Rhododendron kuomeianum (Ericaceae), a new species from northeastern Yunnan (China), based on morphological and genomic data

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**Article info**

**Abstract**

Rhododendron kuomeianum Y.H. Chang, J. Nielsen & Y.P. Ma, a new species of Rhododendron (Ericaceae) within subsect. Maddenia in sect. Rhododendron from Yiliang County, NE Yunnan, China, is described and illustrated. The new species is similar to R. valentinianum, but it can be easily distinguished by its sparse scales on the abaxial surface of the leaf blade, fewer flowers per inflorescence and white corolla with pale red margins. There are also differences in the widths of calyx lobes, leaf blade shape and indumentum characteristics of the petiole between the new species and Rhododendron linearilobum. We confirmed that R. kuomeianum is a new species closely related to R. valentinianum and R. changii with phylogenomic studies of 10 species within this subsection based on restriction site-associated DNA sequencing (RAD-seq) data. These phylogenomic analyses also clarified additional taxonomic problems in this subsection previously raised by morphological analysis. Our findings make a strong case for using next-generation sequencing to explore phylogenetic relationships and identify new species, especially in plants groups with complicated taxonomic problems.

**1. Introduction**

Rhododendron L. is the largest genus in Ericaceae. It contains about 1025 species and can be classified into eight subgenera (Fang and Min, 1995; Chamberlain et al., 1996). Members of this genus are distributed widely in the northern hemisphere, with the Sino-Himalayan as the main center of abundance and diversity (Fang and Min, 1995). Because of its high ornamental value and long history of cultivation, rhododendrons have become popular horticultural plants worldwide. Rhododendron species richness is highest in China, which also contains numerous endemic species (Fang et al., 2005). In fact, an estimated 600 Rhododendron species have been described in China, and large numbers of new taxa are still being discovered since the publication of *Flora of China* (Gao and Zhang, 2008; Gao and Li, 2009; Chen et al., 2010, 2012; Ma et al., 2013, 2015; Liao et al., 2015; Cai et al., 2016; Liu et al., 2018; Tian et al., 2019).

In recent years, field investigations in Yiliang County (Zhaotong prefecture), within the Wumeng Mountain system in northeastern Yunnan, China, have discovered several new species of Rhododendron (Gao and Zhang, 2008; Gao and Li, 2009; Tian et al., 2019), suggesting that Rhododendron diversity in this region has been
underestimated. During field investigations in this region in June 2018, we collected specimens from a *Rhododendron* population with white flowers that were growing on steep cliffs and rocks. After a detailed examination of relevant specimens and literature, we concluded that these specimens represent a species new to science in subsect. *Maddenia* (Hutch.) Sleumer in subgen. *Rhododendron*.

Morphologically, this species is similar to *Rhododendron valentinianum* Forrest ex Hutch. and *Rhododendron changii* (W.P. Fang) W.P. Fang; it has a broadly elliptic to obovate leaf blade, rusty-yellow setae on the petiole and funnelform-campanulate corollas. However, the new species can be distinguished from these species by having sparse scales on the abaxial surface of the leaf blade, 1–2 flowers per inflorescence, and a larger, white corolla. Although both the new species and *Rhododendron linearilobum* R.C. Fang & A.L. Chang have similar corollas, the new species can be distinguished by having a broadly elliptic to obovate leaf blade, dense rusty-yellow setae on the petioles, and wider calyx lobes.

In recent years, as many new species have been described, molecular marker technology has greatly advanced our understanding of *Rhododendron* phylogeny (Gao et al., 2003; Ma et al., 2013; Yan et al., 2014; Liu et al., 2018; Du et al., 2020). However, these studies have mainly relied on a few common markers, which have limited their resolution. Next Generation Sequencing (NGS) offers a solution to these issues (Hohenlohe et al., 2010). For example, restriction site-associated DNA sequencing (RAD-seq) has been widely used in non-model species (e.g., temperate bamboos) to resolve evolutionary relationships (Heckenbauer et al., 2018; Zhang et al., 2018; Liu et al., 2020; Guo et al., 2020).

Here we confirmed that our newly collected specimen is a hitherto undescribed species using RAD-seq data to reconstruct the phylogenetic relationships of 11 *Rhododendron* species.

