



Sedum matsuense (Crassulaceae), a new stonecrop from Matsu Islands

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(Manuscript received 12 July 2022; Accepted 8 March 2023; Online published 23 March 2023)

ABSTRACT: *Sedum matsuense*, a new species from the Matsu Islands, is described. This species is only found in Peigan Island and Dongju Island. Previously, this species has been misidentified as *S. lineare*. However, they can be easily distinguished by phyllotaxy (alternate vs. 3- or 4-verticillate) and leaf shape (linear-lanceolate to oblanceolate, apex acute vs. linear, apex subacute). The phylogenetic analysis also indicates that *S. matsuense* is distantly related to *S. lineare* and to the other resembling species. In this paper, to aid in identification, we provide descriptions, photographs, and line drawings.

KEY WORDS: Crassulaceae, ITS, *Sedum hangzhouense*, *Sedum leptophyllum*, *Sedum lineare*, *Sedum polytrichoides*, Taiwan.

INTRODUCTION

The Matsu Islands are mainly composed of five islands, Dongyin, Peigan, Nangan, Dongju, and Xiju, and numerous small islets. They are located approximately 200 km west of Keelung, Taiwan and approximately 10 km east of Fujian, China (Fig. 1). The vegetation composition of the Matsu Islands is similar to those of both South China and the low-altitude mountains of northern Taiwan (Tzeng *et al.*, 2012).

Sedum L. is the largest and most widespread genus of Crassulaceae, and it comprises 420 species (t Hart and Bleij, 2003; Thiede and Eggl, 2007). The major diversity centers of this genus are in East Asia, the Mediterranean basin, and North America, particularly Mexico (Thiede and Eggl, 2007). Phylogenetic studies reveal that the East Asian *Sedum* members form a monophyletic group (Mayuzumi and Ohba, 2004; Carrillo-Reyes *et al.*, 2009). Fu and Ohba (2001) divide East Asian *Sedum* into three sections: sect. *Filipes* (Fröderström) S.H.Fu, sect. *Oreades* (Fröderström) K.T.Fu, and sect. *Sedum* (H.Ohba) S.H.Fu.

According to Lu *et al.* (2019), 21 species are recognized in Taiwan, including 19 species within sect. *Sedum* and two species within sect. *Filipes*. On the Matsu Islands, two native *Sedum* species of sect. *Sedum* (i.e., *S. formosanum* N.E.Brown, *S. lineare* Thunb.) and one of sect. *Filipes* (i.e., *S. drymarioides* Hance) have been reported (Kuo, 2004; Tzeng *et al.*, 2012). *Sedum lineare* that is not distributed in the main island of Taiwan is a perennial herb characterized by its 3- or 4-verticillate and linear leaves (Fu and Ohba, 2001). On the Matsu Islands, the occurrence of *S. lineare* has been reported in Peigan, Gaodeng and Dongju (Kuo, 2004). However, we observed that the phyllotaxy and flowering seasons of *S. lineare* in the Peigan Island differ from those in China and Japan.

In this study, we conducted morphological comparisons and molecular phylogenetic analysis to

clarify the taxonomic status of plants treated as *S. lineare* in Matsu Islands.

MATERIALS AND METHODS

Sample collection

The plants, historically treated as *S. lineare*, were collected from Peigan Island of the Matsu Islands on April 7, 2021. Some living materials were transplanted to the greenhouse of the Department of Biological Resources, National Chiayi University, Taiwan for further morphological comparison, and some materials were dried by silica gel for subsequent DNA amplification and sequencing. For phylogenetic analysis, we included internal transcribed spacer (ITS) sequences in the samples from 482 taxa of sect. *Sedum*; all samples were stored in GenBank (Table S1). The included taxa were reported in molecular studies by Mayuzumi and Ohba (2004) or Ito *et al.* (2014, 2017a,b, 2020). Following the phylogenetic study of Crassulaceae (Mayuzumi and Ohba, 2004), four taxa were selected as outgroups: *Aeonium castello-paivae* Bolle, *A. gomerense* Praeger, *A. lancerottense* Praeger, and *A. viscatum* Bolle; these specimens were collected by Mort *et al.* (2002) and stored in GenBank (Table S1). In total, 63 operational taxonomic units (OTUs) were included in our molecular phylogenetic analysis (see Table S1). Voucher specimens for the materials were deposited in the Department of Biological Resources, National Chiayi University, Taiwan.

