW	cm
Leaf number	LN	Number
Flower stalk length	FSL	cm
Stigma branch number	SBN	Number
Stamen number	SN	Number
Stamen length	SL	cm
Petal number	PN	Number
Petal length	PL	cm
Stigma length	StL	mm
Calyx number	CN	Number
Calyx length	CL	cm
Calyx width	CW	cm

MATERIALS AND METHODS

Plant materials

Eight populations of seven taxon of medicinal *Paris* were collected separately from their natural habitats (including evergreen broad-leaved forest, forest of *Pinus yunnanensis* rainforest) from China and Vietnam (Table 1). The rhizomes of samples were collected from all sites and then grown together in a medicinal plant resource garden in Kunming, Yunnan. The resource garden was located at 25°10′ N, 102°58′ E. On average, the mean annual temperature is 14.9°C and the annual precipitation is 1011.3 mm in this area.

Morphological description

196 accessions were selected randomly to analyse quantitative and qualitative characters, with being not influenced by the environment in the field. The characters of each accessions recorded were based on the *Paris* descriptors (Wang and Tang, 1978; Li, 1998).

Table 3. Qualitative characters considered in the medicinal *Paris*.

Character	Abbreviation	Scale	Expression
Stem color	SC	1	Green
		3	Light violet
		5	Violet
		7	Dark violet
Stem with powder	SP	0	Absence
		1	Presence
Stamen color	StC	1	Light yellow
		3	Yellow
		5	Orange
		7	Light violet
		9	Violet
Stigma color	StCo	1	White
		3	Green
		5	Red
		7	Violet
		9	Dark violet
Stigma posture	StP	1	Straight
		3	Curl
		5	Curl backward
Petal color	PC	1	Green
		3	Light green
		5	Yellow-green
		7	Orange
Petal shape	PS	1	Acicular
		3	Spatulate
		5	Linear
		7	Zonate
Petal posture	PP	1	Straight
		3	Flat
		5	Droop
		7	Curl
Calyx color	CC	1	Yellow-green
		3	Green
		5	Dark green
		7	Others
Pollen color	PoC	1	Light yellow
		3	Yellow
		5	Orange
Flower stalk color	FSC	1	Green
		3	Light violet
		5	Violet

Table 4. Variation of morphological characters in medicinal *Paris* (*n* = 196).

Character	Mean	Minimum	Maximum	CV (%)
Stem characters				
PH	51.8	12.0	120.5	40.8
SD	6.8	2.7	18.0	32.6
SC	3.2	1.0	7.0	48.9
SP	0.5	0.0	1.0	95.3
Leaf characters				
LSL	2.2	0.4	6.2	48.6
LL	12.7	4.4	24.0	30.6
LW	5.1	1.9	11.1	41.2
LN	6.5	4.0	11.0	21.9
Flower characters				
FSL	15.1	3.0	65.0	58.8
SBN	5.2	2.0	10.0	26.0
SN	5.5	3.0	11.0	23.8
SL	1.5	0.4	2.9	32.3
PN	5.4	3.0	10.0	24.5
PL	5.2	0.9	13.1	48.5
StL	4.5	2.0	10.0	36.6
CN	5.4	3.0	10.0	23.5
CL	4.9	2.0	8.6	28.2
CW	1.7	0.5	3.4	31.9
StC	5.4	1.0	9.0	46.6
StCo	6.8	1.0	9.0	25.2
StP	3.5	1.0	5.0	49.1
PC	4.8	1.0	7.0	32.3
PS	3.8	1.0	9.0	51.4
PP	2.8	1.0	7.0	61.0
CC	2.3	1.0	7.0	74.4
PoC	2.7	1.0	5.0	55.8
FSC	1.8	1.0	5.0	74.5

The parameters studied included plant height (PH), stem diameter (SD), stem color(SC), stem with powder (SP), leaf stalk length (LSL), leaf length (LL), leaf width (LW), leaf number (LN), flower stalk length (FSL), stigma branch number (SBN), stamen number (SN), stamen length (SL), petal number (PN), petal length (PL), stigma length (StL), calyx number (CN), calyx length (CL), calyx width (CW), stamen color (StC), stigma color (StCo), stigma posture (StP), petal color (PC), petal shape (PS), petal posture (PP), calyx color (CC), pollen color (PoC) and flower stalk color (FSC). From these. the 16 characters were quantitative (Table 2), while the rest 11 were qualitative (Table 3).

