Taxonomy of Gesneriaceae in China and Vietnam

Edited by Yu-Min Shui, Wen-Hong Chen, Ming-Xun Ren, Fang Wen, Xin Hong, Zhi-Jing Qiu, Yi-Gang Wei, Ming Kang



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EDITORIAL



Gesneriaceae in China and Vietnam: Perfection of taxonomy based on comprehensive morphological and molecular evidence

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Morphology is fundamental to taxonomy. Specimens in herbaria can provide unique supporting bases for scientific nomenclature. However, they usually reveal some limited variation of the taxa in nature and need to be revised gradually in future taxonomic studies. Because botanists make taxonomic treatments in herbaria without the benefit of molecular verification, many synonyms can occur. Traditionally, morphological treatment needs a combination of detailed herbaria work and extensive fieldwork. In general, the former work is usually dull, requires considerable patience, and tends to be neglected; this leads to unsubstantiated new synonyms. On the converse, field observa-

tions benefit from high-tech tools and equipment, which can reveal more delicate and detailed content in the field and the laboratory. These include detailed images directly from field observation by digital cameras, micro-morphology from SEM, and Vertical microscope work. In a word, the absence of detailed morphology from herbaria and the field cannot support good taxonomic work.

Diligent molecular work can support taxonomic revision. At the species level, molecular phylogeny seldom provides direct evidence to confirm a new species, but only tells us its affinities logically (Chen et al. 2014). Molecular evidence is not usually considered when new species are described. Although morphology seems to work in Gesneriaceae at the genus level, exceptions in morphology often happen, particularly with some of the expanded genera in Asia (*Oreocharis, Petrocodon*, and *Primulina*) (Figure 1; Möller et al. 2011; Wang et al. 2011; Weber et al. 2011a, b). In such cases, molecular evidence is helpful for accurate taxonomic treatment. However, the next crucial question will be how many DNA sequences will support the well-resolved relationships of the taxa above the species level. Based on the recent study, it seems to be that the combination of ITS and *trn*L-F is not enough to resolve the relationship within the above expanded genera. In special cases, we strongly suggest adopting more sequences to issue the taxonomic revision in the future study of Gesneriaceae (Chen et al. 2020), such as *atp*B-*rbc*L, *ndh*H-*rps*15-*ycf*1, *rpl*132, *trn*C-*trn*D, *trn*L-F, *trn*T-trnL of chloroplast DNA.

Some detailed rules are suggested during taxonomic revision in Gesneriaceae. First, the new species' establishment is usually based on morphological differences, with at least two or more different characteristics in diagnosis. It would be better to provide the key to the new species suggested and their related groups and species. Second, the comprehensive observation of morphology is necessary to support the new species, such as staminodes, discs of flowers, and the abaxial surface of leaves. Third, statistical analysis of morphological characters using sufficient samples from multiple populations can provide unbiased evidences for the taxonomic treatment of some species with subtle morphological differences (e.g. Yang et al. 2019). Fourth, chromosomes and pollen grains are important to the taxonomic revision and are strongly encouraged (Pan 1987; Yang et al. 2020). Lastly, more DNA sequences such as *atpB-rbcL*, *ndhH-rps15-ycf1*, *rpl132*, *trnC-trnD*, *trnL-F*, *trnT*-trnL, *psbA-trnH* than should be considered during the taxonomic treatment together with ITS (Qiu et al. 2015; Roalson and Roberts 2016; Chen et al. 2020).

This special issue focuses on China and Vietnam: an essential center of biodiversity worldwide (Myers et al. 2000). Gesneriaceae includes more than 700 accepted species in the area, and thus provides a suitable example for answering the above taxonomic questions (Ho 2000; Myers et al. 2000; Wen et al. 2019). Tan et al. (2020) offer an in-depth look at the updated taxonomy and biogeographical patterns of Asian Gesneriaceae. Hainan Island, one of the biggest islands in China and Vietnam, harbours an extremely high endemism ratio of Gesneriaceae and all *Oreocharis* species on this island are endemic (Ling et al. 2017). With an extensive examination combining both morphological and molecular evidences, Ling et al. (2020a, b) explored the taxonomical treatment of Hainan *Oreocharis* and found a possible new species. In addition to the numerous new species' taxonomic treatments referred to, several studies in this special issue emphasize the use of comprehensive morphological observation and more mo-



Figure 1. Flowers of some species of Gesneriaceae in China and Vietnam A Bournea sinensis Oliv. (photographed by Yu-Min Shui) B Oreocharis guileana (B.L. Burtt) Li H. Yang & F. Wen, comb. nov. (by Li-Hua Yang) C Oreocharis baolianis (Q.W. Lin) Li H. Yang & M. Kang, comb. nov. (by Li-Hua Yang) D Oreocharis jasminina S.J.Ling, F.Wen & M.X. Ren, sp. nov. (by Shao-Jun Ling) E Oreocharis flavovirens Xin Hong (by Xin Hong) F Oreocharis wumengensis Lei Cai & Z.L.Dao, sp. nov. (by Lei Cai) G Oreocharis fulva W.H.Chen & Y.M.Shui, sp. nov. (by Yu-Min Shui) H Allocheilos rubroglandulosus W.H. Chen & Y.M. Shui, sp. nov. (by Yu-Min Shui) I Petrocodon rubiginosus Y.G.Wei & R.L.Zhang, sp. nov. (by Fang Wen) J Petrocodon luteoflorus Lei Cai & F. Wen, sp. nov. (by Fang Wen) K Deinostigma fasciculatum W.H.Chen & Y.M.Shui, sp. nov. (by Yu-Min Shui) L Primulina xuansonensis W.H.Chen & Y.M.Shui, sp. nov. (by Yu-Min Shui) L Oreocharis F.Wen, Xin Hong & W.Y. Xie, sp. nov. (by Jia-Jun Zhou) N Paraboea myriantha Y.M. Shui & W.H. Chen, sp. nov. (by Yu-Min Shui) O Paraboea sinensis var. glabrissima W.H.Chen & Y.M.Shui, var. nov. (by Yu-Min Shui) P Petrocosmea nanchuanensis Z.Y. Liu, Z.Y. Li & Z.J. Qiu, sp. nov. (by Zhi-Jing Qiu).

lecular data to provide convincing conclusions. It would be desirable that all discoveries and taxonomic revisions will be conducted under these strict criteria suggested here in the future.

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RESEARCH ARTICLE



Biogeography and evolution of Asian Gesneriaceae based on updated taxonomy

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Abstract

Based on an updated taxonomy of Gesneriaceae, the biogeography and evolution of the Asian Gesneriaceae are outlined and discussed. Most of the Asian Gesneriaceae belongs to Didymocarpoideae, except *Titanotrichum* was recently moved into Gesnerioideae. Most basal taxa of the Asian Gesneriaceae are found in the Indian subcontinent and Indo-China Peninsula, suggesting Didymocarpoideae might originate in these regions. Four species diversification centers were recognized, i.e. Sino-Vietnam regions, Malay Peninsula, North Borneo and Northwest Yunnan (Hengduan Mountains). The first three regions are dominated by limestone landscapes, while the Northwest Yunnan is well-known for its numerous deep gorges and high mountains. The places with at least 25% species are neoendemics (newly evolved and narrowly endemic) which were determined as evolutionary hotspots, including Hengduan Mountains, boundary areas of Yunnan-Guizhou-Guangxi in Southwest China, North Borneo, Pahang and Terengganu in Malay Peninsula, and mountainous areas in North Thailand, North Sulawesi Island. Finally, the underlying mechanisms for biogeographical patterns and species diversification of the Asian Gesneriaceae are discussed.

Keywords

Didymocarpoideae, endemic, species diversification, limestone landscape, monsoon, long-distance dispersal

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Introduction

Gesneriaceae Rich. & Juss. ex. DC. is a middle-sized family, including about 150 genera and over 3400 species (Weber et al. 2013). Traditionally, the family was divided into two subfamilies, subfamily *Cyrtandroideae* Burnett (palaeotropical group, with superior ovary and two unequal cotyledons) and subfamily *Gesnerioideae* Burnett (neotropical group, with inferior ovary and two equal cotyledons) (Burtt 1998).

Based on recent molecular and morphological data, Weber et al. (2013) put forward a new classification system, consisting of three subfamilies: *Sanangoideae* Weber (monotypic genus, endemic to South America), *Didymocarpoideae* Arn. and *Gesnerioideae* Burnett. In this newest classification system, the monotype genus *Titanotrichum* Solereder in Asia (China and Japan) has been transferred to the subfamily Gesnerioideae, which was formerly treated as "New World Gesneriaceae" (Burtt 1998; Weber et al. 2013). However, Didymocarpoideae is still "Old World Gesneriaceae", consisting of 67 genera and more than 2300 species (Möller et al. 2017, Xu et al. 2017).

Recently, many Asian Gesneriaceae taxa had experienced extensive revision for their systematic positions, such as *Boea* Commerson ex Lamarck (Puglisi and Middleton 2018), *Microchirita* (C.B.Clarke) Y.Z. Wang (Middleton 2018), *Henckelia* Spreng. (Middleton et al. 2013), *Paraboea* (C. B. Clarke) Ridley (Puglisi et al. 2016) and the enlarged genus *Oreocharis* Bentham (Möller et al. 2011, 2014; Chen et al. 2014). Furthermore, several genera were newly established, i.e. *Billolivia* D.J. Middleton (Middleton et al. 2014), *Chayamaritia* D.J. Middleton (Middleton et al. 2014), *Chayamaritia* D.J. Middleton (Middleton 2017), *Rachunia* D.J. Middleton (Middleton (Middleton et al. 2018). Such significant revisions of so many genera call for an updated study about biogeography and evolution of the Asian Gesneriaceae.

In this paper, we collected the species locality data (coordinates) from GBIF (Global Biodiversity Information Facility, https://www.gbif.org/) for all the species of the Asian Gesneraiceae. The species diversity and systematic positions of all genera were determined according to the newest literatures (e.g. Möller et al. 2016a, b, 2017; Roalson and Roberts 2016; Puglisi and Middleton 2018). We used the software DIVA-GIS 7.5 to create a distribution map at $1^{\circ} \times 1^{\circ}$ latitude/longitude grid resolution to reveal distribution patterns of species diversity and endemism. We also analyzed the distribution type of all the genera and identified evolutionary hotspots, i.e. the center of neoendemic species, which is determined when at least 25% of total species are locally endemic. Finally, we discussed the possible mechanisms, including both intrinsic and extrinsic factors, to explain the formation and maintenance of diversification and endemic centers of the Asian Gesneriaceae.

I Distribution type

Based on Wu's (1979, 1991) criterions and Li's (1996) pioneer study on the geographical areal-types of the Asian Gesneriaceae, we identified the Asian Gesneriaceae as belonging to three areal types and 20 subtypes as below.

- I. Pantropics
- I1. Tropic Asia and tropic America disjuncted: Rhynchoglossum Blume.
- Tropic Asia to tropic Australia: *Boea* Comm. ex Lam., *Cyrtandra* J. R. Forster & G. Forster, *Stauranthera* Bentham, *Rhynchotechum* Blume.
- 13. Tropic Asia to tropic Africa: *Epithema* Blume.
- II. Tropical and subtropical Asia
- II1. Widespread in tropical and subtropical Asia: Aeschynanthus Jack, Paraboea (C. B. Clarke) Ridley, Dorcoceras Bunge, Codonoboea Ridl., Didymostigma W. T. Wang, Henckelia Spreng., Didymocarpus Wallich.
- II2. East India to Java: Boeica C. B. Clarke, Leptoboea Benth., Beccarinda Bentham, Microchirita, Middletonia D.J. Middleton.
- II3. Indo-China Peninsula: Tetraphyllum C.B.Clarke, Deinostigma W.T.Wang & Z.Y.Li, Damrongia Kerr ex Craib, Kaisupeea B.L. Burtt, Tribounia D.J. Middleton ex M. Möller, Billolivia, Chayamaritia, Rachunia D.J. Middleton.
- II4. North of Indo-China Peninsula: Pseudochirita W. T. Wang, Anna Pellegrin.
- II5. Subtropic Asia (Southwest and South China): Primulina Hance, Whytockia W. W. Smith, Hemiboea C. B. Clarke, Glabrella M. Möller & W. H. Chen, Gyrocheilos W. T. Wang, Raphiocarpus Chun, Petrocodon Hance, Allostigma W. T. Wang, Allocheilos W. T. Wang, Gyrogyne W. T. Wang, Litostigma Y.G. Wei, Fang Wen & M. Möller.
- II6. Malay Peninsula to Southwest China: Ornithoboea Parish ex C. B. Clarke.
- II7. Hainan Island: Cathayanthe W. Y. Chun, Metapetrocosmea W. T. Wang.
- II8. Sri Lanka and India: Championia Gardn., Jerdonia Wight.
- II9. Malay Peninsula: Orchadocarpa Ridl., Emarhendia R. Kiew, A. Weber & B.L. Burtt, Senyumia R. Kiew, A. Weber & B.L. Burtt, Somrania D.J. Middleton, Spelaeanthus R. Kiew, A. Weber & B.L. Burtt.
- II10. Malay Archipelago: Agalmyla Blume, Monophyllaea R. Br., Loxocarpus R. Br., Loxonia Jack, Didissandra C.B. Clarke, Liebigia, Ridleyandra A. Weber & B.L. Burtt.
- II11. Borneo Island: Hexatheca C.B. Clarke.
- III. North Temperate
- III1. Widespread in East Asia: Oreocharis Bentham.
- III2. Sino-Himalaya: Corallodiscus Batalin, Loxostigma C.B. Clarke.
- III3. Sino-Japan: Conandron Sieb. & Zucc., Titanotrichum Solereder.
- III4. Hengduan Mountains to Yunnan Plateau: Rhabdothamnopsis Hemsl.
- III5. Hengduan Mountains to Central China: Briggsiopsis K. Y. Pan, Petrocosmea Oliver.
- III6. Himalaya: Platystemma Wallich.

2 Geographical distribution patterns

Tropical and subtropical Asia are the distribution centers of the subfamily Didymocarpoideae, harbouring 85% genus and more than 90% species of Didymocarpoideae.



Figure 1. Geographical distribution patterns of the 10 genera of the Asian Gesneriaceae that experienced extensive changes in species compositions.



Figure 2. Distribution localities of 15 genera of the Asian Gesneriaceae that experienced extensive changes in species compositions.

Indo-China Peninsula and Southwest China (Figs 1, 2), which are dominated by limestone landscapes (Clements et al. 2006), are places notable for recording the highest species density (Fig. 1 and Fig. 3). According to the updated phylogeny of the Asian Gesneriaceae (Möller et al. 2016b, 2017; Xu et al. 2017), most basal taxa of the Didymocarpoideae, like *Rhynchotechum* and *Corallodiscus* (Fig. 3), occur at India and Indo-China Peninsula and the nearby regions such as Sino-Vietnam region and Southwest Yunnan. This suggests that modern Didymocarpoideae probably originated in these regions.

2.1 Diversification and endemic centers

Our data recognized four species diversification centers (places with highest values of species density), i.e. Sino-Vietnam Region including boundary areas of Guizhou-Yunnan-Guangxi in Southwest China, Northwest Yunnan (Hengduan Mountains), Malay Peninsula and North Borneo (Fig. 3). In a study focusing on China's Gesneriaceae, Liu et al. (2017) found that richness of Gesneriaceae peaked in Southwest China, and Hengduan Mountains and boundary areas of Guizhou-Yunnan-Guangxi are the most significant hotspots of species diversity and endemism. Our results closely coincided with their findings.

Indo-China Peninsula turned out to be an extraordinary diversification center, harbouring several endemic genera *Tribounia*, *Billolivia*, *Chayamaritia* (Fig. 2). These genera are newly evolved and contain very few species (Fig. 4). The Indian subcontinent has the lowest value of species density, with only two endemic genera, i.e. *Jerdonia* and *Championia* (Sri Lanka). *Boea* was no longer widespread in tropic Asia and its endemic center appeared in Papua New Guinea and the Solomon Islands (Puglisi et al. 2016; Puglisi and Middleton 2018) (Fig. 2). Based on molecular data from Möller and Clark (2013) and Roalson and Roberts (2016), most locally endemic species such as in *Aeschynanthus* and *Cyrtandra* were newly evolved, i.e. neoendemics.



Figure 3. Species distributions pattern of the Asian Gesneriaceae. Black circles indicate diversification centers with highest species richness and the red grids are the evolutionary hotspots (at least 25% species are neoendemics). The species distribution information is obtained from http://www.gbif.org. The map was drawn using DIVA-GIS7.5.

2.2 Evolutionary hotspots

We determined places where at least 25% species are local endemics as 'evolutionary hotspots'. Six evolutionary hotspots were identified, i.e. Northwest Yunnan (Hengduan Mountains), boundary areas of Yunnan-Guizhou-Guangxi in Southwest China, mountains in North Thailand, Malay Peninsula, North Borneo, and North Sulawesi (Fig. 3). These evolutionary hotspots were distributed largely in the diversification centers (Fig. 3), similar to findings of Liu et al. (2017) in which Hengduan Mountains and boundary areas of Yunnan-Guizhou-Guangxi were recognized as two hotspots of species richness of Chinese Gesneriaceae.

The geography of these six evolutionary hotspots was the most complex area in tectonic history, formed by the interaction of Indian, Eurasian, Australian and Pacific Plates (Hall and Spakman 2015). Therefore, the highly fragmented islands and limestone landscapes in Southeast Asia probably facilitated speciation of the Asian Gesneriaceae, similar pattern found in *Begonia* (Chung et al. 2014) and *Alocasia* (Nauheimer et al. 2012).

3 Origin and Evolution of the Asian Gesneriaceae

Burtt (1998) proposed that Gesneriaceae is of southern hemisphere (Gondwana) origin, with the Gondwana broken down and dispersed all over the world. This hypothesis is based on Gesnerioideae spreading to South America via the Antarctic and Didymocarpoideae by migrating northwards, 'dropping' representatives in Africa and



Figure 4. Genera phylogeny with geographical distribution pattern of the Asian Gesneriaceae. The number in the brackets is the species diversity of the genus. Phylogeny tree was redrawn based on Möller and Clark (2013), Middleton et al. (2015), Puglisi et al. (2016), Möller et al. (2016a), Middleton et al. (2018).

Madagascar and finally reaching the Eurasiatic continent and spreading from there to the Malay Archipelago and the Pacific. This hypothesis, however, faces difficulties both from the geological time scale and the molecular data. Perret et al. (2013) reconstructed the biogeography history and suggested an origin of this family in southern America during the late Cretaceous period. The Gondwana break-up, however, began at about 150 Ma (Hall and Spakman 2015). Woo et al. (2011) indicated that there were two independent long-distance dispersals or overland migrations from South America to Australasia via Antarctica, but how they entered Asia or Africa is still unclear. The molecular data from Möller et al. (2009) and Roalson and Roberts (2016) show that the most basal species were found on the Indian subcontinent, such as *Jerdonia* (mountains of SW India), *Corallodiscus* (Himalayas and China), *Tetraphyllum, Leptoboea* and *Boeica* (Himalayas and adjacent areas). Only *Rhynchotechum*, about 18 species, which is widespread from Himalayas to Malay Archipelago, and one other species, even reaches New Guinea. Furthermore, *Boea* was the only endemic genus at the east side of the Huxley's Line. Therefore, the Asian Gesneriaceae may have originated from the Indian subcontinent and/or Indo-China Peninsula, then dispersed to the east and the north and finally reached Southeast Asia and East Asia.

An up-to-date phylogeny indicated that Didymocarpoideae and Gesnerioideae probably separated at about 74 Ma (Roalson and Roberts 2016), when the Indian Plate had been separated from Gondwana (Hall and Spakman 2015). We propose two hypotheses for the origin of Didymocarpoideae. One is in India, whereby the ancestor of Didymocarpoideae dispersed via the south of South America and Antarctica to India, until about 45 Ma when the Indian Plate collided with the Eurasia Plate and rapid speciation occurred. This is in accordance with Burtt's (1998) "Out of India" hypothesis.

The new position of *Titanotrichum* in the Gesnerioideae (Wang et al. 2004) suggests a possible dispersal event from the New World to Asia. Perret et al. (2013) proposed that the ancestor of *Titanotrichum* might either disperse across Beringia from North America to East Asia or originate at East Asia. This genus is distinctive for small and numerous bulbils in the inflorescence, which evolved at about Miocene (Wang and Cronk 2003) and probably facilitated its long-distance dispersal.

The alternative hypothesis is that the Asian Gesneriaceae might follow the 'Malpighiaceae Route' via the 'North Atlantic land bridge' to Eurasia (Davis et al. 2002). However, currently very limited experimental studies focusing on this scenario have been carried out and we cannot speculate on details about this hypothesis. To test such a hypothesis, molecular biogeographic studies on a pantropical genus such as *Epithema and Rhynchoglossum* are needed to figure out their historical dispersal route(s) between tropical America, Africa and Asia.

4 Mechanisms for species diversification

4.1 Growth forms

According to Roalson and Roberts (2016), epiphytism and unifoliate growth are two important growth forms impacting on the diversification rate in Gesneriaceae, like *Monophyllaea* and a section of *Streptocarpus* Lindl. in the Old World. Epiphytes and non-epiphytes have the same speciation rate, but epiphytism has a much lower extinction rate (Roalson and Roberts 2016). This means that epiphytism *per se* is not the driving factor for speciation, it probably can lower the extinction rate or is associated with other traits that promoting speciation. For example, there is a strong correlation between the epiphytic habit and ornithocory (dispersal of seeds by birds) (Weber 2004). In orchids, epiphytism is also often correlated with CAM photosynthesis (Givnish et al. 2015), but in Gesneriaceae only the New World *Codonanthe crassifolia* was confirmed to have some CAM-like characteristics (Guralnick et al. 1986).

