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Microstegium glabratum (Brongn.) A. Camus (Poaceae, Andropogoneae), a New Record for Japan, China, and Taiwan, Based on Morphological and Molecular Evidence

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ABSTRACT: *Microstegium glabratum* (Brongn.) A. Camus (Poaceae, Andropogoneae) is a new record for Taiwan, Japan (SW Ryukyus), and China (Hainan). Many specimens have been collected since its first discovery in 1915, but these were misidentified as *M. ciliatum* (Trin.) A. Camus or *M. glaberrimum* (Honda) Koidz. Its existence in Taiwan is confirmed by the phylogeny of ITS/5.8S sequences and its identity is figured out after examining the related specimens.

KEY WORDS: China, ITS, Japan, *Microstegium glabratum*, new record species, Poaceae, Taiwan.

INTRODUCTION

The genus *Microstegium* Nees belongs to the tribe Andropogoneae of the family Poaceae and contains about 17 species. Its members are widely distributed in the tropical and subtropical Old World (Clayton and Renvoize, 1986; Koyama, 1987; Watson and Dallwitz, 1992; Hsu, 2000). They are creeping or rambling annual or perennial plants with broadly linear to lanceolate leaf-blades. Their inflorescences contains one to many subdigitate racemes usually with a fragile rachis and the rachis internodes are filiform to clavate or inflated. Each lower glume of sessile spikelets has a deep grove or a broadly concave median channel dorsally, and the margins sharply inflexed and usually keeled. Upper glumea are often shortly awned. Lower florets of sessile spikelets can be well-developed, reduced to a palea, or even absent. Upper lemmas of sessile spikelets are linear to cordate and usually with long awns and usually accompanied by a small palea. There are usually three stamens in a floret but rarely two or only one. Pedicelled spikelets resemble the sessile ones and occasionally are smaller and staminate. (Clayton and Renvoize, 1986).

In Taiwan, nine species of *Microstegium* have been described so far. Eight of them have been accounted for by Hsu (2000). They are *M. ciliatum* (Trin.) A. Camus, *M. dilatatum* Koidz., *M. glaberrimum* Koidz., *M. fauriei* (Hayata) Honda, *M. geniculatum* (Hayata) Honda, *M. nudum* (Trin.) A. Camus, *M. somae* (Hayata) Ohwi, and *M. vimineum* (Trin.) A. Camus. One more taxon, *Microstegium japonicum* (Miq.) Koidz., was added by Chen and Kuoh (2007), but this was later found to be a member of the reinstated genus, *Leptatherum* Nees, by Chen et al. (2009), and so were *M. nudum* and *M. somae*. Consequently, the reinstatement necessitated three new combinations, *Leptatherum boreale* (Ohwi) C.-H. Chen, C.-S. Kuoh, Veldk. (= *M. japonicum*), *L. nudum* (Trin.) C.-H. Chen, C.-S. Kuoh, Veldk. (= *M. nudum*) and *L. somae* (Hayata) C.-H. Chen, C.-S. Kuoh, Veldk. (= *M. somae*) (Chen et al., 2009).

Among these taxa, *M. ciliatum* is the most widely distributed and the most variable one in Taiwan, and *M. glaberrimum* is closely related. We can distinguish the other seven species from *M. ciliatum* and *M. glaberrimum* easily and definitely. Hsu (2000) treated *M. glaberrimum* as a distinct species and distinguished *M. glaberrimum* from *M. ciliatum* by its smaller spikelets and shorter ligules though he noted "This species may be a form of *M. ciliatum*." The type specimen of *M. glaberrimum* is deposited in the herbarium of the University of Tokyo (TI), and indeed it has smaller spikelets. However, one more unusual feature is its apparently awnless-like spikelets. In *Microstegium*, the upper lemmas usually have a long awn at the tip which stretches out from the spikelets significantly but the awns of *M. glaberrimum* are very short and enclosed by glumes. We suspected that Hsu (2000) might confuse an unidentified taxon with *M. glaberrimum* because he described a flexuous awn about 15 mm long of *M. glaberrimum* (Hsu, 2000). Thus he identified many specimens of small spikelets, short ligules, and obviously long awns as *M. glaberrimum*. Besides a thorough examination of specimens we discovered identical plants in the field.

