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Oreocharis hainanensis (Gesneriaceae), a new species from karst regions in Hainan Island, South China

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Abstract

Oreocharis hainanensis, a new species of Gesneriaceae is described and illustrated from low-altitudual karst areas in Hainan Island, South China. The new species is easily distinguished from its closely-related *O. jasminina* by campanulate floral tube, zygomorphic corolla and exserted stamens. It also shows different habitats from the four currently-recognized *Oreocharis* taxa on the island. Molecular phylogeny analysis based on plastid *trn*L-*trn*F and nuclear ITS1/2 sequences supported the delimitation of the new species, which forms a monophyletic clade with all the other *Oreocharis* taxa from Hainan Island. The roles of habitat and floral isolation in the evolution of the new species and its affinities are discussed. The species was assessed as Vulnerable (VU C1 and D2) according to IUCN Red List Categories and Criteria.

Keywords: Morphology, Molecular, New taxon, Karst

Introduction

Oreocharis Bentham (1876: 1021) was recently re-delimited to include ten small or monotypic genera and the species number reach to over 160 species, based on extensive molecular phylogenetic studies (Möller *et al.* 2011, Weber *et al.* 2013), while *Bournea* Oliver (in Hooker 1893: 2254) was re-instated as an independent genus (Chen *et al*. 2020). *Oreocharis* belongs to tribe Trichosporeae Nees (1825: 143), subfamily Didymocarpoideae Arnott (1832: 121) of Gesneriaceae, and it is phylogenetic closed to *Aeschynanthus* Jack (1823: 42), *Cyrtandra* Forster & Forster (1776: t. 3) and *Agalmyla* Blume (1826: 766). *Oreocharis* is predominantly distributed in China with approximately 147 species, and some species distributed in the Indochinese Peninsula and Japan (Chen *et al*. 2018, Jin *et al.* 2021, Kong *et al*. 2021). The *Oreocharis* underwent early and rapid explosive radiation in Miocene (Kong *et al.* 2021), it can be divided into two clades. One clade is mainly distributed in Southwest China and characterized by yellow corolla with four stamens, and another clade is mainly distributed in South and Southeast China and characterized by purple corolla, some species evolved with two stamens (Jin *et al.* 2021). All species are rosette plants with spirally arranged leaves, axillary inflorescences, and showed a strikingly-high diversity in floral syndromes with limited differences in habit and fruit structure (Li & Wang 2005, Wei 2010, Jin *et al.* 2021), and the evolution from zygomorphy to actinomorphy in corolla was detected (Jin *et al.* 2021).

As a distinctive part of the globally important Indo-Burma biodiversity hotspot (Myers *et al.* 2000), Hainan Island in South China harbors 14 genera and 25 species of Gesneriaceae, including two endemic genera and ten endemic species (Li & Wang 2005, Ling *et al.* 2017, 2020b). Currently, four taxa of *Oreocharis* were recognized in Hainan Island, i.e., *O. dasyantha* Chun (1946: 287) var. *dasyantha*, *O. dasyantha* var. *ferruginosa* Pan (1987: 283), *O. flavida*

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Merrill (1922: 354) and *O. jasminina* S.J. Ling, F. Wen & M.X. Ren in Ling *et al.* (2020: 157). All of them are endemic to Hainan Island, and mostly restricted to high altitude area (> 1,000 m) (Ling *et al.* 2020a, b).

During field investigations in the past three years, we collected some specimens of an interesting *Oreocharis* species that grows at about 200 m altitude of a karst hill in Hainan Island, South China. The species showed notable differences from the known *Oreocharis* species in various morphological characters, e.g. corolla colour, corolla shape and stamens traits. Based on morphological and molecular analyses, together with literature review (Pan 1987, Li & Wang 2005, Wei 2010, Ling *et al*. 2020a), it is convinced that these specimens represent a new *Oreocharis* species described below.

