Biochemical Genetics, Vol. 41, Nos. 1/2, February 2003 (© 2002)

Allozyme Variation and Population Differentiation of the *Aconitum delavayi* Complex (Ranunculaceae) in the Hengduan Mountains of China

Fumin Zhang,[1] **Hongzhi Kong,**[1] **and Song Ge**[1,2]

Received 22 January 2002—Final 10 April 2002

The Aconitum delavayi *complex is a group of four climbing species with trisect-leaves occurring in the Hengduan Mountains. The species of this complex are highly localized on very narrow regions with quite small population sizes. Because of rapid environmental changes recently in the Hengduan Mountains, this complex shows complicated morphological variability, which makes it difficult to delimit species. In the present study, 10 enzyme systems coding for 14 putative loci were employed to detect the interspecific and intraspecific genetic variation of the complex. In addition to low genetic diversity within all eight populations surveyed, the results indicate that* A. episcopale *is a distinct species because of high genetic identities among its three populations. Very low genetic divergence among populations of* A. stapfianum *and* A. delavayi *suggests that the two species should be treated as a single one.*

KEY WORDS: *Aconitum delavayi* complex; allozyme; taxonomy; Hengduan Mountains.

INTRODUCTION

The genus *Aconitum* L. (Ranunculaceae) is widely distributed throughout Eurasia and North America excluding the arctic and tropic regions (Kadota, 1987; Wang, 1979). More than 300 species have been described in the genus and they are divided into three commonly accepted subgenera: *Gymnaconitum*, *Aconitum*, and

[1] Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, 100093 Beijing, People's Republic of China.
[2] To whom correspondence should be addressed; e-mail: gesong@ns.ibcas.ac.cn or songge@hotmail.com.

0006-2928/03/0200-0047/0

Lycoctonum (Kita and Ito, 2000). As a monotype subgenus, *Gymnaconitum* includes one annual species, *A. gymnandrum* Maxim, which is endemic to western China (Wang, 1979). The largest subgenus, *Aconitum*, is usually distinguished by its biennial and nonlignified tubers, whereas the second largest subgenus *Lycoctonum* is perennial and has lignified rhizomes (Tamura, 1990). Hess *et al*. (1977) considered that East Asia was the center of the genus, and Li (1988) further suggested that the Sino-Himalayan subregion was the center of both diversity and endemism in *Aconitum*. As the main part of Sino-Himalayan subregion, the Hengduan Mountains extend from NW Yunnan, through W Sichuan and E Tibet, to S Qinghai, and are one of three centers of plant endemism in China (Ying and Zhang, 1984). In this area, there occur more than 100 species of *Aconitum*, most of them endemic (Li, 1988; Yang *et al*., 1993).

In the subgenus *Aconitum* about 20 climbing species occur in East Asia, classified into a series *Volubilia* Steinb (Wang, 1979). Of these, the *Aconitum delavayi* complex is a group with trisect-leaves distributed in the Hengduan Mountains that were considered to be the site of the origin of the series (Wang, 1992). Despite there being only four species in the *A. delavayi* complex, the circumscription of and relationship among these have long been in great confusion (Handel-Mazzetti, 1939; Wang, 1979; Yang, 1999). In his recent revision, Yang (1999) indicated that *A. delavayi* Franchet could be identified by higher galeate upper sepals, spreading hairs covering the inflorescence axis, and the pedicels, while *A. episcopale* Léveillé could be separated from *A. delavayi* by having relatively sparse curled hairs covering the pedicels. *A. delavayi* and *A. stapfianum* Handel-Mazzetti are very similar in gross morphology and geographic distribution except that the latter occurs at higher elevation and has the shorter galeate upper sepals. *A. tuguancunense* Q. E. Yang is also quite similar to *A. delavayi* with the main differences being the glabrous inflorescence and pedicels. Although slight morphological differences can be found between species of the *A. delavayi* complex, the intermediate types among the species make it difficult to identify species with certainty. In addition to the morphological complexity, the *A. delavayi* complex species are highly localized in very narrow regions, with quite small population sizes, except that *A. episcopale* is scattered across a relatively large area. For example, both *A. delavayi* and *A. stapfianum* occur only on disjunct regions of the Changshan and Yulong Mountains; *A. tuguancunense* was found only in one locality in Tuguancun.

