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***Oreocharis* × *qianshanensis* (Gesneriaceae), a new natural hybrid from Qianshan City, Anhui, China**

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Abstract

Oreocharis × *qianshanensis*, a new natural hybrid that belongs to Gesneriaceae, is described and illustrated. Morphological evidences hint it is an interspecific hybrid of *O. auricula* and *O. chienii*. Further, molecular studies also showed that *O.* × *qianshanensis* arose from a hybridization between the maternal *O. auricula* and the paternal *O. chienii*. In this report, the morphology, chloroplast genome, and geographic distribution of *O.* × *qianshanensis* are documented.

Keywords: Didymocarpoideae, chloroplast genome, natural hybridization, sympatric distribution

Introduction

Natural hybridization is the successful crossbreeding between individuals from two or more distinct populations based on one or more identifiable heritable traits under natural conditions (Arnold 1997). In the long process of biological evolution, there is a significant correlation between natural hybridization and speciation. Due to the objective existence of natural hybridization, the systematic evolutionary relationship between hybrid offspring and parents fundamentally constitutes a reticulate form different from the “Binary branching” structure. This evolution is called reticulate evolution. Therefore, the way to generate new species is through hybrid speciation.

The importance of inter-species hybridization in plant evolution has been acknowledged for a long time (Stebbins 1950, 1959; Lewis 1966; Grant 1981). Hybridization plays a crucial role in the formation of new species through polyploid or homoploid speciation and introgression (Arnold 2006). Natural hybridization is common but not widespread, and it is concentrated in a few families (Ellstrand et al. 1996; Whitney et al. 2010), such as Primulaceae (Xie et al. 2017), Ericaceae (Zheng et al. 2021) and Orchidaceae (Wu et al. 2021; Solano et al. 2019), and so on. If

polyploidization is not involved, the first-generation plants are completely or almost fully sterile, so hybridization does not necessarily result in the establishment of a new species or hybrid swarm (Rieseberg et al. 2003; Mallet 2007).

Among the families with frequent hybridization, Gesneriaceae is included, and it is ranked 8 among the top 25 families. (Ellstrand et al. 1996; Whitney et al. 2010; Ferreira et al. 2013). They include *Columnea* Linnaeus, *Sinningia* Nees, *Monophyllaea* Brown and *Cyrtandra* Forster & Forster, and so on. Particularly in the African genus *Streptocarpus* Lindley, hybrid origin for some species has been suspected and then demonstrated to play a significant role in the evolution of the genus. Further, intergeneric hybridization within Gesneriaceae is also relatively frequent (Araujo et al. 2021). Although hybridization is common in some genera of Gesneriaceae, there are few examples reported in China, with only sporadic reports in *Oreocharis* and *Petrocosmea* (Puglisi et al. 2011; Qiu et al. 2011).

Oreocharis auricula (S.Moore) C.B.Clarke and *Oreocharis chienii* (Chun) Mich. Möller & A. Weber (Möller et al. 2011) are common in southern Anhui province of Gesneriaceae. Compared to *O. auricula* which is widely distributed in southern China, *O. chienii* is only limited to Anhui, Zhejiang and Jiangxi provinces in eastern China. *O. chienii* affiliated with *Briggsia* Craib (Wang et al. 1998). After *Oreocharis* Bentham was redefined by Möller et al. (2011), it has become a large and morphologically diverse genus including *Briggsia* in Gesneriaceae Rich. & Juss. (Möller et al. 2011, 2014; Chen et al. 2014). There are 152 species and 15 varieties of *Oreocharis* in China, mainly in southern and southwestern China (Wei 2018; Wen et al. 2021; GRC. 2023; Li et al. 2022). Currently, new species are still being discovered of *Oreocharis* in every year (Li et al. 2023).

In October 2021, during a plant diversity investigation in Bancang Provincial Nature Reserve, Qianshan city, Anhui province. We found a sympatric population of *O. auricula* and *O. chienii* but some individual fall somewhere in between. At the same time, we also found there are many naturally self-seedling young plants of this interesting population. We hypothesize that *O. × qianshanensis* might be a hybrid of the putative parents *O. chienii* and *O. auricula*. Unfortunately, the flowering period is over. In June of the second year, we found flowers and seed of *O. × qianshanensis*. This natural hybrid was observed to only a population of about 500 plants. According to its morphological features and morphological evidence we substantiated the *O. × qianshanensis* is a new hybrid between *O. auricula* and *O. chienii*.