Statistical analysis

Summary statistics were calculated on each quantitative and qualitative character using SPSS 17.0 software to discriminate accessions with one way ANOVA and principal component analysis (PCA).

RESULTS AND DISCUSSION

Variation of morphological characters of medicinal *Paris*

The variations of 27 morphological characters observed in medicinal *Paris* are given in Table 4. In general, the coefficients of variation of plant height, stem color, stem with powder, leaf stalk length, leaf width, flower stalk length, petal length, stamen color, stigma posture, petal

Table 5. Comparison of mean values of morphological characters in different populations of medicinal *Paris* ($p<0.05$).

Character	P1	P2	P3	P4	P5	P6	P7	P8
n	**26**	**26**	**28**	**27**	**19**	**24**	**26**	**20**
Stem characters								
PH	59.3 ± 15.3^{b}	43.3 ± 8.2^{c}	47.3 ± 12.3^{c}	82.1 ± 17.8^{a}	59.9 ± 18.8^{b}	53.6 ± 17.5^{bc}	40.8 ± 12^{c}	23.4 ± 7.6^{d}
SD	9.0 ± 1.8^{a}	6.1 ± 1.1^{c}	6.8 ± 1.1^{c}	8.6 ± 2.5^{ab}	6.6 ± 1.2^{c}	7.7 ± 1.4^{b}	4.8 ± 0.9^{d}	3.7 ± 0.8^{e}
SC	4.3 ± 1.4^{ab}	1.8 ± 1.0^{d}	1.9 ± 1.5^{cd}	3.7 ± 1.4^{b}	4.6 ± 1.1^{a}	2.6 ± 1.4^{c}	3.5 ± 0.9^{b}	3.2 ± 0.6^{bc}
SP	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}	0.0 ± 0.0^{b}	1.0 ± 0.0^{a}	0.0 ± 0.0^{b}	1.0 ± 0.0^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}
Leaf characters								
LSL	1.9 ± 0.6^{b}	1.9 ± 0.5^{b}	1.2 ± 0.5^{c}	3.0 ± 1.0^{a}	3.2 ± 1.3^{a}	3.0 ± 1.0^{a}	1.8 ± 0.4^{b}	1.5 ± 0.8^{c}
LL	9.6 ± 1.6^{g}	9.8 ± 1.5^{f}	13.4 ± 1.7^{d}	17.5 ± 3.3^{a}	14.4 ± 3.2^{c}	16.1 ± 2.2^{b}	12.4 ± 2.3^{e}	7.5 ± 1.3^{h}
LW	5.4 ± 1.0^{d}	4.5 ± 0.9^{e}	2.9 ± 0.5^{h}	8.0 ± 1.6^{a}	6.1 ± 1.4^{c}	7.2 ± 1.4^{b}	3.7 ± 0.6^{f}	3.0 ± 0.7^{g}
LN	7.0 ± 1.0^{b}	7.1 ± 1.0^{b}	8.6 ± 1.1^{a}	6.1 ± 0.9^{c}	6.6 ± 0.7^{bc}	5.9 ± 0.7^{c}	5.3 ± 0.8^{d}	5.0 ± 0.9^{d}
Flower characters								
FSL	11.9 ± 3.3^{c}	8.0 ± 1.9^{d}	13.1 ± 4.3^{c}	21.5 ± 6.3^{ab}	18.9 ± 12.0^{b}	24.0 ± 12.1^{a}	16.5 ± 6.2^{bc}	6.7 ± 2.1^{d}
