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A new species of succulent plant discovered in limestone areas of Kyushu, Japan: *Sedum kawaraense* (Crassulaceae)

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Abstract

An unknown species of the genus *Sedum* was discovered in Kawara-dake and Ryu-ga-hana, limestone mountains in Kyushu, Japan. The present study aimed to clarify the taxonomic status of the unknown *Sedum* species through morphological and ecological comparisons and molecular phylogenetic analysis using ITS sequences based on comprehensive taxon sampling of sect. *Sedum* in East Asia. Morphologically and ecologically, the unknown species was most similar to *S. lipingense* in China, but was distinguished by having larger spatulate rosulate leaves and more flowers per flowering stem. Phylogenetically, it belonged to a clade with four Chinese endemics including *S. lipingense* and two other species widely distributed in East Asia. The clade showed polytomy at the base, but the unknown species was genetically differentiated from the other species. Therefore, we describe the unknown species from Kawara-dake as a new species, *S. kawaraense*.

Keywords: Crassulaceae, East Asia, ITS, Karst, Phylogeny, *Sedum*, Stonecrop

Introduction

Sedum Linnaeus (1753: 430) is the largest and the most widespread genus in the family Crassulaceae (Thiede & Eggli 2007). *Sedum* is a group of herbaceous succulent plants with thickened stems and leaves and is mainly distributed in temperate to subtropical zones. Its diversity is centered in the Mediterranean, Mexico and Central America, the Himalayas, and East Asia (Stephenson 1994, Thiede & Eggli 2007). The plants prefer open and arid environments, but some species grow in closed and humid environments in East Asia (Tang & Huang 1993, Ohba 2001, Fu & Ohba 2001). Recent molecular phylogenetic studies showed that the genus *Sedum* in the sense of references cited above is polyphyletic (Carrillo-Reyes *et al.* 2009, Messerschmid *et al.* 2020) and it was redefined to include 14 genera belonging to the tribe Sedeae (= clades *Leucosedum* plus *Acre*)(Messerschmid *et al.* 2020). As redefined, the genus comprises approximately 755 species (Messerschmid *et al.* 2020).

In Japan, 28 taxa have been recorded, all of which are included in sect. *Sedum* except for one species in sect. *Filipes* (Fröderström 1931: 34) Fu, S.H. (1965: 115), *S. drymarioides* Hance (1865: 379)(Ohba 2001, Ito *et al.* 2017b, 2020a, 2020b). Species diversity is the highest on Kyushu Island, with 16 species, two subspecies, and two varieties including eight taxa endemic to Kyushu (Ohba 2001, Ito *et al.* 2018). In 2015, the second author discovered an unknown species of the genus *Sedum* in Kawara-dake, a limestone mountain in Fukuoka Prefecture, Kyushu, Japan (Figs. 1 & 2). In 2021, he carried out an extensive field survey and confirmed that similar-looking plants also grow in Ryu-ga-hana, another limestone mountain near Kawara-dake. According to the literature on the natural history of Kawara-dake (Kumagae 2016), this unknown species was apparently treated as *S. tricarpum* Makino (1891a: 3), but the unknown species differs from *S. tricarpum* in the number of carpels (5 vs. 3). The present study aimed to clarify

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the taxonomic status of the unknown *Sedum* species by morphological and ecological comparisons and molecular phylogenetic analyses based on comprehensive taxon sampling of sect. *Sedum* from East Asia.

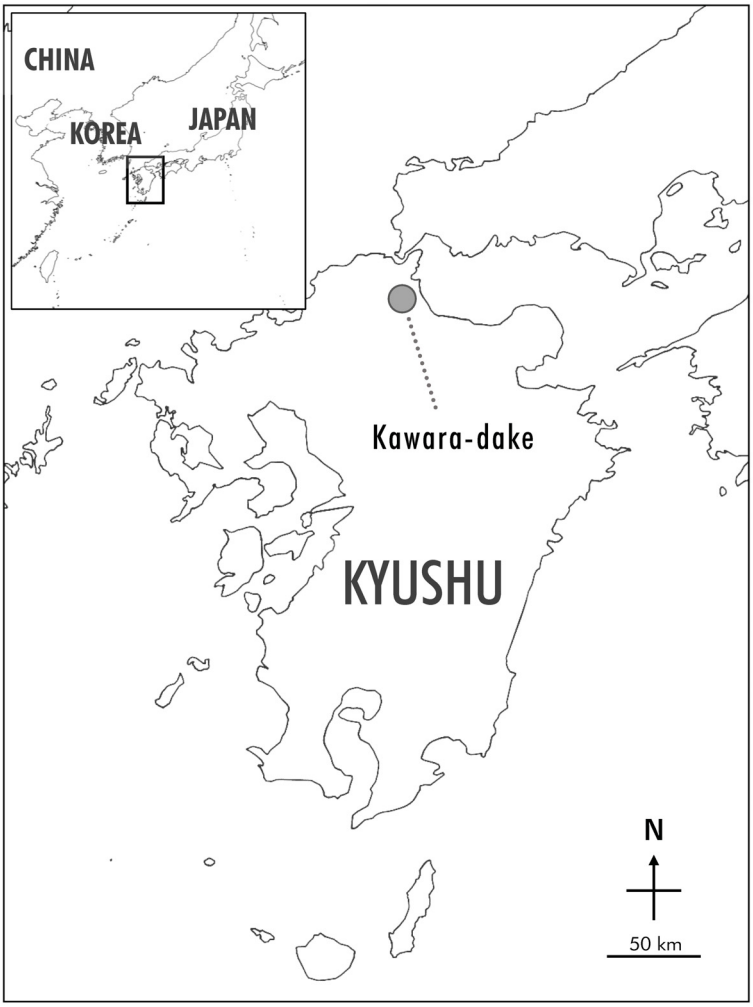

FIGURE 1. Map showing the location of Kawara-dake, Kyushu, Japan.

Materials and methods

Plants materials

We conducted a detailed morphological survey of the unknown *Sedum* species using the specimens collected in the field and the living plants cultivated at the Tohoku University Botanical Garden. This unknown species is morphologically and ecologically similar to *S. bulbiferum* Makino (1891b: 2), *S. erythrospermum* Hayata (1913: 110), *S. hangzhouense* K.T.Fu & G.Y.Rao (1988: 119), *S. lipingense* R.B. Zhang, D. Tan & R.X. Wei (2019: 129), *S. subtile* Miq. (1866: 156) and *S. tricarpum*, and we made morphological and ecological comparisons with these species based on the literature (Tang & Huang 1993, Ohba 2001, Zhang *et al.* 2019) and herbarium specimens, from Kagoshima University Museum (KAG), National Museum of Nature and Science (TNS), and Tohoku University (TUS).

