Lespedeza danxiaensis (Fabaceae), a new species from Guangdong, China, based on molecular and morphological data

Wan-Yi Zhao¹, Kai-Wen Jiang²³*, Zai-Xiong Chen⁴, Bin Tian², Qiang Fan¹

¹ State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China ² Key Laboratory of Biodiversity Conservation in Southwest China, National Forestry and Grassland Administration, Southwest Forestry University, Kunming 650224, China ³ Ningbo Botanical Garden, Ningbo 315201, China ⁴ Administrative Commission of Danxiashan National Park, Shaoguan 512300, China

Corresponding author: Qiang Fan (fanqiang@mail.sysu.edu.cn)

Abstract
Lespedeza danxiaensis (Fabaceae), a new species from Danxiashan National Nature Reserve in Guangdong Province, is described and illustrated. The new species is morphologically similar to Lespedeza pilosa, but it can be easily distinguished by its thin leathery leaflets and long peduncles. Phylogenetic analysis based on ITS confirmed that the new species belongs to Lespedeza subg. Macrolespedeza. The new species is the first known species of Lespedeza endemic to Danxia landform and is currently only known from Mount Danxia, Guangdong.

Keywords
Danxia landform, Guangdong, Leguminosae, new species, taxonomy

* The authors contributed equally to this work.
Introduction

Lespedeza Michx. (Fabaceae) is a member of the subtribe Lespedezinae (Hutch.) Schub. of the tribe Desmodieae (Benth.) Hutch. The genus is characterised by shrubs, sub-shrubs or perennial herbs with tri-foliolate leaves (Huang et al. 2010; Ohashi and Nemoto 2014). Lespedeza has a disjunct distribution being present in both East Asia and North America, and consists of 46 species including the recently described L. pseudomaximowiczii D. P. Jin, Bo Xu bis & B. H. Choi and L. hengduanshanensis (C.J. Chen) Bo Xu bis, X.F. Gao & Li Bing Zhang (Ohashi and Nemoto 2014; Xu et al. 2014; Jin et al. 2018). The genus is traditionally divided into two subgenera, viz. Lespedeza subg. Lespedeza and L. subg. Macrolespedeza (Maxim.) H. Ohashi, based on the presence or absence of cleistogamous flowers (Ohashi 1982; Huang et al. 2010; Xu et al. 2012). Molecular phylogenetic studies, using nrITS and five chloroplast fragments (rpl16, rpl32-trnL, rps16-trnQ, trnL-F and trnK/marK), showed that subg. Lespedeza is paraphyletic since the North America taxa (belonging to L. subg. Lespedeza) are sister to East Asia taxa that included members of both subgenera (Xu et al. 2012). Based on these results, Ohashi and Nemoto (2014) re-circumscribed both subgenera and confined L. subg. Lespedeza to North America, while L. subg. Macrolespedeza was confined to Asia.

During a botanical expedition to Danxiashan National Nature Reserve, Renhua County, Guangdong Province from May to October, 2020, we discovered an unknown species of Lespedeza. It is similar to L. pilosa (Thunb.) Siebold & Zucc. in indumentum (densely villous throughout), procumbent stems and ovate to obovate leaflets, but differs from the latter by its leathery leaflets, pinkish corolla and longer peduncles of chasmogamous flowers. After carefully checking specimens and literature, together with a molecular phylogenetic analysis based on Internal Transcribed Spacers (ITS), we demonstrated it is indeed a new species; thus here, we describe and illustrate it.

Materials and methods

Morphological study

The morphological characters were examined, based on the living plants and specimens kept in the herbaria IBSC, NPH, SWFC and SYS, herbarium acronyms as in Thiers (2021).

