



## New record of an alien plant, *Desmodium paniculatum* (Fabaceae), in Korea based on a morphological examination and DNA barcoding

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**ABSTRACT:** *Desmodium paniculatum* (Fabaceae), native to North America and an alien plant in Japan, was newly found in Hwaseong-si and Daejeon-si, Korea. This species is distinguished from Korean-related taxa based on certain morphological features. Specifically, it is a perennial herb, with a 4–5 jointed loment, and with the basal suture moderately incised between the articles. In the field, *D. paniculatum* grows around rivers and roadsides, suggesting that it is likely to have been introduced through river improvement work and road construction. On the phylogenetic tree based on an internal transcribed spacer, Korean individuals forms a clade with a Japanese individual. Here, we report a morphological description, photographs, an illustration, and the locality of *D. paniculatum*.

**Keywords:** Alien plant, *Desmodium paniculatum*, Fabaceae, new record, plant resource

Genus *Desmodium* Desv. (family Fabaceae, tribe Desmodieae) consists of ca. 275 species, and this genus is widely distributed from tropical to temperate forests around the world. Southeast Asia, Mexico, and South America are considered as the centers of species diversity in this case (Ohashi, 2005). Members of Desmodieae, including *Desmodium*, have been used in traditional medicine and for foraging materials (Jabbour et al., 2018; Li et al., 2019). This genus was traditionally distinguished from related genera by its morphological features, such as its 3-foliolate leaves, non-winged petiole, and distinctly jointed and usually indehiscent fruits (Ohashi et al., 1981). However, molecular phylogenies have constantly indicated polyphyly of *Desmodium* (Kajita et al., 1996; Jabbour et al., 2018; Ohashi et al., 2018). Given these findings, *Desmodium* has been classified into satellite genera, specifically *Grona* Lour. (Ohashi and Ohashi, 2018), *Hylodesmum* H. Ohashi & R. R. Mill (Ohashi and Mill, 2000), and *Ohwia* H. Ohashi (Ohashi, 1999). In Korea, seven taxa of *Desmodium* are recognized (Choi, 2007), whereas six taxa apart from *D. heterocarpon* have recently been considered as members of *Hylodesmum* or *Ohwia* (Lee et al., 2012). In addition to these seven Korean taxa, *H.*

*laxum* (Candolle) H. Ohashi & R. R. Mill subsp. *laxum* was additionally recorded from forest lands of Jeju Island (Lee et al., 2012). In the case of *D. heterocarpon*, the taxonomic position was changed to the genus *Grona*, a resurrected name, according to molecular phylogeny studies (Ohashi and Ohashi, 2018).

*Desmodium paniculatum* (L.) DC. is a perennial herb that is widely distributed in North America (GBIF Secretariat, 2019). This species is known to be difficult to distinguish from related taxa, such as *D. perplexum* B. G. Schub. and *D. glabellum* (Michx.) DC., due to considerable morphological variation (Isely, 1983). Moreover, many hybrids related to *D. paniculatum* have been suggested (Isely, 1998). In this regard, Ohashi (2013) decided to regard species with wide concepts as a group until more evidence is found. There is difficulty in circumscribing the boundary of *D. paniculatum*, but this species has been reported in various provinces of Japan as a naturalized plant (Shimizu, 2003; Mito and Uesugi, 2004). Recently, this species was found in Korea, and this has never been reported. In the present study, we report the morphological characteristics and distribution of *D. paniculatum* in Korea. Also, we provide a

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key to identify it considering related species. This species is differentiated from related taxa through a phylogenetic analysis.

## Material and Methods

### Morphological analysis

The morphological observations of *D. paniculatum* were conducted on living plants in the field and dried specimens in the herbarium of the National Institute of Biological Resources in Korea (KB). In addition, we examined additional specimens deposited in the herbarium of Inha University (IUI). A Nikon D750 (Nikon, Tokyo, Japan) camera was used to take photographs in the field survey. The measurements of the morphological characters were performed with a digital Vernier caliper and with data derived from field notes.

