**Gastrochilus wolongensis** (Orchidaceae): a new species from Sichuan, China, based on molecular and morphological data

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ABSTRACT

*Gastrochilus wolongensis* (Orchidaceae), a new orchid species from Sichuan Province, Southwest China, is described and illustrated. It morphologically resembles *G. sinensis*, but differs markedly from the latter in having black-purple stripes (vs. purplish-red spots) on the adaxial side of the petals and sepals, a reniform epichile densely covered with long papillate hairs (vs. sparsely pubescent) with purplish-red spots (vs. unspotted), and outside the sac of the hypochile with purplish-red stripes (vs. purplish-red spotted). The molecular phylogenetic analysis based on nuclear ribosomal internal transcribed spacer (nrITS) and four chloroplast DNA fragments (*matK, psbA-trnH, psbM-trnD, and trnL-F*) of 36 *Gastrochilus* species showed that *G. wolongensis* was closely related to *G. ciliaris* and *G. formosanus*.

**Introduction**

*Gastrochilus* Don (1825, 32) is a genus of about 69 species mainly distributed in Southeast Asia (Kumar et al. 2014; Liu, Tan, and Gao 2016; Raskoti 2016; Liu et al. 2019; Govaerts et al. 2021). Labellum in this genus is divided into an epichile and a hypochile, with the former extending from the apex of hypochile and the latter firmly adnating to both sides of the staminal column and having two porous pollinia (Seidenfaden 1988; Tsi 1999). Combining recently described species (Liu, Tan, and Gao 2016; Liu and Gao 2018; Rao et al. 2019; Li et al. 2021; Nguyen et al. 2021, 2022), scientific reports of orchid biodiversity (Zhou et al. 2021a, 2021b), and some revisions based on morphology (Tsi 1999) and molecular data (Liu et al. 2019), a total of 43 species have been documented in China, and 23 of them are Chinese endemic species.

In March 2021, a species of *Gastrochilus* attached to the trunk of a tree was discovered during a field trip of the Second Tibetan Plateau Scientific Expedition in Wolong Nature Reserve, Wenchuan County, Sichuan Province, China. Morphologically, it did not fit any of the three known species recorded from Sichuan: *G. fargesii* (Kraenzlin 1903, 423) Schlechter (1919, 288), *G. formosanus* Hayata (1911, 336) Hayata (1917, 78), and *G. nanchuanensis* Tsi (1996, 149). It was neither documented within the local scientific report (Yang, Zhou, and He 2019), nor national updated catalog ([http://www.sp2000.org.cn](http://www.sp2000.org.cn)), nor in any specimen record ([https://www.cvh.ac.cn](https://www.cvh.ac.cn)). Morphological observation and comparison with type specimens of closely related species, e.g., *Gastrochilus sinensis* Tsi (1989, 23), C. P. Tsien et al. 32550 (holotype: PEI), revealed it to be a new species and hereafter it is described as *G. wolongensis*, which could be distinguished from any known species of *Gastrochilus*. Phylogenetic analysis using mainly nuclear ribosomal internal transcribed spacer (nrITS) and chloroplast fragments confirmed its position in this genus.

**Materials and methods**

Morphological description and measurements of *Gastrochilus wolongensis* were based on living plants and dried herbarium specimens. The taxonomic description follows the terminology used by Beentje (2012). Voucher specimens and additional silica-gel dried leaves are stored at CDBI Herbarium (acronym of herbarium follows Thiers 2021). To obtain DNA sequences for phylogenetic analysis, the leaves of *G. sinensis* (voucher ZJY228 at CDBI) were collected from Shunxiwu, Hangzhou City, Zhejiang Province,
China. In addition, the DNA sequences of 40 species from Gastrochilus and closely related genera were downloaded from the GenBank database (see Supplementary Table S1). The sampled taxa cover more than half of the total members of Gastrochilus species.

