



Sinocrassula jiaozishanensis (Crassulaceae), a new species from Yunnan Province, China

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Abstract

Sinocrassula jiaozishanensis, a new species from China, is described and illustrated. Molecular analysis and morphological comparisons were carried out. The new species can be easily distinguished from other *Sinocrassula* species by its basal leaves opposite, not rosulate, turquoise with apex red, flowering stems terminal, and nectar scales oblong to kidney-shaped.

Keywords: Kalanchoideae, Phylogeny, *Sinocrassula*, Taxonomy, New species

Introduction

In 2016, a distinctive plant of Crassulaceae was collected from Jiaozi mountain by us. At first, it was considered a horticultural species that escaped from gardens. However, we found that this plant is morphologically similar to *Adromischus* Lem (1852: 59) and *Cotyledon* Linnaeus (1753: 429), while its flower is similar to *Sinocrassula*. Because of its mixed morphology, we were not sure what genera it belongs to, but the similarities with *Sinocrassula* (e.g flower morphology) were evident. We examined in-depth and made a comparison with some other known Crassulaceae species by DNA barcode, choosing the ITS2 barcode to confirm the preliminary taxonomic placement of the plant (Chen 2010). Sequenced samples were blasted in the Genbank, and the analysis showed that this species is basal to *S. paoshingensis* (S. H. Fu 1965: 114) H. Ohba (2000: 296), *S. yunnanensis* (Franch. 1896: 284) A. Berger (1930: 463), *S. indica* (Decne 1844: 61) A. Berger (1930: 463), and *S. densirosulata* (Praeger 1919: 55) A. Berger (1930: 468).

Sinocrassula A. Berger (1930: 462) contains nine species and five varieties and is mainly distributed in Hengduan Mountains, with few species in Vietnam, Nepal, Pakistan and India (Fu & Hideaki 2001; Wang *et al.* 2012; Averyanov *et al.* 2014).

After carefully morphological comparison for these materials with all document's species in *Sinocrassula*, we confirmed that it represents a distinct new species, described here as *S. jiaozishanensis*.

Materials and methods

All specimens were collected from Luquan County, Yunnan province and sent to the Herbarium of Yunnan University (YUKU). Morphology of plants, leaves, inflorescences, and flowers were examined and photographed with an SMZ1270 stereomicroscope (Nikon, Japan) and D850 Nikon camera.

TABLE 1. Morphological comparison between *S. jiaozishanensis* and other species of *Sinocrassula*.

Character	<i>S. jiaozishanensis</i>	<i>S. ambigua</i>	<i>S. diversifolia</i>	<i>S. techinense</i>	<i>S. stenogrammata</i>	<i>S. indica</i>	<i>S. densirosulata</i>	<i>S. longistyla</i>	<i>S. yunnanensis</i>
Life cycle	Perennial	Perennial	–	–	–	Biennial	Perennial	Annual or Biennial	Annual or Biennial
Basal leaves	Not rosulate	Not rosulate	–	–	Not rosulate	Rosulate	Rosulate	Rosulate	Rosulate
Plant surface	Glabrous	Glabrous	Glabrous, Purple-spotted	Glabrous or sparsely pubescent	Papillate	Glabrate	Glabrate	Glabrate	With short pubescence
Leaf	Turquoise, Red edge	Beige to purplish red	Brown spots on both sides	–	Brown pruinose, with papillae	Green, brown to pruinose	White-green, purple on the top	–	With short pubescence
Bract	Lanceolate, oblanceolate	Linear-oblong	Linear-lanceolate	–	Ovate-lanceolate	Leaf shape and small	–	Oval	Obovoid-lanceolate
length of inflorescence	15–20 cm	1.2–2.5 cm	6–15 cm	7–10 cm	5–15 cm	15–60 cm	5–7.5 cm	20–25 cm	5–10 cm
Flower color	Reddish purple	Reddish purple	Yellow, with pruinose spots	Red	Red	Red	With purple spots on the top	Scarlet or purple	Yellowish green
Nectar scale	Oblong	Subquadrate	Broad-tetragon	Subquadrate	Rectangle	Square	Spathulate-square	Broadly quadrate	Quadrate
Nectar size	0.3 × 0.6 mm	0.5 × 0.5 mm	0.3 × 0.5 mm	0.4 × 0.6 mm	0.6 × 0.2 mm	0.5 × 0.5 mm	–	0.8 mm	–
Phenology	Mar.-June.	May.-Jul.	Aug.-Nov.	Sept.	Sept.	Jul.-Oct.	Jul.-Nov.	Jul.-Oct.	Sept.-Oct.