In the present study, we investigated populations of the *A. delavayi* complex by means of enzyme electrophoresis. Our specific emphasis was given to interspecific and intraspecific genetic variation of the *A. delavayi* complex. Such information may help establish a better classification system of the genus and contribute to understanding the speciation of plants in the Hengduan Mountains.

Table I. Origins of *A. delavayi* Complex Populations Sampled in the Hengduan Mountains of NW Yunnan

Species	Population	Origin	Altitude	Population size[a]	Sample size
A. episcopale Lévl.	EP1	Baishui, Lijiang	2600	≈40	14
A. episcopale Lévl.	EP5	Heishui, Lijiang	2600	≈60	30
A. episcopale Lévl.	EP6	Yunshanping, Yulong Mountain, Lijiang	2900	≈60	29
A. delavayi Franch.	DP4	Huadianba, Cangshan Mountain, Dali	2900	≈40	10
A. delavayi Franch.	DP9	Yulong Mountain, Lijiang	3200	≈50	22
A. stapfianum Hand.-Mazz.	SP7	Yulong Mountain, Lijiang	3600	≈60	29
A. stapfianum Hand.-Mazz.	SP10	Huadianba, Cangshan Mountain, Dali	3400	≈40	11
A. tuguancunense Q. E. Yang	TP11	Tuguancun, Zhongdian	3200	≈20	5

[a] The population sizes were estimated by observation in the field.

MATERIALS AND METHODS

A total of eight populations representing the *A. delavayi* complex was sampled (Table I, Fig. 1). Three populations were collected from *A. episcopale*, the only widespread species in Hengduan Mountains. Two populations from each of *A. delavayi* and *A. stapfianum* were collected from the Cangshan and Yulong Mountains, where these two species form an altitudinal vicariance. *A. tuguancunense* was found only in one locality in Tuguancun with few individuals and thus one population was collected. For each population, tuberous roots were transplanted into pots in the greenhouse in Beijing. Fresh leaves were individually collected and homogenized in a Tris-maleic acid grinding buffer solution (Soltis *et al*., 1983). The homogenate was then immediately absorbed on paper wicks and stored at -80°C before electrophoresis. Horizontal starch gel electrophoresis was employed (Soltis *et al*., 1983).

After screening 16 enzymes, 10 were chosen for further analysis. Gel and electrode buffer system 6 (12% starch gel) was employed for aminopeptidase (AMP; E.C. 3.4.11.1), aspartate aminotransferase (AAT; E.C. 2.6.1.1), NAD(P) H-diaphorase (DIA; E.C. 1.6.2.2), and hexokinase (HEX; E.C. 2.7.1.1). System 11 (14% starch gel) was used for phosphoglucoisomerase (PGI; E.C. 5.3.1.9), phosphoglucomutase (PGM; E.C. 5.4.2.2), 6-phosphogluconate dehydrogenase (6PGD; E.C. 1.1.1.44), glucose-6-phosphate dehydrogenase (G6PD; E.C. 1.1.1.49), shikimate dehydrogenase (SKD; E.C. 4.2.1.3), and malate dehydrogenase (MDH; E.C. 1.1.1.37). Additional putative loci of 6PGD, DIA, HEX, and G6PD were occasionally detected but did not always have good resolution, and therefore were discarded in the analysis. Staining protocols of Soltis *et al*. (1983) were followed.

