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Sedum spiralifolium (Crassulaceae): a new species from Anhui Province, China

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Abstract

A new species of *Sedum, S. spiralifolium* from Anhui Province, eastern China is described and illustrated. Our molecular systematics results (based on nuclear ITS and plastid *trn*L-*trn*F) and morphological analyses indicate that this new species is closest to *S. sarmentosum.* It differs from *S. sarmentosum* by having linear-lanceolate leaves, earlier flowering and twisted upper leaves at the top of its sterile shoots.

Keywords: China, Crassulaceae, molecular systematics, new species

Introduction

Sedum Linnaeus (1753:430) is the largest genus in the family Crassulaceae, including approximately 430 species with the center of diversity in eastern Asia (Thiede and Eggli 2007). *Sedum* sect. *Sedum* includes more than 60 species mainly distributed in Asia and Europe, out of which approximately 49 species (34 endemic) occur in China (Fu *et al.* 2001). There are more than 20 species of sect. Sedum in Anhui Province (Zheng *et al.*1994), most of which have fleshy stems, small flowers, and quite variable habitus of leaves and sepals. However, it is difficult to distinguish them from each other only based on their macro-morphological features (Jin *et al.* 2010, Wu *et al.* 2013). Instead, their micro-morphological characters, such as pollen morphology (Zheng 1997), leaf epidermis morphology (Zheng & Gong 1999), stem anatomy (Zheng *et al.* 2001) and seed morphology (Jin *et al.* 2008) have proven to be good taxonomic characters.

During our field work in the Fourth National Survey on Chinese Material Medical Resources, an unusual population of *Sedum* was discovered in Shucheng County, Anhui Province. Macro-morphological character studies indicated that plants of this population, morphologically, are somewhat similar to *Sedum sarmentosum* Bunge (1833:114) with verticillate leaves and small yellow petals (Figure 3). An integrative taxonomy of this entity was performed, including observation of gross morphological characters, habit, phenology, stem anatomical features and molecular systematics based on ITS and *trn*L-*trn*F sequence data. All lines of evidence indicate that this little known species represent a new species.

Materials and method

Materials

Seven species, including *S. sarmentosum* Bunge, *S. lineare* Thunb. (1784:430) (for illustration, see Rao 1996:623), *S. onychopetalum* Fröd. (1933:199), *S. polytrichoides* Hemsl. (1887:286), *S. bulbiferum* Makino (1903:145), *S. emarginatum* Migo (1937:224) and the new species were collected from fields in eastern China (Table 1). The fresh leaves collected in fields were dried in silica gel for molecular examination. Voucher specimens are deposited in the Herbarium of Anhui University of Chinese Medicine (ACM).

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TABLE 1. Specimens used in the present study

Species	Location	Altitude/m	Latitude North	Longitude East	Voucher
S. polytrichoides	China /AH/Huangshan	601	30°05′12″	118°08′53″	130511hs21
S. polytrichoides	China /AH/Huangshan	455	30°05′22″	118°07′59″	130514hs45
S. polytrichoides	China/AH/Jinzhai	240	31°36′22″	118°83′59″	130503jz05
S. emarginatum	China/AH/Huangshan	461	30°05′29″	118°11′01″	130512hs27
S. emarginatum	China /ZJ/Hangzhou	281	30°14′52″	120°05′33″	130529hz03
S. emarginatum	China /AH/Jinzhai	234	31°35′02″	118°03′59″	130503jz21
S. onychopetalum	China /JS/Nanjin	245	32°07′17″	118°83′50″	130523nj67
S. onychopetalum	China ZJ/Shucheng	100	31°17′34″	116°58′16″	130524sc07
S. lineare	China /ZJ/Longquan	614	28°07′12″	118°57′47″	130529lq02
S. lineare	China /HB/Luotian	450	30°85′22″	115°47′10″	130510lt77
S. lineare	China/CQ/Nanchuan	543	29°35′12″	107°20′09″	130515nc76
S. sarmentosum	China/AH/Suichang	840	28°26′54″	118°54′06″	130526zsc1
S. sarmentosum	China/ZJ/Suichang	811	28°26′39″	118°54′30″	130526sc11
S. sarmentosum	China /ZJ/Longquan	1146	28°05′58″	118°58′11″	130529lq03
S. sarmentosum	China /AH/Huangshan	644	30°05′18″	118°08′50″	130511hs22
S. sarmentosum	China /AH/Lu'an	100	31°17′04″	116°57′16″	130531la22
S. spiralifolium	China /AH/Shucheng	98	31°17′34″	116°58′16″	130425sc01
S. spiralifolium	China /AH/ Shucheng	98	31°17′34″	116°58′16″	130505sc02
S. spiralifolium	China /AH/ Shucheng	98	31°17′34″	116°58′16″	130525sc03
S. spiralifolium	China /AH/ Shucheng	98	31°17′34″	116°58′16″	130525sc04
S. spiralifolium	China /AH/ Zongyan	53	30°56′20″	117°29′11″	130423zy61
S. spiralifolium	China /AH/ Zongyan	53	30°56′20″	117°29′11″	130423zy62
S. bulbiferum	China /ZJ//Suichang	845	28°26′54″	118°54′06″	130526zsc2
S. bulbiferum	China /ZJ/Quzhou	515	28°47′15″	119°04′22″	130524qz09
S. bulbiferum	China /AH/Huangshan	639	30°06′11″	118°10′08″	130514hs41