Morphological comparison

To determine the taxonomic status of *S. lineare* in the Matsu Islands, we consulted works in the literature, such as *Flora of China* (Fu and Ohba, 2001) and *Flora of Japan* (Ohba, 2001), and we investigated specimens (Table S2) from herbaria (e.g. TAI, TAIF, HAST, TNM) and digital herbaria (e.g., the Chinese Virtual Herbarium [<https://www.cvh.ac.cn>]).

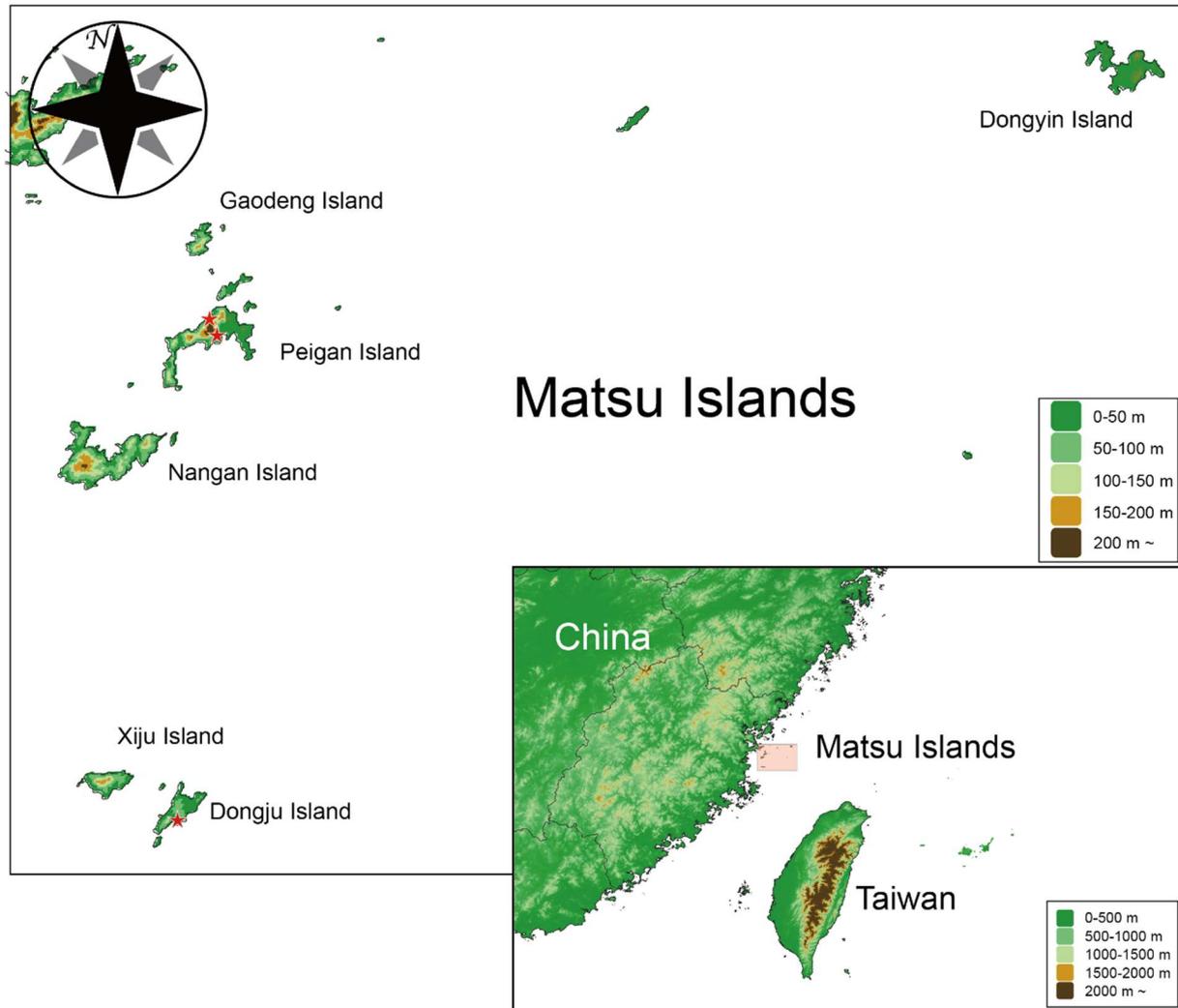


Fig. 1. Geographical distribution of *Sedum matsuense* (red stars).