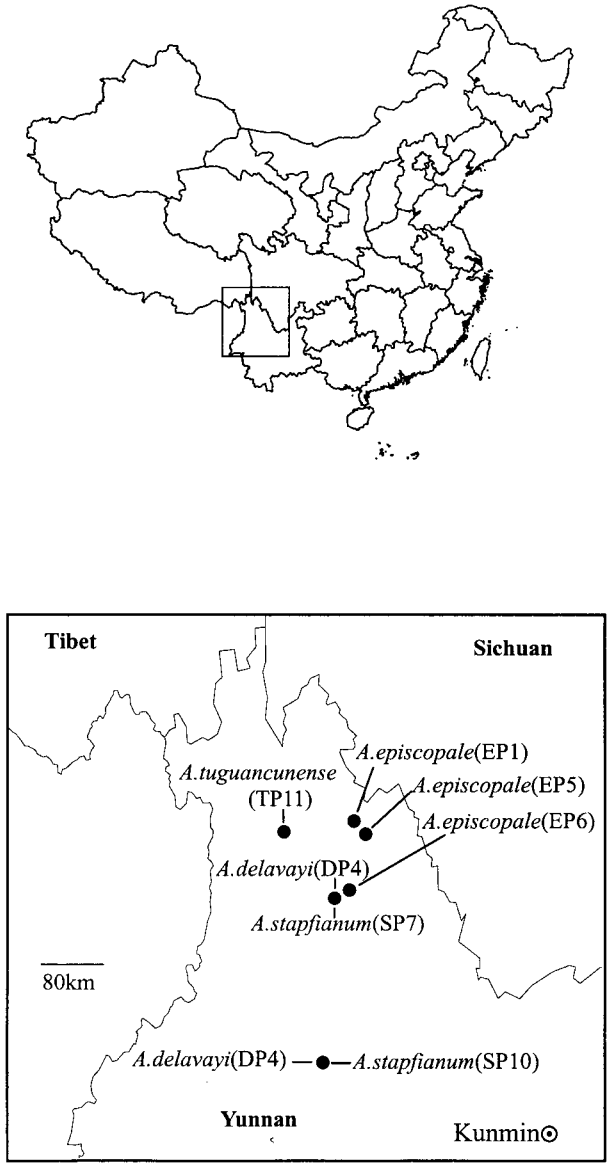

Fig. 1. Sampling localities. Three *A. episcopale* populations were collected in Hengduan Mountains. Two populations from each of *A. delavayi* and *A. stapfianum* were collected from the Cangshan and Yulong Mountains where these two species form an altitudinal vicariance. *A. tuguancunense* was collected in the only known locality in Tuguancun.

We used the computer program BIOSYS 1.7 (Swofford and Selander, 1981) to calculate the allele frequencies, the mean number of alleles per locus (A), the percentage of polymorphic loci (P) (95% criterion), the observed (H_o) and expected (H_e) heterozygosities, as well as Nei's (1972) genetic identity and distance. A dendrogram was generated using unweighted pair group method based on Nei's (1972) genetic identities to exhibit the genetic divergence among populations.

RESULTS

We employed 10 enzyme systems coding for 14 putative loci, of which seven (50%) are polymorphic in at least one population. Allele frequencies are listed in Table II. The statistics of genetic variability are summarized in Table III. The mean number of alleles per locus (A) ranges from 1.1 to 1.4 (average 1.22) and the percentage of polymorphic loci (P) from 7.1 to 35.7 (average 18.73). The observed heterozygosity (H_o) varies between 0 and 0.019 (average 0.01) and the expected heterozygosity (H_e) between 0.022 and 0.152 (average 0.06). The highest diversity was found in populations TP11, DP4, and DP9, while population SP10 shows the lowest diversity as measured by A, P, and H_e.

Genetic identities and distances (Nei, 1972) among populations were 0.897 on the average. A dendrogram was generated based on the genetic identity, and shows that the complex is divided into two distinct clusters, with a genetic identity of near 0.85. Three populations (EP1, EP5, EP6) from *A. episcopale* form a cluster with the mean genetic identity being 0.953. Populations DP4, DP9, SP7, SP10 from *A. delavay* and *A. stapfianum* cluster tightly, but neither *A. delavayi* populations nor *A. stapfianum* populations cluster together. It merits mentioning that the genetic diversity of the *A. delavayi* populations (DP4, DP9), as measured by A, P, H_o, H_e, is higher than those of the *A. stapfianum* populations in either the Changshan or Yulong Mountains. The single *A. tuguancunens* population (TP11) is more similar to populations of *A. delavayi* and *A. stapfianum* than to those of *A. episcopale* (Fig. 2).