Morphological studies

Eighteen quantitative morphological characters of the six fresh specimens were observed. Thirteen qualitative gross morphological characters were observed. Habitat and phenology were also recorded.

Fresh stems of the new species, *S. sarmentosum*, *S. lineare*, *S. onychopetalum*, *S. bulbiferum* and *S. emarginatum* were sectioned into 100–200 μm pieces transversely by hand. These sections were observed and photographed under an Olympus BH-2 microscope.

DNA sequencing and molecular analyses

Total genomic DNA was extracted from leaves (Table 2) dried in silica gel using the Plant Genomic DNA Kit (Tiangen Biotech, Beijing, China) following to the manufacturer's protocols. For each individual, the nuclear (ITS) and chloroplast (*trn*L- *trn*F) regions were amplified using the primers ITS1 (5'TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCGCTTATTGATATGC3') (Gehrig *et al.* 2001), *trn*L (5'CGAAATCGGTAGACGCTACG3') and *trn*F (5' ATTTGAACTGGTGACACGAG 3') (Mayuzumi & Ohba 2004). The selected DNA regions were amplified using a standard polymerase chain reaction (PCR), PCR products were purified using the Biotekes Purification Kit (Bioteke Corporation, Beijing, China) and sequenced with an ABI 3730 DNA sequencer (Applied Biosystems) and ABI BigDye 3.1 terminator cycle sequencing kit (Sangon Co., Ltd, Shanghai, China).

Each sequence was assembled with SeqMan (DNA Star Inc., Madison, WI, USA) alignment using Clustal X version 2.0 (Thompson *et al.* 1997) and manually adjusted using BioEdit version 7.2.0 (Hall 1999) when necessary. Available sequence data were retrieved from GenBank. All sequences generated for the present study are available through GenBank (Table 2).

The phylogenetic relationships were constructed using maximum parsimony (MP) and Bayesian inference (BI). Maximum parsimony analyses were conducted with PAUP* 4.0b10 (Swofford 2002). All of the characters' state changes were equally weighted, and gaps were coded as missing data. Bootstrap values of the internal nodes were obtained with 1,000 replicates (Felsenstein 1985).

Bayesian inference was implemented using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Prior to the Bayesian analysis, the Akaike information criterion (AIC) implemented in ModelTest v3.7 (Posada & Crandall, 1998) was used to select the best-fit model of molecular evolution for each dataset. For the BI analyses, four Markov Chain Monte Carlo (MCMC) chains were run, sampling one tree every 1000 generations for 1,000,000 generations starting with a random tree. When the log-likelihood scores were found to have stabilized, a consensus tree was calculated after omitting the first 25% of the sampled trees as burn-in. The remaining trees were imported into PAUP* and a 50% majority-rule consensus tree was produced to obtain posterior probabilities (PP) of the clades.