### DNA extraction and PCR

Total genomic DNA samples were extracted from the leaves of two dried specimen (Voucher Nos. KIMJH20121 and KIMJH20122) with a MG Plant Genomic DNA Extraction SV Miniprep kit (MGmed, Seoul, Korea) according to the manufacturer's instruction. We used PCR amplification for the internal transcribed spacer (ITS) of nuclear ribosomal DNA, and we chose primers designed in previous studies (White et al., 1990; Jin et al., 2019). We conducted PCR with a GeneAmp PCR System 2700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The PCR reaction mixture included the MG 2× Taq MasterMix with a dye (MGmed), 10 ng of DNA, 0.3 μM primers, and distilled water. Conditions included initial denaturation at 94°C for 3 min followed by 30 cycles each at 94°C for 30 s, 53°C for 45 s, and 72°C for 1 min. The total volume was 50 μL. The PCR products were visualized on 2% agarose gels, which were then processed with a MG PCR purification kit (MGmed). Sequencing was conducted with MACROGEN (Macrogen Inc., Seoul, Korea), and two sequences of Korean *D. paniculatum* were identical. The sequence identified in this study was deposited into the GenBank database (MW740337).

### Phylogenetic analysis

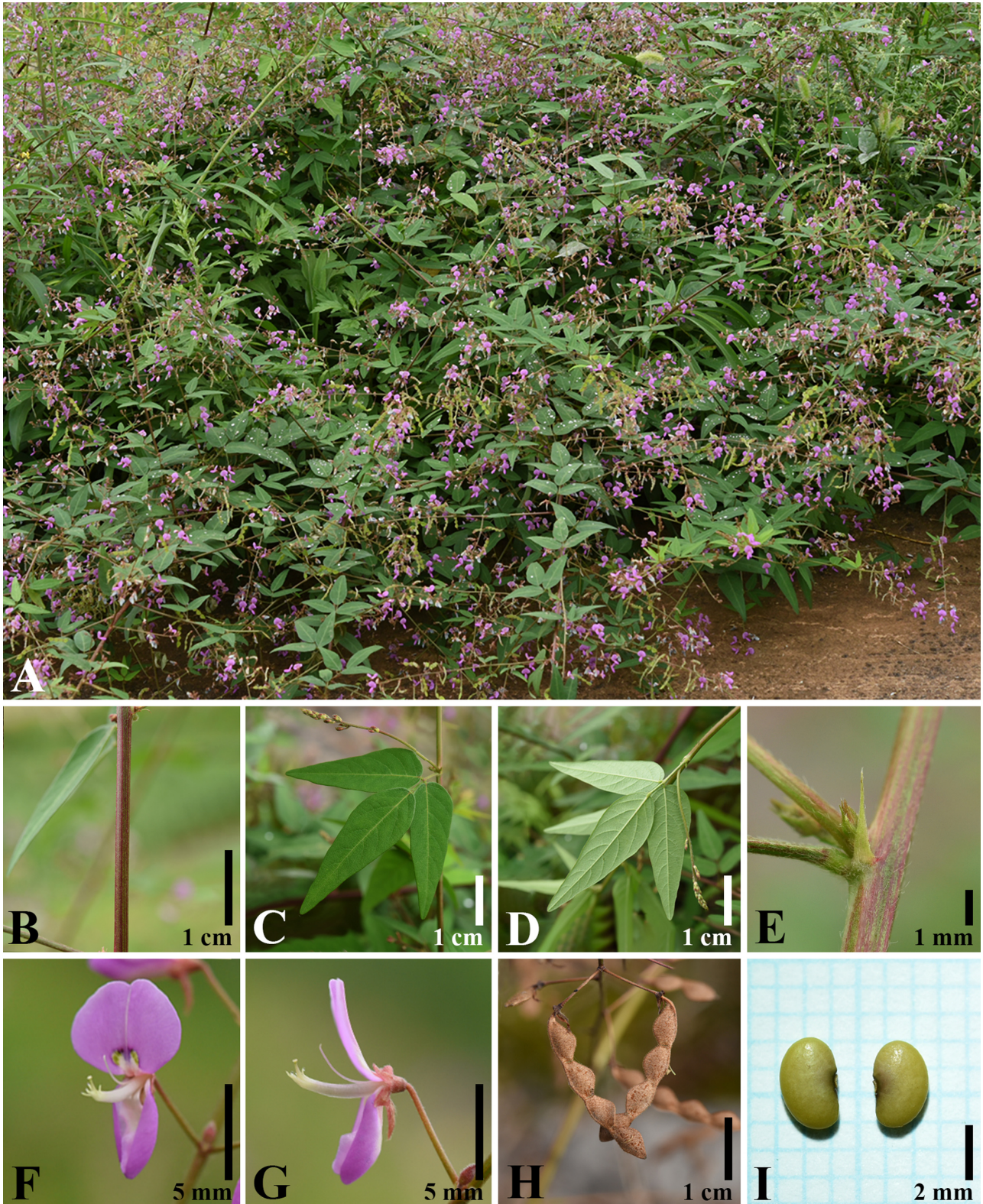
To identify Korean *D. paniculatum*, a neighbor-joining (NJ) tree of tribe Desmodieae was constructed. In total, 33 taxa within the tribe were included considering earlier work on the DNA barcoding of Korean Desmodieae (Jin et al., 2019) and North American *Desmodium* species. Specifically, multiple sequences per taxon were accessed for *D. paniculatum* and taxa belonging to the same subtribe Desmodiinae. Two species [*Acacia ligulata* Aiton ex Steud. and *Glycine max* (L.) Merr.]