For the molecular phylogenetic analyses, we used five accessions of the unknown species collected from Kawara-dake and Ryu-ga-hana by the second author (Table 1). Since previous studies showed that the East Asian species of the genus were monophyletic (Mayuzumi & Ohba 2004), internal transcribed spacer (ITS) sequences of the East Asian *Sedum* species were additionally obtained from NCBI and used for phylogenetic analyses to estimate the phylogenetic position of the unknown species (Table 1) (Mayuzumi & Ohba 2004, Ito *et al.* 2014, 2017a, 2017b, 2020a, 2020b, Zhang *et al.* 2019, Zou *et al.* 2020). The genera *Aeonium* Webb & Berthel. (1840: 184) and *Greenovia* Webb & Berthel. (1841: 198) were selected as outgroups based on previous studies (Mort *et al.* 2002, Mayuzumi & Ohba 2004). Voucher specimens for the materials were deposited in the herbaria TNS and TUS.

TABLE 1. Plant materials of 63 accessions of eastern Asian *Sedum* taxa and five outgroup taxa with their collection locality, voucher information, and accession numbers of ITS sequences.

Taxon	locality	Voucher (Herbarium)	Accession No.	Taxon	locality	Voucher (Herbarium)	Accession No.
S. actinocarpum	Taiwan	*TI 1749* (TNS)	[1]LC229265	*S. polytrichoides* ssp. *polytrichoides*	China	*TI 1057* (TNS)	[1]LC229251
S. alfredii	China	*GK 17190* (IBSC)	[2]AB930259	*S. polytrichoides* ssp. *polytrichoides*	Japan	*TI 2247* (TNS)	[1]LC229252
S. arisanense	Taiwan	*TI 1836* (TNS)	[1]LC229272	*S. polytrichoides* ssp. *yabeanum* var. *yabeanum*	Japan	*TI 396* (TNS)	[2]AB906490
S. baileyi	China	*LBG0064555* (-)	FJ919935	*S. polytrichoides* ssp. *yabeanum* var. *setouchiense*	Japan	*TI 2298* (TNS)	[1]LC229253
S. boninense	Japan	*TI 2371* (TNS)	[1]LC229242	*S. rupifragum*	Japan	*TI 2070* (TNS)	[1]LC229254
S. bulbiferum	Japan	*TI 416* (TNS)	[1]LC229234	*S. sarmentosum*	China	*TI 978* (TNS)	[1]LC229255
S. brachyrinchum	Taiwan	*TI 3118* (TNS)	[1]LC229277	*S. satumense*	Japan	*TI 2295* (TNS)	[1]LC229256
S. danjoense	Japan	*TI 3658* (TNS)	[3]LC260127	*S. sekiteiense*	Taiwan	*TI 1456* (TNS)	[1]LC229295
S. emarginatum	China	*TI 1062* (TNS)	LC530833	*S. subtile*	Japan	*TI 2259* (TNS)	[1]LC229257
S. erici-magnusii	China	*TI 2077* (TNS)	[1]LC229235	*S. taiwanalpinum*	Taiwan	*TI 1823* (TNS)	[1]LC229278
S. formosanum subsp. *formosanum*	Japan	*GK 16712* (TNS)	[2]AB930264	*S. taiwanianum*	Taiwan	*TI 2523* (TNS)	[1]LC229296
S. formosanum subsp. *miyakojimense*	Japan	*TI 1115* (TNS)	[4]LC530813	*S. tarokoense*	Taiwan	*TI 2025* (TNS)	[1]LC229298
S. hakonense	Japan	*TI 623* (TNS)	[2]AB930278	*S. tetractinum*	China	*TI 3623* (TNS)	[3]LC260135
S. hangzhouense	China	*TI 2604* (TNS)	[3]LC260130	*S. tianmushanense*	China	*LP 67* (TNS)	[1]LC229261
S. japonicum ssp. *japonicum* var. *japonicum*	Japan	*TI 723* (TNS)	[1]LC229237	*S. tosaense*	Japan	*TI 655* (TNS)	[1]LC229258
S. japonicum ssp. *japonicum* var. *senanense*	Japan	*TI 2200* (TNS)	[1]LC229238	*S. triactina*	Nepal	*TI 9596091* (TI)	[8]AB088629
S. japonicum ssp. *oryzifolium* var. *oryzifolium*	Japan	*TI 2285* (TNS)	[1]LC229239	*S. triangulosepalum*	Taiwan	*TI 2508* (TNS)	[1]LC229299
S. japonicum ssp. *oryzifolium* var. *pumilum*	Japan	*TI 2287* (TNS)	[1]LC229240	*S. tricarpum*	Japan	*TI 2269* (TNS)	[1]LC229259
S. jiulungshanense	China	*CMQ 76* (TNS)	[1]LC229243		China	*TI 3597* (TNS)	[3]LC260134
S. kiangnanense	China	*TI 1030* (TNS)	[1]LC229244	*S. trullipetalum*	Nepal	*TI 9420132* (TI)	[8]AB088630
S. kwanwuense	Taiwan	*TI 2440* (TNS)	[1]LC229293	*S. truncastigmum*	Taiwan	*TI 2766* (TNS)	[1]LC229305
S. lipingense	China	*ZRB 1479* (ZY)	[5]MN150061	*S. uniflorum*	Japan	*TI 447* (TNS)	[1]LC229241
S. lineare	Japan	*HU 667* (TNS)	[1]LC229245	*S. zentaro-tashiroi*	Japan	*TI 355* (TNS)	[2]AB906491
S. lungtsuanense	China	*TI 3563* (TNS)	[3]LC260131	*Sedum* sp. (*S. kawaraense*)	Japan	*HK 4164* (TUS)	LC731689
S. makinoi	Japan	*TI 2325* (TNS)	[1]LC229246		Japan	*HK 4170* (TUS)	LC731690
S. mexicanum	Japan	*TI 647* (TNS)	[1]LC229247		Japan	*TI 7715* (TUS)	LC731691
S. microsepalum	Taiwan	*TI 2771* (TNS)	[1]LC229282		Japan	*TI 7716* (TUS)	LC731692
S. morrisonense	Taiwan	*TI 2348* (TNS)	[1]LC229289		Japan	*TI 7717* (TUS)	LC731693
S. mukojimense	Japan	*TI 5704* (TNS)	[6]LC530822				
S. multicaule	China	*TI 625* (TNS)	[1]LC229248	Outgroup			
S. nokoense	Taiwan	*TI 3196* (TNS)	[1]LC229294	*Aeonium castello-paivae*	Canary	*MEM 1519* (WS)	[9]AY082236
S. nagasakianum	Japan	*TI 2064* (TNS)	[1]LC229249	*Aeonium gomerense*	Canary	*MEM 1454* (WS)	[9]AY082242
S. nanlingense	China	*MES01* (-)	[7]MN105949	*Aeonium lancerottense*	Canary	*MEM 1518* (WS)	[9]AY082143
S. oligospermum	China	*CMQ 74* (TNS)	[1]LC229250	*Aeonium viscatum*	Canary	*MEM 1432* (WS)	[9]AY082154
S. oreades	Nepal	*TI 9420140* (TI)	[8]AB088632	*Greenovia aizoon*	Canary	*MEM 1425* (WS)	[9]AY082112