DNA extraction, amplification, and sequencing

DNA for polymerase chain reaction (PCR) amplification was extracted from dried leaves using a Plant Genomic DNA Extraction Mini Kit (Viogene BioTek Corp., Taipei, Taiwan) in accordance with the manufacturer's protocol. The ITS region (ITS1, 5.8S ribosomal DNA, and ITS2) was amplified by PCR with a MultiGene OptiMax Thermal Cycler (Labnet International, Inc., NJ, USA) using the forward primer ITS1 and the reverse primer ITS4 (White *et al.*, 1990). The amplification conditions were set based on the method of Ito *et al.* (2017).

Phylogenetic analysis using ITS sequences

Sequences were aligned using MUSCLE (Edgar, 2004) implemented in Seqotron version 1.0.1 (Fourment and Holmes, 2016). The alignment was then checked manually. The best substitution model was determined according to the Akaike information criterion with correction for small

sample size using ModelTest-NG v0.1.7 (Darriba *et al.*, 2019) implemented in raxmlGUI 2.0.8 (Edler *et al.*, 2021). Phylogenetic analyses were conducted using Bayesian inference (BI) and maximum likelihood (ML) estimation. ML analyses were performed using raxmlGUI 2.0.8 (Edler *et al.*, 2021), with 10 runs and with 1000 bootstrap (BS) replicates for each run. The BI analyses were performed in MrBayes version 3.2.5 (Ronquist *et al.*, 2012). Two independent runs were conducted with four differentially heated Metropolis-coupled Monte Carlo Markov chains for 1,000,000 generations started from a random tree. Model parameters were estimated during the analysis. Chains were sampled every 100 generations. Convergence of the analyses was validated by the standard deviation of split frequencies (<0.01). The first 25% of generations were discarded as burn-in. A 50% majority rule consensus tree was constructed from the remaining trees to estimate posterior probabilities (PPs). Trees were visualized using FigTree version 1.4.4 (Rambaut, 2010).



Fig. 2. Bayesian phylogenetic tree based on nrITS sequence for Eastern Asian *Sedum*. Bootstrap percentages from ML analysis (left) and Bayesian posterior probabilities (PPs>0.5: right) indicate above the branches.

**Table 1.** Morphological, phenological and ecological characteristics of *Sedum matsuense* C.T. Lu & Wei Yu Wang and four related species.

