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ORIGINAL RESEARCH

Biodiversity Conservation of the Genus *Incarvillea* Juss. (Bignoniaceae) Based on Molecular Diversity and Species Richness Assessment

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Abstract *Incarvillea* is a genus with considerable ornamental and medicinal value, yet many of its 16 species are threatened in the wild and two (*Incarvillea forrestii* and *Incarvillea altissima*) may be extinct. An extensive field survey of the Chinese species has shown that logging is the greatest threat to woodland margin species, while overgrazing by cattle endangers species from alpine meadows. To aid the setting of conservation priorities, we used phylogenetic weighting of species, based on the molecular phylogeny of both nuclear (nrITS) and chloroplast (*trn*L-F region) gene regions. We estimated molecular diversity with measures for genetic diversity (GD) and phylogenetic diversity (PD). PD/GD values highlighted the importance of *Incarvillea sinensis* and two Central Asian species, *Incarvillea semiretschenskia* and *Incarvillea olgae* from Kazakstan and Kirghizia, for which little is known. This combination of phylogenetic and complementarity analyses focused on the provinces of Sichuan, Yunnan, and Qinghai, with Yunnan having the only adequately protected areas for that genus.

Keywords Conservation · Genetic diversity · *Incarvillea* · Phylogenetic diversity · Species richness

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Plants within the genus *Incarvillea* Juss. have medicinal and ornamental value. Some species have been used for hundreds of years as traditional Chinese medicines by Tibetan, Yi, Lisu, Mongolian, and Han people (Wang et al. 1990; Jiangsu New Medical College 1995). Studies of their chemical constituents and activities have shown that these plants possess anti-pathogenic, anti-inflammatory, and anti-viral properties (Nakamura et al. 1999, 2000, 2001). In addition, most species (especially within subgenus *Pteroscleris*) have showy flowers that are cultivated as ornamentals in Europe, where several garden varieties have been bred (Grey-Wilson 1994; Cullen et al. 2000). The genus is notable as a temperate and herbaceous representative of the primarily tropical and woody family Bignoniaceae (Fig. 1). It comprises 16 species (Grierson 1961; Zhao 1988; Wang et al. 1990; Grey-Wilson 1998) and has wide geographical distribution, from Central Asia to Far East Russia via India, Nepal, and China (Fig. 1).

We have focused on these geographical and taxonomic characteristics in several studies (Chen et al. 2003, 2004, 2005). During that accompanying field work, we have sometimes failed to find *Incarvillea* in some localities where it was previously recorded. Extreme examples are *Incarvillea altissima* and *Incarvillea forrestii*, which have not been collected in the past 40 years. Natural habitats for *Incarvillea* seem to be shrinking, and some species are facing extinction. In view of its ornamental and medicinal properties, conservation of the genus is urgent. Although a traditional biodiversity assessment considers species richness, that method usually values more the group that appears to have evolved over a very short evolutionary time (Meyer 1993). However, increasing numbers of conservation biologists suggest that the phylogenetic distinctiveness of species be taken into account in those assessments and that diversity

