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Sedum danjoense (Crassulaceae), a new species of succulent plants from the Danjo Islands in Japan

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Abstract

We compared *Sedum formosanum* with related species using morphological traits and molecular phylogenetic analysis of nrITS sequences. Morphological comparisons revealed that the plants historically treated as *S. formosanum* in the Danjo Islands of Japan had 4-merous flower; 8 stamens; narrow triangular sepals of equal size; horizontal carpels when matured; and an irregular branching form. These traits differed from those of *S. formosanum* in other regions, which has 5-merous flowers; 10 stamens, thick spatulate sepals of unequal size; erect carpels when matured; and a trichotomous branching form. Phylogenetic analysis indicated that plants known as *S. formosanum* in the Danjo Islands were sister group to *S. tetractinum*, which are endemic to China and belong to a different clade than *S. formosanum*, which are found in other regions. Based on the present morphological comparisons and phylogenetical analyses, we describe plants from the Danjo Islands as a new species, *S. danjoense*, which is distinct from *S. formosanum*.

Keywords: East Asia, ITS, Phylogeny, *Sedum formosanum*, Succulent

Introduction

The Danjo Islands are uninhabited islands in the East China Sea, located about 170 km west of the Kyushu Island of Japan (Fig. 1). The islands comprise five small islets, Oshima, Kurokijima, Yorishima, Hanagurijima and Meshima islands. Despite the small total area of the islands (ca. 4.38 km² [GSI (2013 onward)], Fig. 1) biogeographically significant terrestrial organisms occur in the islands. For example, one snake of *Amphiesma vibakari danjoensis* Toriba (1986: 126), 13 land snail species (Yamamoto & Uozumi 1981) and one Orange Daylily (*Hemerocallis major* (Baker 1895: 62) Hotta 1986: 22) are endemic to the Danjo Islands. The islands also contain a unique mix of temperate and tropical species and an intact primary forest (Uematsu *et al*. 1973). Due to their biological value, the Danjo Islands have been designated as a Japanese national monument area since 1969, and travel to these islands is heavily restricted.

The genus *Sedum* Linnaeus (1753: 430), the largest and most widespread genus of Crassulaceae, consists of ca. 420 species (Thiede & Eggli 2007). *Sedum* includes annual, biennial, and perennial herbs with succulent leaves and stems that are primarily distributed in temperate to subtropical environments, and are most diverse in the Mediterranean Sea, Central America, Himalayas, and East Asia (Stephenson 1994; Thiede & Eggli 2007). Previous phylogenetic study showed that the genus *Sedum* is polyphyletic within the seven American genera (Carrillo-Reyes *et al*. 2009). However, eastern Asian *Sedum* members formed a monophyletic group (Mayuzumi & Ohba 2004, Carrillo-Reyes *et al.* 2009). In 2001, Fu & Ohba divided the East Asian *Sedum* members into three sections, namely, sects. *Sedum*, *Oreades* (Fröderström 1931: 41) Fu, K.T. (1974: 52), and *Filipes* (Fröderström 1931: 34) Fu, S.H. (1965: 115). Following the taxonomic treatment of Fu & Ohba (2001), sect. *Sedum* could be segregated from sects. *Oreades* and *Filipes* based on adaxially gibbous carpels and follicles, whereas sect. *Oreades* differed from sect. *Filipes* based on the absence of spurred leaves spurred at the base. Most members of sect. *Oreades* have yellow or purple-red (rarely red) petals,

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whereas members of sect. *Filipes* have white or reddish purple (rarely yellow) petals (Fu & Ohba 2001). According to the flora of Japan (Ohba 2001), 17 species are distributed in Japan, including 4 subspecies and 4 varieties within sect. *Sedum* and 1 species within sect. *Filipes*. In the Danjo Islands, two native *Sedum* species of sect. *Sedum* have been reported (Uematsu *et al*. 1973): *S. formosanum* N.E. Brown. (1885: 134) and *S. japonicum* Siebold ex Miquel (1866: 156) subsp. *oryzifolium* (Makino 1891: 2) H. Ohba (2003: 300).

Sedum formosanum, which was described based on a type specimen collected from Taiwan (Brown 1885), is a biennial herb that occurs on rocky seashore slopes, and rarely, on rocky inland slopes (Hatusima 1975). It is distributed from the southern part of Kyushu to Batan Island in the Philippines, through the Ryukyu Archipelago and Taiwan (Hatusima 1975, Ohba 1984, 2001). In the Danjo Islands, the occurrence of *S. formosanum* has been reported in Oshima, Yorishima and Meshima islands, representing near the northern limit of its distribution (Uematsu *et al*. 1973) (Fig. 1). However, Itow & Nakanishi (1990) noted that the petal numbers and flowering seasons of *S. formosanum* in the Danjo Islands differ from those of other regions in Japan.

In this study, we conducted morphological comparisons and molecular phylogenetic analysis to clarify the taxonomic status of plants treated as *S. formosanum* in the Danjo Islands, and to aid conservation activities for this endangered species.