TABLE 2. Specimens used in the molecular phylogenetic studies and their GenBank accession numbers

Species	Source	Voucher	Accession number	
			ITS	*trn*L-*trnF*
S. polytrichoides	China/AH/Huangshan	130511hs21(ACM)	KM111142	KM111117
S. polytrichoides	China /AH/Huangshan	130514hs45(ACM)	KM111143	KM111118
S. polytrichoides	China /AH/Jinzhai	130503jz05(ACM)	KM111144	KM111119
S. emarginatum	China /AH/Huangshan	130512hs27(ACM)	KM111145	KM111120
S. emarginatum	China /ZJ/Hangzhou	130529hz03(ACM)	KM111146	KM111121
S. emarginatum	China /AH/Jinzhai	130503jz21(ACM)	KM111147	KM111122
S. onychopetalum	China /JS/Nanjin	130523nj67(ACM)	KM111148	KM111123
S. onychopetalum	China /ZJ/Shucheng	130524sc07(ACM)	KM111149	KM111124
S. lineare	China /ZJ/Longquan	130529lq02(ACM)	KM111150	KM111125
S. lineare	China /HB/Luotian	130710lt77(ACM)	KM111151	KM111126
S. lineare	China /CQ/Nanchuan	130825nc76(ACM)	KM111152	KM111127
S. sarmentosum	China /AH/Jinzhai	130508jz02(ACM)	KM111153	KM111128
S. sarmentosum	China /ZJ/Suichang	130526sc11(ACM)	KM111154	KM111129
S. sarmentosum	China /ZJ/Longquan	130529lq03(ACM)	KM111155	KM111130
S. sarmentosum	China /AH/Huangshan	130511hs22(ACM)	KM111156	KM111131

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TABLE 2. (Continued.)

Species	Source	Voucher	Accession number	
			ITS	*trn*L-*trnF*
S. spiralifolium	China /AH/ Shucheng	130525sc03(ACM)	KM111160	KM111135
S. spiralifolium	China /AH/Zongyang	130525sc04 (ACM)	KM111161	KM111136
S. spiralifolium	China /AH/Zongyang	130423zy61(ACM)	KM111162	KM111137
S. spiralifolium	China /AH/Zongyang	130423zy62 (ACM)	KM111163	KM111138
S. bulbiferum	China /ZJ//Suichang	130526zsc2 (ACM)	KM111164	KM111139
S. bulbiferum	China /ZJ/Quzhou	130524qz09(ACM)	KM111165	KM111140
S. bulbiferum	China /AH/Huangshan	130514hs41(ACM)	KM111166	KM111141
S. makinoi	Japan/Tokyo	Mayuzumi C0003086	AB088627	AB089779
S. bulbiferum	Japan/Mie	Niu 1999	AB088628	AB089776
S. subtile	Japan/Tokyo	Shimizu 1999 (TI)	AB088617	AB089784
S. japonicum	Japan/Fukuoka	Mayuzumi C00030	AB088621	AB089783
S. mexicanum	Japan/Tokyo	Mayuzumi C00001	AB088621	AB089783
S. zentaro-tashiroi	Japan	Ohba 1998 (TI)	AB088619	AB089785
S. hakonense	Japan	Mayuzumi C00005	AB088625	AB089777
S. tosaense	Japan	TI:A.Iwamoto 2000	AB088620	AB089787
Kalanchoe densiflora	Germany	clone pOD5.3–6	AJ231334	X71986
Hylotelephium verticillatum	Japan/Kumamoto	*Ikeda* 16-IV-2000	AB088564	AB089724

Results and conclusion

The macro-morphological and phenological characters of the six *Sedum* species are listed in Table 3. The gross morphological characters suggested that the new species belongs to sect. *Sedum* which differs from *S. sarmentosum* and other similar species with its sterile shoots with twisted leaves and its flower with pedicel of 4–5 mm long. The stem anatomical characters of this new species are distinct and differ greatly from those of *S. sarmentosum, S. lineare, S. onychopetalum, S. polytrichoides* and *S. emarginatum* by having more vascular bundles and a higher ratio of xylem to phloem in cross section (Figure 1). Moreover, the new species is restricted to low elevation and flowers in early April, distinguishing it from the other species.

The partition homogeneity test for ITS + *trn*L-*trn*F shows character incongruence, whereas our visual inspection indicates that there are no 'hard' conflicts between the nuclear and plastid trees (Bull *et al.* 1993, Mason-Gamer & Kellogg 1996, Quicke *et al.* 2007). Therefore, we combined the datasets for simultaneous analyses.

In this study, the sequences of fourteen species (35 samples) were treated as ingroups. *Hylotelephium verticillatum* (L.) H. Ohba (1977:54) and *Kalanchoe densiflora* Rolfe (1919:263) were used as outgroups. Sequence lengths were as follows: 638 bp for the ITS region and 317 bp for the *trn*L-*trn*F intergenic spacer. The combined alignment of the ITS and the *trn*L-*trnF* genes comprised 955 bp, 515 characters were constant, 121 variable characters were parsimony-uninformative and 319 characters were parsimony-informative, and the P value of the partition-homogeneity test was 0.53 with 500 partition-homogeneity test replicates completed with likelihood settings from the best-fit model (GTR+G) selected via AIC in Modeltest v3.7.