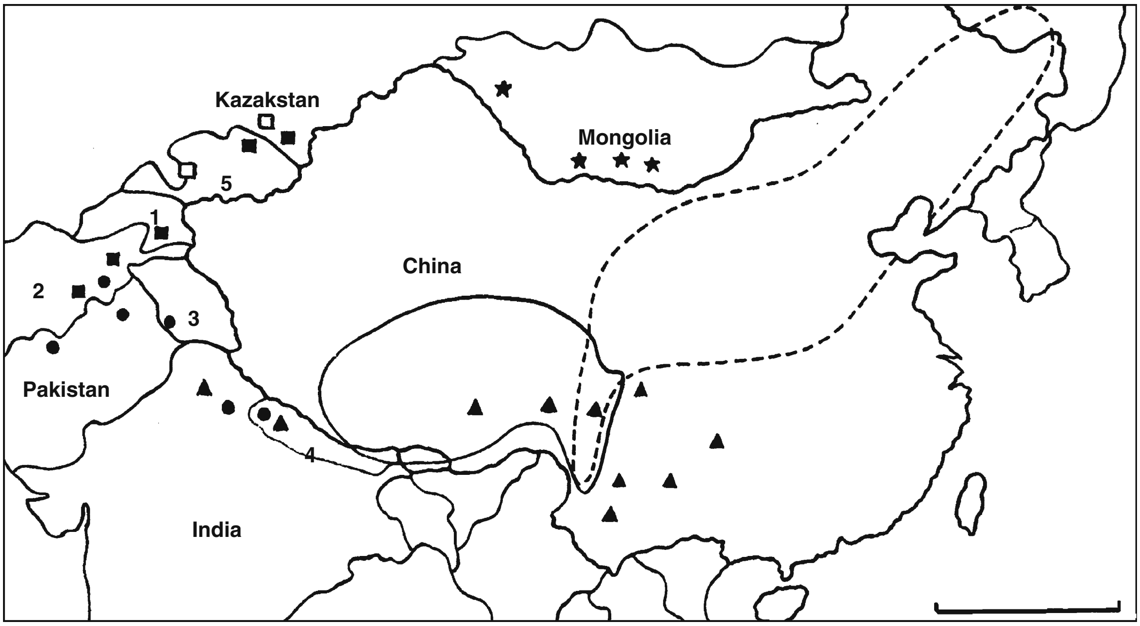

Fig. 1 Distribution of the genus *Incarvillea*, showing the East Asian distribution of subgenus *Pteroscleris* (*solid line*) and *Incarvillea sinensis* (*dotted line*). Other species are scattered through central Asia and Mongolia with the exception of *I. arguta* (subgenus *Amphicome*) in East Asia. *1*, Tadzhikistan; *2*, Afghanistan; *3*, Kashmir; *4*, Nepal; *5*, Kirghizia; *white square*, *I. semiretschenskia*; *black square*, *I. olgae* ; triangle, *I. arguta*; circle, *I. emodi*; *star*, *I. potaninii* (from Chen et al. 2005)

conservation priorities be based on evolutionary distinctiveness using genetic distances to determine whether particular assemblages of species conserve statistically significantly more biodiversity than do other assemblages (Wilson 1992; Crozier 1997a, b; Mace et al. 2003), for example, Conserve 3.2.2 (obtained from the website: http://evolve.bio.ic.ac.uk/software/; Crozier et al. 1999) enables researchers to accomplish this by examining both genetic diversity (GD) (Faith 1992, 1994) and phylogenetic diversity (PD) (Crozier 1992, 1997a, b; Crozier and Kusmierski 1994). This approach combines the merits of those methods based on topology and divergence. Thus, because all species are of interest, including those that are the result of recent developments, phylogenetically weighted scores should never be the sole basis for conservation prioritization.

Because *Incarvillea* species, which differ in their degrees of distinctiveness, seem to have undergone a distribution shift during their history, molecular measures may be better indicators of conservation worthiness than species richness (Crozier 1997a). Using the method outlined by Crozier (1992), we have quantified biodiversity in that genus within the context of evolutionary relationships, inferred by nrITS and *trn*L-F sequence data, as a means of prioritizing conservation efforts (Vane-Wright et al. 1991; Crozier 1997a, b). Those results have then been compared with a biodiversity assessment based on species richness. Here, we present recommendations for the conservation of *Incarvillea* that employ the above approach along with complementarity analyses.

Materials and Methods

Field and Herbaria Surveys and Samples

To assess the conservation status of *Incarvillea* species, we first conducted an extensive herbarium survey using materials from Chinese and European herbaria, including Chengdu Institute of Biology, Chinese Academy of Sciences (CDBI); Kunming Institute of Botany, Chinese Academy of Sciences (KUN); Institute of Botany, Chinese Academy of Sciences (PE); Department of Biology, Sichuan University (SZ); Royal Botanic Garden, Edinburgh, Scotland (E); Royal Botanic Gardens, Kew, England (K); and Muséum national d'Histoire naturelle, Paris, France (P). In addition, field surveys were undertaken of all known localities within China in 2001 and 2002, and local people were consulted about the use of *Incarvillea* species and the human activities that affect them, such as logging, grazing, and local land-use changes. We determined conservation status following 1994 Categories and Criteria (version 2.2; Mace and Stuart 1994).