Materials and methods

Plant material

The plants were collected in the field and subsequently cultivated in the laboratory for morphological observation and molecular analysis.

Morphological data collection

The morphology of *Oreocharis* species presented here is based on field observations. Field traits were recorded on-site, while vegetative and reproductive characteristics

were observed and measured using fresh specimens and pressed-dried material. Digital calipers (Deguqmnt, Shanghai, China) and a fluorescent microscope (Olympus, Hamburg, Germany) were utilized to describe the detailed morphological features of the samples. All photographs were taken in the natural habitat of the species (Bancang Provincial Nature Reserve) using digital camera (Nikon D7200, Tokyo, Japan).

Sequencing, assembly and annotation

Total genomic DNA was isolated from foliage leaves dried over silica-gel by the CTAB method (Doyle and Doyle 1987). Illumina paired end (2×150 bp) libraries were constructed and sequenced at Genesky Biotechnologies Inc. (<http://www.geneskybiotech.com>, Shanghai, China). The chloroplast genome was manually adjusted to remove ambiguous sites. The annotation process was performed following Xin et al (2019).

Phylogenetic analyses

Phylogenetic analyses were conducted based on 14 accessions, including 7 *Oreocharis* species and 6 other members of Gesneriaceae. Except for the new assembly of 3 *Oreocharis* plastomes, the other plastomes were downloaded from the National Center for Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov>). The complete plastome sequences were aligned using MAFFT. Both maximum likelihood (ML) and Bayesian inference (BI) methods were used for phylogenetic tree construction based on the complete plastome sequences.

Results and discussion

Morphological comparison

Characters of the leaves and flowers for the natural hybrids were described and measured on fresh specimens. The flowers of the hybrids were compared with their putative parents, *O. chienii* and *O. auricula*. The morphological characteristics of *O. × qianshanensis* are intermediate between *O. chienii* and *O. auricula*. In the vegetative period, it looks more similar to *O. chienii* and can be easily distinguished from *O. auricula* by leaves. In the reproductive period, it can be easily distinguished from *O. chienii* and *O. auricula* by flowers. (See Table 1, Fig. 4 for details).

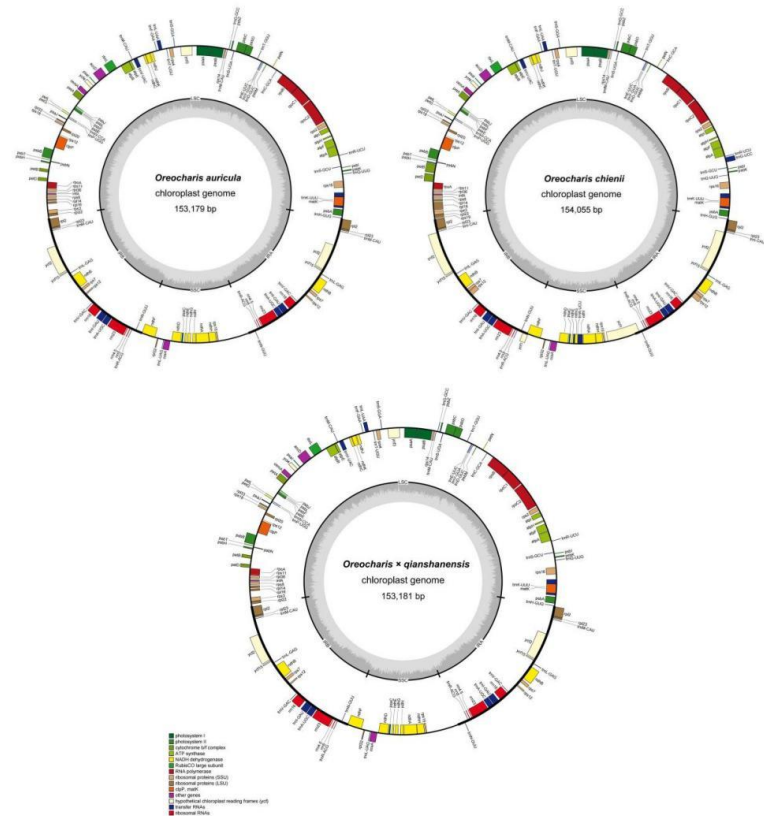
Table 1. A comparison of *Oreocharis × qianshanensis* and its putative parents.

