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**Oxalis wulingensis** (Oxalidaceae), an Unusual New Species from Central China

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**Abstract—** *Oxalis wulingensis*, an unusual new species of Oxalidaceae from Hunan and Hubei provinces of central China, is described and illustrated. Placement of this species within *Oxalis* was assessed based on DNA analyses of the nuclear internal transcribed spacer (ITS) and morphological comparisons with related species. This new species can be easily distinguished by its long, oblong triangular leaves, the two lateral ones arranged at a 180° angle and densely pubescent with brown, villous hairs on both surfaces, pink flowers with lilac veins, and apex of petals obtuse or irregularly denticulate with 3–5 teeth. The chromosome number is 2n = 22. With the addition of *O. wulingensis*, which is the only species endemic to China, a total of seven native *Oxalis* occur in the country. A taxonomic key to all Chinese species of *Oxalis* is also provided.

**Keywords**—chromosome number, ITS, molecular phylogeny, Wuling Mountains.

*Oxalis* L. is a cosmopolitan genus with approximately 500 species, most of which occur in southern Africa and the Americas (De Azkue 2000). A few cosmopolitan and endemic species are found in Eurasia and Oceania (Knuth 1930; Lourteig 1979). Despite many well-known and easily recognized species, members of the genus are often difficult to identify. Huang et al. (1998), applying a narrow species concept, recognized five species (*O. acetosella* L., *O. bouvetii* Ait. ex G. Don, *O. corniculata* L., *O. corymbosa* DC. and *O. pes-caprae* L.) and three subspecies (*O. acetosella* subsp. *griffithii* (Edgew. & Hook. f.) Harv., *O. acetosella* subsp. *japonica* (Franch. & Sav.) Harv. and *O. acetosella* subsp. *leucolepis* (Diels) C. C. Huang & L. R. Xu) of *Oxalis* in China. Lourteig (2000), however, treated these subspecies as three distinct species (i.e. *O. griffithii* Edgew. & Hook. f., *O. leucolepis* Diels, and *O. obtriangularia* Maxim.) and included them in sect. *Oxalis* subsect. *Oxalis*, which also includes *O. acetosella*, *O. oregana* Nutt. and *O. trilliifolia* Hook. By following Lourteig (2000), Liu and Watson (2009) undertook a detailed revision of *Oxalis* in China and recognized eight species, six native (*O. acetosella*, *O. corniculata*, *O. griffithii*, *O. leucolepis*, *O. obtriangularia*, and *O. stricta* L.) and two introduced (*O. corymbosa* and *O. pes-caprae*). *Oxalis acetosella* subsp. *formosana* Terao was not accepted by Lourteig (2000) or Liu and Watson (2009), and they subsumed it into *O. griffithii* as a synonym. Although Lourteig (2000) reduced *O. corymbosa* to a variety of *O. debilis* Kuntz [i.e. *O. debilis* var. *corymbosa* (DC.) Lourteig], we follow Liu & Watson (2009) and recognize it as a distinct species in this study.

The Wuling Mountains region is located in central China, bordering Hunan to the southeast, Hubei to the northeast, Chongqing to the northwest, and Guizhou to the southwest. This region contains high species diversity, is rich in endemic and relic taxa (Chen et al. 2000), and is considered the core area of “western Hubei-eastern Sichuan center”, one of three centers of plant endemism in China. Despite their rich species diversity, the Wuling Mountains are also known as an under-explored region. Therefore, a large number of unique habitats may harbor specialized species, but they have not been studied systematically. A recent, in-depth survey in the region has led to the discovery of many new species (Li and Liu 2002; Li and Chen 2006; Zhang et al. 2008; Liu et al. 2009, 2010, 2011).

During a field excursion to study the biodiversity of the “Wuling Mountains National Nature Reserve”, an unusual isolated population of *Oxalis* was discovered. Subsequent taxonomic studies revealed that plants of this population morphologically differed from all known species of *Oxalis*. Additional expeditions occurred in March 2009 and 2010 to Badongshan Mountain in Hunan and careful observations were made of plants in the field. Some plants were also transplanted to the greenhouse in Jishou University and Kunming Institute of Botany, Chinese Academy of Sciences for further observation. The results of these studies confirmed that the plants represent an undescribed species. Further research was subsequently conducted, including field and herbarium comparisons, karyotype studies, and phylogenetic analysis based on ITS sequence data of this new taxon and related species within the genus. All lines of evidence support the status of these plants as a new species, which is described herein.

