

Reconsiderations of Distribution and Taxonomic Status of Intraspecific Taxa in *Sedum japonicum* (Crassulaceae) Based on Morphological and Molecular Data

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(Received 8 February 2018; accepted 28 March 2018)

Abstract We conducted morphological observations and molecular phylogenetic analyses based on nrITS sequences to reconsider the taxonomic status and distribution of intraspecific taxa of *Sedum japonicum*, particularly *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* and *S. japonicum* subsp. *uniflorum*. Taxonomically, *S. japonicum* subsp. *japonicum* (including two varieties) and *S. japonicum* subsp. *oryzifolium* (including two varieties) could be treated as an intraspecific taxon of *S. japonicum*. Meanwhile, *S. japonicum* subsp. *uniflorum* and *S. japonicum* subsp. *boninense* could be respectively treated as *S. uniflorum* and *S. boninense*. Biogeographically, *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* could be considered to be distributed from the Ryukyu Archipelago northward of Amami Island to Fukushima in Japan, the southern part of the Korean Peninsula and Jeju Island in South Korea, and northeastern Taiwan. Taiwanese plants were traditionally thought to be *S. japonicum* subsp. *uniflorum*, but could be treated as *S. japonicum* subsp. *oryzifolium* var. *oryzifolium*. Therefore, we propose that the revised *S. uniflorum* is an endemic Japanese species distributed throughout the southern regions of Kyushu and the Ryukyus.

Key words : Crassulaceae, Phylogeny, Ryukyus, *Sedum*, Taiwan.

Introduction

The genus *Sedum* L., the largest and most widespread genus of Crassulaceae, consists of ca. 420 species (Thiede and Eggli, 2007) that include annual and perennial herbs with succulent leaves and stems. It is primarily distributed in arid environments in temperate to subtropical regions, and is most diverse in the Mediterranean, Central America, Himalayas, and East Asia (Stephenson, 1994; Thiede and Eggli, 2007). Previously, it was reported that, 18 species (six endemic) with six subspecies and seven varieties are distributed in Japan, and 15 of 25 taxa are

distributed in adjacent countries (Ohba, 2001).

Hooker and Arnott (1838) described *S. uniflorum* based on a type specimen collected from Okinawa Island in the Ryukyu Archipelago (hereafter, the Ryukyus); Makino (1891) described *S. oryzifolium* occurring in Kochi of Shikoku District, and Sagami of Honshu, Japan with an illustration; and Tuyama (1936) described *S. boninense* based on a type specimen collected from Chichijima Island, Bonin Islands, Japan. Subsequently, Ohba (2003) treated these three species as subspecies of *S. japonicum* based on a type specimen collected from Japan (Miquel, 1866), namely *S. japonicum* subsp. *uni-*

Table 1. Comparison of diagnostics of *Sedum japonicum* subsp. *uniflorum* and *S. japonicum* subsp. *oryzifolium* shown in Ohba (2003)

TRAITS \ TAXA	<i>S. japonicum</i> subsp. <i>uniflorum</i>	<i>S. japonicum</i> subsp. <i>oryzifolium</i>
Leaves	Narrowly oblong or liner-spatulate 3.0–6.0 × 1.5–2.5 mm	Widely liner-elliptic to narrowly oblong 3.0–7.0 × 1.7–3.5 mm
Inflorescence	Solitary	Cyme
Flowering Season	May–June	May–July
Habitat	Rocky slopes in seashores	Rocky slopes in seashores

florum, *S. japonicum* subsp. *oryzifolium*, and *S. japonicum* subsp. *boninense*. Ohba (2003) recognized four subspecies as infraspecific taxa of *S. japonicum*: *S. japonicum* subsp. *japonicum* (including var. *japonicum* and var. *senanense*), *S. japonicum* subsp. *oryzifolium* (including var. *oryzifolium* and var. *pumilum*), *S. japonicum* subsp. *uniflorum*, and *S. japonicum* subsp. *boninense*. These taxa were primarily distinguished using inflorescence morphology, cymose or solitary inflorescence, and subtle differences in leaf morphology (Table 1). However, the taxonomic status of the infraspecific taxa remain unclear, because they have morphological similarities of slightly compressed cylindrical leaves and some floral characteristics (Ohba, 1981, 2001). Consequently, further morphological and phylogenetic studies were required to reconsider their taxonomic status (Mayuzumi and Ohba, 2004).

Therefore, in the present study, we performed morphological investigations and molecular analyses of infraspecific taxa of *S. japonicum* collected from Japan, South Korea, and Taiwan to assess the taxonomic status and distribution range of the infraspecific taxa, particularly *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* and *S. japonicum* subsp. *uniflorum*.

Materials and Methods

Tentative taxonomic treatment

We tentatively follow the taxonomic treatment of Ohba (2003) for infraspecific taxa of *Sedum japonicum*, and then reconsider these taxonomic status in Discussion below. We identified plants following Ohba (2001, 2003), especially identified plants with cyme inflorescence as *S. japoni-*

cum subsp. *oryzifolium* var. *oryzifolium* (Fig. 1A and B); and those with solitary inflorescence as *S. japonicum* subsp. *uniflorum* (Fig. 1C and D).

Plant materials and morphological observation

In the present study, we examined in total 96 herbarium specimens of the infraspecific taxa of *S. japonicum* including *S. japonicum* subsp. *uniflorum* and *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* collected from Japan, South Korea and Taiwan in herbaria of National Museum of Nature and Science (TNS), Kagoshima University Museum (KAG), National Taiwan University (TAI) and Taiwan Forestry Research Institute (TAIF). Out of them, we extracted DNA from nine plants collected in the present studies.