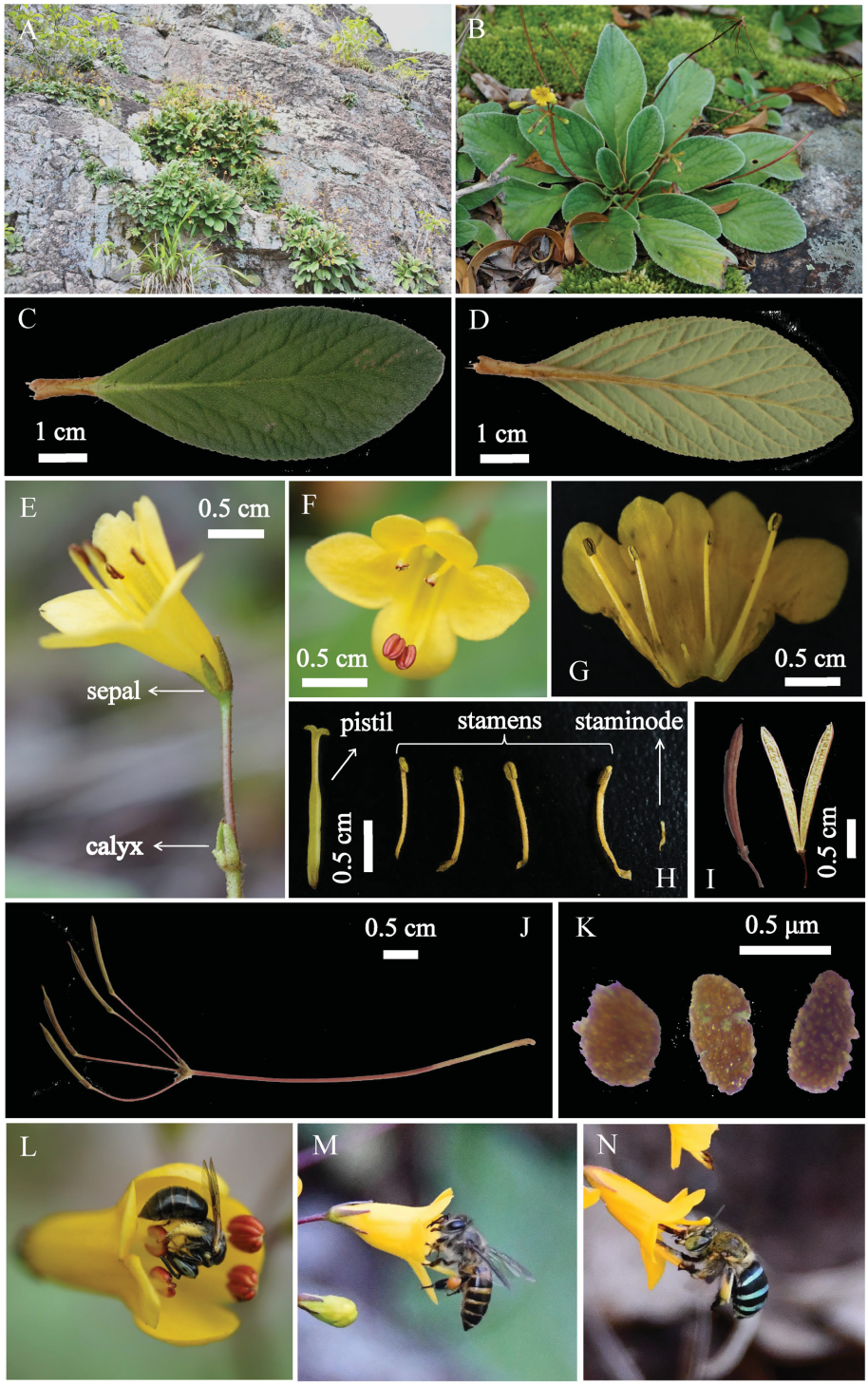

FIGURE 1. *Oreocharis hainanensis* S.J.Ling & M.X. Ren *sp. nov.* **A.** Habitat; **B.** habit; **C.** adaxial leaf surface; **D.** abaxial leaf surface; **E.** lateral view of corolla, sepal and calyx; **F.** face view of corolla; **G.** opening flower showing stamens and staminode; **H.** pistil, stamens and staminode; **I.** capsule; **J**. fruit pods; **K**. seeds; L–N. Main floral visitors: **L**. *Braunapis* sp.; **M.** *Apis* sp.; **N**. *Amegilla* sp.; All photos by Shao-Jun Ling.