Trait	<i>O. auricula</i>	<i>O. chienii</i>	<i>O. × qianshanensis</i>
Petiole	2–7 cm	1–6 cm	1–10 cm
leaf	2–12 cm long, 1–5 cm wide	4–15 cm long, 2–6 cm wide	6–22 cm long, 7–10 cm wide
Cymes	4–11 flowered	1–5 flowered	3–20 flowered
Peduncle length	6–15 cm	11–20 cm	12–15 cm
Calyx	ca. 3 mm long, ca. 1 mm wide	8–10 mm long, 3–4 mm wide	3.5–4 mm long, ca. 2 mm wide

Corolla tube	thin tubular, constricted larynx	thick tubular, swollen	tubular, without swollen
Stamens	upper stamens ca. 10 mm, lower stamens ca. 7 mm long	upper stamens ca. 16 mm long, lower stamens ca. 12 mm long	upper stamens ca. 12 mm long, lower stamens ca. 10 mm long
Pistils	pistil 8–13 mm long, staminode 1	pistil 14 mm long, staminode 2	pistil 10–14mm long, staminode 2
Flower colour	blue-purple to purple or white	purplish-red, inner purple spots	purplish, without spots
Corolla length	1.6–2.8 cm	3.5–4.2 cm	2.5–3.8 cm
Flowering time	May–September	September–October	July–September

Sequences alignment and phylogenetic analyses

The complete chloroplast genome of *O. × qianshanensis* was 153,181 bp in length, comprising a large single copy (LSC) region of 84,521 bp, a small single copy (SSC) region of 17,844 bp and two inverted repeat (IR) regions of 25,408 bp, and its GC content was 37.60%. The chloroplast genome contained 130 genes, including 86 protein-coding genes, 36 transfer RNAs and 8 ribosomal RNAs (Fig. 1).



The total chloroplast genome length of *O. × qianshanensis* is between that of *O. auricula* and *O. chienii*, slightly longer than *O. auricula*, but shorter than *O. chienii* (<200bp). And all characters tended to be in favors of *O. auricula* (Table 2).

Table 2. Comparison of complete chloroplast genomes of three taxa of *Oreocharis* in the study.

Characters	<i>O. auricula</i>	<i>O. chienii</i>	<i>O. × qianshanensis</i>
Total length	153179	154055	153181
LSC region	84519	84996	84521
SSC region	17844	18151	17844
IR region	25408	25454	25408
Total genes	130	134	130
CDS	86	88	86
tRNA	36	38	36
rRNA	8	8	8

The majority-rule consensus tree with both maximum likelihood (ML) and Bayesian inference (BI) analyses was shown in Fig. 2. There is high support for the *O. auricula* is the maternal parent of *O. × qianshanensis* (Bootstrap value of 100% in ML analysis and posterior probability of 1.0 in BI analysis).