## 2. Materials and methods

### 2.1. Morphological analysis

During field investigations in June 2018 in Xiaocaoba Nature Reserve, Zhaotong prefecture, northeastern Yunnan, China, specimens of the new species were collected. Some living plants were introduced and cultivated at the Kunming Botanical Garden, Kunming Institute of Botany, CAS. The microscopic morphology of leaves was observed through a KEYENCE VHX-6000 stereoscopic microscope (KEYENCE Corp., Osaka, Japan). In addition, three similar species were sampled for morphological comparison. All voucher specimens in this study were deposited in the herbarium of the Kunming Institute of Botany (KUN), CAS.

### 2.2. Restriction site-associated DNA sequencing and SNP identification

A total of 11 individuals were sampled to construct the phylogram (Appendix A). Because the morphological characters of the new species are very similar to species within subsect. *Maddenia* in *Rhododendron*, we sampled nine additional species within the subsection and selected *Rhododendron simii* Planch. as the outgroup for subsequent phylogenetic analysis. High quality genomic DNA was extracted from silica gel dried young leaves using a modified CTAB protocol (Doyle, 1991).

The RAD-seq library was prepared using EcoRI to digest DNA following the adapted protocol of Miller et al. (2007). The de novo assembly of the RAD-seq library and SNP genotyping from short-read sequences was performed using the STACKS 2.5 pipeline (Rochette, 2017, 2019). The raw data was demultiplexed and filtered using the process_radtags program (key parameters included -c -q -E -t 135). For each sample, short-reads were merged into loci based on a maximum likelihood framework using the program ustacks, and were then aligned into exactly-matching stacks. The stack—depth parameter was set to three (m = 3) and the within-individual distance parameter was five (M = 5). The catalog of loci from all samples was built using cstacks with a between-individual distance parameter of three (n = 3). Lastly, the loci of each sample were matched against the catalog to confirm alleles via the program stacks. To reconstruct a phylogenetic tree, we randomly selected one SNP from each locus via the program popstubs. The SNP obtained from the previous STACKS pipeline was first applied by VCFtools (Danecek, 2011) with the following key parameters: --min-alleles 2 --max-alleles 2 --remove-indels --maf 0.05 --max-missing 0.2, --minGQ 30 --minDP 3.

After filtering with VCFtools, 42,083 SNPs were obtained and then tested for neutrality using the key parameter Tajima’s D with the sliding windows of 2500 and a 95% confidence interval (Tajima, 1989). The VCF file of neutral site was converted to phy format using Tassel 5.0 (Bradbury et al., 2007). Phylogenetic analysis of SNP matrices in phy format was performed using the maximum likelihood method of concatenation using IQTREE (Minh et al., 2020; Kalyaanamoorthy et al., 2017; Hoang et al., 2018) on partition A of the Beijing Supercloud Computing Center, and the best base substitution model selected from 242 DNA base substitution models was TVM+F+ASC, and the bootstrap was set as 1000. Phylogenetic trees were graphically visualized using FigTree (Price et al., 2010).

To capitalize on phylogenetic information from the sequencing data and obtain a robust taxonomic status for the new species, SNP loci were genotyped based on the de novo assembly of the RAD-seq data. After non-linkage filtering and neutral tests, we genotyped 12,380 loci. By calculating and sorting the BIC scores for these genotyped sites, the TVM+ASC model was selected as the best-fit model for the maximum likelihood method of the phylogenetic tree.

### 3. Results

#### 3.1. Morphological characters

The morphological characters of the new species differ from all species previously described in *Rhododendron*. Several traits of the new species indicate that it belongs to the subsect. *Maddenia*; specifically, it has a large corolla, the abaxial surface of the leaf is scaly, and a quarter of the base of the style has scales (Figs. 1 and 2). The new species can be distinguished from the two most similar species (i.e., *R. valentinianum* and *R. changii*) by having sparse scales (ca. 1–2 × their own diameter apart) on the abaxial surface of leaf blade (Fig. 3), fewer flowers (1–2) per inflorescence, and a larger, white corolla (3.5–4.5 cm) (Table 1). *R. linearilobum* has a corolla with similar morphology and color as the new species, but can be easily distinguished from the new species by having a narrowly obovate leaf blade, densely rusty-red-villosus petiole, and calyx lobes ca. 2 mm in width (Table 1).