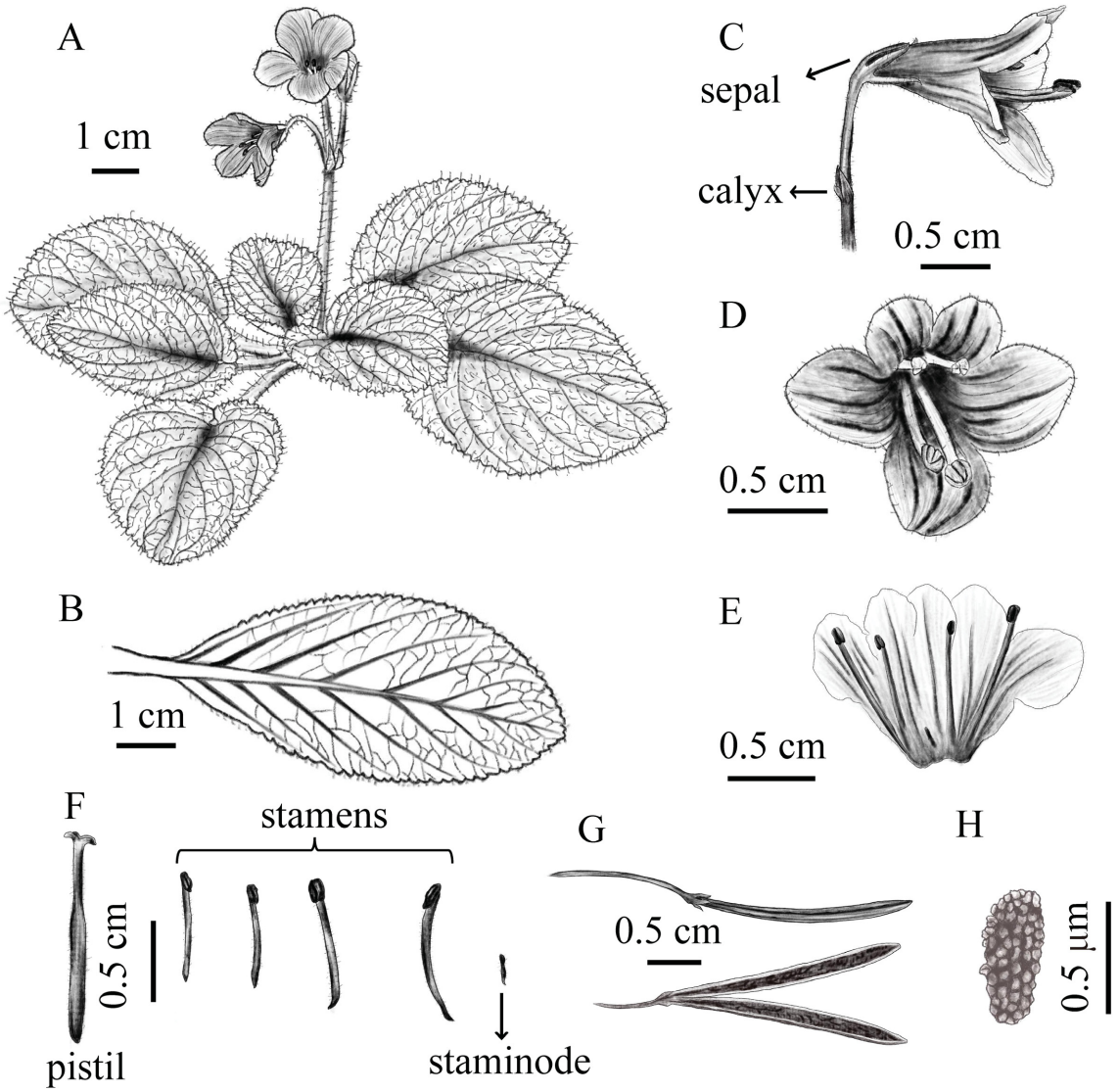

FIGURE 2. *Oreocharis hainanensis* S.J.Ling & M.X. Ren *sp. nov.* **A.** Habit; **B.** abaxial leaf surface; **C.** lateral view of corolla, sepal and calyx; **D.** front view of corolla; **E.** opening corolla showing stamens and staminode; **F.** pistil, stamens and staminode; **G.** capsule; **H.** seed. (Drawn by Shu-Ping Guan based on the holotype *S.J.Ling 2020091701* in HUTB).

Materials & methods

Morphological observations

Morphological observations and measurements were conducted based on the field work, and we studied *Oreocharis* collections deposited in the following herbaria: IBK, IBSC, KUN, and PE. The images of type specimens of most *Oreocharis* species were gathered from JSTOR Global Plants (http://plants.jstor.org) and Chinese Virtual Herbarium (http://www.cvh.ac.cn) to compared detailed morphological traits between the new species and the currently accepted species of *Oreocharis*. We analyzed at least 30 collections of the new species and other taxa of *Oreocharis* in Hainan Island, and pollinators of the new species were observed and photographed in the wild. Specimens were kept under cultivation in herbarium of Hainan University (HUTB) to photograph and analyze fresh flowers, fruits, and seeds under a stereomicroscope. A morphological description was made based on the terminology of Stevens (2009). A comparative table of the morphologically similar species was prepared based on the herbarium specimens and protologues of the new species and other species of *Oreocharis* in Hainan Island. The informal conservation assessment was based on the criteria proposed by the IUCN (2019). Voucher specimens of new species are deposited in HUTB, Kunming Institute of Botany, Chinese Academy of Sciences (KUN), and Guangxi Institute of Botany, Chinese Academy of Sciences (IBK).

TABLE 1. Comparison of diagnostic characters of *Oeocharis hainanensis* and all its congeners in Hainan Island.

