Chrysosplenium sangzhiense (Saxifragaceae), a new species from Hunan, China

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Abstract

Chrysosplenium sangzhiense Hong Liu, a new species from Hunan, China, is described and illustrated. The phylogenetic analysis revealed that the new species belongs to subgen. Chrysosplenium and is closely related to C. grayanum, C. nepalense and C. sinicum. The chromosome number of the new species is 2n = 46, indicating a novel basic number x = 23 in Chrysosplenium that is different from other species. This also suggests that C. sangzhiense is probably an allopolyploid derivative of a species with x = 11 and one with x = 12. Morphologically, C. sangzhiense can be easily distinguished from C. grayanum, C. nepalense, C. sinicum and C. cavaleriei, a species not included in our phylogenetic analysis by a suite of characters relating to the sterile shoots, basal leaves, cauline leaves, flowering stem, sepals, disc, capsule and seed. A global conservation assessment is performed, and classifies C. sangzhiense as Least Concern (LC).

Keywords

Chrysosplenium, cytology, phylogeny, Saxifragaceae, subgen, taxonomy

Introduction

Chrysosplenium L. (1753) is a perennial herbaceous genus in Saxifragaceae and comprises more than 70 species (Kim et al. 2019; Fu et al. 2020). Chrysosplenium is distributed in Asia, America and Europe (Pan and Ohba 2001; Soltis 2007).

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The latest revision of Chinese *Chrysosplenium* included 35 species (Pan and Ohba 2001). Although no particular infra-generic classification was adopted in this revision, use of leaf arrangement as the primary character in the key to species reflected the recognition of two subgenera in previous taxonomic revisions (Pan 1986a, b). In addition, seed surface has been used as an important character to delimit sections (Pan 1986a, b). Soltis et al. (2001) showed that the two subgenera are both monophyletic and sister to each other using *matK* sequence data, thereby confirming that leaf arrangement is a phylogenetically informative morphological character. Subsequent taxonomic research on Chinese *Chrysosplenium* has been undertaken by Liu et al. (2016), Kim et al. (2019) and Fu et al. (2020), bringing the total diversity of the Chinese flora to 38 species, of which 23 (60%) are endemic.

Previous studies have demonstrated that *Chrysosplenium* has a diverse basic chromosome number with $x = 7, 8, 9, 10, 11, 12$ and 13 at species level indicating cytological data provides important evidence for the delimitation and evolution of *Chrysosplenium* (Hara and Kurosawa 1963; Funamoto and Tanaka 1988a, b, 1989; Funamoto et al. 1997, 1999, 2000, 2004; Funamoto and Zhou 2010).

As part of ongoing research into the diversity of Chinese *Chrysosplenium*, the authors undertook an extensive fieldtrip in Hunan, China. During the trip an unknown species of *Chrysosplenium* was collected. Following a thorough literature survey (Hara 1957; Pan 1992; Pan and Ohba 2001; Liu et al. 2016; Kim et al. 2019; Fu et al. 2020) along with the molecular and cytological evidence, we confirmed that it is a distinct and undescribed species.

**Materials and methods**

**Morphology observations and conservation assessments**

All morphological characters were studied based on the material from field and herbarium specimens using a dissecting microscope (SMZ171, Motic, China). For seed morphology, we also undertook scanning electron micrograph (SEM) observation; seeds were collected from the field and dried by silica gel. The pre-treatment including impurities removing, air-drying and gold-coating was performed, following Fu et al. (2020). Observations and photographs were taken under a Hitachi SU8010 scanning electron microscope. At least 15 seeds were used to determine the size and surface. Conservation assessment was undertaken following IUCN (2019).

**Genomic DNA extraction, PCR amplification, and Sequencing**

To confirm the systematic position of this unknown species, we conducted phylogenetic studies using *matK* sequence data. We chose this DNA region due to its highest species coverage within the genus (Soltis et al. 2001; De Vere 2012;
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Saarela et al. 2013; Ebersbach et al. 2017; Kim et al. 2018) so that we could trace the most closely related species. Forty-eight species of *Chrysosplenium* as in-group and three species of *Saxifraga* and *Itea* as out-group were sampled. Of these, 15 sequences were obtained from the Genbank (https://www.ncbi.nlm.nih.gov/), while 36 sequences were newly generated. Their species names and GenBank accession numbers are listed in Table 1. DNA extraction, PCR amplification, and sequencing were performed following Soltis et al. (2001).