DISCUSSION

Although *Aconitum* is a large genus and is distributed across the world, relatively few studies on its population diversity have been conducted so far. In their allozyme studies on *Aconitum lycoctonum* complex, Utelli *et al.* (1998) found that the mean values of genetic diversity are 1.4 (A), 26.3 (P), 0.08 (H_e) within populations. Cole and Kuchenreuther (2001) conducted an allozyme survey on two North America *Aconitum* species (*A. noveboracense* and *A. columbianum*) and detected slightly higher within-population diversity with the mean values of 1.45 (A), 40.8 (P), and 0.15 (H_e). In comparison, therefore, the *A. delavayi* complex maintains lower genetic diversity at the population level ($A = 1.22$, $P = 18.7$, $H_e = 0.06$). This

Table II. Allele Frequencies at 14 Loci in Eight Populations of the *A. delavayi* Complex

Locus	Population (N)[a]							
	EP1 (14)	EP5 (30)	EP6 (29)	DP4 (10)	DP9 (22)	SP7 (29)	SP10 (11)	TP11 (5)
Aat								
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Amp-1								
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Amp-2								
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6pgd								
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgi								
a	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000
b	0.000	0.000	0.000	0.450	0.886	1.000	1.000	1.000
c	1.000	1.000	1.000	0.500	0.114	0.000	0.000	0.000
Skd								
a	0.000	0.000	0.000	0.000	0.000	0.069	0.000	0.000
b	0.000	0.000	0.069	0.450	0.227	0.845	0.000	0.600
c	1.000	1.000	0.931	0.550	0.773	0.086	1.000	0.400
Mdh-1								
a	0.000	0.000	0.000	0.150	0.136	0.000	0.182	0.200
b	1.000	0.950	1.000	0.750	0.864	1.000	0.818	0.800
c	0.000	0.050	0.000	0.100	0.000	0.000	0.000	0.000
Mdh-2								
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgm-1								
a	0.500	0.833	0.931	1.000	1.000	0.862	1.000	0.600
b	0.500	0.167	0.069	0.000	0.000	0.138	0.000	0.400
Pgm-2								
a	1.000	1.000	1.000	1.000	0.932	1.000	1.000	0.800
b	0.000	0.000	0.000	0.000	0.068	0.000	0.000	0.200
Hex								
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Dia-1								
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Dia-2								
a	0.000	0.000	0.897	1.000	1.000	1.000	1.000	1.000
b	1.000	1.000	0.103	0.000	0.000	0.000	0.000	0.000
G6pd								
a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.200
b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.800

[a] N indicates the sample size in each population. Population numbers are the same as in Fig. 1 and Table I.

may arise from their narrow and isolated distribution as well as small population sizes, as many endemic species possess lower genetic variability than widespread species (Hamrick and Godt, 1990).

Generally, genetic identity among populations decreases with the increase of taxonomic ranking. As summarized in their reviews, Gottlieb (1977) and Crawford

Table III. Genetic Diversity of Eight Populations of the *A. delavayi* complex

Population	$A \pm$ SE	P^a	$H_o \pm$ SE	$H_e^b \pm$ SE
EP1	1.1 ± 0.1	7.1	0 ± 0	0.037 ± 0.037
EP5	1.1 ± 0.1	14.3	0.002 ± 0.002	0.027 ± 0.021
EP6	1.2 ± 0.1	21.4	0.005 ± 0.005	0.032 ± 0.017
SP7	1.2 ± 0.2	14.3	0.017 ± 0.013	0.037 ± 0.025
SP10	1.1 ± 0.1	7.1	0 ± 0	0.022 ± 0.022
DP4	1.4 ± 0.2	21.4	0.05 ± 0.029	0.109 ± 0.058
DP9	1.3 ± 0.1	28.6	0.019 ± 0.011	0.067 ± 0.032
TP11	1.4 ± 0.1	35.7	0 ± 0	0.152 ± 0.059
Mean	1.22 ± 0.13	18.73 ± 10	0.01 ± 0.017	0.06 ± 0.046

Note: A, mean number of alleles per locus; P, Percentage of loci polymorphic; H_o, observed heterozygosity; H_e, expected heterozygosity.
[a] A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95.
[b] Unbiased estimate (see Nei, 1978).