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We thought that the abberant individuals belonged to a supplementary taxon we have never known in Taiwan.

In this study, we have conducted molecular analyses to elucidate whether the suspected supplementary taxon of *Microstegium* exists in Taiwan, and provide information on its identity.

MATERIALS AND METHODS

Plant materials for DNA analysis

A total of 27 accessions were sampled by determining their nrITS sequences. Seven were from the suspected taxon, and six of *M. ciliatum*, two of *M. vimineum*, two of *M. fauriei*, one of *M. geniculatum*, four of *L. boreale*, one of *L. nudum*, and four of *L. somae* (see Table 1 for details). It is necessary to mention that the seven accessions of the suspected supplementary taxon were misidentified as *M. ciliatum* in our previous publication (Chen et al., 2009). They were all collected in Taiwan. All vouchers were deposited in the herbaria of the Endemic Species Research Institute (TAIE) and the National Museum of Natural Science (TNM), Taiwan.

The internal transcribed spacer (ITS) region of the nuclear ribosomal cistron (18S-5.8S-26S) has been used for plant molecular systematic research at the species level (Alvarez and Wendel, 2003) with an effectiveness of species-level discrimination, and a large body of sequence data already exists in the GenBank for this region (Kress et al., 2005). In addition, it has been suggested as a potential plant barcode locus (Kress et al., 2005; Stoeckle, 2003). Therefore we chose it as the marker in our studies.

DNA extraction, PCR amplification and DNA sequencing

Leaves dried in silica gel or taken from herbarium specimens were frozen with liquid nitrogen and crushed using a mortar and pestle. The total DNA was extracted using a modified cetyltrimethyl-ammonium bromide (CTAB) extraction procedure (Murray and Thompson, 1980).

The ITS region, which includes the ITS1, 5.8S rDNA, and ITS2, was amplified by PCR with primers we designed based on the 26S rRNA gene and 18S rRNA gene of some other grasses. The sequence of forward primer, IT-11: 5'-TCG TAA CAA GGT TTC CGT AGG T-3', is located at the 3' end of 18S rRNA gene, and the reverse one, IT-8: 5'-GTA AGT TTC TTC TCC GCT-3', at the beginning sequence of 26S rRNA gene.

The protocols for the PCR were as follows: we used a 50-µl mixture containing 40 mM Tricine-KOH (pH 8.7), 15 mM KOAc, 3.5 mM $Mg(OAc)_2$, 3.75µg/ml BSA, 0.005% Tween 20, 0.005% Nonidet-P40, four dNTPs (0.2 mM each), primers (0.5 µM each), 2.5 units of Advantage 2 DNA polymerase (Clontech), 10 ng genomic DNA, and a 50-µl volume of mineral oil. The PCR mixture for amplifying the ITS region included 10% dimethyl sulfoxide (DMSO) to reduce problems related to the secondary structure and efficiency of PCR primer binding. Amplification reactions were completed in a dry-block with two-step thermal cycles (Biometra). In the first step, the mixture was incubated at 94ºC for 3 min, then it underwent 10 cycles of denaturation at 94ºC for 45 s, annealing at 58ºC for 45 s, and extension at 72ºC for 1 min. The second step was carried out by the following process: 30 cycles of denaturation at 94ºC for 45 s, annealing at 54ºC for 45 s, and extension at 72ºC for 1 min, with a final extension for 10 min at 72ºC. These PCR products were detected by agarose gel electrophoresis (1.0%, w/v in TBE), stained with 0.5 µg/ml ethidium bromide, and finally photographed under UV light exposure.

These DNAs were directly sequenced following the method of dideoxy chain-termination using an ABI377 automated sequencer with the Ready Reaction Kit (PE Biosystems, California) of the BigDye™ Terminator Cycle Sequencing. Sequencing primers were the same as those used for PCR. Each sample was sequenced two or three times to confirm the sequences. These reactions were performed as recommended by the manufacturers.

Sequence alignment and phylogenetic analysis

In addition to the 27 accessions of Microstegium/ Leptatherum, the ITS sequences of 16 other accessions of Andropogoneae were obtained from GenBank. Alignment of obtained sequences was first aided by using the program Clustal W multiple alignment in BioEdit (Hall, 1999), and adjusted manually. The aligned data matrix and tree files are available from the first author.