**Phylogenetic analysis**

We performed phylogenetic analyses of *Chrysosplenium* based on *matK* sequence data-set using Bayesian inference (BI) and maximum likelihood (ML). For BI analysis, we employed MrBayes v.3.2.6 (Ronquist et al. 2012) to obtain a maximum clade credibility (MCC) tree. The matrix of *matK* sequence was aligned by MAFFT. Bayesian inference was performed using one million generations, four runs, four chains, a temperature of 0.001, 25% trees discarded as burn-in, and trees sampled every 1,000 generations (1,000 trees sampled in total) with GTR+F+G4 model.

We conducted the ML analysis using IQ-TREE v 2.0.6 (Nguyen et al. 2015) with 1,000 bootstrap replicates, and default ModelFinder (Kalyaanamoorthy et al. 2017) to find TVM+F+R3 as the best-fit substitution model. Tree visualization was achieved in FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

**Chromosome preparations**

Living plants of the new species were cultivated in the green house of South-Central University for Nationalities. Actively growing root tips were harvested after 1–2 weeks. Cytological examination was performed following Funamoto and Zhou (2010). The best metaphase plates were photographed using an imager microscope with a camera attachment. At least 3–5 cells from 3–5 root tips of five individuals of the new species at somatic metaphase were counted to determine the chromosome numbers.

**Results**

**Molecular phylogenetic studies**

The aligned matrix of *matK* sequence was 1,644 characters. Of the 154 variable characters, 90 (58.44%) were parsimony-informative, including indels. BI and ML analyses resulted in the same tree topology which showed the undescribed species as belonging to a strongly supported clade (BP = 89%, PP = 1) that included *Chrysosplenium grayanum* Maxim. (1877), *C. nepalense* D.Don (1825) and *C. sinicum* Maxim. (1877) (Fig. 1).
Table 1. Species names and GenBank accession numbers of matK DNA sequences used in this study (* newly generated sequences).