Taxon sampling and molecular analyses

Three individuals of L. danxiensis were collected from Danxiashan National Park, Guangdong province, China from July to September in 2020 (Fig. 1). Voucher specimens were deposited in the Herbarium of Sun Yat-sen University (SYS). The nuclear DNA Internal Transcribed Spacers (ITS) was used for reconstructing the phylogeny of the new species and its related taxa (Xu et al. 2012). A total of 45 ac-
A new species of *Lespedeza* from China is described

cessions, representing 33 species of *Lespedeza* [including two nominal species viz. *L. nipponica* Nakai and *L. japonica* L. H. Bailey, which had been synonymised with *L. formosa* (Vogel) Koehne (Hatusima 1967) or *L. thunbergii* (DC.) Nakai (Ohashi et al. 2009)] and one species of a related genus, *Campylotropis macrocarpa* (Bunge) Rehder was sampled for outgroup comparison. The GenBank accession numbers are listed in Appendix I. Most sequences were downloaded from GenBank, except for the new species, which was newly sequenced in the present study. Three samples of the new species were sequenced and were identical, of which only one sequence (MZ468553) was selected for the phylogenetic analysis. Genomic DNA was extracted from silica-gel-dried leaves using the modified 2 × CTAB procedure of Doyle and Doyle (1987). The ITS sequences were amplified with primer pairs ITS4/ITSA, with PCR amplification and sequencing following Xu et al. (2012). The phylogenetic relationships were assessed using the Maximum Likelihood (ML) method, which was constructed using the programme IQ-TREE (Nguyen et al. 2015).

**Results**

**Molecular phylogenetics**

The aligned sequences of ITS for phylogenetic analyses are 702 bp in length. *Lespedeza* was recovered as monophyletic in the resulting phylogenetic tree in this study (LP: 100, Fig. 2). The North American *Lespedeza* taxa were clustered into a clade

---

Figure 1. Satellite image for the location of *Lespedeza danxiaensis* Q. Fan, W.Y. Zhao & K.W. Jiang.
(clade D) as sister to the Asian taxa, of which were divided into three clades (viz. clade A, B and C) (LP: 100, Fig. 2). The putative new species is deeply nested within the clade C and was strongly supported as a member of subclade C-1 consisting of L. caraganae Bunge, L. cuneata G. Don, L. hispida (Franch.) T. Nemoto & H. Ohashi, L. lichyiuniae T. Nemoto, H. Ohashi & T. Itoh and L. pilosa (Thunb.) Siebold & Zucc. (LP = 94, Fig. 2).

**Morphological comparison**

A detailed morphological comparisons of the new species with the five closely related species within subclade C-1 are summarized in Table 1. In morphology, the putative new species is most similar to L. pilosa, sharing such features as procumbent stem, ovate to obovate leaf blades, and plant covered densely villous indumentum. However, the new species differs from the latter by leathery leaflets, longer peduncles of chasmogamous flowers, and pink to pale purple corolla (Table 1, Fig. 3). The other four species included in subclade C-1 could be easily distinguishable from the new species by their habits (stem erect vs. stem procumbent), narrow leaf shape (oblong-linear to narrowly obovate leaf vs. ovate, obovate to subrounded), and shorter peduncles (0.5–1.0 mm vs. 11–28 mm) (Table 1).
A new species of *Lespedeza* from China is described

**Lespedeza danxiaensis** Q. Fan, W.Y. Zhao & K.W. Jiang, sp. nov.
urn:lsid:ipni.org:names:77222602-1

**Taxonomic treatment**

*Lespedeza danxiaensis* Q. Fan, W.Y. Zhao & K.W. Jiang, sp. nov.

**Type.** China. Guangdong: Renhua County, Danxiashan National Nature Reserve, 24°56′N, 113°45′E, 290 m a.s.l., 30 Sept 2020, *Q. Fan 18409* (holotype, SYS!; isotypes IBSC!, NPH!, SWFC!, SYS!). (Figs 3, 4)

**Diagnosis.** *L. danxiaensis* is most similar to *L. pilosa* morphologically both being densely villous throughout, and having procumbent stems with ovate to obovate leaflets, but differs from the latter by its leathery leaflets with obviously concave veins (vs. leaflets papery, veins slightly concave), pink to pale purple corolla (vs. corolla yellowish-white to white, with purple spots at base of the standard) and longer peduncles of chasmogamous flowers (1.1–2.8 cm vs. peduncles of chasmogamous flowers rather short, 0.5–1.0 mm in *L. pilosa*).