Reported by [1]Ito *et al.* (2017b), [2]Ito *et al.* (2014), [3]Ito *et al.* (2017a), [4]Ito *et al.* (2020a), [5]Zhang *et al.* (2019), [6]Ito *et al.* (2020b), [7]Zou *et al.* (2020), [8]Mayuzumi and Ohba (2004) and [9]Mort *et al.* (2002).

DNA extraction, PCR amplification, and sequencing

Total DNA was extracted from silica-dried leaves by using the modified CTAB method (Murray & Thompson 1980). The ITS region (ITS1, 5.8S rDNA, and ITS2) was amplified by polymerase chain reaction (PCR) with an iCycler (Bio-Rad, Hercules, CA, USA) using the forward primer ITS1 and the reverse primer ITS4 (White *et al.* 1990). EmeraldAmp PCR Master Mix dye (Takara, Otsu, Japan) was used for amplification. After an initial 3 min denaturing

at 94°C, the PCR profile comprised 35 cycles of 30 s at 94°C, 30 s at 50°C, and 1.5 min at 72°C. The PCR products were purified with an ExoStar clean-up kit (USB, Cleveland, OH, USA). Cycle sequencing was performed using a BigDye Terminator Cycle Sequencing Kit ver. 3.1 (Applied Biosystems, Foster City, CA) and the abovementioned PCR primers. The PCR products were purified by ethanol precipitation and then electrophoresed on an Applied Biosystems 3130xl Genetic Analyzer. The electropherograms were assembled using ATGC ver. 6 software (GENETYX, Tokyo, Japan). Sequence data from this study were deposited in the DNA Data Bank of Japan (DDBJ; extant since 1983).

Molecular phylogenetic analyses

The ITS sequences were aligned with the online version of MAFFT v7.49 software (Kato *et al.* 2019) and ClustalW 1.8 (Thompson *et al.* 1994), and then adjusted manually by Bioedit v7.0.5 (Hall 1999). Phylogenetic analyses with a bayesian approach and a maximum-likelihood (ML) method were conducted using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and RAxML-NG (Kozlov *et al.* 2019), respectively. In the Bayesian approach, Akaike's Information Criterion (AIC) implemented in MrModeltest 2.2 (Nylander 2004) was used to obtain an appropriate evolutionary model of nucleotide substitutions. Subsequently, two separate runs of Metropolis-coupled Markov chain Monte Carlo (MCMCMC) analysis, each with a random starting tree and four chains (one cold and three hot) based on the selected model were performed. The MCMCMC length was 10,000,000 generations, and the chain was sampled every 1,000th generation from the cold chain. The first 2,500 sample trees (25% of the total 10,000 sample trees) were discarded as burn-in after checking that the average standard deviation of split frequencies (ASDSF) reached a stationary state at < 0.01 thereafter. In the ML method, ModelTest-NG was run to determine the best model for nucleotide substitutions under the Bayesian information criterion (BIC)(Darriba *et al.* 2020). The ML tree and bootstrap proportions (BPs) were obtained by simultaneously running rapid bootstrapping with 10,000 iterations followed by a search for the most likely tree. The output tree files were generated using FigTree ver. 1.4.4 (Rambaut 2018)

Results

Morphological and ecological comparisons

Morphologically and ecologically, the unknown species was not identical to any of the six species (*S. bulbiferum*, *S. erythrospermum*, *S. hangzhouense, S. lipingense*, *S. subtile* and *S. tricarpum*) that share similar features in leaf shape and life cycle (Figs. 2 & 3; Table 2).

Phylogenetic relationships based on ITS region

Five individuals of unknown *Sedum* species from Kawara-dake and Ryu-ga-hana had identical ITS sequences and were used as one operational taxonomic unit (OTU) for phylogenetic analyses. In total, 64 OTUs including 59 ingroup and five outgroup OTUs were used for Bayesian and ML analyses. After alignment, a matrix of 628 bp was obtained. The GTR+I+G and SYM+I+G4 models were selected as the best nucleotide substitution models for Bayesian and ML phylogenetic analyses, respectively. The 50% majority-rule consensus tree was shown with Bayesian posterior probabilities (PPs) in Fig. 4. The topology of the ML tree was highly compatible with that of the Bayesian tree, and the BPs were plotted on a Bayesian tree (Fig. 4).

In both the Bayesian and ML trees (Fig. 4), the unknown *Sedum* species belonged to a clade supported by PP/BS = 0.99/72, including four Chinese endemic species (*S. baileyi* Praeger (1919: 4), *S. emarginatum* Migo (1937: 224), *S. hangzhouense*, and *S. lipingense*) and two widely distributed species in East Asia (*S. bulbiferum* and *S. makinoi* Maxim. (1888: 487) and). This clade contained a subclade supported by PP/BS =1.00/100, composed of *S. baileyi*, *S. emarginatum* and *S. makinoi* (Fig. 4). This subclade, *S. bulbiferum*, *S. hangzhouense*, *S. lipingense*, and the unknown species were polytomous at the base, and the unknown species was not sister to any other species (Fig. 4). In addition, *S. erythrospermum*, *S. subtile* and *S. tricarpum*, which were morphologically and ecologically similar to the unknown species in terms of leaf shape and life cycle, were located in different clades (Fig. 4; Table 2).

TABLE 2. The diagnostic of *Sedum* sp. (*S. kawaraense*) and its related species.