Our sampling represented all five subgenera (Grierson 1961; Chen et al. 2005, 2006). The three exceptions included two species that may be extinct (*I. altissima* and

I. forrestii), and the Mongolian *Incarvillea potaninii*, which closely resembles *Incarvillea sinensis*. The percentage of sampling coverage was 81.25 (or 92.86% if *altissima* and *forrestii* are extinct). The sample for *Incarvillea semiretschenskia* was collected from an herbarium specimen and DNA for *Incarvillea emodi* was provided by Dr. Mark Chase (Royal Botanic Gardens, Kew). Other materials were taken from the field and from Kunming Botanic Garden (Table 1). DNA extraction, target sequence amplification, sequencing, and sequence alignments were performed as described by Chen et al. (2005).

Phylogenetic Analysis

We constructed a "total evolution tree" that reflected the best hypothesis of evolutionary relationship for the following diversity assessment. This entailed the combined data of ITS and *trn*L-F sequences. To assess the degree of phylogenetic incongruence between ITS and *trn*L-F partitions, we performed an incongruence-length difference (ILD) test (Mickevich and Farris 1981; Farris et al. 1994) in PAUP* 4.0b10 (Swofford 2001), using 1,000 replications with an heuristic tree search and ten addition sequence replicates. To ensure the accuracy of the *P* value in the ILD test, only parsimony informative positions were considered. We then applied the program SEQBOOT from the phylogeny inference package PHYLIP (Felsenstein 1989) to make 1,000 bootstrap subsamples of the dataset. Afterward, 1,000 Jukes–Cantor distance matrices were derived using DNADIST in the PHYLIP program. We obtained 1,000 neighbor-joining phylogenetic trees from the distance matrices via NEIGHBOR, and the resulting

Table 1 Materials and vouchers used in the phylogenetic study

Subgenus	Species	Location	Voucher	Sequence No.
Niedzwedzkia	*I. semiretschenskia*	Kasakh	Joffe and Titov 446(E)	AY695852 AY695875
Amphicome	*I. arguta* (YN)	Zhongdian, Yunnan	Chen ST 200103	AY695847 AY695870
	I. arguta (SC)	Markang, Sichuan	Chen ST 200104	AY695848 AY695871
	I. emodi	No location	M. W. Chase 11753(K)	AY695849 AY695872
Olgaea	*I. olgae*	Kunming Garden	Botanical Chen ST 200112	AY695846 AY695869
Incarvillea	*I. sinensis* var. *sinensis*	Markang, Sichuan	Chen ST 200106	AY695850 AY695873
	I. sinensis var. *przewalskii*	Kunming Botanical Garden	Chen ST 200113	AY695851 AY695874
Pteroscleris	*I. beresowskii*	Markang, Sichuan	Chen ST 200109	AY695854 AY695877
	I. compacta	Shiqu, Sichuan	Chen ST 200111	AY695855 AY695878
	I. delavayi	Lijiang, Yunnan	Chen ST 200108	AY695860 AY695883
	I. dissectifoliola	Yanyuan, Sichuan	Chen ST 200107	AY695858 AY695881
	I. lutea	Daocheng, Sichuan	Chen ST 200110	AY695853 AY695876
	I. mairei var. *mairei*	Dali, Yunnan	Chen ST 200101	AY695857 AY695880
	I. mairei var. *grandiflora*	Lijiang, Yunnan	Chen ST 200105	AY695856 AY695879
	I. younghusbandii	Nielamu, Tibet	Chen ST 200114	AY695861 AY695884
	I. zhongdianensis	Zhongdian, Yunnan	Chen ST 200102	AY695859 AY695882

trees were saved in a treefile produced by NEIGHBOR, whose restrict strict consensus tree was produced by CONSENSE of the PHYLIP program (Felsenstein 1989).