Unifoliate growth form strongly suggests that growth form positively influences speciation rate (Roalson and Roberts 2016). Previous work on *Streptocarpus* shows the evolution of growth forms, especially rosulate and unifoliate growth and they believed this was an adaptation for deep shade and unifoliate growth increasing diversification rate (Möller and Cronk 2001). *Cyrtandra* has, however, also undergone a significant increase in speciation rate, probably associated with other characteristics such as seeds' dispersal mode.

4.2 Specialized pollination adaptations

Normally, most Gesneriaceae species exhibiting zygomorphic flowers are thought to be more specialised in pollination adaptation, since it restricts pollinator behaviour and can therefore increase pollination efficiency (Gong and Huang 2009; Ling et al. 2017). Wang et al. (2010) and Ling et al. (2017) proposed that actinomorphy probably is a derived trait in the Asian Gesneriaceae, which is associated with shifts in pollination strategies, such as nectar- to pollen-rewards and/or from specialist to generalist pollinators.

Most Asian Gesneriaceae has the fused anthers, normally anthers are united by pairs, such as *Loxostigma* and *Anna* (Weber 2004). In some species, all the four anthers are fused or adnated, such as *Beccarinda* and *Stauranthera* (Weber 2004). Anther fusion can assemble the anthers to the same position and facilitate all the anthers touching the same locality of the pollinator's body, which can greatly enhance the precision of cross-pollination (Ren 2008; Ren and Tang 2010). Wang (1990) indicated that anthers fusion with all stamens in the flower is late evolved than anther fusion in pairs and show a relatively higher level of pollination efficiency and consequently probably facilitate species diversifications.

There is a highly specialized pollination mechanism in the Asian Gesneriaceae, i.e. mirror-image flowers (Gao et al. 2006; Lu et al. 2019). Mirror-image flowers can be found in five genera in the Asian Gesneriaceae, i.e. *Paraboea* (Gao et al. 2006), *Or-nithoboea, Didymocarpus, Rhabdothamnopsis*, and *Henckelia* (Lu et al. 2019). Mirror-image flowers are a sexual polymorphism in which the style deflects either to the left or the right side of the floral axis (Jesson and Barrett 2002; Gao et al. 2006). Jesson and Barrett (2002, 2003) pointed out that mirror-image flowers can increase the precision of cross-pollen transfer and may play an important role in pollination isolation and speciation. Normally, mirror-image flowers have reciprocal deflecting stamen(s) to the other side as compared with the deflecting style (reciprocal mirror-image flowers), which greatly increases pollen transfer efficiency (Jesson and Barrett 2003; Ren et al.

2013), but most mirror-image flowers in the Asian Gesneriaceae are non-reciprocal, without a deflecting stamen (Lu et al. 2019). Such unusual floral syndromes indicate unusual pollination adaptations in these species, but are awaiting further study.

4.3 Fruit adaptations to long-distance dispersal

In angiosperms, fruits significantly increase adaptive ability to withstand harsh environments and facilitate seed dispersal (Weber 2004). Fruit trait probably is a key trait for the high speciation rate and widespread range of both *Aeschynanthus* and *Cyrtandra* (Roalson and Roberts 2016). The hair-like appendages of seed in *Aeschynanthus* provide a favourable surface area to mass ratio (Denduangboripant et al. 2001), adapting to wind dispersal.

In *Cyrtandra* and *Rhynchotechum*, soft-fleshy (a true berry) fruits facilitate their colonisation throughout the Southeast Asia islands and nearby Pacific islands via bird dispersals (Cronk et al. 2005; Liu et al. 2017), and numerous islands in this area then promoted the speciation of the genus.

4.4 Extrinsic (environmental) factors

Compared to other regions, Asia is mostly dominated by the monsoon climate. There are three main types of summer monsoons in Asia, i.e. East Asia Monsoon, South Asia Monsoon and North-west Pacific Ocean Monsoon (Jiang et al. 2017; Kong et al. 2017). These three monsoons interact at Southwest China and Indo-China Peninsula and bring a large quantity of warm and wet air, thus providing a precondition for tropical plants to exist and facilitate the long-distance dispersal of the propagules. Monsoons do not only facilitate the northwards spread of tropical plants, but also aggravate the isolation of local habitat via the alternate dry and rainy seasons (Jiang et al. 2017), which might be related with the formation of diversification centers and evolutionary hotspots in Southwest China and Sino-Vietnam regions (Wang et al. 2011). More specifically, temperature changes since the Last Glacial Maximum had stronger effects on richness of rare species (Kong et al. 2017; Liu et al. 2017) while richness of common species was determined largely by current temperature seasonality such as monsoon climate (Liu et al. 2017).

Many studies had pointed out that microhabitat isolation caused by various landscapes such as limestone is the main factor for speciation in the Asian Gesneriaceae (Wang 1990; Ren 2015; Liu et al. 2017; Shui and Chen 2017). Especially for the three diversification and endemic centers, i.e. Sino-Vietnam Region, Malay Peninsula, North Borneo, the local landscapes are characterised by the various types of limestone landscapes (Clements et al. 2006; de Bruyn et al. 2014). Northern Borneo and Malaysia Peninsular were separated by the South China Sea, but they were connected during the glacial period and acted as a "land bridge" for plant

Table 1. List of present genera of Asian Gesneriaceae.

Gernus	Distribution	Habitat	Species number	Taxonomic status	Reference	
Subfamily Gesnerioideae						
Tribe Titanotriche	ae					
Titanotrichum	E China (Fujian and Taiwan) and Japan	Shaded areas in valleys; altitude 100–1200 m.	1	Placed in subfam. Gesnerioideae	Perret et al. 2013; Weber et al. 2013	
Subfamily Didyme	ocarpoideae					
Tribe Epithematea	le					
Epithema	Central tropical Africa, India, Sri Lanka, Nepal, southern China and through Southeast Asia and Malesia to the Solomon Islands.	Shaded limestone rocks or caves in valleys.	20	No change at genus level	Bransgrove and Middleton 2015	
Gyrogyne	S China (Bose Xian, W Guangxi)	Shaded waysides in hilly regions at low elevations.	1	Position in Epithemateae- Loxoniinae uncertain		
Loxonia	Southeast Asia (Sumatra, Malay Peninsula, Borneo, Java)	Damp places and humid rocks in deep shade.	3	No change		
Monophyllaea	Throughout Sumatra to New Guinea and from S Thailand and Luzon to Java	Limestone rocks, in shady forests, at cave entrances and below rocks.	>40	No change	Möller et al. 2016b	
Rhynchoglossum	From India and S China to New Guinea, but one species distributed in Central America (Mexico, Colombia, Venezuela)	Wet and shady (preferably limestone) rocks, in forest or open, shady places; usually in the lowlands.	16	No change		
Stauranthera	from NE India and S China throughout Malaysia to New Guinea	Wet rocks and damp places in lowland rain forest.	7	No change		
Whytockia	S China (Sichuan, Guangxi, Hunan, Hubei, Guizhou, Yunnan and Taiwan)	Shaded and moist areas in valleys, shaded streamside rocks and stream banks, altitude 500–2200 m.	8	No change		
Tribe Trichosporea	ae	1				
Aeschynanthus	From S China, N & S India throughout Malesia to New Guinea and the Solomon Islands	Epiphytically on trees (rarely on rocks or bare soil), lowland or montane rain forest.	~185	Emended by inclusion of <i>Micraeschynanthus</i>		
Agalmyla	Malaysia (Sumatra, Malay Peninsula, Borneo, Java, Sulawesi, New Guinea)	Lowland and montane rainforest, mostly climbers.	96	No change		
Allocheilos	S China (Guizhou, E Yunnan)	Rocks in limestone hills, altitude ca. 1400 m.	2	No change		
Allostigma	S China	Limestone pavements; altitude ca. 200 m.	1	No change		
Anna	China, N Vietnam	Grassy slopes or forests, rock crevices in limestone hills.	4	No change		
Beccarinda	NE India, Burma, S China, Vietnam, Sumatra	Probably growing on humid rocks.	8	No change		
Billolivia	E Vietnam (Lam Dong)	Submontane tropical evergreen closed forest at 1550 m alt.	7	Re-established for five species of <i>Cyrtandra</i>	Middleton et al. 2014	
Boea	Eastern Indonesia, Papua New Guinea, the Solomon Islands and Queensland (Australia)	Limestone, moorstone and argillite montane cliffs or shady places under the forest, altitude 100–3300 m.	11	Redefined; Chinese spp. now in <i>Dorcoceras</i> and <i>Damrongia</i>	Puglisi et al. 2016; Puglisi and Middleton 2018	

Gernus	Distribution	Habitat	Species number	Taxonomic status	Reference
Boeica	Bhutan, S China, N & NE India, Myanmar, N Vietnam, NW Malaya	Shady and damp places and on humid rocks in forests, altitude 200–1400 m.	14	No change	Möller et al. 2017
Briggsiopsis	S China (C & S Sichuan, NE Yunnan, Guizhou)	Forests, at stream sides and on rocks in shady places, altitude 250–1500 m.	1	No change	
Cathayanthe	S China (Hainan)	Rocks, in wet valleys and ravines; altitude ca. 1800 m.	1	No change	
Championia	Sri Lanka	Undisturbed forest, in shady places and loose soil along stream beds.	1	No change	
Chayamaritia	Central and eastern Thailand, Laos.	Evergreen and submontane forest in deep shade at 150– 1200 m altitude.	2	Genus recently established	Middleton et al. 2015
Codonoboea	S Thailand and throughout Malesia, S Japan, E China and Taiwan	Primary forest granite, sandstone and quartz derived soils or rocks.	120	New combination for particular species of <i>Henckelia</i> and <i>Loxocarpus</i>	Kiew and Lim 2011; Weber et al. 2011a
Conandron	E China, Taiwan region of China, S Japan	Humid and wet rocks in forests, altitude 500–1300 m.	1	No change	
Corallodiscus	Bhutan, China, N & NE India, Nepal, Thailand	Rocks and rock crevices within forests or above the forest line, from 700 to nearly 5000 m.	5	No change	
Cyrtandra	Nicobar Islands and S Thailand through Malesia include Taiwan region of China and the S Pacific to the Hawaiian Islands	Lowland and montane rain forests.	>800	No change	
Damrongia	China to Sumatra	Limestone rocks, usually in shade.	11	Re-established for particular species of erstwhile <i>Chirita</i> ; inclusion of <i>Boea</i> <i>clarkeana</i> and the Asian species described in <i>Streptocarpus</i>	Weber et al. 2011a; Puglisi et al. 2016
Deinostigma	Southern China and Vietnam.	Forests rocks and along trails and roadsides in forested areas; altitude 650–1200 m.	7	Expanded to included several species previously ascribed to <i>Primulina</i>	Möller et al. 2016a
Didissandra	W Malesia (Sumatra, Malay Peninsula, Borneo, Java)	Lowland and montane rain forests.	8	No change	
Didymocarpus	From N and NE India, Nepal and S China southwards to the Malay Peninsula and N Sumatra	Damp (usually acid) rocks or earth banks, in forest or above the forest line, altitude (rarely) sea level to 3500 m.	98	Some spp. transferred to <i>Petrocodon</i>	Weber et al, 2011b
Didymostigma	SE China (Guangdong, Fujian, Guangxi) & Vietnam	Forests rocks and along trails and roadsides in forested areas; altitude 650–1200 m.	3	No change	
Dorcoceras	China, Thailand, Cambodia, Vietnam, Philippines and Indonesia	Shady and damp rocks along trails and roadsides in forests; 100–1500 m.	4	Re-established for particular (non- Australasian) species of <i>Boea</i>	Puglisi et al. 2016
Emarhendia	Malay Peninsula	Damp limestone rocks, especially at cave entrances.	1	No change	
Glabrella	S China, Taiwan region of China	Forests damp rocks and crevices of rocks; 600-1800 m.	3	New genus estblished for 3 spp. of <i>Briggsia</i> not to be included in <i>Oreocharis</i> or <i>Loxostigma</i>	Möller et al. 2014; Wen et al. 2015a,b

Gernus	Distribution	Habitat	Species number	Taxonomic status	Reference
Gyrocheilos	S China (Guangxi, Guangdong, SE Guizhou), Vietnam	Forests wet places, in valleys and on rocks beside streams; altitude 400–1600m.	5	No change	
Hemiboea	C & S China, Taiwan region of China, N Vietnam, S Japan, NE India	Forests and at forest margins rock crevices by streams and wet, shady places in karst regions; altitude 80–2500 m.	34	Inclusion of <i>Metabriggsia</i> (2 spp.)	Weber et al. 2011c
Henckelia	India, S China, Indo-China Peninsula, Malay Peninsula	Acidic soils and rocks but not on limestone.	~60	Redefined to include Chirita p. p. (excl. Microchirita and Primulina) and Hemiboepsis, and to exclude Codonoboea	Weber et al. 2011a; Middleton et al. 2013
Hexatheca	Borneo (W Kalimantan, Sarawak to Sabah)	Sandstone or limestone rocks.	4	No change	
Jerdonia	India (Nilghiri and Anamally Hills)	Rocks in mountains	1	No change	
Kaisupeea	Myanmar, Thailand, S Laos	Wet rocks and rocks crevices along streams and waterfalls	3	No change	
Leptoboea	Bhutan, N and NE India, S China (Yunnan), Myanmar, Thailand	Hills and mountains	2	No change	
Liebigia	Sumatra, Java and Bali	Forest plants, also in disturbed forest, open places and forest margins, river banks etc.; probably growing in acid soil (limestone not recorded, but ecological information generally scanty)	12	Raised from <i>Chirita</i> sect. <i>Liebigia</i> to generic rank	Weber et al. 2011a
Litostigma	China (Guizhou, Yunnan)	Wet limestone rocks and at the entrance to caves.	2	Genus recently established	Wei et al. 2010
Loxocarpus	S Thailand and E Malesia	Primary forests, often on sloping ground, river banks or on damp rocks.	20	New combinations	Middleton et al. 2013
Loxostigma	S China (Sichuan, Yunnan, Guizhou, Guangxi), N Vietnam	Damp, mossy rocks or on tree trunks in forests	11	Recently inclusion of caulescent <i>Briggsia</i> species	Möller et al. 2014
Lysionotus	From N India and Nepal eastwards through N Thailand, N Vietnam and S China to S Japan	Epiphytically on trees in forest or on damp mossy rocks; 300–3100 m.	29	No change	
Metapetrocosmea	S China (Hainan)	Forests and stream sides, altitude 300–700 m.	1	No change	
Microchirita	From the Western Ghats of India to the foothills of the Himalayas, through continental SE Asia to Sumatra, Borneo and Java	Wet, light to moderately shady places at cliff bases, on cliff walls in crevices and cracks, or at cave entrances.	37	Raised from <i>Chirita</i> sect. <i>Microchirita</i> to generic rank	Wang et al. 2011; Weber et al. 2011a; Middleton 2018
Middletonia	India, Bangladesh, Bhutan, China, Burma, Thailand, Laos, Cambodia, Vietnam, Malaysia	Limestone or granite	5	Genus recently established for four species of <i>Paraboea</i>	Puglisi et al. 2016
Orchadocarpa	Malay Peninsula (Main Range)	Montane forests, on acid soil.	1	No change	
Oreocharis	China, Thailand, Vietnam, Myanmar, Bhutan, NE India, Japan	Shady and damp rocks by streams, in valleys or in forests on slopes or cliffs, dry shaded rocks, altitude 200–3600 m.	>120	Expanded to include Ancylostemon, Bournea, Briggsia, Dayaoshania, Deinocheilos, Isometrum, Opithandra, Paraisometrum, Thamnocharis and Tremacron; Inclusion offurther ten spp. of Briørsia	Möller et al. 2011b; Möller et al. 2014; Chen et al. 2014

Gernus	Distribution	Habitat	Species number	Taxonomic status	Reference
Ornithoboea	From S China and Vietnam southwards to N Peninsular Malaysia	Rocks, in shaded, humid places; some (possibly all) species confined to limestone.	16	No change	Scott and Middleton 2014
Paraboea	Bhutan, China, Indonesia, Malaysia, Myanmar, Philippines, Thailand, Vietnam	Usually growing on limestone (rarely quartzitic) rocks, in forest or sun-exposed places, altitude 100–3200 m.	141	Expanded by inclusion of <i>Phylloboea</i> and <i>Trisepalum</i> ; removal of four species and placement in the new genus <i>Middletonia</i>	Puglisi et al. 2011; Puglisi et al. 2016
Petrocodon	China, N Vietnam, NE Thailand	Shady places on rocks cliffs and rocks crevices of limestone hills or in broad-leaved evergreen forests; altitude sea level to 3500 m.	33	Expanded to include Calcareoboea, Didymocarpus, Dolicholoma, Lagarosolen, Paralagarosolen, Tengia and Wentsaiboea p. p. (exclude type)	Wang et al. 2011; Weber et al. 2011b
Petrocosmea	NE India, S China, Myanmar, Thailand, S Vietnam	Damp rocks and shaded cliffs in forest and above the forest line, altitude 500–3100 m.	49	No change	
Platystemma	Nepal, Bhutan, N India, SW China	Shady and damp rocks in valleys or dry cliffs, altitude 2300–3200 m.	1	No change	
Primulina	Essentially southern half of China and Vietnam	Limestone	>190	Enormous expansion of the previously monotypic genus by inclusion of <i>Chirita</i> sect. <i>Gibbosaccus</i> , <i>Chiritopsis</i> , <i>Deltocheilos</i> , and <i>Wentsaiboea</i>	Wang et al. 2011; Weber et al. 2011a
Pseudochirita	S China (C & W Guangxi), Vietnam	Forests on limestone hills.	1	No change	
Rachunia	Thailand, Kanchanaburi province, Thong Pha Phum district, Ban E Tong, near the Thai-Myanmar border at 900 m.	Moist evergreen forest on a slope in shade.	1	Genus recently established	Middleton et al. 2018
Raphiocarpus	S China and N & C Vietnam	Montane regions, in shady and damp places under forests, on slopes near streams or in rock crevices.	14	No change since Weber 2004, but changes to be expected	
Rhabdothamnopsis	S China	Dense forests, at streamsides in forested areas and in thickets along roadsides, altitude 1600– 2200(–4600) m.	1	No change	
Rhynchotechum	NE India, Nepal, Bhutan, SW & S China, SE Asia and Malesia to New Guinea	Under broad-leaved forest in valley, shady places near the stream, on the rocks, distributed from coast to 2200 m.	16	No change	
Ridleyandra	Malay Peninsula and Borneo	Lowland and (more frequently) montane rain forests.	31	No change	Yunoh and Dzulkafly 2017
Senyumia	Malay Peninsula (Pahang: Gunung Senyum and adjacent localities)	Rock faces in damp limestone caves	1	No change	
Somrania	Peninsular Thailand	limestone	2	Genus recently established	Middleton and Triboun 2012
Spelaeanthus	Malay Peninsula (Pahang), Batu Luas	Damp rock faces, especially at the entrance to limestone caves.	1	No change	
Tetraphyllum	NE India, Bangladesh, Myanmar, Thailand	Damp rocks in forest	3	No change	
Tribounia	Thailand	Crevices of karst limestone in deciduous forest.	2	Genus recently established	Middleton and Möller 2012

dispersal across Southeast Asia (Hall and Spakman 2015). Frequent alternation of transformation between sea and continent intensified speciation in this area (de Bruyn et al. 2014).

Southwest China has the largest continuous limestone areas in the world, which includes Guizhou, Yunnan, Guangxi provinces and the Sino-Vietnam region (Clements et al. 2006; Hou et al. 2010; Ren 2015; Kong et al. 2017). South Yunnan is not only rich in limestone landscapes, but also comprises a series of spectacular north-south trending ridges along three major rivers of Asia: the Salween, Mekong and Red River, which have formed many unique microhabitats and microclimates, such as dry and hot valleys in Yunnan (Jiang et al. 2017). These diverse landscapes, forming 'microhabitat islands', greatly facilitated plant speciation (Clements et al. 2006; Ren 2015). Clements et al. (2006) and Chung et al. (2014) also proposed that these kinds of limestone microhabitats were formed largely by the East Asian monsoon (Jiang et al. 2017).

Northwest Yunnan (Hengduan Mountains), located at the eastern fringe of the Tibetan Plateau, is widely recognised as a globally important biodiversity hotspot (Myers et al. 2000; Liu et al. 2017) and the cradle of new species with an extraordinarily high ratio of recently evolved endemic species (neoendemics) that resulted from the uplift of the Himalayas and surrounding mountains (López-Pujol et al. 2011). It is noteworthy that not only neoendemics of Gesneriaceae have been found here, but most of the basal taxa also appeared here (Möller et al. 2009; Möller and Clark 2013; Roalson and Roberts 2016). Such a distribution pattern may relate to the Asian Gesneriaceae migration route (Möller et al. 2009).

In conclusion, we have discussed the biogeographic and diversification patterns of the Asian Gesneriaceae, along with underlying mechanisms of the family's dispersal, adaptation and evolution. The family is still undergoing quick diversification and is awaiting further detailed studies not only about ecological adaptations but also evodevo examinations on relationships between micro- and macro-evolution. Molecular biogeographic studies on the typical pantropic taxa using updated techniques such as sequenced restriction-site associated DNA (Baird et al. 2008; Feng et al. 2017) are also suggested to explore the historical dispersal patterns and evolutionary diversification of the family from tropical America to Africa and Asia.

