SEDUM PLUMBIZINCICOLA X.H. GUO ET S.B. ZHOU EX L.H. WU (CRASSULACEAE), A NEW SPECIES FROM ZHEJIANG PROVINCE, CHINA

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Abstract

Sedum plumbizincicola X.H. Guo et S.B. Zhou ex L.H. Wu (Crassulaceae), a new species restricted to lead–zinc mining areas in Zhejiang Province, China, is described and illustrated. This taxon belongs to sect. *Sedum* (H. Ohba) S.H. Fu based on the adaxially gibbous carpels and follicles. It superficially resembles *S. alfredii* Hance and three other *Sedum* species found in the same area, but differs from these other taxa in bearing 4-merous flowers. Differences in geographical distribution, growth habit, phenology, macromorphology, leaf and stem anatomy, as well as seed micromorphology among *S. plumbizincicola*, *S. alfredii* and other related taxa in the genus *Sedum* are also reported. nrDNA ITS sequences from seven populations of *S. plumbizincicola* support the recognition of this as a taxonomic entity distinct from *S. alfredii*.

KEYWORDS

Sedum plumbizincicola - Crassulaceae - new species – morphological data - nrDNA ITS sequence data

INTRODUCTION

Sedum L. is the largest genus in the family Crassulaceae and as currently circumscribed includes about 430 species, with major centres of diversity in eastern Asia, the Mediterranean basin, and northern America and Mexico (’t Hart and Bleij, 2003; Thiede and Eggli, 2007). The genus appears to be paraphyletic and is consequently taxonomically rather problematic, and a large number of sectional names and segregate genera have been published (’t Hart and Bleij, 2003; Carrillo-Reyes et al. 2009). Approximately 121 species (91 endemics) occur in China, which on the basis of morphological characters (gibbous carpels, spurred leaf bases and petal colour) have been assigned to three sections: sect. *Filipes* (Fröderström) S.H. Fu, sect. *Oreades* (Fröderström) K.T. Fu, and sect. *Sedum* (H. Ohba) S.H. Fu (Fu and Ohba, 2001). Section *Filipes* includes eight species and is distributed in Bhutan, China, Japan, Myanmar, Nepal and Sikkim Province in India, with three species endemic to China; section *Oreades* contains about 67 species distributed in Bhutan, China, India, Myanmar, Nepal and Pakistan, with 64 species (54 endemic) in China; and section *Sedum* comprises more than 60 species, occurring mainly in Asia and Europe, with 49 species (34 endemics) in China.

In 2005 the senior author undertook extensive field research in Zhejiang Province and collected many specimens in a search for metal hyperaccumulator plants. Some unusual and isolated populations that superficially resembled *S. alfredii* Hance, but which produced 4-merous flowers, were found in Lin'an and Chun'an counties, together with four other species, *S. alfredii*, *S. emarginatum* Migo, *S. hangzhouense* K.T. Fu & G.Y. Rao and *S. bailey* Praeger, that were identified from the same areas. After comparison with the taxonomic accounts of *Sedum* in the *Flora of China* (Fu and Ohba, 2001), the *Flora of Zhejiang* (He, 1993), the *Flora of Jiangsu* (Jiangsu Institute of Botany, 1982), the *Flora of Anhui* (Xue, 1986), and the *Flora of Jiangxi* (Jiangxi Institute of Botany, 2004), the authors found that the populations were distinctly different from *S. alfredii* and proposed that they should be recognized as a new species, *Sedum plumbizincicola* X.H. Guo et S.B. Zhou, in a brief report (without Latin or English description) in the Chinese journal *Soils* (Wu et al., 2006). The specimens were lodged in the Herbarium of Anhui Normal University, China (ANU!).

The identification of *Sedum* is traditionally based on macromorphological characters of vegetative and generative organs (Fu and Ohba, 2001). However, micromorphological characters such as pollen morphology (Zheng, 1997), leaf epidermis (Zheng and Gong, 1999), stem anatomy (Zheng et al., 2001) and seed morphology (Jin et al., 2008) have also proved useful, whilst DNA characters have been used to reveal the relationships among *Sedum* species (Wu et al., 2008; Carrillo-Reyes et al., 2009; Li et al., 2010). The aim of this paper is to describe formally the new species *S. plumbizincicola* X.H. Guo et S.B. Zhou ex L.H. Wu and to clarify the affinities between *S. plumbizincicola* and closely related taxa on the basis of geographical distribution, growth habit, phenology, macromorphological characters, stem and leaf anatomical features, seed micromorphology and nrDNA ITS sequence data.

MATERIALS AND METHODS

Sixteen populations comprising *S. hangzhouense* (1 population), *S. alfredii* (3 populations), *S. emarginatum* (3 populations), *S. bailey* (2 populations) and *S. plumbizincicola* (7 populations) from Zhejiang Province, China were sampled during the summer of 2011 (Table 1) and more than 10 individuals were sampled per population for morphological, anatomical and molecular research. Voucher specimens are deposited in the Herbarium of Anhui Normal University (ANU!), China. Fifty-six ITS sequences were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/: Table 2).

Eighteen quantitative macromorphological characters were measured in the sampled taxa (Table 3). Ten measurements were made for each population and then averaged at species level. Eight qualitative macromorphological characters, together with four habit, habitat and phenology characters were also determined. Seeds of *S. plumbizincicola* and *S. alfredii* were collected from mature capsules of living specimens. Dried seeds were arranged on stubs and sputter-coated with gold for observation by scanning electron microscopy (SEM). Seed micromorphology was photographed and morphometry of seeds was examined using an FEI Quanta 200 ESEM scanning electron microscope at Nanjing Forestry University. Ten measurements were made for each population and then averaged at species level.

Ten fresh leaves and stem sections of *S. alfredii* and *S. plumbizincicola* were fixed and preserved in formaldehyde–acetic acid–alcohol (FAA) solution (Stern and Judd, 2002). They were dehydrated in a graded ethanol series, embedded in paraffin and sectioned transversely to 8 μm with a KD-2508 Rotary Microtome (Zhejiang Jinhua Kedi Instrument Equipment Co., China). These sections were stained with methylene blue and fixed in neutral resin. Finally, all specimens were observed and photographed under an Olympus stereomicroscope (DP71) to compare the main anatomical characters.