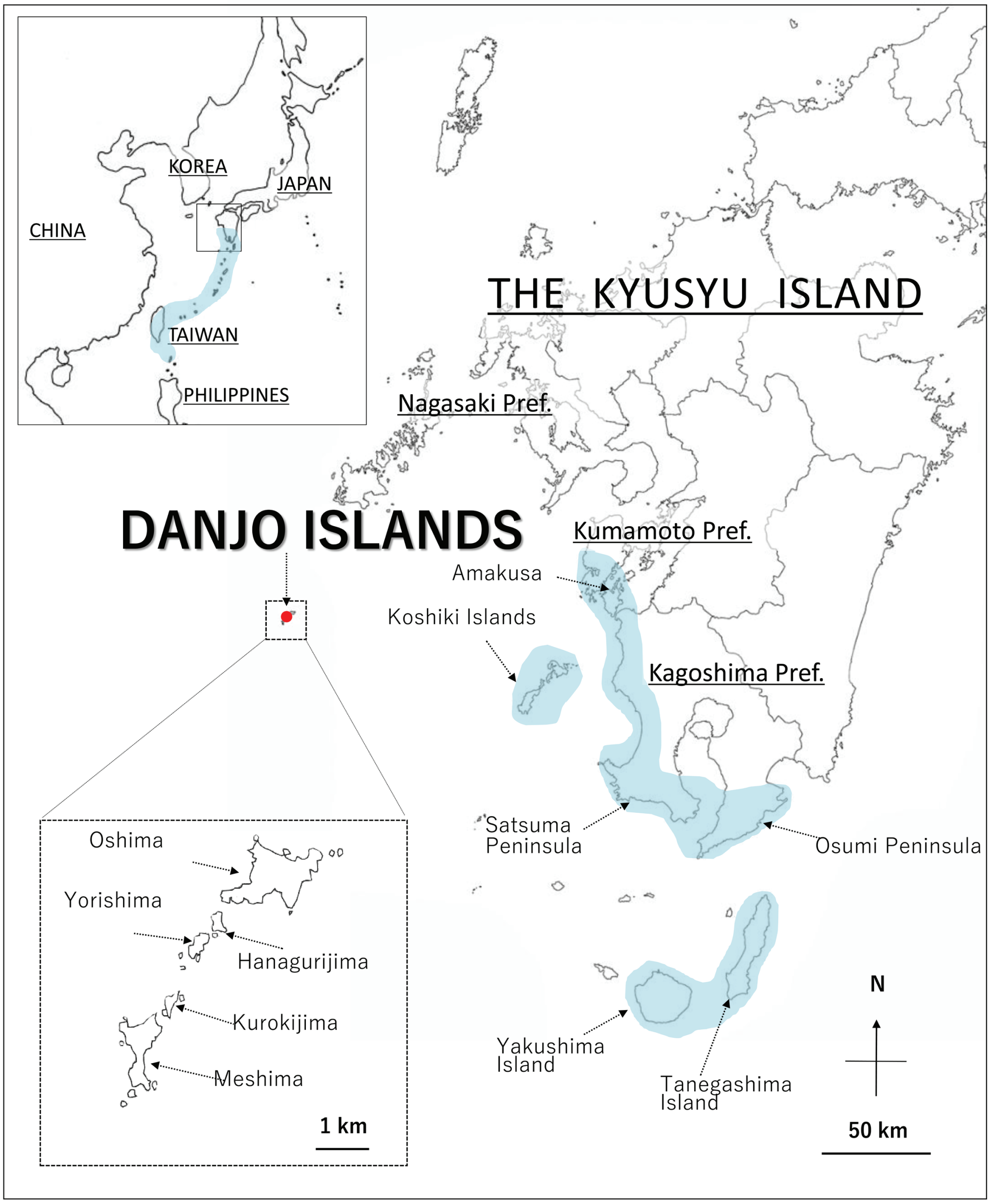

FIGURE 1. Map showing the location of Danjo Islands and the adjacent area. The blue shaded area indicates the distribution of *S. formosanum* (see Tables 1 and 2 for collection localities).

Materials and Methods

Sample collection

The plants, historically treated as *S. formosanum*, were collected by the third author from Yorishima Island within the Danjo Islands as part of a special field survey on 24 May 1989. Then the plants were cultivated in the Nagasaki Subtropical Botanical Garden of Japan for about 30 years prior to morphological and molecular phylogenetic analyses. Their voucher specimens are deposited in the herbarium of National Museum of Nature and Science (TNS; *T. Ito 3658*). For morphological comparison, we additionally used specimens of *S. formosanum* collected from Meshima Isl. (TNS; *Masao Ejima 15108*) of Danjo Islands, but it is too old to extract DNA. To determine the phylogenetic position of the plant from the Danjo Islands known as *S. formosanum* (*T. Ito 3658*), we used 32 species of sect. *Sedum* and two species of sect. *Oreades* in East Asia including 19 accessions of *S. formosanum* from Japan (excluding the Danjo Islands), Taiwan and Philippines (Tables 1 and 2). We included internal transcribed spacer (ITS) sequences from 26 species of sect. *Sedum*, including 13 *S. formosanum* accessions and 2 species of sect. *Oreades* from Eastern Asia in the samples, which were reported in previous molecular studies by Mayuzumi & Ohba (2004) and Ito *et al*. (2014, 2017) and stored in GenBank (Table 2). Following previous phylogenetic study of Crassulaceae (Mayuzumi & Ohba 2004), *Aeonium castello-paivae* Bolle (1859: 240), *A. gomerense* Praeger (1929: 473), *A. lancerottense* Praeger (1932: 190), *A. viscatum* Bolle (1859: 241), and *Greenovia aizoon* Bolle (1859: 242), which were collected by Mort *et al*. (2002) and stored in GenBank (Table 1) were selected as outgroup. In total, 59 operational taxonomic units (OTUs) were included in our molecular phylogenetic analysis (Tables 1 and 2). Voucher specimens for the materials were deposited in the TNS herbarium.

DNA extraction, amplification, and sequencing

DNA for polymerase chain reaction (PCR) amplification was extracted from dried leaves using a DNeasy Kit (QIAGEN, Valencia, CA, USA) following the manufacturer's protocol. The ITS region (ITS1, 5.8S rDNA, and ITS2) was amplified by 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 for the ITS sequence was performed using a BigDye Terminator Cycle Sequencing Kit ver. 3.1 (Applied Biosystems, Foster City, CA) (Table 5) and the abovementioned PCR primers with the internal reverse primer N2 (5'-GGC GCA ACT TGC GTT CAA-3') and forward primer N3 (5'-GCT CTC GCA GCA TCG ATG AAG-3') designed by T. Yukawa (TNS, personal communication). The samples 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).

TABLE 1. List of plant material collected 8 *Sedum* taxa for sequenced in the present study with their respective locality, vouchers and DDBJ Accession numbers.