Characters	***O. hainanensis***	***O. jasminina***	***O. dasyantha***	***O. dasyantha* var. *ferruginosa***	***O. flavida***
Corolla colour	yellow	yellow	orange-red to yellow	orange-red to yellow	orange
Corolla tube	campanulate, 1–1.5 cm long, corolla width 1–1.7 cm, corolla mouth width 3–4.5 mm	narrowly tubular, 1.7–2.2 cm, long, corolla width 1.8–2.2 cm, corolla mouth width 3–4.5 mm	conical, 1.6–2.4 cm long, corolla width 0.9–2 cm, corolla mouth width 6–7 mm	conical, ca. 1.6 cm, tube 9–1.1 mm, corolla width 0.9–1.9 cm, corolla mouth width 6–7 mm	campanulate-tubular, 1.7–1.9 cm long, corolla width 1.6–1.8 cm, corolla mouth width 6–8 mm
Corolla symmetry	zygomorphic	actinomorphic	zygomorphic	zygomorphic	actinomorphic
Leaf blade shape	obovate, ovate to broadly ovate, rarely broadly ovate	ovate to broadly ovate, rarely elliptic or obovate	ovate-elliptic to broadly ovate	ovate-elliptic to broadly ovate	ovate-elliptic to broadly ovate, rarely broadly elliptic
Leaf base shape	cuneate, rarely subrounded	cordate to rounded	oblique, cuneate to subrounded or cordate	sometimes oblique, cuneate to subcordate	oblique, subrounded
Leaf base margin	shallowly crenate-serrate	nearly entire to shallowly crenate, apex rounded	serrulate or crenate-serrate, apex acute to rounded	crenate-serrate	shallow crenate
Stamens	exserted, didynamous, staminode 1	included, didynamous, staminode 1	exserted, equivalent, staminode absent	exserted, didynamous, staminode absent	included, equivalent, staminode 1
Anthers	ovate, 2-loculed, dehiscing longitudinally	ovate, 2-loculed, dehiscing transversely	broadly oblong, 2-loculed, dehiscing longitudinally	broadly oblong, 2-loculed, dehiscing longitudinally	horseshoe-shaped, 1-loculed, dehiscing transversely
Filaments	pubescent	pubescent	pubescent	pubescent	glabrous
Pistil	ca. 16 mm long	ca. 9 mm long	ca. 22 mm long	ca. 22 mm long	ca. 9 mm long
Habitat	Karst at low altitude	Mountain top, non karst	Mountain top, non karst	Mountain top, non karst	Mountain top, non karst

Taxonomic sampling, DNA extraction, PCR and sequencing

Leaf materials for DNA extraction of *O. dasyantha* var. *dasyantha*, *O. dasyantha* var. *ferruginosa*, *O. flavida*, *O. jasminina* and the putative new species were dried in a vascular bag with silica gel in the field. Total genomic DNA extraction was carried out using standard CTAB methods (Doyle & Doyle 1987). The chloroplast DNA (cpDNA) intron-spacer region *trn*L-*trn*F (Taberlet *et al*. 1991) and one nuclear ribosomal DNA (nrDNA) sequence, the ITS region comprising spacer 1, the 5.8S gene and spacer 2 (White *et al*. 1990) were used in this study. PCR and sequencing procedures followed Ling *et al*. (2020a). Vouchers and sequences information are listed in Table 2.

Sequence alignment and phylogenetic analyses

To identify the systematic position of the putative new species, we followed Möller *et al*. (2011) and Ling *et al*. (2020a) and used 57 other *Oreocharis* species with available DNA sequences in the study. Finally, a total of 62 species were included in the phylogenetic analysis (Table 2). For base confirmation and contiguous sequences editing, the acquired original chromatograms from both directions of the *trn*L-F and ITS1/2 sequences were checked and evaluated using Bioedit (Hall 1999). We manually adjusted and aligned sequences, excluded ambiguous positions from alignments, where necessary, using MEGA v.6.5 (Kumar *et al*. 2008). After a congruency test, the *trn*L-*trn*F and ITS1/2 sequences were concatenated to a single matrix by PAUP* 4.0a164 (Swofford 2003). The optimal model of nucleotide substitution was inferred for two gene using MRMODELTEST v. 2.3 (Nylander 2004), based on Akaike Information Criteria (AIC, Akaike 1981). The most suitable model GTR + I + G was used in BI and ML analyses. Bayesian Inference (BI) analysis was conducted using MrBayes version 3.1.2 (Huelsenbeck & Ronquist 2001), with two independent Markov Chain Mounte Carlo (MCMC) analyses were run for 10 million generations, and sampled every 10,000 generations. The first 25% trees were discarded as burn-in, the remaining trees were summarized in a 50% majority-rule consensus tree with the posterior probabilities (PP). The maximum clade credibility tree was then visualized in FigTree v. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). Maximum Likelihood (ML) analyses were employed to reconstruct the phylogeny in MEGA v.6.5 (Kumar *et al*. 2008), with the optimal substitution models to carry out 1000 bootstrap (BS) replicates.

TABLE 2. List of Hainan *Oreocharis* taxa and 57 *Oreocharis* species used in the phylogenetic analysis, including respective Genbank accession and voucher numbers.