Genomic DNA from 160 individuals belonging to 16 populations of five *Sedum* species (Table 1) was extracted from silica gel-dried leaves using the modified 2× hexadecyltrimethylammonium bromide (CTAB) method (Hellwig, 1999). ITS-F (TGAACCTGCGGAAGGATCAT) and ITS-R (GGTAGTCCCGCCTGACCTG) primers to the conserved domain of the ITS sequence were synthesized by the Beijing Genomics Institute. Each reaction solution contained 10 μl of 2 EasyTaq PCR SuperMix, 10 μmol μl^{-1} of each primer, and 1 μl of DNA template in a total volume of 20 μl. PCR amplifications were carried out on a TaKaRa PCR Thermal Cycler Dice using the following programme: 300 s of initial denaturation at 94 C, followed by 34 cycles of 45 s denaturation at 94 C, 45 s annealing at 58 C and 90 s elongation at 72 C, and finishing with 10 min elongation at 71 C. PCR products were subcloned with the pEASY-T3 Cloning Kit (TransGen Biotech) according to the manufacturer's instructions, and five colonies for each sample were screened. Products were sequenced at the Beijing Genomics Institute. The ITS sequence data for 16 populations, together with 56 ITS sequences downloaded from GenBank, were edited and aligned using Lasergene version 7.0 (Griffin and Griffin, 1996) and DNAMAN version 6.0.40 (Altschul et al., 1990). Phylogenetic trees were inferred using maximum parsimony (MP) criteria as implemented in MEGA version 5.0 (Tamura et al., 2011). All positions containing gaps and missing data were eliminated. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) (Felsenstein, 1985) was calculated. The MP tree was obtained using the Close-Neighbor-Interchange algorithm (Nei and Kumar, 2000) with search level 3, in which the initial trees were obtained with the random addition of sequences (10 replicates).

RESULTS AND DISCUSSION

Description of the new species *S. plumbizincicola*

Type: China, Zhejiang Province, Hangzhou city, Chun'an county, Zitong town, 29°32′08″ - 29°37′00″ N, 118°34′48″ - 118°39′51″ E, alt. 220 m , Lead–zinc mining area, 10 June 2005, *Bide05061028* (holotype ANU!; isotype IBK!).

Description: Sedum plumbizincicola X.H. Guo et S.B. Zhou ex L.H. Wu *sp. nov.* Perennial, light green, or yellowish green herb. Roots fibrous. Rhizomes slender and horizontal, yellowish brown or dark brown, to 7 cm long, *ca.* 4–8 mm diameter. Sterile stems several-branched, erect, 10–25 cm, densely caespitose. Leaves of foliage branches alternate, usually deciduous, crowded distally on stem; leaf blade ovate to obovate-spatulate, 1–5 0.5–1.5 cm, glabrous, apex blunted, base cuneate, pseudopetiolate; adaxial surface bright green, abaxial surface jade green, the midrib not convex abaxially. Inflorescence yellow, erect, much-branched, the peduncles up to 35 cm tall, *ca.* 0.8 cm in diameter. Cyme corymbiform, *ca.* 8 cm in diameter, many flowered, bracts linear to linear-lanceolate, 5–10 3–8 mm, apex blunted. Flowers sessile, unequally 4-merous. Sepals 4, narrowly triangulate, *ca.* 1–2 mm long apex blunted. petals 4, yellow, lanceolate, 4–6 1–1.5 mm, apex acute. Stamens 8, slightly shorter than the petals, antesepalous ones *ca.* 3.5–4.5 mm long, antepetalous ones ca.

3 mm long, inserted *ca*. 1 mm from petal base; filaments greenish, anthers oblong, yellow. Nectar scales inverted trapezia, *ca*. 0.3–1.0 0.8 mm. Carpels erect, ovoid-lanceolate, *ca*. 4–5 mm long, connate about one-third at base. Styles *ca*. 1 mm. Follicles split divergent, tetra-aristiform. Seeds numerous, brown, obovoid-oblong, *ca*. 0.7–1 mm long, mammillate. Flowering early June–August, fruiting July–September. See Figs 1 and 2.

Ecology, distribution and importance: This new species is only known from the type locality, Zitong town (lead–zinc mining areas) northwest of Hangzhou city in western Zhejiang Province of eastern China (see Figure 3). Annual rainfall varies from 980 to 2000 mm and occurs mainly in the summer, with a mean annual temperature of 15–18 C. Soils in this area are usually sandy, acidic, highly leached and often shallow (http://baike.baidu.com/view/2341.htm). This species has a strong ability to hyperaccumulate zinc and cadmium and is a promising taxon in the restoration of metal-polluted soils by phytoremediation

Etymology: The specific epithet '*plumbizincicola*' refers to the plant's distribution in the lead and zinc mining areas of western Zhejiang Province, China.

Diagnosis: S. plumbizincicola is placed in sect. *Sedum* according to the adaxially gibbous carpels and follicles. *S. alfredii*, *S. emarginatum*, *S. hangzhouense* and *S. bailey* are also distributed in the same areas as *S. plumbizincicola*, but they produce 5-merous flowers while the latter bears 4-merous flowers. *S. tetractinum* Fröderström, *S. hakonense* Makino and *S. dongzhiense* D.Q. Wang & Y.L. Shi in sect. *Sedum* also bear 4-merous flowers, but they differ from *S. plumbizincicola* in leaf blade shape (oblanceolate in *S. hakonense* and *S. dongzhiense,* orbicular in *S. tetractinum*, and ovate to obovate-spathulate in *S. plumbizincicola*) and in their habitats.