were added as outgroups. All sequences were aligned with MAFFT (Kato and Standley, 2013). To assess the confidence of the phylogenetic relationships, a bootstrap test was conducted with 1,000 resamplings for the NJ analysis. Kimura's two-parameter model (Kimura, 1980) was selected as the substitution model.

## Results and Discussion

In the present study, we observed *D. paniculatum* in the field (Hwaseong-si and Daejeon-si), after which we determined this species as an alien plant in Korea. These habitats are located around rivers and roadsides, and we considered that *D. paniculatum* was therefore introduced into Korea through river improvement work and/or road construction. In terms of morphology, this species harbors a non-winged petiole, a subsessile and 4–5 jointed loment, and a moderately incised basal suture of the fruit (Figs. 1, 2), which are distinguished from related genera *Ohwia* and *Hylodesmum* (Choi, 2007). Within Korean *Desmodium*, the erect and ascending stem of *D. paniculatum* is distinguishable from the procumbent and ascending stem of *D. heterocarpon* (Lee, 1980). Regarding the terminal leaflets, the lanceolate to ovate shape of *D. paniculatum* is different from the obovate or elliptic leaflets of *D. heterocarpon* (Figs. 1, 2) (Lee, 1980).

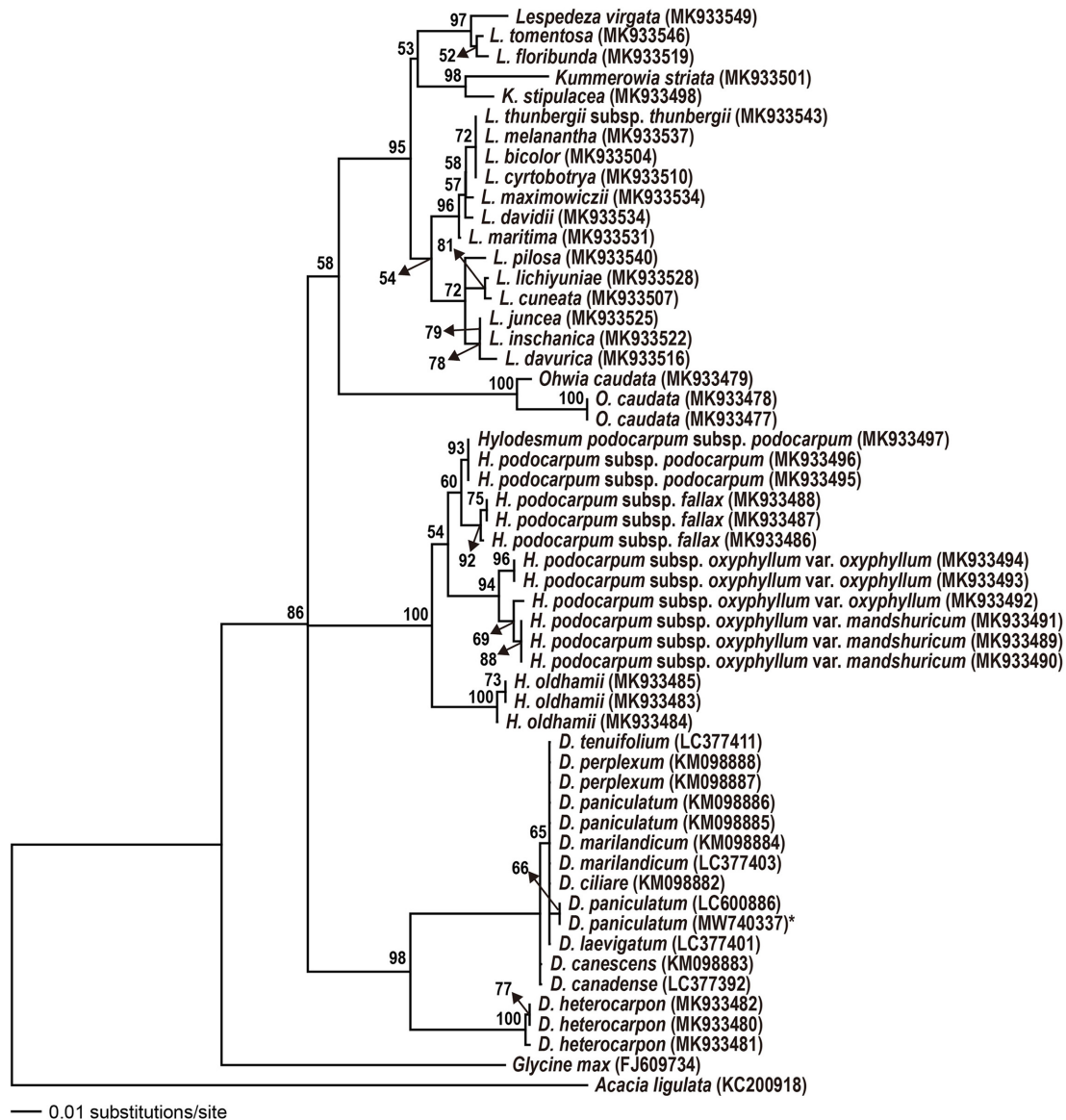