Taxon	Source locality	Abbreviation*	Voucher (herbarium)	Accession no.
Section *Sedum*				
S. formosanum N.E.Br.	Japan: Kyusyu, Kagoshima, Minami-Osumi.	K-OSM	*T. Ito 3238* (TNS)	LC260123
	Japan: Kyusyu, Kagoshima, Mt. Isoma.	K-ISM	*T. Ito 2296* (TNS)	LC260124
	Japan: Kyusyu, Kagoshima, Shimo-Koshiki Island.	K-SKS	*T. Ito 3200* (TNS)	LC260125
	Japan: Kyusyu, Kumamoto, Reihoku.	K-KMM	*T. Ito 637* (TNS)	LC260126
	Japan: Kyusyu, Nagasaki, Danjyo Islands, Yorijima.	K-DJO	*T. Ito 3658* (TNS)	LC260127
	Japan: Ryukyu, Kagoshima, Okinoerabu Island.	R-OKE	*T. Ito 2611* (TNS)	LC260128
	Japan: Ryukyu, kagoshima, Yakushima Island.	R-YKS	*T. Ito 2648* (TNS)	LC260129
S. hangzhouense K.T.Fu & G.Y.Rao	China: Zhejiang, Hangzhou City, Mt. Ling	–	*T. Ito 2604* (TNS)	LC260130
S. lungtsuanense H. Fu,	China: Zhejiang.	–	*T. Ito 3563* (TNS)	LC260131
S. makinoi Maxim.	Japan: Shikoku, Tokushima, Naka.	–	*T. Ito 709* (TNS)	LC260132
S. morrisonense Hayata	Taiwan: Nantou, Ren'ai.	–	*T. Ito 1274* (TNS)	LC260133
S. tricarpum Makino	China: Zhejiang.	CHN	*T. Ito 3597* (TNS)	LC260134
S. tetractinum Fröderström	China: Zhejiang.	–	*T. Ito 3623* (TNS)	LC260135
S. zentaro-tashiroi Makino	Japan: Kyusyu, Nagasaki, Tsushima.	–	*T. Ito 441* (TNS)	LC260136

*Abbreviation are used for Fig. 2.

TABLE 2. 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	Abbreviation*	Voucher (herbarium)	Accession no.
INGROUP		–		
Section *Oreades*		–		
S. oreades Raym.-Hamet	Nepal.	–	*F. Miyamoto et al. TI9420140* (TI)	AB088632[1]
S. trullipetalum Hook.f. *et* Thomson	Nepal.	–	*F. Miyamoto et al. TI9420132* (TI)	AB088630[1]
Section *Sedum*				
S. alfredii Hance	China: Guangdong.	–	*G. Kokubugata 17190* (IBSC)	AB930259[2]
S. bulbiferum Makino	Japan: Kyusyu, Nagasaki, Tsushima.	–	*T. Ito 435* (TNS)	LC229234[3]
S. erythrospermum Hayata	Taiwan: Kaohsiung, Taoyuan Hsiang.	–	*C. Tsutsumi 504* (TNS)	AB906473[2]
S. formosanum N.E.Br.	Japan: Kyusyu, Kagoshima, Minami-Satsuma.	K-MST	*G. Kokubugata 16768* (TNS)	AB930262[2]
	Japan: Ryukyu, Kagoshima, Amami Island.	R-AMM	*G. Kokubugata 16712* (TNS)	AB930264[2]
	Japan: Ryukyu, Kagoshima, Tanegashima Island.	R-TNG	*G. Kokubugata 15602* (TNS)	AB930265[2]
	Japan: Ryukyu, Okinawa, Iheya Island.	R-IHY	*G. Kokubugata 10726* (TNS)	AB930267[2]
	Japan: Ryukyu, Okinawa, Iriomote Island.	R-IRO	*T. Ito 598* (TNS)	AB930270[2]
	Japan: Ryukyu, Okinawa, Izena Island.	R-IZN	*G. Kokubugata 12224* (TNS)	AB930266[2]
	Japan: Ryukyu, Okinawa, Kume Island.	R-KMJ	*G. Kokubugata 12755* (TNS)	AB930269[2]
	Japan: Ryukyu, Okinawa, Tonaki Island.	R-TNK	*G. Kokubugata 13049* (TNS)	AB930268[2]
	Philippines: Batanes, Batan Island.	P-BTN	*G. Kokubugata 15715* (TNS)	AB930273[2]
	Taiwan: New Taipei, Ruifang.	T-TPI	*G.Kokubugata 16446* (TNS)	AB930272[2]
	Taiwan: Pingtung, Hengchun.	T-PNT	*T. Ito 1260* (TNS)	LC229279[3]
	Taiwan: Taitung, Lanyu Island.	T-LNY	*G. Kokubugata 6132* (TNS)	AB930271[2]
	Taiwan: Yilan, Gueishan Island.	T-GSN	*T. Ito 1921* (TNS)	LC229280
S. japonicum Siebold *ex* Miq. ssp. *japonicum* var. *senanense* Makino	Japan: Tohoku, Akita, Misato.	–	*T. Ito 2200* (TNS)	LC229238[3]
S. japonicum Siebold *ex* Miq. ssp. *japonicum* var. *japonicum*	Japan: Shikoku, Kochi, Kuroshio.	–	*T. Ito 723* (TNS)	LC229237[3]
S. japonicum Siebold *ex* Miq. ssp. *oryzifolium* (Makino) H.Ohba	Japan: Kyusyu, Nagasaki, Nagasaki.	–	*T. Ito 2285* (TNS)	LC229239[3]
S. jiulungshanense Y. C. Ho	China: Zhejiang.	–	*CMQ20150076* (TNS)	LC229243[3]
S. kiangnanense D.Q.Wang & Z.F.Wu	China: Zhejiang, Hangzhou, Lin'an.	–	*T. Ito 1030* (TNS)	LC229244[3]
S. hakonense Makino	Japan: Chubu, Yamanashi, Narisawa.	–	*T. Ito 623* (TNS)	AB930278[2]
S. lineare Thunb.	Japan.	–	*S. Mayuzumi C00120* (TI)	AB088623[1]
S. multicaule Wall. *ex* Lindl.	Nepal.	–	*F. Miyamoto et al. TI9596136* (TI)	AB088631[1]
S. mexicanum Britt.	Japan: Shikoku, Kochi, Kochi.	–	*T. Ito 647* (TNS)	LC229247[3]
S. nagasakianum H.Ohba	Japan: Kyusyu, Nagasaki, Nagasaki.	–	*T. Ito 2064* (TNS)	LC229249[3]
S. nokoense Yamam.	Taiwan: Hualien, Nenggao.	–	*G. Kokubugata 10426* (TNS)	AB906478[2]
S. polytrichoides Hemsl. ssp. *polytrichoides*	Japan: Kyusyu, Oita, Kitsuki.	–	*T. Ito 2247* (TNS)	LC229252[3].
S. polytrichoides Hemsl. ssp. *yabeanum* (Makino) H.Ohba var. *yabeanum* H.Ohba	Japan: Kyusyu, Nagasaki, Tsushima.	–	*T. Ito 396* (TNS)	AB906490[2]
S. polytrichoides Hemsl. ssp. *yabeanum* (Makino) H.Ohba var. *setouchiense* H.Ohba	Japan: Shikoku, Kagawa, Syodo Island.	–	*T. Ito 2298* (TNS)	LC229253[3]
S. rupifragum Koidz.	Japan: Sanin, Shimane, Izumo.	–	*T. Ito 2070* (TNS)	LC229254[3]
S. sarmentosum Bunge	Japan: Tohoku, Iwate, Morioka.	–	*T. Ito 978* (TNS)	LC229255[3]
S. satumense Hatus.	Japan: Kyusyu, Kagoshima, Minami-satsuma.	–	*T. Ito 2295* (TNS)	LC229256[3]
S. subtile Miq.	Japan: Kyudyu, Oita.	–	*T. Ito 2259* (TNS)	LC229257[3]
S. tianmushanense Y. C. Ho & F. Chai	China: Zhejiang.	–	*LP 67* (TNS)	LC229261[3]
S. tosaense Makino	Japan: Shikoku, Kochi, Kochi.	–	*G. Kokubugata 16726* (TNS)	AB906483[2]
S. triactina A.Berger	Nepal.	–	*F. Miyamoto et al. TI9596091* (TI)	AB088629[1]
S. tricarpum Makino	Japan: Kyusyu, Oita, Yamakuni.	KYU	*T. Ito 2269* (TNS)	LC229259[3]
OUTGROUP				
Aeonium castello-paivae Bolle	Canary.	–	*M.E. Mort 1519* (WS)	AY082127[4]
Aeonium gomerense (Praeger) Praeger	Canary.	–	*M.E. Mort 1454* (WS)	AY082133[4]
Aeonium viscatum Webb *ex* Bolle	Canary.	–	*M.E. Mort 1432* (WS)	AY082154[4]
Aeonium lancerottense (Praeger) Praeger	Canary.	–	*M.E. Mort 1518* (WS)	AY082143[4]
Greenovia aizoon Bolle	Canary.		*M.E. Mort 1425* (WS)	AY082112[4]