Additional collections: CHINA. Zhejiang Province: Chun'an county, Zitong town, alt. 250 m, 10 June 2011, *Y.j.Liu 201106021*(ANU!); ibid., 251 m, *Y.j.Liu 201106022*(ANU!); ibid., 234 m, *Y.j.Liu 201106023*(ANU!); ibid., 258 m, *Y.j.Liu 201106024*(ANU!); Zhejiang province: Chun'an county, Panjia town, alt. 134 m, *Y.j.Liu 201106025*(ANU!); ibid., 188 m, *Y.j.Liu 201106026*(ANU!); ibid., 199 m, *Y.j.Liu 201106027*(ANU!).

Affinities between *S. plumbizincicola* and closely related taxa

Because we found that *S. plumbizincicola* mostly resembled *S. alfredii* and that *S. hangzhouense, S. emarginatum* and *S. baileyi* were distributed in the same areas as these two species, our research work focused on the relationships among these five taxa.

Macromorphological, phenological and ecological characters of the five *Sedum* species are listed in Table 3. Macromorphological characters show that *S. plumbizincicola* is differentiated from *S. alfredii* and other similar *Sedum* species by its tetramerous flowers and thick sterile stems. Its restriction to metalliferous mining areas and low altitude in distribution also support recognition of *S. plumbizincicola* as a distinct taxonomic entity.

The leaf and stem anatomical characters of *S. alfredii* and *S. plumbizincicola* are shown in Table 4 and Figure 4. Leaves of *S. plumbizincicola* are thicker than those of *S. alfredii*. The stems of *S. alfredii* are narrower than those of *S. plumbizincicola*, whilst *S. alfredii* stems have more cortical parenchyma cells and fewer pith cells than *S. plumbizincicola*. *Sedum plumbizincicola* produces more vascular bundles and has a higher ratio of xylem to phloem cross-sectional area than *S. alfredii*. The leaf and stem anatomical data support *S. alfredii* and *S. plumbizincicola* as separate taxa.

The seed micromorphological characters of *S. alfredii* and *S. plumbizincicola* are displayed in Figure 5 and their comparisons with *S. hangzhouense*, *S. emarginatum* and *S. baileyi* are listed in Table 5. The epidermal cells of *S. alfredii* and *S. hangzhouense* seeds are uplifted, whereas those of the other three species are not. The surfaces of *S. alfredii* and *S. plumbizincicola* seeds are both loosely mammillate, but the mammillae differ in shape. Those of *S. alfredii* are spherical whereas those of *S. plumbizincicola* are prolate-spheroidal. The seed micromorphological characters also suggest that *S. alfredii* and *S. plumbizincicola* are different species.

The nrDNA ITS sequences of 16 populations of *S. plumbizincicola*, *S. alfredii*, *S. emarginatum*, *S. hangzhouense* and *S. baileyi* (Table 1) were determined. These ITS sequence data together with 56 ITS sequence data of *Sedum* species and 3 outgroups downloaded from NCBI (Table 2) were used to reconstruct a nrDNA ITS phylogeny for these taxa using maximum parsimony. The sequence alignment consisted of 682 characters, of which 443 were variable sites and 352 were parsimony-informative sites. Twenty equally parsimonious trees were obtained (length = 1852, consistency index = 0.42, retention index = 0.78), of which one is shown in Figure 6. The seven *S. plumbizincicola* accessions (top of Figure 6) form a strongly supported monophyletic clade (99 % bootstrap support) sister to *S. alfredii*. Together, *S. plumbizincicola* and *S. alfredii* form a strongly supported clade (99 % bootstrap support) sister to a monophyletic *S. emarginatum*. Thus, the nrDNA ITS phylogeny supports the concept of *S. plumbizincicola* as a monophyletic entity.

At the infrageneric level, there is still uncertainty about taxonomic relationships within *Sedum*. Praeger (1921) recognized 10 sections within the genus *Sedum* whereas Berger (1930) recognized 22, of which ten have now been transferred to other genera (Carrillo-Reyes et al., 2009). Fu and Ohba (2001) assigned the Chinese species of *Sedum* to three sections, i.e. sect. *Filipe* and sect. *Oreades* (Fu, 1965, 1974) and sect.

Sedum, the largest in the genus ('t Hart, 1991; Carrillo-Reyes et al., 2009). Figure 6 shows that the 69 accessions of 49 *Sedum* species can be split into two strongly supported clades (99 % bootstrap support): the upper one in Figure 6 comprises 24 accessions of 7 species from China, 11 accessions of 11 species from Japan, and 4 accessions of 4 species from Nepal, whereas the lower one contains 30 accessions of 29 species from Mexico. This result suggests that *Sedum* species in East Asia are phylogenetically distinct from those in Mexico. Among the 39 East Asia accessions, *S. oreades* (Decaisne) Raym.-Hamet and *S. trullipetalum* J.D. Hooker & Thomson belong to sect. *Oreades*, while all the other accessions belong to sect. *Sedum* (Fu and Ohba, 2001). Figure 6 does not support a sister-group relationship between *S. oreades* and *S. trullipetalum* , although relationships are not well resolved in this part of the tree. Nevertheless, this result lends weight to the view that the phylogeny of *Sedum* inferred from nucleotide sequence data does not accord with classifications of the genus based on morphological characters (Carrillo-Reyes et al., 2009). Greater taxon sampling and additional markers will be required to arrive at a more definitive consensus on infrageneric relationships within *Sedum*, but we place the new species *S. plumbizincicola* in sect. *Sedum* on the basis of its adaxially gibbous carpels and follicles.

In summary, the ecological, macromorphological, micromorphological and molecular data show that *S. plumbizincicola* should be recognized as a new species in sect. *Sedum*.

ACKNOWLEDGEMENTS

This research was supported jointly by the International Scientific Collaborative

Programme of the Ministry of Science and Technology of China (2010DFA92360), the National Natural Science Foundation of China (40821140539, 40871155), and the Program of Innovative Engineering of the Chinese Academy of Sciences (KSCX2-YW-G-053). A grant from Anhui Normal University is gratefully acknowledged. We extend special thanks to research scientists Xiao-quan Wang and Xian-zhao Kan. We thank Xia Pan, Jing Ren, Jie-xue Huang, Feng-jiao Nai, Li-ke Cheng, Li-qiang Zhou, Qi Luo, Ying Wang, Gui-jun Wang, Benqi Yu, Jinrong Hu, Jinghua Li and Naidong Chen for useful discussion regarding populations, habitat, and conservation. We also thank Dr S.A. Harris, Curator of the Oxford University Herbaria, UK, for helpful advice and discussions.