Molecular analysis

We examined 49 taxa of *Sedum* sect. *Sedum* and two species of *Sedum* sect. *Oreades* in East Asia including eight and five accessions of *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* and *S. japonicum* subsp. *uniflorum*, respectively (Tables 2 and 3). After identification based on morphological characteristics, we sequenced internal transcribed spacer (ITS) region of nine samples, including five samples of *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* from Japan, South Korea, and Taiwan, and four samples of *S. japonicum* subsp. *uniflorum* from Japan (Table 2).

ITS sequences reported in previous molecular studies by Mayuzumi and Ohba (2004), Ito *et al.* (2014a, 2014b, 2017a, 2017b) and stored in GenBank were used for the others samples, including the other four infraspecific taxa of *S. japonicum* (Tables 2 and 3). Following previous phyloge-



Fig. 1. Habit of *Sedum japonicum* subsp. *oryzifolium* var. *oryzifolium* and *S. japonicum* subsp. *uniflorum*. **A:** *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* in Kagoshima, Kyushu Dist.. **B:** *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* in Fukushima, Tohoku Dist. (*T. Ito 2061*) photo by Shuichi Nemoto. **C:** *S. japonicum* subsp. *uniflorum* in Kagoshima, Kyushu Dist. (*T. Ito 3244*). **D:** *S. japonicum* subsp. *uniflorum* in Kagoshima, Yakushima Isl. The Ryukyus. (*T. Ito 2639*). **E:** *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* in Taiwan (*T. Ito 1444*).

netic study of Crassulaceae (Mayuzumi and Ohba, 2004), ITS sequences of four *Aeonium* and one *Greenovia* species reported by Mort *et al.* (2002) were used as outgroup. In total, 61 operational taxonomic units (OTUs) were included in

our molecular phylogenetic analysis (Tables 2 and 3). Voucher specimens for the materials were deposited in the TNS herbarium.

Genomic DNA was extracted from silica-dried leaves using a DNeasy Kit (QIAGEN, Valencia,

Table 2. List of plant material collected *Sedum japonicum* subsp. *uniflorum* and *S. japonicum* subsp. *oryzifolium* with their respective locality, vouchers and DDBJ Accession numbers

Taxon	Source locality	Abbreviation*	Voucher (herbarium)	Accession no.
<i>S. japonicum</i> subsp. <i>oryzifolium</i> var. <i>oryzifolium</i>	Japan: Kyushu, Nagasaki, Tsushima	J-KN1	<i>T. Ito 392</i> (TNS)	AB906482 ¹
	Japan: Shikoku, Kochi	J-SK	<i>T. Ito 692</i> (TNS)	—
	Japan: Tohoku, Fukushima	J-TF	<i>T. Ito 2061</i> (TNS)	—
	Japan: Kyushu, Nagasaki	J-KN2	<i>T. Ito 2073</i> (TNS)	—
	Korea: Jeollanam-do	K-JL	<i>T. Ito 2106</i> (TNS)	—
	Japan: Kyushu, Nagasaki	J-KN3	<i>T. Ito 2285</i> (TNS)	LC229239 ²
	Korea: Jeju-do	K-JJ	<i>G. Kokubugata 16854</i> (TNS)	AB906481 ¹
<i>S. japonicum</i> subsp. <i>uniflorum</i>	Japan: Ryukyu, Okinawa, Okinawa Isl.	J-RO	<i>T. Ito 447</i> (TNS)	LC229241 ²
	Japan: Ryukyu, Kagoshima, Yakushima Isl.	J-RY	<i>T. Ito 2639</i> (TNS)	—
	Japan: Kyushu, Kagoshima	J-KK1	<i>T. Ito 3244</i> (TNS)	—
	Japan: Kyushu, Kagoshima	J-KK2	<i>T. Ito 3483</i> (TNS)	—
	Japan: Ryukyu, Okinawa, Sesoko Isl.	J-RS	<i>T. Ito 3856</i> (TNS)	—
	Taiwan: Yilan	T-YL	<i>T. Ito 1444</i> (TNS)	—

* Reported by ¹ Ito *et al.* (2014a), ² Ito *et al.* (2017a).

** Abbreviations are used for Fig. 2.

CA, USA) following the manufacturer's protocol. 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). Amplifications were performed using EmeraldAmp PCR Master Mix dye (Takara, Otsu, Japan). 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 checked by electrophoresis before purification 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). The Sanger sequencing product 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 in this study were registered in the DNA Data Bank of Japan (DDBJ, extant since 1983).

The ITS sequences were aligned using ClustalW 1.8 (Thompson *et al.*, 1994) and then adjusted manually. Phylogenetic analyses were conducted based on a Bayesian approach using

MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) and maximum-likelihood (ML) phylogenetic analysis using RAxML (Stamatakis, 2014). In the Bayesian phylogenetic analysis, we used the Akaike Information Criterion (AIC) implemented in MrModeltest 2.2 (Nylander, 2004) to obtain an appropriate evolutionary model of nucleotide substitutions. Then we performed 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. The MCMCMC was 10 million generations long, 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. A 50% majority consensus tree of the output tree file from MrBayes was generated using FigTree ver. 1.3.1 (Rambaut, 2009). The ML phylogenetic analyses were implemented in RAxML 8 (Stamatakis, 2014) with a GTRGAMMA substitution model. The ML bootstrap proportions (BPs) and trees were obtained by simultaneously running rapid bootstrapping with 10,000 iterations followed by a search for

Table 3. Taxon, locality, vouchers, accession number and reference for ITS sequences of *Sedum*, *Aeonium* and *Greenovia* species registered in the DDBJ/ENA/NCBI database, and additionally used for the present molecular analyses