**Description.** Perennial herbs, evergreen, with densely erect or ascending villous hairs throughout, turn sparse when old. **Stems** procumbent or ascending, woody at base, 50 cm tall. **Leaves** alternate, 3-foliolate; stipules persistent, ovate-triangular to triangular-lanceolate, apex acute, 3.5–4.5 mm, with 3–5 veins, sparsely pubescent; petioles 1.4–3.8 cm, densely pubescent; rachis 0.5–1.3 cm, densely pubescent, leaflets leathery, adaxially green, pubescent with ± adpressed hairs, more dense along the margin, abaxially greyish-green, more densely pubescent with ± adpressing hairs and more dense along the veins, lateral veins 8–12 pairs, obviously concave adaxially and prominent abaxially; terminal leaflets slightly larger than lateral ones, ovate to obovate, 2.2–3.8 × 1.5–2.5 cm, obtuse at apex, apiculate, rounded at base; lateral leaflets ovate to sub-

---

**Table 1.** Morphological comparison of *Lespedeza danxiaensis* with its closest relatives.

| Characters | *L. danxiaensis* | *L. pilosa* | *L. caraganae* | *L. cuneata* | *L. hispida* | *L. lichiyuniae* |
|------------|-----------------|------------|----------------|--------------|--------------|-----------------|
| Habit      | Procumbent      | Procumbent | Erect          | Erect or ascending | Erect or ascending | Erect or ascending |
| Leaf texture | Leathery or thin leathery | Papery | Papery | Papery | Papery | Papery |
| Leaf shape  | Ovate, obovate to subrounded | Broadly obovate or obovate | Oblong-linear | Cuneate or linear-cuneate | Narrowly obtriangular or narrowly obovate |
| Adaxial surface of leaflet | Pubescent with ± adpressing hairs, more dense along the margin | White ascending-pilose | Subglabrous | Subglabrous | Glabrous | Glabrous |
| Abaxial surface of leaflet | Densely pubescent with ± adpressing hairs and more dense along the veins | White ascending-pilose | Adpressed hairy | Densely adpressed hairy | Densely adpressed or ascending pubescent | Densely appressed hairy |
| Peduncles of chasmogamous flowers (mm) | (2–) 11–28 | 0.5–1 | 0.5–1 | Short | Ca. 1 | Short |
| Flower color | Pink to pale purple | Yellowish white or white | White or yellow | Yellowish or white | White | Pink or pale purple |
Figure 3. *Lespedeza danxiaensis* Q. Fan, W.Y. Zhao & K.W. Jiang and *L. pilosa* (Thunb.) Siebold & Zucc. *L. danxiaensis* (A–J) A habit, bushwood on the mountaintop of Danxia landform B plant, stems procumbent C adaxial view of leaf, surface green, leather D abaxial view of one leaflet, surface greyish-green with densely pubescent E flowering branchlet with flower bud, stipule triangular-lanceolate, apex acute F front view of flower G lateral view of flower, bracteoles long ovate, sepals narrowly lanceolate H fruiting branchlet, show the long peduncles I fruit, densely pubescent, stamens persistent J flowering branchlet, peduncles usually longer than 1 cm, flower pink to pale purple, young branch reddish brown *L. pilosa* (K–L) K branchlet with unripe fruit, leaf papery L flowering branchlet, peduncles short, flower white, young branch green. (Photographs: A-J by Qiang Fan K–L by Kai-Wen Jiang).
A new species of *Lespedeza* from China is described