Total genomic DNA was extracted from fresh plant materials using DNA quick Plant System Kit (Tiangen Biotech Beijing, China) CO., LTD.), following the manufacturer's protocols. Four plastid regions (*rbcL*, *trnL-F*, *matK* and *psbA-trnH*) and one nuclear gene (ITS) were amplified and sequenced. The PCR conditions of Zhang *et al.* (2015) and Kress *et al.* (2007) were followed and the primer sequences were listed in Table 1. PCR products were purified and sequenced in Sangon Biological Technology (Shanghai, China).

Ten sequences of the new species were generated. In total, 111 accessions representing 42 species of Crassulaceae were used to explore the phylogenetic position of the new species (see Appendix 1 for a full list of specimens and GenBank accession numbers).

Sequences of each marker were initially aligned with MAFFT v.7 (Katoh & Standley 2013) and manually adjusted in BioEdit 7.0.5.3 (Hall 1999).

For each marker and simultaneous, jModeltest2 (Darriba *et al.*, 2012) was used to select the best-fitting likelihood model for maximum likelihood (ML) and Bayesian analyses (BI). Six species representing three genera [*Adromischus* Lem (1852: 59), *Cotyledon* Linnaeus (1753: 429), *Kalanchoe* Adans (1763: 248) in subfamily Kalanchoideae were used as outgroups following Messerschmid (2020)].

ML tree searches and ML bootstrapping were conducted using RAxML-HPC2 on TG ver. 7.2.8 on CIPRES webserver (Stamatakis *et al.* 2008, Miller *et al.* 2010), with GTR +I+G model and 1000 rapid bootstrap (BS) analyses followed by a search for the best-scoring tree and with 1,000 bootstrap replicates.

Bayesian inference (BI) was conducted using MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003) on CIPRES (Miller & al., 2010). Two independent runs, each with four chains (one cold, three heated) and priors set to their default values, were conducted, each beginning with a random tree and sampling one tree every 1000 generations of 10 million generations. The posterior was sampled every 1,000 generations, and the first 25% discarded as "burn-in". Convergence was estimated by inspection of the parameters in TRACER v 1.4 (Rambaut *et al.* 2007).

Result and discussion

Morphologically, we compared all the other known species of *Sinocrassula*, with the new species, almost all *Sinocrassula* have rosulate basal leaves, except for *S. ambigua* which differs from all other species of *Sinocrassula* by calyxes longer than perianths. Basal stem leaves opposite and others alternate, making the new species easily be confused with *S. ambigua*. However, the new species differs from the *S. ambigua* in having perianths nearly as long as calyxes, leaves with red tip, longer inflorescence, and nectar scale oblong (See also Table 2).

TABLE 2 Primers used in this study

Primer	Primer sequence (5'–3')	reference
ITS (F)	TCCGTAGGTGAACCTGCGG	Mayuzumi 2004.
ITS (R)	TCCTCCGCTTATTGATATGC	
psbA-trnH (F)	GTTATGCATGAACGTAATGCTC	Sang 1997.
psbA-trnH (R)	CGCGCATGGTGGATTCAAAATC	
trnL-trnF (F)	CGAAATCGGTAGACGCTACG	Taberlet 1991.
trnL-trnF (R)	ATTTGAACTGGTGACACGAG	
rbcL (F)	ATGTCACCACAAACAGA-GACTAAAGC	Kress 2007.
rbcL (R)	GTAAAATCAAGTCCACCRCG	
matK (F)	CGTACAGTACTTTTGTGTTTACGAG	Hollingsworth 2009.
matK (R)	ACCCAGTCCATCTGGAAATCTTGGTTC	

To determine the systematic position of the new species, we reconstructed the phylogeny of subfam Sempervivoideae, the results were generally consistent with that of Messerschmid *et al.* (2020). Phylogenetic analysis showed the new species is resolved as sister to the accession *Sinocrassula* (MLBS = 100%, BIPP = 0.6), and they together form a distinct subclade being sister to other species of the *Kungia* clade (MLBS = 99%, BIPP = 0.6). The new species and *Sinocrassula* could be treated as the same genus given their sister relationship (Fig. 4).

Though the overall morphology of the new species is different from almost all species of *Sinocrassula*, the new species was resolved in the *Sinocrassula* clade with a relatively long branch by phylogenetic analysis. Morphologically, it still has a few features of *Sinocrassula* such as corolla campanulate, 5-merous, petals initially erect then spreading above middle, S-shaped in longitudinal section. Here we described the new species as *S. jiaozishanensis*.