Taxon	Source locality	Voucher (herbarium)	Accession no.
INGROUP			
Section <i>Oreades</i>			
<i>S. oreades</i>	Nepal	<i>F. Miyamoto et al. TI9420140</i> (TI)	AB088632 ¹
<i>S. trullipetalum</i>	Nepal	<i>F. Miyamoto et al. TI9420132</i> (TI)	AB088630 ¹
Section <i>Sedum</i>			
<i>S. actinocarpum</i>	Taiwan: New Taipei	<i>T. Ito 1732</i> (TNS)	LC229264 ³
<i>S. alfredii</i>	China: Guangdong	<i>G. Kokubugata 17190</i> (IBSC)	AB930259 ²
<i>S. arisanense</i>	Taiwan: Taichung	<i>T. Ito 1836</i> (TNS)	LC229272 ³
<i>S. brachyrinchium</i> var. <i>brachyrinchium</i>	Taiwan: Yilan	<i>T. Ito 1633</i> (TNS)	LC229275 ³
<i>S. brachyrinchium</i> var. <i>taiwanalpinum</i>	Taiwan: Taichung	<i>T. Ito 1823</i> (TNS)	LC229278 ³
<i>S. bulbiferum</i>	Japan: Kyushu, Nagasaki	<i>T. Ito 416</i> (TNS)	LC229234 ³
<i>S. erici-magnusii</i>	China	<i>T. Ito 2077</i> (TNS)	LC229235 ³
<i>S. formosanum</i>	Taiwan: New Taipei	<i>G. Kokubugata 16446</i> (TNS)	AB930272 ²
<i>S. japonicum</i> subsp. <i>japonicum</i> var. <i>senanense</i>	Japan: Tohoku, Akita	<i>T. Ito 2200</i> (TNS)	LC229238 ³
<i>S. japonicum</i> subsp. <i>japonicum</i> var. <i>japonicum</i>	Japan: Shikoku, Kochi	<i>T. Ito 723</i> (TNS)	LC229237 ³
<i>S. japonicum</i> subsp. <i>oryzifolium</i> var. <i>pumilum</i>	Japan: Kanto, Tokyo	<i>T. Ito 2287</i> (TNS)	LC229240 ³
<i>S. japonicum</i> subsp. <i>boninense</i>	Japan: Kanto, Tokyo, Ogasawara Isl.	<i>T. Ito 2371</i> (TNS)	LC229242 ³
<i>S. jiulungshanense</i>	China: Zhejiang	<i>CMQ20150076</i> (TNS)	LC229243 ³
<i>S. kiangnanense</i>	China: Zhejiang	<i>T. Ito 1030</i> (TNS)	LC229244 ³
<i>S. hangzhouense</i>	China: Zhejiang	<i>T. Ito 2604</i> (TNS)	LC260130 ⁴
<i>S. hakonense</i>	Japan: Chubu, Yamanashi	<i>T. Ito 623</i> (TNS)	AB930278 ²
<i>S. lineare</i>	Japan	<i>S. Mayuzumi C00120</i> (TI)	AB088623 ¹
<i>S. lungtsuanense</i>	China: Zhejiang	<i>T. Ito 3563</i> (TNS)	LC260131 ⁴
<i>S. multicaule</i>	China	<i>T. Ito 625</i> (TNS)	LC229248 ³
<i>S. makinoi</i>	Japan: Kansai, Okayama	<i>T. Ito 2325</i> (TNS)	LC229246 ³
<i>S. mexicanum</i>	Japan: Shikoku, Kochi	<i>T. Ito 647</i> (TNS)	LC229247 ³
<i>S. morrisonense</i> var. <i>morrisonense</i>	Taiwan: Nantou	<i>T. Ito 2348</i> (TNS)	LC229289 ³
<i>S. morrisonense</i> var. <i>kwanzuense</i>	Taiwan: Hsinchu	<i>T. Ito 2440</i> (TNS)	LC229293 ³
<i>S. nagasakiianum</i>	Japan: Kyushu, Nagasaki	<i>T. Ito 2064</i> (TNS)	LC229249 ³
<i>S. nokoense</i>	Taiwan: Nantou	<i>T. Ito 3196</i> (TNS)	LC229294 ³
<i>S. oligospermum</i>	China: Zhejiang	<i>CMQ 74</i> (TNS)	LC229250 ⁴
<i>S. polytrichoides</i> subsp. <i>polytrichoides</i>	Japan: Kyushu, Oita	<i>T. Ito 2247</i> (TNS)	LC229252 ³
	China: Zhejiang	<i>T. Ito 1057</i> (TNS)	LC229251 ³
<i>S. polytrichoides</i> subsp. <i>yabeantum</i> var. <i>yabeantum</i>	Japan: Kyushu, Nagasaki	<i>T. Ito 396</i> (TNS)	AB906490 ²
<i>S. polytrichoides</i> subsp. <i>yabeantum</i> var. <i>setouchiense</i>	Japan: Shikoku, Kagawa	<i>T. Ito 2298</i> (TNS)	LC229253 ³
<i>S. rupifragum</i>	Japan: Sanin, Shimane	<i>T. Ito 2070</i> (TNS)	LC229254 ³
<i>S. sarmentosum</i>	Japan: Tohoku, Iwate	<i>T. Ito 978</i> (TNS)	LC229255 ³
<i>S. satumense</i>	Japan: Kyushu, Kagoshima	<i>T. Ito 2295</i> (TNS)	LC229256 ³
<i>S. sekiteiense</i>	Taiwan: New Taipei	<i>T. Ito 1456</i> (TNS)	LC229295 ³
<i>S. subtile</i>	Japan: Kyushu, Oita	<i>T. Ito 2259</i> (TNS)	LC229257 ³
<i>S. taiwanianum</i>	Taiwan: Taichung	<i>T. Ito 2523</i> (TNS)	LC229296 ³
<i>S. tarokoense</i>	Taiwan: Hualien	<i>T. Ito 2025</i> (TNS)	LC229298 ³
<i>S. tetractinum</i>	China: Zhejiang	<i>T. Ito 3623</i> (TNS)	LC260135 ⁴
<i>S. tianmushanense</i>	China: Zhejiang	<i>LP 67</i> (TNS)	LC229261 ³
<i>S. tosaense</i>	Japan	<i>T. Ito 655</i> (TNS)	LC229258 ³
<i>S. triactina</i>	Nepal	<i>F. Miyamoto et al. TI9596091</i> (TI)	AB088629 ¹
<i>S. triangulosepalum</i>	Taiwan: Yilan	<i>T. Ito 2508</i> (TNS)	LC229299 ³
<i>S. tricarpum</i>	Japan: Kyushu, Oita	<i>T. Ito 2269</i> (TNS)	LC229259 ³
<i>S. truncastigium</i>	Taiwan: Hualien	<i>T. Ito 2766</i> (TNS)	LC229305 ³
<i>S. zentaro-tashiroi</i>	Japan: Kyushu, Nagasaki, Tsushima	<i>T. Ito 441</i> (TNS)	LC260136 ⁴
<i>Sedum</i> sp.	China	<i>JP 404</i>	LC229262 ³
OUTGROUP			
<i>Aeonium castello-paivae</i>	Canary	<i>M.E. Mort 1519</i> (WS)	AY082127 ⁵
<i>Aeonium gomerense</i>	Canary	<i>M.E. Mort 1454</i> (WS)	AY082133 ⁵
<i>Aeonium viscatum</i>	Canary	<i>M.E. Mort 1432</i> (WS)	AY082154 ⁵
<i>Aeonium lancerottense</i>	Canary	<i>M.E. Mort 1518</i> (WS)	AY082143 ⁵
<i>Greenovia aizoon</i>	Canary	<i>M.E. Mort 1425</i> (WS)	AY082112 ⁵