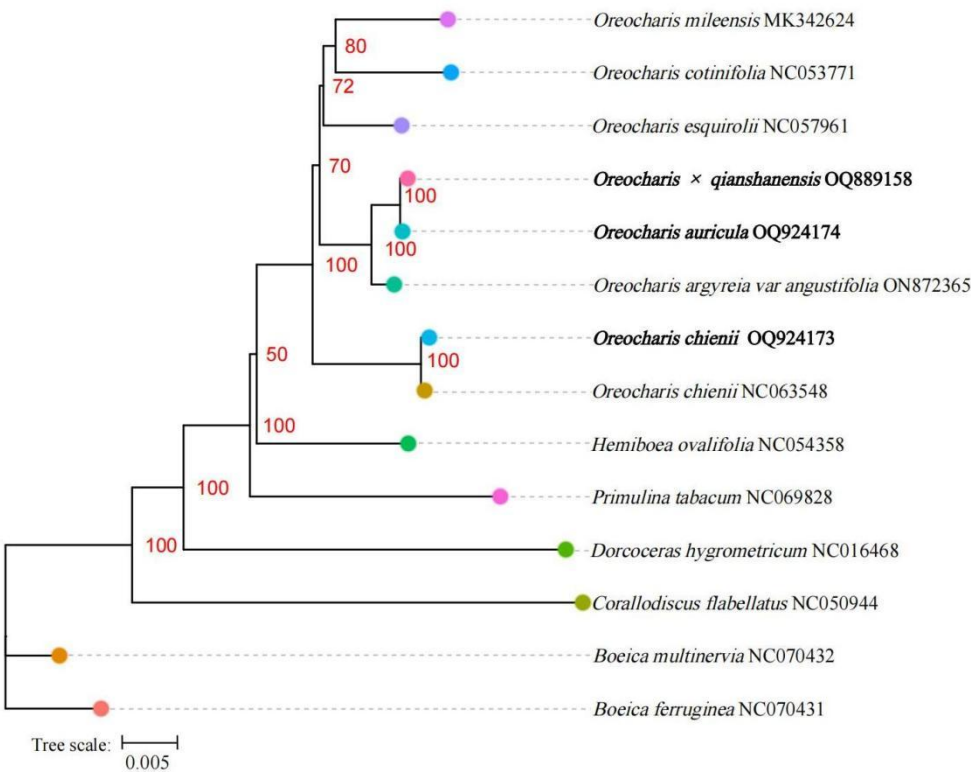


Figure 2. Phylogenetic consensus tree of the *O. × qianshanensis* based on Complete chloroplast genome dataset with maximum likelihood and Bayesian analyses. The new natural hybrid species and parents is highlighted in bold.