Phylogenetic Weighting of Biodiversity

In our biodiversity assessment, the treefile with 1,000 trees was read by Conserve 3.2.2 (obtained from the website: http://evolve.bio.ic.ac.uk/software/; Crozier et al. 1999). Based on the evolutionary relationship reflected by that phylogenetic tree, the contribution to diversity of a taxon or an area when added to the group is measured by GD (probability of more than one allele) (Faith 1992, 1994) and by PD (length of evolutionary history) (Crozier 1992, 1997a, b; Crozier and Kusmierski 1994). This uses the combined data matrix, and confidence limits of $P=0.05$ are based on 1.000 bootstrap replicates. Conservation priorities of areas are set according to two approaches for diversity assessment as well as the 'c' analysis, which estimates the gain in biodiversity representation and persistence when an area is added to an existing set of areas (Margules and Pressey 2000; Vane-Wright et al. 1991).

Results

Field Survey

We re-found all the Chinese species in 2001 to 2002, except for *I. forrestii* and *I. altissima*. These are believed to be apparently extinct because no specimen of either species has been collected for about 40 years, and recent searches of known localities have not been successful. Both grow at forest margins, but no forests remain at the localities recorded in old specimen annotations. Logging seems to have led to complete habit loss, although further searches are needed. The other 11 Chinese species grow in alpine meadows and shrub land that are subject to cattle grazing, and reduction or disappearance of *Incarvillea* populations is the norm, for instance, we found only two mature and a few young plants of *Incarvillea beresowskii* in Markang, Sichuan. This population will probably vanish in the near future without conservation efforts. The development of commercial farming ventures has resulted in increased land exploitation, e.g., cultivation, logging, and grazing, so that habitats of *Incarvillea* are now threatened (Table 2). In this context, changes in land use are likely to be a main threat factor.

Based on International Union for Conservation of Nature (IUCN) criteria (version 2.2; Mace and Stuart 1994) and excluding three species with insufficient data (*I. semiretschenskia*, *Incarvillea olgae*, and *I. emodi*), we classified all *Incarvillea* species into four categories: critically endangered (CR; four species), endangered (EN; one species), vulnerable (VU; five species), and at lower risk (LR; two species). These are presented in detail in Table 2.

Phylogenetic Analysis

In the homogeneity test, the combined data provided a *P* value of 0.597: the null hypothesis of congruence is not

Table 2 IUCN status of *Incarvillea* species (version 2.3, 1994)

Species	IUCN category	Threat status	Threat factor
I. semiretschenskia (NCS) *I. olgae* (NCS) *I. emodi* (NCS)	DD	Current information on these species is inadequate to make an assessment. Further data are needed from Kazakstan, Kirghizia, Tadzhikistan, Afghanistan, and Pakistan.	
I. arguta *I. sinensis*	LR, lc	These widely distributed species do not satisfy the criteria for categories critically endangered, endangered, or vulnerable.	
I. forrestii, *I. altissima* (possibly extinct)	CR	A population reduction of at least 80% can be inferred based on direct observation.	Logging and grazing
I. lutea *I. dissectifoliola*	CR	Area of occupancy is less than 10 km^2; plants are known only from single locations where they are declining.	Cultivation, logging, and grazing
I. beresowskii	EN	A population reduction of at least 50% over the last 10 years can be inferred based on a decline in the area of occupancy, a decline in the extent of occurrence, and a decline in the quality of habitat.	Logging and grazing
I. delavayi *I. compacta* *I. mairei* *I. younghusbandii*	VU	A population reduction of at least 20% over the last 10 years can be inferred based on a decline in the area of occupancy, extent of occurrence, and the quality of habitat.	Grazing
I. zhongdianensis	VU	The area of occupancy is estimated to be less than 2,000 km^2 (probably less than 500 km^2), and estimates indicate a continuing decline in the quality of habitat, with fewer than 10 localities.	Grazing