| Species                                          | Location        | Voucher specimens | Herbarium      | Genbank number |
|--------------------------------------------------|-----------------|-------------------|----------------|----------------|
| Chrysosplenium album Maxim.                       | Nikkou-shi, Japan | HSN09815          | HSN            | MW402998      |
| Chrysosplenium aureobracteatum Y.I.Kim & Y.D.Kim | Gangwon, South Korea | KYI-2009032      | –              | AXY64019      |
| Chrysosplenium biondianum Engl.                  | Shanxi, China   | HZ2017050107362  | HSN            | MW402999      |
| Chrysosplenium carnosum Hook.f. et Thoms.        | Sichuan, China  | HSN013113         | HSN            | MW403000      |
| Chrysosplenium davidianum Deone. ex Maxim.       | Sichuan, China  | HSN06442          | HSN            | MW403001      |
| Chrysosplenium delavayi Franch.                  | Hunan, China    | SZZ2016080907105  | HSN            | MW403002      |
| Chrysosplenium echiurus Maxim.                   | Nikkou-shi, Japan | HSN09817          | HSN            | MW403005      |
| Chrysosplenium fauriae Franch.                   | Nikkou-shi, Japan | HSN09823          | HSN            | MW403004      |
| Chrysosplenium flagelliferum Fr. Schmidt.        | Nikkou-shi, Japan | HSN09816          | HSN            | MW403005      |
| Chrysosplenium forrestii Diels                   | Nikkou-shi, Japan | HSN7797           | HSN            | MW403006      |
| Chrysosplenium gracilidanum Engl.                | Sichuan, China  | JZ2018042507981   | HSN            | MW403007      |
| Chrysosplenium glaucephylum Hara                 | Sichuan, China  | QCS2017102608035  | HSN            | MW403008      |
| Chrysosplenium grayanus Maxim.                   | Nikkou-shi, Japan | HSN09810          | HSN            | MW403009      |
| Chrysosplenium griffithii Hook.f. et Thoms.      | Shanxi, China   | HSN7760           | HSN            | MW403010      |
| Chrysosplenium hensleyi Franch.                  | Hunan, China    | HSN7505           | HSN            | MW403011      |
| Chrysosplenium hydrangeoides (Lév.) et Vant.     | Hubei, China    | HSN09188          | HSN            | MW403012      |
| Chrysosplenium japonicum (Maxim.) Makino         | Zhejiang, China | HSN7909           | HSN            | MW403013      |
| Chrysosplenium kamtschaticum Fisch. ex Seringe    | Shimane-ken, Japan | DG2019032310004   | HSN            | MW403014      |
| Chrysosplenium kiotense Ohwi.                    | Nikkou-shi, Japan | HSN09818          | HSN            | MW403015      |
| Chrysosplenium lanuginosum Hook.f. et Thoms.     | Anhui, China    | BD2017030507343   | HSN            | MW403016      |
| Chrysosplenium lectus-cochlear Kitagawa          | Jilin, China    | HSN7379           | HSN            | MW403017      |
| Chrysosplenium macrophyllum Oliv.                | Hubei, China    | BD2017030507344   | HSN            | MW403018      |
| Chrysosplenium macrostemon Y.I.Kim & Y.D.Kim     | Jilin, China    | CBS2016062466656  | HSN            | MW403019      |
| Chrysosplenium macrostemon Maxim. ex Franch. et Sav. | Nikkou-shi, Japan | HSN09820          | HSN            | MW403020      |
| Chrysosplenium nepalense D.Don                   | Yunnan, China   | GLGH20170607375   | HSN            | MW403021      |
| Chrysosplenium medicule Bunge                    | Gansu, China    | HSN07772          | HSN            | MW403022      |
| Chrysosplenium picton Maxim.                     | Nikkou-shi, Japan | HSN09819          | HSN            | MW403023      |
| Chrysosplenium pilosum Maxim.                    | Sichuan, China  | HSN7980           | HSN            | MW403024      |
| Chrysosplenium quinlingense Z.P.Jien ex J.T.Pan  | Sichuan, China  | SJH2017052107372  | HSN            | MW403025      |
| Chrysosplenium ramousum Maxim.                   | Jilin, China    | SJH2017052107371  | HSN            | MW403026      |
| Chrysosplenium serreanum Hand.-Mazz.             | Jilin, China    | SJH2017052107371  | HSN            | MW403026      |
| Chrysosplenium sincinum Maxim.                   | Hunan, China    | TPS2017042407594  | HSN            | MW403027      |
| Chrysosplenium talbaishanense J.T.Pan            | Shanxi, China   | HSN7761           | HSN            | MW403028      |
| Chrysosplenium uniiflorum Maxim.                 | Tibet, China    | HSN7380           | HSN            | MW403029      |
| Chrysosplenium zhouchaense Hong Liu              | Shanxi, China   | HSN13356          | HSN            | MW403030      |
| Chrysosplenium zangebienense Hong Liu sp. nov.   | Hunan, China    | TPS2017042307449  | HSN            | MW403032      |
| Chrysosplenium alternifolium L.                  | Shimane-ken, Japan | DG2019032310003   | HSN            | MT362050      |
| Chrysosplenium maximowiczi Franch. et Sav.       | Kanagawa, Japan | –                 | –              | AB003053      |
| Chrysosplenium nagasei Wakab. & H.Ohba           | Gifu, Japan     | –                 | –              | AB003054      |
| Chrysosplenium rhodopernum Maxim.                | Nagasaki, Japan | –                 | –              | AB003058      |
| Chrysosplenium tosaense Makino                   | Saitama, Japan  | –                 | –              | AB003059      |
| Chrysosplenium xinensis Rydb.                    | Iowa, USA       | –                 | –              | L34120        |
| Chrysosplenium oppositifolium L.                 | Wales, UK       | –                 | –              | JN894973      |
| Chrysosplenium ruendahilii Packter               | Northwest Territories, Canada | – | – | KC474470 |
| Chrysosplenium tetrandrum (N. Lund) Th. Fries    | Nunavut, Canada | Brysting_01-065_CAN  | CAN        | KC474473      |
| Chrysosplenium wrightii Franch. & Sav.           | Yukon, Canada   | Bennett_08-125_CAN | CAN        | KC474474      |
| Chrysosplenium americanum Schwein. ex Hook.      | Hartford, New Hampshire, USA | – | – | KU524206 |
| Chrysosplenium valdivicum Hook.                  | Chile           | –                 | –              | KU524208      |
| Chrysosplenium zhanglejaense                      | Hunan, China    | ZJ2016031506369   | HSN            | MW402997      |
| X.L.Yu, Hui Zhou & D.S.Zhou X.                   | Anhui, China    | HSN07355          | HSN            | MW403031      |
| Saxifraga stolonfera Curt.                       | –               | –                 | –              | NC_037884     |
| Itea chinensis C.K.Schneider                     | –               | –                 | –              | MF350096      |
| Itea virginica L.                                | –               | –                 | –              |               |
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Chromosome characteristics

The chromosome number of *Chrysosplenium sangzhiense* was observed to be 2n = 46 (Fig. 2). The chromosome size fell into the range 0.93–2.43 μm, suggesting slight size variation. A detailed karyotype analysis was not possible because the chromosomes are small, and the position of centromere could not be determined.