A new species of *Lespedeza* from China is described (with rachis 2–5 mm long; terminal leaflets obovate, 1.2–1.8 × 0.8–1.7 cm, apex obtuse or emarginate, broadly cuneate at base, lateral ones rounded to obovate, 0.9–1.5 × 0.7–1.2 cm). **Inflorescence** a pseudoraceme, 1–2 axillary, with 2–4 flowers per inflorescence, 2-flowered per node; peduncles of chasmogamous flowers slender and pubescent, (0.2–)1.1–2.8 cm, those of cleistogamous flowers reduced to 1–4 mm, on upper part of stems sometimes reduced; bracts 2 per node, narrowly ovate-triangular to broadly triangular, acute at apex, 1.5–3.3 mm, sparsely pubescent adaxially, glabrous abaxially, 3–5-veined. **Pedicel** 0.5–2.0 mm, pubescent; bracteoles 2, adnate to the base of the calyx, shorter than the calyx tube, oblong-ovate to ovate-lanceolate, 3.5–5.5 mm, sparsely pubescent, 5(–7)-veined. **Calyx** deeply 5-lobed almost to the base, densely pubescent adaxially, glabrous abaxially; tube ca. 1 mm; lobes lanceolate, sub-equal, 7–8 × ca. 1 mm, acute at apex. **Corolla** exserted (absent in cleistogamous flowers), pink to pale purple, with dark purple spots at base, longer than wings and keels, inflexed-auriculate at base, lamina 7.5–8.0 × 6.5–7.0 mm, broadly elliptic to sub-orbicular, apex obtuse or emarginate, attenuate to a claw ca. 1 mm long at base; wings pale purplish-white, slightly shorter than keels, 7.5–8.3 mm with lamina 5.5–6.0 × 2.3–2.6 mm, narrowly ovate, obtuse at apex, slightly auriculate at base, with a basal claw ca. 2.5 mm; keel petals white to pale purplish-white, 7.5–8.5 mm with lamina 5.5–6.0 × 2.8–3.0 mm, obovate to elliptic, obtuse at apex, attenuate to a claw ca. 2.5 mm at base. **Stamens** glabrous, (9+1) diadelphous, ca. 9 mm, curved upwards in distal part; staminal tubes ca. 5 mm; anthers uniform, ovate, ca. 0.5 mm. **Pistils** ca. 10 mm, longer than stamens (shorter than stamens in cleistogamous flowers); ovary narrowly elliptic, shortly stipitate, style filiform, curved upwards in distal part, ascending-pubescent; stigma terminal, capitate. **Pods** brownish, 1-seeded, elliptic, style persistent at apex, rostrate, 7–9 × ca. 3 mm, densely ascending-pubescent; those of cleistogamous flowers not seen. **Seeds** ovate, ca. 3.0 × ca. 1.4 mm.

**Phenology.** Flowering from June to October, fruiting from September to December.

**Etymology.** The specific epithet refers to Mount Danxia, the locality of the type collection. The Chinese name of the new species is here given as 丹霞铁马鞭 (Dān xiá tiě mǎ biān), in which “丹霞” is the Chinese name for Mount Danxia, as well as “铁马鞭” being the common name for *Lespedeza pilosa* and its allies.

**Distribution, ecology and habitat.** *Lespedeza danxiaensis* is currently known only from a few populations on Mount Danxia in Renhua County, Guangdong Province of China. It was observed to occur in bushwood on the mountaintop of Danxia landform at elevations between 270 and 310 m; plants in association included *Osteomeles subrotunda* K. Koch, *Abelia chinensis* R. Br., *Lagerstroemia indica* L., *Selaginella tamariscina* (P. Beauv.) Spring etc.

**Conservation status.** The known localities of *Lespedeza danxiaensis* are in Danxiashan National Nature Reserve where they are well protected. However, its population size is quite small. There are fewer than 100 individuals surviving in an area of about 200 m² in the currently known localities. We carried out several field surveys in 2020...
Figure 4. *Lespedeza danxiaensis* Q. Fan, W.Y. Zhao & K.W. Jiang A upper portion of plant B lower portion of plant C chasmogamous flower D cleistogamous flower E staminal tube F pistil G standard H wing-petal I keel-petal J chasmogamous fruit K abaxial view of calyx, flattened L bracts M bracteoles. (Drawn by Rong-En Wu).
A new species of Lespedeza from China is described from May to October, but no other populations were found. Due to its limited distributional range and small population size, Lespedeza danxiaensis is here recommended as Critically Endangered (CR, B2a) according to IUCN Categories (IUCN Standards and Petitions Subcommittee 2019).