Phylogenetic trees were constructed using two methods, neighbour-joining (NJ) (Saito and Nei, 1987) and maximum parsimony (MP) (Swofford et al., 1996). Both NJ and MP analyses were conducted using *MEGA* version 4 (Tamura et al., 2007), and rooted by the 16 other Andropogoneae accessions. For the NJ analysis, a rooted tree was generated from the distance matrix calculated by the two-parameter method of Kimura (1980). The MP tree was obtained using the Close-Neighbour-Interchange algorithm (Nei and Kumar, 2000) with search level 3 (Felsenstein, 1985; Nei and Kumar, 2000) in which the initial trees were obtained with the random addition of sequences (10 replicates). The strict consensus parsimonious tree was then constructed. Supporting levels for nodes in both analyses were assessed with a bootstrap analysis (Felsenstein, 1985) using 1000 replicates.

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Table 1. Information of the 27 *Microstegium* samples used in this study.

Taxa	Sample number	Voucher information	Deposit herbarium	GenBank accession number
Accessions of the suspected supplementary taxon (=*Microstegium glabratum*)	S22	*Wu 17-033*	TNM	EU489066
	S28	S90933	TNM	EU489068
	S42	*Huang 62*	TNM	EU489069
	S46	S90921	TNM	EU489070
	W39	*Chen 5639*	TAIE	EU489077
	W53	*Chen 5737*	TAIE	EU489086
	W56	*Chen 5741*	TAIE	EU489087
Microstegium ciliatum	S48	*Huang & Chiu 83*	TNM	FJ170271
	W12	*Chen 5774*	TAIE	FJ170272
	W30	*Chen 6031*	TAIE	FJ170273
	W31	*Chen 6040*	TAIE	FJ170274
	W37	*Chen 5637*	TAIE	FJ170275
	W58	*Chen 5745*	TAIE	FJ170276
Microstegium fauriei	W42	*Chen 5642*	TAIE	EU489079
	W60	*Chen 5799*	TAIE	EU489088
Microstegium geniculatum	W61	*Chen 5802*	TAIE	EU489071
Microstegium vimineum	W44	*Chen 5644*	TAIE	EU489080
	W50	*Chen 5628*	TAIE	EU489083
Leptatherum boreale	W45	*Chen 5623*	TAIE	EU489081
	W46	*Chen 5624*	TAIE	EU489082
	W52	*Chen 5630*	TAIE	EU489085
	W65	*Chen 5690*	TAIE	EU489076
Leptatherum nudum	S24	*Liu 890367*	TNM	EU489067
Leptatherum somae	W32	*Chen 5631*	TAIE	EU489072
	W35	*Chen 5635*	TAIE	EU489074
	W36	*Chen 5636*	TAIE	EU489075
	W51	*Chen 5629*	TAIE	EU489084

Morphological examination

We examined all the specimens identified as *Microstegium* in Biodiversity Research Center, Academia Sinica (HAST), National Cheng-Kung University (NCKU), National Pingtung University of Science and Technology (PPI), National Taiwan University (TAI), Endemic Species Research Institute (TAIE), Forestry Research Institute (TAIF), National Museum of Natural Science (TNM). We also examined the specimens, especially the types, in Natural History Museum (BM), Australian National Herbarium (CANB), Royal Botanic Garden Edinburgh (E), Royal Botanic Gardens Kew (K), Kyoto University (KYO), Nationaal Herbarium Nederland, Leiden University branch (L), Muséum National d'Histoire Naturelle (P), University of Tokyo (TI).

RESULTS

Molecular phylogenetics

The 27 sequences produced during this study have been submitted to GenBank (see Table 1 for their accession numbers). The dataset had a total of 672 bp of aligned ITS sequence for each taxon with 197 variable characters, 140 of which were parsimony-informative (20.8% of the total ITS sequence length). The MP-analysis resulted in 54 most parsimonious trees (length = 342), with consistency index (CI) of 0.59, and retention index (RI) of 0.85, for parsimony-informative sites. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

The topologies of NJ tree and MP bootstrap consensus tree are given in Figure 1 and Figure 2, respectively. In both of the two trees, the species names and accession numbers of all the OTUs are given. The topologies of the two trees are similar. The seven accessions of the suspected supplementary taxon form a monophyletic clade (marked in Figs. 1 and 2) with strong bootstrap support (97% in NJ tree and 91% in MP trees) while the six *M. ciliatum* accessions form another separate monophyletic clade with very strong bootstrap support, too (100% in NJ tree and 99% in MP trees). Based to this result, we can confirm that the suspected unknown taxon do exist in Taiwan.