	***Sedum* sp. (*S. kawaraense*)**	***S. bulbiferum***	***S. erythrospermum***	***S. hangzhouense***	***S. lipingense***	***S. subtile***	***S. tricarpum***
Life cycle	annual or biennial	biennial	annual	annual	biennial (or perennial?)	perennial	perennial
Rosulate leaves	present during flowering, spatulate, 0.7–2.4×0.1–0.7 cm	present during flowering, widely ovate-suborbicular, 1.0–1.5×0.2–0.4 cm	present during flowering, spatulate, 0.5–2.4×0.3–0.8 cm	present during flowering, spatulate, 0.7–2.5×0.3–0.7 cm	present during flowering, broadly obovate, 0.5–1.5×0.4–0.7 cm	absent during flowering, spatulate, 0.5–1.5×0.3–0.6 cm	absent during flowering, spatulate, 2.0–2.5×0.5–0.8 cm
Cauline leaves	spatulate to oblanceolate, 0.3–1.6×0.1–0.7 cm	ovate to oblanceolate, 1.0–1.5×0.2–0.4 cm	obovate to spatulate, 0.7–1.2×0.3–0.6 cm	narrowly obovate to spatulate-oblong, 2.0–3.0×0.3–0.7 cm	spatulate, obovate to oblanceolate, 0.7–1.2×0.3–0.4 cm	spatulate, liner to oblanceolate, 0.5–2.0×0.1–0.3 cm	spatulate to oblanceolate, 2.0–3.0×0.5–1.0 cm
Bulb at leaf axil	absent	present	absent	absent	absent	absent	absent
Stolon	absent	absent	absent	absent	absent	present	absent
Flowering stem	extends from rosette center and leaf axil, 2–8 cm	only extends from rosette center, 10–30 cm	only extends from rosette center, 6–8 cm	only extends from rosette center, 8–20 cm	extends from rosette center and leaf axil, 3–7 cm	only extends from rosette center, 4–10 cm	only extends from rosette center, 8-25 cm
Flowers	4–18	10–20	12–20	many	2–6	3–9	many
Carpels	5	5	5	5	5	5	3
Habitat	shady, mossy limestone	road side	shady moist rock	shady moist rock	moist limestone	rock along stream	rocky slope

Discussion

Phylogenetic evidence based on ITS sequences showed that the unknown species is related to four Chinese endemic species (*S. baileyi*, *S. emarginatum*, *S. hangzhouense*, and *S. lipingense*) and two widely distributed species in East Asia (*S. bulbiferum* and *S. makinoi*), but not sister to any of them (Fig. 4). Among these species, the unknown species is morphologically and ecologically similar to *S. lipingense* in the monocarpic life cycle, presence of rosette leaves during flowering, leaf shape (spatulate-oblanceolate cauline leaves), absence of bulbs and stolons, position where flower stems branch (from rosette center and leaf axil), and habitat preference (growing on moist limestone). However, the two species differ in the morphology of rosulate leaves and number of flowers per flowering stem (Figs. 2 & 3; Table 2). While the unknown species is restricted to two localities of Japan, *S. lipingense* is endemic to SE Guizhou, China (Zhang *et al.* 2019). On the basis of this evidence, the unknown species is described below as a new species.

Taxonomic treatment

Sedum kawaraense Takuro Ito & Kanemitsu, *sp. nov*. (Figs. 2 & 3)

Type:—JAPAN. Kyushu, Fukuoka: Tagawa-gun, Kawara-machi, Mt. Kawara-dake, 16 May 2021, *Hironobu Kanemitsu* 4170 (holotype TUS!).

Diagnosis:—*Sedum kawaraense* differs from its close relatives (Table 2) in life history, rosulate and cauline leaf morphology, presence of stolons and bulbs at the leaf axil, flowering stem extension pattern, length, number of flowers, and habitat. Among these species, *S. kawaraense* is morphologically most similar to *S. lipingense* in the monocarpic life cycle, presence of rosette during anthesis and spatulate-oblanceolate cauline leaves, absent bulbs and stolons, and flowering stem extending from the rosette center and leaf axil, but differs in having larger spatulate rosulate leaves and more flowers per flowering stem (Table 2).

Description:—Winter annual or biennial herbs, fleshy, glabrous. Rarely forms bulbils on leaf axils for vegetative propagation. Roots, fibrous, partially lignified. Rosettes 1.5–4.7 cm across; rosulate leaves alternate, reddish or greenish, sessile, thick, spatulate, entire, apex obtuse, base attenuated and shortly spurred, 0.7–2.4 cm long, 0.1–0.7 cm wide. Stem, single shoot emerging from rosette center, other shoots from the rosulate leaf axils, reddish or greenish, erect, 2.2–5.8 cm tall; cauline leaves mostly alternate, reddish or greenish, sessile, thick, spatulate to oblanceolate, entire, apex obtuse, base attenuated and shortly spurred, 0.3–1.6 cm long, 0.1–0.7 cm wide.; Flowering stems 1 to 7, fleshy, 2.0–8.2 cm tall, base ca. 0.2 cm broad, reddish or greenish, erect. Inflorescences terminal, cymes, 2 to 3 branched, each branch usually 2–6 flowered. Bracts leaf-like, oblanceolate, apex obtuse, 0.3–1.6 cm long, 0.1–0.5 cm wide. Sepals 5, free, green, fleshy, subequal, lanceolate, 1–3 mm long, 0.8–1.2 mm wide, base shortly spurred, apex obtuse. Petals 5, blight yellow, lanceolate 4.5–6.5 mm long, 1.6–2.2 mm wide, apex acuminate, base slightly connate. Stamens 10, shorter than petals, 3.5–5.2 mm long, erect at flowering, 2–whorled; anthers oblong, ca. 0.5 mm long, red before dehiscence. Carpels 5, free, ascending, connate at the base, gibbous ventrally, 3.2–4.5 mm long. Fruits star-shaped, follicle, ascending, 5.0–6.5 mm long. Flowering in May to June.

This new species is categorized in sect. *Sedum* due to the presence of adaxially gibbous carpels (Fu & Ohba 2001)(Fig. 3).

Etymology:—Epithet refers to the Japanese name of the type locality, Kawara-dake.

Distribution and habitat:—Endemic to Kawara-dake and its adjacent areas, Kyushu, Japan, on shady and mossy limestone rock.

Japanese common name:—Kawara-mannen-gusa (nov.).

Additional specimens examined (paratype):—JAPAN. Kyushu, Fukuoka: Tagawa-gun, Kawara-machi, Mt. Kawara-dake, *Hironobu Kanemitsu 1121, 4163, 4164, 4165* (TUS), *Hironobu Kanemitsu 4166, 4173* (TNS), Miyako-gun, Miyako-machi, Ryu-ga-hana, *Takuro Ito 7715, 7716, 7717* (TUS).