Figure 1. Phylogenetic tree of *Chrysosplenium* generated from maximum likelihood (ML) of *matK* data-set. Numbers on the branches indicate bootstrap values (≥50%) of the ML and the posterior probability (≥0.5) of Bayesian inference analyses.
Taxonomic treatment

Chrysosplenium sangzhiense Hong Liu, sp. nov.
urn:lsid:ipni.org:names:77216564-1
Figs 3–5

Remarks. Similar to Chrysosplenium grayanum, C. nepalense, C. sinicum and C. cavaleriei (Table 2). C. sangzhiense differs from C. grayanum in its usually fewer cauline leaves, a square flowering stem and red-brown seeds; from C. nepalense it differs in its usually fewer cauline leaves, a square flowering stem and conspicuously unequal capsule lobes; from C. sinicum it differs in producing sterile shoots from all leaf axils, an absence of basal leaves, larger cauline leaves, and red-brown seeds; and from C. cavaleriei it differs in its erect sepals and absent disc.

Type. China. Hunan: Badagongshan National Nature Reserve, Sangzhi County, 29°47’10”N, 110°5’33”E, under broadleaved forests and near the stream in a mountain area at ca 1,220 m altitude, 22 April 2017, Hong Liu HSN07449 (holotype HSN; isotypes HSN, IBK).

Table 2. Morphological comparison of Chrysosplenium sangzhiense, C. cavaleriei, C. grayanum, C. nepalense and C. sinicum.

| Characters            | C. sangzhiense | C. cavaleriei | C. grayanum | C. nepalense | C. sinicum       |
|-----------------------|----------------|---------------|-------------|--------------|------------------|
| Sterile branch        | from all leaf axils | from near stem base | from all leaf axils | from all leaf axils | only from basal leaf axils | |
| Basal leaves          | absent          | absent        | absent      | absent       | present          | |
| Cauline leaves        | 2–3 pairs, 10–30 × 10–25 mm | 1–3 pairs, 9–13 × 10–14 mm | 2–7 pairs, 4–17 × 4–17 mm | 3–5 pairs, 3–18 × 5–18 mm | 1–2 pairs, 6–10.5 × 7.5–11.5 mm |
| Flowering stem (upper part) | square | unknown | rounded | rounded | square | |
| Sepals                | erect           | spreading     | erect       | erect        | erect             | |
| Disc                  | absent          | distinct      | somewhat inconspicuous | absent   | absent | |
| Capsule lobe          | lobes conspicuous unequal | lobes conspicuous unequal | lobes conspicuous unequal | lobes subequal | lobes conspicuous unequal | |
| Seed                  | red brown, papillose | dark brown, papillose | dark brown, papillose | red brown, smooth | dark brown, papillose | |