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Table 1 *Sedum* material used in the present study.

Taxon	Location	Altitude /m	Latitude North	Longitude East	Voucher
S. alfredii 1	Fuyang	177-198	30°06′40″ -30°06′41″	119°49′57″	*P.C.Tsoong 19363935*(WUK)
S. alfredii 2	Hangzhou	18-52	30°11′58″ -30°12′02″	120°06′57″ -120°11′58″	*G.j.Li 198211777*(IBK)
S. alfredii 3	Lin'an	996-1040	30°17′29″ -30°17′36″	119°11′36″ -119°11′37″	*Z.r.Liu 19562314*(WUK)
S. emarginatum 1	Hangzhou	15-52	30°11′58″ -30°12′02″	120°06′57″ -120°11′58″	*M.x.Nie 1957661*(LBG)
S. emarginatum 2	Hangzhou	60-68	30°08′42″ -30°14′32″	120°03′30″ -120°05′53″	*M.x.Nie 1957661*(LBG)
S. emarginatum 3	Lin'an	181-702	30°12′56″ -30°16′08″	119°11′18″ -119°11′31″	*M.x.Nie 1957661*(LBG)
S. hangzhouense	Hangzhou	59-77	30°08′42″ -30°14′32″	120°03′30″ -120°05′53″	*Y.j.Liu 201106011*(ANU!)
S. baileyi 1	Lin'an	997-1024	30°17′29″- 30°17′30″	119°11′35″- 119°11′37″	Q.h.Li 19591357 (LBG)
S. baileyi 2	Chun'an	135-211	29°32′15″ -29°35′14″	118°34′47″ -118°38′53″	Q.h.Li 19591357 (LBG)
S. plumbizincicola 1	Chun'an	134	29°32′08″ -29°32′09″	118°34′48″ -118°39′05″	*Y.j.Liu 201106021*(ANU!)
S. plumbizincicola 2	Chun'an	188-220	29°35′43″ -29°35′53″	118°34′51″ -118°34′52″	*Y.j.Liu 201106022*(ANU!)
S. plumbizincicola 3	Chun'an	199-220	29°35′42″ -29°35′55″	118°34′48″ -118°34′51″	*Y.j.Liu 201106023*(ANU!)
S. plumbizincicola 4	Chun'an	251-270	29°36′50″ -29°36′55″	118°39′53″ -118°39′56″	*Y.j.Liu 201106024*(ANU!)
S. plumbizincicola 5	Chun'an	239-267	29°36′56″ -29°37′00″	118°39′49″ -118°39′53″	*Y.j.Liu 201106025*(ANU!)
S. plumbizincicola 6	Chun'an	234-282	29°36′56″ -29°36′59″	118°39′49″ -118°39′53″	*Y.j.Liu 201106026*(ANU!)
S. plumbizincicola 7	Chun'an	234-282	29°36′56″ -29°36′58″	118°39′50″ -118°39′51″	*Y.j.Liu 201106027*(ANU!)

Table 2 Accession information relating to ITS sequences for 56 taxa downloaded from NCBI.

Species	Source	Voucher	Taxon number	Accession number	Base pairs
S. alfredii Hance	China:	*G.j.Li*	taxon: 439688	FJ919949	642

	Chunan	*198212006*(IBK)			
S. alfredii Hance	China: Fuyang	*P.C.Tsoong 19363935*(WUK)	taxon: 439688	FJ919948	642
S. alfredii Hance	China: Guangzhou	*G.j.Li 198010620*(IBK)	taxon: 439688	FJ919951	647
S. alfredii Hance	China: Hangzhou	*G.j.Li 198211777*(IBK)	taxon: 439688	FJ919950	646
S. baileyi Praeger	china	*Q.h.Li 19591357* (LBG)	taxon: 650621	FJ919935	644
S. emarginatum Migo	China: Anhui	*M.x.Nie 1957661*(LBG)	taxon: 516554	EU592006	671
S. lineare Thunberg	China	*P.y.Li 19592215*(WUK)	taxon: 114260	FJ980313	691
S. lineare Thunberg	Japan: Ohita	*Mayuzumi* C00030 (TI)	taxon: 114260	AB088623	678
S. sarmentosum Bunge	China: Shanghai	*L.d.Nan 19585214*(IBSC)	taxon: 91146	EU592003	678
S. sarmentosum Bunge	Japan	*Mayuzumi* C00008 (TI)	taxon: 91146	AB088624	677
S. makinoi Maximowicz	Japan	*Mayuzumi* C00086 (TI)	taxon: 203022	AB088627	672
S. yabeanum Makino	Japan: Nagasaki	*Mayuzumi* C00029 (TI)	taxon: 203030	AB088626	671
S. bulbiferum Makino	Japan: Mie	*Niu* 1999 (TI)	taxon: 91125	AB088628	677
S. subtile Miquel	Japan	*Shimizu* 1999 (TI)	taxon: 203026	AB088622	676
S. oryzifolium Makino	Japan: Kanagawa	*Mayuzumi* C00016 (TI)	taxon: 91144	AB088618	669
S. japonicum Siebold ex Miquel	Japan: Fukuoka	*Mayuzumi* C00030 (TI)	taxon: 203021	AB088617	664
S. mexicanum Britt.	Japan: Tokyo	*Mayuzumi* C00001 (TI)	taxon: 203023	AB088621	674
S. tosaense Makino	Japan: Kochi	*Iwamoto* 2000 (TI)	taxon: 203027	AB088620	676
S. zentaro-tashiroi Makino	Japan	*Ohba* 1998 (TI)	taxon: 203031	AB088619	666
S. triactina A. Berger	Nepal	*Miyamoto* 9596091 (TI)	taxon: 203028	AB088629	675
S. multicaule subsp. *multicaule* Wall. ex Lindl.	Nepal	*Miyamoto* 9596136 (TI)	taxon: 203024	AB088631	673
S. oreades (Decaisne) Raym.-Hamet	Nepal	*Miyamoto* 9420140 (TI)	taxon: 203025	AB088632	671