Conservation. IUCN Red List category:—Critically Endangered (CR). The distribution of *S. kawaraense* is restricted to limestone areas < 10 km^2 (~ca. 0.056 km^2) in Kawara-dake and Ryu-ga-hana, Kyushu, Japan, and is anticipated to decline in the future because most of the distribution area of the species is privately owned, and limestone quarrying is still being carried out. Until now, one of the peaks of Kawara-dake has disappeared because of limestone mining activities. Based on Criterion B2 (IUCN 2022), *S. kawaraense* qualifies as a CR. However, based on Criterion D1, *S. kawaraense* is qualified as NT because the populations in the two localities amount to ca. 2,400 mature

individuals (ca. 1500 individuals in Kawara-dake and ca. 900 individuals in Ryu-ga-hana). Criterion B2 was adopted based on the precautionary principle.

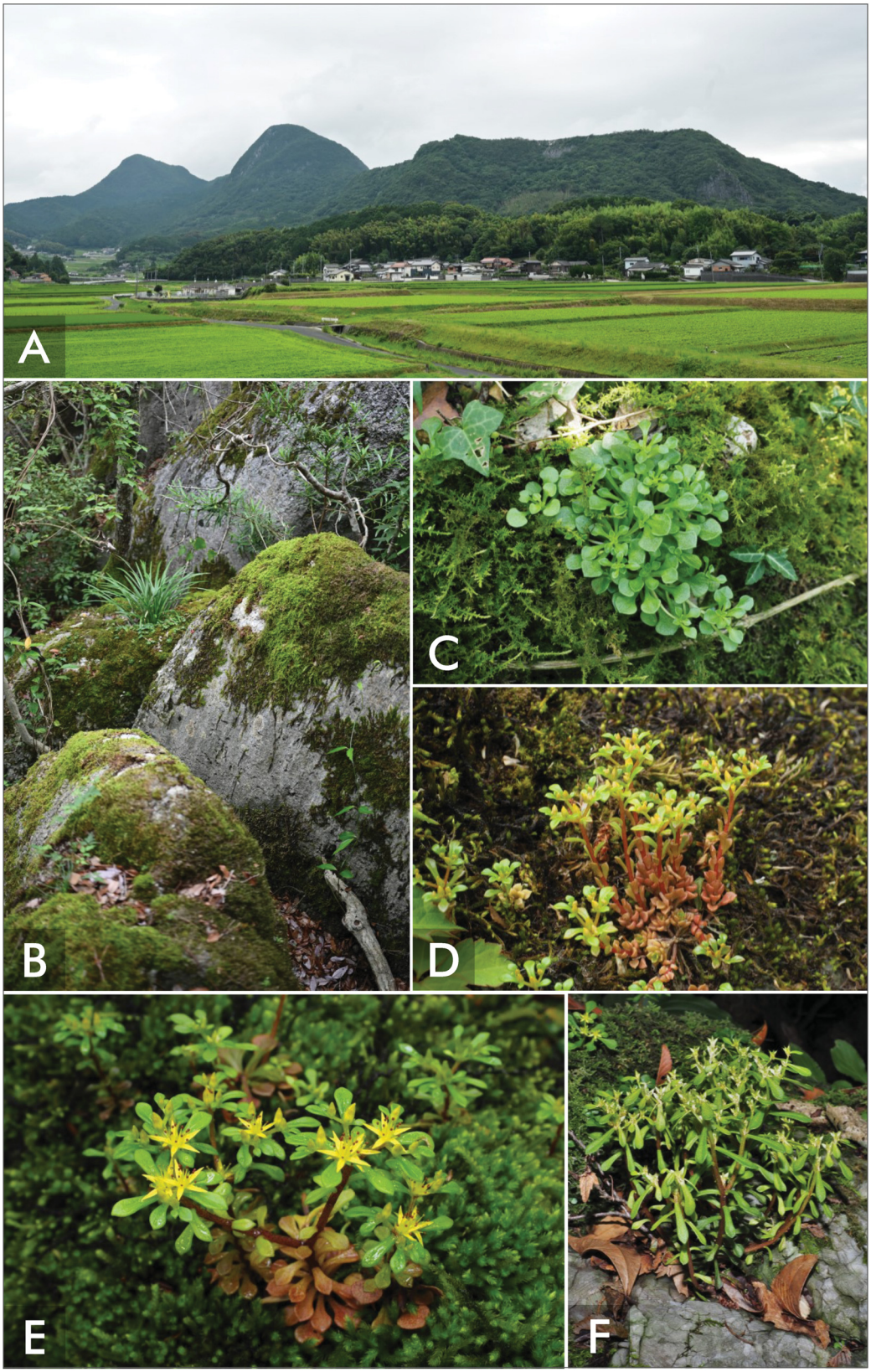

FIGURE 2. The native habitats and habits of *Sedum* sp. (*S. kawaraense*). **A.** Kawara-dake, consists of three peaks. The first of the three peaks (Ichi-no-dake) has lost its upper half due to limestone mining. **B.** Habitat. On shady mossy limestone around the summit. **C.** Rosettes. **D.** Flower buds. **E.** Flowers. **F.** Fruits. [Photographed on 25 July 2022 (A & B), 29 August 2021 (C), 4 May 2021 (D), 16 May 2021 (E), 12 June 2022 (F) in Kawara-dake (A, B, E & F) and Ryu-ga-hana (C & D), Kyushu.]