Figure 2. Somatic chromosomes at metaphase of C. sangzhiense Hong Liu, sp. nov. from three different individuals. Scale bar: 10 μm.
**Description.** Perennial herbs, 10–25 cm tall. **Root** fibrous and robust. **Rhizome** long creeping without stolons or bulbs. **Basal leaves** absent. **Sterile shoots** well developed, arising from all leaf axils, round in cross-section, 5–15 cm long at anthesis, later elongate and decumbent, up to 50 cm long, rooting at nodes, without forming...
a rosette. *Leaves* of sterile shoots opposite, isophyllous, always ca 8 at anthesis, dark purple, petiole 6–10 mm long, blade 10–30 × 10–25 mm, rounded, glabrous, apex obtuse, margin obtusely dentate (10–16 teeth), base broadly cuneate; post-anthesis 10–30 or more, green, petiole 6–10 mm long, blade 20–35 × 15–20 mm, rounded or ovate, glabrous, apex obtuse, margin obtusely dentate (12–20 teeth), base broadly cuneate. *Cauline leaves* 4–6 (2–3 pairs), opposite, petiole 6–10 mm long; blade 6–13 × 5–12 mm, rounded or broadly ovate, glabrous, apex obtuse, margin obtusely dentate (10–14 teeth), base broadly cuneate. *Flowering stem(s)* erect, branched, 10–23 cm tall, glabrous, purple, square in cross-section. *Inflorescence* 8–25-flowered cyme, dense, 1.4–9 cm long, 5–10 cm in diam.; *bracteal leaves* yellow-green, triangular arrangement and unequal, the middle one larger, petiole 2–8 mm long, blade 4–15 × 7–10 mm, subrounded, glabrous, apex obtuse, margin obtusely dentate (6–12 teeth), base broadly cuneate; *Flowers* tetramerous, actinomorphic; *sepals* 4 (2 pairs), erect, yellow in flowering phase but turn green in fruiting time, 2–3 × 2–3 mm, broadly ovate, apex obtuse; disk absent; *stamens* 8, homostylic, 1–2 mm long, shorter than
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sepals; filaments slender, ca 1 mm long; anther yellow, 2-locular, longitudinally dehiscent; ovary 2-locular, semi-inferior; stigma 2; styles erect, ca 1–2 mm long. **Fruit** a capsule, 5–7 mm long, green, smooth, 2-lobed (horn-shaped), conspicuous unequal, dehiscent along the adaxial suture; seeds numerous, reddish brown, sub-ovoid, a raphe on one side, 650–800 × 600–750 μm, papillose.

**Etymology.** *Chrysosplenium sangzhiense* is named after the type locality, Sangzhi County, Hunan Province, China.

**Vernacular name.** sāng zhí jīn yāo (Chinese pronunciation); 桑植金腰 (Chinese name).

**Conservation status.** At present, *Chrysosplenium sangzhiense* is only known from a single locality (IUCN criterion D2). At this locality, the population is ca 500 mature individuals (IUCN criterion D1) growing in at least ten patches within a nature reserve. Using the IUCN methodology, *C. sangzhiense* would be classed as Vulnerable (VU), however no plausible threat could be found to confirm its status as the population is located within a protected area and not under threat in the near future. In addition, considering that the surrounding area has not been completely explored, there may be hitherto undocumented additional populations. For these reasons the Global Species Conservation Assessment for *C. sangzhiense* is Least Concern (LC).
Discussion

Our phylogenetic analysis is consistent with previous studies (Soltis et al. 2001) that *Chrysosplenium* is monophyletic and comprises two strongly supported clades namely subgen. *Gamosplenium* (with alternate leaves) and subgen. *Chrysosplenium* (with opposite leaves). *C. sangzhiense* is recovered as a member of subgen. *Chrysosplenium* and falls into a strongly supported clade that includes *C. grayanum*, *C. nepalense* and *C. sinicum*. In addition, *C. cavaleriei* H.Lév. & Vaniot (1911) is also a morphologically similar species despite that it is not included in our phylogenetic analysis. All five species are closely related species despite the fact that it is endemic to Japan, while *C. nepalense*, *C. sinicum* and *C. cavaleriei* are widespread in China.

The basic chromosome number of Japanese *Chrysosplenium* species is x = 11 or x = 12, but in China there is more diversity with x = 7, 8, 9, 10, 12 and 13 (Hara and Kurosawa 1963; Funamoto and Tanaka 1988a, b, 1989; Funamoto et al. 1997, 1999, 2000, 2004; Funamoto and Zhou 2010). Our cytological studies support this. The chromosome number of *C. sangzhiense* is 2n = 46 indicating its basic number to be x = 23. Given the relationship of reported basic chromosome number of *Chrysosplenium*, it suggests that the new species is probably an allopolyploid derivative of a species with x = 11 and one with x = 12. Furthermore, this is a novel basic number for the genus, and different from the closely related species such as *C. grayanum* (x = 11), *C. sinicum* (x = 12) and *C. nepalense* (x = 12) (Hara and Kurosawa 1963; Funamoto and Tanaka 1989; Funamoto et al. 1999; Funamoto and Zhou 2010).

Conclusion

In this study, we confirm and describe a new species of *Chrysosplenium* based on morphological, molecular and cytological evidence. The newly generated molecular data contributes to reconstruct a robust phylogenetic framework for further studies on the aspects of biogeography and character evolution of *Chrysosplenium*. In addition, a novel basic chromosome number for *Chrysosplenium* reported here will be useful data to evaluate the evolutionary pattern of chromosome number change and to estimate the basic chromosome number of clades of the genus.

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