SBN	6.8 ± 1.0^{a}	3.8 ± 0.5^{d}	4.3 ± 0.7^{c}	5.9 ± 1.0^{b}	6.0 ± 0.7^{b}	5.9 ± 0.8^{b}	4.5 ± 1.1^{c}	4.1 ± 0.8^{cd}
SN	6.8 ± 1.0^{a}	4.5 ± 0.9^{cd}	4.7 ± 0.8^{cd}	6.0 ± 0.^{9b}	6.2 ± 0.5^{b}	6.3 ± 1.5^{a}	4.8 ± 0.7^{c}	4.2 ± 0.7^{d}
SL	1.7 ± 0.1^{c}	1.8 ± 0.3^{b}	0.8 ± 0.2^{g}	1.9 ± 0.2^{b}	2.0 ± 0.4^{a}	1.2 ± 0.3^{e}	1.4 ± 0.3^{d}	1.0 ± 0.2^{f}
PN	6.8 ± 1.0^{a}	4.0 ± 1.0^{d}	4.7 ± 0.8^{cd}	6.0 ± 0.9^{b}	6.3 ± 0.8^{b}	6.1 ± 1.1^{b}	5.0 ± 0.7^{c}	4.3 ± 0.7^{d}
PL	4.8 ± 0.9^{e}	5.8 ± 1.3^{d}	1.8 ± 0.4^{f}	6.2 ± 0.9^{c}	9.1 ± 1.9^{a}	2.2 ± 1.0^{f}	7.1 ± 1.2^{b}	5.8 ± 1.4^{d}
StL	5.5 ± 1.4^{b}	3.8 ± 1.4^{c}	2.6 ± 0.6^{d}	5.1 ± 1.0^{b}	6.2 ± 1.9^{a}	3.4 ± 1.5^{c}	4.8 ± 1.0^{b}	5.1 ± 0.9^{b}
CN	6.9 ± 1.0^{a}	4.1 ± 1.1^{d}	4.8 ± 0.8^{c}	6.1 ± 0.9^{b}	6.4 ± 0.6^{ab}	5.9 ± 0.7^{b}	5.0 ± 0.6^{c}	4.2 ± 0.7^{d}
CL	4.3 ± 0.6^{c}	6.0 ± 1.0^{a}	5.3 ± 1.1^{b}	5.8 ± 0.8^{ab}	5.5 ± 1.3^{b}	4.6 ± 0.8^{c}	4.5 ± 1.5^{c}	2.6 ± 0.6^{d}
CW	1.6 ± 0.3^{d}	2.5 ± 0.4^{a}	1.5 ± 0.3^{de}	1.7 ± 0.3^{c}	1.9 ± 0.6^{b}	1.4 ± 0.3^{e}	1.5 ± 0.5^{de}	1.2 ± 0.4^{f}
StC	4.6 ± 2.7^{b}	7.2 ± 0.5^{a}	7.0 ± 0.0^{a}	1.0 ± 0.0^{c}	5.5 ± 2.8^{b}	5.0 ± 0.0^{b}	6.7 ± 1.8^{a}	6.7 ± 1.8^{a}
StCo	6.5 ± 1.6^{c}	7.0 ± 0.6^{b}	7.3 ± 0.7^{b}	8.9 ± 0.5^{a}	5.0 ± 0.0^{d}	5.0 ± 0.0^{d}	8.8 ± 0.7^{a}	4.7 ± 1.0^{d}
StP	4.0 ± 1.6^{a}	2.8 ± 1.8^{b}	4.0 ± 1.3^{a}	4.6 ± 0.8^{a}	2.5 ± 2.0^{bc}	3.0 ± 1.0^{b}	4.3 ± 1.5^{a}	1.8 ± 1.6^{c}
PC	5.8 ± 1.4^{a}	4.1 ± 1.0^{c}	4.1 ± 1.8^{c}	6.4 ± 0.9^{a}	4.9 ± 0.5^{b}	2.9 ± 1.5^{d}	4.9 ± 0.4^{b}	4.7 ± 0.7^{bc}
PS	3.0 ± 0.6^{cd}	5.0 ± 1.5^{ab}	3.7 ± 1.0^{c}	2.3 ± 1.0^{d}	5.5 ± 2.9^{a}	4.8 ± 1.9^{ab}	4.7 ± 0.7^{b}	1.0 ± 0.0^{e}
PP	2.4 ± 0.9^{cd}	2.2 ± 1.0^{d}	5.0 ± 0.5^{a}	1.1 ± 0.5^{e}	3.7 ± 1.8^{b}	3.7 ± 1.7^{b}	2.8 ± 1.4^{c}	1.0 ± 0.0^{e}
CC	3.8 ± 1.6^{a}	1.5 ± 0.9^{cd}	2.1 ± 1.0^{c}	4.4 ± 2.4^{a}	1.5 ± 0.9^{cd}	1.2 ± 0.8^{d}	1.0 ± 0.0^{d}	3.0 ± 0.0^{b}
PoC	3.1 ± 1.5^{b}	5.0 ± 0.0^{a}	1.0 ± 0.0^{d}	2.9 ± 0.5^{b}	1.5 ± 0.9^{d}	2.9 ± 1.4^{b}	2.8 ± 0.5^{b}	2.2 ± 1.8^{c}
FSC	1.0 ± 0.0^{d}	1.0 ± 0.0^{d}	2.5 ± 0.9^{b}	1.0 ± 0.0^{d}	1.9 ± 1.0^{c}	1.0 ± 0.0^{d}	4.5 ± 0.9^{a}	1.0 ± 0.0^{d}