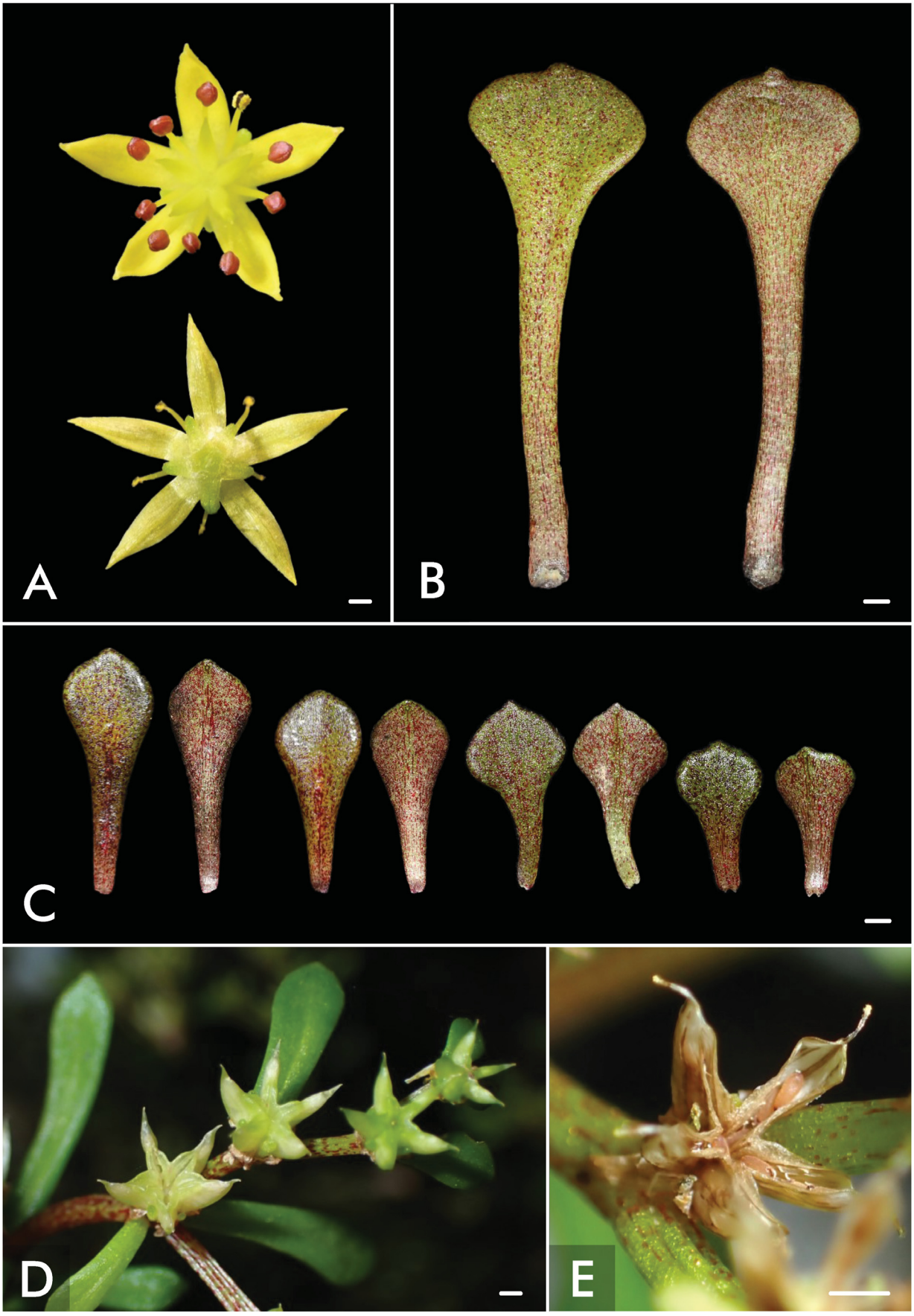

FIGURE 3. The detailed morphology of *Sedum* sp. (*S. kawaraense*). **A.** Flower. **B.** Rosulate leaves. **C.** Cauline leaves. **D.** Young fruits. **E.** Mature fruit. Scale bars are 1 mm.

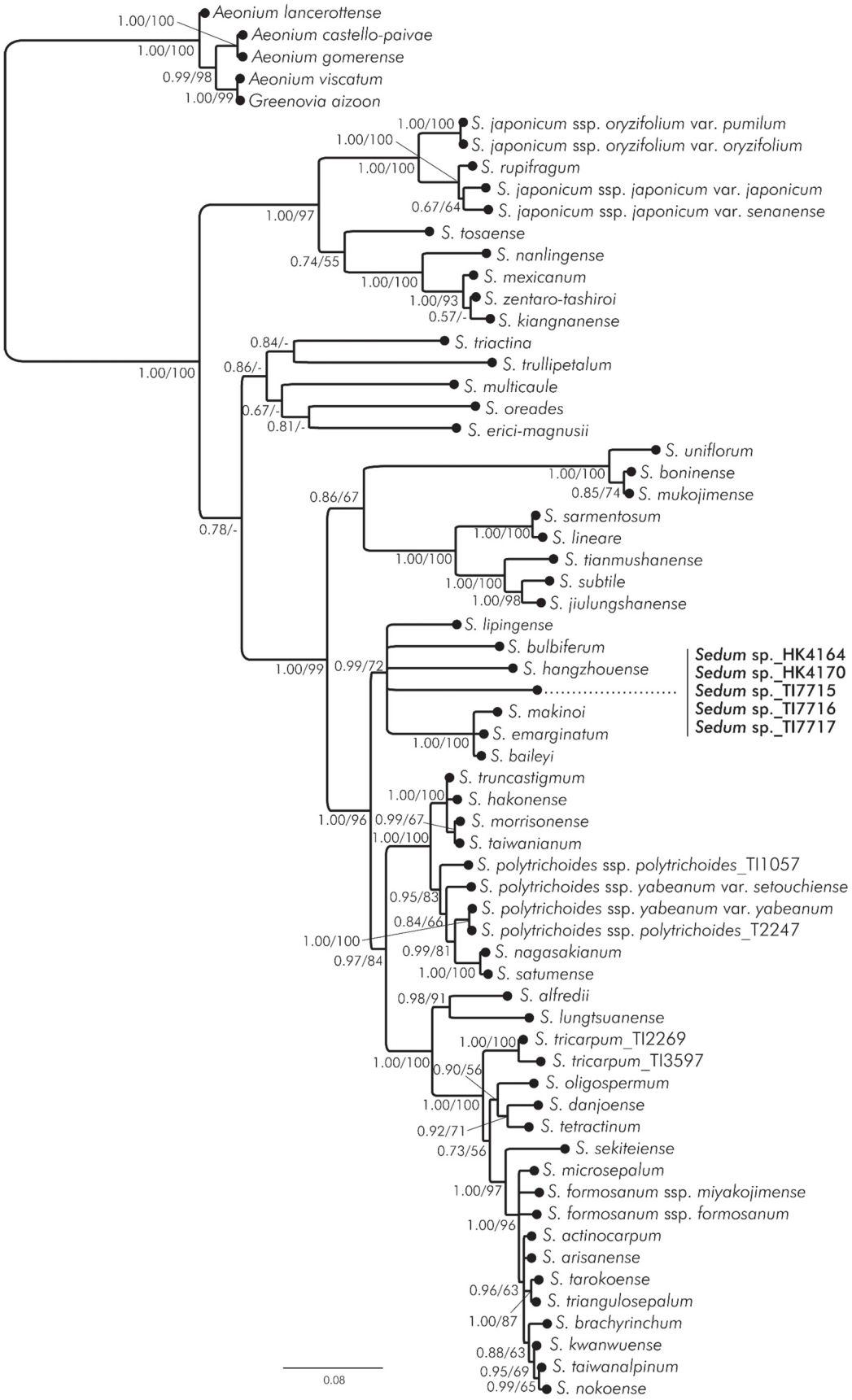

FIGURE 4. The Bayesian 50% majority rule consensus tree based on ITS sequences for Eastern Asian *Sedum*. The topology of the ML tree was highly compatible with the Bayesian tree. Bayesian posterior probabilities (PPs: left) and bootstrap percentages from the ML analysis (BP: right) are shown (See Table 1 for the abbreviations).