On the NJ tree based on ITS (Fig. 3), *Desmodium* taxa were constructed as monophyletic, and Korean *D. paniculatum* was classified as a clade independent from other members of Korean Desmodieae. It appears to be possible to identify *D. paniculatum* from other Desmodieae in Korea. However, it is difficult to identify *D. paniculatum* from other North American taxa. This may be related to the complex morphological variation within *D. paniculatum* due to recent speciation or putative hybridization (Isely, 1983, 1998; Ohashi, 2013). Short sequences of ITS (total aligned 374 bp) also may only provide insufficient information with regard to distinguishing the relationships among *D. paniculatum* and other species. The analysis of a combined DNA barcode regions (ITS + *rbcL* + *matK*) for Korean Desmodieae showed a higher resolution than that of only one DNA barcode (Jin et al., 2019). Unfortunately, employing a combined sequence in this study was limited due to the absence of an available sequence acquired from one individual. Instead, we found that Korean *D. paniculatum* (MW740337) formed a clade with Japanese *D. paniculatum* (LC600886) (bootstrap value = 66%); accordingly, we suggest the hypothesis that Korean individuals were introduced from Japan or from the same locality at which Japanese individuals were originally introduced.



**Fig. 1.** Photographs of *Dsemodium paniculatum* (L.) DC. **A.** Habit. **B.** Stem. **C.** Leaf (adaxial surface). **D.** Leaf (abaxial surface). **E.** Stipule. **F.** Flower (frontal view). **G.** Flower (lateral view). **H.** Fruit. **I.** Seed.