Taxonomic treatment

Sinocrassula jiaozishanensis Chao Chen, J.G. Wang & Z.R. He *sp. nov.* (Fig. 1, 2, 3)

Type:—CHINA. Yunnan Province, Jiaozi Mountain, 102.7828E, 26.1803N, 2373m, 5 October 2016, C. Chen *et al.* JZS1001 (holotype YUKU!, isotypes YUKU!).

Diagnosis:—This species is somewhat similar to *S. ambigua* (Praeger 1921:13) A. Berger (1930: 462), but differs in having turquoise leaves and the tip of the leaves red (Fig. 1 A, D, E) (vs. beige to purplish-red leaves and the tip no colour change in *S. ambigua*), length of sepals and perianth segments subequal (Fig. 1 H, I, J) (vs. sepals significantly longer than perianth in *S. ambigua*), inflorescence 10–20 cm (Fig. 1 A) (vs. 1.2–2.5 cm in *S. ambigua*), nectar scale oblong 0.3 × 0.6 mm (vs. 0.5 × 0.5 mm, subquadrate in *S. ambigua*).

Description (Fig. 1): Perennial, glabrous, succulent. Roots fibrous. Stems creeping, erect or somewhat pendulous, terete. Basal leaves opposite, not rosulate, stem leaves alternate, round obovate or oblanceolate to oblong, acute or cuspidate, turquoise with red apex, 2–3.5 × 1.3–2 cm. Flowering stems terminal, erect, apex bending or drooping ca. 10–20 cm tall; inflorescences corymbiform, ca. 20–45-flowered cyme to 10 cm in diam; bracts sessile, alternate or opposite, obovate or lanceolate, 0.15–1.5 × 0.2–0.7 cm. Flowers bisexual, ca. 6 mm in diam; sepals narrowly triangular, ca. 3.5 × 0.8 mm, minutely papillate; petals base white, central and above rose to purple, oblong-lanceolate, apex cuspidate, ca. 1.8 × 4.2 mm; stamens ca. 4mm; anthers purple, orbicular, pollen yellow. Nectar scales yellow, oblong or kidney-shaped, 0.55–0.65 × 0.25–0.3 mm. Carpels 5, distinct, ovate-lanceolate. ca. 4 mm. Styles erect, short, ca. 1.2 mm, apex red. Fl. Mar–May, fr. Apr–Jun.

Paratypes:—CHINA. Yunnan Province, Jiaozi Mountain, 102.7828E, 26.1803N, 2373m, 5 October 2016, C. Chen *et al.* JZS1002 (YUKU), C. Chen *et al.* JZS1003 (YUKU).

Distribution and Habitat:—*Sinocrassula jiaozishanensis* is only known from Jiduo village, Xueshan Township, Luquan County, in the crevice of cliffs with epilithic plants (e.g., *Selaginella pulvinata* (Hook. et Grev.) Maxim, *Incarvillea arguta* (Royle) Royle, *Arthraxon lanceolatus* (Roxb.) Hochst var. *lanceolatus*) (Fig. 2).

Phenology:—Flowering time March–May, fruiting time April–June.

Conservation status: In the type locality of *Sinocrassula jiaozishanensis*, only 10 plants were found, and we failed to find more populations or individuals besides type locality. Because of the restricted distribution and the peculiar habitat, we assessed *S. jiaozishanensis* as Critically Endangered (CR) according to criterion D (IUCN 2017).

Etymology:—The specific epithet “*jiaozishanensis*” refers to Jiaozi mountain where the species was discovered. Jiaozi Mountain is a famous mountain in Kunming, Yunnan province.