Morphological examination

After examining the specimens in the European and

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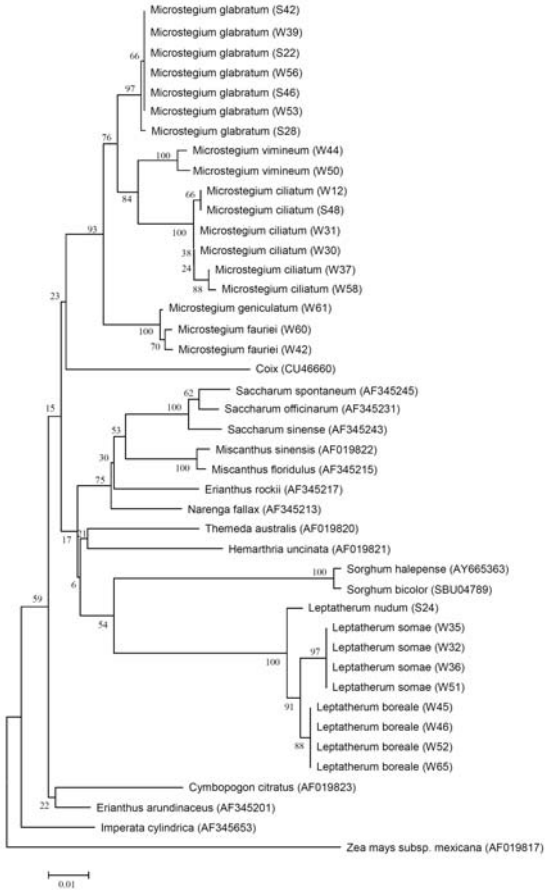


Fig. 1. Phylogenetic tree resulted from NJ analysis inferred from the ITS/5.8S sequences of *Microstegium* and some other Andropogoneae species. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

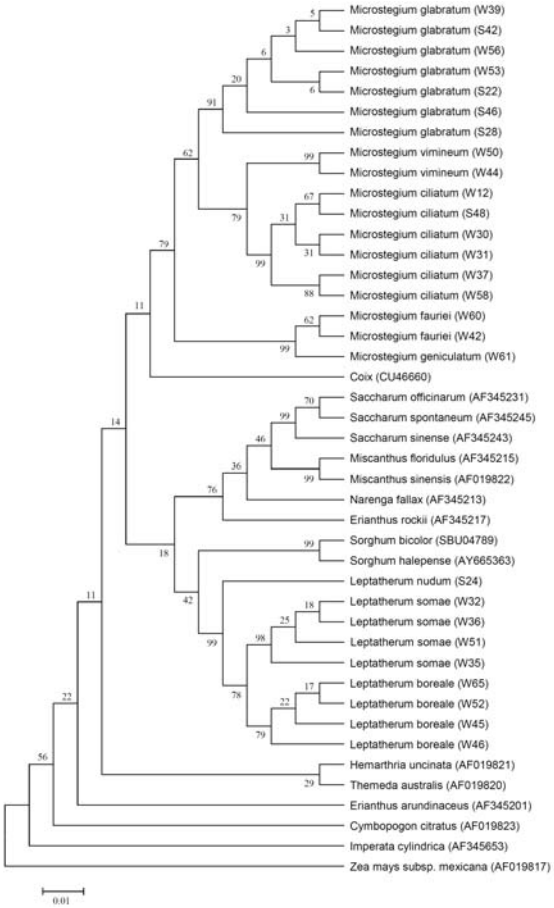


Fig. 2. Consensus of bootstrap parsimonious trees of *Microstegium* and some other Andropogoneae species using the ITS/5.8S sequences.