(1983, 1990) indicate that mean genetic identities of conspecific populations are usually above 0.90, whereas the genetic identities of congeneric taxa are usually below 0.7. In their study on *Aconitum lycoctonum* complex, Utelli *et al.* (1999) found very high genetic identity (average 0.958) among all investigated populations, and

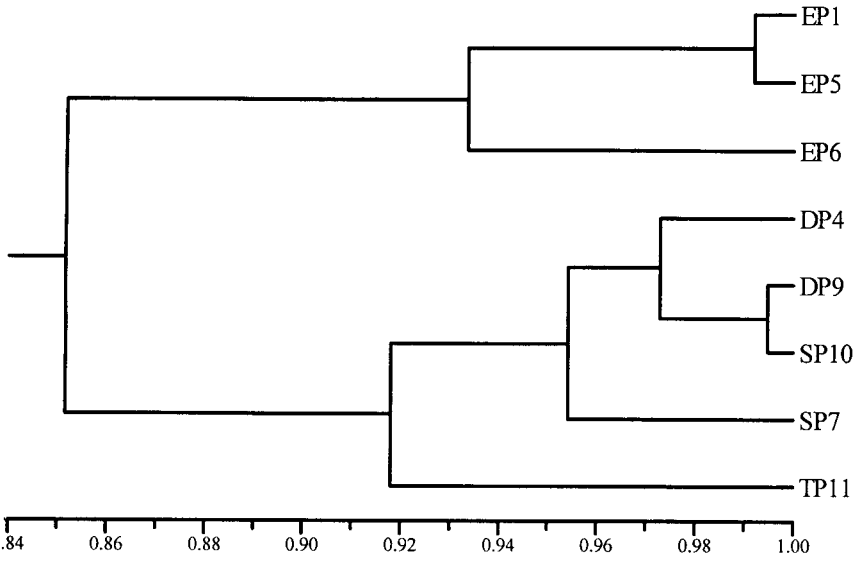

Fig. 2. Dendrogram using unweighted pair group method based on Nei's (1972) genetic identities. Population numbers are the same as in Fig. 1 and Table I.

suggested that the complex should be treated as one species. Similarly, Cole and Kuchenreuther (2001) provided further evidence supporting the previous treatment of *A. noveboracense* and *A. columbianum* as a single species because of the high allozyme identity and strong RAPD similarity among populations of the two species. In the present study, we obtained high genetic identities among populations of the *A. delavayi* complex. In particular, three *A. episcopale* populations from a distinctive cluster with high genetic identities (average 0.953). *A. episcopale* has recently been recognized as a distinct species based on morphological and chromosome data (Yang, 1999). Given the fact that the populations from *A. stapfianum* and *A. delavayi* cluster tightly with high genetic identities (average 0.967) but populations from either species don't cluster together, we suggest that the two species may be a single species. According to our geographic survey, *A. stapfianum* and *A. delavayi* form an altitudinal vicariance with the former existing in the higher altitude. More importantly, *A. delavayi* populations (DP4 and DP9) maintain higher genetic diversity than those of *A. stapfianum* (SP7 and SP10) on both localities sampled (Table III), indicating that the two "species" may be two ecological races within a single species.

Aconitum tuguancunense was described as a new species by Yang and Gong (1995) based mainly on its morphology and distinctive B chromosomes, which are seldom found in other *Aconitum* species (Shang and Li, 1984; Yang and Gong 1995). Although the present study shows that *A. tuguancunense* has relatively high genetic identity with *A. delavayi* populations, its taxonomic status awaits further study because only a single small population of this species has been found so far.

ACKNOWLEDGMENTS

We thank Professor Qing-Er Yang for helpful suggestions. This work was supported by the National Natural Science Foundation of China (30070057) and the Chinese Academy of Sciences (kscxz-sw-101A).