* Reported by ¹ Mayuzumi and Ohba (2004), ² Ito *et al.* (2014b), ³ Ito *et al.* (2017a), ⁴ Ito *et al.* (2017b) and ⁵ Mort *et al.* (2002).

the most likely tree.

Results

Identification using morphological characteristics

We identified 49 specimens collected from Tohoku, Kanto, Chubu, Kansai, Shikoku, Kyushu, and the Ryukyus northward of Amami Island in Japan, and the southern part of the Korean Peninsula and Jeju Island in South

Korea, and northeastern Taiwan as *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* based on their cyme inflorescence (Fig. 1, Table 2). In the present survey, we newly collected a plant identified, as *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* from Fukushima, Tohoku District, Japan, as the northernmost population (Fig. 1, Table 2).

We identified specimens collected from Minamisatsuma and Shibushi of Kagoshima, Kyushu, and the Ryukyus as *S. japonicum* subsp. *uniflorum* because of their solitary inflorescence, and

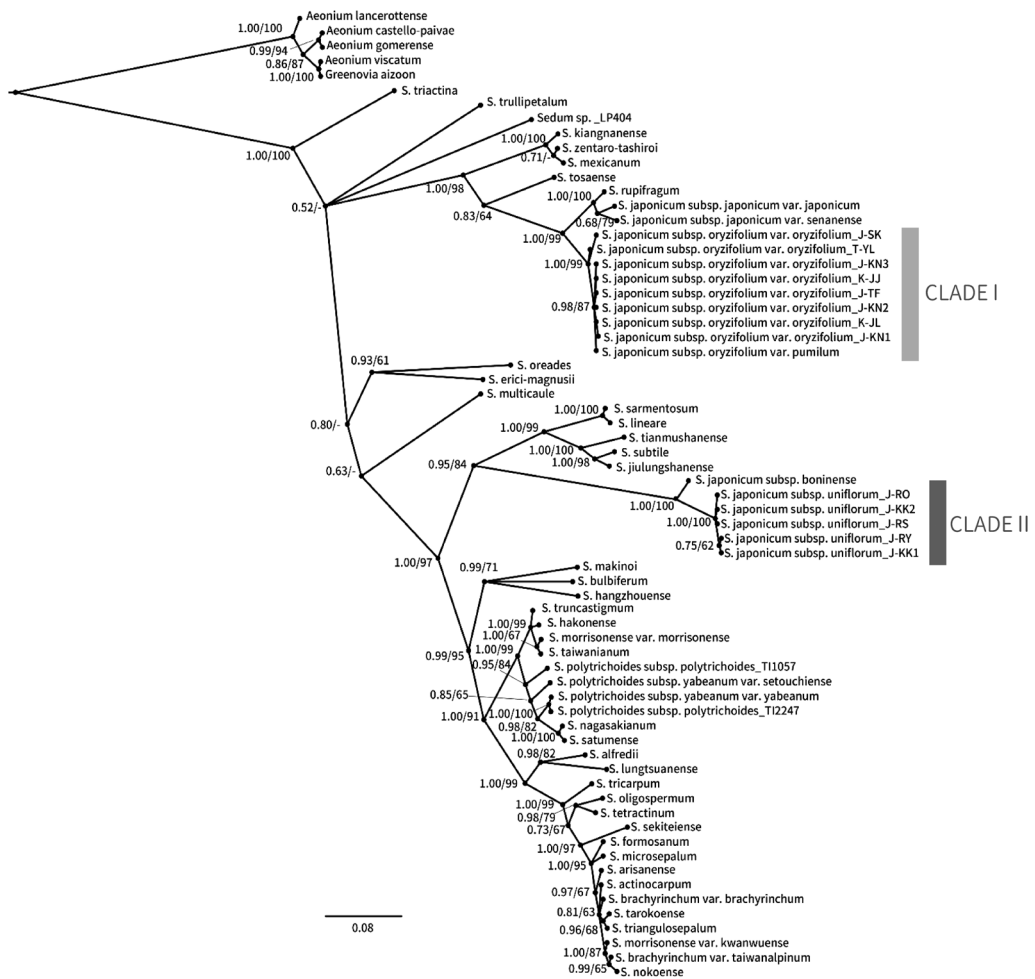


Fig. 2. Bayesian phylogenetic tree based on nrITS of eastern Asian *Sedum* species and phylogenetic position of *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* and *S. japonicum* subsp. *uniflorum*. The topology of the maximum likelihood (ML) tree was highly compatible with the Bayesian tree. Bayesian posterior probabilities (PPs: left) and bootstrap percentages from ML analysis (BP: right) are shown (see Table 2 for the abbreviations of localities).

did not find any plants identified as *S. japonicum* subsp. *uniflorum* in Japan northward of Kagoshima Prefecture and Taiwan (Fig. 1, Table 2). We additionally identified one specimen collected from Bonin Island as *S. japonicum* subsp. *boninense* based on the leaf morphology and underground bulb formation.

Phylogenetic analyses using nrITS sequences

The GTR+I+G model was used for the Bayesian analysis. A 50% majority rule consensus tree of all post-burn-in trees was created with mean branch lengths and posterior probabilities (PPs; Fig. 2). The bootstrap percentages (BSs; 1,000 replicates) were plotted on the Bayesian tree (Fig. 2) because the topology of the maximum likelihood tree (not shown) was compatible with that of the Bayesian 50% majority rule consensus tree.

Both of Bayesian and ML analyses supported two clades, i.e., one comprising plants identified herein as *S. japonicum* subsp. *oryzifolium* var. *oryzifolium*, and the other, *S. japonicum* subsp. *uniflorum* (Fig. 2). Five Japanese plants, two South Korean plants, and one Taiwanese plant identified as *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* based on the inflorescence morphological characteristics, formed a well-supported clade together with *S. japonicum* subsp. *oryzifolium* var. *pumilum* (Clade I: PP/BS = 1.00/99%). Clade I was sister to a clade comprising *S. japonicum* subsp. *japonicum* var. *japonicum*, *S. japonicum* subsp. *japonicum* var. *senanense* and *S. rupifragum* (Fig. 2). Meanwhile, five Japanese plants identified as *S. japonicum* subsp. *uniflorum* formed a clade with strong statistical support (Clade II: 1.00/100). Clade II was located in an apparently different lineage from Clade I to *S. japonicum* subsp. *boninense* with strong statistical support (1.00/100; Fig. 2).