Diagnostic key of *S. kawaraense* and related species

1. Carpels 3 ..... *S. tricarpum*
– Carpels 5 2
2. Perennial, a stolon present, rosulate leaves absent during flowering ..... *S. subtile*
– Annual or biennial, a stolon absent, rosulate leaves present during flowering 3
3. Bulb present at leaf axils ..... *S. bulbiferum*
– Bulb absent at leaf axils 4
4. Flowering stem extends only from rosette center 5
– Flowering stem extends from rosette center and leaf axils 6
5. Cauline leaves obovate to spatulate, 0.7–1.2×0.3–0.6 cm ..... *S. erythrospermum*
– Cauline leaves narrowly obovate to spatulate-oblong, 2.0–3.0×0.3–0.7 cm ..... *S. hangzhouense*
6. Flowers 2–6, rosulate leaves broadly obovate ..... *S. lipingense*
– Flowers 4–16, rosulate leaves spatulate ..... *S. kawaraense*

Probable biogeographic history and biological importance of Sedum kawaraense

Molecular phylogenetic analysis showed that *S. kawaraense* formed a clade with four Chinese endemic species (*S. baileyi*, *S. emarginatum*, *S. hangzhouense*, and *S. lipingense*) and two widely distributed species in East Asia (*S. bulbiferum* and *S. makinoi*), suggesting that it may have diverged from a common ancestor with any of these species.

Sedum kawaraense is endemic to the limestone areas of northern Kyushu. In general, limestone soils tend to cause edaphic and geographic isolation of plant populations owing to their alkaline properties and fragmented distribution (Nakamura *et al.* 2014), and often play a role as a refuge for plants (Chung *et al.* 2014). Consequently, many plants specific to limestone areas have been reported to be locally endemic worldwide (Chung *et al.* 2014, Wang *et al.* 2017). Therefore, the common ancestral population of *S. kawaraense* with either of the four Chinese endemics or the two widely distributed species in East Asia might have been stranded in the limestone areas of northern Kyushu, and subsequently, speciation may have been promoted by edaphic and geographic isolation. Further studies of this species, including estimates of the detailed interspecific relationships and its divergence time using more genetic markers, will help us understand the formation of the flora of Kyushu.

Similar to many limestone-specific plant species threatened by modern destructive land uses (Clements *et al.* 2006), *S. kawaraense* is threatened by habitat loss from limestone mining. To meet international efforts to minimize species loss (Belote *et al.* 2021), measures are urgently needed to conserve its native habitat.

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References

Belote, R.T., Barnett, K., Dietz, M.S., Burkle, L., Jenkins, C.N., Dreiss, L., Aycrigg, J.L. & Aplet, G.H. (2021) Options for prioritizing sites for biodiversity conservation with implications for "30 by 30". *Biological Conservation* 264: 109378. https://doi.org/10.1016/j.biocon.2021.109378

Carrillo-Reyes, P., Sosa, V. & Mort, M.E. (2009) Molecular phylogeny of the Acre clade (Crassulaceae): Dealing with the lack of definitions for *Echeveria* and *Sedum*. *Molecular Phylogenetics and Evolution* 53: 267–276. https://doi.org/10.1016/j.ympev.2009.05.022

Chung, K.F., Leong, W.C., Rubite, R.R., Repin, R., Kiew, R., Liu, Y. & Peng, C.I. (2014) Phylogenetic analyses of *Begonia* sect. *Coelocentrum* and allied limestone species of China shed light on the evolution of Sino-Vietnamese karst flora. *Botanical Studies* 55 (1): 1–15. https://doi.org/10.1186/1999-3110-55-1

Clements, R., Sodhi, N.S., Schilthuizen, M. & Ng, P.K. (2006) Limestone karsts of Southeast Asia: imperiled arks of biodiversity. *Bioscience* 56 (9): 733–742. https://doi.org/10.1641/0006-3568(2006)56[733:LKOSAI]2.0.CO;2

Darriba, D., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B. & Flouri, T. (2020) ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular Biology and Evolution* 37 (1):291–294.