Species	*trn*L-*trn*F	ITS1/2	Voucher Number
Oreocharis acaulis	HQ633012	HQ632916	*M.Möller MMO 09-1605*
Oreocharis amabilis	KM232654	KJ475433	*Carles 587*
Oreocharis argyreia	HQ632919	HQ633015	*M.Möller MMO 07-1131*
Oreocharis aurea	KM062914	KM063154	*M.Möller MMO 06-980*
Oreocharis auricula	FJ501482	DQ912664	*M.Möller MMO 03-304*
Oreocharis begoniifolia	KM062926	KM063166	*M.Möller MMO 08-1221*
Oreocharis benthamii	JF697584	JF697572	*M.Möller MMO 08-1317*
Oreocharis brachypodus	KR476564	KR337019	*Jia-Mei Li 2304*
Oreocharis burttii	JF697582	JF697570	*F.Wen 2010-05*
Oreocharis chienii	KM062908	KM063148	*JXU0008123*
Oreocharis cinnamomea	KM062921	KM063161	*PE-02053073*
Oreocharis concava	KM062930	KM063170	*PE-02053062*
Oreocharis convexa	FJ501337	FJ501506	*M.Möller MMO 01-176*
Oreocharis cordatula	KM062922	KM063162	*PE-02053432*
Oreocharis cotinifolia	HQ632914	HQ633010	*Q.M.Chuan 01*
Oreocharis craibii	HQ632921	HQ633017	*M.Möller MMO 07-1072*
Oreocharis dalzielii	JF697583	JF697571	*F.Wen 2010-06*
Oreocharis dasyantha	MK587993	MK587954	*S.Jun Ling & M.X. Ren 2015011803*
Oreocharis dasyantha var. *ferruginosa*	MK587992	MK587956	S.Jun Ling 2015102203
Oreocharis dentata	KM062916	KM063156	*GH00353683*
Oreocharis dimorphosepala	KM062925	KM063165	*Y. M.Shui & al. 85333*
Oreocharis dinghushanensis	GU350643	GU350675	*Lin Q.B. LQB06-01*

.....continued on the next page

TABLE 2. (Continued)

Species	*trn*L-*trn*F	ITS1/2	Voucher Number
Oreocharis duyunensis	MG722858	MG722856	*PE-02114626*
Oreocharis elliptica	KM063155	KM062915	*CDBI0130369*
Oreocharis esquirolii	HQ633011	HQ632915	*D.W.Zhang 723*
Oreocharis eximia	KM062919	KM063159	*PE-02052811*
Oreocharis farreri	JF697585	JF697573	*Zhou Ping ZP 2010-020*
Oreocharis flavida	MK587990	MK587947	*S.Jun Ling 2018112901*
Oreocharis georgei	KM062917	KM063157	*PE-02053075*
Oreocharis hainanensis	OK040231	OK040233	*S.Jun Ling 2020091701*
Oreocharis hekouensis	KM062934	KM063174	*KUN-1219106*
Oreocharis henryana	JF697586	JF697574	*CSH0017984*
Oreocharis heterandra	KM232655	KJ475432	*PE-02052999*
Oreocharis hirsuta	KM062913	KM063153	*Put 3428*
Oreocharis humilis	GU350633	GU350665	*Liang R.H.SC-YB*
Oreocharis jasminina	MK587987	MK587948	*S.Jun Ling 2018112601*
Oreocharis jiangxiensis	HQ633029	HQ632933	*M.Möller MMO 09-1451*
Oreocharis jinpingensis	KM062923	KM063163	*Y.M. Shui et al. 91309*
Oreocharis lancifolia	HQ632924	HQ633020	M.Möller and P.Zhou MMO 09-1624
Oreocharis leiophylla	GU350676	GU350644	*Zhou X.R. ZXR-05-01*
Oreocharis longifolia	HQ632934	HQ633030	*M.Möller MMO 08-1239*
Oreocharis lungshengensis	HQ632917	HQ633013	*M.Möller MMO 06-916*
Oreocharis magnidens	HQ632930	HQ633026	*PE-02052989*
Oreocharis mileensis	KM063145	KM063182	*KUN-1385472*
Oreocharis muscicola	DQ912665	FJ501548	*Kew (1995-2229)*
Oreocharis nanchuanica	KM062924	KM063164	*KUN-1385365*
Oreocharis pankaiyuae	HQ632925	HQ633021	*PE-02053064*
Oreocharis primuliflora	HQ633019	HQ932923	*PE-02053071*
Oreocharis primuloides	FJ501546	FJ501364	*PE-01270488*
Oreocharis rhombifolia	GU350632	GU350664	*PE-02053532*
Oreocharis ronganensis	HQ633023	HQ632927	*PE-00030693*
Oreocharis rosthornii	KM062928	KM063168	*ZY0001346*
Oreocharis rotundifolia	KM062911	KM063151	*PE-00030861*
Oreocharis saxatilis	KM062932	KM063172	*JIU05295*
Oreocharis sericea	KM232656	KJ475407	*CSFI059560*
Oreocharis sinensis	HQ632912	HQ633008	*IBSC-0548658*
Oreocharis sinohenryi	HQ632913	HQ633009	*M.Möller MMO 07-1150*
Oreocharis speciosa	KM062909	KM063149	*K000858093*
Oreocharis stewardii	HQ632926	HQ633022	*M.Möller MMO 06-917*
Oreocharis urceolata	KM062920	KM063160	*M.Möller MMO 09-1633*
Oreocharis wangwentsaii	GU350658	GU350689	*Liang R.H.YN-Qj*
Oreocharis xiangguiensis	HQ632932	HQ633028	*JIU04686*