Our analyses strongly indicate that *S. spiralifolium* belongs to sect. *Sedum* (Table 3). Based on combined ITS and *trn*L-*trn*F data, the trees produced with maximum parsimony (MP) and Bayesian inference (BI) are consistent in overall topology. The Bayesian tree is presented in Figure 2 with MP bootstrap and PP support values for each clade. Within clade II, *S. spiralifolium* is sister to *S. sarmentosum* (MP bootstrap = 100%, PP = 100%). The phylogenetic relationships estimated using the MP and BI methods were found to be almost identical.

Both morphological studies and phylogenetic analyses demonstrate that *S. spiralifolium* is a new species of *Sedum* sect. *Sedum*.

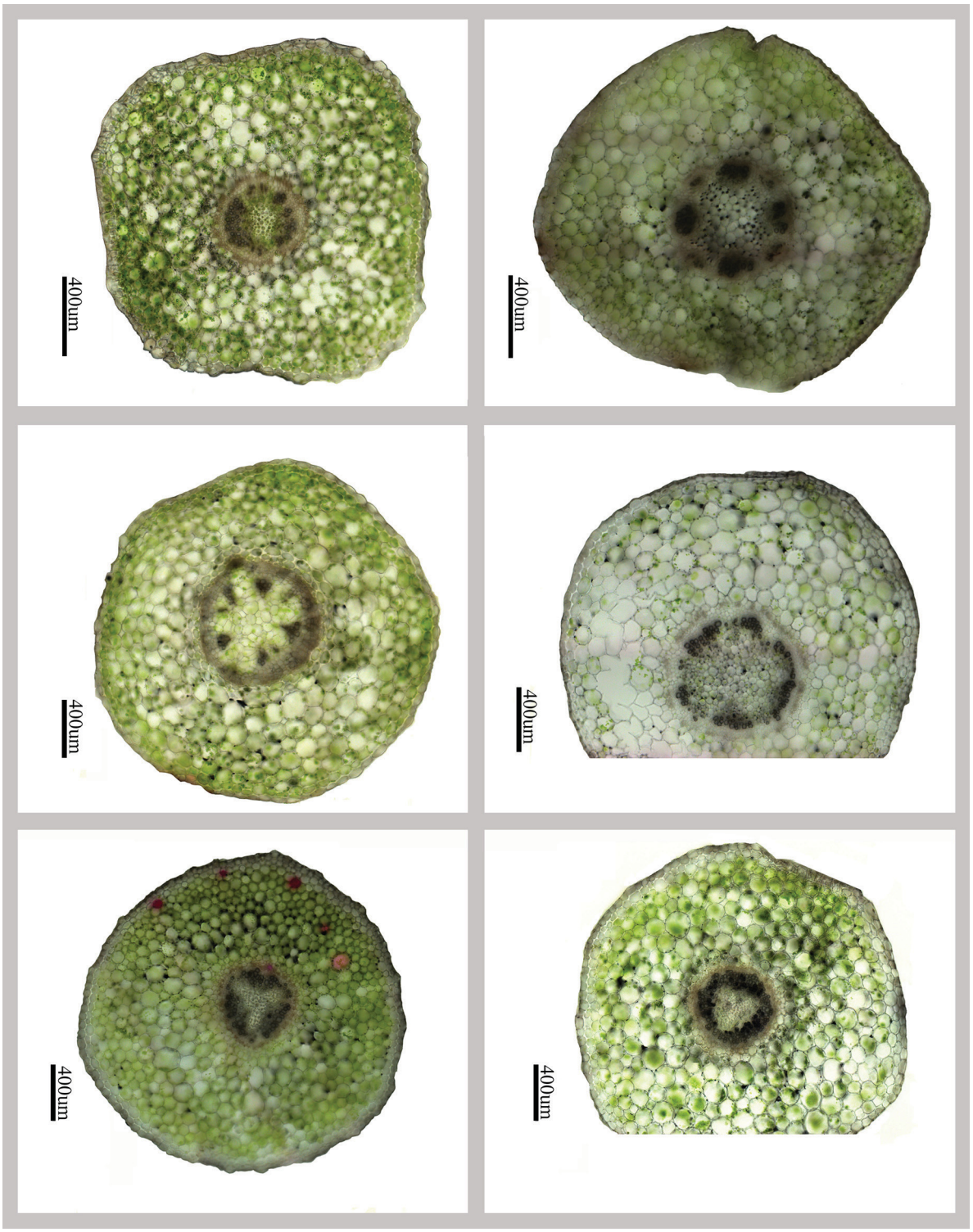

FIGURE 1. Morphological comparison of transverse stem sections between *S. spiralifolium* and other species within *sect. Sedum* of the same developmental age. A. *S. emarginatum*; B. *S. lineare*; C. *S. polytrichoides*; D. *S. onychopetalum*; E. *S. sarmentosum*; F. *S. spiralifolium*.