Taxonomic treatment

***Oreocharis* × *qianshanensis* H.J.Ma & S.B.Zhou, hybr. nov.**

Fig. 3, 4

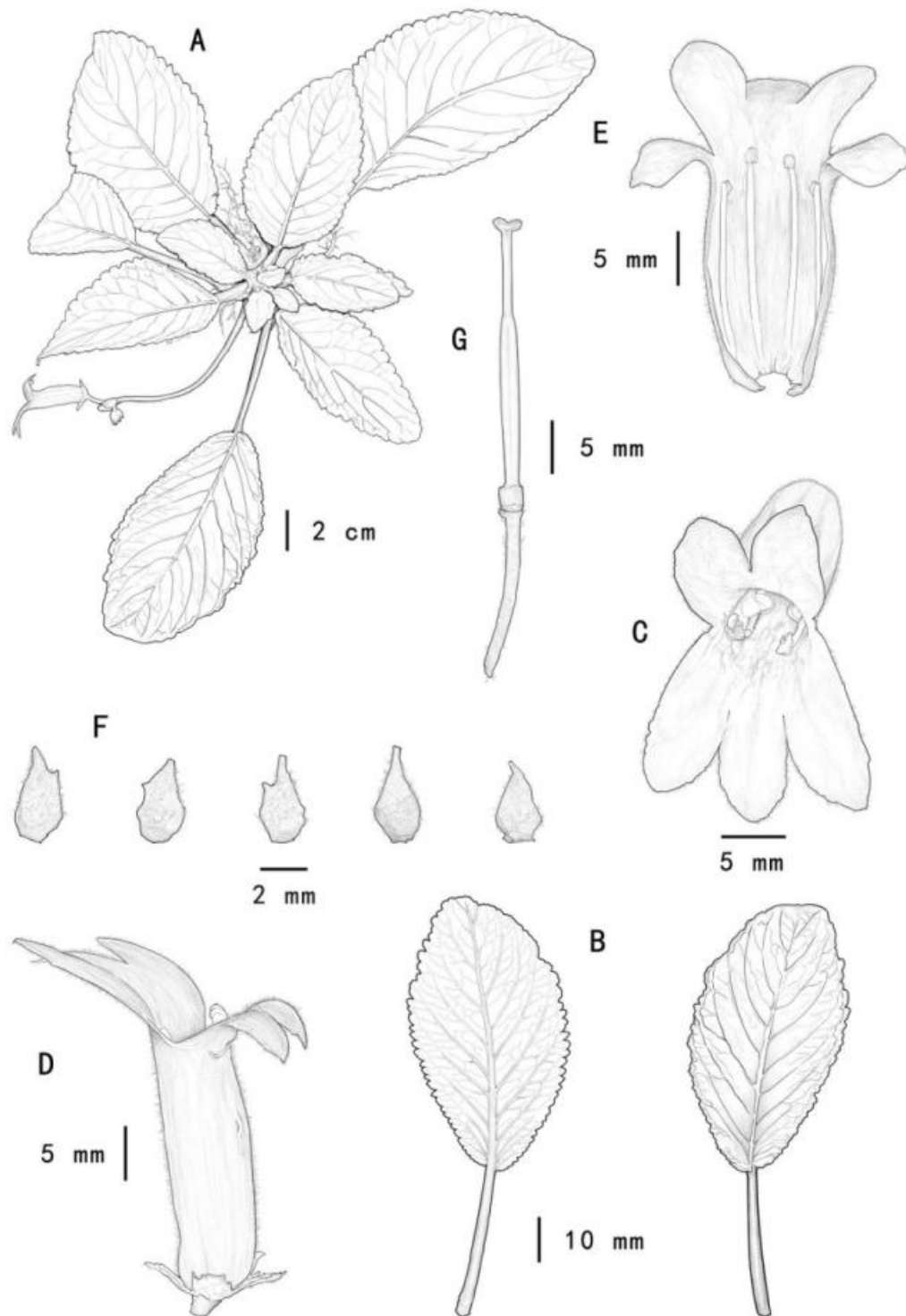


Figure 3. Line drawings of *Oreocharis* × *qianshanensis* based on photos and the type specimens (Drawn by Di Hu).

Diagnosis. The new hybrid species intermediate between *O. chienii* and *O. auricula*. It shares morphological similarities with *O. chienii* during the nutritional period, but it

differs from *O. chienii* in several aspects. The calyx of the new hybrid species is notably smaller, with a length of around 3.5–4 mm and a width of approximately 2 mm, in contrast to *O. chienii*, which possesses a larger calyx measuring 8–10 mm in length and 3–4 mm in width. Additionally, the flower color of the new hybrid species is purplish red, whereas *O. chienii* has a purplish color. Furthermore, the corolla tube of the new hybrid species is tubular without swelling.

The new hybrid species also bears similarities to *O. auricula*, but it can be distinguished by certain characteristics. It has brownish hairs on its leaves, petioles, and pedicels, which are not present in *O. auricula*. Moreover, the calyx of the new hybrid species is longer and wider than that of *O. auricula*. the corolla tube of the new hybrid species is tubular and lacks the constricted larynx observed in *O. auricula*.

Type. CHINA. Anhui province: Qianshan City, Bancang Provincial Nature Reserve, ca. 569 m (30°59'58.17"N, 116°32'33.01"E), *H. J. MA 21061401* (holotype: IBK; isotype: ANUB).