#### 3.2. Data generation and molecular phylogeny

We constructed a RAD-seq libraries and genotyped SNPs from 11 species of *Rhododendron*. A total of 4.6 gigabase pairs (Gb) of data was obtained, with an average of 428.22 megabase pairs (Mb) of data per sample (see Appendix B for details).

Phylogenetic trees based on the de novo assembly of our RAD-seq library reveal that all 10 species within subsect. *Maddenia* are clustered into one clade, with *Rhododendron maddendii* subsp. *crassum* (Franch.) Cullen as the basal species (Fig. 4). One subclade includes *R. valentinianum*, *R. changii*, *Rhododendron pachypodium*...
Balf. f. & W.W. Smith and *Rhododendron kuomeianum* Y.H. Chang, J. Nielsen & Y.P. Ma sp. nov., which verifies that *R. kuomeianum* is genetically distinct and supports its status as a new species. Notably, the topological structure of the ML tree indicates that the phylogenetic position of *R. valentinianum* is very close to that of *R. changii*. The remaining five species (*Rhododendron liliiorum* Levl., *R. chunienii* Chun & W.P. Fang, *R. maddenii* subsp. *maddenii* (Batalin) H. Hara, *R. excellens* Hemsl. & Wils. and *R. ciliatum* Hook. f.) cluster into a separate subclade, although *R. liliiorum* and *R. chunienii* cannot be distinguished from each other. In addition, *R. maddenii* subsp. *maddenii* is genetically distant from the subspecies *R. maddenii* subsp. *crassum* (Fig. 4).

4. Discussion

In the present study, morphological comparisons and phylogenetic analysis based on *de novo* assembly of a RAD-seq library confirmed that *Rhododendron kuomeianum* is a new species. *R. kuomeianum* has similar floral traits as *R. linearilobum*; however, the new species has an oblong-elliptic leaf blade similar to *R. valentinianum* and *R. changii*. Due to unavailability of sequencing materials of *R. linearilobum*, which was estimated to be < 50 plants and hence evaluated to be critically endangered by IUCN criteria (Gibbs et al., 2011), the most genetically close relative remains to be unclear.

*Rhododendron* is the largest genus of seed plants in China, and recognized as one of the most taxonomically challenging plants due to recent adaptive radiations and natural hybridization (Yan et al., 2014). One major taxonomic problem for this genus is that many hybrids have been incorrectly identified as new taxa. Previous

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**Fig. 1.** Illustrations of *Rhododendron kuomeianum* Y.H. Chang, J. Nielsen & Y.P. Ma sp. nov. Drawn by R.M. Zhang. (A) habit; (B) corolla; (C) dissected corolla; (D) inflorescence bud; (E) flower bud; (F) stamen; (G) pistil and calyx; (H) leaf adaxial surface; (I) leaf abaxial surface; (J) distribution of scales on the adaxial surface of the leaf blade; (K) single scale on the abaxial surface of leaf blade; (L) capsule.

**Fig. 2.** *Rhododendron kuomeianum* Y.H. Chang, J. Nielsen & Y.P. Ma sp. nov. (A–B) plant and habitat; (C–D) leaf; (E) inflorescence bud; (F) corolla anatomy; (G) fruit. Scale bar = 1 cm.
studies at the Baili Rhododendron Nature Reserve in Guizhou have confirmed that many of the new taxa described in this area are actually different genotypes produced by natural hybridization between *Rhododendron delavayi* Franch. and *Rhododendron irroratum* Franch. (Marczewski et al., 2016; Zhang et al., 2017).

Morphological evidence has been used to describe taxa for over a century; however, this approach often leads to uncertainty and debate. For instance, *R. changii* was initially described as a variety of *R. valentinianum*; subsequently, it was upgraded to species status because of its larger, scaleless corolla, glabrous style, and the absence of hairs on the pedicel or calyx (Fang, 1983). After comparing holotypes and native plants, Geng (2014) restored *R. changii* as a variety of *R. valentinianum* based on morphological similarities and habitats. Our phylogenetic tree clusters these two species together, indicating that *R. changii* can reasonably be treated as a variety of *R. valentinianum*. Similarly, Li (1995) and Geng (2004) proposed that the holotype of *R. chunienii*, which had previously been described as having five stamens (the most unique character in this subsection), had been misdiagnosed because of damage to the corolla. Both living plants and specimens of *R. chunienii* collected at the type locality have 10 stamens; furthermore, other characteristics of *R. chunienii* are the same as in *R. lilii flororum*. Thus, *R. chunienii* has been treated as a synonym for *R. lilii flororum*. Our phylogenetic analysis supports this classification.