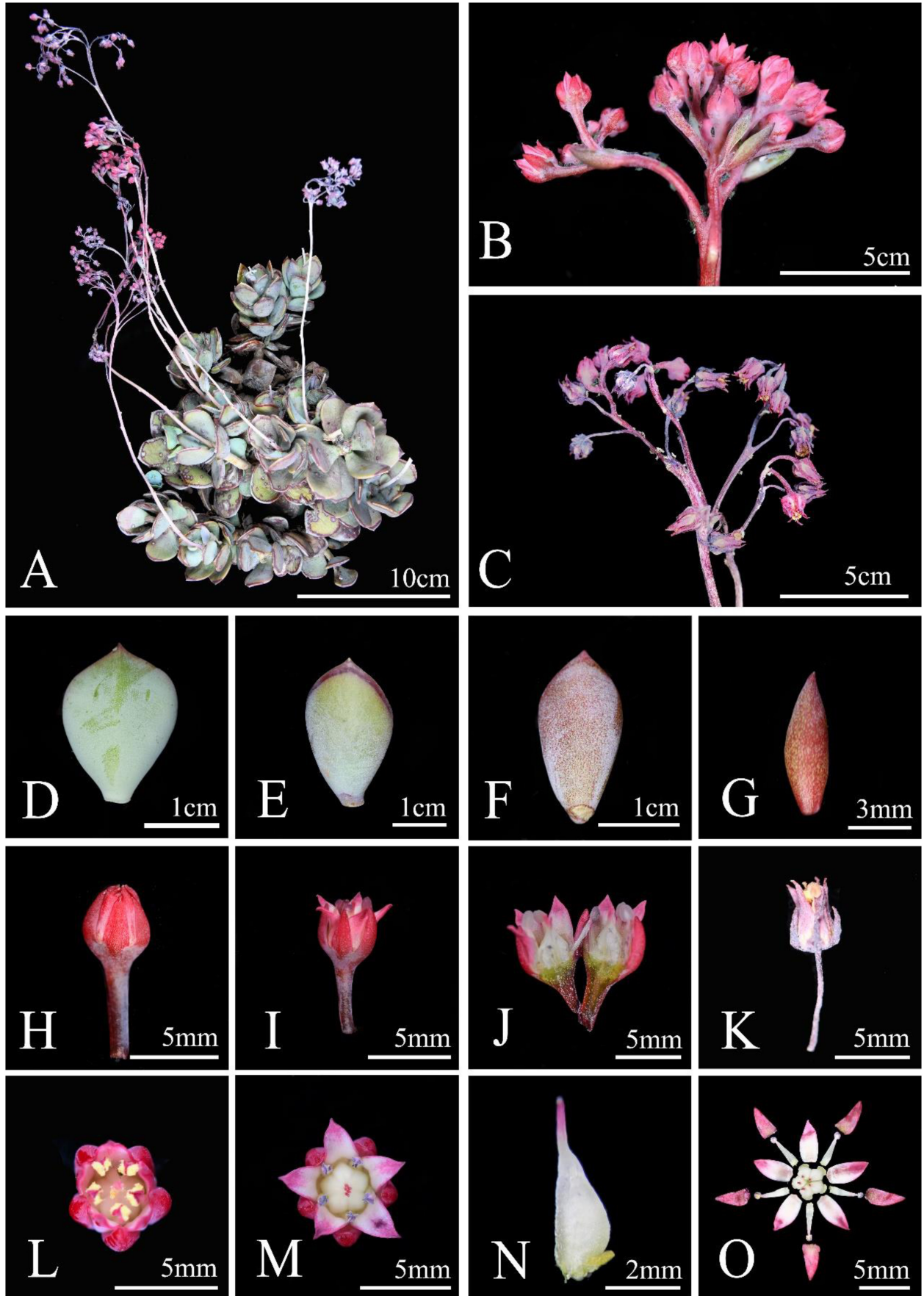


FIGURE 1. *Sinocrassula jiaozishanensis*.—A. Habit.—B. Inflorescence.—C. Infructescence.—D, E. Leaf.—F, G. Bract.—H, I, J, K, L, M. Flowers.—N. Ovary.—O. Flower structure. Photographed by C. Chen.