Japanese herbaria mentioned above, we discovered that the confirmed unknown taxon belonged to *M. glabratum* (Brongn.) A. Camus. This species differs from *M. ciliatum* or *M. glaberrimum* by the distinctly smaller spikelets, subequal sessile and pedicelled spikelets, lanceolate to elliptic lower glumes of the sessile spikelets and the raceme internodes which are at least as long as the sessile spikelets. The differences between *M. ciliatum* and *M. glabratum* are summarized as Table 2.

Besides Taiwan, the species occurs in the Philippines, Melanesia, Micronesia, Polynesia. We also found some specimens from Japan and China that belong to this species. SW Ryukyus, Japan: Yonaguni-jima (24˚ 27’ N, 123˚ E), (*Hatusima 24275*: L) and Iriomote-jima (24˚ 20’ N, 123˚ 50’ E) (*Furuse 4479*: K; *Hatusima 23061 and 24402*: L), and Hainan, China (*McClure 7834*: BM, L). Apparently these also are new country records for the name which is not mentioned by Koyama (1987) for Japan, Walker (1976) for the Southern Ryukuys, and Chen and Phillips (2006) for China, and nor its synonyms.

Table 2. Diagnostic morphological characters between *Microstegium ciliatum* (Trin.) A. Camus and *M. glabratum* (Brongn.) A. Camus.

	M. ciliatum	*M. glabratum*
Shape of lower glumes of sessile spikelets	Elliptic to oblanceolate	Lanceolate to elliptic
Shape of awn at tip of upper lemma	Straight to flexuous	Flexuous
Size of sessile and pedicelled spikelets	Pedicelled spikelets variously reduced	Subequal
Length of raceme internodes	Shorter than the 4/5 length of sessile spikelet	Subequal

DISCUSSION

After examining and comparing the specimens in the herbaria mentioned above, we discovered that the accessions of suspected supplementary taxon actually

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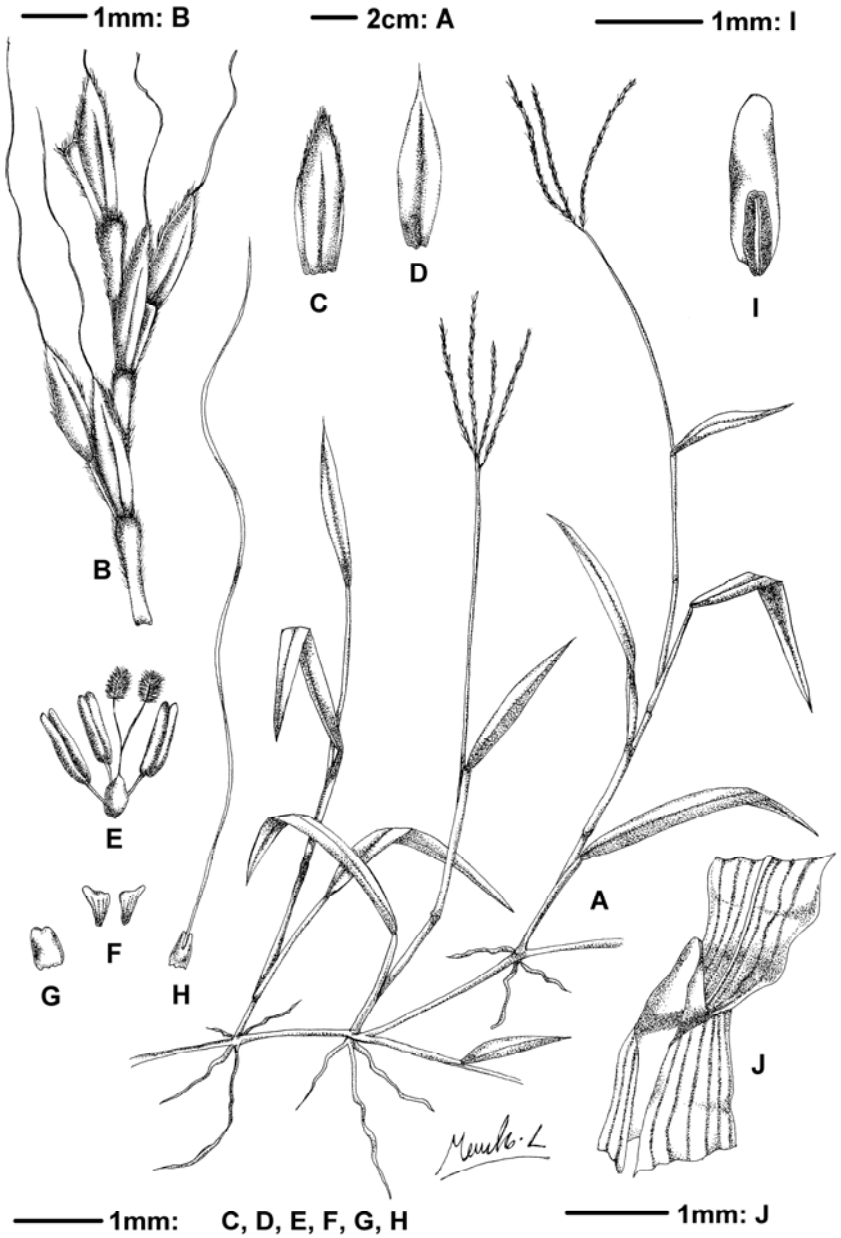