Discussion

Taxonomic relationships

Based on the present data, it is reasonable to treat *S. japonicum* subsp. *japonicum* (including

two varieties) and *S. japonicum* subsp. *oryzifolium* (including two varieties) as infraspecific taxa following Ohba (2003) because of their morphological similarities and close phylogenetic relationship. Conversely, a previous molecular phylogenetic study (Ito *et al.*, 2017a) and the present study indicated that *S. japonicum* subsp. *uniflorum* had a different lineage from the other infraspecific taxa of *S. japonicum*. Therefore, we suggest that *S. japonicum* subsp. *uniflorum* can be treated as *S. uniflorum* Hooker & Arnott (1838), and thus we treat *S. japonicum* subsp. *uniflorum* as *S. uniflorum* hereafter in this paper. Furthermore, the results of a molecular phylogenetic study (Ito *et al.*, 2017a) and the present study suggested that *S. japonicum* subsp. *boninense* had a different lineage from the other infraspecific taxa of *S. japonicum*, and was sister to *S. uniflorum*. Morphologically, *S. japonicum* subsp. *boninense* and *S. uniflorum* share similar inflorescence features, such as a small number of flowers in each branch, differing from the other infraspecific taxa of *S. japonicum* (Ohba, 2001, 2003). Meanwhile, *S. japonicum* subsp. *boninense* is distinguishable from *S. uniflorum* in that the aboveground part of the plant withers in summer and it forms underground bulbs (Ohba, 2001). Therefore, we suggest that *S. japonicum* subsp. *boninense* can be treated as *S. boninense* Tuyama (1936). The taxonomic treatment of the present study is not in agreement with the treatments of Ohba (1981, 2001). In addition, *S. rupifragum* is phylogenetically closely related to two varieties of *S. japonicum* subsp. *japonicum*; however, this species has flattish, linear-lanceolate or linear leaves, differing from the two varieties of *S. japonicum* subsp. *japonicum*. From these results, we conclude that *S. rupifragum* should not be included as an infraspecific taxon of *S. japonicum*. Further studies are required for reconsidering *S. rupifragum*.

Taxonomic treatment

Sedum japonicum Siebold ex Miq. subsp. *oryzifolium* (Makino) H. Ohba var. *oryzifolium* (Makino) H. Ohba in J. Jpn. Bot. 78: 300 (2003).

– *Sedum oryzifolium* Makino, Ill. Fl. Jpn. 8: 2, tab. 50 (1891) – *Sedum uniflorum* subsp. *oryzifolium* (Makino) H.Ohba in J. Jpn. Bot. 56: 184 (1981); in Fl. Jap. Iib: 25 (2001).

Distribution: Japan: Honshu (southward of Fukushima), Shikoku, Kyushu and the Ryukyus (northward of Amami Island).

Japanese name: Taito-gome.