Table 6. Results of the PCA (for the first four principle components) of the medicinal *Paris* accessions.

Eigenvalues	7.278	3.278	3.065	2.405
Total variance (%)	26.957	12.142	11.353	8.906
Cumulative (%)	26.957	39.099	50.452	59.358

	Factors			
Characters	**PC1**	**PC2**	**PC3**	**PC4**
PH	0.835	0.227	0.049	0.059
SD	0.722	-0.008	0.295	-0.233
SC	0.392	-0.266	-0.219	0.458
SP	0.562	0.018	-0.102	-0.683
LSL	0.660	0.286	-0.044	0.014
LL	0.604	0.415	0.254	0.183
LW	0.819	0.169	-0.104	-0.147
LN	0.094	0.141	0.550	-0.216
FSL	0.486	0.249	0.261	0.262
SBN	0.739	-0.428	0.258	0.048
SN	0.717	-0.375	0.259	-0.015
SL	0.653	0.277	-0.468	0.023
PN	0.703	-0.424	0.353	0.108
PL	0.280	0.179	-0.649	0.456
StL	0.408	-0.203	-0.526	0.308
CN	0.718	-0.429	0.333	0.124
CL	0.419	0.762	0.055	-0.037
CW	0.211	0.717	-0.291	-0.288
StC	-0.660	0.097	0.108	0.035
StCo	0.146	0.322	-0.126	0.295
StP	0.287	0.202	0.171	0.281
PC	0.344	-0.199	-0.390	0.282
PS	0.033	0.562	0.316	0.105
PP	-0.148	0.322	0.740	0.176
CC	0.351	-0.383	-0.214	-0.135
PoC	0.157	0.206	-0.421	-0.461
FSC	-0.293	0.240	0.127	0.751

shape, petal posture, calyx color, pollen color and flower stalk color had a wide range and were all above 40%. Moreover, the coefficients of variation of stem diameter, leaf length, leaf number, stigma branch number, stamen number, stamen length, petal number, stigma length, calyx number, calyx length, calyx width, stigma color and petal color and were all below 37% with a narrow range.