**Materials and Methods**

**Morphological Assessment**—Morphology of the newly collected specimens was examined and compared to that of *Oxalis acetosella*, *O. griffithii*, *O. leucolepis* and *O. obtriangularia* based on specimens from PE and KUN (acronyms follow Holmgren et al. 1990), as well as fresh materials of *O. acetosella* and *O. griffithii* obtained from the field. Ecological traits of these species were also included in the comparative analysis. A total of 36 individuals of the new species were examined from two populations, (Deng, Zhang, & Nie 544 from Hunan Province and Deng, Zhang, & Nie 259 from Hubei Province).

**Chromosomal Analysis**—Ten plants from the Hunan Province population and five plants from the Hubei Province population were sampled to determine the chromosome number of this new taxon. Living plants were collected from these two populations and cultivated in pots in the glasshouse at KUN. Root tips were pretreated with 0.002 M 8-hydroxyquinoline at room temperature for 3–5 h before being fixed in Carnoy’s solution (glacial acetic acid: absolute ethanol = 1:3), followed by maceration in a 1:1 mixture of 45% acetic acid and 1 M HCl at 60°C for 2.5 min, then stained and squashed in Carbol Fuchsine. Karyotype formulae were
based on measurements of mitotic-metaphase chromosomes taken from photographs. For karyotypic analysis, measurements of five cells were made in most cases. The symbols used to describe the karyotypes followed Levan et al. (1964). Voucher specimens are deposited in KUN.

**DNA Sequencing and Molecular Analyses**—Phylogenetic reconstruction was based on DNA sequence data obtained from the nuclear Internal Transcribed Spacer (ITS). Total genomic DNA was extracted from dried leaves following a modified CTAB method (Rogers and Bendich 1985) and used as a template in the polymerase chain reaction (PCR). Sequence alignment was initially performed using the program CLUSTALX version 1.81 (Thompson et al. 1997) in the multiple alignment routine using default settings and the accurate search option followed by manual adjustment in the program BioEdit version 7.0 (Hall 1999). Available sequence data were retrieved from GenBank. Voucher information and accession numbers are provided in Appendix 1. Sequence alignments of the ITS dataset and phylogenetic trees are available from TreeBASE (study number S13321).

Phylogenetic trees were constructed using maximum parsimony (MP), Maximum Likelihood (ML), and Bayesian inferences (BI). The MP analyses were conducted using PAUP* version 4.0b10 (Swofford 2003). Character state changes were equally weighted and gaps were treated as missing data. The most parsimonious trees were obtained with heuristic searches of 1,000 replicates, with random stepwise sequence addition, tree bisection-reconnection (TBR) branch swapping, collapse of zero-length branches, multiple tree option in effect, and saving 100 trees from each random sequence addition. Bootstrap values of the internal nodes were obtained with 1,000 replicates (Felsenstein 1985). In each replicate, 10 random sequence additions were performed, followed by TBR swapping, keeping no more than 1,000 trees per replicate.

Prior to these analytical approaches, the best fit model of nucleotide substitutions for the ITS sequence data was explored. A hierarchical likelihood ratio test as implemented in MrModeltest 2.3 (Nylander 2004) suggested the generalized time reversible model (GTR + I + G) as the best model. In the ML and BI analyses these substitution models and parameters were adjusted according to the estimates of MrModeltest. ML analysis was performed in GARLI 2.0 (Zwickl 2006) and bootstrap support values were estimated from 100 replicates.

Bayesian inference was implemented using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001). Bayesian tree topology was determined from two independent Markov chain Monte Carlo (MCMC) runs of four incrementally heated chains. Runs were performed for 5 million generations with sampling of trees every 500 generations. When the log-likelihood scores were found to have stabilized, a consensus tree was calculated after omitting the first ca. 10% of 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 clad.

### Results

**Morphological Assessment**—Morphological data suggested that the new species is related to members of sect. *Oxalis* subsect. *Oxalis*. Characters shared by species in this group include: habit acauliculent; bulbs absent; rhizome creeping at or just below the soil surface and covered with persistent, scale-like leaf bases; leaves 3-foliolate; flowers solitary with petals white to purple, but not yellow (Lourteig 2000). Numerous features, including leaflet shape, arrangement, and indumentum, plus petal length, collectively differentiate the new taxon from other species in this subsection (Table 1). Within the two populations examined, only a single style morph of flowers was found for the new species, i.e. long pistil (ca. 2 cm) with short stamens (ca. 1.0 cm). This contrasts with other *Oxalis* species from China (e.g. *O. pes-caprae*), which have three style morphs.