*Reported by [1]Mayuzumi & Ohba (2004) , [2]Ito *et al.* (2014), [3]Ito *et al.* (2017) and [4]Mort *et al.* (2002).

Phylogenetic analysis using ITS sequences

The ITS sequences obtained by PCR were aligned using ClustalW 1.8 (Thompson *et al.* 1994) and then adjusted manually. Phylogenetic analyses were based on a Bayesian approach using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and maximum-likelihood (ML) phylogenetic analysis using RAxML (Stamatakis, 2014). In the Bayesian phylogenetic analysis, we used the Hierarchical Likelihood Ratio Tests (hLRT) implemented in MrModeltest 2.2 (Nylander 2004) to obtain an appropriate evolutionary model of nucleotide substitutions. Based on the selected model, 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). The MCMCMC was 10 million generations long, and the chain was sampled every 1000th 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 the most likely tree.

Morphological comparison

The two plants known as *S. formosanum* from Yorishima Isl. (TNS; *T. Ito 3658*) and Meshima Isl. (TNS; *Masao Ejima 15108*) of the Danjo Islands, were used for morphological comparisons. In addition, herbarium specimens of *S. formosanum* from 20 localities in Japan excluding the Danjo Islands, Taiwan, and the Philippines deposited the TNS were examined (Table 1). Sedum species revealed to be closely related to the Danjo plants treated as *S. formosanum* in the present analysis were also included in the morphological comparisons.

In our morphological surveys, we compared numbers of carpels, sepals, petals, and stamens; the shapes of leaves, sepals, and carpels; and the branching form and anther color. We also compared habitat, life cycle, and flower season among these plants based on our field survey and previous references.

Results

Phylogenetic relationships based on ITS

For the Bayesian and ML phylogenetic analyses, we obtained 59 OTUs, including 54 accessions as ingroups and 5 outgroup accessions obtained from GenBank (Tables 1 and 2). After alignment, we obtained a matrix of 639 base pairs (bp). 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 posterior probabilities (PPs, Fig. 2). The topology of the ML tree was highly compatible with that of the Bayesian tree, and the BPs are plotted on the Bayesian tree (Fig. 2). In both the Bayesian and ML analyses (Fig. 2), the Danjo plant treated as *S. formosanum* formed a well-supported clade, with *S. tetractinum* Fröderström (1931:103) being an endemic species to China (Fu & Ohba 2001) (PP/BS = 1.00/96; clade I). Clade I was the sister of a well-supported clade (1.00/100; clade II) including two subclades. The first clade comprised two species endemic to Taiwan, namely, *S. erythrospermum* Hayata (1913:110) and *S. nokoense* (Yamamoto 1926:21) (1.00/96); and the second clade comprised 19 accessions of *S. formosanum* from Japan (excluding Danjo Island), Taiwan, and the Philippines (0.70/83).