**DNA sequence acquisition**

For 40 species, nucleotide sequences including the nrITS and the four chloroplast DNA fragments (matK, psbA-trnH, psbM-trnD and trnL-F), were retrieved from GenBank database directly. Three cp genomes (MN124437, MN124438, MN124439) representing three Gastrochilus species were also downloaded for the extraction of the corresponding chloroplast DNA fragments. The information of these DNA fragments and the three complete plastid genomes used in this study were listed in Supplementary Table S1. For G. sinensis and two individuals of the new species, total genomic DNA was extracted exclusively from silica-gel dried leaves via a Plant DNA Isolation Kit (Cat.No.DE-06111). Based on the phylogenetic study of Gastrochilus by Liu et al. (2019), we applied the same primers to amplify the nrITS and the four chloroplast DNA fragments (matK, psbA-trnH, psbM-trnD and trnL-F) by polymerase chain reaction (PCR). All DNA samples were sent to TSINGKE Biotech Co. Ltd (Chengdu, China) for sequencing and then deposited to GenBank, with the following accession numbers: G. wolongensis, nrITS (OM985810, OM985811), matK (OK172400, OM974209), psbA-trnH (OK172402, OM974211), psbM-trnD (OK172403), trnL-F (OK172404, OM974210); G. sinensis, nrITS (OM985813), matK (OK042953), psbA-trnH (OK172399), trnL-F (OK172401).

**Phylogenetic analyses**

All sequences were edited by Sequencher v4.1.4 (Gene Codes, Ann Arbor, Michigan, USA) and aligned by MAFFT v7.475 (Katoh and Standley 2013) with default parameters. We performed phylogenetic analyses based on the dataset of nrITS, the four chloroplast DNA fragments, and the combined dataset of the nrITS and the four chloroplast DNA fragments, respectively. The nucleotide substitution models for the three data matrices were estimated using jModeltest 2.1.6 (Posada 2008) software and the evolutionary best fit model (GTR, GTR and GTR + I + G, respectively) was selected using the corrected Akaike Information Criterion (AICc). Two different methods including Maximum likelihood (ML) and Bayesian inference (BI) methods were employed. The ML analysis was performed using IQ-TREE v.1.4.241 (Nguyen et al. 2014) with branch support estimated using 2,000 replicates. The BI analysis was conducted using MrBayes 3.2.7a (Ronquist and Huelsenbeck 2003) with two parallel runs (20 million generations). The first 25% percent of trees from all runs were discarded as burn-in.

**Results**

The three molecular phylogenetic trees showed that the 36 species of Gastrochilus formed a well-supported monophyletic group (BI/ML = 1/98, Figure 1; BI/ML = 0.99/91, Supplementary Fig. S1; BI/ML = 0.90/93, Supplementary Fig. S2). Two accessions of G. wolongensis from different localities were resolved as sister to each other. Gastrochilus wolongensis and G. ciliaris Maekawa (1936, 92) formed a well-supported (BI/ML = 0.91/84, Figure 1; BI/ML = 0.93/91, Supplementary Fig. S2) monophyletic group, which was sister to G. formosanus (BI/ML = 0.87/95, Figure 1; BI/ML = 0.89/92, Supplementary Fig. S2). Gastrochilus sinensis was resolved as sister to a subclade consists of the three above mentioned species and three Gastrochilus species based on the data-sets of combined nrITS and chloroplast regions with moderately support (BI/ML = 0.82/73, Figure 1). Within the sub-clade, G. sinensis (Figure 2c,di) is morphologically closest to G. wolongensis (Figure 2a,b); G. formosanus (Figure 2ef) and G. ciliaris (Figure 2gh) also are similar to G. wolongensis in general morphology, though both have relatively smaller epichile and hypocile (Table 1). A thorough morphological comparison of G. wolongensis, C. sinensis, C. formosanus, and G. ciliaris is also summarized in Table 1.

**Taxonomy**

Gastrochilus wolongensis JUN.Y.Zhang, B.Xu & Yue. H.Cheng, sp. nov. (Figures 2a–b & 3).

**Type**

CHINA. Sichuan: Wenchuan, Wolong, evergreen broad-leaved forest, on tree trunk, elev. ca. 1700 m, 29 March 2021, Jun-Yi Zhang & Yue-Hong Cheng ZJY140 (holotype CDIB).