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RESEARCH ARTICLE



Reassessment of Bournea Oliver (Gesneriaceae) based on molecular and palynological evidence

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Abstract

The former genus *Bournea* is endemic to China, including two species, has been under consideration for incorporation into the expanded genus *Oreocharis* s.l. in Gesneriaceae. The phylogenetic tree inferred from two DNA sequences (*trnL*-F and ITS) showed that this genus is deeply nested into *Oreocharis* s.l. However, the new tree from seven ones (*atpB-rbcL*, *ndhH-rps15-ycf1*, *rpl132*, *trnC-trnD*, *trnL-F*, *trnT-trnL* of chloroplast DNA and ITS regions) revealed that *Bournea* is the sister group of other of *Oreocharis* s.l. Furthermore, *Bournea* is morphologically different from other *Oreocharis* based on existing data. We suggest keeping *Bournea* as an independent genus in Gesneriaceae.

Keywords

Bournea, morphological characters of flowers, Oreocharis, phylogeny, pollen grains

Introduction

The genus *Bournea* Oliver was established in 1893 based on the type species *Bournea* sinensis Oliv., which was endemic to Guangdong province, Southeast China (Oliver 1893). Wang et al. (1990) transferred another species from Fujian province next to Guangdong province, *B. leiophylla* (W.T.Wang) W.T.Wang & K. Y. Pan, to this genus. *Bournea* is easily recognized by the combination of white and actinomorphic flowers and the verrucate exine of pollen grains (Pan 1987; Wang et al. 1990, 1998; Ying et al. 1993; Li and Wang 2004; Weber 2004). The genus is similar to the monotypic genus *Thamnocharis* in the expanded *Oreocharis* in actinomorphic and dissected corollas, but different in its white flowers (vs. blue in *Thamnocharis*) and verrucate exine of pollen grains (vs. spiny) (Wang et al. 1998 onw.; Ying et al. 1993; Zhang 2018). With the inclusion and exclusion of more species in *Oeocharis* s.l., the considerable variation in morphology would become better understood than before in the expanded genus (Möller et al. 2011; Yang et al. 2020). At this time, *Bournea* includes two endemic species in China (Fig. 1; Wang et al. 1998 onw.; Shui and Chen 2018, 2020).

The preliminary phylogenetic analysis revealed that *Bournea* ought to be combined into the expanded genus Oreocharis s.l. in Gesneriaceae. Möller et al. (2011) sampled 55 samples of 51 species and sequenced the chloroplast *trn*L-F intron-spacer and the nuclear ribosomal ITS regions and reconstructed the phylogenetic relationships of the Oreocharis-dominated clade in Gesneriaceae. Chen et al. (2014) sampled 64 samples of 52 species of *Oreocharis* to locate the systematic position of an endangered species in the karst region in Southwestern China, *Paraisometrum mileense* W. T. Wang $\equiv Oreo$ charis mileensis (W.T.Wang) Mich.Möller & A.Weber]. Both of the above phylogenetic trees had shown that Bournea was deeply nested inside Oreocharis s.l., which seems to support that Bournea was treated as a member of the expanded genus Oreocharis in Gesneriaceae (Möller et al. 2011). However, the above two DNA markers help to resolve the relationship within the expanded genus, and so these two sequences do not seem to be enough to support the phylogenetic analysis in the expanded Oreocharis. It is the reason why the above taxonomic treatment has not been updated in the recent publications of Gesneriaceae (Wang et al. 1998 onw.; Weber and Skog 2007 onw.; Shui and Chen 2018, 2020). In a word, it is premature to make the taxonomic combination in the expanded genus.

Our recent study based on six chloroplast sequences has revealed a more wellresolved relationship of *Bournea* with the expanded genus. In fact, the low resolution from the above two DNA regions within the expanded *Oreocharis* s.l. has been troubling us. Here, we adopted more DNA sequences to explore the precise phylogenetic position of the former *Bournea* within the expanded genus to reassess the necessity of the taxonomic combination made by Möller et al. (2011). Furthermore, due to the positive value of pollen grains in the expanded *Oreocharis* (Pan 1987; Guo and Wang 2013), we made the additional palynological observation of *Bournea* to support the taxonomic reassessment of the genus *Bournea* in Gesneriaceae.



Figure 1. The morphology of *Bournea sinensis* Oliv. (**A–E**) and *B. leiophylla* (W. T. Wang) W. T. Wang (**F–J**). **A** plant **B** inflorescence **C** calyx and disc **D** stigma **E** front view of corolla showing the anthers and the style and stamens **F** plant **G** inflorescence **H** pistil and disc **I** stigma **J** front view of corolla showing the anthers.

Materials and methods

Molecular approach

Molecular materials. First, we sampled 52 samples of 46 species in the expanded *Oreocharis* and two outgroup taxa (Suppl. material 1: Table S1), which approximately matches the sample list in the previous publication (Möller et al. 2011; Tan et al. 2011; Chen et al. 2014; Yang et al. 2020). The voucher specimens are deposited in the herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences (KUN). Second, we downloaded the nuclear ITS regions of 43 samples of 39 species (including two outgroup taxa) from the National Center for Biotechnology Information (NCBI) nucleotide database (http://www.ncbi.nlm.nih.gov/) (Suppl. material 1: Table S2). Thirdly, the additional cpDNA and nuclear data from the two new combinations proposed by Yang et al. (2020) have been downloaded and incorporated into our phylogenetic analysis (Suppl. material 1: Tables S1, S2).

DNA extraction and sequence assembly of the complete cp DNA. Total genomic DNA of Oreocharis using a modified CTAB (Doyle and Doyle 1987; Yang et al. 2014) from about 100 mg fresh leaves. Moreover, DNA amplified by the PCR method from Yang et al. (2014). DNA was sequenced by an Illumina Miseq (Illumina, San Diego, CA, USA) at GBOWS (Kunming, China). Available contigs are assembled into the scaffold files by SPAdes (Bankevich et al. 2012). The scaffold files are aligned to the sequence in Blast and manually conducted a complete chloroplast genome sequence.

Abstract of the cp DNA markers and matric preparation. First, we produced individual gene trees of the six cp DNA markers and ITS. Then, we compared the similarity of these gene trees and further decided which sequences can be combined or not. Next, we compared the different combinations of cp DNA markers and ITS. We confirmed that five sequences *atpB-rbcL*, *ndhH-rps15-ycf1*, *rpl132*, *trnL-F*, *trnT-trnL*, and ITS seem to provide strong support to resolve the relationship of *Bournea* within the expanded *Oreocharis*. Furthermore, six cp DNA markers with additional cp DNA marker *trnC-trnD* can provide more robust support than the above five cp DNA markers. The above sequences were abstracted separately under the Geneious v10.2.3 (Kearse et al. 2012) by comparing their respective sequence from NCBI and combined into a matrix by Sequence Matrix (Vaidya et al. 2011). The matrix has been aligned with MAFFT v. 7.409 (Katoh and Standley 2013; Katoh et al. 2015) and then manually adjusted in Geneious v10.2.3 (Kearse et al. 2012). All the original sequences are uploaded on NCBI (Suppl. material 1: Tables S1, S2).

Methods of phylogenetic analysis. The best-fitting models (GTR) of molecular evolution for Bayes inference (BI) and the model of the Maximum Likelihood (ML) were determined by the Akaike Information Criterion (AIC) in MrModelTest within MrMTgui (https://www.softpedia.com/get/Science-CAD/MrMTgui.shtml). Bayes Inference (BI) analyses were conducted with MrBayes v3.2.3 on Windows 7 (Huelsenbeck and Ronquist 2001), two independent runs, each with four chains were conducted, each beginning with a random tree and sampling one tree every 1000 generations of 6,000,000 generations. The convergence was checked using the average standard deviation of split (<0.01). The first 1500 trees were discarded as burn-in, and the remaining trees were used to construct majority-rule consensus trees. ML analysis was conducted with the GTR+I+G model with RAxMLGUI on Windows 7 (i.e., Silvestro and Michalak 2012; Stamatakis 2014, depending on the version used). ML tree's boot-strap values are evaluated with nonparametric bootstrapping by using 1,000 replicates.

Palynological approach

The methods on SEM for pollen grains followed Chen et al. (2009) and Hong et al. (2015), and terminology about pollen morphology follows Yan et al. (1997), Li and Wang (2004), Weber (2004), Punt et al. (2007) and Chen et al. (2009). Two samples of *B. sinensis* are from the different individuals of the same population at Boluo county of Guangdong province, China (*Y. M. Shui et al. B2015-284*, KUN). Two samples of *B. leiophylla* are respectively from the different populations at Liancheng county (*Y. M. Shui et al. B2015-272*, KUN) and Yong'an county (*Y. M. Shui et al. B2015-275*, KUN), Fujian province, China. The micro-morphology of pollen grains was observed by using Zeiss Sigma 300 (Germany). We also collected the pollens dataset of 51 samples of 48 species from the previous study and compared the difference among the expanded genus (Pan 1987; Xi 1987; Ying et al. 1993; Guo and Wang 2013; Hong et al. 2015; Zhang 2018).

Results

Molecular analysis

Six plastid markers are enough to resolve the relationship of Bournea within Oreocheris s.l. The expanded genus Oreocharis s.l. can be divided into two clades in Bayes tree (100% posterior probability value, PPV=100%) and Raxmil tree (92% bootstrap value, BTV=92%) based on the six cp DNA markers (atpB-rbcL, ndhH-rps15-vcf1, rpl132, trnC-trnD, trnL-F, trnT-trnL) (Fig. 2). The first clade is the minor clade, including two species of Bournea (B. sinensis and B. leiophylla). The second clade is the major clade, including all the sampled species within the expanded genus except for Bournea. Within the second clade, however, there is no well-solved topography among the numerous clades (80%≤PPV≤88%, BSV<50%). Nevertheless, the group dominated by yellow flowers is resolved very well (PPV=100%, BSV=97%). As to the former genus Thamnocharis with actinomorphic flowers, its unique species, now Oreocharis espuirolii, is strictly nested with O. speciosa and O. pingfaensis and O. farreri, all of which are deeply involved in the expanded Oreocharis (Fig. 2). Besides, O. baolianis (B.L. Burtt) Li H. Yang & F. Wen and O. guiliana (Q.W. Lin) Li H. Yang & M. Kang, two new combinations from other genera, are a sister group and involved in the second clade, which supports their taxonomic treatment (Yang et al. 2020).






Figure 3. The Bayes inference (BI) and Maximum likelihood (ML) tree inferred from six cp DNA markers (*atpB-rbcL*, *ndhH-rps15-ycf1*, *rpl*132, *trnC-trnD*, *trnL-F*, *trnT-trnL*) and ITS of the expanded genus *Oreocharis* s.l. in Gesneriaceae. Note 1) the red clade indicates the position of *Bournea* in phylogenetic trees; 2) the number of the node respectively indicates posterior probability values in BI and bootstrap values in ML, \times indicates < 50%.

Six plastid markers together with one nuclear marker (ITS) are enough to resolve the relationship of *Bournea* within *Oreocharis* s.l. Within the combined analysis of six plastid markers (*atpB-rbcL*, *ndhH-rps15-ycf1*, *rpl132*, *trnC-trnD*, *trnL-F*, *trnT-trnL*) and one nuclear marker (ITS), all the two species in *Bournea* form a monophyletic group, and the genus *Bournea* becomes a sister clade to the other *Oreocharis* s.l. (Fig. 2). In other words, *Oreocharis* s.l. is splitting into two clades with strong support (PPV: 100%, BSV: 100%). Within clade I, *Bournea sinensis* and *Bournea leiophylla* form another clade with strongly-support monophyletic (PPV: 1, BSV: 100). Within the clade II, the remaining species of *Oreocharis* s.l., form a clade with strongly-support



Figure 4. The morphology of pollen grains of *Bournea sinensis* Oliv. (**A–D**) and *B. leiophylla* (W. T. Wang) W. T. Wang (**E–H**) by SEM. **A** polar view showing pollen grain with three equatorial, colporus apertures **B** equatorial view showing single free, prolate pollen grain **C** equatorial view show apertures and granular aperture membrane **D** detail showing verrucate tectum with granular **E** polar view showing pollen grain **G** equatorial view show apertures and granular aperture membrane **H** detail showing verrucate tectum with granular showing verrucate tectum with granular showing verrucate tectum with granular aperture applies free, oblate pollen grain **G** equatorial view show apertures and granular aperture membrane **H** detail showing verrucate tectum with granular.

monophyletic (PPV: 1, BSV: 93). The yellow-flowered group and the former genus *Thamnocharis* with now *Oreocharis espuirolii* show the same case as the above phylogenetic result inferred from the six cp DNA markers (Figs 2, 3). Besides, *O. baolianis* and *O. guiliana*, although not a sister group, are involved in the second clade, which supports their taxonomic treatment (Yang et al. 2020).

However, five plastid markers and its combination with one nuclear marker (ITS) cannot completely resolve the relationship of *Bournea* within *Oreocheris* s.l. As to five cp DNA markers (*atpB-rbcL*, *ndhH-rps15-ycf1*, *rpl132*, *trnL-F*, *trnT-trnL*), the relationship of *Bournea* seems to be resolved (PPV=100, BSV=100). As to the combination of 5 cp DNA markers and ITS, the relationship of *Bournea* is not completely resolved (PPV=100%, BSV=65%). At the above second clade sister to the *Bournea* clade, the groups have been resolved with weak support (BSV<50%). Nevertheless, the yellow-flowered group and the former genus *Thamnocharis* with now *Oreocharis espuirolii* show the same case as the above phylogenetic result inferred from the above more markers (Figs 2, 3). So, the first necessary step is to add more sequences of cp DNA markers to resolve the relationship within the expanded *Oreocharis*.

Palynological observation

The two species in the genus show almost the same characteristics. In the two species, the pollen grains single-grained, isopolar, radial symmetry, prolate, amb circular, tricol-

porate, aperture membrane granulum, exine verrucate, tectum verrucate, supratectal elements granulum (Fig. 4). The difference between them is polar axis 14–18 μ m diam. in *B. sinensis*, 12–14 μ m diam. in *B. leiophylla*.

Discussion

The phylogenetic position of *Bournea* inferred from chloroplast genes seems to be more convincing than those from the combination of chloroplast and nuclear gene (ITS). The relationship of *Bournea* has been completely resolved by the phylogenetic tree inferred from six cp DNA markers (Fig. 2; PPV = 100%, BSV = 93%) and the combination of six cp DNA markers and nuclear ITS (Fig. 3; PPV = 100%, BSV = 92%). Furthermore, it seems to be resolved by the phylogenetic tree inferred from five cp DNA markers (Suppl. material 2: Fig. S1; PPV = 100%, BSV = 81%) and partly from the combination of five cp DNA make and nuclear ITS (Suppl. material 3: Fig. S2; PPV = 100%, BSV = 65%). As the yellow-flowered group, on the other hand, BSV of the analysis from six and five cp DNA markers are respectively 82% and 74%, while BSV from the combined analysis up to 99% and 82% at the terminal of the Raxmil tree, which implies the apparent increase of the bootstrap values (BSV) in ML trees (Figs 2, 3, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2). The yellowdominated clade is mainly distributed in high-altitude regions in Western China with abundant narrowly-distributed species, while the former genus Bournea is distributed in low-altitude regions, mainly in Eastern China with lower endemism (Wang et al. 1990, 1998; Li and Wang 2004; Weber 2004). In the high-altitude regions, the phylogenetic analysis of the expanded genus without ITS region is less affected by hybrids and so better resolved than the analysis of the ITS region. In low-altitude regions, however, the species of Oreocharis s.l. in Southeast China, excluding the yellow-dominated group, are usually widely distributed and easily breed with each other. So, as to the expanded genus, high endemism in the high-altitude regions may result in the inconsistency of the phylogenetic trees with ITS and without ITS.

It is pending that floral actinomorphy can be considered as one of the diagnostic characteristics between *Bournea* and *Thamnocharis* within *Oreocharis* s.l. In the expanded genus, both of the two species of *Bournea* are morphologically very similar to the monotypic genus *Thamnocharis* Burtt in actinomorphic corolla (Wei et al. 2010; Möller et al. 2011). Based on our phylogenetic tree, the genus *Bournea* is the sister to the other species in the expanded genus, while *Thamnocharis* is deeply nested into the expanded genus (Figs 2, 3, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2). The previous study seems to imply that floral actinomorphy can be considered to be apomorphy (Zhou et al. 2008; Wang et al. 2010; Weber 2011a, b; Yang et al. 2012). It is reasonable that the floral actinomorphy. (Figs 2, 3, Suppl. material 2: Fig. S1, Suppl. material 2: Fig. S1, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S1, Suppl. material 3: Fig. S2).

considered as one of the diagnostic characteristics between *Bournea* and *Thamnocharis*. If *Bournea* remains free from *Oreocharis* s.l. (Shui and Chen 2018, 2020) we prefer to adopt the vertucate exine of pollen grains as a diagnostic characteristic separating *Bournea* from *Thamnocharis* within *Oreocharis* s.l. More work needs to be carried out to decide if *Bournea* can be combined into the expanded genus.

Conclusion

More chloroplast markers provide useful data to resolve the phylogenetic relationship within the expanded genus *Oreocharis* s.l. The two DNA markers (*trn*L-F and ITS) cannot resolve any above relationship (Möller et al. 2011). The five chloroplast markers (or including ITS data) have almost resolved the phylogenetic relationship of the former genus *Bournea* within the expanded genus (Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2), which provide the first step to resolve the phylogenetic relationship within the expanded genus *Oreocharis* s.l. Furthermore, six cp DNA markers (or including ITS data) well resolved the phylogenetic relationship of the former genus *Bournea* within *Oreocharis* s.l. Our above results show that *Bournea* is sister to *Oreocharis* s.l. and indicate that *Bournea* cannot be combined into the expanded *Oreocharis*.

The verrucate exine of pollen grains can differentiate the former *Bournea* from other of the *Oreocharis* s.l. The case seems to match the above relationship inferred from the more chloroplast markers. At present, *Bournea* can be diagnosed by the verrucate exine of pollen grains within the expanded genus. However, only one more than 40% (50 out of 130) species of the expanded genus have been sampled to explore their pollen grains in the expanded genus. It is pending if the unique characteristics of the pollen grains happen to some un-sampled species. So, more palynological evidence may be necessary to the taxonomic treatment within the expanded genus.

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Supplementary material I

Tables S1, S2

Authors: Wen-Hong Chen, Ya-Mei Zhang, Shi-Wei Guo, Zhi-Rong Zhang, Li Chen, Yu-Min Shui

Data type: dataset

- Explanation note: **Table S1.** Sample list of the species and their ITS sequences in the expanded genus *Oreocharis* in Gesneriaceae. **Table S2.** Sample list of the species and their chloroplast sequences in the expanded genus *Oreocharis* in Gesneriaceae.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
- Link: https://doi.org/10.3897/phytokeys.157.55254.suppl1

Supplementary material 2

Figure S1

Authors: Wen-Hong Chen, Ya-Mei Zhang, Shi-Wei Guo, Zhi-Rong Zhang, Li Chen, Yu-Min Shui

Data type: phylogenetic tree

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Link: https://doi.org/10.3897/phytokeys.157.55254.suppl2

Supplementary material 3

Figure S2

Authors: Wen-Hong Chen, Ya-Mei Zhang, Shi-Wei Guo, Zhi-Rong Zhang, Li Chen, Yu-Min Shui

Data type: phylogenetic tree

- Explanation note: The Bayes inference (BI) and Maximum likelihood (ML) tree inferred from 5 cp DNA markers (*atpB-rbcL*, *ndhH-rps15-ycf1*, *rpl*132, *trnL*-F, *trnTtrnL*) and ITS of the expanded genus *Oreocharis* s.l. in Gesneriaceae. Note 1) the red clade indicates the position of *Bournea* in phylogenetic trees; 2) the number of the node respectively indicates posterior probability values in BI and bootstrap values in ML, \approx indicates < 50%..
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Supplementary material 4

Supporting materials

Authors: Wen-Hong Chen, Ya-Mei Zhang, Shi-Wei Guo, Zhi-Rong Zhang, Li Chen, Yu-Min Shui

Data type: phylogenetic

- Explanation note: **Data 1** DNA sequences of 6 cp genes + ITS from 52 samples and 48 species of *Oreocharis* in Gesneriaceae. https://figshare.com/s/23027089ad5af90fc3b3
- Data 2 DNA sequences of 6 cp genes from 52 samples and 48 species of *Oreocharis* in Gesneriaceae. https://figshare.com/s/455c93000b474faee7d2
- Data 3 DNA sequences of 5 cp genes + ITS from 52 samples and 48 species of *Oreo-charis* in Gesneriaceae. https://figshare.com/s/3a8fe0f8923ccc120c68
- Data 4 DNA sequences of 5 cp genes from 52 samples and 48 species of *Oreocharis* in Gesneriaceae. https://figshare.com/s/76c56fd0ff6fcaa26485

Data 5 DNA sequences of ITS-from 52 samples and 48 species of *Oreocharis* in Gesneriaceae. https://figshare.com/s/8c045c93b02389fd6777

Data 6 DNA sequences of *atp*B-*rbc*L from 52 samples and 48 species of Oreocharis in Gesneriaceae. https://figshare.com/s/b177b05cbcc08d22d9c3

Data 7 DNA sequences of *rpl*132 from 52 samples and 48 species of *Oreocharis* in Gesneriaceae. https://figshare.com/s/a2930a767a1411dc49c2

Data 8 DNA sequences of ndhH-rps15-ycf1 from 52 samples and 48 species of *Oreo-charis* in Gesneriaceae. https://figshare.com/s/8ed14b5f7de0552107cc

Data 9 DNA sequences of *trn*C-*trn*D from 52 samples and 48 species of *Oreocharis* in Gesneriaceae. https://figshare.com/s/93609c5db11bc52b7d80

Data 10 DNA sequences of *trn*L-F from 52 samples and 48 species of *Oreocharis* in Gesneriaceae. https://figshare.com/s/ee47f235d59d00f3e89a

Data 11 DNA sequences of *trn*T-*trn*L from 52 samples and 48 species of *Oreocharis* in Gesneriaceae. https://figshare.com/s/b55363c06cfd1235e435.