Specimens examined: **JAPAN TOHOKU** Fukushima: Iwaki, *T. Ito 2061* (TNS). **KANTO** Ibaraki: Hitachinaka, *T. Ito 2594* (TNS); Chiba: Choshi, *J. Haginiwa 6559*, *T. Ito 3121* (TNS), Minami-boso, *G. Kokubugata 19490* (TNS); Tokyo: Izu-Oshima Isl., *T. Yamada 32* (TNS), *S. Kobayashi 1754* (KAG), Toshima Isl., *H. Noguchi 4495* (KAG), Kozu Isl., *T. Ito 2382* (TNS), Miyake Isl., *G. Kokubugata 19801* (TNS), Mikura Isl., *T. Yamada 306-1* (TNS). **CHUBU** Shizuoka: Shimoda, *F. Konta & H. Noguchi 23153*, *T. Ito 4641* (TNS), Minami-Izu, *H. Noguchi 4495* (KAG). **KANSAI** Wakayama: Susami, *T. Ito 4053* (TNS); Shimane: Izumo, *T. Ito 3136* (TNS), Okinoshima Isl., *T. Ito 3171* (TNS). **SHIKOKU**: Kochi: Muroto, *T. Ito 692*, *T. Yamada 432* (TNS), *S. Hatusima 22074* (KAG); Ehime: Niihama, *M. Furuse 48309* (KAG). **KYUSHU** Nagasaki: Tsushima, *J. Haginiwa 6557*, *T. Ito 375*, *392*, *431* (TNS), Nagasaki, *T. Ito 2073*, *2285* (TNS), Saikai, *T. Ito 4225* (TNS); Saga: Karatsu, *T. Ito 2915*, *2916* (TNS); Kumamoto: Amakusa, *S. Hatusima 29595* (KAG); Kagoshima: Minamisatsuma, *T. Ito 4018* (TNS), Satsumasendai, *T. Ito 4032* (TNS). **RYUKYU** Kagoshima: Ujimukae Isl., *T. Shiuchi 99* (KAG), Uchima Isl., *T. Shiuchi 195* (KAG), Kusagaki Isls., *S. Sako 8303* (KAG), Mage Isl., *S. Ohuchiyama 66* (KAG), Gaja Isl., *S. Hatusima 15780*, *S. Sako & K. Kawanabe 2335* (KAG), Taira Isl., *T. Shiuchi 2685* (KAG), Akuseki Isl., *T. Shiuchi 2857* (KAG), Amami Isl., *G. Kokubugata 18185*, *18968* (TNS), Kakeroma Isl., *G. Kokubugata 18970* (TNS), Yoro Isl., *T. Ito 4585* (TNS). **SOUTH KOREA JEOLLANAM-DO**: Sani-myeon, *T. Ito 2106* (TNS), **JEJU-DO**: Jeju Isl., *G. Kokubugata 16824*, *16854*, *T. Ito 2972* (TNS). **TAIWAN NEW TAIPEI**: Gongliao, *S.W. Chung 10319*, *P.F.*

Lu 23790 (TAIF); **ILAN**: Su'ao, *T. Ito 1444* (TNS), *C.F. Chen 3642* (TAIF), *W.S. Tang 1804*, *S.F. Huang 5094* (TAI), Toucheng, *P.F. Lu 15833* (TAIF), *H.N. Yang 2737* (TAI).

Sedum uniflorum Hook. & Arn. in Bot. Beechey Voy. 263 (1838) – *Sedum japonicum* subsp. *uniflorum* H.Ohba, J. Jpn. Bot. 78: 300 (2003).

Holotype: *Arnott s.n.* (K). Japan, Loo Choo (the Ryukyus).

Synonym: *Sedum sasakii* Hayata, Icon. Pl. Form. 3: 111 (1913).

Distribution: Japan, Kyushu (southward of Kagoshima) and the Ryukyus.

Japanese name: Kogome-mannen-gusa.

Specimens examined: **JAPAN KYUSHU** Kagoshima: Minamisatsuma, *T. Ito 3244*, *3483* (TNS). Shibushi, *T. Naito 9490* (KAG). **RYUKYU** Kagoshima: Mage Isl., *S. Ohuchiyama 67* (KAG), Yakushima Isl., *T. Ito 2639* (TNS), *S. Hatusima 41368* (KAG), Kuchinoshima Isl., *S. Sako 7379*, *T. Shiuchi & M. Hotta 206*, *T. Shiuchi 975* (KAG), Takarajima Isl., *S. Hatusima 15851*, *T. Shiuchi 3395* (KAG), Kikai Isl., *S. Hatusima & S. Sako 23698*, *31061*, *K. Yoshinaga 292* (KAG), Amami Isl., *H. Ohno 31*, *S. Ohuchiyama 50*, *S. Hatusima 20259* (KAG), Tokunoshima Isl., *G. Kokubugata 17598*, *17635*, *T. Ito 961* (TNS), Okinoerabu Isl., *T. Ito 2609* (TNS), *S. Hatusima 21400*, *31006*, *S. Hatusima & S. Sako 21400* (KAG), Yoron Isl., *S. Hatusima & S. Sako 30880* (KAG); Okinawa: Okinawa Isl., *G. Kokubugata 18322*, *T. Ito 447*, *850*, *861*, *H. Umemoto 1370* (TNS), *S. Hatusima 17556*, *17914* (KAG), Sesoko Isl., *T. Ito 3856* (TNS), Iheya Isl., *T. Ito 4646* (TNS), Aguni Isl., *T. Ito 2531* (TNS), Kume Isl., *S. Tawada 18427* (KAG), Ishigaki Isl., *J. Haginiwa 32465*, *T. Ito 3973* (TNS), *S. Kawagoe 4674* (KAG).