Fig. 3. *Microstegium glabratum* (Brongn.) A. Camus. A: Habit. B: Spikelets. C: Lower glume. D: Upper glume. E: Pistil and stamens. F: Lodicules. G: Upper palea. H: Upper lemma with long flexuous awn. I: Caryopsis. J: Joint of leaf sheath and leaf blade.

belong to *M. glabratum*. The ITS phylogeny confirmed its identity and showed to be distinct from other taxa. Combining the results of these morphological and molecular studies, it is apparent that *M. glabratum* occurs in Taiwan, too. This species is originally known to the Philippines, New Guinea, Melanesia, Micronesia, and Polynesia. Its distribution map now can be expanded more northward.

The earliest record of *M. glabratum* in Taiwan dates back to 1915 (*Sasaki s.n. Oct. 15, 1915*: TAI). However, taxonomists never identified this and subsequent specimens correctly. Most of them were identified as *M. glaberrimum* in herbaria of Taiwan because of Hsu's (2000) incorrect use of that name. Some others were misidentified as *M. ciliatum*.

Microstegium glabratum has never been reported for Japan or China, either. Nevertheless, during this study we encountered some specimens of *M. glabratum* from the south-western Ryukyus, Japan (Yonaguni-jima and Iriomote-jima), and Hainan, China.

Microstegium glabratum (Brongn.) A. Camus Fig. 3

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Microstegium glabratum (Brongn.) A. Camus, Ann.Soc. Linn. Lyon II, 68 (1921) 201; Hosok., J. Soc. Trop. Agric. 6 (1934) 663, isonym. – *Eulalia glabrata* Brongn. in Duperrey, Voy. Monde, Phan. (1831) 93. – *Pollinia glabrata* (Brongn.) Trin., Bull. Sci. Acad. Imp. Sci. Saint-Pétersbourg 1 (1836) 70. – *Andropogon glabratus* (Brongn.) Steud, Nomencl. Bot., ed. 2, 1 (1840) 91, *non* Roxb. [*Herb. Roxburgh in Wallich Cat. 8805-A* (in syn.; *microfiche IDC 7394)*]. – *Pogonatherum glabratum* (Brongn.) Roberty, Boissiera 9 (1960) 388. – Type: *Dumont d'Urville s.n. 'Borabora'* (P, holo).

Nemastachys taitensis Steud., Syn. Pl. Glumac. 1 (1854) 357. – *Microstegium taitense* (Steud.) A. Camus, Ann. Soc. Linn. Lyon II, 68 (1921) 201. – Type: *Dumont d'Urville s.n. "Taiti"* (P, holo).

Pollinia gracillima Hack. in A. DC., Monogr. Phan. 6 (1889) 179. – *Eulalia gracillima* (Hack.) Kuntze, Revis. Gen. Pl. 2 (1891) 775. – *Microstegium gracillima* (Hack.) A. Camus, Ann. Soc. Linn. Lyon n.s. 68 (1921) 201. – Type: *Anon.* (G, holo) sub "*Leptatherum royleani*", Taïti.