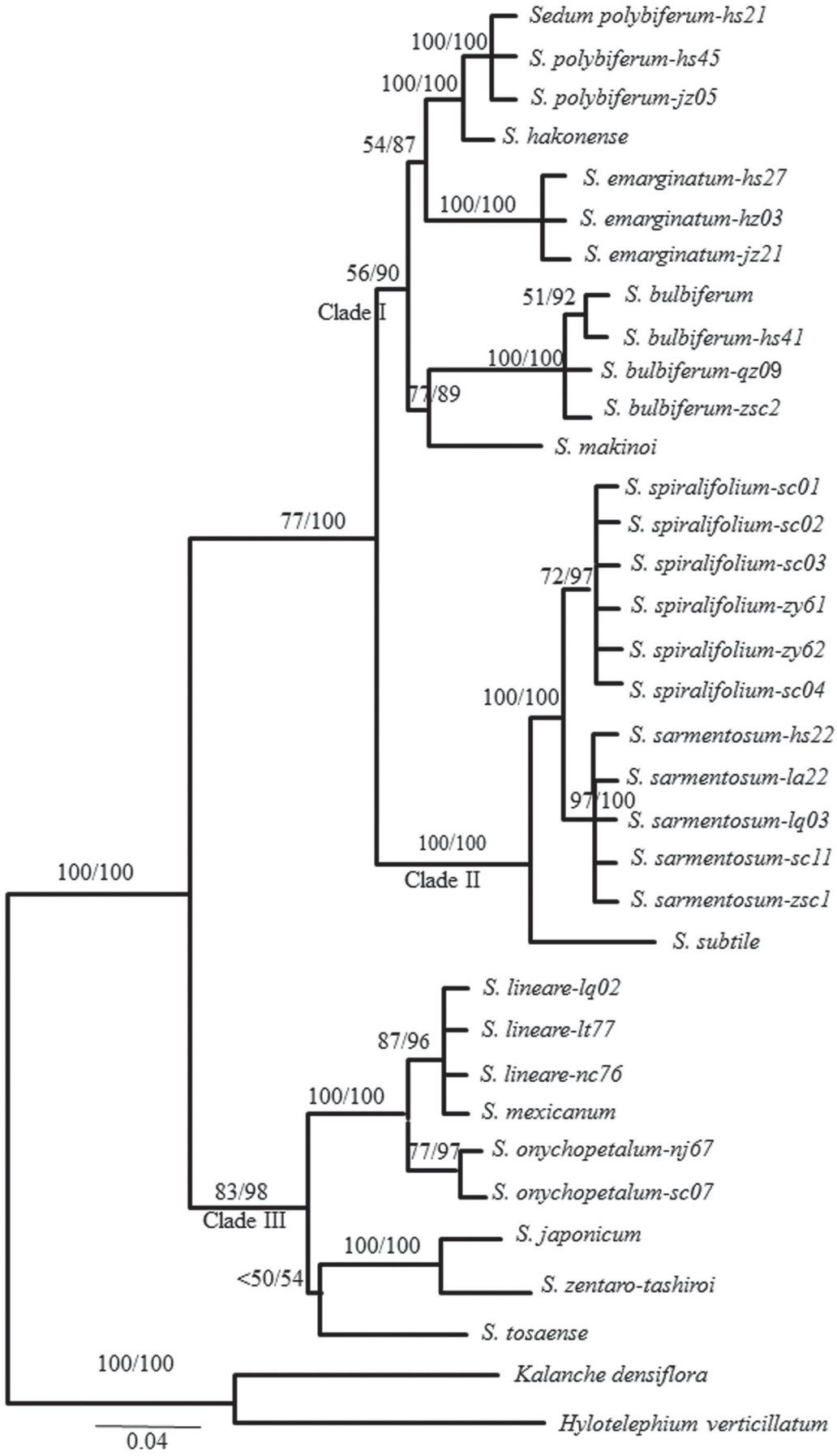

FIGURE 2. Phylogenetic relationships of *Sedum* sect. *Sedum* species related to *S. spiralifolium* inferred from a combined nuclear (ITS) and plastid region (*trn*L-*trn*F) dataset. The numbers above the branches are bootstrap values (BS) and posterior probabilities (PP).

Taxonomic treatment

Sedum spiralifolium D. Q. Wang, D. M. Xie & L. Q. Huang, *sp. nov*. (Figure 4)

Type:—CHINA. Anhui Province, Lu'an City, Shucheng County, Shucha Township, on rocks and along road banks, elevation ca. 50–100 m, 20 April 2012, *D. Q. Wang & D. M. Xie 120420* (fl., holotype ACM!, isotype ACM! & PE!)

Herbs perennial. Root fibrous. Sterile stems erect, 5–20 cm tall. Flowering stems erect or slightly bent, 10–30 cm. Leaves 3-verticillate, leaf blade linear-lanceolate, 10–25 × 2–3 mm, apex sub-acute, base shortly spurred, mostly 3-lobed, middle tilted 1 mm. Cyme 3–4 branched, corymbiform, ca. 4–7 cm in diameter, many flowered. Flowers sessile except the central flower (4–5 mm long), unequally 5-merous; bracts resembling stem leaves, 5–15 mm. Sepals linear-lanceolate, base shortly spurred, ca. 3–6 mm, apex subacute. Petals yellow, narrowly ovate or broadly lanceolate, 4–7 × 1–2 mm, apex acute, 0.2–0.5 mm. Stamens 10, 4–5 mm; antepetalous ones ca. 4 mm, inserted ca. 1 mm from petal base; antesepalous ones ca. 5 mm. Nectar scales nearly fan-shaped, apex obtuse, yellow-white, ca. 0.2 × 0.5 mm.