Sedum boninense Yamam. ex Tuyama in Bot. Mag. Tokyo 50: 428 (1936) – *Sedum uniflorum* subsp. *boninense* (Yamam. ex Tuyama) H.Ohba, J. Jpn. Bot. 56: 184 (1981); Fl. Jap. Iib: 26 (2001) – *Sedum japonicum* subsp. *boninense* (Yamam. ex Tuyama) H.Ohba, J. Jpn. Bot. 78:

300 (2003).

Holotype: *T. Soma s.n.* (TI). Japan, Chichijima Island of Bonin Islands.

Distribution: Japan, Bonin Islands.

Japanese name: Munin-taito-gome.

Specimens examined: **JAPAN KANTO** Tokyo: Chichijima Isl., *J. Haginiwa 40201* (TNS).

Distribution range of Sedum japonicum subsp. oryzifolium var. oryzifolium

According to the regional flora of East Asian countries, *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* was thought to be distributed in coastal areas of Honshu (westward of Kanto District), Shikoku, and Kyushu Districts, extending to Amami Island of the Ryukyus in Japan, and the southern part of the Korean Peninsula and Jeju Island in South Korea (Tang and Huang, 1993; Ohba, 2001; Korea National Arboretum, 2018).

Our data newly indicate that, in Japan, the northernmost limit of the distribution range of *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* is Fukushima, Tohoku District, Japan (Fig. 3). Meanwhile, we did not find any *S. japonicum*

subsp. *oryzifolium* var. *oryzifolium* plants in either herbarium surveys or field surveys (data not shown) in the area from Okinawa Island to Yonaguni Island of the Ryukyus. This indicates that *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* is distributed from Japan proper to Taiwan, being absent from Okinawa to Yonaguni Island of the Ryukyus.

Only a few cases of such disjunct distribution patterns have been reported, including *Premna microphylla* Turcz. (Lamiaceae) in the coastal area of northeastern Taiwan and partially from the Amami islands to Kansai District in Japan (Hatusima, 1975). These disjunctive distribution patterns might be caused by approximately 2.0–3.0°C higher average temperature in winter (December–February) in the Ryukyus (Okinawa to Yonaguni Island) than the Amami Islands and northeastern coastal area of Taiwan (Central Weather Bureau, 2018; Japan Meteorological Agency, 2018), because we personally observed that *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* requires several weeks at low temperatures to flower (vernalization) under cultivation conditions. This affects the fitness of the species, and

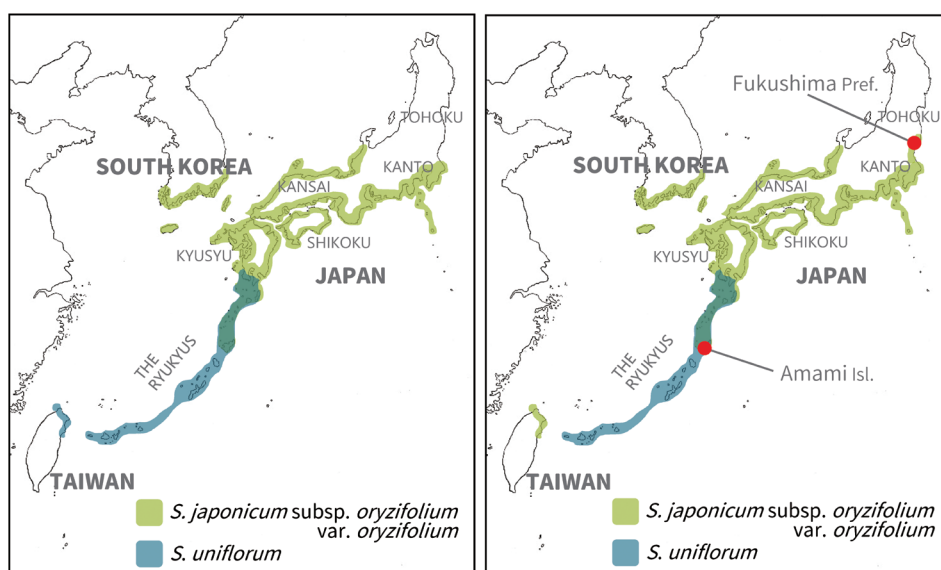


Fig. 3. Distribution map of *Sedum japonicum* subsp. *oryzifolium* var. *oryzifolium* and *S. uniflorum*. Previous distribution map based on Flora of Taiwan (Tang and Huang, 1993) and Flora of Japan (Ohba, 2001) (left), and revised distribution map in the present study (right).

possibly caused a population decline in the Ryukyus, leading to the present distribution pattern. Further investigations, including population genetic and physiological analyses, could help clarify the historical process of this disjunctive distribution pattern.

Furthermore, the Fukushima population currently has an extremely small population size, and coastal revetment work is ongoing near its natural habitat. Therefore, we suggest that the Fukushima population should be designated as a conservation target at the prefecture level.

Distribution range of Sedum uniflorum

Sedum uniflorum was previously thought to be distributed in coastal areas from northeastern Taiwan to southern Kyushu and throughout the Ryukyus (Liu and Chung, 1977; Tang and Huang, 1993; Ohba, 2001). By contrast, Lin (1999) mentioned that *S. uniflorum* subsp. *oryzifolium* (= *S. japonicum* subsp. *oryzifolium* var. *oryzifolium*) was distributed, and *S. uniflorum* was not distributed, in Taiwan based on morphological observation.

In the present study, we did not find any specimens of *S. uniflorum*, but rather all specimens were identified as *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* based on morphological and molecular data. *Sedum uniflorum* and *S. japonicum* subsp. *oryzifolium* var. *oryzifolium* have similar leaf morphologies and habitat, such as coastal rocky slopes (Ohba, 2001); therefore, these morphological and habitat similarities might have resulted in the misidentification of Taiwanese plants. We suggest that the Taiwanese plant treated as *S. uniflorum* could be *S. japonicum* subsp. *oryzifolium* var. *oryzifolium*. Therefore, we conclude that *S. uniflorum* could be an endemic Japanese species distributed from the Ryukyus to Kagoshima, Kyushu District, Japan (Fig. 3 right).