S. trullipetalum J.D. Hooker & Thomson	Nepal	*Miyamoto* 9420132 (TI)	taxon: 203029	AB088630	665
S. guatemalense Hemsl.	Mexico	*Ruiz-Sanchez* 145 (XAL)	taxon: 665369	FJ753945	697
S. oaxacanum Rose 1	Mexico	*Carrillo-Reyes & Cabrera-Toledo* 5092 (XAL)	taxon: 91142	EF632176	687
S. oaxacanum Rose 2	Mexico	*Kimnach* 5365 (HBG)	taxon: 91142	AY545716	641
S. bourgaei Hemsl.	Mexico	*Perez & Platas* 3224 (IEB)	taxon: 665357	FJ753932	699
S. alexanderi Eggli	Mexico	*Carrillo-Reyes & Vaz-de-Mello* 4257 (IEB, XAL)	taxon: 467245	EF632174	692
S. obcordatum R.T. Clausen	Mexico	*Carrillo-Reyes & Nicolalde* 4440 (XAL)	taxon: 91143	FJ753957	688
S. compactum Rose	Mexico	*Lau s.n.* (HBG, NY)	taxon: 264182	EF632175	690
S. treleasei Rose	Mexico	*Cabrera-Toledo & Nicolalde* 4 (XAL)	taxon: 665387	FJ753966	695
S. retusum Hemsl.	Mexico	*Zamudio & Perez* 9908 (IEB)	taxon: 665386	FJ753964	697
S. pachyphyllum Rose	Mexico	*Carrillo-Reyes & Vaz-de-Mello* 4912 (IEB, XAL)	taxon: 665382	FJ753960	693
S. pacense J. Meyran	Mexico	*Perez* 2750 (IEB)	taxon: 665381	FJ753959	694
S. allantoides Rose	Mexico	*Rose s.n.* (HBG)	taxon: 264180	AY545712	643
S. grandisepalum R.T. Clausen	Mexico	*Carrillo-Reyes & Vaz-de-Mello* 4964 (XAL, IEB)	taxon: 665365	FJ753942	687
S. quevae Raym.-Hamet	Mexico	*Carrillo-Reyes & Cabrera-Toledo* 4496 (XAL)	taxon: 665384	FJ753962	687
S. jurgensenii (Hemsl.) Moran	Mexico	*Carrillo-Reyes & Nicolalde* 4488 (XAL, MEXU)	taxon: 264186	FJ753949	688
S. oxypetalum Kunth	Mexico	*Perez* 3509 (IEB)	taxon: 665380	FJ753958	697
S. greggii subsp. *angustifolium* R.T. Clausen	Mexico	*Rodriguez s.n.* (IBUG)	taxon: 665368	FJ753944	698
S. catorce G.L. Nesom	Mexico	*Perez s.n.* (IEB)	taxon: 665360	FJ753935	701
S. clavatum R.T. Clausen	Mexico	*Meyran* 1306 (HBG)	taxon: 264181	AY545713	643
S. reniforme (H.	Mexico	*Carrillo-Reyes &*	taxon: 665385	FJ753963	691

Jacobsen) Thiede & 't Hart		*Chocce* 5174 (IEB, USM)			
S. nussbaumerianum Bitter	Mexico	*Ruiz-Sanchez* 9 (XAL)	taxon: 665379	FJ753956	686
S. jurgensenii subsp. *attenuatum* Moran	Mexico	*Ruiz* 93 (XAL)	taxon: 665372	FJ753950	697
S. commixtum Moran & Hutchison	Mexico	*Carrillo-Reyes & Vaz-de-Mello* 4954 (IEB, XAL)	taxon: 91128	FJ753938	688
S. carinatifolium (R.T. Clausen) Pérez-Calix	Mexico	*Perez & Carranza* 3155 (IEB)	taxon: 665359	FJ753934	694
S. versadense C.H. Thomps.	Mexico	*Carrillo-Reyes & Cabrera-Toledo* 4498 (IEB, XAL)	taxon: 665389	FJ753968	688
S. plicatum Thiede & 't Hart	Mexico	*Carrillo-Reyes & Chocce* 5173 (IEB, USM)	taxon: 665383	FJ753961	691
S. andinum Ball	Mexico	*Carrillo-Reyes & Ortega* 5178 (IEB, USM)	taxon: 665356	FJ753930	691
S. fuscum Hemsl.	Mexico	*Perez & Ocampo* 4188 (IEB)	taxon: 665364	FJ753941	709
S. corynephyllum Fröd.	Mexico	*Acevedo* 1713 (XAL, NY)	taxon: 264183	AY545715	643
S. palmeri S. Watson	Mexico	*Acevedo & Hernandez-Galavaz* 1721 (XAL, NY)	taxon: 264184	AY545717	631
Rhodiola rosea L.	Japan:Hokkaido	*Midorikawa*. 1999 (TI)	taxon: 203015	AB088599	670
Kalanchoe beharensis Drake	Germany (cultivated)	clone pOG5.3-6	taxon: 80894	AJ231314	695
Hylotelephium verticillatum (L.) H. Ohba	Japan: Kumamoto	*Ikeda* 16-IV-2000 (TI)	taxon: 202978	AB088564	678

Table 3 Macromorphological, phenological and ecological characters of five *Sedum* species.