REFERENCES

Cole, C. T, and Kuchenreuther, M.A. (2001). Molecular markers reveal little genetic differentiation among *Aconitum noveboracense* and *A. columbianum* (Ranunculaceae) populations. *Am. J. Bot.* **88**(2):337–347.

Crawford, D. J. (1983). Phylogenetic and systematic inferences from electrophoretic studies. In Tanksley, S. D., and Orton, T. J. (eds.), *Isozymes in Plant Genetics and Breeding*, part A, Elsevier, Amsterdam, The Netherlands, pp. 257–287.

Crawford, D. J. (1990). *Plant Molecular Systematics: Macromolecular Approaches*, Wiley, New York.

Gottlieb, L. D. (1977). Electrophoretic evidence and plant systemtics. *Ann. Mo. Bot. Gard.* **64**:161–180.

Hamrick, J. L., and Godt, M. J. W. (1990). Allozyme diversity in plant species. In Brown, A. H. D., Clegg, M. T., Kahler, A. L., and Weir, B. S. (eds.), *Plant Population Genetics, Breeding and Genetic Resources*, Sinauer Associates, Sunderland, MA, pp. 43–63.

Handel-Mazzetti, H. F. (1939). Plantae Sinenses XXXIII. *Acta Hort. Gotob.* 13:125.
Hess, H. E., Landolt, F., and Hirzel, R. (1977). *Flora der Schweiz und angrenzender Gebiete*, Birkhäuser Verlag, Basel.
Kadota, Y. (1987). *A Revision of Aconitum Subgenus Aconitum (Ranunculaceae) of East Asia*, Sanwa Shoyaku Company, Utsunomiya, Japan.
Kita, Y., and Ito M. (2000). Nuclear ribosomal ITS sequences and phylogeny in East Asian *Aconitum* Subgenus *Aconitum* (Ranunculaceae), with special reference to extensive polymorphism in individual plants. *Plant Syst. Evol.* **225**:1–13.
Li, L. Q. (1988). On distributional features of the genus *Aconitum* in Sino-Himalayan Flora. *Acta Phytotax. Sin.* **26**(3):189–204.
Nei, M. (1972). Genetic distance between populations. *Am. Nat.* **6**:283–293.
Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**:583–590.
Shang, X. M., and Li, Z. L. (1984). A karyotype study of 10 species of *Aconitum* L. from China. *Acta Phytotax. Sin.* **22**(5): 378–385.
Soltis, D. E., Haufler, C. H., Darrow, D. C., and Gastony, G. J. (1983). Starch gel electrophoresis of ferns: A compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Am. Fern. J.* **73**:9–27.
Swofford, D. L., and Selander, R. B. (1981). BIOSYS-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* **72**:281–283.
Tamura, M. (1990). A new classification of the family Ranunculaceae 1. *Acta Phytotax. Geobot.* **41**:93–101.
Utelli, A. B., Roy, B. A., and Baltisberger, M. (1999). History can be more important than 'pollination syndrome' in determining the genetic structure of plant populations: The case of *Aconitum lycoctonum* (Ranunculaceae). *Heredity* **82**:574–584.
Wang, W. T. (1979). *Flora Reipublica Popularis Sinicae*, Tomus 27, Beijing.
Wang, W. T. (1992). On some distribution patterns and some migration routes found in the eastern Asiatic region. *Acta Phytotax. Sin.* 30(1):1–24.
Yang, Q. E. (1999). Taxonomic notes on some species of *Aconitum* L. (Ranunculaceae) from Yunnan, China. *Acta Phytotax. Sin.* **37**(6):545–590.
Yang, Q. E., and Gong X. (1995). A new species of the genus *Aconitum* L. from Yunnan, with an observation on its B chromosomes. *Acta Phytotax. Sin.* **33**(6):572–575.
Yang, Q. E., Gu, Z. J., Wu, Z. Y., and Hong, D. Y. (1993). A karyomorphological study in *Aconitum* subgen. *Aconitum* (Ranunculaceae) from Yunnan, China. *Cathaya* **5**:89–114.
Ying, J. S., and Zhang, Z. S. (1984). Endemism in the flora of China—Studies on the endemic genera. *Acta Phytax. Sin.* **22**(4):259–268.