**Chromosome Data**—In the interphase nuclei, a few darkly stained, condensed bodies were observed, but their boundaries were not clear because the other part was also stained fairly well but unevenly (Fig. 1A). The prophase chromosomes displayed a distinctly continuous condensation pattern (Fig. 1B). The metaphase chromosomes of all individuals were determined to be 2n = 22 (Fig. 1C). Based on the nomenclature of Levan et al. (1964), *O. wulingensis* has 18 median centromeric (m) and four submedian centromeric (sm) chromosomes (Fig. 1D); i.e. 2n = 22 = 18m + 4sm.

**Phylogenetic Position**—The Bayesian tree is presented in Fig. 2 with MP, ML bootstrap and PP support values for each clade. *Oxalis* sect. *Oxalis* subsect. *Oxalis* is supported as a monophyletic group (MP bootstrap = 100%, PP = 100%, ML bootstrap = 99%). Within this clade, *O. wulingensis* is sister to *O. griffithii* (MP bootstrap = 82%, PP = 98%, and ML bootstrap = 89%) (Fig. 2).

### Taxonomic Treatment

Morphological data clearly confirm the newly collected taxon represents a distinct new species of *Oxalis*. Both

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**Table 1. Morphological comparison between *Oxalis wulingensis* and other species within sect. Oxalis subsect. Oxalis.**

<table>
<thead>
<tr>
<th>Characters</th>
<th><em>O. wulingensis</em></th>
<th><em>O. acuticula</em></th>
<th><em>O. griffithii</em></th>
<th><em>O. leucophae</em></th>
<th><em>O. obliquangula</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizomes (including scales)</td>
<td>Ca. 10 mm diameter</td>
<td>Ca. 3 mm diameter</td>
<td>6–12 mm diameter</td>
<td>1.5–2 mm diameter</td>
<td>5–8 mm diameter</td>
</tr>
<tr>
<td>Two lateral leaflets arrangement</td>
<td>180° angle</td>
<td>120° angle</td>
<td>120° angle</td>
<td>120° angle</td>
<td>120° angle</td>
</tr>
<tr>
<td>Leaflet shape</td>
<td>Long obliquangular</td>
<td>Obovate</td>
<td>Obovate</td>
<td>Rounded-obovate</td>
<td>Broadly obliquangular</td>
</tr>
<tr>
<td>Leaflet size</td>
<td>2.2–3.1 × 1.6–2.5 cm</td>
<td>0.5–2 × 0.8–3 cm</td>
<td>1–2.5(–4.5) × 1.5–3.3(–5.3) cm</td>
<td>1–1.8 × 1.1–2 cm</td>
<td>1–1.8 × 1.4–2.2 cm</td>
</tr>
<tr>
<td>Leaflet apex</td>
<td>Broadly emarginate</td>
<td>Deeply emarginate</td>
<td>Broadly emarginate to subtruncate</td>
<td>Deeply emarginate</td>
<td>Truncate to subtruncate</td>
</tr>
<tr>
<td>Leaflet indumentum</td>
<td>Both surfaces villous, densely covered, adaxially glabrous</td>
<td>Abaxially pubescent, adaxially glabrous</td>
<td>Abaxially pubescent, adaxially glabrous</td>
<td>Abaxially pubescent, adaxially glabrous</td>
<td>Abaxially pubescent, glabrous, adaxially glabrous</td>
</tr>
<tr>
<td>Leaflet abaxial surface color</td>
<td>Purple</td>
<td>Green</td>
<td>Green</td>
<td>Purple</td>
<td>Green</td>
</tr>
<tr>
<td>Petal length</td>
<td>Ca. 2.5 cm</td>
<td>(1.2–) 1.5–2.2 cm</td>
<td>1.2–1.6 (–2) cm</td>
<td>0.9–1.4 cm</td>
<td>Ca. 1.6 cm</td>
</tr>
<tr>
<td>Petal apex</td>
<td>Obtuse or irregularly denticulate</td>
<td>Retuse to deeply emarginate</td>
<td>Retuse to deeply emarginate</td>
<td>Unknown</td>
<td>Emarginate</td>
</tr>
<tr>
<td>Petal color</td>
<td>Pink</td>
<td>White</td>
<td>White, rarely pink (Hubei and Hunan)</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Capsules</td>
<td>Ovoid, 3–5 mm long</td>
<td>Angular-ovoid, 3–4 mm long</td>
<td>Oblong-conic, 5–13 × 5–6 mm</td>
<td>Unknown</td>
<td>Long-conical, 3–4 × 0.6–0.8 cm</td>
</tr>
<tr>
<td>Flowering time</td>
<td>March</td>
<td>July to August</td>
<td>April to September</td>
<td>July to August</td>
<td>May -June</td>
</tr>
</tbody>
</table>
cytological and molecular data indicate a close affinity with *O. acetosella* and *O. griffithii* in sect. *Oxalis* subsect. *Oxalis*. It is thus described as follows:


_Haec species similis Oxalis wulingensis et O. griffithii foliola longorum obtriangularis, ad angulum 180° dispositus, ramis dese utrinque brunneus villosus, petala roseus, apex obtusus aut 3–5 irregulariter denticulatus, florescentia martius differt._

Perennial herb, apparently acaulescent, 8–10 cm tall; rhi-

zome creeping underground, densely covered by dark brown, scale-like remains of leaf bases, ca. 11 mm thick including scales, root system fibrous. Leaves basal, 3-foliolate, the two lateral leaflets arranged at a 180° angle; petioles 5–7 cm long, densely covered with long, brown, villous hairs over their entire length; leaflets without calli, long obtriangular, 2.2–3.1 × 1.6–2.5 cm (length to width ratio 1.3–1.6), both surfaces densely villous with long, brown, villous hairs; occasionally glabrescent on abaxial surface with scattered hairs restricted to leaflet margin and veins in mature leaves, apex broadly emarginate, base cuneiform, lobe apices obtuse. Inflorescence 1-flowered, nodding; peduncles 10–12 cm long, longer than leaves; bracts at middle of flowering stalk, broadly triangular, ca. 3.0 mm long, with dense trichomes along midvein and margins. Flowers with sepals lacking calli, oblong-lanceolate, ca. 5.0 × 2.0 mm, green, surface and margins with some hairs, persistent; petals pink with lilac veins, oblong, ca. 2.5 × 1.0 cm, apex obtuse or irregularly denticulate with 3–5 teeth; stamens 10, alternately long and short, the longer ones ca. 1.5 cm, the shorter ones ca. 1.0 cm, all basally connate, filaments aubergine, glabrous, anthers white; gynoe-

cium ca. 2.0 cm long, ovary glabrous, locules 5, each with a single ovule, styles 5, separate, stigmas green, capitulate. Fruit an oblong capsule, 5.0–7.0 mm long, nodding, with 5 alar ridges; seeds ovoid, ca. 2.1 mm long, with 4–5 distinct ridges on both surfaces, dark brown when dry (Figs. 3, 4).

**Additional Specimen Examined—**

CHINA. Hubei Province: Xuan'en, Changtan, Banzhuyuan, humid hillside, ca. 1100 m, 5 March 2010, Deng, Zhang, & Nie 259 (KUN).

**Distribution and Ecology**

The distribution of species within sect. *Oxalis* subsect. *Oxalis* in China is presented in Fig. 5. Both *O. acetosella* and *O. griffithii* are widely distributed and have colonized multiple habitats between 800 and 3,700 m. *Oxalis acetosella* mainly occurs in northeast, north, northwest and southwest China, whereas *O. griffithii* occurs throughout eastern, central and southwest China. *Oxalis obtriangulata* is restricted to lowlying areas of northeast China. In contrast, *O. leucolepis* is very limited in distribution in the Hengduan Mountains region, occurring at higher elevations from 2,800–4,000 m in humid habitats under the mixed forests or among moss and boulders. The newly discovered taxon is the only endemic species of *Oxalis* in China, and is so far known only from a restricted area in northwestern Hunan and southwestern Hubei Provinces in the Wuling Mountains region of central China. Furthermore, it is currently only known from limestone habitat. Despite an apparently restricted distribution range, the plants were found to be locally abundant. Plants were scattered on moist rocks in the valley or in shady crevices below rocks, at elevations between 250–1,200 m. *Oxalis wulingensis* flowers in March, and fruits from April to May.

**Discussion**

The morphological attributes (Table 1), chromosome number, and molecular phylogeny confirm a close affinity of *Oxalis wulingensis* with taxa of sect. *Oxalis* subsect. *Oxalis*. Similar to members of this subsection, *O. wulingensis* possesses an acaulescent habit, bulbs are absent, rhizomes creep at or just...
below the soil surface, flowers are solitary and the petals are white to purple, but not yellow; this combination of features differs from other species of *Oxalis* in China.