Acknowledgment

We thank S. Nemoto, C. Park, A. Abe, T. Yamada, H. Umemoto and M. Yokota for assist-

ing with field work; M. Kato for valuable comments; and herbaria of Kagoshima University Museum (KAG), National Taiwan University (TAI) and Taiwan Forestry Research Institute (TAIF) for herbarium survey in the present study. This study was partly supported by JSPS KAKENHI Grant Numbers 16J08504 (TI) and 16K14798 & 17H04609 (GK).

References

- Central Weather Bureau 2018 onward. Climate Statistics (1941 onward). Available from: <http://www.cwb.gov.tw/V7e/climate/dailyPrecipitation/dP.htm> [accessed: 4 February 2018].
- DDBJ 1983 onward. DNA Data of Bank of Japan, National Institute of Genetics, Japan, Mishima. Available from: <http://www.ddbj.nig.ac.jp/> [accessed: 30 January 2018].
- Hatusima, S. 1975. Flora of the Ryukyus, added and corrected edition. Okinawa Association of Biology Education, Naha.
- Hooker, W. J. and Arnott, G. A. 1838. The Botany of Captain Beechey's Voyage, part 7. 485 pp. Henry G. Bohn, London.
- Ito, T., Nakamura, K., Park, C. H., Song, G. P., Maeda, A., Tanabe, Y. and Kokubugata, G. 2014a. Nuclear and plastid DNA data confirm that *Sedum tosaense* (Crassulaceae) has a disjunct distribution between Pacific mainland Japan and Jeju Island, Korea. *Phytotaxa* 177(4): 221–230.
- Ito, T., Chen, R., Yang, Q., Saito, Y., Yokota, M. and Kokubugata, G. 2014b. Taxonomic reexamination of *Sedum formosanum* (Crassulaceae) in Japan, Taiwan, and the Philippines based on molecular data. *Journal of Phytogeography and Taxonomy* 62: 1–9.
- Ito, T., Yu, C., Nakamura, K., Chung, K., Yang, Q., Fu, C., Qi, Z. and 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.
- Ito, T., Nakanishi, H., Chichibu, Y., Minoda, K. and Kokubugata, G. 2017b. *Sedum danjoense* (Crassulaceae), a new species of succulent plants from the Danjo Islands in Japan. *Phytotaxa* 309(1): 23–34.
- Japan Meteorological Agency 2018. Meteorological observation data (1956 onward). Available from: <http://www.jma.go.jp/jma/indexe.html> [accessed: 4 February 2018].
- Korea National Arboretum 2018. Korean Plant Name Index (2004 onward). Available from: <http://www.nature.go.kr/kpni/english/index.jsp> [accessed: 30 Janu-

- ary 2018].
- Lin, H. 1999. A taxonomic study of *Sedum* L. (Crassulaceae) of Taiwan. Master course thesis. National Taiwan Normal University, Taipei.
- Liu, T. and Chung, N. 1977. Crassulaceae. In: Editorial Committee Flora of Taiwan (ed.), Flora of Taiwan vol. 3. 1st ed., pp. 10–34. Editorial Committee Flora of Taiwan, Taipei.
- Makino, T. 1891. Illustrated Flora of Japan 18. 3 pp. with 6 figure plates. Keigyosya, Tokyo.
- Mayuzumi, S. and Ohba, H. 2004. The phylogenetic position of Eastern Asian Sedoideae (Crassulaceae) inferred from chloroplast and nuclear DNA sequences. Systematic Botany 29: 587–598.
- Miquel, F. A. W. 1866. Prolusio florum japonicarum. Annales Musei Botanici Lugduno-Batavi 2: 69–212.
- Mort, M. E., Soltis, D. E., Soltis, P. S., Francisco-Ortega, J. and 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.
- Nylander, J. A. A. 2004. MrModeltest ver 2. Distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Ohba, H. 1981. Nomenclatural changes and notes on Japanese Sedoideae. Journal of Japanese Botany 56: 181–187.
- Ohba, H. 2001. Crassulaceae. In: Iwatsuki, K., Boufford, D. E. and Ohba, H. (eds.), Flora of Japan 2b. pp. 21–29. Kodansha, Tokyo.
- Ohba, H. 2003. New names of *Sedum uniflorum* Hook. and Arn. and its infraspecific taxa (Crassulaceae). Journal of Japanese Botany 78: 300–301.
- Rambaut, A. 2009. FigTree v1.3.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh.
- Ronquist, F. and Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.
- Stephenson, R. 1994. *Sedum*. Cultivated Stonecrops. 355 pp. Timber Press, Portland, Oregon.
- Stamatakis, A. 2014. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313.
- Tang, W. and Huang, T. 1993. Crassulaceae. In: Editorial Committee of the Flora of Taiwan (ed.), Flora of Taiwan, vol. 3, pp. 15–34. Editorial Committee of the Flora of Taiwan, Taipei.
- Thiede, J. and Eggli, U. 2007. Crassulaceae. In: Kubitzki, K. (ed.), The Families and Genera of Vascular Plants, vol. 9. pp. 83–118. Springer, Berlin.
- Thompson, J. D., Higgins, D. G. and 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.
- Tuyama, T. 1936. Plantae Boninenses Novae vel Criticae. VII. Botanical Magazine, Tokyo 50: 425–430.
- White, T. J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (eds.), PCR Protocols, A Guide to Methods and Applications, pp. 315–322. Academic Press, San Diego.