	<i>Sedum matsuense</i>	<i>S. hangzhouense</i>	<i>S. leptophyllum</i>	<i>S. lineare</i>	<i>S. polytrichoides</i>
Life cycle	perennial	annual	perennial	perennial	perennial
Phyllotaxy	alternate	alternate	alternate, less often verticillate	3 (or 4)-verticillate	alterante
Leaves blade	linear-ob lanceolate, apex acute	narrowly obovate to spatulate-oblong, apex obtusely rounded	narrowly linear-lanceolate to oblanceolate, apex obtuse	linear, apex subacute	linear to linear-lanceolate, apex acute
Leaf length and width (mm)	12.8–30(–40) × 0.2–0.4	20–30 × 0.3–0.7	20–23 × 0.1–0.2	20–25 × ca. 0.2	5–11 × 0.1–0.2
Inflorescence	Cyme, 3-branched, ca. 4 cm in diam.	Cyme, large, 3-branched	Cyme corymbiform, ca. 8 cm in diam.	Cyme 2–3-branched, lax, 4–8 cm in diam.	Cyme, 2–4-branched
Flower	sessile	sessile	sessile	sessile	shortly pedicellate
Petal shape	narrowly lanceolate, apex acute	linear-lanceolate, base connate for ca. 0.5 mm	narrowly lanceolate, apex mucronate	lanceolate, apex acute	narrowly lanceolate, apex acuminate
Petal length and width (mm)	3.7–4.0 × ca. 1	4–4.5 × 0.8–1.2	4–4.5 × ca. 1.2	4–6	5–7
Sepal	free	free	base connate	free	base connate
Sepal shape	ovate-lanceolate, unequal, apex acute	broadly linear-ovate, subequal, apex obtuse	slightly spreading, narrowly triangular, apex obtuse	linear-lanceolate, unequal, apex obtuse	lanceolate-ovate, equal, apex acute
Sepal length and width (mm)	1.5–2.2 × 0.5–0.6	1.5–2.4 × 0.3–0.6	ca. 1 × 0.4	1.5–7	1.5–2.5
Carpel number	5	5	3	5	5
Phenology	Apr.–Jul.	May–Jun.	Jul.–Aug.	Apr.–May	Jul.–Aug.
Ecology	Sunny rocky slopes, 40–100 m	Forested slopes, shady moist rock crevices, rocks along trials	ca. 1,300 m	low mountains, rock on grassy slopes	Rocky slopes, ca. 1,000 m

RESULTS AND DISCUSSION

Morphological comparison

Sedum lineare from China and Japan can be distinguished from the stonecrop from the Peigan Island and Dongju Island, generally identified as *S. lineare* in Flora of Matsu (Kuo, 2004), by phyllotaxy (alternate vs. 3- or 4-verticillate) and leaf shape (linear-lanceolate to oblanceolate, apex acute vs. linear, apex subacute). We argue that this is a misidentification. After consulting the relevant literatures and herbarium specimens, we discovered that this plant resembles three species from China and Japan: *S. hangzhouense* K.T.Fu & G.Y.Rao, *S. leptophyllum* Fröd. and *S. polytrichoides* Hemsley. These species share linear to lanceolate or spatulate-oblong leaves, and they share alternate phyllotaxy. However, the plant from the Matsu Islands differs from *S. hangzhouense* in leaf shape and size (linear-ob lanceolate, 12.8–30[–40] × 0.2–0.4 mm vs. narrowly obovate to spatulate-oblong, 20–30 × 0.3–0.7 mm), and sepal shape (ovate-lanceolate, unequal, apex acute vs. broadly linear-ovate, subequal, apex obtuse) (Table 1). They also occurred in different environments (sunny rocky slopes vs. forested slopes). The stonecrop from the Matsu Islands can be distinguished also from *S. leptophyllum* and *S. polytrichoides* by sepal morphology (free vs. base connate), flowering season (April–July vs. July–August), and elevation distribution (less than 100 m vs. more than 1,000 m). In addition, it also differed from *S.*

leptophyllum by carpel number (5 vs. 3) and differed from *S. polytrichoides* by leaf length (10–40 vs. 5–11 mm) (Table 1).

Phylogenetic analysis using ITS sequences

For the BI and ML phylogenetic analyses, we obtained 63 OTUs, including the sequence of *Sedum matsuense* (accession number: OQ658619) and other 58 accessions as ingroups and four outgroup accessions obtained from GenBank (Table S1). After alignment, we obtained a matrix of 668 base pairs. The GTR+I+G was selected for Bayesian analysis. The 50% majority rule consensus tree of all of the post-burn-in trees is depicted with Bayesian PPs (Fig. 2). The topology of the ML tree was highly compatible with that of the Bayesian tree, and the BS percentages are plotted on the Bayesian phylogenetic tree (Fig. 2). In both the BI and ML analyses (Fig. 2), *S. lineare* from the Matsu Islands, with 13 species from Taiwan and five species from China or Japan (*S. danjoense* Takuro Ito, Nakanishi & Kokub., *S. formosanum* ssp. *miyakojimense* Takuro Ito, Yokota & Kokub., *S. oligospermum* Maire, *S. tetractinum* Fröd. And *S. tricarpum*), formed a well-supported clade (BS/PPs = 98/1; Clade I), and it was deemed to be the sister group of these species. In Clade I, one Japanese species (*S. formosanum* ssp. *miyakojimense*) and 13 Taiwanese endemic species, namely, *S. actinocarpum* Yamam.; *S. arisanense* Yamam.; *S. brachyrinchium* Yamam.; *S. erythrospermum* Hayata; *S. kwanwuense*