NCS non-Chinese species, *lc* least concern

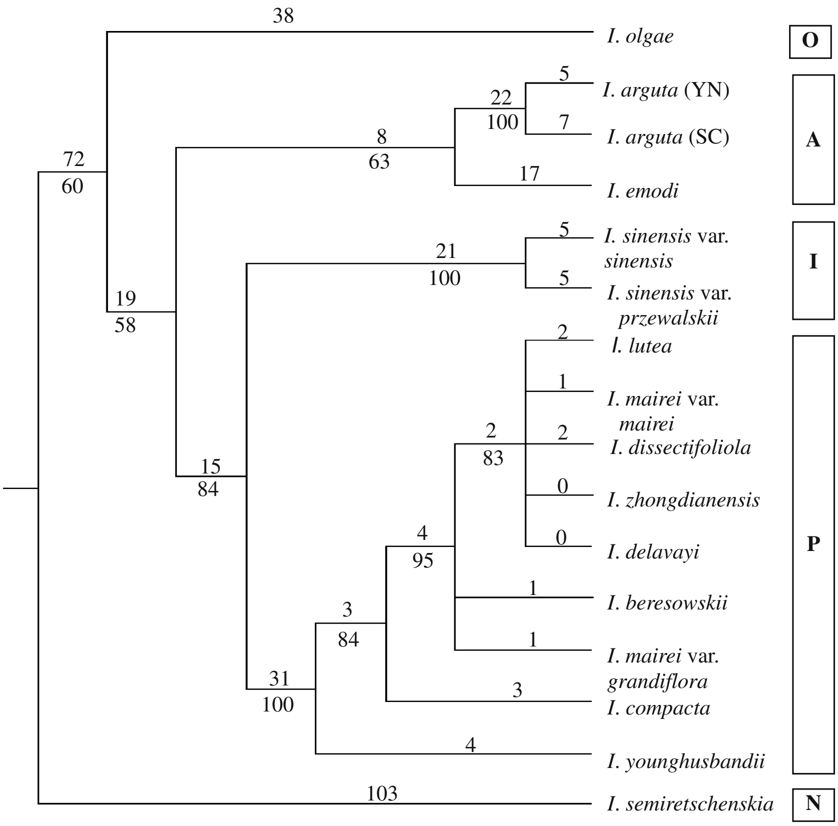

Fig. 2 Phylogenetic relationships of species based on combined data of ITS and *trn*L-F sequences. *A* subgenus *Amphicome*, *I* subgenus *Incarvillea*, *P* subgenus *Pteroscleris*, *O* subgenus *Olgaea*, *N* subgenus *Niedzwedzkia*. *Numbers below* branches indicate bootstrap values (percent). Branch length is *above* branches. Names of subgenera are shown *near* the tree

rejected. Therefore, we combined the ITS and *trn*L-F data sets into a single matrix for phylogenetic analysis.

The strict consensus tree (Fig. 2) of 1,000 trees generated by NEIGHBOR in PHYLIP is identical to that produced in maximum parsimony on topology in the genus (Chen et al. 2005). Five major clades occur within the genus, and ten clades/subclades have bootstrap support >50%. The five major clades are equivalent to the five subgenera in that system, and the relationships among them are fully resolved. We considered this tree to be the "total