Results

The systematic position of the putative new species

Based on the incongruence length difference (ILD) test, there was no significant incongruence between the *trn*L-*trn*F and ITS1/2 ($p > 0.05$). So the *trn*L-*trn*F and ITS1/2 sequences were concatenated. The combined *trn*L-*trn*F and

ITS1/2 datasets were 886 and 561 bp long, of which 96 bp and 236 bp were variable, and 35 bp and 162 bp were parsimony informative, respectively. The aligned dataset was 1447 bp long, a total number of 332 polymorphic sites were measured, of which 197 bp were parsimony informative.

Molecular phylogeny indicated the 62 *Oreocharis* species can be divided into six clades, including two major and four minor clades (Fig. 3). The proposed new species is the sister to the Hainan-endemic *O. jasminina* and forms a monophyletic clade with all the other *Oreocharis* taxa from Hainan Island, with PP (posterior probability) = 1 and BS (bootstrap value) = 97% (Fig. 3).

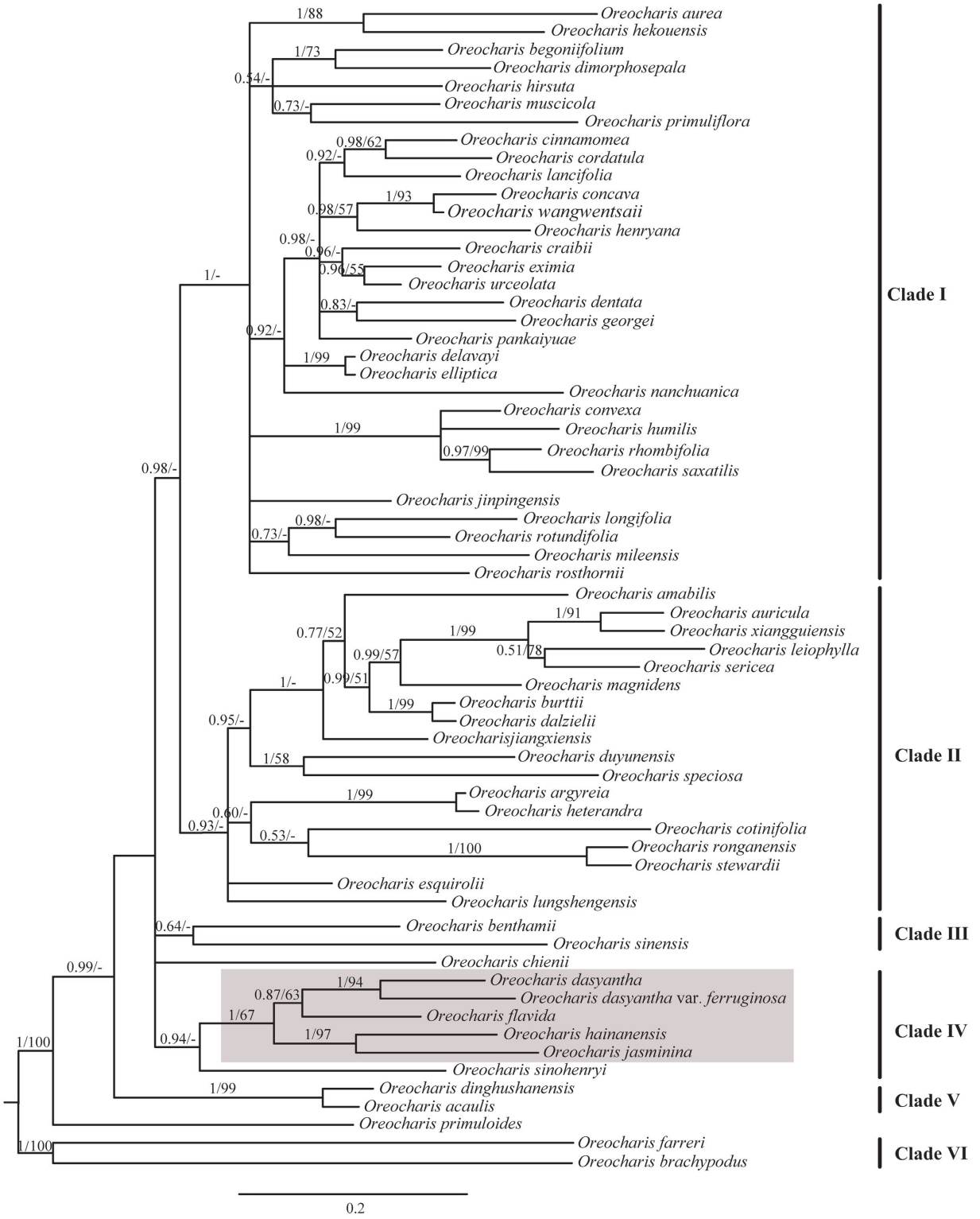

FIGURE 3. Molecular phylogeny of Hainan *Oreocharis* taxa and 57 *Oreocharis* species, based on the combined chloroplast gene *trn*L-*trn*F and nuclear ribosomal DNA (nrDNA) sequence ITS1/2 data matrices. Posterior probability (PP) and Bootstrap value (BS) are showed above branches (only shown if BS > 50%). Hainan *Oreocharis* taxa were showed in grey.

Taxonomic treatment

Oreocharis hainanensis S.J.Ling & M.X.Ren, *sp. nov.* (Figs 1, 2)

Diagnosis:—The new species is easily distinguished from *O. jasminina* by campanulate (not narrowly tubular) floral tube, zygomorphic (not actinomorphic) corolla, exserted (not included) stamens (Fig. 1, 2, Table 1).

Type:—CHINA. Hainan Island: Dongfang City, Baobaimiao Village, north to the Changhua River, 18°55'N, 109°02'E, elev. ca. 280 m a.s.l., on karst rocks, 17 September 2020, *S.J.Ling 2020091701* (holotype HUTB!; isotypes HUTB!, KUN!, IBK!).