Description. Perennial herbs, rhizome short, ca. 2 cm long, ca. 0.4 cm in diameter; *leaves* basal, spirally arranged, 6–12; petiole 1–10 cm long, young leaves densely brown pilose and pubescent, getting mature gradually glabrescent; thickly herbaceous, oblong to lanceolate, 10–22 cm long, 7–10 cm wide at the middle, abaxially sparsely to densely dark grey or pale brown pubescent, sparsely brown villous along veins, veins adaxially sunken and abaxially ridged, lateral veins 6–7 on each side of midrib, base often slightly asymmetrical, wedge-shaped to elliptical, margin coarsely serrate, apex obtuse to rounded. *Cymes* axillary, 1–3 inflorescence, 3–6 branched, 6–20 flowered; peduncle 12–15 cm long, ca. 1 mm in diameter, sparse pale brown villous. bracts 2, opposite, lanceolate, outside densely villous, apex acuminate, cuneate to triangular, margin serrate; ca. 4 mm long, ca. 1 mm wide, densely brown pilose; pedicel 1.2–4 cm long, ca. 0.8 mm mm in diameter, densely rust-brown pilose and pubescent. *Calyx* 5-lobed to base, sharply triangular, serrated edges ca. 4 mm long, ca. 2 mm wide, outside brown fleece, inside glabrous. *Corolla* purplish, 2.5–3.8 cm long, densely glandular pubescent on both sides; tube cylindrical, slightly curved from the middle upwards the top, limb barely 2-lipped, adaxial lip shallowly 2-lobed near to the base, abaxial 3-lobed to the middle, slightly equal; upper lip lobes ca. 8 mm long, ca. 3 mm wide at the base, lower lobes ca. 12 mm long, ca. 4 mm wide at the base. *Stamens* 4, paired, included, adnate to ca. 4 mm above the corolla base; filaments glabrous, 10–11 mm long in anterior pair, of posterior pair 12–13 mm long; anthers white, slightly reniform, slightly constricted. *Pistil* mature 10–14 mm long; style 4–6 mm long; ovary cylindrical, glabrous, 6–8 mm long; stigma with 2 lobes, equal, suborbicular, each lobe ca. 1 mm long. *Capsule* brownish red, 1.2–1.5 cm long, 1–2 mm wide, sparsely pubescent, narrowly oblong.

Phenology. Flowering from July to September, and fruits in September.

Etymology. Named after the its type locality, Qianshan city.

Vernacular name. Qián Shān Mǎ Líng Jù Tái (Chinese pronunciation); 潜山马铃苣苔 (Chinese name).

Preliminary conservation assessment. Currently, the new natural hybrid species has been observed only from the type locality (Bancang Provincial Nature

Reserve). Only one population about 500 mature individuals was growing on the rock wall by the road. The locations of populations are easily accessible and frequently passed by passersby. The natural habitat may be disturbed or altered by human activities such as road extensions and other construction projects. In the meanwhile, we believe that the *O. × qianshanensis* is highly likely occurrence in sympatric populations of *O. chienii* and *O. auricula* elsewhere. Therefore, we propose that *O. × qianshanensis* should be considered as Vulnerable (VU), according to the IUCN Red List Categories and Criteria (IUCN 2022).

Distribution and habitat. The hybrid is only known to exist at its type locality, growing on rocks covered with humus along streams. It is found mixed with the sympatric population of its parents at an elevation of 500–600 m.