Character	**Species**				
	S. alfredii	***S. emarginatum***	***S. hangzhouense***	***S. baileyi***	***S. plumbizincicola***
Habit	Perennial	Perennial	Annual	Perennial	Perennial
Rhizome	Absent	Absent	Absent	Present	Present
Sterile stems	Slender	Slender	Slender	Absent	Thick
Stem length (cm)	10-20	10-15	8-20	3-7	10-25
Phyllotaxy	Alternate	Opposite	Alternate	Opposite	Alternate
Leaf blade	Linear -cuneate, spatulate, or obovate	Spatulate-obovate to broadly obovate	Narrowly obovate to spatulate-oblong	Obovate-spatulate	Ovate or obovate-spatulate
Leaf length width (cm)	1.2-3 2-6	1-2 5-10	2-3 3-7	1.5 6	1-5 5-15
Inflorescence	Cyme corymbiform	Cyme corymbiform	Cyme corymbiform	Cyme corymbiform	Cyme corymbiform
Bract shape	Leaf-like	Leaf-like	Leaf-like	Obovate	Linear to linear-lanceolate
Bract length width (mm)	3-10 1-4	2-6 2-6	6-20 2-5	1-3 1-4	5-10 3-8
Sepal shape	Linear-spatulate	Lanceolate to narrowly oblong	Broadly linear-ovate	Oblong-linear	Narrowly triangle, apex blunted
Sepal length width (mm)	3-5 1-1.5	2-5 0.7-2	1.5-2.4 0.3-0.6	4-5 1.5	1-2 0.16-1.08
Sepal number	5	5	5	5	4
Petal shape	Lanceolate to lanceolate-oblong	Linear-lanceolate to lanceolate	Linear-lanceolate	Lanceolate	Lanceolate
Petal length width (mm)	4-6 1.6-1.8	6-8 1.5-2	4-4.5 0.8-1.2	4-5 1.5	4-6 1-1.5
Petal number	5	5	Unequally 5	5	4

Stamen number	10	10	10	10, shorter than petals	8
Stamen (length)	Antesepalous (4 mm)	Antesepalous (3-6mm)	Antesepalous (3.5-4.5 mm	Antesepalous (2.5-3mm)	Antesepalous (3.5-4.5 mm)
Style (length)	Antepetalous (2.5 mm)	Antepetalous (2-4 mm)	Antepetalous (2-3 mm)	,Antepetalous (2.5-3 mm)	Antepetalous (3-4 mm)
Scale shape	Spatulate-quadrangular, apex obtusely truncate	Oblong	Subspatulate	Oblong-spatulate	Inverted trapezia
Squama length	0.2-1.2 mm	0.3-0.6 mm	0.2-0.4 mm	0.4-0.6 mm	0.3-1.0 mm
Squamae number	5	5	5	5	4
Carpel shape	Ovoid-lanceolate	Oblong	Ovoid-lanceolate	Oblong	Ovoid-lanceolate
Carpel length	4 mm	4-5 mm	4-4.5 mm	4 mm	4-5 mm
Carpel number	5	5	5	5	4
Follicles	Divergent	Divergent	Stellately divaricate	Divergent	Split divergent
Seed colour	Brown	Brown	Brown	Brown	brown
Flowering	April-May	May-June	May-June	April	June-August
Fruiting	June-August	July	June-July	July	July-September
Habitat	Shady, moist rocks on forested slopes	Shady and moist slopes	Forested slopes, shady, moist crevices	Rock crevices on slopes.	Mining area
Altitude (m)	< 1400	600-1800	600	c. 900	134-290

Table 4 Comparison of leaf and stem anatomy of *S. alfredii* and *S. plumbizincicola*. Where indicated, errors are ± 1 standard error (*n* = 10 samples).

Characters	*S. alfredii*	*S. plumbizincicola*
Thickness of leaves (μm)	625 ± 28.4	911 ± 65.6
Thickness of upper epidermis (μm)	14 ± 2.05	35 ± 4.69
Thickness of lower epidermis (μm)	10 ± 0.73	24 ± 2.24
Thickness of mesophyll (μm)	601 ± 25.6	852 ± 58.7
Stem diameter (μm)	1372 ± 22.4	2416 ± 61.9
Occupancy of cortex parenchyma cells in stem (%)	64.1	45.9
Occupancy of pith parenchyma cells in stem (%)	10.9	30.2
Number of vascular bundles	5	11
Ratio of xylem to phloem cross-sectional area	1.99	6.82

Table 5 Seed micromorphology of *S. plumbizincicola* and S. *alfredii* compared to *S. baileyi*, *S. emarginatum* and *S. hangzhouense*.

Species	Shape	Seed size (length width) mm	Size index	Seed sculpture	Number of samples	Reference
S. plumbizincicola	Fusiform or oval	(0.818 0.087) (0.352 0.032)	0.288	Epidermal cell edges not obviously uplifted; surface loosely mammillate; mammillae uniform, prolate-spheroidal	10	
S. alfredii	Oval-elliptic	(0.668 0.048) (0.274 0.028)	0.183	Epidermal cell edges obviously uplifted; surface loosely mammillate; mammillae uniform, spherical	10	
S. baileyi	Long round spherical	(0.519 0.011) (0.210 0.007)	0.109	Epidermal cell edges not obviously uplifted; surface densely mammillate; mammillae uniform, spherical or	10	Jin (2008)

				prolate-spheroidal		
S. emarginatum	Ellipsoidal	(0.664 0.010) (0.283 0.005)	0.188	Epidermal cell edges not obviously uplifted; surface loosely mammillate; mammillae uniform, spherical or prolate-spheroidal	10	Jin (2008)
S. hangzhouense	Ellipsoidal	(0.473 0.005) (0.188 0.005)	0.089	Epidermal cell edges obviously uplifted; surface densely mammillate; mammillae uniform, spherical or prolate-spheroidal	10	Jin (2008)

Fig. 1 *Sedum plumbizincicola* in its vegetative (a) and flowering (b) states in the original habitat (Wu et al., 2006).

Fig. 2 *Sedum plumbizincicola*. a: flowering states; b: vegetative states; c: leaf; d: petal with two stamens; e: sepal; f: sepal at frutescence; g: two of four carpels; h: two of four follicles; i: bract; j: squama (scale bars: a, c = 10 mm; b = 6 mm; d, i = 5 mm; e–g = 2 mm; h = 4mm; j = 0.5 mm). Illustration by Shoubiao Zhou.