H.W.Lin, J.C.Wang & C.T.Lu; *S. microsepalum* Hayata; *S. nokoense* Yamam.; *S. sekiteiense* Yamam.; *S. tarokoense* H.W.Lin & J.C.Wang; *S. taiwanalpinum* H.W.Lin, J.C.Wang & C.T.Lu; and *S. triangulisepalum* T.S.Liu & N.J.Chung ex T.C.Hsu & S.W.Chung (Hsu and Chung, 2022), also form a well-supported clade (BS/PPs = 96/1). Four species from Japan and China that form two monophyletic clades were sister to this clade. One clade comprised three accessions of *S. tricarpum* and the other is composed of *S. danjoense*, *S. tetractinum* and *S. oligospermum*. Finally, clade I was the sister of a well-supported clade (BS/PPs = 93/1; Clade II) including two China endemic species (three accessions of *S. alfredii* Hance and one accession of *S. lungtsuanense* Fu).

The morphological survey indicates that *S. lineare* from the Matsu Islands differ from *S. lineare*, and is similar to *S. hangzhouense*, *S. leptophyllum*, and *S. polytrichoides*. However, with reference to our ITS phylogeny (Fig. 2), they were observed to be in different clades and they were all observed to be far from Clade I. *Sedum hangzhouense*, located at Clade V, forms a well-supported clade with *S. bulbiferum* Makino, *S. emarginatum* Migo and *S. makinoi* Maxim. (BS/PPs = 90/1). *Sedum lineare* (from Japan) and *S. sarmentosum* Bunge formed a well-supported clade in Clade VI (BS/PPs = 100/1). Two accessions of *S. polytrichoides* were distributed in Clade III. This result indicates that *S. lineare* from the Matsu Islands is distantly related to these species. Although we did not sample *S. leptophyllum*, the morphological and ecological information indicate that they were different. For example, *S. lineare* from the Matsu Islands has free sepal, but the sepals of the *S. lineare* from China were connate at the base (Table 2).

On the basis of these morphological and phylogenetic results, we conclude the plants from the Matsu Islands historically treated as *S. lineare* should be considered as a new species. The new species is described below.

TAXONOMIC TREATMENT

Sedum matsuense C.T. Lu & Wei Yu Wang, *sp. nov.*

馬祖佛甲草 Figs. 3 & 4

Sedum lineare auct. non Thunb., Kuo, Flora of Matsu p. 378, 2004.

Type: Matsu Islands, Peigan Island, Peigan road, near Shang Village, elev. ca. 100 m, 6 June 2022, C.T. Lu 3030 (holotype: TAI, isotype: TAIF)

Diagnosis: *Sedum matsuense* resembles *S. lineare* in flower morphology, but can be distinguished by phyllotaxy (alternate vs. 3- or 4-verticillate); also resembles *S. leptophyllum* in alternate phyllotaxy and linear-lanceolate to oblanceolate leaves but differs in leaf blade size (12.8–30[–40] × 0.2–0.4 vs. 20–23 × 0.1–0.2 mm), sepal morphology (free, lanceolate vs. base connate, lobes broadly linear-ovate), and carpel number (5 vs. 3).