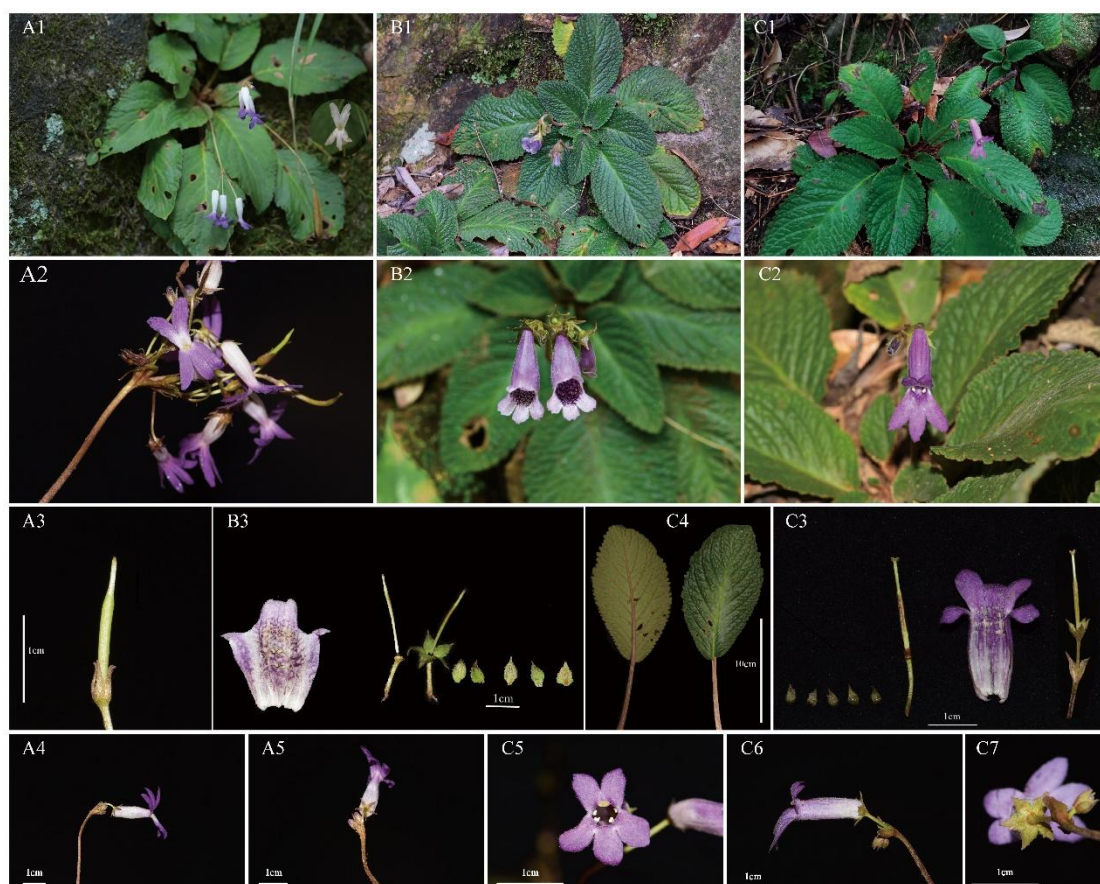


Figure 4. Colorful compared photographic images of *O. auricula* (A), *O. chienii* (B) and *O. × qianshanensis*(C). 1 plant 2 flowers A3 fruit pods B3, C3 flower anatomy A4, C6flower side C4 adaxial leaf surface and abaxial leaf surface A5, C7 Calyx C5 flower front.

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References

- Arnold ML (2006) Evolution through genetic exchange. Oxford University Press, New York, 252 pp.
- Arnold, ML (1997) Natural hybridization and evolution. Oxford University Press, New York, 215 pp.
- de Araujo AO, Peixoto M, de Souza CN, Gasparino EC, Faria JT, Lombello RA (2021) A natural intergeneric hybrid of Gesneriaceae from Brazil. *Phytotaxa* 497(2): 79-96. <https://doi.org/10.11646/phytotaxa.497.2.2>
- Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochem Bull* 19: 11–15.
- Ellstrand NC, Whitkus R, Rieseberg LH (1996) Distribution of spontaneous plant hybrids. *Proceedings of the National Academy of Sciences USA* 93: 5090–5093. <https://doi.org/10.1073/pnas.93.10.5090>
- Ferreira GE, Waechter JL, Chautems A (2013). *Sinningia* × *vacariensis* (Gesneriaceae) from Southern Brazil, the first natural hybrid described for the genus. *Phytotaxa*, 119(1), 45-50. <https://doi.org/10.11646/phytotaxa.119.1.4>
- Grant V (1981) Plant speciation. Columbia University Press, New York, 563 pp.
- GRC (2023) [continuously updated] Gesneriaceae Resource Centre. Royal Botanic Garden Edinburgh. <https://padme.rbge.org.uk/GRC> [Accessed: 20 April 2023]
- IUCN (2022) Guidelines for Using the IUCN Red List Categories and Criteria. Ver. 15.1 Prepared by the Standards and Petitions Subcommittee of the IUCN Species Survival Commission. <https://www.iucnredlist.org/resources/redlistguidelines>
- Lewis H (1966) Speciation in Flowering Plants. *Science, New Series*, 152: 167-172.
- Li RF, Le XG, Xu L, Maciejewski S, Chen B, Wen F (2023) *Oreocharis yangjifengensis* (Gesneriaceae), a new species from Yangjifeng National Nature Reserve of Yingtan City, Jiangxi Province, China. *Phytotaxa* 583(2): 213-218. <https://doi.org/10.11646/phytotaxa.583.2.10>
- Li ZL, Ma HJ, Ye ZR, Meng DC, Wen F, Hong X (2022) *Oreocharis guangwushanensis*, a new species of Gesneriaceae from Sichuan Province, China. *PhytoKeys* 201: 123–129. <https://doi.org/10.3897/phytokeys.201.77574>
- Mallet J (2007) Hybrid speciation. *Nature* 446: 279-283.
- Möller M, Chen WH, Shui YM, Atkins H, Middleton DJ (2014) A new genus of Gesneriaceae in China and the transfer of *Briggsia* species to other genera. *Gardens' Bulletin (Singapore)* 66: 195-205. <https://biostor.org/reference/217698>
- Möller M, Middleton D, Nishii K, Wei YG, Sontag S, Weber A (2011) A new delineation for *Oreocharis* incorporating an additional ten genera of Chinese Gesneriaceae. *Phytotaxa* 23(1): 1-36. <https://doi.org/10.11646/phytotaxa.23.1.1>
- Puglisi C, Wei, YG, Nishii K, Moeller M (2011) *Oreocharis* × *heterandra* (Gesneriaceae): a natural hybrid from the Shengtangshan Mountains, Guangxi,