Fig. 3 Distribution map of *Sedum plumbizincicola.*

Fig. 4 Leaf (a, b) and stem (c, d) transverse sections of *S. alfredii* (a, c) and *S. plumbizincicola* (b, d). The organs of the two species were of comparable developmental age.

Fig. 5 Seed morphology (a, b) and surface detail (c, d) of *S. alfredii* (a, c) and *S.*

plumbizincicola (b, d).

Fig. 6 nrDNA ITS maximum parsimony tree for 69 *Sedum* accessions with three outgroups (*Hylotelephium verticillatum*, *Kalanchoe beharensis* and *Rhodiola rosea*). Numbers associated with nodes are percentage bootstrap support values; nodes where values are not shown received < 50 % support.

Figure 1

Figure 2


ANHUI
Hangzhou
Jinhua
Quzhou
Taizhou
JIANGXI
Lishui
Wenzhou
FUJIAN
50 km
30 mi
© Daniel Dalet / d-maps.com

Figure 3

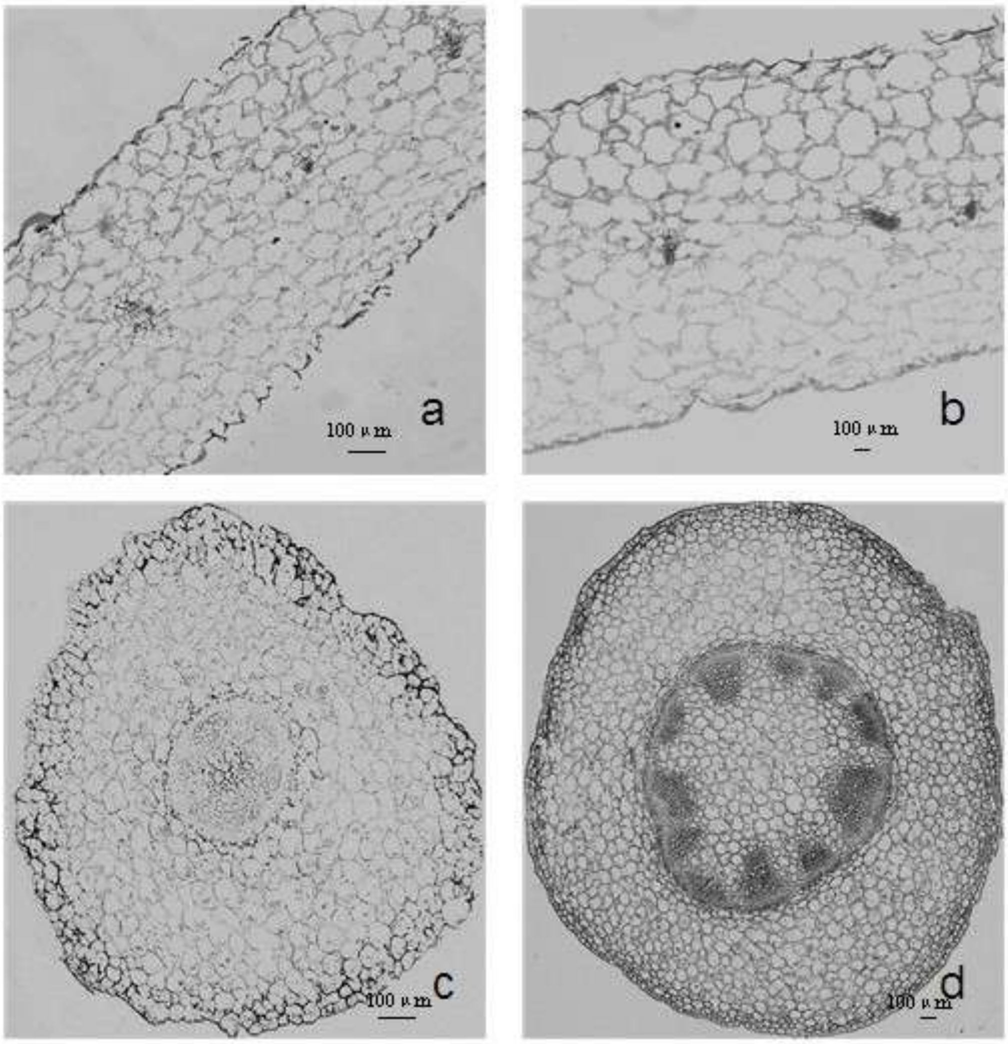

Figure 4

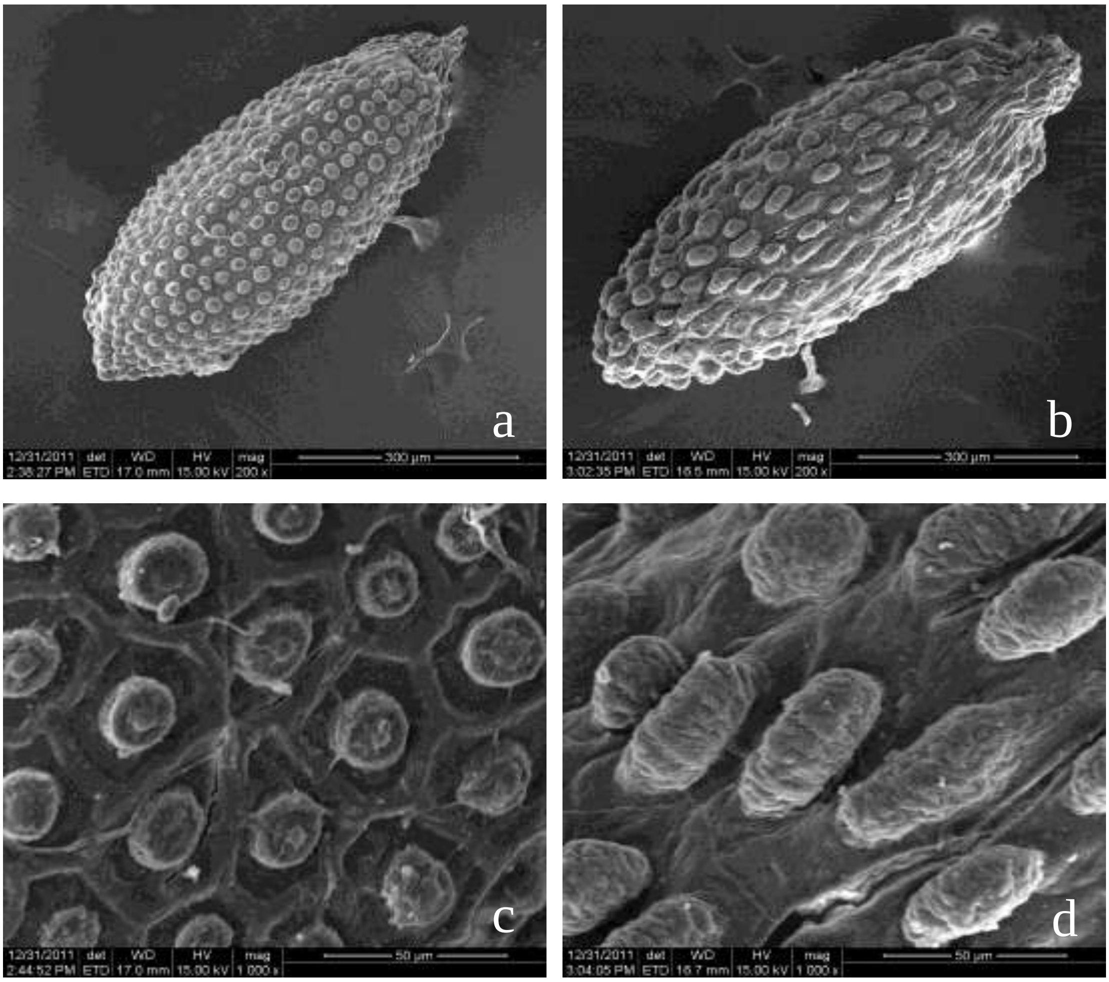

Figure 5

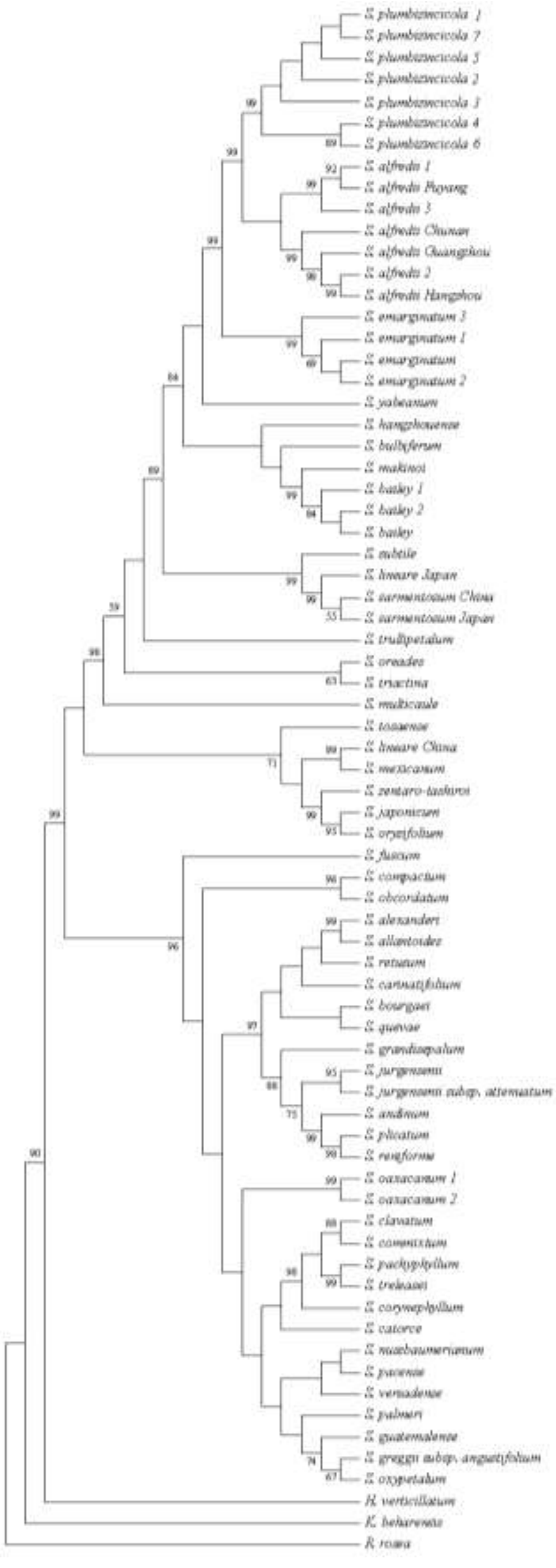