Morphological comparison

In floral morphology, the plants treated as *S. formosanum* (TNS; *T. Ito 3658* and *Masao Ejima 15108*) from the Danjo Islands and those from the other regions in Japan, Taiwan, and the Philippines had similar leaf shapes: thick, and spatulate to obovate (Figs. 3 & 4). They also shared similar habitats, inhabiting rocky slopes on seashores. However, the Danjo Islands plants differed from the others in branching form (irregular vs. trichotomous); numbers of carpels, petals, sepals (4 vs. 5), and stamens (8 vs. 10); sepal shapes (equal in size and narrowly triangular vs. thick, spatulate, and unequal in size); carpel shapes when matured (horizontal vs. erect); and anther color (red only vs. yellow or red) (Table 3; Figs. 3 & 4). The number of carpels, petals, sepals, and stamens of the Danjo Islands plants were consistent with a previous report (Itow & Nakanishi 1990). However, the differences of branching form, anther color, and sepal and carpel shapes were reported for the first time in this study. On the other hand, plants from the Danjo Islands historically treated as *S. formosanum* shared several traits with *S. tetractinum*, the species indicated as the closest relative based on molecular analysis. Both species shared similar branching forms (irregular); numbers of carpels, petals, sepals (4), and stamens (8); sepal shapes (equal in size; narrowly triangular); and anther color (red). However, the former differed from the latter in leaf shape (thick and succulent, spatulate, obovate vs. flat, ovate to orbicular; Table 3, Figs. 3 & 4); carpel shapes when matured (horizontal vs. oblique). They also occurred in different environments (dry rocky slopes on seashores vs. moist rocky slopes near streams). We confirmed that these morphological characters of the plants from Japan, Taiwan and the Philippines were consistent between natural habitats and under cultivations.

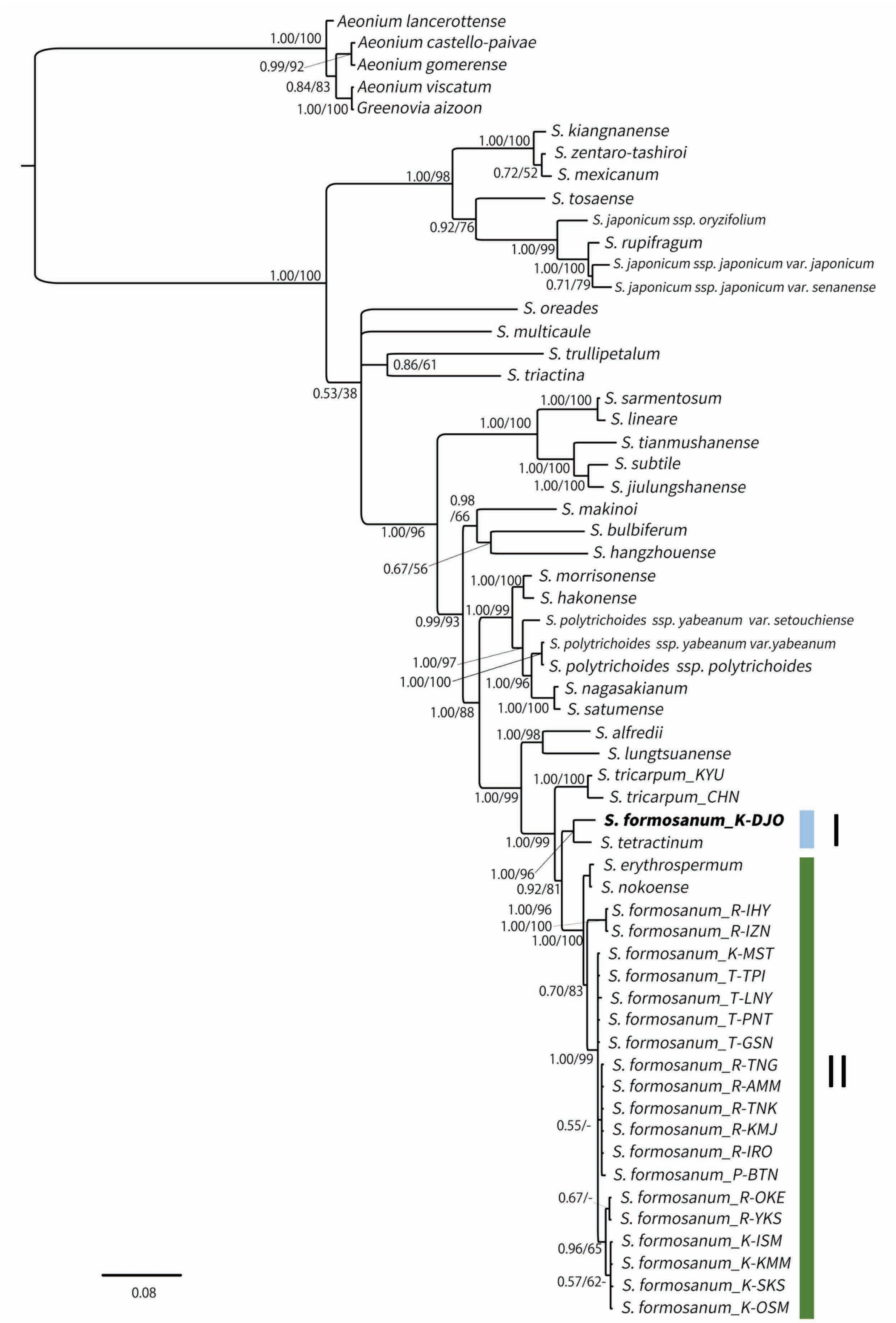

FIGURE 2. Bayesian phylogenetic tree based on ITS sequence for Eastern Asian *Sedum*. The topology of the maximum likelihood (ML) tree was highly compatible with the Bayesian tree. Bayesian posterior probabilities (PPs: left) and bootstrap percentages from ML analysis (BP: right) are shown (See Tables 1 and 2 for the abbreviations of localities).