Description: Perennial herbs. Seedling: stem erect, with rootstock, radical leaves spatulate, stem leaves

linear-oblanceolate. Mature plants: Stems ascending, branch near from base. Leaves alternate, sessile, linear-oblanceolate, 12.8–30(–40) × 2.6–4.3 mm, base attenuate, shortly spurred, apex acute, midvein on adaxial surface sunken. Flowering stems erect to ascending, 10–20 cm. Cyme 3-branched, ca. 4 cm in diam.; 2-forked per branch. Bract lanceolate, 6.4–13 × 1.6–1.8 mm, apex acute. Flowers sessile, 5-merous. Sepals 5, free, ovate-lanceolate, unequal (one larger than the other four), 3.0–4.3 × 1.0–1.3 mm, base slightly spurred, apex acute. Petals yellow, narrowly lanceolate, subequal, 3.7–4.0 × ca. 1.0 mm, apex acuminate. Stamens 10, two wheels, antesealous ones ca. 3.2 mm long; antepetalous ones ca. 2.6 mm long, inserted from petal base; anther orange-yellowish. Carpels ovoid-lanceolate, ca. 3 mm long, style ca. 0.9 mm. Nectar scales orange, cuneate, ca. 0.35 × 0.3 mm. Follicles stellate, divaricate. Seeds 8 per follicle.

Etymology: The epithet refers to the Chinese name of Matsu Islands; the location of the type specimen collected.

Phenology: Flowers from April to July and fruits from May to November.

Distribution and habitat: This species is known to be distributed in the Peigan Island and Dongju Island (Fig. 1). It grows on sunny rocky slopes at the elevation of 40–100 m.

Proposed IUCN conservation status: We proposed the IUCN conservation status of *S. matsuense* as Data Deficient (IUCN, 2012) until further research.

Additional specimens examined: Lienchiang County: Peigan, elev. 50–250 m, 28 April 2001, *Her-Long Chiang 2139* (TAIF); Matsu, Peigan, 26°12'N, 119°53', elev. 50–250 m, 27 April 2001, *Yu-Pin Cheng 3666* (TAIF); Peigan Island, Peigan road, near Shang Village, elev. ca. 100 m, 24 May 2021, *C.T. Lu 2919* (TAIF); Peigan Township, Mt. Chin, elev. ca. 100 m, 18 May 2019, *Tian-Chuan Hsu 11657* (TAIF); Peigan Township, Chinpi, 11 November 2020, *Ming-Jer Jung 6386* (TAIF); Peigan Township, Qiaozi, elev. ca. 40 m, 17 May 2019, *Tian-Chuan Hsu 11619* (TAIF); Jyuguang Township, Dapu, elev. ca. 40 m, 14 May 2019, *Tian-Chuan Hsu 11575* (TAIF); same location, 10 November 2020, *Ming-Jer Jung 6379, 6380* (TAIF).

Note: *Sedum matsuense* resembles *S. hangzhouense* K.T.Fu & G.Y.Rao in alternate phyllotaxy and free sepal but can be distinguished by life cycle (perennial vs. annual), leaf shape (linear-oblanceolate vs. narrowly obovate to spatulate-oblong), and sepal shape (ovate-lanceolate, unequal, apex acute vs. broadly linear-ovate, subequal, apex obtuse). It is similar to *S. polytrichoides* but can be distinguished by leaf length (12.8–30 mm vs. 5–11 mm), flower pedicellate (sessile vs. short) and sepal (free vs. base connate). Additionally, this new species is relatively close to *S. alfredii* Hance and *S. lungtsuanense* Fu in the ITS phylogenetic tree. It can be distinguished from *S. alfredii* by stem (branched near from base, ascending vs. simple or apically branched, ascending) and leaf shape (linear-oblanceolate vs. linear-cuneate, spatulate, or obovate). It differs from *S. lungtsuanense* by habit (perennial vs. annual), leaves and branch (glabrous vs. sparsely pubescent) and sepals (ovate-lanceolate, glabrous vs. lanceolate to spatulate, sparsely puberulent).