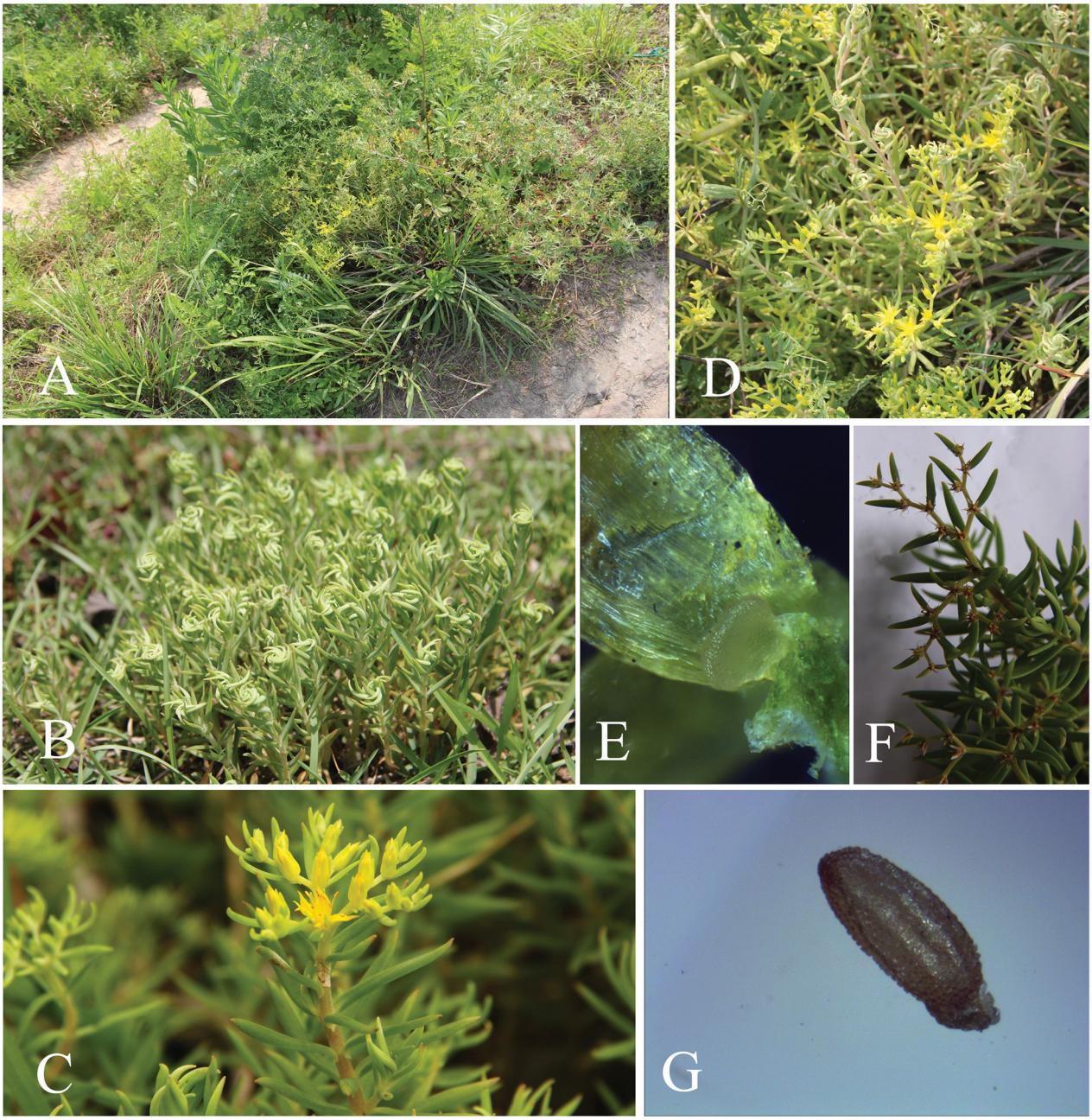

FIGURE 3. *Sedum spiralifolium* sp. nov. A. Habit in flowering period; B. Sterile shoots (twisted upper leaves at the top of the sterile shoots); C. Inflorescence; D. Flowering plant; E. Nectar scale; F. Follicles; G. Seed.

FIGURE 4. *Sedum spiralifolium* sp. nov. A. Flowering plant; B. Petal with stamen; C. Twisted leaves of the sterile shoot; D. Flower; E. Nectar scale; F. Carpels; G. Seed. (Drawn by Hai-Yan Cao from the holotype in ACM)

Carpels divergent, lanceolate, slightly split ends, ca. 5 mm, base connate for ca. 1 mm, apex slightly divergent. Style ca. 2 mm long. Follicles divergent, many seeded. Seeds brown, ovoid, 0.3–0.8 × 0.2–0.4 mm. Flowering early April, fruiting April–May. (Figure 3 and 4).

Distribution & Habitat:—*S. spiralifolium* is endemic to Anhui Province. It is known from Shucha Township of Shucheng County and Zongyang County of Anqing City in central Anhui Province of eastern China. It grows in valleys, on rocks and along road banks at an elevation of ca. 50–100 m.

Etymology:—The specific epithet '*spiralifolium*' refers to the habitus of the upper leaves on the sterile shoots.

Additional collection (paratypes):—CHINA. Anhui Province, Lu'an City, Shucheng County, Shucha Township, along road banks, elevation ca. 100 m, 25 April 2013, *D. M. Xie & L. Q. Huang130425*(PE!); the same locality, road banks, elevation ca. 95 m, 5 May 2013, *D. M. Xie & L. Q. Huang130505*(ACM!), *D. Q. Wang & D. M. Xie130506*(ACM!). Zongyang County of Anqing City, roadside, elevation ca. 50 m, 23 April 2013, *D. M. Xie & Q. S. Yang1304231* (ACM!).

Relationships:—*S. spiralifolium belongs to S. sect. Sedum* characterized by leaves 3-verticillate, yellow flowers, carpels and divergent follicles. It is most similar to *S. sarmentosum* except for its sterile shoots erect with twisted leaves, its shortly spurred and mostly 3-lobed leaf base, its central flower with 4–5 mm long pedicel, its fan-shaped scales and its earlier flowering time. *S. sarmentosum* is characterized by leaves that are not twisted, sterile stems creeping and rooting at nodes, leaves oblanceolate to oblong with base abruptly narrowed and spurred, flowers sessile, nectar scales cuneate-quadrangular. (Table 3)