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RESEARCH ARTICLE



Two new combinations in Oreocharis (Gesneriaceae) based on morphological, molecular and cytological evidence

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Abstract

The newly-circumscribed genus *Oreocharis* is recently enlarged by incorporating ten other genera with high floral diversity. In this study, our morphological, molecular and cytological evidence supports our adding two species from other two different genera (*Boeica* and *Beccarinda*) to *Oreocharis*. The special corolla shape (campanulate or flat-faced) and related short filament of these two new combinations, *Oreocharis guileana* and *O. baolianis*, further enrich the diversity of floral characters of the enlarged *Oreocharis*. Meanwhile, some supplementary and amended descriptions of these two species are made here. Our morphological, molecular and geographical data indicate that *O. guileana* is related to *O. pilosopetiolata* to a certain extent. For *O. baolianis*, however, our current dataset does not allow conclusions on the species relationship within *Oreocharis*.

Keywords

Beccarinda baolianis, Boeica guileana, ITS, Oreocharis, taxonomy, trnL-F

Introduction

Recent phylogenetic studies on the Old World Gesneriaceae have greatly advanced our understanding of species relationships and generic delimitations in this plant group (Wang et al. 2010; Möller et al. 2011a). One of the typical cases is the re-circumscription of Oreocharis Bentham. Molecular phylogenetic analyses indicate that species of the original *Oreocharis* are phylogenetically intertwined with species of ten other small or monotypic genera (Möller et al. 2011b). After considering both the phylogenetic conclusion and the morphological evaluation, Möller et al. (2011b, 2014) formally transferred all species of nine genera (i.e. Ancylostemon Craib, Bournea Oliver, Dayaoshania Wang, Deinocheilos Wang, Isometrum Craib, Opithandra Burtt, Paraisometrum W.T. Wang, Thamnocharis Wang and Tremacron Craib) and rosette species of Briggsia Craib (except B. longipes and B. mihieri) to Oreocharis. This expansion not only made the Oreocharis a large genus within subfamily Didymocarpoideae (tribe Trichosporeae, subtribe Didymocarpinae) of the Gesneriaceae (Weber et al. 2013), but also made it one of the florally-diverse groups. Species of the newly-circumscribed Oreocharis all represent rosette herbaceous plants with spirally-arranged leaves, scapose inflorescences and loculicidally dehiscent capsules; however, their floral characters are extremely variable (Möller et al. 2011b). For instance, the characters corolla shape and colour, stamen number, placement of fertile stamens and anther coherence and shape are highly varied in this genus. The expansion of the morphological boundary of Oreocharis calls for reconsideration of the generic status of some problematic species; in particular, species whose generic placements are heavily based on flower characters, such as Boeica guileana B.L. Burtt and Beccarinda baolianis Q.W. Lin.

Boeica guileana was first described in 1977 by Burtt based on cultivated plants without description of mature fruits. The species has been regarded as endemic to Hong Kong for a long time (Burtt 1977; Wang et al. 1998; Li and Wang 2004); however, it was later discovered in Shenzhen, southern China (Wei et al. 2010). Based on corolla shape (short tube and spreading limb) and morphological resemblance to two existing species (*Boe. brachyandra* Ridl. and *Boe. nutans* Ridl.), Burtt (1977) assigned the plant to *Boeica* Clarke. However, *Boe. guileana* is distinctly different from most of other *Boeica* species by its rosette herbaceous habit, spirally-arranged leaves and longitudinally dehiscent anthers. Thus, in the original protologue, Burtt (1977) emphasised that the inclusion of *Boe. nutans*) into *Boeica* is questionable. The generic status of all these three species is furthermore doubted by Weber and Skog (2007).

Beccarinda baolianis was newly described in 2016 from southern China, based on two specimens (Lin 2016). At present, only one population of this species can be found at its type locality (Lin 2016). Based on floral characters, such as the campanulate corolla and four stamens and capsule at a 90° angle to the pedicel, Lin (2016) assigned this species to *Beccarinda* Kuntze and compared it with *Be. minima* K.Y. Pan. However, our field observations found that the 90° angle between capsule and pedicel is not a stable character in the population. In addition, the inconspicuous rhizome and rosette habit of this species allow us to assume that it may only be distantly related to *Beccarinda*. Moreover, other important characters, i.e. the way of dehiscence in anthers and fruits, were not even described by Lin (2016). In the original protologue, Lin (2016) excluded this species from the original *Oreocharis* for its ovate anthers with apically confluent thecae, but this character does exist in the enlarged *Oreocharis*.

Summarising, the generic placements of *Boe. guileana* and *Be. baolianis* need reconsideration. Therefore, this study aims to clarify the generic status of these two species and discuss their species relationships in the enlarged *Oreocharis* using cytological, molecular and morphological analyses.

Materials and methods

Morphological observation

The previous descriptions of *Boe. guileana* and *Be. baolianis* were based on only one or two specimens from a single location; thus, the described characters do not reflect the whole range of variation and other characters were not described at all. For example, the dehiscent way of fruit of both of these two species had not been mentioned in the original protologue. To revise and amend the morphological descriptions and clarify the morphological affinities between these two species and its congeners, detailed morphological studies were made on living plants in the field (Shenzhen and Fujian, China) and on plants cultivated in greenhouses (South China Botanical Garden, SCBG and Gesneriad Conservation Center of China, GCCC). Furthermore, dried specimens were investigated. Checking of specimens was undertaken at IBSC, IBK, SZG and FAFU. Additionally, high-resolution images of specimens were critically checked using the web service of E (http://data.rbge.org.uk/search/herbarium/) and HK (http:// www.herbarium.gov.hk/index.aspx).

Molecular taxon sampling

In the recent classification of Gesneriaceae, *Boeica* and *Beccarinda* were deposited in subfamily Didymocarpoideae, tribe Trichosporeae, subtribe Leptoboeinae and the enlarged *Oreocharis* was deposited in subtribe Didymocarpinae (Weber et al. 2013). To verify the generic placements of *Boe. guileana* and *Be. baolianis*, we sampled a total of 82 ingroup samples, comprising almost all genera of the subtribe Leptoboeinae and 24 out of 34 genera in subtribe Didymocarpinae. Two species, *Rhynchoglossum obliquum* Blume and *Stauranthera grandifolia* Benth., were selected as outgroup, according to the phylogeny of the Old World Gesneriaceae (Möller et al. 2011a).

DNA extraction, PCR and sequencing

The plastid *trnL-F* and nuclear ribosome internal transcribed spacer (ITS) sequences were used for the phylogenetic analysis. Most of these DNA sequences were acquired

from GenBank and the sequences of six species, *Boe. guileana, Boe. ornithocephalantha* F. Wen, T.V. Do & Y.G. Wei (Wen et al. 2016), *Be. minima, Be. baolianis, Oreocharis pilosopetiolata* L.H. Yang & M. Kang (Yang et al. 2015) and *O. benthamii* var. *reticulata* Dunn, were newly amplified and sequenced in this study. DNA was extracted from dried leaves using a modified CTAB procedure described by Doyle and Doyle (1987). The polymerase chain reaction (PCR) amplification procedures and the PCR primers of these regions were described in Kong et al. (2017). Newly-amplified sequences were deposited in GenBank. The GenBank accession numbers used in this study are listed in Suppl. material 1.

Sequence alignment and phylogenetic analysis

Sequence matrices of trnL-F and ITS were separately aligned using the programme MUSCLE implemented in the software MEGA7 (Kumar et al. 2016) with minor manual adjustments. A combined ITS and trnL-F matrix was generated using SequenceMatrix (Vaidya et al. 2011). The incongruence length difference test (Farris et al. 1994) was implemented in PAUP*4.0B10 (Swofford 2002) to assess potential congruence between ITS and trnL-F. Phylogenetic analyses were performed on a highperformance computer cluster available in the CIPRES Science Gateway 3.3 (www. phylo.org, Miller et al. 2015). Bayesian Inference (BI) in MRBAYES 3.2.6 (Ronquist et al. 2012) and Maximum Likelihood (ML) in RAxML8.2.10 (Stamatakis 2014) were calculated for each of *trnL-F*, ITS and the combined sequence matrix. Best-fitting models for MrBayes were obtained separately for *trnL-F* and ITS spacers through AIC in Smart Model Selection (SMS) by its web server (www.atgc-montpellier.fr, Lefort et al. 2017) and were GTR+G and GTR+I+G, respectively. A total of 10,000,000 generations were run in two independent analyses, each with four Markov Chain Monte Carlo (MCMC) chains. One tree was sampled every 1,000 generations and the first 2,000 trees discarded as burn-in. Posterior probabilities (PP) obtained from the analysis were used to indicate the credibility of various branches. The ML tree was evaluated by non-parametric bootstrapping (1,000 replications) with the thorough bootstrap option of RAxML under the general time-reversible (GTR) model with a gamma model (Γ) of the rate of heterogeneity for each of *trnL-F*, ITS and the combined sequence matrix.

Chromosome preparations

Four species, *Boe. guileana*, *Boe. stolonifera* K.Y. Pan, *Be. baolianis* and *Be. tonkinensis* (Pellegr.) B.L. Burtt, were investigated cytologically. The plants for chromosome studies were collected from the field and cultivated in Gesneriad Conservation Center of China (GCCC). Actively growing root tips were collected and pretreated with 2 mM 8-hydroxyquinoline at 20 °C for about 2 hrs, then fixed with Farmer's solution (absolute alcohol: glacial acetic acid 3:1) at 4 °C for about 2 hrs. After hydrolysis for 30

min in 1 M HCl at room temperature, followed by washing through several changes of distilled water, the roots were transferred to carbol fuchsin for about 2 hrs.

Results

Morphological affinities

The results of morphological observation indicated that both of *Boe. guileana* (Fig. 1A–H) and *Bec. baolianis* (Fig. 2) should be deposited into the enlarged *Oreocharis*. Both of these two species possessed these characters: rosette herbaceous habit (Figs 1A, E, 2A–C), longitudinally dehiscent anther (Figs 1D, 2G) and loculicidal dehiscent capsule (Figs 1G, H, 2J), which showed distinct relationships to *Boeica* or *Beccarinda* and the equivalent characters of these two genera were discussed below. Our morphological comparisons showed *Boe. guileana* was similar to *Ore. pilosopetiolata* in the texture, shape and margin of leaf and the pubescence on most part of the plants. Nevertheless, *Bec. baolianis* showed a special combination of characters in the enlarged *Oreocharis*, such as small plant size, campanulate corolla and short filaments. The morphological affinities of *Bec. baolianis* are discussed below. Additionally, we amended or supple-



Figure 1. Photographs of *Oreocharis guileana* (**A–H**), *O. pilosopetiolata* (**I**), *Boeica ferruginea* (**J**) and *Beccarinda tonkinensis* (**K**). **A, E, I** habit **B, C** flower **D** opened corolla, showing stamens and staminodes **F** pistil **G, H, J, K** mature fruit.



Figure 2. Photographs of *Oreocharis baolianis*. A–C Habit D, E flower F, H opened corolla, showing stamens and staminodes G anthers I pistil J mature fruit.

mented the description of some characters (such as dehiscent way of anther and fruit, corolla lobes margin and calyx) of these two species here and the detailed amended descriptions were given below.

Matrix characteristics

The aligned *trnL-F* and ITS datasets were 966 and 824 characters long, thereof, 195 and 138 were parsimony-uninformative and 139 and 388 parsimony-informative characters, respectively. The combined dataset was 1790 characters long with 527 (29.4%) parsimony-informative characters. The incongruence length different (ILD) test showed no significant incongruence between the ITS and *trnL-F* (p = 0.53).

Phylogenetic analyses

In the combined DNA sequence analysis, the BI tree was less resolved but congruent (virtually identical) where the ML branches received bootstrap support > 50%



Figure 3. Bayesian (> 50%) tree resulting of the combined nuclear (ITS) and plastid (*trnL-F*) data matrices. Posterior probability (PP) from the BI analysis are indicated above branches and Bootstrap value (BS) from the ML analysis are indicated below. The asterisk indicates a BS < 50. The dash indicates the topological discordance between ML and Bayesian tree. The two species, *O. baolianis* and *O. guileana*, are highlighted in bold.

(Fig. 3). The phylogenetic relationships reconstructed from the concatenated matrix were generally congruent with those reported in Möller et al. (2011a, b). The subtribe Leptoboeinae (BS = 100%, PP = 100%) and Didymocarpinae (BS = 100%, PP = 92%) were both monophyletic with high support values. The enlarged *Oreocharis* formed a well-supported (BS = 100%, PP = 100%) monophyletic clade within the subtribe Didymocarpinae (Fig. 3). As expected, *Boe. guileana* and *Be. baolianis* fell into the enlarged *Oreocharis* clade (Fig. 3). Although with relatively low support (BS = 56%, PP = 79%), *Boe. guileana* is sister to *Oreocharis pilosopetiolata* in the current phylogeny (Fig. 3). However, the phylogenetical position of *Be. Baolianis* in the enlarged *Oreocharis* was difficult to discuss here. *Beccarinda baolianis*, together with two other larger clades, formed a polytomy in our phylogenetical analyses (Fig. 3).

Chromosome cytology

In the four investigated species, the somatic chromosomes were determined to be diploid with 2n = 34 in *Boe. guileana* (Fig. 4A) and *Be. baolianis* (Fig. 4B) and 2n = 20 in *Be. tonkinensis* (Fig. 4C) and *Boe. stolonifera* (Fig. 4D).

Discussion

Traditionally, the classification of the Old World Gesneriaceae is heavily based on floral characters (e.g. Burtt 1977; Wang et al. 1998). However, recent phylogenetic studies reveal that the diversity of floral characters within this family has a complex evolutionary background, i.e. multiple convergences and parallel changes (Wang et al. 2010; Möller et al. 2011b; Weber et al 2011; Lu et al. 2017). Therefore, wrong generic placements have been made for some species based on the homoplasious floral traits. For example, symmetrical corolla was regarded as an original character in the Old World Gesneriaceae (Wang et al. 1998) and several genera have been established by this character such as Bournea Oliv. (Hooker 1893), Tengia Chun (Chun 1946) and Thamnocharis Wang (Wang 1981); but the recent phylogenetic studies indicate that the symmetrical corolla convergently evolve in different alliances of Old World Gesneriaceae (Wang et al. 2010) and all these three genera have been incorporated into other genera (Möller et al. 2011b; Weber et al. 2011). In the recent case of Wentsaiboea tiandengensis Yan Liu & B. Pan (Liu et al. 2010) and *Primulina guangxiensis* Yan Liu & W.B. Xu (Liu et al. 2011), molecular phylogenetical research reveals that these species belong to other genera as Petrocodon tiandengensis (Yan Liu & B. Pan) A. Weber & Mich. Möller (Weber et al. 2011) and Petrocodon guangxiensis (Yan Liu & W.B. Xu) W.B. Xu & K.F. Chung (Xu et al. 2014), respectively.

In the present study, we show a similar finding for *Boe. guileana* and *Be. baolianis*. We combine detailed morphological studies, cytological examinations and phylogenetic analyses to show that *Boe. guileana* and *Be. baolianis* are two species belong-



Figure 4. Somatic metaphase chromosome spreads of *Oreocharis guileana*, 2n = 34 (**A**), *O. baolianis*, 2n = 34 (**B**), *Beccarinda tonkinensis*, 2n = 20 (**C**) and *Boeica stolonifera*, 2n = 20 (**D**). Scale bar: 10 µm.

ing to the enlarged Oreocharis. The morphological characters of these two species, i.e. growth habit, anther and fruit dehiscence are clearly distinct from its original genera. Boeica guileana and Be. baolianis are both rosette plants with inconspicuous rhizome (Figs 1A, E, 2A-C), which is distinctly different from most of other Boeica (caulescent habit; Burtt 1977; Wang et al. 1998; Weber and Skog 2007) and Beccarinda (long rhizomatous, caulescent or stoloniferous habit; Burtt 1955; Wang et al. 1998; Weber and Skog 2007) species. The anthers of Boe. guileana (Fig. 1D) and Be. baolianis (Fig. 2G) are longitudinally dehiscent which is different from other Boeica and Beccarinda species (poricidally- or transversely-dehiscent anthers; Burtt 1955, 1977; Wang et al. 1998; Weber and Skog 2007). In addition, fruits of both Boe. guileana (Fig. 1G, H) and Be. baolianis (Fig. 2J) are loculicidally dehiscent, which is a character compatible to the enlarged Oreocharis (Möller et al. 2011b). In other Boeica species, fruits dehisce septicidally or loculicidally (Fig. 1J; Weber and Skog 2007) and in other Beccarinda species, the fruits dehisce septicidally (Fig. 1I; Weber and Skog 2007). The somatic chromosome numbers of Boe. guileana and Be. baolianis were determined as diploid with 2n = 34 (Fig. 4A, B), which is consistent with previous findings for species within the enlarged Oreocharis (Ratter 1963; Ratter and Prentice 1964; Fussell 1958; Wang and Gu 1999; Lu et al. 2002; Zhou et al. 2003; Tan et al. 2011). The somatic chromosome numbers of *Boeica* and *Beccarinda* species studied here were 2n = 20 (Fig. 4C-D) which is also consistent with previous studies (Ratter and Prentice 1964), except one case of *Boe. brachyandra* was recorded as 2n = +/-22 (Kiehn et al. 1998). Ultimately, our phylogenetic analyses prove with high support rate (Fig. 3) that Boe. guileana and Be. baolianis belong to the enlarged Oreocharis clade. Additionally, the further phylogenetic analysis, based on some additional plastid sequences, revealed the above relationship (pers. Yu-Min Shui).

After incorporation of ten additional genera, the genus *Oreocharis* becomes a large group with high flower diversity. Flowers of this group are extremely variable in corolla shape, colour, number of stamens and anthers being free or fused (Möller et al. 2011b). However, the corolla shapes and the filament length of *Boe. guileana* (filament 3–4 mm long) and *Be. baolianis* (filament 2.5–3 mm long) are rather uncommon in

the enlarged *Oreocharis*. Therefore, the addition of these two species further enriches the diversity of flower morphology within the genus *Oreocharis*.

Taxonomic treatment

Oreocharis guileana (B.L. Burtt) Li.H.Yang & F.Wen, comb. nov.

urn:lsid:ipni.org:names:77211180-1 Figure 1A–H

≡Boeica guileana B.L. Burtt (1977: 371).

Type. China. Hong Kong: New territories, Ma On Shan, 690 m alt., ravine in montane forest, on rocks in humid shade, July 1974, Guile; culture in R.B.G. Edinb. 1976, *C.8467* (holotype: E!).

Amended description. Perennial herbs with inconspicuous rhizome. Leaves in basal rosette, 8–22; petiole 1–3 cm long, with densely white villous; leaf blade elliptic to ovate or obovate, $2-6 \times 1.1-3.2$ cm, white villous and pubescent on both sides, abaxially more densely villous along veins, base cuneate, margin serrate, apex acute to rounded; lateral veins 4–6 on each side of midrib. Cymes 1–5, axillary, 1–4-flowered. Peduncles 2.5–5.5 cm long, with brown villous; bracts 2, lanceolate, $2.5-3 \times ca.1 \text{ mm}$, margins entire, outside brown villous; pedicel 1–2 cm long, with brown villous. Calyx 5-lobed near base, lobes equal, lanceolate to linear, $3-5 \times ca$. 1 mm, outside covered by brown villous, margin entire. Corolla blue-purple, outside with sparely-brown villous; tube short and not swollen, 1.5–2.5 mm long; adaxial lip 2-lobed near base, lobes obovate-oblong, margin entire or slightly erose, apex obtuse, $5-7 \times 4-5$ mm; abaxial lip 3-lobed near base, lobes obovate-oblong, margin entire or slightly erose, apex obtuse, $9-12 \times 4.5-6$ mm. Stamens 4, adnate to corolla base, filaments linear, with sparsely pubescent, adaxial stamens ca. 3 mm long, abaxial stamens ca. 4 mm long, anthers dorsifixed, free, ovate-oblong, dehiscing longitudinally, glabrescent; staminode absent; disc not obvious; pistil 8-12 mm long; ovary conical, 2-3.5 mm, with densely-white villous; style 6-8 mm long, with pubescent. Stigma 1, disc-shaped. Capsule ca. 1.5 cm long, villous, dehiscing loculicidally to base, initially on one side, valves 2, capsule straight in relation to pedicel, not twisted.

Distribution and habitat. *Oreocharis guileana* was once recognised as an endemic species and only recorded at Ma On Shan, Hong Kong. However, recent field works reveal that it can be found from several sites in Shenzhen, such as Wu Tong Shan, Pai Ya Shan, Tian Xin Shan, Dakeng reservoir and Xigong village (Fig. 5). The plants grow on moist rock surfaces under evergreen broad-leaved forests, at an altitude of 300–900 m.

Note. Burtt (1977) thought that *Oreocharis guileana* is morphologically similar to *Boe. brachyandra* and *Boe. nutans* by its rosette habit, short corolla tube and spreading limb. However, as mentioned above, the currently taxonomic status of these two *Boeica* species is doubtful and need further studies to confirm it. Additionally, the

distinctively disjunctive distribution between *Oreocharis guileana* and *Boe. brachyandra* and *Boe. nutans* may indicate a distant relationship between them (*Oreocharis guileana* is a species occurring in S China, but *Boe. brachyandra* and *Boe. nutans* are distributed on the Malayan Peninsula). Although with low support, our phylogenetic results show that *Oreocharis guileana* and *O. pilosopetiolata* have a sister relationship (Fig. 3). These two species have similar leaf blade (size, shape, texture and margin) and indumentum on most of organs and it is difficult to distinguish these two species without flowers. Additionally, the related adjacent geographic distribution also indicates the close relationship between these two species (Fig. 5).