- China. Phytotaxa 38(1): 1-18. <https://doi.org/10.11646/phytotaxa.38.1.1>
- Qiu, ZJ, Yuan ZL, Li ZY, Wang YZ (2011) Confirmation of a natural hybrid species in *Petrocosmea* (Gesneriaceae) based on molecular and morphological evidence. *Journal of Systematics and Evolution* 49(5): 449-463. <https://doi.org/10.1111/j.1759-6831.2011.00151.x>
- Rieseberg LH, Raymond O, Rosenthal DM, Lai Z, Livingstone K (2003) Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* 301: 1211-1216.
- Solano R, Huerta-Espinoza H, Cruz-García G (2019) A new natural hybrid in the genus *Laelia* (Orchidaceae) from Oaxaca, Mexico. *Phytotaxa* 402(5): 232-240. <https://doi.org/10.11646/phytotaxa.402.5.2>
- Stebbins GL (1950) Variation and evolution in plants. Columbia University Press, New York, 643 pp.
- Stebbins GL (1959) The role of hybridisation in evolution. *Proceedings of the American Philosophical Society* 103: 231-251.
- Wei YG (2018) The Distribution and Conservation Status of Native Plants in Guangxi, China. China Forestry Publishing House, Beijing, 876 pp.
- Wen F, Wei YG, Fu LF, Xin ZB, Li S, Ge YZ (2021) The Checklist of Gesneriaceae in China. <http://gccx.gxib.cn/cn/about-68.aspx> [Accessed: 29 August 2021] [in Chinese]
- Whitney KD, Ahern JR, Campbell LG, Albert LP, King MS (2010) Patterns of hybridization in plants. *Perspectives in Plant Ecology, Evolution and Systematics* 12: 175-182. <https://doi.org/10.1016/j.ppees.2010.02.002>
- Wu PP, Wang Z, Jia NX, Guo SW, Liu CC, Jin XH, Guo K (2021) *Calanthe* × *yarlungzangboensis*, a new natural hybrid in genus *Calanthe* (Orchidaceae) from China. *Phytotaxa* 518(2): 167-174. <https://doi.org/10.11646/phytotaxa.518.2.5>
- Xiao YE, Yu FY, Zhou XF (2021) A new natural hybrid of *Iris* (Iridaceae) from Chongqing, China. *PhytoKeys* 174: 1-12. <https://doi.org/10.3897/phytokeys.174.62306>
- Xie Y, Zhu X, Ma Y, Zhao J, Li L, Li Q (2017) Natural hybridization and reproductive isolation between two *Primula* species. *Journal of Integrative Plant Biology* 59(8): 526-53. <https://doi.org/10.1111/jipb.12546>
- Xin ZB, Fu LF, Fu ZX, Li S, Wei YG, Wen F (2019) Complete chloroplast genome sequence of *Petrocodon jingxiensis* (Gesneriaceae). *Mitochondrial DNA Part B* 4(2): 2771-2772. <https://doi.org/10.1080/23802359.2019.1624208>
- Zheng W, Yan LJ, Burgess KS, Luo YH, Zou JY, Qin HT, Gao LM (2021) Natural hybridization among three *Rhododendron* species (Ericaceae) revealed by morphological and genomic evidence. *BMC Plant Biology* 21(1): 1-12. <https://doi.org/10.1186/s12870-021-03312-y>