**Diagnosis**

It has similar habit dimension and leaves (two columns alternate, elliptic or oblong in outline and leaf blade green with a few purplish-red spots) with Gastrochilus sinensis, but it differs in shorter branched stems (4–
9 cm vs. 10–20 cm in the latter), one or two flowered racemes with larger sized flowers (1.2 × 1.3 cm vs. 1.0 × 1.1 cm in the latter), and black-purple striped sepal and petals. Moreover, the labellum with larger and reniform epichile (4.5–5.2 × 10.0–12.3 mm vs. 2.5 × 4.0–5.0 mm in the latter) densely papillate, distinctly purplish-red spotted while yellow-green centered, and with hypochile distinctly purplish-red striped on outside sac.

Epiphytic herbs, prostrate, 4.0–9.0 cm tall, leafy. Roots veriform, slender, up to 8.0 cm long. Stems short branched, green, glabrous, up to 9.0 cm long, covered with sheathing leaf bases; sheaths with purplish-red spots; internodes of stems 3.2–4.0 mm long. Leaves alternate, distichous, elliptic or oblong, plump, both surfaces green with few purplish red spots, 1.1–1.5 × 0.5–0.8 cm, margin smooth, apex acute and leathery. Inflorescence in racemes with 1 or 2 flowers, ca. 2.8 cm long; Peduncle 4.0–7.0 mm long. Flowers 1.2 × 1.3 cm, light yellowish green, with black-purple stripes on petals and sepals, elevated on abaxial midrib; pedicel and ovary 6.0–8.3 mm long, green at the base and blackish green toward the apex; dorsal sepal elliptic-oblong, 6.8–8.9 × 4.2–5.8 mm, with 3 veins, only the midvein reaching the apex; lateral sepals elliptic-oblong, slightly pointed at the apex, 6.2–8.6 × 4.2–5.8 mm, with 1 vein; petals obovate, 7.1–8.5 × 4.5–6.0 mm, with 3 veins, none reaching the apex; labellum 7.0–9.8 × 6.2–7.7 mm; hypochile

Figure 1. Maximum likelihood tree of Gastrochilus from phylogenetic analysis of combined nrITS, matK, psbA–trnH, psbM–trnD and trnL–F sequence data. Numbers before slash indicate Bayesian posterior probabilities and numbers after slash indicate ML bootstrap supports for major lineages. The new species inferred are indicated in red.
attached to base of column wing, subcupular, laterally compressed, ca. 3.7 × 5.8 mm, opening cordate, base saccate, sac with lavender patches inside, long papillate hairs near epichile side, outside of sac with conspicuous purplish-red stripes; epichile reniform, apically concave, densely covered with long papillate hairs, 4.5–5.2 × 10.0–12.3 mm, with purplish-red spots and a yellow-green center; column cylindrical, ca. 3.0 mm; anther cap subhemispheric, with two chambers, 1.2 × 1.4 mm, hanging from both ends of the stipe; pollinia 2, 0.7 × 0.6 mm, yellow, full and nearly spherical, with a depression in the center; stigma deeply sunken, inverted V-shaped, 1.2 mm long, yellow, apically forked, forked in a subtriangular outline. Capsule long ellipsoid, 14–18 mm long, green with sparse purplish-red spots, prominently 3-ridged.

Figure 2. Comparison of Gastrochilus wolongensis (A, B) with G. sinensis (C, D), G. formosanus (E, F) and G. ciliaris (G, H). [Images C & D courtesy of Feng Li; image E referring to website (http://www7a.biglobe.ne.jp/~flower_world/Orchids/); image F cited from Kumar et al. (2014); images G & H reproduced from website (http://www7a.biglobe.ne.jp/~flower_world/Orchids/)].
Table 1. Morphological comparison of *Gastrochilus wolongensis* with three related species: *G. sinensis*, *G. formosanus*, and *G. ciliaris*.