Within *Oxalis*, the haploid chromosome number (*n*) is often 5, 6, 7, or 9, and *n* = 11, as observed in *O. wulingensis*, is very rare (De Azkue 2000). Marks (1956) identified a basic chromosome number of *x* = 7 as the most common condition in the genus. De Azkue (2000) advocated that species with the basic number *n* = 11 may have a polyploid or aneuploid origin and are likely derived. The chromosome numbers of *O. acetosella* and *O. griffithii* are also *n* = 11 (Nakajima 1936; Dobeš et al. 1997; Lövkvist and Hultgård 1999), which support their close relationship to *O. wulingensis* in subsect. *Oxalis*.

Furthermore, the Bayesian consensus tree based on ITS sequence data clearly places the new species in sect. *Oxalis* subsect. *Oxalis* (Fig. 2), corresponding to the *O. acetosella* clade in Oberlander et al. (2009, 2011). The results support a sister-species relationship between *O. wulingensis* and *O. griffithii* (Fig. 2). The two species, especially in central China, share the unique feature of having pink to pinkish-purple petals. Nevertheless, the two species are distinct morphologically, especially with respect to leaf architecture, petal shape, and capsule shape. *Oxalis wulingensis* has two lateral leaflets arranged at a 180° angle, long obtriangular in shape, densely pubescent with brown, villous hairs on both surfaces (vs. 120°, obtriangular, abaxially pubescent, adaxially glabrous), apex of petals obtuse or irregularly denticulate (vs. apex broadly emarginate to subtruncate), and capsules ovoid (vs. oblong-conic).

It is noteworthy that both *O. wulingensis* and *O. griffithii* were found to occur on the same mountains in the Wuling
Fig. 3. *Oxalis wulingensis* T. Deng, D. G. Zhang & Z. L. Nie. A, Habit; B, Individuals; C, Upper surface of leaves; D, Lower surface of leaves; E, Inflorescence; F, Flower in front view; G, Flower in side view; H, Fruits; I, Seed.
Fig. 4. *Oxalis wulingensis* T. Deng, D. G. Zhang & Z. L. Nie. a, Habit; b, Flower; c, Fruit; d, Seed. (All from Deng, Zhang, & Nie 544, KUN).
Mountains region (Badagongshan Mountain in Sangzhi County of Hunan and Qizimeishan Mountain in Xuan’en County of Hubei), but it seems that the two species never occur sympatrically. Both species grow in quite different habitats: *O. griffithii* occurs in sandy areas within forests and thickets or open places along stream sides; *O. wulingensis*, in contrast, is narrowly restricted to a limestone habitat and grows on moist rocks in mountain valleys or in shady crevices below rocks. The two species also differ in phenology, with *O. griffithii* usually flowering from April to September, and *O. wulingensis* blooming in early March. No intermediate forms between the two species were found on these mountains.

As shown in Table 1, *O. wulingensis* may be confused with *O. acetosella* and *O. obtriangulata* due to similarities in rhizome size, leaflet shape, and leaf size. However, they both differ from *O. wulingensis* in leaflet arrangement and indumentum, petal morphology and capsule shape, and generally occur in more northern regions. The new species is also somewhat similar to *O. leucolepis* by having adaxially purple leaflets, but differs in leaflet shape (long obtriangular vs. rounded-obcordate) and size (2.2–3.1 × 1.6–2.5 cm vs. 1–1.8 × 1.1–2 cm), and the latter species is endemic to the Himalayan region. All species of *Oxalis* in China can be readily identified using the following key:

**Key to Oxalis in China**

1. Bulbs present.
2. Petals yellow; leaves glabrous, semi-succulent ................................................................. *O. pes-caprae*
3. Petals pink; leaves hairy above and below, membranous .................................................. *O. corymbosa*
4. Plants creeping to caespitose, stolons absent; flowers in an umbellate inflorescence or solitary; fruiting pedicels deflexed to horizontal .......................................................... *O. corniculata*
5. Two lateral leaflets arranged at 180° angle, both surfaces densely covered with brown villous hairs; petals pink ........................................... *O. wulingensis*
6. Two lateral leaflets arranged at 120° angle, abaxially pubescent and adaxially glabrous; petals white (*O. griffithii* rarely pink in Hubei and Hunan).

![Distribution map of Oxalis wulingensis and other species within Oxalis sect. Oxalis subsect. Oxalis in China.](image)
6. Rhizome less than 3 mm thick (including scales).
7. Rhizome less than 2 mm thick; leaves abaxially purple
6. Rhizome more than 5 mm thick (including scales).
8. Leaflet blade obtriangular; capsules subglobose to oblong, less than 1.5 cm long
8. Leaflet blade broadly obtriangular; capsules long-conical, more than 2.5 cm long

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Literature Cited


De Azkue, D. 2000. Chromosome diversity of South American of Science and Technology (Grant no. 2005DKA21404).