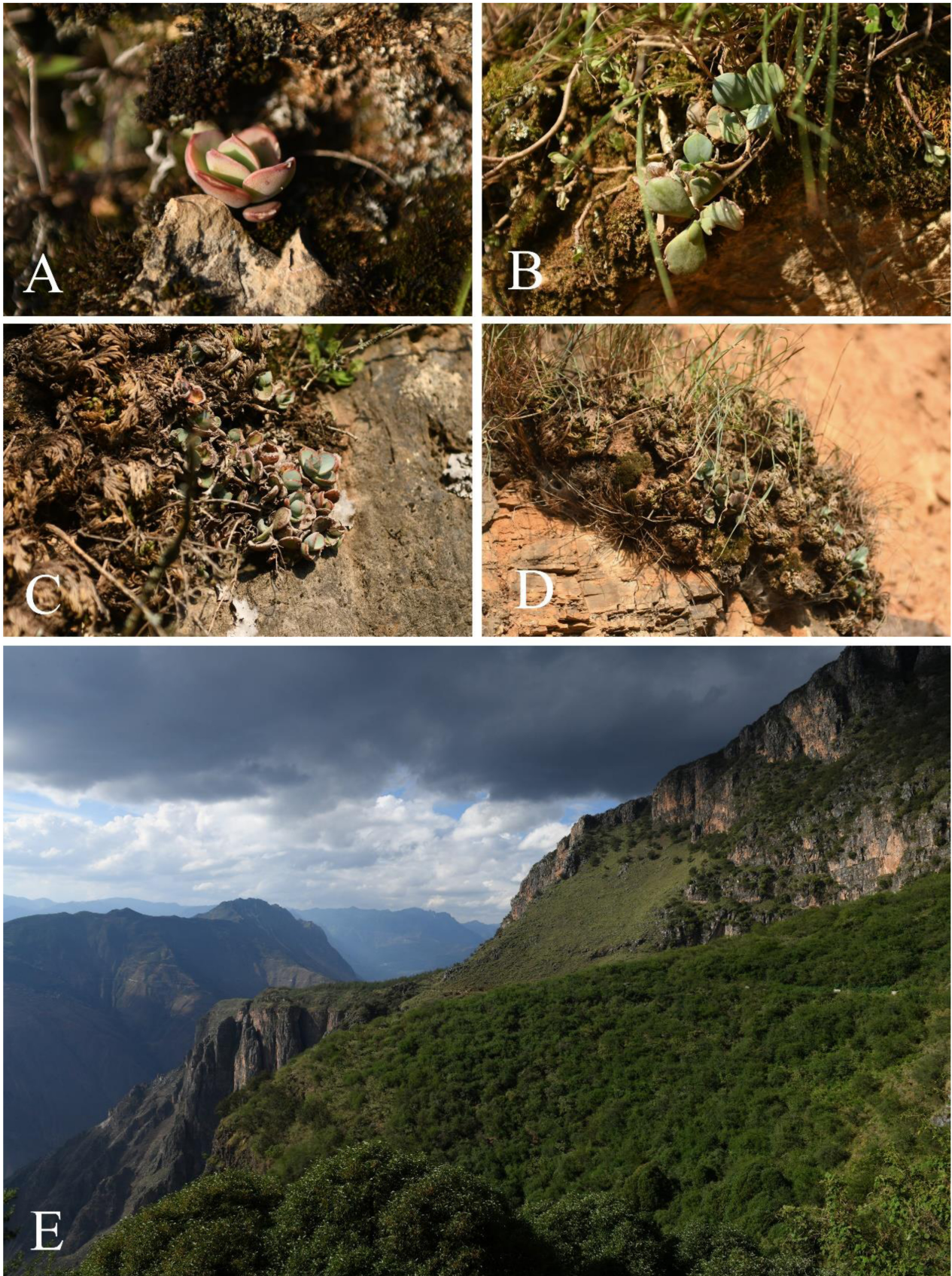


FIGURE 2. Field information of *Sinocrassula jiaozihanensis*.—A. Seedling.—B. Mature plant.—C, D. Population.—E. Habitat. Photographed by C. Chen.