https://doi.org/10.1093/molbev/msz189
Fröderström, H. (1931) The genus *Sedum* L. A systematic essay. *Acta Horti Gothoburgensis* 6, appendix: 1–111.
Fu, S.H. (1965) Species et Combinationes Novae Crassulacearum Sinicarum. *Acta Phytotaxonomica Sinica Additamentum* 10: 111–128.
Fu, K.T. & Ohba, H. (2001) Crassulaceae. *In*: Editorial Committee of Flora of China (ed.) *Flora of China* 8. Missouri Botanical Garden Press, St. Louis, pp. 244–401.
Fu, K.T. & Rao, G.Y. (1988) zhong guo jing tian ya shu de xin fen lei qun he ba bao shu yi xin zu he. *Acta Botanica Boreali-Occidentalia Sinica* 8 (2): 116–124.
Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
Hayata, B. (1913) *Icones Plantarum Formosanarum nec non et Contributiones ad Floram Formosanam 3.* The Bureau of Productive Industry, Government of Formosa, Taihoku, 222 pp. + 32 figs.
Hance, H.F. (1865) Descriptions of four new plants from southern China. *Journal of Botany, British and Foreign* 3: 378–381.
Ito, T., Chen, R., Yang, Q., Saito, Y., Yokota, M. & Kokubugata, G. (2014) Taxonomic reexamination of *Sedum formosanum* (Crassulaceae) in Japan, Taiwan, and the Philippines based on molecular data. *Journal of Phytogeography and Taxonomy* 62: l–9.
Ito, T., Yu, C.C., Nakamura, K., Chung, K.F., Yang, Q.E., Fu, C.X., Qi, Z.C. & Kokubugata, G. (2017a) Unique parallel radiations of high-mountainous species of the genus *Sedum* (Crassulaceae) on the continental island of Taiwan. *Molecular Phylogenetics and Evolution* 113: 9–22.
https://doi.org/10.1016/j.ympev.2017.03.028
Ito, T., Nakanishi, H., Chichibu, Y., Minoda, K. & Kokubugata, G. (2017b) *Sedum danjoense* (Crassulaceae), a new species of succulent plants from the Danjo Islands in Japan. *Phytotaxa* 309 (1): 23–34.
https://doi.org/10.11646/phytotaxa.309.1.2
Ito, T., Yu, C.C., Yokota, M. & Kokubugata, G. (2020a) *Sedum formosanum* subsp. *miyakojimense* (Crassulaceae), a new subspecies from Miyako-jima Island of the Ryukyu Islands, Japan. *PhytoKeys* 148: 51–70.
https://doi.org/10.3897/phytokeys.148.48957
Ito, T., Goto, M., Nakano, H. & Kokubugata, G. (2020b) A new species of succulent plants from the Muko-jima group of the Bonin Islands, Japan: *Sedum mukojimense* (Crassulaceae). *Phytotaxa* 450 (2): 188–198.
https://doi.org/10.11646/phytotaxa.450.2.4
IUCN (2022) *The IUCN Red list of the threatened species*, version 2022-1. Available from: http://www.iucnredlist.org (accessed: 22 July 2022).
Katoh, K., Rozewicki, J. & Yamada, K.D. (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in bioinformatics* 20 (4): 1160–1166.
https://doi.org/10.1093/bib/bbx108
Kozlov, A.M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* 35 (21): 4453–4455.
https://doi.org/10.1093/bioinformatics/btz305
Kumagae, N. (2016) *Natural history of Kawara-dake*. kaichosha, Fukuoka, 231 pp. [in Japanese]
Linnaeus, C. (1753) *Species Plantarum* 1. Laurentii Salvii, Holmiae, 560 pp.
Makino, T. (1891a) *Illustrated flora of Japan* I7. Keigyosya, Tokyo, 3 pp.
Makino, T. (1891b) *Illustrated flora of Japan* I10. Keigyosya, Tokyo, 2 pp.
Maximowicz, C. (1888) Diagnoses des plantes nouvelles asiatiques. VII. *Bulletin de l'Académie Impériale des Sciences de Saint-Pétersbourg* xxxii: 477–629.
Mayuzumi, S. & Ohba, H. (2004) The phylogenetic position of Eastern Asian *SeDOI: deae* (Crassulaceae) inferred from chloroplast and nuclear DNA sequences. *Systematic Botany* 29: 587–598.
https://doi.org/10.1600/0363644041744329
Messerschmid, T.F., Klein, J.T., Kadereit, G. & Kadereit, J.W. (2020) Linnaeus's folly–phylogeny, evolution and classification of *Sedum* (Crassulaceae) and Crassulaceae subfamily Sempervivoideae. *Taxon* 69 (5): 892–926.
https://doi.org/10.1002/tax.12316
Migo, H. (1937) Notes on the flora of south-eastern China. (Section III). *The Journal of the Shanghai Science Institute* 3: 221–228.
Miquel, F.A.G. (1866) Prolusio florae Iaponicae. *Annales Musei botanici lugduno-batavi* 2: 69–212.
Mort, M.E., Soltis, D.E., Soltis, P.S., Francisco-Ortega, J. & Santos-Guerra, A. (2002) Phylogenetics and evolution of the Macaronesian clade of Crassulaceae inferred from nuclear and chloroplast sequence data. *Systematic Botany* 27: 271–288.
Murray, M.G. & Thompson, W.F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 8: 4321–4325.
https://doi.org/10.1002/tax.12316
Nakamura, K., Chung, S.W., Kono, Y., Ho, M.J., Hsu, T.C. & Peng, C.I. (2014) *Ixeridium calcicola* (Compositae), a new limestone

endemic from Taiwan, with notes on its atypical basic chromosome number, phylogenetic affinities, and a limestone refugium hypothesis. *PloS one* 9 (10): e109797.
https://doi.org/10.1002/tax.12316

Nylander, J.A.A. (2004) *MrModeltest ver2*. Distributed by the author. Evolutionary Biology Centre, Uppsala University.

Ohba, H. (2001) Crassulaceae. *In:* Iwatsuki, K., Boufford, D.E. & Ohba, H. (eds.) *Flora of Japan 2b*. Kodansha, Tokyo, pp. 21–29.

Praeger, R.Li. (1919) On species of *Sedum* collected in China by L. H. Bailey in 1917. *In Proceedings of the Royal Irish Academy* 35 B (1): 1–8, + 3 plates.

Rambaut, A. (2018) *FigTree* v1.4.4. Available from: http://tree.bio.ed.ac.uk/software/figtree/ (accessed 22 July 2022)

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.
https://doi.org/10.1093/bioinformatics/btg180

Stephenson, R. (1994) *Sedum. cultivated stonecrops*. Timber Press, Portland, Oregon, 355 pp.

Tang, W.S. & Huang, T.C. (1993) Crassulaceae. *In*: Huang, T.C. (eds.) *Flora of Taiwan* vol. 3. Department of Botany, National Taiwan University, Taipei, pp. 15–34.

Thiede, J. & Eggli, U. (2007) Crassulaceae. *In*: Kubitzki, K. (ed.) *The Families and Genera of Vascular Plants* vol. 9. Springer, Berlin Heidelberg, pp. 83–118.
https://doi.org/10.1093/bioinformatics/btg180

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673−4680.
https://doi.org/10.1093/nar/22.22.4673

Wang, J., Ai, B., Kong, H. & Kang, M. (2017) Speciation history of a species complex of *Primulina eburnea* (Gesneriaceae) from limestone karsts of southern China, a biodiversity hot spot. *Evolutionary applications* 10 (9): 919–934.
https://doi.org/10.1111/eva.12495

Webb, P.B. & Berthelot, S. (1836–1841) *Histoire naturelle des Îles Canaries. Tome troisième. Deuxième partie. Phytographia canariensis. Sectio I*. Bethune, Paris, 220 pp.
https://doi.org/10.5962/bhl.title.60795

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.) *In PCR Protocols, A Guide to Methods and Applications*. Academic Press, San Diego, pp. 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhang, R.B., Deng, T., Dou, Q.L., He, L., Lv, X.Y. & Jiang, H. (2019) *Sedum lipingense* (Crassulaceae) identifying a new stonecrop species in SE Guizhou, China, based on morphological and molecular evidence. *PhytoKeys* 134: 125–133.
https://doi.org/10.3897/phytokeys.134.38287

Zou, C.Y., Meng, S.Y., Lu, Z.C. & Liu, Y. (2020) *Sedum nanlingense* (Crassulaceae), a new species from Guangxi, China. *Phytotaxa* 447 (3): 176–184.
https://doi.org/10.11646/phytotaxa.447.3.3