Perennial herb, rhizomatous, acaulescent. Leaves basal, rosette; petiole 1.5–5 cm long, 2–3 mm in diameter, brown woolly; blade obovate, ovate to broadly ovate, rarely broadly ovate, 2.5–9 × 2–7 cm, adaxially gray pubescent, abaxially brown pubescent, densely brown villous along veins, apex subrounded, rarely rounded, margin shallowly crenate-serrate, base often cuneate, rarely subrounded, lateral veins 5–7 pairs, distinct, conspicuously prominent adaxially and conspicuously elevated abaxially. Inflorescence cymose, axillary, cymes 2–7, 3–8-flowered; peduncle 5–17 cm long, brown, sparsely pale gray villous. Bracts 2, linear to narrowly triangular, 2–3 × ca. 1 cm, abaxially pale gray villous, apex acuminate, margin entire; pedicel to 4 cm long, sparsely pale gray villous. Calyx green, 3–4 mm long, 5-parted near to base, lobes narrowly lanceolate, 2.5–4 × ca. 1 mm, apex acuminate, margin entire, adaxially pale gray pubescent, abaxially glabrous. Corolla yellow, 1–1.5 cm long, adaxially pubescent; tube campanulate, 1–1.7 cm × 3–4.5 mm; limb 2-lipped, adaxial lip shallowly 2-lobed, lobes ca. 3.5 × 5 mm, abaxial lip 3-lobed almost to base, lobes equal, subsemiorbicular, 5.5–8 × 5–7 mm, apex acuminate. Stamens 4, didynamous, adnate to corolla 3–4 mm from base, exserted; filaments of adaxial pairs ca. 0.8 cm long, abaxial pairs ca. 1.4 cm long; anthers ovate, bithecous, dehiscing longitudinally, adaxial thecae 1–1.5 × 0.7–1.1 mm, downward, abaxial thecae 1.8–2.8 × 1–1.5 mm, upward; staminode 1, adnate to corolla 2–3 mm above base, ca. 2 mm long. Disc ca. 1 mm high, entire. Pistil ca. 1.6 cm long, glabrous; ovary narrowly oblong, ca. 8 mm long, glandular-puberulent; style cylindrical and glandular-puberulent, ca. 0.5 cm long; stigma 2, equal, suborbicular. Capsule linear, 2.5–4 cm long, glabrous to sparsely puberulent.

Distribution and habitat:—*Oreocharis hainanensis* is currently only known on low-altitudual karst cliffs and rocks at the edge of evergreen broad-leaved forests near Changhua River, in the southwestern Hainan Island, South China.

Phenology:—Flowering from August to October and fruiting from September to November.

Etymology:—The specific epithet refers to the type location, Hainan Island, South China.

Vernacular name:—海南马铃苣苔 (Chinese, Hǎi Nán Mǎ Líng Jù Tái).