Furthermore, It was revealed that all of 27 characters varied significantly among taxa by ANOVA analysis (Table 5). The results showed that the highest values of stem diameter, stem with powder, stigma branch number, stamen number, petal number, calyx number, calyx length, calyx width, stamen color, calyx color and pollen color were found in *P. polyphylla* var*. yunnanensis*. The highest values of plant height, stem with powder, leaf length, leaf width, stigma color, stigma posture, petal color were found in *P. vietnamensis*. The highest values of stem color, leaf stalk length, stamen length, petal length, stigma length, and petal shape were found in *P. cronquistii* var *cronquistii*. However, 15 out of 27 characters were obtained the lowest values in *P. forrestii*.

Multivariate analysis of morphological characters

Principal component analysis (PCA) can determine which of the characters most strongly contributes to the principal components. The first four principal components in the PCA of the morphological characters accounted for 26.957, 12.142, 11.353 and 8.906% of the total variation

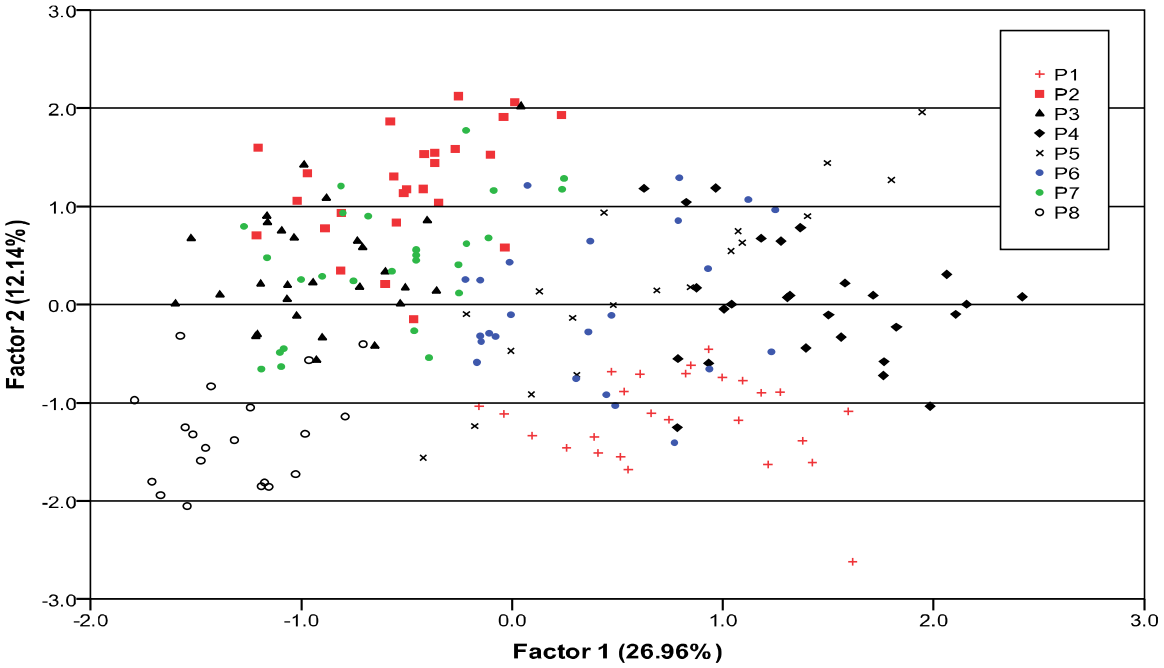

Figure 1. Scores plot of PCA of 196 medicinal *Paris* accessions using 27 morphological characters.

respectively and together explained 59.358% of the total variation among the 8 medicinal *Paris* taxon (Table 6). The relationships among the 196 medicinal *Paris* accessions were revealed by PCA based on 27 morphologicalcharacters are presented in Figure 1. The clustering of the accessions based on morphological similarity reflected their geographic region of origin.

Conclusion

To our knowledge, this is the first study comparing the morphological diversity of medicinal *Paris* accessions. The results showed that the medicinal *Paris* studied was presented a high diversity in both qualitative and quantitative characters. These investigations could be useful for further exploitation of wild medicinal *Paris* in genetic improvement of domestication and cultivation.

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