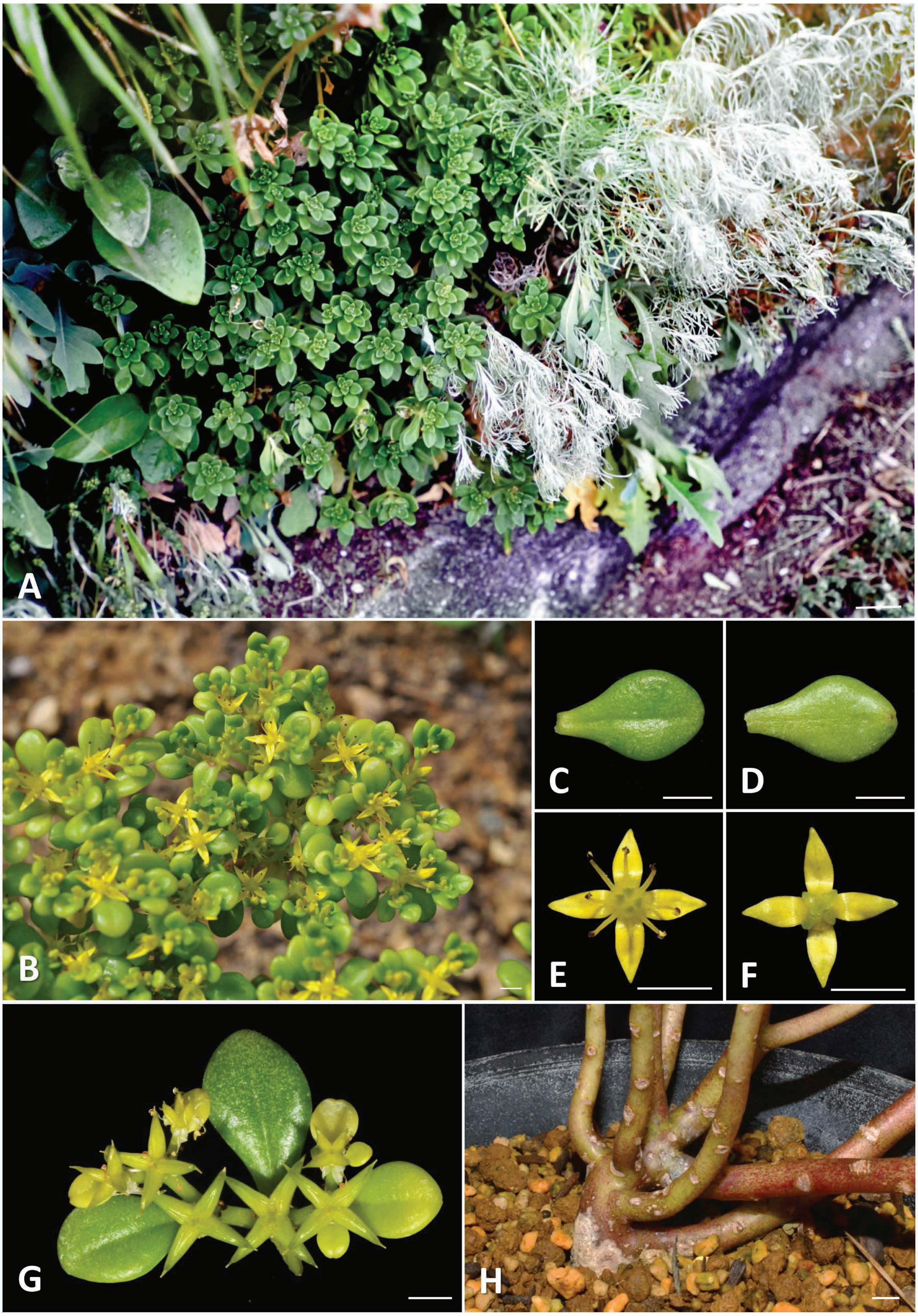

FIGURE 3. *Sedum danjoense.* A. Habitat and habit. B. Inflorescence. C. Adaxial surface. D. Abaxial surface. E. Flower. F. Sepals. G. Carpels. H. Branching. Scale bars are 25 mm for A, 5 mm for B–H [A. Wild individuals in Yorishima island photo by Yoshiro Chichibu in May 1989; B. Cultivated in Nagasaki Subtropical Botanical Garden photo by Kiyotaka Minota in Sep. 2011; C-H. *Takuro Ito 3658* in Oct. 2016]