S. plumbizincicola 1
S. plumbizincicola 7
S. plumbizincicola 5
S. plumbizincicola 2
S. plumbizincicola 3
S. plumbizincicola 4
S. plumbizincicola 6
S. alfredii 1
S. alfredii Fuyang
S. alfredii 3
S. alfredii Chunan
S. alfredii Guangzhou
S. alfredii 2
S. alfredii Hangzhou
S. emarginatum 3
S. emarginatum 1
S. emarginatum
S. emarginatum 2
S. hangzhouense
S. makinoi
S. bailey 1
S. bailey 2
S. bailey
S. lineare Japan
S. oreades
S. triactina
S. multicaule
S. tosaense
S. lineare China
S. mexicanum
S. zentaro-tashiroi
S. japonicum
S. fuscum
S. compactum
S. obcordatum
S. alexanderi
S. retusum
S. carinatifolium
S. bourgaei
S. grandisepalum
S. jurgensenii
S. jurgensenii subsp. attenuatum
S. andinum
S. plicatum
S. reniforme
S. oaxacanum 1
S. oaxacanum 2
S. clavatum
S. commixtum
S. treleasei
S. corynephyllum
S. catorce
S. pacense
S. versadense
S. palmeri
S. guatemalense
S. oxypetalum
H. verticillatum
K. beharensis
R. rosea

Figure 6