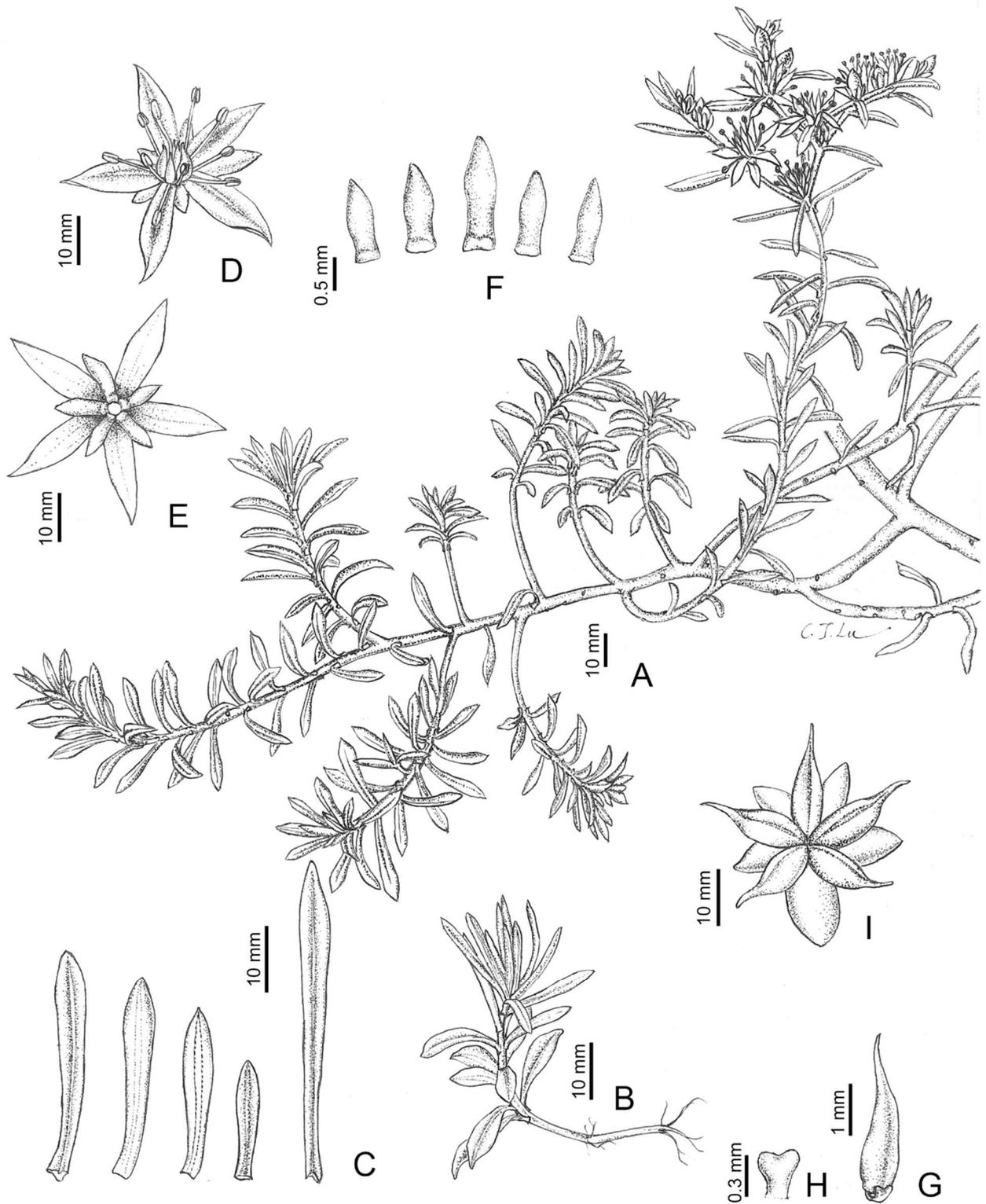


Fig. 3. Illustration of *Sedum matsuense* C.-T. Lu & Wei Yu Wang. A. Habit; B. Seedling; C. Leaves; D. Flower; E. Flower (back view); F. Sepals; G. Carpel; H. Nectary scale; I. Fruits.