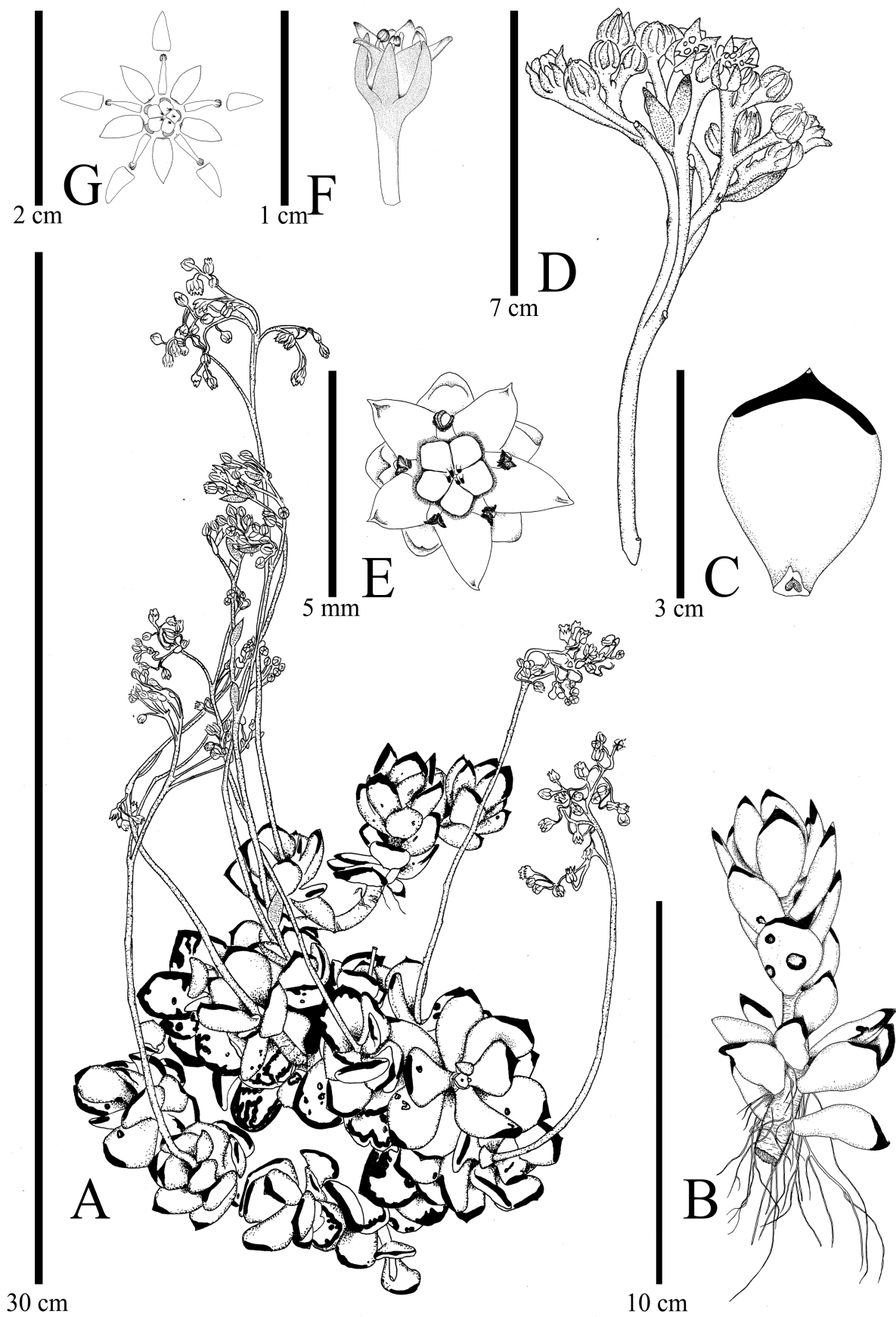


FIGURE 3. *Sinocrassula jiaozishanensis*.—A, B. Habit.—C. Leaf.—D. Inflorescence.—E, F. Flower. (Based on Chen *et al.*, JZS1001, drawn by Z.L.Liang.)