**Additional specimens examined (paratypes).** China. Guangdong: Renhua County, Danxiashan National Nature Reserve, 24˚56’N, 113˚45’E, 290 m a.s.l., 3 July 2020, Q. Fan 18027 (IBSC, NPH, SWFC, SYS); ibid., 14 August 2020, Q. Fan & Y. S. Huang 18130 (IBSC, NPH, SYS).

**Discussion**

It is obvious that the new species belongs to Lespedeza due to its persistent bracts with two flowers inside, non-articulate pedicels, and 1-seeded pods (Fig. 3). Our molecular phylogenetic results further support the inclusion of the new species within Lespedeza subg. Macrolespedeza re-circumscribed by Ohashi and Nemoto (2014) (Fig. 2). The most conspicuous character of L. danxiaensis is its procumbent stems. There are only three procumbent Lespedeza species formerly recorded in China, i.e., L. fasciculiflora, L. hengduanshanensis, and L. pilosa. However, the former two species, occurring in western China (northwestern Yunnan, western Sichuan and Tibet) (Huang et al. 2010; Xu et al. 2014), are distantly related to the new species in the phylogenetic tree (Fig. 2). The third species L. pilosa is close to the new species, but they differ in the leaf texture, flower color, and the peduncle length of the chasmogamous flowers as described above. In addition, the ITS sequences of the three individuals of the new species are identical and no heterozygous sites were detected in these sequences, indicating that L. danxiaensis is not of hybrid origin, but a distinct species.

Lespedeza danxiaensis is current only known from the type locality, i.e. Mount Danxia, and only one population with fewer than 100 individuals was found by the authors. They grow in the special habitat of the Danxia landform, confined to the sub-top area of a peak. The special habitat may lead the phenomenon in which the number of this species is extremely small, thus the conservation of the species, including ex situ and in situ conservation, is urgently needed. Lespedeza danxiaensis has a procumbent habit, usually growing in patches on the ground, and is drought-tolerant. Our observations found that the above-ground part of the species survives drought by dropping many leaves during the dry season. Thus, this species may be suitable as a slope protection or soil-and-water conservation plant, which has potential development and application value.

**Acknowledgements**

We are deeply grateful to Mrs Rong-En Wu for her excellent illustrations in the manuscript. We thank the curators and staff of herbaria IBSC, NPH, SWFC and SYS for accepting and storing the type specimens. This study was supported by Guangdong Provincial Construc-
tion Project of Agricultural Science and Technology Innovation and Extension System (2020KJ264), Guangdong Special Fund for Natural Resources Management and Ecological Forestry Construction (2021GJGY001), the Foundation of Administrative Committee of Danxiashan National Nature Park (2016-0293) and the project of the Fourth Survey of Chinese Traditional Medicine Resources (2019-302-001 and 2019-303-001).

References

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Hatusima S (1967) Lespedeza: Sects. Macrolespedeza and Heterolespedeza from Japan, Corea and Formosa. Memoirs of the Faculty of Agriculture Kagoshima University 6: 1–17.

Huang P, Ohashi H, Nemoto T (2010) Lespedeza Michaux. In: Wu CY, Raven PH, Hong DY (Eds) Flora of China. Vol. 10. Science Press, Beijing & Missouri Botanical Garden Press, St. Louis, 302–311. http://www.iplant.cn/info/Lespedeza?i=foc

IUCN Standards and Petitions Subcommittee (2019) Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Subcommittee. https://www.iucnredlist.org/resources/redlistguidelines

Jin DP, Xu B, Chio BH (2018) Taxonomic reconsideration of Chinese Lespedeza maximowiczii (Fabaceae) based on morphological and genetic features, and recommendation as the independent species L. pseudomaximowiczii. Korean Journal of Plant Taxonomy 48(3): 153–162. https://doi.org/10.11110/kjpt.2018.48.3.153

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Ohashi H (1982) Nomenclatural changes in Leguminosae of Japan. Shokubutsu Kenkyu Zasshi 57(1): 29–30.

Ohashi H, Nemoto T (2014) A new system of Lespedeza (Leguminosae Tribe Desmodieae). Shokubutsu Kenkyu Zasshi 89(1): 1–11.