Additional specimens examined. China. Shenzhen Special Economic Zone, Pingshan District, Tian Xin Shan, alt. 300 m, 22 May 2017, 114°25'E, 22°41'N, *L.H. Yang & F. Wen YLH383* (IBSC!); in the same place, alt. 300 m, 20 October 2005, S.Z. Zhang et al. 4658 (SZG!); 20 April 2005, S.Z. Zhang et al. 0384 (SZG!); 5 November 2004, S.Z. Zhang et al. SCAUF490 (SZG!), SCAUF491 (SZG!), SCAUF583 (SZG!); Longgang District, Pai Ya Shan, alt. 600 m, 25 October 2006, G.D. Wang et al. 6896 (SZG!); 13 October 2005, S.Z. Zhang et al. 4533 (SZG!); 17 December 2005, S.Z. Zhang et al. 2144 (SZG!); 8 June 2005, S.Z. Zhang et al. 2340 (SZG!); Xi Chong, Xigong village, 8 November 2004, S.Z. Zhang et al. SCAUF469 (SZG!), SCAUF470 (SZG!); Luohu District, Wu Tong Shan, alt. 900 m, 7 October 2005, S.Z. Zhang et al. 4288 (SZG!); 23 March 2005, Team of Flora of Shenzhen 013556 (SZG!); Longgang District, Dakeng reservoir, 16 July 2005, S.Z. Zhang et al. 2932(SZG!).



Figure 5. Geographical distribution of *Oreocharis guileana* (green dot), *O. baolianis* (red triangle) and *O. pilosopetiolata* (blue square).

Oreocharis baolianis (Q.W. Lin) Li.H.Yang & M.Kang, comb. nov.

urn:lsid:ipni.org:names:77211181-1 Figure 4

≡Beccarinda baolianis Q.W. Lin, (2016: 651).

Type. China. Fujian: Changting County, Sidu Town, Xiaojin, 600 m alt., 25°35'12"N, 116°7'56"E, on moist rock surfaces under evergreen broad-leaved forests, 27 March 2015, *Q.W. Lin et al. 0016* (holotype: FAFU!).

Amended description. Perennial herbs with inconspicuous rhizome. Leaves in basal rosette, 6–12; petiole 5–20 mm long, with densely-white villous; leaf blade ovate, $10-35 \times 6-32$ mm, with white villous and puberulent on both sides, abaxially more densely-white villous along veins, base broadly cuneate or rounded, margin shallowly crenate or serrate, apex subacute to rounded; lateral veins 4-5 on each side of midrib, adaxially not obvious, abaxially prominent. Cymes 3-5, axillary, 1-4-flowered; peduncles 5-40 mm long, with densely-white villous; bracts 2, narrowly lanceolate, ca. 2 × ca. 1 mm, margins entire, outside covered with white villous. Pedicel 15-30 mm long, with densely-white villous. Calyx 5-lobbed near base, lobes equal, lanceolate, ca. 3 × ca. 1 mm, outside covered with white villous, margin entire. Corolla blue-purple, the inner face at tube throat with several longitudinally purple striations, campanulate, ca. 15 mm long, outside puberulent, inside glabrescent; tube ca. 8 mm long, ca. 7 mm in diameter at the mouth, gibbous abaxially; limb distinctly 2-lipped, upper lip 2-lobbed near base, folded backward, lobes oblong, apex obtuse, ca. 5 × ca. 4 mm, lower lip 3-lobbed near base, lobes oblong, apex rounded, ca. $5.5 \times$ ca. 4 mm; stamens 4, adnate to corolla base, nearly equal in length; filaments 2.5–3 mm, glabrescent; anthers dorsifixed, free, ovate to horseshoe-shaped, thecae divaricate and confluent at apex, dehiscing longitudinally, glabrescent; staminode 1, ca. 0.5 mm long. Disc not obvious. Pistil 8–10 mm long; ovary conical, 2–3.5 mm, with densely-white villous; style ca. 5 mm long, with densely-white villous. Stigma 1, disc-shaped. Capsule 8–12 mm long, villous, dehiscing loculicidally to base, initially on one side, valves 2, capsule straight in relation to pedicel, not twisted.

Distribution and habitat. Currently, *Oreocharis baolianis* has only been found at its type locality, Sidu Twon, Changting County, Fujian Province, China (Fig. 5). Plants grow on moist rock surfaces under evergreen broad-leaved forests, at an altitude of ca. 600 m.

Note. The results of our morphological comparisons show a special status of *Oreocharis baolianis* in the enlarged *Oreocharis*. It is difficult to find a congeneric species with small plant size, campanulate corolla and short filaments. At present, we reservedly consider morphological affinities exist between *O. baolianis*, *O. guileana* and *O. pilosopetiolata* by their similar leaf blade texture, shape and margin and pubescence on most of organs and, especially, the similar pistil (short ovary densely covered with villous) between *O. baolianis* (Fig. 2I) and *O. guileana* (Fig. 1F). Although, the combined molecular data do not support a close phylogenetic relationship between *O. baolianis*

and the latter two species, we cannot rule out the above speculation due to the general low resolution in the backbone of the phylogeny of *Oreocharis* (Fig. 3). In fact, the analysis of ITS indicate that these three species form a monophyletic clade (data not shown). Further field and molecular works are needed to verify this hypothesis and to discuss the deep species relationships in the enlarged *Oreocharis*.

Additional specimens examined. The same place as type locality, 2 June 2015, *Q.W. Lin & X.B. Wang 01-03* (FAFU!); 4 July 2017, *L.H. Yang* et al. *YLH400* (IBSC!); 11 April 2018, *L.H. Yang and H.H. Kong YLH460* (IBSC!).

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Supplementary material I

Taxon, voucher information and GenBank accession number of the samples used in the phylogenetic analyses.

Authors: Lihua Yang, Fang Wen, Hanghui Kong, Zhixia Sun, Lanying Su, Ming Kang Data type: phyogenetic

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RESEARCH ARTICLE



Genetic delimitation of Oreocharis species from Hainan Island

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Abstract

Hainan Island harbours an extraordinary diversity of Gesneriaceae with 14 genera and 23 species, amongst which two species and one variety are recognised in the genus *Oreocharis*. These three *Oreocharis* taxa are all Hainan-endemics and show a complex geographical distribution pattern with considerable morphological intermixtures. In this study, we combined DNA (nuclear ITS sequences and cpDNA *trnL-trnF* and *ycf*1b) to evaluate genetic delimitation for 12 *Oreocharis* populations from the island, together with morphological similarity analysis using 16 morphological traits. The results showed Hainan *Oreocharis* taxa were monophyletic with relative low genetic diversity within populations, highly significant genetic differentiation amongst populations and a significant phylogeographical structure. The 12 populations formed three genetically distinct groups, roughly correspondent to the currently recognised two species and one unknown lineage. The PCA analyses of morphological traits indicate three distinctive groups, differing mainly in petal colour and corolla shapes. The roles of river and mountain isolations in the origin and distribution of these three lineages are discussed.

Keywords

genetic differentiation, genetic diversity, morphological similarity, Oreocharis

Introduction

Hainan Island is the largest tropical island in China, with an area of 33,920 km². As a biodiversity hotspot in the world (Myers et al. 2000), Hainan Island has a species-rich and remarkable endemic flora (Francisco-Ortega et al. 2010), which is remarkably richer in endemic genus than Taiwan Island (36,193 km²) and contains almost twice the number of Gesneriaceae species than Sri Lanka (65,610 km²), which is twice the size of Hainan Island (Ranasinghe et al. 2019). The richest biodiversity is concentrated in the southcentral mountains of the island (Li and Wang 2005; Xing 2012; Yang 2013), such as Mt. Wuzhi (the highest peak with 1867 m) and Mt. Yingge (1812 m). Xing (2012) and Ling et al. (2017) identified 14 genera and 23 species of Gesneriaceae on Hainan Island, amongst which two genera (*Metapetrocosmea* W.T. Wang and *Cathayanthe* Chun) and eight species (including one variety) are endemic (Ling et al. 2017; Jiang et al. 2017).

Interestingly, there are three recognised taxa of *Oreocharis* Bentham on Hainan Island (*O. flavida* Merrill, *O. dasyantha* Chun and *O. dasyantha* Chun var. *ferruginosa* Pan) and all are endemic to the island (Wei 2010; Xing 2012; Yang 2013; Ling et al. 2017). During three years' observation, we found these *Oreocharis* taxa to possess diverse floral syndromes in a single currently recognised species and mixed distribution of different species (Wei 2010; Xing 2012; Yang 2013), together with considerable genetic differentiations amongst populations (Xing et al. 2018). The presence of a variety, i.e. *O. dasyantha* var. *ferruginosa*, further complicates the taxonomy classification of Hainan *Oreocharis*.

Here, we sampled 12 populations of *Oreocharis* taxa covering its entire distribution range on Hainan Island and examined their molecular phylogenetic relationships with one nuclear DNA fragment and two combined chloroplast DNA sequences separately. We also quantitatively analysed 16 morphological traits with principal component analysis (PCA). We aim to determine (1) whether or not the currently recognised three species or variety can be supported by genetic data (2) what factors (e.g. geographic isolation, pollination isolation, climate or intrinsic traits) are responsible for the evolution and maintenance of these Hainan-endemic *Oreocharis*?

Materials and methods

Materials collection and DNA extraction

Twelve geographic populations of *Oreocharis* taxa covering all the suitable habitats of the genus on the island were collected, including populations DW (Dongwu in Bawangling), DE (Donger in Bawangling), FT (Futou in Bawangling), NG (Nangao), HM (Mt. Houmi), JF (Mt. Jianfeng) and CH (Chahe at the foot of Mt. Jianfeng) from *O. dasyantha*, populations QX (Mt. Qixian), WZA (Wuzhi A in Mt. Wuzhi) and WZB (Wuzhi B in Mt. Wuzhi) from *O. flavida* and populations YG (Mt. Yingge) and LM (Mt. Limu) from unidentified *Oreocharis* sp. (Table 1, Fig.1). Fresh leaves were collected from the south-central mountains in Hainan Island in 2015, 2016 and 2017 and dry

stored in silica gel. In total, 238 leaf samples from 12 populations that represented the whole geographical range of *Oreocharis* taxa on Hainan Island were collected (Fig.1).

Total genomic DNA for each individual was extracted using CTAB methods (Doyle and Doyle 1987) and served as a template for the polymerase chain reaction. AL2000 DNA marker (Aidlab Biotechnologies Co. Ltd) was used to detect DNA quality and quantity on 0.8% agarose gels stained with 2.5 μ l Goldview (Aidlab Biotechnologies Co. Ltd) in DTU-48 spectrophotometer (Hangzhou Miu Instruments Co. Ltd, China).

PCR amplification and sequencing

One nuclear ribosomal DNA (nrDNA) sequence, the ITS region comprising spacer 1, the 5.8S gene and spacer 2 (White et al. 1990) and two chloroplast DNA (cpDNA) intron-spacer region trnL-trnF (Taberlet et al. 1991) and ycflb (Dong et al. 2015) were used in this study (Table 2). PCR reactions were set up in a volume of 25 µl consisting of 20 µl ddH₂O, 2.5 µl 10×Buffer, 0.5 µl dNTPs (10 mM), 0.5 µl each 5 µM primer, 0.5 µl DNA template and 0.5 µl 5 U/µl Taq polymerase (Aidlab Biotechnologies Co. Ltd). PCR was conducted in a 2720 Thermal cycler (Applied Biosystems by Life Technologies, made in Singapore) and Veriti 96-Well Thermal Cycler (Applied Biosystems by Life Technologies, made in Singapore). The PCR programme for nrDNA and trnL-trnF was designed for an initial denaturation at 94 °C 5 min, followed by 35 cycles of 1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C and with a final extension of 10 min at 72 °C. Amplification of ycflb used the following protocol: 4 min at 94 °C, 35 cycles of 30 s at 94 °C, 40 s at 58 °C and 1 min at 72 °C, ending with 10 min at 72 °C. All the PCR products were checked by electrophoresis. Then purification and sequencing of PCR products were finally sequenced by an ABI 3730 DNA Analyzer based on the BigDye Terminator Cycle Sequencing Ready Kit (Applied Biosystems, Foster City, CA) in BGI (Beijing Genomics Institution), the chemistry and primers being used above in BGI.

The systematic position of Oreocharis taxa in Hainan Island

In order to explore the systematic position of *Oreocharis* taxa in Hainan Island, we followed Möller et al. (2011a, b) and Chen et al. (2014) and used 57 other *Oreocharis* species with suitable DNA sequences in the study. Finally, a total of 60 species were included in the phylogenetic analysis. We manually aligned all sequences using MEGA v.6.5 (Kumar et al. 2008) and excluded ambiguous positions from the alignments. The two no-coding gene ITS1/2 and *trnL-trn*F were concatenated to a single matrix by the programme SequenceMatrix v.1.7.8 (Vaidya et al. 2011) after a congruency test by PAUP* 4.0a164 (Swofford 2003). We inferred the optimal model of nucleotide substitution by MR-MODELTEST 2.3 (Nylander 2004), based on the AIC (Akaike Information Criteria) (Akaike 1981). In addition, the most suitable model GTR+I+G was used in both ML and BI analysis. Maximum Likelihood (ML) analysis was conducted using MEGA v.6.5 (Kumar et al. 2008) with the optimal substitution models to carry out 1000 bootstrap (BS) replicates. Bayesian Inference (BI) analysis was conducted using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001). The Markov Chain Monte Carlo (MCMC) was analysed for 10 million generations and sampling every 10000 generations for two independent Bayesian runs. The first 2500 trees (25% of total trees) were discarded as burn-in and the remaining trees were summarised in a 50% majority-rule consensus tree with the posterior probabilities (PP). The mean and posterior of each branch were visualised by FIGTREE v.1.4.2 (Rambaut 2009). Sequences used are showed in Appendix 2.

Genetic diversity and differentiation

The original chromatograms from both directions of the ITS and cpDNA sequences obtained were evaluated with the software BioEdit (Hall 1999) for base confirmation and contiguous sequences editing, then sequences were manually aligned, where necessary, using MEGA v.6.5 (Kumar et al. 2008) and ambiguous positions were excluded from the alignments. All sequences have been deposited in GenBank (MK587942–MK588003). Subsequently, we combined the two no-coding cpDNA regions as a single locus by the programme SequenceMatrix v.1.7.8 (Vaidya et al. 2011). Then, we performed a Partition Homogeneity Test based on the combined cpDNA and an Incongruence Length Difference Test, based on combined ITS and cpDNA using PAUP* v. 4.0a164 (Swofford 2003).

The number of nucleotypes/haplotypes, number of nucleotypes/polymorphic sites (S), nucleotype/haplotype diversity (h), nucleotide diversity (π) and measures of DNA divergence (K) values were analysed by the programme DNASP v. 6.12.01 (Rozas et al. 2017) for each population and Fu's *Fs* (Fu 1997) and Tajima's *D* (Tajima 1989) values were tested for vital deviations from the null hypothesis of neutral evolution and constant population size, based on the ITS and cpDNA sequences separately. We generated the geographical distribution of nucleotypes/haplotypes according to sampling information (Table 1).

Genetic diversity within populations (*Hs*; Nei 1973), in total populations (H_T), total gene diversity index (N_{ST}) and genetic differentiation index within populations (G_{ST}) were measured using Haplonst (Pons and Petit 1996) and G_{ST} and N_{ST} compared by the U test (Pons and Petit 1996) based on the ITS and cpDNA sequences separately.

The Analysis of Molecular Variance (AMOVA) was conducted to estimate genetic variation which was assigned within and amongst populations using GENALEX v. 6.503 (Peakall and Smouse 2012), based on the ITS and cpDNA sequences separately.

Phylogenetic relationships

Phylogenetic relationships of nucleotypes/haplotypes were inferred with BI using Mr-Bayes v. 3.2.6 (Ronquist et al. 2012). According to test above, *O. sinohenryi*, which had the closest phylogenetic relationships with the Hainan *Oreocharis* taxa, were used as outgroups with sequences of nrDNA. Prior to Bayesian analysis, the optimal model of nucleotide substitution was detected for each gene using MRMODELTEST v. 2.3 (Nylander 2004), based on the AIC (Akaike 1981). Two independent Bayesian runs of MCMC were performed for 10 million generations, sampling every 10000 generations. We accessed the Chain convergence in Tracer v. 1.7.1 (Rambaut and Drummond 2007) by checking the effective sample size (ESS) that was larger than 200 for each parameter. To further explore the relationships amongst unique nucleotypes, genealogical relationships were inferred from Median-Joining network (MJ) of NETWORK v. 5.001 (http://www.fluxus-Engineering.com/).

Neighbour-joining (NJ) tree and structure

All sequences of each population were chosen to represent effective geographic populations themselves. The method for the Neighbour-joining (NJ) tree was selected to build the phylogenetic relationship of *Oreocharis* taxa populations in Hainan Island by MEGA v.6.5, with Kimura two-parameter model (Kimura 1980), based on the ITS and two combined cpDNA sequences separately.

A Bayesian clustering approach conducted in STRUCTURE v. 2.3.4 (Earl and Vonholdt 2012) was used to detect the population genetic structure of the Hainan *Oreocharis* taxa, based on ITS and combined cpDNA sequences separately. The number of possible clusters (*K*) was set from 1 to 10 and each *K* run 10 times. Each run comprised a burn-in period of 1×10^5 interactions with 1×10^5 MCMC steps after burning. The most suitable value of *K* was determined from Structure Harvester (Pritchard et al. 2000; http://taylor0. biology.ucla.edu/structureHarvester/) by using ΔK and the log-likelihood value. Finally, the result from programme STUCTURE for the best *K* value was drawn in CLUMPAK server (Jakobsson and Rosenberg 2007; http://clumpak.tau.ac.il/index.html).

Isolation by distance (IBD)

To detect whether there was local genetic variation under geographically limited dispersal, isolation by distance (IBD) for each population was tested by a Mantel test in GENALEX between pairwise genetic distance (uncorrected sequence divergence (Dxy) for nuclear DNA and cpDNA) and geographical distance.

Morphological traits

To characterise phenotype diversity and differences amongst populations, we measured and observed 16 morphological characters, including floral and leaf traits of at least 30 individuals for each population. The measured floral traits were, (i) corolla colour (yellow tube with orange lip, yellow, orange), (ii) corolla shape and type (tubular, thin tubular, campanulate), (iii) corolla width (< 1.49 cm, 1.5 cm - 1.99 cm, > 2.0 cm), (iv) corolla mouth width (< 0.5 cm, > 0.5 cm), (v) floral tube length (< 0.99 cm, 1 cm - 1.49 cm, > 1.5 cm), (vi) sepal length (short, long) and (vii) number of petals (five, six).

Five stamen traits were included in the analyses: (i) anther position (includedanthers hidden inside the floral tube, floral throat-anthers lying in the throat of floral tube, exerted-anthers are exposed outside the floral tube), (ii) stamen type (monomorphic, didynamous), (iii) pollen presentation (simultaneous, separately for each pair), (iv) anther shape (oval, horseshoe) and (v) hair on filament (absent, present).

Two stigma characters and two leaf traits were also included in the analyses: (i) location of stigma (included-stigma hidden inside the floral tube, throat-stigma lying in the throat of floral tube, exerted-stigma is exposed outside the floral tube), (ii) number of stigma (one, two), (iii) serration of leaf edge (present, absent) and (iv) leaf epidermal hair in abaxial side (absent, present). Measurements were taken with a rectilinear scale and rounded to the nearest 0.1 mm.

Principal Component Analysis (PCA) was conducted in SPSS v. 19.0 (Liu and Li 2014) to determine the traits with the highest value for classification and the plotting map.

Results

Monophyly of the Hainan Oreocharis taxa

The combined ITS1/2 and *trn*L-F datasets of Hainan *Oreocharis* taxa with other 57 *Oreocharis* species were 568 and 871 bp long, amongst which 233 and 89 were polymorphic sites and 141 and 38 were parsimony informative sites, respectively. The aligned dataset was 1439 bp long with a total number of 305 polymorphic sites measured, of which 160 were parsimony informative sites. There was no significant incongruence, based on the incongruence length difference (ILD) test between the ITS1/2 and *trn*L-F (p > 0.05).

Both the BI and ML analysis showed Hainan *Oreocharis* taxa being monophyly with PP (posterior probability) = 0.79 and BS (bootstrap value) = 38% (Appendix 1). In addition, *O. sinohenryi*, whose regions are restricted in South China (Guangxi and Guangdong Province), is the sister to Hainan *Oroecharis* taxa in the current tree with relatively high support (Appendix 1).

Genetic diversity and differentiation

The aligned ITS sequence matrix comprised in total of 670 basepairs (bp). A total number of 56 polymorphic sites were present, of which 48 were parsimony-informative, which allowed the identification of 16 different nucleotypes from a size of 238 samples (Table 1, Fig.1a). Four nucleotypes, H1, H2, H8 and H12, were shared amongst populations, the other 12 nucleotypes were private, i.e. present in only one population (Table 1, Fig. 1a).

The combined alignment of the two cpDNA regions was in total 1615 bp long (858 and 757 bp for *trnL-trn*F and *ycf*lb, respectively) with a significant rate of homogeneity

Table 1. Sampled populations and nucleotypes/haplotypes information calculated from nrDNA and cpDNA of 12 Oreacharis populations. Private nucleotypes/ haplotypes (nucleotype/haplotype occurs in only one population) are given in Bold.