*Rhododendron maddenii* contains two subspecies, *R. maddenii* subsp. *maddenii* and *R. maddenii* subsp. *crassum*. Although these subspecies are morphologically similar, they are geographically isolated from one another. *R. maddenii* subsp. *maddenii* is mainly distributed in Cuona County and Naidong District in southern Tibet, China, as well as in Bhutan and northeastern India; in contrast, *R. maddenii* subsp. *crassum* is mainly distributed in western Yunnan. In this study, the high-confidence phylogenetic tree

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**Table 1**

Main morphological comparisons of *Rhododendron kuomeianum* with similar *Rhododendron* species.

| Characters                  | *R. kuomeianum* | *R. valentinianum* | *R. changii* | *R. linealilobum* |
|----------------------------|-----------------|--------------------|--------------|-----------------|
| Geographic distribution     | NE Yunnan       | SE W Yunnan        | Chongqing    | SE Yunnan       |
| Elevation                  | 1800–2000 m     | 2400–3000 m        | 1600–2000 m  | 2200 m          |
| Shape of the leaf blade     | broadly elliptic| obovate            | 3–4 × 1.5–2 cm| elliptic        |
| Size of leaf blade          | 3.5–5.5 × 2.3–3.5 cm | 0.5 or contiguous | 3–4.5 × 2–2.5 cm| nearly contiguous |
| Scale density (as own diameter apart) | 1–2 | 0.5                | 1–2          | 1–2             |
| Inflorescence               | 1 or 2-flowered | 2–4-flowered       | 3 or 4-flowered | 2–4-flowered    |
| Pedicel                    | glabrous        | setose             | glabrous     | pubescent       |
| Calyx lobes                 | 4–7 × 3–5 mm, ovate | 8 × 4–5 mm, ovoid or oblong-ovate | light to bright yellow | white with pale red margins |
| Color of the corolla        | light to bright yellow | 3.5–4 cm long, tube 1.8–2 cm | light to bright yellow | white with pale red margins |
| Corolla size                | 3.5–4.5 cm long, tube 2–3 cm | long | 3.5–4 cm long, tube 1.8–2 cm | 2–4 cm long, tube 1.8–2 cm |
| Proportion of scales to style | 1/4 | 1/8 | 1/8 | 2/5             |
| Indumentum characteristics of the style | glabrous | glabrous | glabrous | base pubescent |

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Fig. 3. The micrograph of the leaf surface of *Rhododendron kuomeianum* Y.H. Chang, J. Nielsen & Y.P. Ma sp. nov. (A) distribution of scales on the adaxial surface of the leaf blade; (B) distribution of scales on the abaxial surface of leaf blade; (C) single scale on the on the adaxial surface of the leaf blade; (D) single scale on the abaxial surface of leaf blade; (E) indumentum characteristics of the petiole.
reveals that the genetic difference between these two subspecies is very large, which has also been confirmed by DNA barcoding (Yan et al., 2014). One explanation for the genetic differences between these two subspecies is that long-term geographic isolation has allowed mutations to accumulate in their respective populations.

**Taxonomic treatment**

**Rhododendron kuomeianum** Y.H. Chang, J. Nielsen & Y.P. Ma sp. nov. (Figs. 1 and 2).

**Type.** CHINA. Yunnan: Yiliang county Xiaocaoba Nature Reserve, 25°50′01″N, 104°17′41″E, alt. 2000 m, 15 Apr. 2019, Y.H. Chang, Y.P. Ma & D.T. Liu, Cyh20190402 (holotype KUN! 1498888; isotype KUN! 1498889, 1498890).