**Fig. 2.** Illustrations of *Desmodium paniculatum* (L.) DC. **A.** Habit. **B.** Flower (frontal view). **C.** Flower (lateral view). **D.** Standard petal. **E.** Wing petal. **F.** Keel petal. **G.** Pistil. **H.** Stamens (diadelphous). **I.** Stipule. **J.** Fruit. **K.** Seed.



**Fig. 3.** The Neighbor-Joining phylogenetic tree of tribe Desmodieae based on internal transcribed spacer. Asterisk indicates newly sequenced individuals. GenBank accession number is shown behind scientific name. The number from node indicate bootstrap value (>50%).

Consequently, we provide a description of *D. paniculatum* and an identification key of this species and members of related genera in Korea. Regarding the Korean name of this species, we have referenced the National Species list of Korea (National Institute of Biological Resources, 2019).

### Taxonomic Treatment

*Desmodium paniculatum* (L.) DC., Prodr. 2: 329, 1825.—  
TYPE: USA. Virginia, Clayton 184 (lectotype, BM, photo!)  
(Figs. 1, 2).

*Hedysarum paniculatum* L. Sp. Pl. 2: 749, 1753; *Meibomia paniculata* (L.) Kuntze, Revis. Gen. Pl. 1: 198, 1891.

*H. glabellum* Michx., Fl. Bor. Am. 2: 73, 1803; *Desmodium glabellum* (Michx.) DC., Prodr. 2: 329, 1825; *M. glabella* (Michx.) Kuntze, Revis. Gen. Pl. 1: 198, 1891.

*D. dillenii* Darl., Fl. Cestr. ed. 2: 414–415, 1837; *D. paniculatum* var. *dillenii* (Darl.) Isely, Am. Midl. Nat. 49: 927, 1953.

*D. perplexum* B. G. Schub., Rhodora 52: 154, 1950.

**Korean name:** Mi-guk-jan-di-gal-go-ri (미국잔디갈고리).

Perennial herb, 50–150 cm in height. Stem erect or ascending, often branched, terete, striated, pubescent with short hairs and long appressed hairs; stipule narrowly triangular, apex

acuminate, semipersistent, 2–5 mm long. Leaves trifoliate, petiolate, 7–11 cm long at middle; leaflets lanceolate, 4–6 cm × 1.5–2.5 cm wide, apex acute and spined-tip, both surfaces pubescent with short and long appressed hairs. Inflorescence raceme, terminal or axil. Flowers chasmogamous, pink or reddish purple, 6–7 mm long; pedicels densely pubescent with slightly hooked and straight hair. Calyx campanulate, green to brown, ca. 2 mm long, 4-lobed, abaxial surface densely pubescent with short hairs and long appressed hairs; lobes longest at lower lobe, slightly clefted at upper lobe. Standard petals obovate, pink or reddish purple with green dotted inside, apex emarginate, base not clawed; wing petals narrowly obovate, apex curved, base auriculate and shortly clawed; keel petal narrowly obovate, paler than standard and wing, base shortly clawed. Stamens diadelphous (9 + 1), one free stamen diverged basally, exposed at maturing. Pistil narrowly oblong, sparsely curved hair from base to middle. Fruit legume, narrowly oblong, densely curved hair outside, ca. 3 × 0.4 cm, basal suture incised to upper, isthmus about 1/3 as broad as segment. Seed reniform, glabrous, ca. 4 × 2 mm.