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	Haplotype (No. individuals)	H1(17)	(6) IH	H1(11)	H1(19)	(/-)	H8(51)	H8(51)	H8(51) H8(15)	H8(51) H8(51) H8(15) H9(9), H10(1	H8(51) H8(15) H8(15) H9(9), H10(1 H7(17)	H8(51) H8(15) H9(9), H10(1 H7(17) H2(32)	H8(51) H8(15) H9(9), H10(1 H7(17) H2(1), H3(2), H H5(1), H6(2)	H8(51) H8(51) H8(15) H9(9), H10(1 H7(17) H7(17) H2(1), H3(2), H H1(1), H12(1 H13(3), H14(1 H15(1) H15(1)	H8(51) H8(51) H8(15) H9(9), H10(1 H7(17) H7(17) H2(1), H6(2) H12(1), H6(2) H15(1) H15(1) H15(1) H15(1)	H8(51) H8(51) H8(15) H9(9), H10(1 H7(17) H7(17) H2(1), H3(2), H H1(1), H12(1 H13(3), H14(1 H13(3), H14(1 H12(27), H16(1) H12(27), H16(1)
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Population		DW	DE	FT	DNG		MH	MH	JF	HM JF CH	HM JF CH	HM JF CH QX WZA	HM JF QX WZA WZB	HM JF QX WZA WZB YG	HM JF WZA WZB VG YG	HM JF WZA WZB VG LM
Sampling site		Dongwu in Mt. Bawang	Donger in Mt. Bawang	Mt. Futou	Mr Nangao	IVIL: 1 VALIGAO	Mt. Houmi	Mt. Houmi	Mt. Houmi Mt. Jianfeng	Mt. Houmi Mt. Jianfeng Riverside at Chahe	Mt. Houmi Mt. Jianfeng Riverside at Chahe Mt. Qixian	Mt. Houmi Mt. Jianfeng Riverside at Chahe Mt. Qixian Mt. Wuzhi	Mt. Houmi Mt. Jianfeng Mt. Qixian Mt. Wuzhi Mt. Wuzhi	Mt. Houmi Mt. Jianfeng Riverside at Chahe Mt. Qixian Mt. Wuzhi Mt. Wuzhi Mt. Yingge	Mt. Houmi Mt. Jianfeng Riverside at Chahe Mt. Qixian Mt. Wuzhi Mt. Wuzhi Mt. Yingge	Mt. Houmi Mt. Jianfeng Riverside at Chahe Mt. Qixian Mt. Wuzhi Mt. Yingge Mt. Limu
Putative	populations	0.dasyantha					O.dasyantha	O.dasyantha var. ferruginosa	O.dasyantha var.ferruginosa	O.dasyantha var.ferruginosa	O.dasyantha var.ferruginosa O.flavida	O.dasyantha var.ferruginosa O.flavida	O.dasyantha var.ferruginosa O.flavida	O.dasyantha var.ferruginosa O.flavida	O.dasyantha var.ferruginosa O.flavidat Oreocharis sp.	O.dasyantha var. ferruginosa O.flavida Oreocharis sp.

Genetic delimitation of Oreocharis species from Hainan Island



Figure 1. Sampling sites and nucleotype and haplotype distribution of nuclear ITS (**a**) and cpDNA *trnL*-F and *ycf*1b (**b**) of *Oreocharis* lineages in Hainan Island.

(P = 1) in the congruency test, indicating that there was no significant difference in the laboratory between the two cpDNA regions. The alignment contained 55 polymorphic sites and 8 indels (Table 2). A total of 23 chloroplast haplotypes were present amongst the 238 samples (Fig. 1b, Table 1). Of these, only two haplotypes, H2 and H20, were shared amongst several populations, whereas the other 21 haplotypes were private (Table 1, Fig. 1b). The combined dataset, based on ITS and cpDNA as pairwise ILD tests, showed that the two DNA regions were not significantly different from each other (P > 0.05).

Haplotypes diversity (Hd) and nucleotide diversity (Pi) for each population are summarized in Table 1 and there is little difference between nrDNA and cpDNA. Generally, except for population WZB, YG and LM presented high genetic diversity in both nrDNA and cpDNA, nuclear gene ITS in population CH and chloroplast gene *trn*L-F and *ycf*1b in population DE, NG and HM showed variable genetic diversity, the rest of the populations having very low nucleotide and haplotype diversity (Table 1).

In total, the average intrapopulation diversity $H_{\rm S}$ was lower than the genetic diversity $H_{\rm T}$. Both in ITS and cpDNA sequences, total gene diversity index $(N_{\rm ST})$ was not significantly greater than the genetic differentiation index within populations ($G_{\rm ST}$, P > 0.05), revealing that Hainan *Oreocharis* taxa have no correspondence between haplo-type comparability and geographic distribution (Appendix 3).

The AMOVA indicated that almost all variation (99% and 97%) was partitioned amongst populations, which was higher than the variation (1% and 3%) within populations, based on the ITS and cpDNA data, respectively, revealing highly significant genetic differentiation amongst populations (Table 3).

Phylogenetic relationship

Both phylogenetic trees, based on ITS nucleotypes and cpDNA haplotypes, indicated that nucleotypes/haplotypes can be separated into three main groups with strong bayesian probabilities (> 0.95) (Figs 2, 3). The nucleotype/haplotype network of nuclear DNA and cp-DNA was concordant with the phylogenetic relationship, which presented three centrally located nodes, representing possible ancestral haplotypes with a high frequency (Figs 2b, 3b). The rest of the haplotypes were connected to the central haplotypes by one to four steps in a star-like network. In the network of nuclear DNA, nucleotypes H1 and H8 occurred at the highest frequency, indicating they probably are ancestral nucleotypes of *O. dasyantha*. In the network of cpDNA, haplotypes H12 may be the ancestral haplotypes of *O. flavida* since it was at the centrally located nodes with highest occupied frequency.

Neighbour-joining (NJ) tree and Population structure

The results of the NJ tree, based on nrDNA and cpDNA, suggested 12 populations were clearly clustered into three major groups, which well corresponded to the three defined *Oreocharis* taxa in Hainan Island, i.e. *O. dasyantha* (includes *O. dasyantha* var. *ferruginosa*),

DNA fragment	Primers sequences	S	h	PН	π	K	Fragment size	Tajima's D	Fu's Fs	Reference
ITS	ITS4: 5'TCCTCCGCTTATTGATATGC 3'	56	16	0.820 (0.02178	14.067	670 bp	1.53380	17.662***	White et al. 1990
	ITS5 HP: 5'GGAAGGAGAAGTCGTAACAAGG 3'									
trnL-F	c: 5'CGAAATCGGTAGC GCTACG 3'	16	11	0.805 (0.00428	3.460	843 bp	0.77872	2.529	Taberlet 1991
	f: 5'ATTTGAACTGGTGA CACGAG 3'									
yeftb	<i>Juft</i> bF: 5'ACATATG CCAAAGTGATGGAAAA 3'	29	12	0.871	0.01243	8.890	725 bp	2.05208	12.821^{***}	Dong et al. 2015
	yc/tbR: 5'CCTCGCCGAAAATCTGATTGTTGTGTGAAT 3'									
trnL-F and ycflF		55	23	0.887 (0.00845	13.214	1568 bp	1.33642	8.426*	

Table 2. Primers used for DNA amplification of *Oreocharis* taxa and genetic diversity. S, polymorphic sites, h, number of haplotypes, Hd, haplotypes diversity, π , nucleotide diversity, K, average number of nucleotide difference.

equences in Oreocharis taxa.
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DNA fragment	H_s	H_{T}	$G_{_{ST}}$	$N_{_{ST}}$	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation (%)	$F_{ m ST}$	r
ITS	0.170	0.884	0.808	0.982	Amongst populations	11	2353.579	213.962	11.176	%66	
					Within populations	226	28.698	0.127	0.127	1%	2382.277
cpDNA	0.215	0.913	0.765	0.977	Amongst populations	11	2497.917	227.083	11.848	97%	
					Within populations	226	88.752	0.393	0.393	3%	2586.668


Figure 2. Bayesian Inference tree (**a**) using MrBayes and network (**b**) showing the genetic relationships amongst the observed ITS nucleotypes of Hainan *Oreocharis* populations. Numbers on branches indicate the bootstrap values for MP/MB and posterior probability. The relative sizes of the circles in the network are proportional to the nucleotype frequencies and missing nucleotypes are represented by a small black spot.

O. flavida and *Oreocharis* sp. Additionally, the analyses also presented a close relationship between *O. flavida* and *Oreocharis* sp., then with *O. dasyantha*.

Although the signal was stronger for cpDNA (Rxy = 0.473, P < 0.001) than for nuclear DNA (Rxy = 0.257, P < 0.001), the relationship between genetic and geographical distance for 12 populations was significant both in nuclear DNA and cpDNA (Appendix 3).

Ordinations of morphological traits

According to the floral syndromes, the Principal Component Analysis of 16 floral characters of Hainan *Oreocharis* populations can be divided into three clusters (Fig. 5): (1) tubular, zygomorphic flowers with yellow tube but orange limbs, monomorphic stamens, pol-



Figure 3. Bayesian Inference tree (**a**) and network (**b**) of *trn*L-F and *ycf*1b haplotypes of *Oreocharis* populations in Hainan Island. Posterior probabilities are given above branches. The relative sizes of the circles in the network are proportional to the haplotype frequencies and missing haplotypes are represented by a small black spot.

len presentation separated (populations DW, DE, FT, NG, HM, CH and JF of *O. dasyantha* and *O. dasyantha* var. *ferruginosa*); (2) campanulate, zygomorphic, orange flower with included stamen and stigma (populations WZA, WZB and QX of *O. flavida*); (3) thin tubular, zygomorphic yellow flower with included didynamous stamens (populations YG and LM of *Oreocharis* sp.). The corolla colour and corolla shapes may play a key role in ordinations of morphological traits with high values of 45.41% and 34.04%, followed by location of stigma and length of the corolla tube with the values 9.293% and 5.272%.

Discussion

Monophyly of the Hainan Oreocharis taxa

The phylogenetic tree showed that Hainan *Oreocharis* taxa are monophyly (Appendix 1), suggesting a single dispersal of *Oreocharis* into Hainan Island. The sister species to Hainan *Oreocharis* is *O. sinohenryi*, which is restricted to South China including Guangxi and

Guangdong provinces. Hainan Island is only about 30 km from these provinces, thus such observed pattern can be simply explained by geographic relationships.

Genetic diversity and structure

Most *Oreocharis* populations hold very low nucleotide and haplotype diversity (Table 1) and overall populations revealed a high level of genetic differentiation (Table 3) and a significant phylogeographical structure. The three groups, i.e. *O. flavida*, *O. dasyantha* (including the variety *O. dasyantha* var. *ferruginosa*) and an unknown species showed clear geographical isolations associated with three different river ranges. *O. dasyantha* are found mostly in the watershed of the Changhua River (the secondly largest river on the island), *O. flavida* distributes in Mt. Wuzhi and Mt. Qixian, the upper reaches of the Wanquan River. The unknown species is restricted in the upper reaches of the largest river on the island, i.e. Nandu River. We concluded that these three groups may have evolved and maintained largely through allopatric differentiations.

Mountains can also probably explain such observed pattern with geographic isolation of these groups. Almost all *Oreocharis* populations in Hainan Island were restricted in > 1000 m high-elevation mountains with massive humidity, such that the islandlike habitat became fragmented caused by a deep and wide valley in the complicated mountains system, which resulted in blocking of gene flow of *Oreocharis* populations with weak seed dispersal ability even at the fine scale (Xing et al. 2018).

Genetic differentiation and species delimitation

Li et al. (2019) found that geographic isolation by Changhua River is a driving force for the strong population differentiation in the Hainan-endemic *Primulina heterotricha* Merr. and *Metapetrocosmea peltata* (Merr. et Chun) W. T. Wang. Our results can also be explained by the isolation of Changhua River (Figs 1, 4), which indicated that Changhua River may play a key role in driving population divergence and speciation of the Hainan *Oreocharis* taxa (Xing et al. 2018; Li et al. 2019).

Secondly, the 'sky island' caused by high mountains may also cause such genetic differentiation for montane species (Palma-Silva et al. 2011; Tapper et al. 2014; Robin et al. 2015). Mountain tops in Hainan Island have tropical mountain cloud forests (> 1200 m) in Mt. Wuzhi, Mt. Yingge, Mt. Bawang, Mt. Jianfeng and Mt. Limu (Wang et al. 2016) which fragmented and restricted the island-like habitat of *Oreocharis* taxa. Alpine plant radiations have accelerated speciation with trait diversification (Sanderson 1998; Colin and Ruth 2006; Hughes and Atchison 2015) and, in general, these radiations are geared to be recent and rapid (Linder 2008). Almost all *Oreocharis* taxa in Hainan Island lived in high mountains except Population CH, which grew in a low-altitude habitat and held a distinct structure from other high-altitude populations of *O. dasyantha*, indicating the sky-island effect may drive population divergence and speciation.



Figure 4. Neighbour-joining (NJ) tree based on ITS (**a**) and combined *trn*L-F and *ycf*1 (**c**) with the results of STRUCTURE, based on ITS (**b**) and combined *trn*L-F and *ycf*1 (**d**).

According to morphological traits, all the 12 *Oreocharis* populations were also grouped into three clusters and corolla colour, shape and types are the main characters for distinguishing groups (Fig. 5; Ling et al. 2017). Such differences in floral



Figure 5. Principle Component Analysis of 16 morphological traits for the Hainan *Oreocharis* populations. Different clusters are shown in red circles.

syndromes further indicate the *Oreocharis* on Hainan Island should be recognised as three different lineages (species). Besides two species (includes one variety) currently recognised in Hainan Island, populations from Mt. Limu and Mt. Yingge should be treated as a new species or subspecies, which is still in need of further illumination.

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Appendix I



Molecular phylogeny for Hainan *Oreocharis* taxa and 57 *Oreocharis* species based on combined ITS1/2 and *trnL-trnF*, there was no significant incongruence based on the incongruence length different (ILD) test between the ITS1/2 and *trnL-trnF*. Bayesian posterior probability (PP) and ML bootstrap values (BS) are showed above branches. Hainan *Oreocharis* taxa were showed in grey.

Appendix 2

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

Species	trnL-trnF	ITS1/2	Voucher number
Oreocharis acaulis(Merr.) Mich.Möller & A.Weber	HQ633012	HQ632916	M.Möller MMO 09-1605
Oreocharis amabilis Dunn	KM232654.1	KJ475433.1	Carles 587
Oreocharis argyreia Chun ex Pan	HQ632919.1	HQ633015.1	M.Möller MMO 07-1131
Oreocharis aurea Dunn	KM062914.1	KM063154.1	M.Möller MMO 06-980
Oreocharis auricula (S.Moore) C.B.Clarke	FJ501482.1	DQ912664.1	M.Möller MMO 03-304
<i>Oreocharis begoniifolia</i> (H.W.Li) Mich.Möller & A.Weber	KM062926.1	KM063166.1	M.Möller MMO 08-1221
Oreocharis benthamii C.B.Clarke	JF697584.1	JF697572.1	M.Möller MMO 08-1317
Oreocharis brachypodus J.M. Li & Z.M.Li	KR476564.1	KR337019.1	Jia-Mei Li 2304
<i>Oreocharis burttii</i> (W.T.Wang) Mich.Möller & A.Weber	JF697582.1	JF697570.1	F.Wen 2010-05
Oreocharis chienii (Chun) Mich.Möller & A.Weber	KM062908.1	KM063148.1	JXU0008123
Oreocharis cinnamomea Anthony	KM062921.1	KM063161.1	PE-02053073
<i>Oreocharis concava</i> (Craib) Mich.Möller & A.Weber	KM062930.1	KM063170.1	PE-02053062
<i>Oreocharis convexa</i> (Craib) Mich.Möller & A.Weber	FJ501337.1	FJ501506.1	M.Möller MMO 01-176
Oreocharis cordatula (Craib) Pellegrin	KM062922.1	KM063162.1	PE-02053432
<i>Oreocharis cotinifolia</i> (W.T.Wang) Mich.Möller & A.Weber	HQ632914	HQ633010	Q.M.Chuan 01
Oreocharis craibii Mich.Möller & A.Weber	HQ632921	HQ633017	M.Möller MMO 07-1072
<i>Oreocharis dalzielii</i> (W.W.Sm.) Mich.Möller & A.Weber	JF697571	JF69783	F.Wen 2010-06
Oreocharis dentata A.L.Weitzman & L.E.Skog	KM062916.1	KM063156.1	GH00353683
Oreocharis dimorphosepala (W.H. Chen & Y.M. Shui) Mich.Möller	KM062925.1	KM063165.1	Y. M.Shui & al. 85333
Oreocharis dinghushanensis (W.T.Wang) Mich. Möller & A.Weber	GU350643	GU350675	Lin Q.B. LQB06-01
<i>Oreocharis duyunensis</i> Z.Y. Li, X.G. Xiang &Z.Y. Guo	MG722858.1	MG722856.1	PE-02114626
Oreocharis elliptica Anthony	KM063155.1	KM062915.1	CDBI0130369
Oreocharis esquirolii Léveillé	HQ633011	HQ632915	D.W.Zhang 723
<i>Oreocharis eximia</i> (Chun ex K.Y.Pan) Mich.Möller & A.Weber	KM062919.1	KM063159.1	PE-02052811
Oreocharis farreri (Craib) Mich.Möller & A.Weber	JF697585	JF697573	Zhou Ping ZP 2010-020
Oreocharis georgei Anthony	KM062917.1	KM063157.1	PE-02053075
Oreocharis hekouensis (Y.M.Shui & W.H.Chen) Mich.Möller & A.Weber	KM062934.1	KM063174.1	KUN-1219106
Oreocharis henryana Oliver	JF697586.1	JF697574.1	CSH0017984
Oreocharis heterandra D.Fang & D.H.Qin	KM232655.1	KJ475432.1	PE-02052999
Oreocharis hirsuta Barnett	KM062913.1	KM063153.1	Put 3428
<i>Oreocharis humilis</i> (W.T.Wang) Mich.Möller & A.Weber	GU350633	GU350665	Liang R.H.SC-YB

Species	trnL-trnF	ITS1/2	Voucher number
<i>Oreocharis jiangxiensis</i> (W.T.Wang) Mich.Möller & A.Weber	HQ633029	HQ632933	M.Möller MMO 09-1451
Oreocharis jinpingensis W. H. Chen & Y. M.	KM062923.1	KM063163.1	Y.M. Shui et al. 91309
<i>Oreocharis lancifolia</i> (Franch.) Mich.Möller & A.Weber	HQ632924	HQ633020	M.Möller and P.Zhou MMO 09-1624
Oreocharis leiophylla Wang	GU350676	GU350644	Zhou X.R. ZXR-05-01
<i>Oreocharis longifolia</i> (Craib) Mich.Möller & A.Weber	HQ632934	HQ633030	M.Möller MMO 08-1239
Oreocharis lungshengensis (W.T.Wang) Mich.Möller & A.Weber	HQ632917	HQ633013	M.Möller MMO 06-916
Oreocharis magnidens Chun ex Pan	HQ632930.1	HQ633026.1	PE-02052989
<i>Oreocharis mileensis</i> (W.T.Wang) Mich.Möller & A.Weber	KM063145.1	KM063182.1	KUN-1385472
<i>Oreocharis muscicola</i> (Craib) Mich.Möller & A.Weber	DQ912665	FJ501548	Kew (1995-2229)
<i>Oreocharis nanchuanica</i> (K.Y.Pan & Z.Y.Liu) Mich. Möller & A.Weber	KM062924.1	KM063164.1	KUN-1385365
Oreocharis pankaiyuae Mich.Möller & A.Weber	HQ632925	HQ633021	PE-02053064
<i>Oreocharis primuliflora</i> (Batalin) Mich.Möller & A.Weber	HQ633019	HQ932923	PE-02053071
<i>Oreocharis primuloides</i> (Miq.) Benth. & Hook.f. ex Clarke	FJ501546.1	FJ501364.1	PE-01270488
<i>Oreocharis rhombifolia</i> (K.Y.Pan) Mich.Möller & A.Weber	GU350632	GU350664	PE-02053532
<i>Oreocharis ronganensis</i> (K.Y.Pan) Mich.Möller & A.Weber	HQ633023	HQ632927	PE-00030693
<i>Oreocharis rosthornii</i> (Diels) Mich.Möller & A.Weber	KM062928.1	KM063168.1	ZY0001346
Oreocharis rotundifolia Pan	KM062911.1	KM063151.1	PE-00030861
Oreocharis saxatilis (Hemsl.) Mich.Möller & A.Weber	KM062932.1	KM063172.1	JIU05295
Oreocharis sericea Léveillé	KM232656.1	KJ475407.1	CSF1059560
Oreocharis sinensis (Oliv.) Mich.Möller & A.Weber	HQ632912	HQ633008	IBSC-0548658
Oreocharis sinohenryi (Chun) Mich.Möller & A.Weber	HQ632913.1	HQ633009.1	M.Möller MMO 07-1150
Oreocharis speciosa (Hemsl.) Mich.Möller & W.H. Chen	KM062909.1	KM063149.1	K000858093
<i>Oreocharis stewardii</i> (Chun) Mich.Möller & A.Weber	HQ632926	HQ633022	M.Möller MMO 06-917
<i>Oreocharis urceolata</i> (K.Y.Pan) Mich.Möller & A.Weber	KM062920.1	KM063160.1	M.Möller MMO 09-1633
Oreocharis wangwentsaii Mich.Möller & A.Weber	GU350658	GU350689	Liang R.H.YN-Qj
Oreocharis xiangguiensis W.T.Wang & K.Y.Pan	HQ632932.1	HQ633028.1	JIU04686
Oreocharis dasyantha Chun	MK587993	MK587954	S.Jun Ling 20181124-02
Oreocharis flavida Merr.	MK587990	MK587947	S.Jun Ling 20181126-01
Oreocharis dasyantha Chun var. ferruginosa K.Y. Pan	MK587992	MK587956	S.Jun Ling 20181124-05
Oreocharis sp.	MK587948	MK587987	S.Jun Ling 20181205-01