Conservation status:—So far, *Oreocharis hainanensis* is only known from two locations (less than 5 locations) with about 3,000–4,000 individuals. The populations are under threat due to the limited and fragmented karst habitats, with a very restricted area of occupancy (typically less than 20 km^2). Therefore, we propose that *O. hainanensis* should be considered as Vulnerable (VU C1 and D2) according to the IUCN Red List Categories and Criteria (IUCN 2019).

Key to the species of *Oreocharis* in Hainan Island

1. Anthers horseshoe-shaped, 1-loculed, dehiscing transversely ..... ***O. flavida***
- Anthers broadly oblong, 2-loculed, dehiscing longitudinally **2**
2. Stamens included, floral tube thin tubular ..... ***O. jasminina***
- Stamens exserted, floral tube conical or campanulate **3**
3. Corolla yellow ..... ***O. hainanensis***
- Corolla orange-red **4**
4. Leaf blade adaxially grey pubescent, base oblique, subrounded to cordate, margin serrulate; petiole to 14.5 cm, densely pale brown villous; cymes 1–3 (or 4)-flowered; corolla 1.7–2.4 cm, tube 1.1–2 cm ..... ***O. dasyantha***
- Leaf blade adaxially grey to brown pubescent and villous, base sometimes oblique, cuneate to subcordate, margin crenate-serrate; petiole to 6 cm, densely pale brown woolly; cymes 3–8-flowered; corolla ca. 1.6 cm, tube 9–11 mm ..... ***O. dasyantha* var. *ferruginosa***

Discussion

Our Molecular phylogeny showed that *Oreocharis* could be separated into six main clades, Clade I was mainly distributed in the mountains of Southwest China, Clades II-VI were mainly distributed in South to Southeast China (Fig. 3), which is roughly congruent with Ling *et al*. (2020a), Kong *et al*. (2021) and Lv *et al.* (in press). However,

some phylogenetic relationships in our result are different from previously works, which may cause by incomplete sampling of *Oreocharis* and the unequal aligned matrix information sites among studies. This phenomenon indicates that the *Oreocharis* underwent an early and rapid evolution radiation (Möller *et al*. 2011, Kong *et al*. 2021).

The new species *Oreocharis hainanensis* forms a monophyletic group with all the other *Oreocharis* taxa from Hainan Island with high support values (Fig. 3), indicating these species from Hainan (all are Hainan-endemic) had a common origin. All species from Hainan form the sister groups with *O. sinohenryi*, which is restricted to South China, suggesting a single dispersal of *Oreocharis* from mainland China to Hainan, and *Oreocharis* in Hainan Island probably have experienced evolutionary radiation.

The DNA evidence confirmed that Hainan *Oreocharis* taxa can be divided into two groups (Fig. 3). One group includes *O. dasyantha*, *O. dasyantha* var. *ferruginosa* and *O. flavida*, which dominated by orange corolla or corolla lobes. Another group includes *O. hainanensis* and *O. jasminina*, which dominated by yellow corolla. Our new species *O. hainanensis* is closely related to *O. jasminina* with high support (Fig. 3), however, *O. hainanensis* can be easily distinguished from *O. jasminina* by the characters of campanulate floral tube, zygomorphic corolla and exserted stamens (Table 1).

Generally, floral shape played a key role in generating floral isolation (Castellanos *et al*. 2004; Muchhala 2007), which has a considerable association with the expected pollinators in Gesneriaceae (Martén-Rodríguez *et al*. 2009). *Oreocharis hainanensis* has campanulate corolla, with various floral visitors including *Braunapis* sp., *Apis* sp. and *Amegilla* sp. (Fig. 1), differing from *Oreocharis jasminina* (thin-tubular corolla), *O. dasyantha* var. *dasyantha*, *O. dasyantha* var. *ferruginosa* (both are conical corolla) and *O. flavida* (campanulate-tubular corolla). These differences in floral syndromes indicated possible pollination isolations between the new species *O. hainanensis* and other *Oreocharis* taxa in Hainan Island.

Hainan Island has ca. 400 km^2 of karst topography, with an abundance of endemic plants, including two Gesneriaceae species endemic to Hainan, i.e., *Paraboea hainanensis* (Chun 1974: 3) Burtt (1984: 41) and *P. changjiangensis* Xing & Li (1993: 15). *Oreocharis hainanensis* is the unique species from karst regions in Hainan-endemic *Oreocharis* taxa, indicating that karst habitats offered excellent environments for the speciation and adaptation of *Oreocahris* in Hainan Island. Secondly, *O. hainanensis* grows at low-altitudinal areas of about 200 m, which has the closest phylogenetic relationship with *O. jasminina* with PP = 1 and BS = 97%, while its closest congener *O. jasminina* is only found on mountain tops, above 1,200 m in Mt. Yingge and Mt. Limu (Ling *et al*. 2020a, b). Therefore, the different elevation of mountains probably facilitated species differentiation of *Oreocharis* on the island, besides the habitat heterogeneity associated with karst landscapes.

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