Ohashi H, Nemoto T, Ohashi K (2009) A revision of Lespedeza subgenus Macrolespedeza (Leguminosae) in China. Shokubutsu Kenkyu Zasshi 84(4): 197–223.

Thiers B (2021) [continuously updated] Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden’s Virtual Herbarium. http://sweetgum.nybg.org/science/ih/ [accessed 30.05.2021]

Xu B, Wu N, Gao XF, Zhang LB (2012) Analysis of DNA sequences of six chloroplast and nuclear genes suggests incongruence, introgression, and incomplete lineage sorting in the evolution of Lespedeza (Fabaceae). Molecular Phylogenetics and Evolution 62(1): 346–358. https://doi.org/10.1016/j.ympev.2011.10.007

Xu B, Gao XF, Zhang LB (2014) Lespedeza hengduanshanensis comb. & stat. nov. (Leguminosae: Papilionoideae: Desmodieae) from the Hengduan Mountains of SW China. Phytotaxa 202(2): 165–168. https://doi.org/10.11646/phytotaxa.202.2.10
A new species of *Lespedeza* from China is described

### Appendix I

**Table A1.** List of the GenBank accession numbers of the ITS sequences of sampled species in this study.

| Species                          | GenBank Accession Number |
|----------------------------------|--------------------------|
| *Campylotropis macrocarpa* (Bunge) Rehder | JN402492                |
| *Lespedeza bivolor* Turcz.        | JN402403                |
| *Lespedeza buergeri* Miq.         | JN402407                |
| *Lespedeza caraganae* Bunge       | JN402410                |
| *Lespedeza chinensis* G. Don      | JN402415                |
| *Lespedeza cuneata* G. Don        | JN402418                |
| *Lespedeza cyriformis* Miq.       | JN402422                |
| *Lespedeza danxianensis* Q. Fan, W. Y. Zhao & K. W. Jiang | MZ468553 MZ468554 MZ468555 |
| *Lespedeza davidi* Franch.        | JN402428                |
| *Lespedeza davurica* (Laxm.) Schindl. | JN402425               |
| *Lespedeza dunnii* Schindl.       | JN402431                |
| *Lespedeza fasciculiflora* Franch. | JN402452                |
| *Lespedeza floribunda* Bunge      | GU572179                |
| *Lespedeza fordii* Schindl.       | JN402440                |
| *Lespedeza formosa* (Vogel) Koehne | GU572180                |
| *Lespedeza forrestii* Schindl.    | JN402448                |
| *Lespedeza frutescens* (L.) Hornem. | JN402454               |
| *Lespedeza hengduanshanensis* (C.J. Chen) Bo Xu bis, X.F. Gao & Li Bing Zhang | KY174667               |
| *Lespedeza hirta* (L.) Hornem.    | JN402449                |
| *Lespedeza hirta* (Franch.) T. Nemoto & H. Ohashi | JN402450               |
| *Lespedeza homoleoba* Nakai       | JN402451                |
| *Lespedeza inschanica* Schindl.   | JN402452                |
| *Lespedeza japonica* L.H. Bailey  | GU572186                |
| *Lespedeza juncoa* (L. f.) Pers.  | JN402457                |
| *Lespedeza lichiyuniae* T. Nemoto, H. Ohashi & T. Itoh | KY174750               |
| *Lespedeza maritima* Nakai        | GU572190                |
| *Lespedeza melanantha* Nakai      | KY174778                |
| *Lespedeza nipponica* Nakai       | GU572193                |
| *Lespedeza patens* Nakai          | KY174785                |
| *Lespedeza pilosi* Siebold & Zucc. | KY174795               |
| *Lespedeza potaninii* V.N, Vassil. | KY174804               |
| *Lespedeza repens* W.P.C. Barton  | JN402473                |
| *Lespedeza rupestris* Nutt.       | JN402474                |
| *Lespedeza thunbergii* (DC.) Nakai | GU572186               |
| *Lespedeza tomentosa* Siebold ex Maxim. | JN402476               |
| *Lespedeza virginica* DC.         | JN402481                |
| *Lespedeza virginica* (L.) Britton | JN402483                |