The results graph of the relationship between genetic and geographical distance for 12 populations based on the (a) ITS1/2 and (b) cpDNA

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	DW I	DE	FT	H DN	M	JF (W HC	ZM WZ	B Q	X	TIN	1 Total Variance Explained
corolla color, coded as (0) yellow with orange, (1) yellow, (2) orange	0	0	0	0	0	0	0	2 2	2	1	1	45.406%
corolla shape and type, coded as (0) conical, (1) thin tubular, (2) campanulate	0	0	0	0	0	1	0	2	2	1	1	34.040%
corolla size, coded as (0) <1.49 cm, (1) 1.5 cm<-<1.99 cm, (2) >2.0 cm	1	1	1	1	1	1	1	1 1	0	0	2	0
corolla mouse width, coded as $(0) < 0.5$ cm, $(1) > 0.5$ cm	1	1	1	1	1	0	1	1 1	1	0	0	0
length of tube, coded as (0) <0.99 cm, (1) 1 cm <-<1.49 cm, (2) >1.5 cm	2	2	5	2	2	2	2	1 1	0	1	1	5.272%
length of sepal, coded as (0)short, (1) long	0	0	0	0	0	0	0	0 (1	0	0	0
number of petal, coded as (0) five, (1) six	0	0	0	0	0	0	0	0 (0	0	1	0
location of stamens, coded as (0) included, (1) throat and (2) exerted	2	2	5	2	2	0	5	0 (0	1	1	0
types of stamens, coded as (0) equal length, (1) didynamous stamens	0	0	0	0	0	0	1	0 (0	1	1	0
pollen presentation, coded as (0) simultaneous, (1) separated	0	0	0	0	0	0	-	0 (0	0	0	0
anther shape, coded as (0) oval shape, (1) horseshoe stage	0	0	0	0	0	0	0	1 1	1	1	1	0
hair on stamen, coded as (0) absent, (1) exist	1	1	1	1	1	1	0	1 1	1	0	0	0
location of stigma, coded as (0) included, (1) throat and (2) exerted	2	2	5	2	5	1	5	0 (0	0	0	9.293%
number of stigma, coded as (0) one, (1) two	0	0	0	0	0	0	0	0 (0	1	1	0
serration pf leaves edge, ceded as (0) absent, (1) exist	0	0	0	0	0	0	0	1	1	1	1	3.280%
leaf epiderrmal hair on abaxial side , ceded as (0) absent, (1) exist	1	1	1	1	1	1	-	0 (0	1	1	0
Note: DW (Dongwu in Mt. Bawang), DE (Donger in Mt. Bawang), FT (Futor	u in Mt	. Baw	ang),	NG (I	Mt. N	angao), HM	(Mt. Ho	umi), J	F (M	t. Jianf	eng), CH (Riverside at Chahe),

WZA (Mt. Wuzhi A), WZB (Mt. Wuzhi B), QX (Mt. Qixian), YG (Mt. Yingge), LM (Mt. Limu)

RESEARCH ARTICLE



Four new species of Oreocharis (Gesneriaceae) in Yunnan province, China

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Abstract

Four new species of *Oreocharis* (Gesneriaceae) are described and illustrated. These new species grow in pairs in montane forests in Yunnan province, China. One pair grows in Wenshan county, Southeast Yunnan, viz. *Oreocharis eriocarpa* W.H. Chen & Y.M. Shui and *O. wenshanensis* W.H. Chen & Y.M. Shui and another pair grows in Yongde county, Southwest Yunnan, viz. *O. fulva* W.H. Chen & Y.M. Shui and *O. lacerata* W.H. Chen & Y.M. Shui. Their morphological and geographical relationship with similar species is discussed and the IUCN endangered status is provided, based on the available data.

Keywords

Flora of Yunnan; Montane forests; New species; South-western China; Subtropic regions; Yellow flowers

Introduction

In China, Southeast Yunnan and Southwest Yunnan are rich in species diversity of the genus *Oreocharis* s.l. (Gesneriaceae) (Fig. 1, Li and Wang 2005, Möller et al. 2011). Firstly, bordering North Myanmar, SW Yunnan includes Baoshan, Dehong Dai and Jingpo Autonomous Prefecture, Lincang and Pu'er districts with 11 species of the genus, viz. *O. begoniifolia* (H.W.Li) Mich.Möller & A.Weber, *O. concava* (Craib) Mich.



Figure 1. The localities of the four new species and the other species of *Oreocharis s.l.* from SW Yunnan and SE Yunnan in SW China. The districts are shown in Yunnan province, SW China.

Möller & A.Weber var. angustifolia (K.Y.Pan) Mich.Möller & A.Weber, O. convexa (Craib) Mich.Möller & A.Weber, O. flabellata (C.Y.Wu ex H.W.Li) Mich.Möller & A.Weber, O. longifolia (Craib) Mich.Möller & A.Weber, O. shweliensis Mich.Möller & W.H.Chen, O. rhytidophylla C.Y.Wu ex H.W.Li, O. tsaii Y.H.Tan & Jian W.Li and O. yunnanensis Rossini & J.Freitas (Li and Wang 2005, Möller et al. 2011, Tan et al. 2013, Rossini and Freitas 2014, Tan et al. 2015). Amongst them, O. concava var. angustifolia, O. flabellata, O. begoniifolia, O. rhytidophylla, O. tsaii and O. yunnanensis are endemic to SW Yunnan. Amongst the two species without morphology of flowers in the protologue (Li 1983), O. rhytidophylla has been supplemented (Zhang et al. 2019) and O. flabellata has still not been confirmed up to now. Secondly, bordering Vietnam, SE Yunnan includes Honghe Hani and Yi Autonomous Prefecture and Wenshan Zhuang and Miao Autonomous Prefecture with 10 species of the genus, viz. O. amabilis Dunn, O. aurea Dunn, O. aurea var. cordato-ovata C. Y. Wu ex H. W. Li, Oreocharis dimorphosepala (W. H. Chen & Y. M. Shui) Mich. Möller, O. hongheensis W.H. Chen & Y.M. Shui, O. jinpingensis W.H. Chen & Y.M. Shui, O. mileensis (W.T.Wang) Mich. Möller & A.Weber, O. obliqua C. Y. Wu ex H. W. Li, O. rosthornii (Diels) Mich. Möller & A.Weber var. wenshanensis (K.Y.Pan) Mich. Möller & A.Weber and O. rotundifolia K. Y. Pan. Amongst these O. amabilis, O. aurea var. cordato-ovata, O. dimorphosepala, O. hongheensis, O. jinpingensis, O. obliqua, O. rosthornii var. wenshanensis and O. rotundifolia are endemic to SE Yunnan (Li and Wang 2005, Möller et al. 2011, Chen et al. 2012, 2013, 2017, Cai et al. 2015). Nevertheless, the recent exploration reveals that there are an additional four new species needing to be described in the genus from Yongde county of Lincang District in SW Yunnan and Wenshan county of Wenshan



Figure 2. Oreocharis eriocarpa W.H.Chen & Y.M.Shui, sp.nov. (A–F) A habit B leaf adaxial surface C leaf abaxial surface D bracts E ovary and disc F dense public entropy and disc; O. wenshanensis (G–K)
G habitat H plant I leaf adaxial surface J leaf abaxial surface K flower. Photography by He De-Ming (A–E, K), Zhang Ting (E), Chen Li (F), Shui Yu-Min (G, I, J).

Zhuang and Miao Autonomous Prefecture in SE Yunnan (Figs 2, 3). The new findings complement the species richness of the genus in the above regions in China (Fig. 1; Wang et al. 1990, Wang et al. 1998 onwards, Li and Wang 2005, Liu and Peng 2010, Shui and Chen 2010).



Figure 3. *Oreocharis fulva* W.H.Chen & Y.M.Shui, sp.nov. (**A–F**) **A** habitat **B** leaf abaxial surface **C** leaf abaxial surface **D** flower **E** open corolla **F** calyx and ovary; *O. lacerata* (**G–M**) **G** plant **H** leaf adaxial surface **J** lateral view of flower **K** lateral opened flower **L** disc and ovary **M** seeds. Photography by Li Yong-Liang (**A**, **G**), Shui Yu-Min (**B–G**, **H**, **I**, **L**), Chen Li (**J–M**).

The four new species have been recently confirmed, based on the morphological evidence instead of molecular data in the expanded genus. Firstly, the recent combined analysis of ITS and *trn*L-F revealed the possible rapid radiation and low resolution of the phylogenetic trees (Möller et al. 2011, Chen et al. 2014), implying that the molecular data from few molecular makers just provide affinity between similar endemic species rather than whether or not they are conspecific. Therefore, more molecular makers, transcriptome or genome data will need to be adopted to resolve the above question. Secondly, based on the recent phylogenetic study, the genus seems to be divided into two groups, which are dominated respectively by diandrous or tetrandrous flowers with purple flowers, south-eastern China distribution and usually less than 1600 m elevation and by tetrandrous flowers with yellow flowers, south-western China and usually more than 2000 m elevation (Möller et al. 2011, Chen et al. 2014, Zhang et al. 2018). Thus, the four new species which we proposed should be a member of the second group because of the tetrandrous, yellow-flowered and more than 2000 m elevation. Thirdly, amongst the second group, we consulted the actual specimens and on-line images in the important herbaria in China (KUN, PE) and worldwide (BM, E, K, P) and confirmed the potential similarity of the new species we proposed. Furthermore, due to the high endemism in the genus, we paid more attention to the species growing in the same regions and designed an identification key to differentiate the new species and the other species of the two species groups, respectively from SW Yunnan and SE Yunnan, China. Finally, we provided both the tables showing the differences between the new species and the most similar species, as well as colour figures showing their detailed and actual morphology besides their illustration.

Key to the species of Oreocharis from SW Yunnan and SE Yunnan, China

Flower purple
Flower yellow or orange
Leaf blade round, base cordate
Leaf blade lanceolate or elliptic, base cuneate
Stamens exserted (SW Yunnan)
Stamens included (SE Yunnan) O. dimorphosepala
Leaf blade lanceolate, acuminate on the top (SE Yunnan)5
Leaf blade elliptic, obtuse on the top (SE Yunnan)
Corolla tube-form, ovary and fruit glabrous (SE Yunnan)
Corolla narrowly campanulate, ovary and fruit pubescent
Leaf blade surfaces white pubescent, staminode 1–2.2 mm (SE Yunnan)
O. rosthornii var. wenshanensis
Leaf blade rust-brown villous, staminode 2.5–3 mm (SW Yunnan)
O. shweliensis
Corolla narrowly campanulate, yellow with purple dots inside (SE and SW
Yunnan) O. longifolia

_	Corolla tubiformis, yellow without purple dots inside 8
8	Ovary and fruit pubescent
-	Ovary and fruit glabrous10
9	Leaf blade ovate, base cordate (SW Yunnan)
_	Leaf blade narrowly elliptic, base cuneate (SW Yunnan)
	O. concava var. angustifolia
10	Leaf blade broadly ovate or ovate, base cordate11
-	Leaf blade elliptic or lanceolate, base cuneate or shallow cordate13
11	Corolla tube constricted at throat12
-	Corolla tube not constricted at throat (SW Yunnan)
12	Calyx lobes more than 1/2 longer than corolla tube (SE Yunnan)
	O. rotundifolia
_	Calyx lobes less than 1/5 longer than corolla tube (SW Yunnan) O.tsaii
13	Stamens not exserted
_	Stamens exserted (SE Yunnan)
14	Calyx connate
-	Calyx free
15	Leaf blade lobed up to 1/3 (SW Yunnan) U. <i>lacerata</i> sp. nov.
-	
16	Adaxial corolla lips emarginate to undivided
_ 17	Adaxial corolla lips 2-lobed (SE funnan)
1/	Filaments glabrous, disc 2–3 mm, 3-tobed (3 w Tumnan)
	Filaments sparsely puberulent disc ca. 1 mm. entire (SW Vunnan)
_	I hamenes sparsely publication, disc ca. I min, entite (5 w Tulman)
18	Abavial lins 2-lobed adavial lins 3-lobed 19
	Abayial lip emarginate to undivided adayial lips 4-lobed (SW Yunnan)
	O mileensis or O amabilis
19	Leaf blade apex acute 20
_	Leaf blade apex retuse
20	Filaments pubescent, staminode ca. 0.5 mm (SE and SW Yunnan)
20	O. aurea var. aurea
_	Filaments white villous, staminode ca. 2 mm (SE Yunnan)
	O. aurea var. cordato-ovata
21	Plants golden-brown villous (SW Yunnan)
_	Plants dense brown pubescent
22	Leaf blade adaxially sparsely villous (SE Yunnan)
_	Leaf blade adaxially glabrous (SW Yunnan)

The above new discovery depended on the long-term field exploration from the local forestry staff. In general, most of the species in *Oreocharis* are prone to grow on the north-facing shady slope nearby the summit, especially in SW China (Li and Wang 2005) and so it is difficult to find them in the field except in inaccessible localities. For example, as for *Orocharis wenshanensis* W.H.Chen & Y.M.Shui in the core region of Wenshan Laojunshan National Nature Reserve, the staff (DMH in authorship) of the Natural Reserve had searched for it for several years since 2008 and found it in 2013 even if the preliminary record was from the previous intergraded surveys (Shui et al. 2008). Another example is from the staff member (YLL) of the forestry department of Yongde county. He also went to the core regions of Yongde Daxueshan National Nature Reserve to search for it in 2013 even if the information on record was from the previous intergraded surveys in 2003 (Liu and Peng 2010). Therefore, during the exploration of the genus, the local staff provided considerable contributions to the new discovery for science and to the conservation of the regional biodiversity.

Taxonomy

Oreocharis eriocarpa W.H.Chen & Y.M.Shui, sp. nov. urn:lsid:ipni.org:names:77211183-1 Fig. 4

Diagnosis. The new species is similar to *Oreocharis concava* (Craib) Mich.Möller & A.Weber, but different in broadly ovate bracts (*vs.* narrowly oblong to obovate), corolla strigose outside (*vs.* pubescent), calyx 5-sect up to 1/3 from base (*vs.* above middle) and ovary and fruit pubescent (*vs.* glabrous).

Type. CHINA. Yunnan Province: Wenshan Zhuang and Miao Autonomous Prefecture, Wenshan county, Laohuilong community, Laowuji village, 103°51'13.17"E, 23°20'29.35"N, alt. 2168 m, on rocks in the forests, 30 July 2013, *Shui Y.M., He D.M. et al. B2013-304* (holotype, KUN; isotype, PE).

Description. Herb perennial and stemless, rhizomatous. Leaves basal; petiole 4-8 cm, densely brown villous; leaf blade ovate, thickly chartaceous, $4.0-5.2 \times 3.0-$ 3.5 cm, adaxially setulose and rugose, abaxially glabrous amongst veins, sparsely brown villous along veins, base cordate, apex acute, margin biserrate, lateral veins 5-6 on each side of midrib, indistinct adaxially and distinct abaxially. Inflorescences axillary; peduncle 9-13 cm, densely brown villous; bracts 2, leaf-like, broadly ovate, 7-10 × ca. 5 mm, adaxially glabrous and abaxially villous, margin serrate. Pedicel 1-2.5 cm, villous. Calyx ca. 6 mm, 5-sect from 1/3; segments equal, triangular, ca. $4 \times 1.5-2$ mm, margin entire below top, top crenate, adaxially glabrous, abaxially pubescent. Corolla yellow, 1.6-2.9 cm, outside strigose and inside glabrous; tube campanulate-cylindric, gradually slightly widening from middle of tube, 1.3-2 cm, 0.3-0.4 cm in diam., throat not constricted; limb 2-lipped; adaxial lip smaller, 3-5 mm, emarginate; abaxial lip larger, 3-lobed, lobes oblong, apex rounded, central lobe ovate, ca. 7×5 mm, lateral lobes rotund, ca. 5×5 mm. Stamens 4, coherent in 2 pairs, included, adaxial stamens 3–6 mm, adnate to corolla tube 7-13 mm from base, abaxial stamens 3-4 mm, adnate to corolla tube 13-16 mm from base; filaments tender, glabrous; anthers basifixed, oblong, 2-loculed, dehiscing longitudinally; staminode ca. 0.5 mm, adnate to corolla tube ca. 8 mm. Disc ring-like, 1–2 mm, 5-lobed shallowly. Pistil 0.9–1.8 cm, pubescent; ovary oblong, pubescent, 0.5–1.2 cm, 1-loculed; style glabrous, 0.4-0.6 cm; stigma 1, 2-lobed. Capsule straight, narrowly oblong, 3.4-4 cm × 0.8-0.9 cm, existing style ca. 0.7 cm. Seeds not seen.



Figure 4. Oreocharis eriocarpa W.H.Chen & Y.M.Shui, sp. nov. **A** plant **B** lateral view of flower **C** frontal view of flower **D** opened corolla showing stamens, anthers, disc and staminode **E** pistil showing ovary, disc and stigma. (Drawn by Ling Wang from holotype).

Distribution, habitat and phenology. The new species is endemic to SE Yunnan of China, on rocks or limestone cliffs. Flowering is July–September; and fruiting is October–January the following year.

Conservation status. So far, there are two populations of the new species observed in the field (Fig. 1). One is in the type locality at the core position of the nature reserve with ca. 500 mature individuals and ca. 10, 000 m² (100 m × 100 m) area, the other is the Pingbian county with ca. 120 mature individuals and ca. 1, 200 m² (20 m × 60 m) area. According to the IUCN Red List Categories and Criteria, the new species is hereby assessed as "Vulnerable (VU)" (D1+D2). (IUCN 2012).

Characters	O. eriocarpa sp.nov.	O. concava
leaves	thick-chartaceous	thin chartaceous
abaxial surface of leaf	glabrous amongst veins	densely white pubescent amongst veins
corolla	strigose outside	pubescent outside
bracts	broadly ovate, 7–10 × ca. 5 mm	narrowly oblong to obovate, $4-7 \times 1-2$ mm
calyx	ca. 6 mm, 5-sect up to 1/3 from base, lobes	7–10 mm, 5-sect up to 1/4 from middle,
	3–4 mm long	lobes 1–2.5 mm long
ovary	densely pubescent	glabrous
fruit	pubescent	glabrous
elevation	ca. 2100 m	3100–3600 m
distribution	Southeast Yunnan, China	Northwest Yunnan, China

Table 1. Differences in characters between Oreocharis eriocarpa and O. concava in Gesneriaceae.

Additional specimens examined (paratype). CHINA, Yunnan Province: Honghe Hani and Yi Autonomous Prefecture, Pingbian county, Heping community, Baige village,103°52'36.71"E, 23°17'24.68"N, 26 August 2015, *Shui Y.M. et al. B2015-315A* (KUN). Wenshan Zhuang and Miao Autonomous Prefecture: Wenshan county, Laohuilong community, Laowuji village, Matangqing, 103°51'14.33"E, 23°20'29.96"N, in fruits, 20 October, 2012, *De-Min He and Ting Zhang WSLJS558* (KUN); the same locality, 103°51'13.17"E, 23°20'29.35"N, on rocks in the forests, alt. 2168 m, in flower, 16 August 2018, *Ting Zhang, De-Min He and Yan-Fei Feng 18CS17589* (KUN).

Etymology. The species is named after the pubescent fruits (Fig. 2F).

Note. The new species is endemic to the border regions of Honghe Hani and Yi Autonomous Prefecture and Wenshan Zhuang and Miao Autonomous Prefecture in SE Yunnan (Fig. 1), at ca. 2100 m elevation. It is unique in its pubescent ovary and fruits (Fig. 2A–F). Its similar species (*Oreocharis concava*) is distributed in Northwest Yunnan at 2,800–3,600 m elevation, viz. Dali Bai Autonomous Prefecture, Lijiang District and Deqin Zang Autonomous Prefecture (Fig. 1). Table 1 shows the other differences between the above two species. Thus, there is an obvious geographical substitution between the above two species. Besides, after the examination of type specimens in PE and consulting the literature (Pan 1987, Li and Wang 2005, Möller et al. 2011), the new species seems similar to its another variety [*Oreocharis concava* var. *angustifolia* (K.Y.Pan) Mich.Möller & A.Weber] in the pubescent ovary, but obviously different from it in its ovate blade (*vs.* elliptic). It is possible that the latter variety might be a different species from the original variety and needs to be explored in the future.

Oreocharis fulva W.H.Chen & Y.M.Shui, sp. nov.

urn:lsid:ipni.org:names:77211184-1 Fig. 5

Diagnosis. The new species is similar to *Oreocharis georgei* J. Anthony, but different in shallowly cordate leaf base (*vs.* narrowly cuneate), corolla ca. 2.4 cm long (*vs.* 1.4–1.9 cm), abaxial lip of corolla ca. 12 mm long (*vs.* 5–6 mm), the corolla throat not constricted (*vs.* constricted) and stamens coherent in 2 pairs (*vs.* separated).



Figure 5. *Oreocharis fulva* W.H.Chen & Y.M.Shui, sp. nov. **A** plant **B** opened corolla showing stamens and staminode **C** lateral view of flower **D** fruit **E** face view of the corolla, showing the large abaxial limb lobes. (Drawn by Ling Wang from holotype).

Type. CHINA. Yunnan Province: Lincang district, Yongde county, Daxue Mt., on rocks at forest margins along slope, 99°41'25"E, 24°11'50"N, elev. 2,000 m, 13 September 2013, in flower, *Shui Y.M. et al. B2013-579* (holotype, KUN; isotype, KUN, PE).