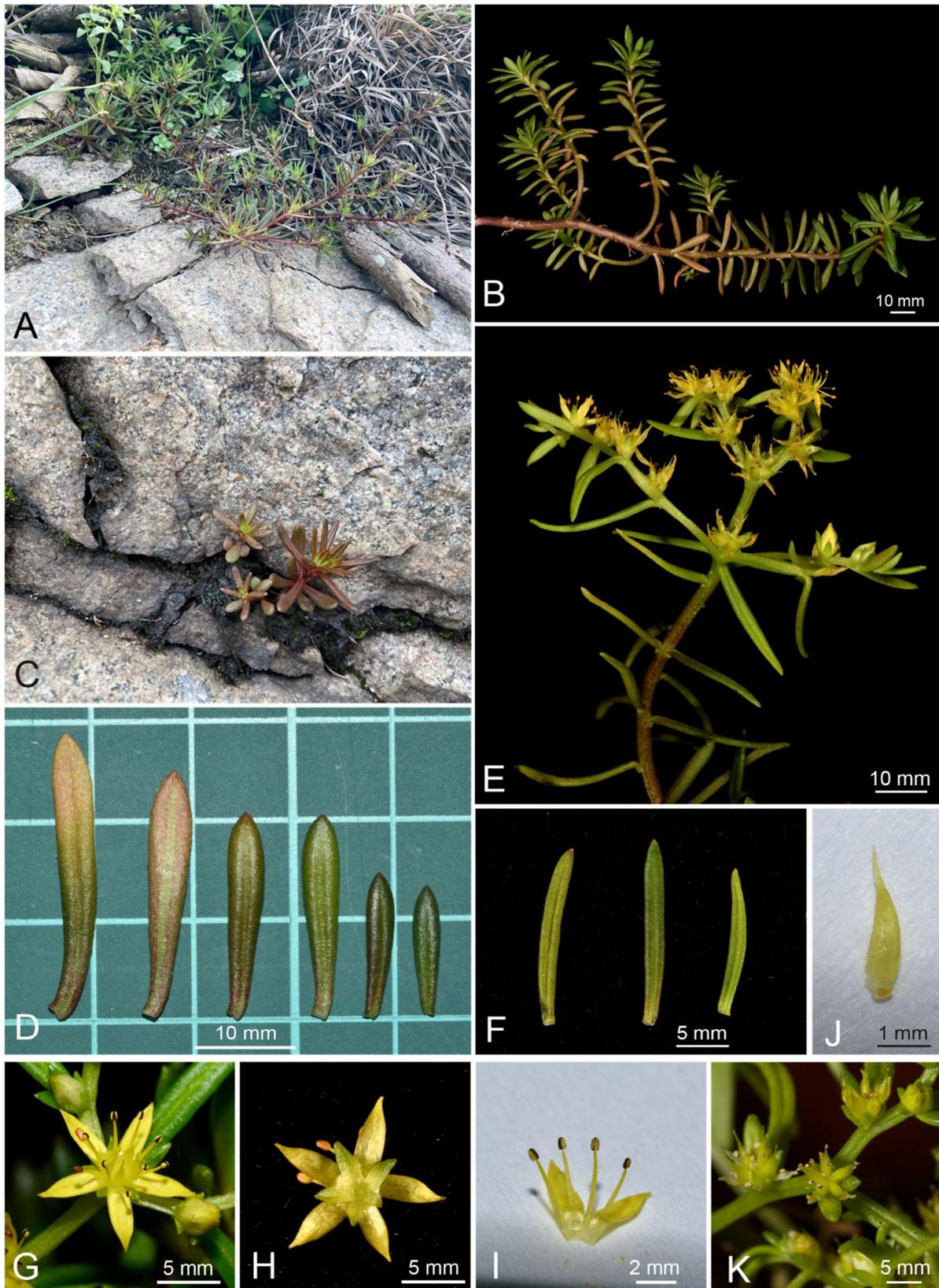


Fig. 4. Habit and Morphology of *Sedum matsuense* C.T. Lu & Wei Yu Wang. **A.** Habit (mature individual); **B.** Vegetation branch; **C.** Habit (seedlings); **D.** Leaves; **E.** Flowering branch; **F.** Bracts; **G.** The front view of flowers; **H.** The back view of flower, showing sepals; **I.** Petals and stamens; **J.** Carpel; **K.** Fruits.



ACKNOWLEDGMENTS

We thank Dr. Yu-Feng Hsu for telling us about the distribution of this new species and thank Dr. Yuan-Ching Tsai and Mr. Kai-Bing Chang for their molecular work. We especially thank two anonymous reviewers for their providing constructive comments to improve our manuscript.

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