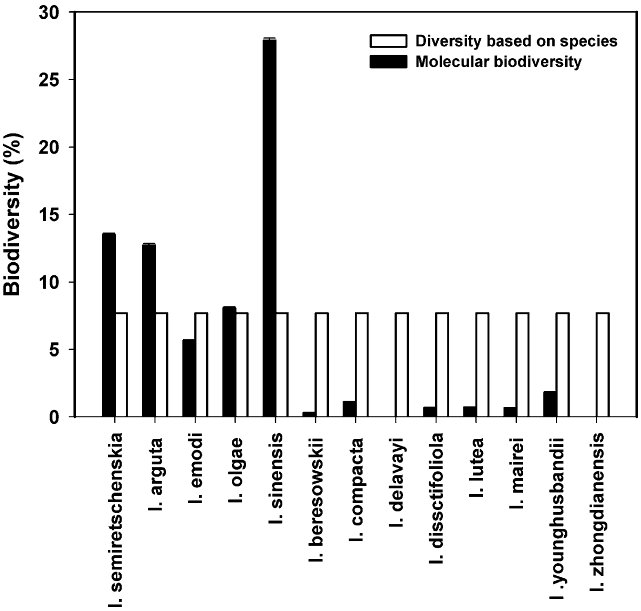

Fig. 3 The contribution to diversity of a taxon when added to the group

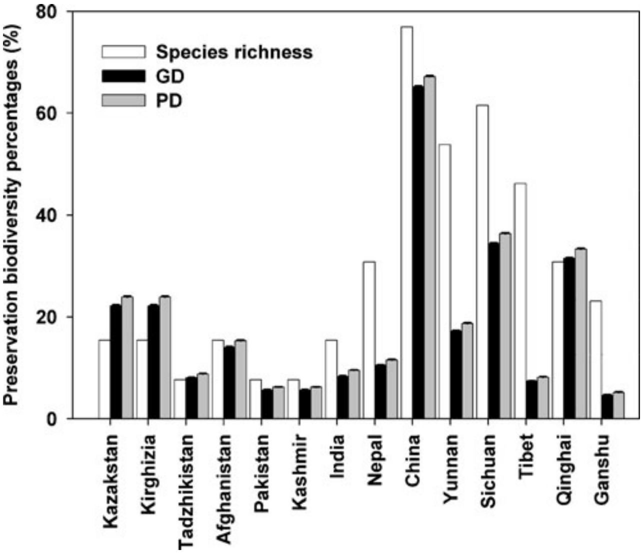

Fig. 4 The contribution to diversity of an area when added to the group

Table 3 Results of complementarity analysis and priorities of areas

	Priority based on species richness	Priority based on molecular biodiversity	Complementarity analysis
Kazakstan	3	2	2/0
Kirghizia	3	2	0/2
Tadzhikistan	4	6	0
Afghanistan	3	3	0
Pakistan	4	7	0
Kashmir	4	7	0
India	3	5	0
Nepal	2	4	0
China	1	1	11
China			
Yunnan	2	3	1
Sichuan	1	1	9
Tibet	3	4	1
Qinghai	4	2	0
Ganshu	5	5	0

evolution tree" reflecting the best hypothesis of evolutionary relationships in order to assess molecular biodiversity in the following analysis.

Phylogenetically Weighted Diversity

Under a traditional framework of biodiversity, the number of species in an area (species richness) is used to evaluate its diversity, and all species are considered of equal value—these covered 7.692% here. Using the combined data, species varied from 0.004±0.0003% to 27.899±0.168% in GD and from 0.005±0.0003% to 29.577±0.171% in PD. The biodiversity loss per species extinction is shown in Fig. 3.

Assessment of Biodiversities and Conservation Priorities of Areas Based on Two Approaches

We used species richness and phylogenetic and genetic diversities to assess the diversity of various distribution areas, based on combined data (Fig. 4). As inferred from PD/GD, China has the highest diversity followed by Kazakstan, Kirghizia, Afghanistan, Nepal, and India. The ranking of species richness is similar except that Nepal is second rather than Kazakstan. China has 11 species that are found in five provinces; other provinces, e.g., Guizhou, Hebei, and Peking, have only single species. Sichuan province is ranked first because of its high PD/GD, followed by Qinghai, Yunnan, Tibet, and Ganshu. In contrast, Yunnan is ranked second based on the assessment using species richness, followed by Qinghai. Priorities and

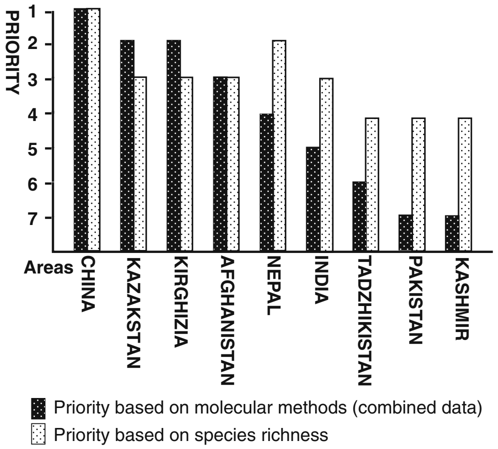

Fig. 5 Comparison of priorities among countries based on molecular method and species richness

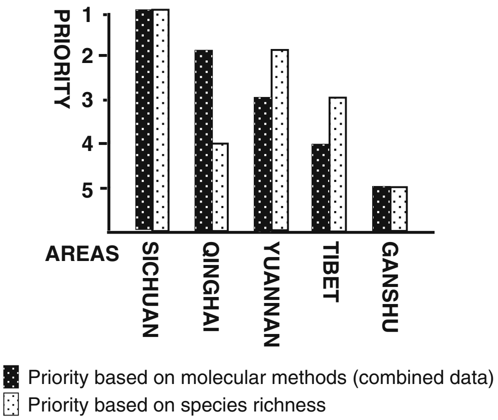

Fig. 6 Comparison of priorities among provinces of China based on molecular method and species richness

results of complementarity analysis are shown in Table 3 and in Figs. 5 and 6.