**Diagnosis.** *Rhododendron kuomeianum* resembles *R. valentinianum* in having a broadly elliptic to obovate leaf blade, dense rusty-yellow setae on the petiole, and a funnelform-campanulate corolla. It differs from *R. valentinianum* in having a broadly elliptic to obovate leaf blade, dense rusty-yellow setae on the petiole, and a funnelform-campanulate corolla. It differs from *R. valentinianum* in having a white corolla with pale red margins (versus light to bright yellow), fewer flowers (1–2 per inflorescence) (versus 2–4), and sparser distribution of scales on the abaxial surface of the leaf blade (1–2 × scales diameter apart) (versus 0.5 × scales diameter apart) (Table 1).

**Description.** Multi-branched shrubs, evergreen, 40–100 cm tall; branches short, old branches deep red to brown, young shoots scaly, hispid. Petiole 5–9 mm, scaly, hispid; leaf blade leathery, broadly elliptic to obovate, 3.5–5.5 × 2.5–3.5 cm; base broadly cuneate or rounded; margin entire and slightly revolute, sparsely setose; apex obtuse or rounded, apiculate; abaxial surface pale green, scales 1–2 × their own diameter apart; about equal in size, golden, seldom contiguous, adaxial surface deep green and shiny, densely scaly, brown hispid along midrib; midveins raised adaxially and lateral veins hardly raised; midrib concave adaxially. Inflorescence terminal, cymose, 1 or 2-flowered. Pedicel stout, 0.6–0.9 cm, scaly, without hairs; calyx 4–5-lobed to the half, lobes pale green, 4–7 × 3–5 mm, ovate or broadly elliptic, persistent in fruit, scaly abaxially and on margin, margin sparsely white-ciliate; corolla funnelform-campanulate, white with pale red margin and without blotch, 3.5–4.5 cm, tube 2–3 cm, outer surface glabrous, lobes orbicular or broadly elliptic, ca. 1.8–2.5 × 2.0–2.5 cm; stamens 10, 1.5–3.7 cm, unequal, shorter than corolla; filaments densely pubescent in lower 1/5; anthers oblong-elliptic, red brown, ca. 2.5–3 mm; ovary 5-locular, ca. 4.5 mm, densely scaly; style slightly arched, as long as corolla or exserted from corolla, 3.5–4.2 cm base densely scaly in lower 1/4. Capsule ovoid, ca. 1 cm, dehiscing from top, densely scaly. Flowering: April–May. Fruiting: after June.

**Paratype.** China. Yunnan: Yiliang county Xiaocaoba Nature Reserve, 25°50′06″N, 104°17′39″E, alt. 1950 m, 5 Apr. 2019, Y.H. Chang & F.M. Yang, Cyh20190401 (KUN! 1498891). Same locality, alt. 2030 m, 6 June 2018, Y.P. Ma, Y.H. Chang & J. Nielsen, Ma20180603 (KUN! 1498892, 1498893).

**Distribution and habitat.** To date, *Rhododendron kuomeianum* has only been found in the type locality at Xiaocaoba Nature Reserve, Zhaotong City, NE Yunnan, China, where it is represented by one population at elevations of 1800–2000 m (Fig. 5). In addition, we found all plants growing on rocks and cliffs, which are steep and barren (Fig. 2A). Based on the known occurrences, we conclude that *R. kuomeianum* is well-adapted to cold and wet environments.

**Conservation status.** During many field investigations since 2018, we have only found this species in the type locality, and there are about 200 mature individuals in this population. Detailed examination of relevant specimens and literature available for *Rhododendron* species collected from Xiaocaoba Nature Reserve and adjacent regions revealed no additional information about the distribution of this species; therefore, this species is likely to be endemic to this region. Based on the limited information currently available, we thus tentatively assign this species to an IUCN Red List status of Data Deficient (DD) (IUCN, 2019). Further field investigation is urgently needed.
Etymology. The new species *Rhododendron kuomeianum* was named after Professor Kuo Mei Feng, a botanist and horticulturist in China. In pinyin, the Chinese name is “Guó méi dù juān”.

Author contributions

Y.P. Ma conceived and designed experiments. Y.H. Chang, Y.P. Ma, D.T. Liu, and J. Neilsen performed field investigations and collected the specimens. G. Yao and L. Zhang analyzed the molecular data. Y.H. Chang and Y.P. Ma revised the manuscript.

Declaration of competing interest

We declare that the named authors have no conflicts of interest, financial or otherwise.

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Appendix A and B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pld.2021.04.003.
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