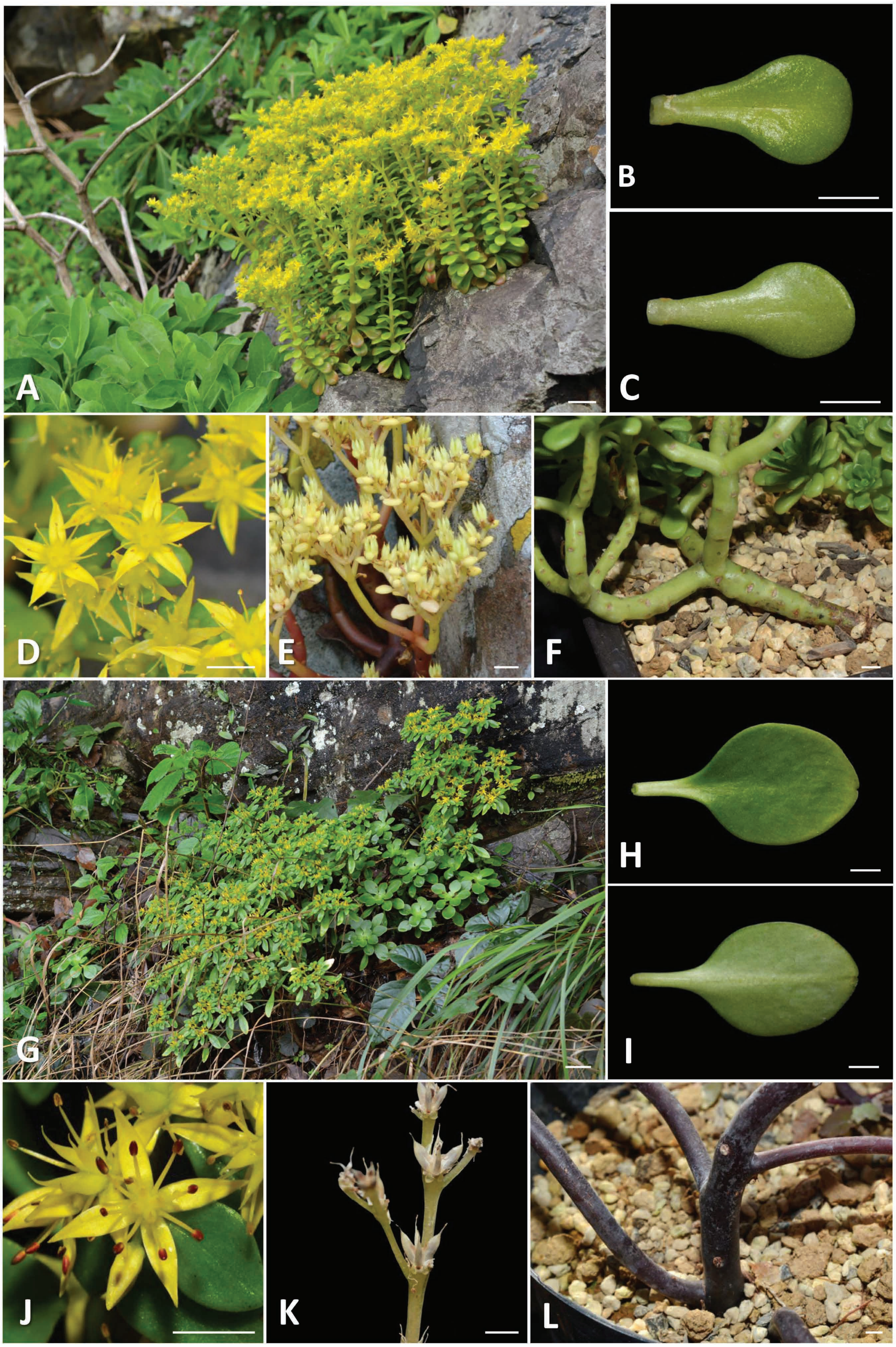

FIGURE 4. *Sedum formosanum* (A–F) and *S. tetractinum* (G–L). **A** and **G**. Habitat and habit. **B** and **H**. Adaxial surface. **C** and **I**. Abaxial surface. **D** and **J**. Flower. **E** and **K**. Carpels. **F** and **L**. Branching. Scale bars are 25 mm for A and G, 5 mm for B–F and H–K [A–D. Tokunoshima island in May. *Takuro Ito 3623*; E. Tanegashima Island in June. *Takuro Ito 3456*; In December. *Takuro Ito 832*; G–J. Zhejiang in June. *Takuro Ito 3623*; K–L. In December. *Takuro Ito 3623*]

TABLE 3. Comparison of diagnostics of *Sedum formosanum* and *S. tetractinum*.

TRAITS \ TAXA	***S. formosanum*** (the Danjo Islands)	***S. formosanum*** (other regions in Japan, Taiwan and Philippines)	***S. tetractinum***
Life cycle	Biennial	Biennial	Perennial
Branching form	Irregular	Trichotomous	Irregular
Leaves	Thick and succulent, spatulate to obovate 1.2–3.2 × 0.5–1.6 cm	Thick and succulent, spatulate to obovate 1.5–2.5 × 0.8–1.2 cm	Flat, ovate to orbicular 1.8–4.2 × 1–2.3 cm
Carpels, Sepals and Petals	4	5	4
Sepals shape	Equally narrow triangular	Unequally thick spatulate	Equally narrow triangular
Stamens	8	10	8
Anther color	Red	Yellow, Red	Red
Fruits shape	Horizontal	Erect	Oblique
Primary flowering Season	October–November	April–June	August–September
Habitat	Dried rocky slopes in seashores	Dried rocky slopes in seashores	Moist rocky slopes near streams

Discussion

Taxonomic status of plants historically treated as S. formosanum *from the Danjo Islands*

The molecular phylogenetic analysis in this study indicates that plants from the Danjo Islands, currently considered *S. formosanum*, belong to a separate clade from *S. formosanum* collected from other regions of Japan, Taiwan (type locality), and the Philippines. Our morphological comparisons revealed that the plants from the Danjo Island were clearly distinguishable from *S. formosanum* and *S. tetractinum*. Although Brown (1885) did not mention the number of carpels, petals, sepals, and stamens in the description of *S. formosanum*, the observations of this study were consistent with previous reports (Ohba 2001, Tang & Huang 1993). The flat and ovate to orbicular leaf shape, as well as the numbers of carpels, petals, sepals, and stamens of *S. tetractinum* observed in this study were consistent with previous observations (Fröderström 1931, Fu & Ohba 2001). Furthermore, there were phenological differences between the plants from Danjo Island and *S. formosanum* from the other regions: the primary flower season of the former is autumn (October to November) (Itow & Nakanishi 1990), whereas the latter blossoms in spring (April to June) (Ohba 2001). The primary flower season of the plants from Danjo Island also differs from that of *S. tetractinum*, which flowers in summer (August to September) (Fu & Ohba 2001). Therefore, we conclude that the plants from Danjo Island historically treated as *S. formosanum* should be considered as a different taxonomic entity. The new species is described below.

Taxonomic Treatment

Sedum danjoense Takuro Ito, H. Nakanishi & G. Kokub., *sp. nov*. (Fig. 3)

Type:—JAPAN. The Kyusyu, the Danjo Islands, Yorishima Island, 25 October 2016 (cultivated in Nagasaki Subtropical Botanical Garden after collecting its natural habit in 1989), *Takuro Ito 3658*. (holotype TNS!).