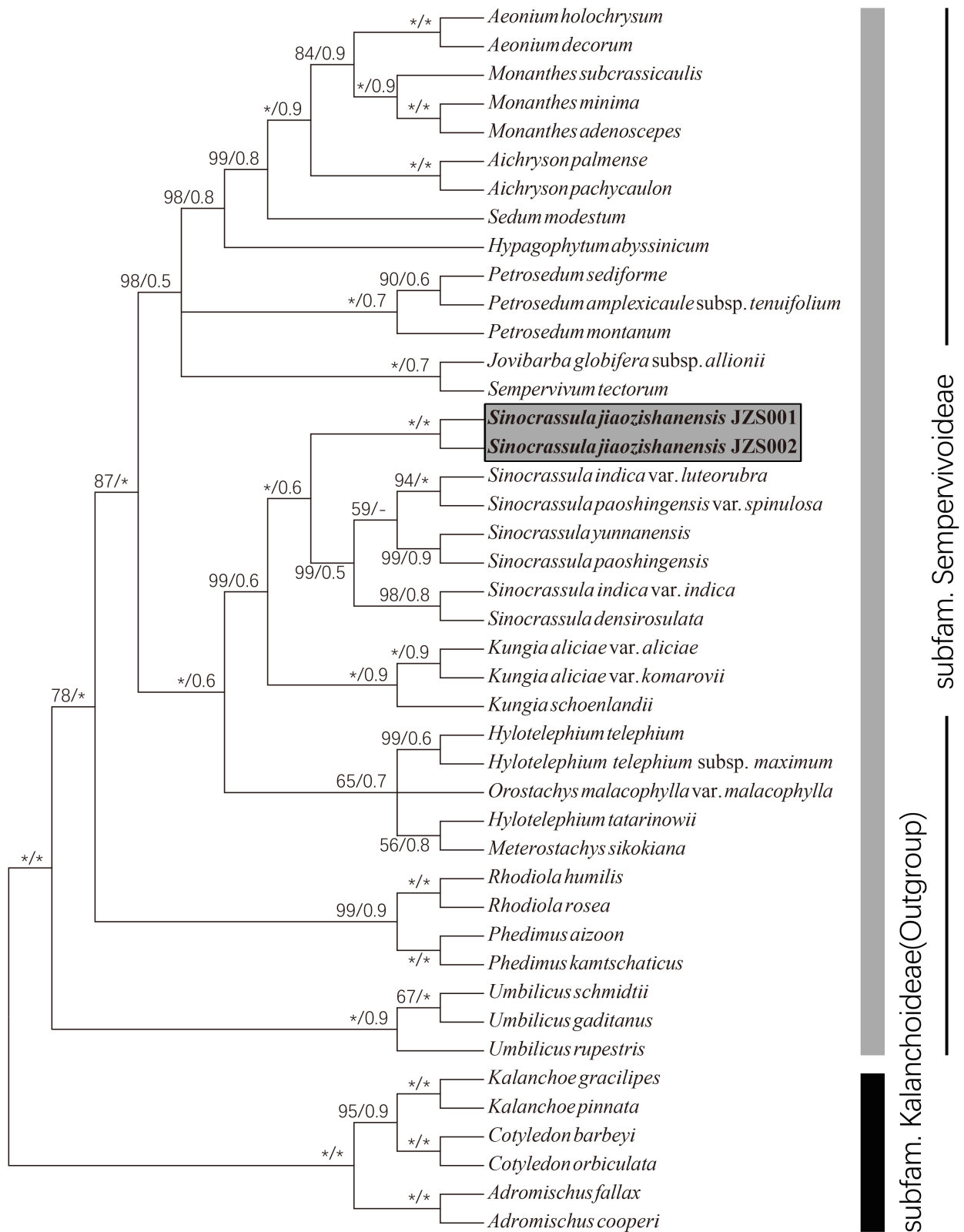


FIGURE 4. The maximum likelihood phylogeny of *Sinocrassula jiaozishanensis* and its allies based on molecular data. Support values [maximum likelihood bootstrap support (MLBS) \geq 50%, Bayesian inference posterior probability (BIPP) \geq 0.5] are shown above the main branches; the dash (-) indicates MLBS < 50%, BIPP < 0.5; the asterisk indicates MLBS = 100%, BIPP = 1.00.

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APPENDIX 1

Detailed information about specimens and GenBank accession numbers used in the molecular phylogenetic analyses. Species, voucher (herbarium code or Botanical Garden), country (locality and additional information): *RbcL*, ITS, *trnL-trnF*, *MatK*, *psbA-trnH*. “–” indicates missing data or missing information and “*” newly generated sequences. A part of Herbarium acronyms followed Index Herbarium (Thiers 2018).

Ingroup

Aeonium decorum Webb ex Bolle., Mort 1435 (WS), Spain (Gomera): –, AY082130, AY082239, AY082165, AY082197; *A. holochrysum* Webb & Berthel., Mort 1430(WS), Spain (Tenerife, Gomera, La Palma, El Hierro): –, AY082138, AY082297, AY082269, AY082283; *Aichryson pachycaulon* Bolle., Mort 1404 (WS), Spain (Tenerife, Gran Canaria, La Palma, Fuerteventura, Gomera): –, AY082105, AY082223, AY082157, AY082182; *A. palmense* Webb ex Bolle., Mort 1482 (WS), Spain (La Palma): –, AY082104, AY082222, AY082156, AY082181; *Hylotelephium tatarinowii* (Maxim.) H. Ohba., J. Q. Zhang 100717–08(PEY), China: –, KF113681, KF113787, –, KF113734; *H. telephium* (L.) H. Ohba., AP412 (UOG), Canada (Ontario): HQ590138, –, –, HQ593328, HQ596731; *H. telephium* subsp. *maximum* (L.) H. Ohba., ZPL: 04509(MIB), Italy: HE963514, –, –, HE967419, HE966664; *Hypagophytum abyssinicum* (Hochst.) A. Berger., I. Friis, G.S. Bidgood, W. Abebe, E. Getachew 12294 (WAG), Ethiopia: –, MT336090, MT155885, MT181557, –; *Jovibarba globifera* subsp. *allionii* (Jord. & Fourr.) J. Parn., J. Klein B 100825–05–164 (MJG), Italy: –, KJ884135, –, –, *Kungia aliciae* var. *komarovii* (Raymond-Hamet) K. T. Fu., S. Mayuzumi CH00062 (TI), China: –, AB480592, AB480633, –, –; *K. aliciae* (Raym-Hamet) K. T. Fu., S. Mayuzumi CH00061 (TI), China: –, AB480591, AB480632, –, –; *K. schoenlandii* (Raym-Hamet) K. T. Fu., S. Mayuzumi CH00063 (TI), China: –, AB480593, AB480634, –, –; *Meterostachys sikokianus* (Makino) Nakai., S. Mayuzumi *et al.* C00028 (TI), Japan; Nagasaki: –, –, AB480670, –, –; *Monanthes adenoscepes* Svent., Santos s.n. (WS), Spain (Tenerife): –, AY082118, AY082291, AY082264, AY082277; *M. minima* (Bolle) Christ., Santos s.n. (WS), Spain (Tenerife): –, AY082119, AY082233, AY082160, AY082191; *M. subcrassicaulis* (Kuntze) Praeger., Mort 1483 (WS), Spain (Tenerife): –, AY082117, AY082232, AY082159, AY082190; *Orostachys malacophylla* (Pall.) Fisch., S. Mayuzumi CH00054B (TI), Russia (Primorsky): –, AB480580, AB480617, –, –; *Petrosedum amplexicaule* subsp. *tenuifolium* (Sm.) Velayos., F. J. Valtueña, C. G. Relinque (MJG), Spain: –, MT336100, –, MT181567, –; *P. montanum* (L.) E.H.L. Krause., Stephenson 5.27.15 (–), –: –, –, KX452241, –; *P. sediforme* (Jacq.) Grulich, HRT-15429 (Utrecht University Botanic Gardens), Portugal; Beira Litoral, Vaguira Praia: –, –, AF115640, –; *Phedimus aizoon* (L.) ‘t Hart., J. Q. Zhang *et al.* 120613–03(PEY), China (Hebei): –, KF113682, KF113788, –, KF113735; *P. kamtschaticus* (Fisch.) ‘t Hart., J. Q. Zhang *et al.* 120613–04(PEY), China (Hebei, Xiaowutai Mt): –, KJ569919, KJ570285, –, KJ570047; *Rhodiola humilis* (Hook. f. & Thomson) S.H. Fu., J. Q. Zhang *et al.* 110804-03-03(PEY), China (Xizang, Mangkang Xian): KP115042, KP114742, KP115148, KP114838, KP114937; *R. rosea* L., J. Q. Zhang 100717-02(PEY), China (Beijing, Dongling Mt): KP115070, KP114772, –, KP114859, KP114967; *Sedum modestum* Boiss., UT 33112 (WS), Morocco: –, AY082101, AY082221, –, AY082180; *Sempervivum tectorum* L., CCDB-18313-F08 (–), Canada: MG249291, MG237296, –, –, –; *Sinocrassula jiaozishanensis* Chaochen, J.G. Wang *sp. nov.*, C. Chen *et al.* JZS001(YUKU), China (Yunnan): MZ343263, MZ343260, MZ343264, MZ343261, MZ343262; *S. jiaozishanensis* Chaochen, J.G. Wang *sp. nov.*, C. Chen *et al.* JZS002(YUKU), China (Yunnan): MZ343268, MZ343265, MZ343269, MZ343266, MZ343267; *S. paoshingensis* var. *spinulosa* H. Ohba *et al.*, Akiyama *et al.* 105516 (TI), China: –, AB088583, AB089739, –, –; *S. densirosulata* (Praeger) A. Berger., H. Hara *et al.* 1967 (TI), Bhutan: –, AB088580, AB480664, –, –; *S. indica* (Decne.)