TABLE 3. Macro-morphological and phenological comparison of six *Sedum* species

Character	Species					
	S. polytrichoides	*S. emarginatum*	*S. onychopetalum*	*S. lineare*	*S. sarmentosum*	*S. spiralifolium*
Habit	Perennial	Perennial	Perennial	Perennial	Perennial	Perennial
Sterile stems	Ascending, tufted, slender, woody	Creeping and rooting at nodes	Suberect, slender	Suberect	Creeping and rooting at nodes	Erect
Flowering stems	Shoots tufted, slender, woody	Suberect, slender	Suberect, tufted	Erect, slender	Creeping and rooting at nodes	Erect or slightly bent
Phyllotaxy	Alternate	Opposite	3(4)-verticillate	3(4)-verticillate	3-verticillate	3-verticillate
Leaf blade	Linear to linear-lanceolate, base spurred, apex acute	Spatulate-obovate to broadly obovate, apex rounded and emarginate	Broadly linear to lanceolate, base shortly spurred, apex obtuse	Sessile, linear, base shortly spurred, apex subacute	Oblanceolate to oblong, base shortly spurred, apex obtuse	Linear-lanceolate, base spurred, mostly 3-lobed, middle tilted
Leaf length × width	5-15 × 1-2 mm	10-2 × 0.5-1 mm	6-11 × 1-1.5 mm	10-15 × 2 mm	15-28× 3-7 mm	10-25 × 2-3 mm
Inflorescence	Cyme 2-4-branched	Cyme 3-branched	Cyme 2-3-branched, scorpioid	Cyme 2-3-branched, corymbiform	Cyme 3-5-branched, corymbiform	Cyme 3-4-branched, corymbiform
Inflorescence diam. (cm)	3-5	3-7	3-6	4-8	5-10	4-7
Flower stalks	Shortly pedicellate	Sessile	Sessile	Sessile, but dichasial central flower pedicellate, 1-2 mm	Sessile	Sessile, the central flower pedicellate, 4-5 mm long
Sepal shape	Lanceolate-ovate, equal	Lanceolate to narrowly oblong	Broadly linear to suboblong	Linear-lanceolate, unequal	Lanceolate to oblong	Linear-lanceolate, equal
Sepal length	1.5-2.5 mm	2-5 mm	2-3 mm	1.5-6 mm	3.5-5 mm	3-6 mm
Sepal number	5	5	5	5	5	5

…… *continued to the next page*

TABLE 3. (continued)

Character	Species					
	S. polytrichoides	*S. emarginatum*	*S. onychopetalum*	*S. lineare*	*S. sarmentosum*	*S. spiralifolium*
Petal shape	Narrowly lanceolate	Linear-lanceolate to lanceolate	Lanceolate	Lanceolate	Lanceolate to oblong	Narrowly ovate
Petal length × width	5-7 × 0.5-1 mm	6-8 × 1.5-2 mm	5 × 1.5-2 mm	4-6 × 1-1.5 mm	5-8 × 1-2 mm	4-7 × 1-2 mm
Stamen size	Slightly shorter than petals	Shorter than petals	Shorter than petals	Shorter than petals	Shorter than petals	Shorter than petals
Nectar scales	Base cuneate, apex broadly rounded	Oblong	Sub-quadrangular	Broadly cuneate to sub-quadran-gular	Cuneate-quadrangular	Nearly fan-shaped
Nectar length	ca. 0.4 mm	ca. 0.6 mm	ca. 0.5 mm	ca. 0.5 mm	ca. 0.5 mm	ca. 0.5 mm
Carpel shape	Base connate, apex divergent	Base connate, oblong	Base connate, apex divergent	Divergent, apex shortly beaked	Divergent, oblong	Lanceolate, apex slightly divergent
Carpel length	3-4 mm	4-5 mm	4-5 mm	3-4 mm	5-6 mm	4-5 mm
Follicles	Spreading	Divergent	Divergent	Spreading	Divergent	Divergent
Style length	3-4 mm	4-5 mm	4-5 mm	3-4 mm	5-6 mm	2-4 mm
Seed shape	Oblong	Ellipsoid	Sub-ovoid	Long-ellipsoid	Ovoid	Ovoid
Seed length	ca. 0.6 mm	ca. 0.65 mm	ca. 0.5 mm	ca. 0.45 mm	ca. 0.7 mm	0.3-0.8 mm
Flowering	July-August	May-June	April-May	April-May	May-July	April
Fruiting	August-September	July	May-June	June-July	August	April-May

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