Discussion

Land-Use Changes and the Conservation of *Incarvillea*

In parts of Yunnan, Sichuan, and Tibet, the interests of logging have been served, and the complete disappearance of forest from some areas may have caused the extinction of two species, *I. altissima* and *I. forrestii*. Conservation of remaining shrub lands and the planting of forest are necessary conservation actions if living material of those "extinct" species is to be found. Species on subalpine and alpine meadows are threatened by increased cattle grazing, a result of a shift from subsistence farming to commercial operations. Therefore, careful land management that considers the local environment will be required in order to preserve diversity within those areas.

In Yunnan province, China, *Incarvillea arguta*, *I. sinensis*, *Incarvillea zhongdianensis*, *Incarvillea mairei*, *Incarvillea lutea*, and *Incarvillea delavayi* are all preserved at one or more of three protected sites: the Alpine Botanical Garden areas of Lijian, Dali, and Zhongdian. However, the other Chinese species, *I. beresowskii*, *Incarvillea compacta*, *Incarvillea younghusbandii*, and *Incarvillea dissectifoliola*, are more problematic because they separately occur only in Sichuan, Qinghai, or Tibet where there are no protected sites. Because their habitats are fragmented, it is difficult to designate potential reserve areas that would ensure their protection.

Use of Phylogenetic Diversity as a Conservation Tool

"Biodiversity" encompasses the variety of all living forms on the planet, extending from genes to species to ecosystems. Authoritative estimates for the number of species in the world are around 10 million (May 1992; Magurran 2005). Therefore, conservation decisions must ultimately prioritize the use of limited resources for preserving taxa based on biodiversity assessments. Objective criteria when selecting the sites and populations necessary to protect individually selected species are relatively well developed, but broad-scale solutions clearly are needed when the focus is on millions of species. Ecologists typically regard species richness, i.e., the number of species on sites being considered for preservation, as the currency of conservation (Justus and Sarkar 2002). However, the consideration of species numbers alone may be insufficient because of such factors as general imperfect taxonomic knowledge and variations in the level of this knowledge from one group to another.

It has been suggested that the phylogenetic distinctiveness of a species also be taken into account (Wilson 1992; Crozier 1997a; Mace et al. 2003) when biodiversity is defined as the information content within the world's genomes. Most general frameworks for objective assessments of conservation worthiness also include evaluating evolutionary distinctiveness such as GD (Crozier 1992), PD (Faith 1992), and evolutionary history (Nee and May 1997), for example, the biodiversity assessment of the East African Great Lakes shows that relying on species richness alone would place the most value on the group that appears to have evolved over a very short evolutionary time (Meyer 1993). By comparison, it would be intuitively more correct to apply a system that weights species by their distinctiveness.

In our study, we assessed the biodiversity of the genus *Incarvillea* using criteria for both species richness and phylogenetic distinctiveness. Phylogenetic analysis, together with complementarity analysis (Table 3), serves the following purposes here. First, it highlights the importance of two basal and distinctive species, *I. semiretschenskia* and *I. olgae* from Central Asia, about which little is known. Further conservation data must be collected for these species, particularly from Kazakstan and Kirghizia. Second, it focuses on the importance of Sichuan and Qinghai, where there are few protected areas compared with Yunnan, a province protection is good.

Our study supplies the basal data for conservation strategy of the genus *Incarvillea*. It also demonstrates that phylogenetic weighting should not be the only criterion for setting conservation priorities, an approach that has been useful in generating new conservation perspectives and highlighting additional priorities. In the conservation of biodiversity, both the traditional method based on species richness and the molecular method that utilizes phylogenetic distinctiveness should be taken into consideration when making objective decisions for preserving taxa.

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