Pollinia monantha Nees var. *leptathera* Hack., Philipp. J. Sci. 3 (1908) 167. – Type: *Merrill 5212* (W, holo, not found; L, P).

Pollinia glabrata Trin. var. *luzonensis* Hack. in Kneuck., Allg. Bot. Z. Syst. 20 (1914) 3. – Type: *Kneucker Exsicc. 785 (Fénix and Ramos)* (KR, holo, lost; K, L, WAG, not found in W).

Perennials, mat-forming. Culms rambling, weak, 25-60 cm long, rooting from the lower nodes. Nodes glabrous. Sheaths glabrous, outer margin glabrous or hairy, oral hairs scanty or absent. Ligule a glabrous membrane, 0.5-0.8 mm long. Blades lanceolate, 5-16 cm by 2-10 mm, flaccid, base attenuate to slightly rounded, pseudopetiole absent, margins scaberulous, apex acute to acuminate, glabrous or sparsely puberulous on both sides, without tubercle-based hairs. Panicle 4-10 cm long, common axis 0.5-1 cm long. Racemes 4-11, digitate, ascending, straight, 4-9 cm long. Rhachis fragile, flattened, margins glabrous, internodes linear, 2.5-3.5 mm long. Sessile spikelets without a short stalk, deciduous with the adjacent joint and pedicel, oblong to lanceolate, 2.5-3 mm long, callus glabrous or puberulous. Lower glume oblong, coriaceous, midrib glabrous, dorsally longitudinally grooved, smooth, glabrous, margins glabrous, apex obtuse, entire. Upper glume elliptic, back acute, glabrous, midrib ciliate, apex entire, attenuate, mucro c. 0.5 mm long. Lower florets absent. Upper lemma oblong, 0.5-1 mm long, without veins, glabrous, apex entire, awn flexuous, column absent, 10-15 mm long. Upper palea ovate, 0.5-1 mm long, as long as the lemma, membranous, apex entire. Anthers 3, 0.7-1 mm long. Pedicels linear, flattened, 2-2.5 mm long, margins glabrous. Pedicelled spikelets subequal to the sessile ones.

Distribution: Taiwan, SW Ryukyus (Iriomote-jima and Yonaguni-jima), China (Hainan), Malesia: Philippines (Luzon, Mindanao, Mindoro, Samar) to Melanesia, Micronesia, Polynesia.

Habitat: Thickets, ravines, old clearings, up to 1,500m alt.

Taiwan specimens examined: TAIPEI: Hsienchiyen, *Hsu 244* (TAI); *Wuku, Sasaki s.n.* Nov. 12, 1933 (TAI); Hsienkungmiao, *Kuo 2391* (TAI). HSINCHU: *Suzuki s.n.* Dec. 10, 1936 (TAI). NANTOU: Hsitou, *Huang s.n. Oct. 28, 1958* (TAI); *Chen 5639* (TAIE); Tili, *Chen 5737* (TAIE). CHIAYI: Alishan, *Kuoh 15948* (NCKU). TAINAN: Paiho, *Wang s.n. Nov. 22, 1970* (TAI); Kuantzuling, *Chen 5741* (TAIE); Yuching, *Kuoh 12787* (NCKU); Hsinhua, *Kuoh 10957* (NCKU). PINGTUNG: Hengchun, *Hsu 13014* (TAI); Wutai, *Huang 80* (TNM); Shenchihshan, *Huang 75* (TNM); Yichangshan, *Huang 62* (TNM); Machia, *Ku 164* (PPI). TAITUNG: *Sasaki s.n.* Oct. 15, 1915 (TAI); Tulanshan, *Wu 17-033* (TNM).

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日本、中國及臺灣之新紀錄種禾草 – 光莠竹(禾本科蜀黍族)

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摘要：本文報導中國、日本及臺灣之一種新紀錄種禾草：光莠竹（禾本科蜀黍族）。本種最早採自1915年，而後陸續有採集紀錄，惟皆被錯誤鑑定為剛莠竹或短軸莠竹。本研究依據本屬各種類之核醣體內轉錄區格間序列之親緣分析，並配合標本館模式標本之檢閱，確認本種為臺灣之新紀錄種。

關鍵詞：中國、核醣體內轉錄區格間、日本、光莠竹、新紀錄種、禾本科、臺灣。

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