| Character                  | *G. wolongensis* | *G. sinensis* | *G. formosanus* | *G. ciliaris* |
|----------------------------|------------------|---------------|-----------------|--------------|
| Plant length               | 4–9 cm           | 10–20 cm      | 10–40 cm        | ca. 10 cm    |
| Leaf outline               | elliptic or oblong | elliptic or oblong | oblong         | elliptic or oblong |
| Leaf dimensions            | 1.1–1.5 × 0.5–0.8 cm | 2.0 × 0.7 cm | ca. 2.5 × 0.7 cm | 0.8–2.5 × 0.4–0.5 cm |
| Leaf apex                  | acute and leathery | acute and with three short awns | retuse, minutely setaceous in the sinus | Acute or apiculate |
| Inflorescence              | raceme to 2.8 cm long | raceme to 2.0 cm long | raceme to 3.0 cm long | subumbellate to 1.2 cm long |
| Flower color               | yellowish green, with purplish red stripes | yellowish green, with purplish red spots | yellowish, with purplish speckles | yellowish green, with brown spots |
| Flower dimensions          | 1.0–1.5 cm       | 2.0–2.5 cm    | 3.0–4.0 cm      | 3–4 flowers  |
| Peduncle length            | 0.4–0.7 cm       | 0.3–0.4 cm    | 0.3–0.4 cm      | 0.3–0.4 cm   |
| Dorsal sepal               | elliptic-oblong, 6.8–8.9 × 4.2–5.8 mm, apex slightly pointed, with 1 vein | elliptic-oblong, concave, 4.2–5.0 × 2.0–2.5 mm, apex obtuse | elliptic-oblong, concave, 4.2–5.0 × 2.0–2.5 mm, apex obtuse | elliptic-oblong, concave, 4.2–5.0 × 2.0–2.5 mm, apex obtuse |
| Lateral sepals             | obliquely oblong, apex obtuse, with 3 veins, ± elevated on abaxial midrib, only midvein reaching apex | obliquely oblong, apex obtuse, with 1 vein, ± elevated on abaxial midrib | obliquely oblong, apex obtuse | obliquely oblong, apex obtuse or acute |
| Petals                     | suborbiculate, 7.1–8.5 × 4.5–6.0 mm, apex subrounded, with 3 veins, none reaching apex | suborbiculate, 3.5–4.5 × 1.5–2.0 mm, apex subrounded, with 3 veins, only midvein reaching apex | oblong, 4.0–5.0 × 2.8–3.0 mm, apex rounded | rhombic-elliptic, 2.4–2.7 × ca. 1.3 mm |
| Flower dimensions (height and width) | 1.2 × 1.3 cm | 1.0 × 1.1 cm | 0.8 × 0.9 cm | 0.4 × 0.5 cm |
| Hypochile                  | suborbiculate, laterally compressed, ca. 3.7 × 5.8 mm | subconic, laterally compressed, 3.5–4.0 × ca. 3.0 mm | subcylindrical, 3.0 × 4.0 mm | cylindrical conical, 1.5–2.0 × ca. 2.0 mm, obtuse at bottom |
| Epichile                   | margin and adaxial surface densely hairy, reniform, with purplish red spots, 4.5–5.2 × 10.0–12.3 mm, with a central cushion, apex broadly emarginate | margin and adaxial surface densely shortly hairy, reniform, ca. 2.5 × 4.0–5.0 mm, with a central cushion, apex broadly emarginate | suborbicular, 2.2–3.2 × 7.0–9.0 mm, thick, margin not alate, hirsute | semiorbicular to reniform, 2.5–3.0 × 3.5–4.0 mm, apex rounded |
| Flowering period           | March and April  | October       | Throughout year | September    |
Additional specimens examined
CHINA. Sichuan: Wenchuan, Wolong, evergreen broad-leaved forest, on tree trunk, elev. ca. 1700 m, 2 March 2022, Jun-Yi Zhang & Yue-Hong Cheng ZJY142 (CDBI).

Distribution, habitat and phenology
The new species in currently known only from Wenchuan County, Sichuan Province, Southwest China. It is found epiphytic on tree trunks, at elevation ca. 1700 m. It is in flowering from March to April.
Etymology
– The specific epithet is derived from Wolong Nature Reserve, which was one of the earliest reserves dedicated to preserve the habitat of wild pandas. A Chinese name, wo long peng ju lan (卧龙盆距兰), is suggested here.

Conservation status
Due to the probably need of more extensive fieldwork, we assessed the conservation status of Gastrochilus wolongensis as DD (Data Deficient) according to the IUCN (2019).

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Disclosure statement
No potential conflict of interest was reported by the author(s).

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