A. Berger., S. Akiyama 2000 (TI), China: –, AB480610, AB480665, –, –; *S. indica* var. *luteorubra* (Praeger) S.H. Fu., Akiyama *et al.* 105516 (TI), China: –, –, AB480668, –, –; *S. paoshingensis* (S.H. Fu) H. Ohba, S. Akiyama & S.K. Wu., Wu *et al.* 103555 (TI), China: –, AB088581, AB089741, –, –; *S. yunnanensis* (Franch.) A. Berger., S. Mayuzumi C00115 (TI), China: –, AB088582, AB480669, –, –; *Umbilicus gaditanus* Boiss., S. Rivas Martinez s.n. (MA), Spain (Cadiz): –, –, MK725859, MK725846, –; *U. rupestris* (Salisb.) Dandy., T. Menezes 5 (LISC), Portugal (Azores, Sao Miguel): –, –, MK725868, MK725855, –; *U. schmidtii* Bolle., M. Romeiras & M. Carine 3170 (LISC), Cape Verde (Santo Antao, Delgadinho da Corda): –, –, KP279339, KP279381, KP279450;

Outgroup

Adromischus cooperi (Baker) A. Berger., Bruyns 11921 (UPS), Saudi Arabia: –, MH503496, MH503093, MH503363, –; *A. fallax* Toelken., Bruyns 2997 (BOL), Saudi Arabia: –, MH503497, LN878814, MH503364, LN878728; *Cotyledon barbeyi* Schweinf. ex Baker., Bruyns 12754 (BOL), Kenya: –, MH503623, MH503217, MH503487, –; *C. orbiculata* L., Bruyns 12929 (BOL), Saudi Arabia: –, MH503624, MH503218, MH503488, –; *Kalanchoe gracilipes* (Baker) Baill., Bruyns 6232 (BOL, MO), Madagascar: –, MH503625, MH503219, MH503489, –; *K. pinnata* (Lam.) Pers., S.B. Davis 1290 (FLAS), USA (Florida): GU135277, –, –, GU135118, GU135449.