**Flowering:** August to October.

**Fruiting:** October to November.

**Distribution and habitat:** *Desmodium paniculatum* is native in North America, whereas this species is non-native in Korea and Japan. This species occurs in open, sunny places at 10–100 m a.s.l. on river and roadside. The population was composed of 30 individuals within 10 × 10 m<sup>2</sup> in size. It grows together with herbaceous plants such as *Equisetum arvense* L., *Capsella bursa-pastoris* (L.) Medik., *Draba nemorosa* L., *Chamaecrista nomane* (Siebold) H. Ohashi, *Vigna minima* (Roxb.) Ohwi & H. Ohashi, *Oenothera biennis* L., *Acalypha australis* L., *Euphorbia hypericifolia* L., *Metaplexis japonica* (Thunb.) Makino, *Mosla punctulata* (J. F. Gmel.) Nakai, *Artemisia indica* Willd., *Aster pilosus* Willd., *Bidens biternata* (Lour.) Merr. & Sherff, *Lactuca indica* L., *Cyperus microiria* Steud., *Arthraxon hispidus* (Thunb.) Makino, *Digitaria ciliaris* (Retz.) Koeler, *Miscanthus sacchariflorus* (Maxim.) Hack., *Phragmites japonica* Steud., *Setaria faberi* R. A. W. Herrm., *S. pumila* (Poir.) Roem. & Schult., and *S. viridis* (L.) P. Beauv.

**Specimens examined:** KOREA. Gyeonggi-do: Geumgok-ri, Dongtan-myeon, Hwaseong-si, 30 Sep 2010, B.-H. Choi and W.-B. Cho 99502 (IUI), 99503 (IUI), 99504 (IUI), 99505 (IUI); Yeongcheon-dong, Hwaseong-si, 15 Oct 2019, J.-H. Kim KIMJH19233 (3 sheet KB); same locality, 17 Sep 2020, J.-H. Kim KIMJH20117 (KB), KIMJH20118 (KB), KIMJH20119 (KB), KIMJH20120 (KB), KIMJH20121 (2 sheet (KB)), KIMJH20122 (3 sheet (KB)); same locality, 3 Nov 2020, J.-H. Kim KIMJH20123 (KB), KIMJH20124 (3 sheet (KB)).

Chungcheongnam-do: Mannyeon-dong, Seo-gu, Daejeon-si, 19 Sep 2019, G.-H. Nam KIMJH19228 (KB).

JAPAN. Chiba: Teganomori, Kashiwa, 25 Sep 2010, I. Tanabe INM-2-063142 (KB). Ibaraki: Toyookamachiinuma, Joso, 3 Sep 2010, E. Gokita INM-2-063138 (KB); Dochi, Moriya, 4 Sep 2010, Y. Wada INM-2-063140 (KB).

## Key to *D. paniculatum* and related taxa in Korea

1. Petioles winged; flower pale yellow or greenish white ..  
..... *Ohwia* 된장풀속
1. Petioles not winged; flower purple or reddish purple.
  2. Loment distinctly stipitate, lower suture deeply incised between segments, isthmus less than 1/5 as broad as legume ..... *Hylodesmum* 갈고리속
  2. Loment sessile or subsessile, lower suture not deeply incised between segments, isthmus more than 1/3 as broad as legume ..... *Desmodium* 잔디갈고리속
  3. Subshrub; stem prostrate; leaflet elliptic or obovate; loment sessile ..... *D. heterocarpon* 잔디갈고리
  3. Perennial herb; stem erect or ascending; leaflet narrowly ovate or lanceolate; loment subsessile ..  
..... *D. paniculatum* 미국잔디갈고리

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## Conflict of Interest

The authors declare that there are no conflicts of interest.

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## 형태와 DNA 바코드에 근거한 한국 미기록 외래식물의 보고, 미국잔디갈고리(콩과)

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**적 요:** 북아메리카 원산이며, 일본에서는 외래식물로 알려진 미국잔디갈고리(*Desmodium paniculatum*)가 국내의 화성시와 대전시에서 발견되었다. 본종은 다년생 초본이며, 협과는 4-5개의 소절과로 연결되고, 소절과 사이의 배측면이 완만하게 좁아지는 점에서 국내의 근연분류군들과 구분된다. 미국잔디갈고리는 강변과 도로변에서 자라고 있어, 하천정비나 도로 공사를 통해 유입된 것으로 추측된다. Internal transcribed spacer 계통수상에서 한국산 미국잔디갈고리는 일본산 개체와 단계통을 형성하였다. 본 연구는 미국잔디갈고리의 형태적 특징에 대한 기재와 사진, 도해, 분포 위치에 대해 보고하였다.

**주요어:** 외래식물, 미국잔디갈고리, 콩과, 미기록, 자원식물