Description:—Biennial herb, fleshy, glabrous. Roots fibrous adventitious at the basal node of each branch. Stem stout, erect with irregular branching, 10–25 cm tall. Leaves mostly alternate, sessile, thick, spatulate to obovate, 1.2–3.2 cm long, 0.5–1.6 cm wide, apex rounded, base long, attenuate, margins entire. Flowering stems fleshy, 10–20 cm tall, base ca. 5 mm broad, usually yellowish, erect or sprawling with irregular branching at base. Inflorescences terminal cymes, flowers usually 10–22 sparsely distributed per branch. Sepals 4, free, green-yellow, fleshy, equal in size, narrowly triangular, 1–3 mm long, 0.8–1.2 mm wide, apex round or obtuse. Petals 4, blight yellow, lanceolate 5–6 mm long, 1.4–1.7 mm wide, apex acuminate, base slightly connate. Stamens 8, shorter than petals, 4.8–5 mm long, erect at flowering, 2- whorled arranged; anthers oblong, ca. 0.5 mm long, red before dehiscence. Carpels 4, free, horizontal, connate at the base, gibbous ventrally, 5.5–6.5 mm long. Fruits star-shaped, follicle, spreading, 6–7 mm long. Flowering in October to November.

This new species is categorized in sect. *Sedum* because it has adaxially gibbous carpels (Fu & Ohba 2001) (Fig. 3).

Etymology:—The epithet refers to the Japanese name of type locality of the Danjo Islands.

Distribution and habitat:—Endemic to the Danjo Islands (Kyusyu), on sunny, coastal rocky slopes exposed to direct sunlight; in typical coastal vegetation within "a community of *Miscanthus condensatus* Hackel (1899: 639)–*Crepidiastrum lanceolatum* (Houtt.) Nakai (1920: 150)" similar to those in other regions of Japan, Taiwan and the Philippines. Itow & Nakanishi (1990) mentioned that this species was distributed throughout the Danjo Islands (as *S. formosanum*), and thus further field surveys are required on the islands.

Additional specimens examined (paratypes):—JAPAN. The Kyushus, the Danjo Islands: Meshima Island, *Masao Ejima 15108* (TNS)

IUCN Red list category:—According to the IUCN red list categories criteria (IUCN 2016), this species should be considered Vulnerable, because it occurs in small islets in the Danjo Islands. At present, the habitat of this species is relatively stable because the Danjo Islands are protected as a Japanese natural monument. However, islets such as the Danjo Islands are easily influenced by subtle climatic changes and competition with naturalized plant species. In fact, naturalized plant species are known to invade the habitats of native plant species in the Danjo Islands (Toyama 1980). Therefore it is necessary to maintain the environment of these islands and continue to protect it as a national monument.

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

***Convergence of leaf morphology between* S. danjoense *and* S. formosanum**

Both of *S. danjoense* and *S. formosanum* have thick leaves and were found in similar habitats on coastal rocks, which are xeric, saline, and exposed to direct sunlight, although the two species were not sister clades in this study. In the Danjo Islands, coastal plants have a high tolerance to drought because the islands lack major streams and are generally dry (Uematsu *et al*. 1973). In coastal plants, it was well known that certain plants have thick and succulent leaves to protect against high levels of light intensity and salinity (Rozema *et al*. 1985, Hesp 1991, Maun 1994). Thus, the morphological similarity in leaf thickness between *S. danjoense* and *S. formosanum* could be caused by convergent evolution to tolerate the distinctive stresses of coastal environments. Alternatively, an ancestral species of *S. danjoense*, *S. formosanum*, and *S. tetractinum* might have had thick and succulent leaves, and only *S. tetractinum* might have developed flat leaves as an adaptation to moist, riparian areas.

***Possible biogeographical history of* S. danjoense**

It was previously reported that the biota of the Danjo Islands are heavily influenced by adjacent regions, including the southern part of Kyusyu and the Ryukyu archipelago (Yamaguchi & Ejima 1973). In animals, disjunctive patterns of distribution have been reported at the intraspecific or species level between continental China and the Danjo Islands. For example, the terrestrial snake *Amphiesma vibakari* occurs in China and Taiwan as the subspecies *vibakari*, and in the Danjo Islands as the subspecies of *danjoensis* (Toriba 1986). Similarly, one species of 13 Danjo endemic species of land snails (including 2 unclear species) lacking long-distance dispersal ability species is thought to be most closely related to Chinese species (Yamamoto & Uozumi 1981). These previous reports support the notion that the Danjo Islands were once physically connected with the continental China. On the other hand, among the plants of the Danjo Islands, this study is the first to identify a disjunctive distribution pattern between islands and continental China at the species level.

In general, Crassulaceae species are mostly dispersed by gravity and wind (Thiede & Eggli 2007). Furthermore, Nakanishi (2002) reported that *S. danjoense* (as *S. formosanum*) dispersed by raindrops. Therefore they are thought to lack long-distance dispersal ability because their seeds are much larger than typical anemochorous dust seeds (Thiede & Eggli 2007). Therefore, it is likely that the *S. danjoense* ancestor migrated to the Danjo Islands from continental China via a land bridge, and that the subsequent separation of the islands resulted in anagenesis for *S. danjoense*, such as the Danjo Islands snake mentioned above. It is plausible that many animals and plants in the Danjo Islands share similar biogeographical histories, including migration routes and dates of divergence. Further studies including estimated divergence dates based on molecular markers are needed to shed more light on the biogeographical history of *S. danjoense*.

A diagnostic key of the new *Sedum* species from the Danjo Islands and the related species

1. Pentamerous flowers; 10 stamens; trichotomous branching; unequally sized, thick, spatulate sepals; erect carpels when matured .... *S. formosanum*
– Tetramerous flowers; 8 stamens; irregular branching; equally sized, narrowly triangular sepals; horizontal or oblique carpels when matured 2
2. Perennial; leaf flat and ovate to orbicular; oblique carpels; primary flower season summer .... *S. tetractinum*
– Biennial; leaf thick and succulent, spatulate to obovate; horizontal carpels; primary flower season autumn .... *S. danjoense*

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