Description. Herbs perennial, stemless, rhizomatous. Leaves basal. Petiole 1–3 cm long, golden-brown villous; leaf blade elliptic, thickly chartaceous or nearly leathery, 2.8-4 × 1.2-1.6 cm, adaxially green, sparsely long golden-brown villous, abaxially red-brown and with densely long golden multi-articulate hairs (especially on midrib), base shallowly cordate, apex obtuse, margin widely crenate and ciliate; lateral veins ca. 5 pairs on each side of midrib. Inflorescences axillary, multi-flowered. Peduncles 4.5–7 cm, golden-brown villous; bracts 2, very small, linear, ca. 6 × 1 mm. Calyx 5-sect to base, lobes lanceolate or linear, ca. 8 × 1 mm, adaxially green and glabrous, abaxially red-brown and golden-brown villous. Corolla yellow, ca. 2.4 cm long, outside white glandular pubescent and inside glabrous; corolla tube cylindrical, not constricted at throat, ca. 1.2 cm long, ca. 0.2 cm in diam., more or less curving; limb 2-lipped, adaxial lip 0.3-0.4 cm, 2-lobed, much shorter than abaxial lip, lobes oblong or subround, ca. 0.2 × 0.15-0.25 cm, apex rounded; abaxial lip ca. 1.2 cm, 3-lobed, middle lobe oblong or obovate, $0.7-0.9 \times 0.4-0.6$ cm, lateral lobes oblong or obovate, closely equal, $0.6-0.7 \times 0.3-0.5$ cm, apex rounded. Stamens 4, coherent in 2 pairs, included, adaxial stamens ca. 7 mm, adnate to corolla tube ca. 4 mm from base, abaxial stamens ca. 8 mm, adnate to corolla tube ca. 6 mm from base; filaments white, adaxial ca. 0.7 cm, abaxial ca. 0.8 cm; anthers ca. 1 mm long, oblong,

Characters	<i>O. fulva</i> sp.nov.	O.georgei
leaf blade	elliptic	narrowly ovate to elliptic or narrowly obovate
adaxial surface of leaf	glabrescent	pubescent
abaxial surface of leaf	golden-brown villous between veins	glabrescent between veins
leaf base	shallowly cordate	narrowly cuneate
leaf apex	obtuse	acute to obtuse or acuminate
calyx lobe	ca. 8 mm long	2–4 mm long
corolla tube	not constricted at throat	constricted at throat
corolla lip	adaxial lip 3–4 mm, abaxial lip ca. 12 mm	adaxial lip 2–3 mm long, abaxial lip 5–6 mm
	long	long
stamens coherent	2 pairs	separated
elevation	ca. 2000 m	2300–3400 m
distribution	SW Yunnan, China	NW Yunnan and SW Sichuan, China

Table 2. Differences in characters between Oreocharis fulva and O.georgei in Gesneriaceae.

basifixed, dehiscing longitudinally; staminode 1, ca. 0.4 cm long, completely adnate to tube. Pistil included, ca. 0.7 cm long, glabrous; ovary columned, ca. 0.3 cm long, 2-loculed, glabrous; style ca. 0.3 cm, glabrous; stigmas 1, retuse; disc ring-like, ca. 0.1 cm high, margin dentate. Capsule straight, oblong, 2.0–2.5 cm long, existing style ca. 0.2 cm. Seeds not seen.

Distribution, habitat and phenology. This species is only distributed in Yongde, Yunnan Province and grows on the rocks in montane forests. Flowering is September– October and fruiting is September–November.

Etymology. The epithet "fulva" is named after the golden-brown villi on the plants. **Conservation status.** There is only a population with ca. 200 mature individuals and ca. 20, 000 m² area (400 m \times 500 m) from the type locality outside the nature

reserve. Due to the vicinity of the local villages, the population is extremely affected by walnut planting. According to the IUCN Red List Categories and Criteria, the new species is assessed as "Critically Endangered (CR)" (B1ab(iii)+C2a(ii)). (IUCN 2012)

Note. *Oreocharis fulva* is different from the other species in the tetrandrous and yellow-flowered group of *Oreocharis* s.l. and unique in its expanding corolla lips and narrow and short corolla tubes, with slight similarity to *Oreocharis georgei* in the morphology and texture of leaves (Fig. 3A–F, Table 2). Additionally, the new species and its similar species are respectively distributed in Southwest Yunnan (Lincang District) and Northwest Yunnan (Dali Bai Autonomous Prefecture and Lijiang District) without overlapping geographical distribution (Fig. 1, Li and Wang 2005).

Oreocharis lacerata W.H.Chen & Y.M.Shui, sp. nov.

urn:lsid:ipni.org:names:77211185-1 Fig. 6

Diagnosis. The new species is similar to *Oreocharis concava* (Craib) Mich.Möller & A.Weber, but different in leaf margin lacerate (*vs.* unlobed), corolla strigose or glandular outside (*vs.* glabrous), abaxial lip much larger than the adaxial corolla lip (*vs.* nearly equal).



Figure 6. *Oreocharis lacerata* W.H.Chen & Y.M.Shui, sp. nov. **A** plant **B** flower **C** opened corolla showing stamens **D** fruit **E** abaxially leaf blade **F** pistil showing calyx, ovary, stigma and disc. (Drawn by Ling Wang from holotype).

Type. CHINA. Yunnan Province: Lincang district, Yongde county, Womulong xiang, Ganhe village, Daliang Mt., on rocks along slope, alt. 2700 m, riverside, rare, 5 August 2003, *Zi S. S. 208* (holotype, KUN; isotype, PE).

Description. Herbs perennial, stemless, rhizomatous. Leaves basal. Petiole 4–6 cm, covered with long golden multi-articulate hairs; leaf blade broadly lanceolate or elliptic, $3-4 \times 2-2.5$ cm, adaxially sparely puberulent, abaxially setal and with long golden multi-articulate hairs on ribs, base cordate, margin lacerate, lobes oblong and serrate, apex obtuse; lateral veins 3–4 pairs on each side of midrib. Inflorescences axillary, with many flowers. Peduncles 8–10 cm, golden-brown villous; bracts 2, ovate, ca. 2 × 1 cm. Calyx ca. 0.5 cm, 5-sect from middle, lobes triangular, 0.1–0.2 × ca. 0.15 cm, glabrous, apex acute, margin crenate. Corolla yellow, campanulate-cylindrical, 2.3–2.4 cm long, outside sparely multi-articulate strigose, inside glabrous; tube 1.8–1.9 cm long, ca. 1.5 mm in diam. at base and ca. 5 mm in diam. at throat, inflated above the middle; limb 2-lipped, adaxial lip 2-lobed, lobes semi-rounded, 3.1–3.4 × 0.3–3.2 mm, apex obtuse; abaxial lip explanate and 3-lobed, middle lobes oblong, 5–6 × 2.0–2.6 mm,

Characters	O. lacerata sp.nov.	O. concava
leaf blade	margin lacerate, base cordate	margin un-lobed, base cuneate
adaxial surface of leaf	sparely puberulent	densely white puberulent and sparsely brown
		Villous
corolla	campanulate-cylindrical, outside sparely	cylindrical, outside densely pubescent
	multi-articulate strigose	
corolla tube	ca. 1.5 mm in diam. at base and ca. 5 mm in	1.7–2.2 mm in diam. at base and ca. 2.0–2.6
	diam. at throat, inflated above the middle	mm in diam. at throat, slightly inflated above
		the middle
adaxial corolla lip	apex obtuse, 2-lobed, lobes semi-rounded,	apex acute, emarginate to undivided, lobes
	3.1–3.4 × 0.3–3.2 mm	less than 1 mm or lacking
elevation	2700–2902 m	3100–3600 m
distribution	SW Yunnan, China	NW Yunnan, China

Table 3. Differences between Oreocharis lacerata and O. concava in Gesneriaceae.

glabrous, apex obtuse. Stamens 4, coherent in 2 pairs, included, adaxial stamens ca. 1.2 cm, adnate at the throat of corolla, abaxial stamens ca. 1.7 cm, adnate to corolla tube ca. 1.2 cm from base; filaments white, glabrous; anthers ca. 0.1 cm, oblong, basifixed, dehiscing longitudinally; staminode 1, ca. 0.1 cm long. Pistil included, ca. 1.2 cm long, glabrous; ovary columned, glabrous, ca. 0.7 cm long, 2-loculed; style glabrous, ca. 0.2 cm; stigma 1, undivided, oblate; disc ring-like, ca. 0.15 cm high, margin dentate. Capsule straight, oblong, 2–3.8 cm long, existing style ca. 0.2 cm. Seeds ovate, $0.6-0.63 \times 0.21-0.24$ mm.

Distribution, habitat and phenology. This species is distributed in Yongde county, Yunnan Province, SW China. Flowering is August and fruiting is September–November.

Etymology. The species is named after the lacerate leaves.

Conservation status. The new species has been observed only from the type locality. The preliminary observation revealed that there are 300 mature individuals and ca. $600 \text{ m}^2 (20 \text{ m} \times 30 \text{ m})$ area nearby the summit in the core area of the nature reserve, almost never to be affected by the activity of the local people. According to the IUCN Red List Categories and Criteria, the new species is hereby assessed as "Vulnerable (VU)"(D). (IUCN 2012)

Additional specimens examined (paratypes). CHINA. Yunnan province: Lincang district, Yongde county, Wumulong community, Ganhe village, Daliang Mt., 99°38'58"E, 24°08'56"N, on rocks in shrubs, elev. 2,902 m, flowers yellow, common, 11 August 2003, in flower, *Zi S.S. 261* (KUN, PE); the same locality, elev. 2,900 m, rare,16 September 2013, *Li Yong-Liang YDDXS 1137* (KUN).

Note. Oreocharis lacerata is more similar to O. concava in the morphology of flowers than other species in the group with tetrandrous and yellow flowers, but differs mainly in the lacerate leaf margin (vs. unlobed) and obviously longer inferior lip of corolla than the superior lip (vs. equal between the two lips of corolla) (Fig. 3G–M, Table 3). With its pinnatilobate leaves, the new species is slightly more similar to Oreocharis eximium in the yellow-flowered group in Oreocharis and to O. pinnatilobata (K.Y.Pan) Mich.Möller & A.Weber in the purple-flowered group, but differs considerably in the morphology of flowers and fruits (Wang et al. 1990, 1998, Li 1991, Li and Wang 2005, Möller et al. 2011).

Oreocharis wenshanensis W.H.Chen & Y.M.Shui, sp. nov.

urn:lsid:ipni.org:names:77211186-1 Fig. 7

Diagnosis. The new species is most similar in leaves to *Oreocharis concava* (Craib) Mich. Möller & A.Weber, but differs in broadly ovate leaf blade (*vs.* oblong-ovate), remotely pubescent adaxial leaf surface (*vs.* villous), calyx margin crenate (*vs.* irregularly dentate), the shorter corolla (1.5–1.6 cm long *vs.* 2.2–2.8 cm), adaxial corolla lip bilobed (*vs.* emarginate to undivided) and disc subentire (*vs.* 5-lobed).

Type. CHINA. Yunnan Province: Wenshan county, Bozu Mt., 23°21'1.41"N, 103°55'6.20"E, in dense forests, elev. 2,700 m, 27 July 1993, in flower, *Shui Y.M. 3126* (holotype, KUN!; isotype, PE!).

Description. Herb perennial, stemless, rhizomatous. Leaves many, basal; petiole 5-9 cm, densely pubescent; leaf blade broadly ovate, chartaceous, $5.0-9.0 \times 3.7-7.0$ cm, adaxially sparsely pubescent and abaxially along veins, base cordate, apex acuminate, margin biserrate, lateral veins 4-5 on each side of midrib, indistinct adaxially and distinct abaxially. Inflorescences axillary, peduncle 6-10 cm, densely pubescent; bracts narrowly oblong, ca. 0.9 × 0.2 cm, adaxially glabrous, abaxially sparsely pubescent, apex acuminate, margin serrate above middle and entire below middle. Inflorescences axillary, densely cymose. Peduncles 5-7 cm, pubescent; Pedicels 2.0-2.8 cm, pubescent. Calyx 6–7 mm, 5-sect from 2/3; segments equal, triangular, ca. 3 × 2 mm, adaxially glabrous, abaxially pubescent, margin serratulate. Corolla yellow, 1.5–1.6 cm long, outside pubescent and inside glabrous; tube cylindrical, gradually slightly widening from middle of tube, 0.7–1.0 cm long, ca. 0.3 cm in diam., throat not constricted; limb 2-lipped; adaxial lip smaller, ca. 3 mm long, 2-lobed, lobes oblong, apex obtuse, $1-1.5 \times \text{ca.} 1.5 \text{ mm}$; abaxial lip larger, 4-5 mm long, 3-lobed, lobes oblong, apex acute, central lobe $4-5 \times ca.3$ mm, lateral lobes ca. 3×3 mm. Stamens 4, coherent in 2 pairs, included, adaxial stamens ca. 2 mm, adnate to corolla tube ca. 3 mm from base, abaxial stamens ca. 4 mm, adnate to corolla tube ca. 3 mm from base; filaments tender, glabrous; anthers basifixed, oblong, 2-loculed, dehiscing longitudinally; staminode 1, 1–2 mm, adnate to corolla tube 2–3 mm from base. Disc ring-like, 1–2 mm, subentire. Pistil 2.5–6 mm, glabrous; ovary oblong, glabrous, 1–4 mm, 1-loculed; style glabrous, 1.5-2 mm; stigma 1, top retuse. Capsule straight, oblong, 1.3-1.8 cm, existing style ca. 0.2 cm. Seeds not seen.

Distribution, habitat and phenology. The new species only grows in the montane forest in Wenshan county, Yunnan Province of China. Flowering is July–September; and fruiting is October–January the following year.

Etymology. The species is named after the type locality of the new species.

Conservation status. Currently, the new species has been observed only from the type locality. The more than two years observation revealed that there are ca. 50 mature individuals and ca. 300 m² (10 m × 30 m) area nearby the summit in the core area of the nature reserve, similarly to the above species (*O. lacerata*). According to the IUCN Red List Categories and Criteria, the new species is hereby assessed as "Critically Endangered (CR)" (D1+D2). (IUCN 2012)



Figure 7. Oreocharis wenshanensis W.H.Chen & Y.M.Shui, sp. nov. A plant B opened corolla showing stamens and staminode C pistil showing ovary, disc and stigma D calyx. (Drawn by Ling Wang from holotype).

Additional specimens examined (paratypes). CHINA. Yunnan Province: Wenshan county, Laojun Mt., on rocks in forests, 23°21'1.41"N, 103°55'6.20"E, 31 August 2012, in flower, *Shui Y.M., He D.M. et al. B2012-099* (KUN); the same locality, on rocks in bamboo, 23°21'1.45"N, 103°55'6.24"E, 24 June 2013, *Shui Y.M. & Xiao B. B2013-100C* (KUN).

Note. Oreocharis wenshanensis was collected first in 1993 by Y. M. Shui in Wenshan county, SE Yunnan, China (Fig. 2G–K). In 2005, the new species was wrongly identified as Oreocharis aurea Dunn var. cordato-ovata (C.Y. Wu ex H.W. Li) K.Y. Pan, A.L. Weitzman & L.E. Skog, based on the photo in Li and Wang (2005) possibly because of their similar locality in SE Yunnan. However, the latter species endemically grows in the limestone forests in Xichou county, the neighbouring county of Wenshan county, a very different habitat from Oreocharis wenshanensis. Furthermore, the new species we proposed can be easily distinguished from Oreocharis aurea Dunn var. cordato-ovata by its broadly ovate blade, smaller corolla without contracted throat and bilobed adaxial corolla lips (Li and Wang 2005). In fact, due to its 2700 m elevation, it is morphologically more similar to O. concava from NW Yunnan with 3100–3400 m

Characters	O. wenshanensis sp.nov.	O. concava
leaf blade	broadly ovate	oblong-ovate
adaxial leaf surface	remotely pubescent	villous
Inflorescences	densely cymose, flowers fascicular	sparely cymose, flowers sparse
corolla	1.5–1.6 cm long	2.2–2.8 cm long
calyx	ca. 3 mm, margin irregularly dentate	7–10 mm, margin crenate
corolla lip	adaxial lips slightly smaller than abaxial lips,	adaxial lips much smaller than abaxial lips,
	adaxial ca. 3 mm long, abaxial lip 4–5 mm	adaxial 1.5–3 mm long; abaxial ca. 7 mm long
	long	
adaxial lips	2-lobed	emarginate to undivided
disc	subentire	5-lobed
elevation	2700 m	3100–3400 m
distribution	SE Yunnan, China	NW Yunnan, China

Table 4. Differences between Oreocharis wenshanensis and O. concava in Gesneriaceae.

elevation (Fig. 7, Table 4). After more than 20 years of observation, with the support of local staff of the Nature Reserve, we made a long-term observation from 1993 to 2018 and confirmed its taxonomic novelty.

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RESEARCH ARTICLE



Oreocharis flavovirens, a new species of Gesneriaceae from Southern Gansu Province, China

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Abstract

Oreocharis flavovirens is a new species of Gesneriaceae from Gansu, China and is described and illustrated here. It is morphologically similar to *O. glandulosa*, *O. humilis* and *O. farreri*, but those congeners of this new taxon can be distinguished by several salient characters. A description of *O. flavovirens*, together with illustrations and photos, are presented.

Keywords

Gansu of China, new taxon, Oreocharis

Introduction

In the summer of 2018, two of the authors (QWH and GYF) encountered an unknown Gesneriaceae species with young flowers during a botanical survey in Gansu Province. Subsequently, the plants were monitored in the field and flowering specimens were collected in autumn. The gross morphology, such as leaves in a basal rosette with spiral leaf arrangement, shape of the corolla and pistil, including stigma, indicates that this taxon can be assigned to *Oreocharis* Benth., which now includes species from eleven former genera (Möller et al. 2011, 2014, 2015). Many new taxa of this genus have been discovered and published in recent years (e.g. Cai et al. 2017, Chen et al. 2016, 2017a,b, 2018, Do et al. 2017, Guo et al. 2018, Li et al. 2017, Wei et al. 2016, Yang et al. 2017, Möller et al. 2016, 2018).

After thorough comparisons of diagnostic morphological and anatomical features of similar taxa from China, Vietnam and Thailand (Pan 1987, Wang et al. 1998, Li and Wang 2005, Wei et al. 2010) and herbarium specimens also being consulted, it was concluded that it was a species new to science and thus described and illustrated here.

Material and methods

Measurements and morphological character assessments of the new species were performed and described, using specimens worked on by the authors. All available specimens of *Oreocharis* stored in the following herbaria in China, Russia, the United States and the United Kingdom were examined (codes according to Thiers 2015+): E, GH, IBK, K, KUN, MO, PE and US. In addition, images of other type specimens were obtained from Tropicos (http://www.tropicos.org) and JSTOR Global Plants (http:// plants.jstor.org). All morphological characters were studied under dissecting microscopes and are described using the terminology presented by Wang et al. (1998) and Li and Wang (2005).

Taxonomic treatment

Oreocharis flavovirens Xin Hong, sp. nov. urn:lsid:ipni.org:names:77211187-1 Figures 1, 2

Diagnosis. *Oreocharis flavovirens* can be diagnosed as a new species from all others in the genus by the upturned corolla tube combined with its rare greenish-yellow colour.

Type. CHINA. Gansu Province: Yuhe Provincial Nature Reserve, Longnan City, 33.08426°N, 105.27858°E, 1,193 m a.s.l., 5 September 2018, flowering, Xin Hong: *HX18090510* (holotype: IBK; isotype: PE).

Description. Perennial, rosette herbs. *Leaves* basal, spirally arranged, 4–20, petiolate; petioles terete, 1.4–4 cm long, ca. 2 mm in diameter, densely reddish-brown long woolly and white glandular hairy; leaf blades ovate to obovate or elliptic, $3-6 \times 1.5-3$ cm, bases cuneate, slightly unequal, margins crenate to lobulate, apices obtuse, papery, adaxially sparsely rust-brown villous hairy, green, abaxially densely brown villous along veins, pale green; midrib usually vivid when fresh, lateral veins 3–5 on each side of



Figure 1. *Oreocharis flavovirens* Xin Hong in natural habitat **A** habitat, growing on the surface of rocks **B** vegetative part of plants **C** top view of corolla **D** lateral view of corolla **E** bud, showing the shape and indumentum of calyx **F** young capsule. Scale bars: 2 cm (**B**); 1 cm (**C–F**).

midrib, distinct, concave adaxially, prominent abaxially. *Inflorescences* cymes, axillary, 1-2(-3)-branched, 1-6(-10)-flowered; peduncles 4-10 cm long, pale green, densely white glandular hairy and sparsely brown pilose; pedicels 1-3(-5) cm long, with indumentum as on the peduncle. *Bracts* 2, ca. 3×1 mm, lanceolate, margins entire, green, glabrous inside, brown pilose and sparsely glandular hairy outside; bracteoles similar but smaller, ca. 1.5×0.5 mm. *Calyx* actinomorphic, 5- sect from base, segments oblong to linear-lanceolate, $2-3.5 \times$ ca. 1.0 mm, green, glandular hairy outside and glabrous inside, margins entire, sometimes revolute when flowering. *Corolla* zygomorphic, ca. 2 cm long, greenish-yellow to greenish, lobes greenish, becoming white at tube base, outside densely glandular-pubescent, inside glabrous; tube cylindrical, dilated and slightly narrowing gradually ventricose from base to throat and constricted at the throat; ca. 15 mm long, ca. 3 mm in diameter at base and ca. 2 mm in diameter at the throat; limb slightly 2-lipped; adaxial lip rounded, $2.5-3 \times$ ca. 3 mm. *Stamens* 4, adnate elliptic, apex rounded, central longer than laterals, 3×4 -ca. 3 mm.



Figure 2. Morphological character of *Oreocharis flavovirens* Xin Hong **A–B** different sizes of leaves (**A** adaxial leaves **B** abaxial leaves) **C** indumentum of petiole **D** abaxial leaf blades, showing veins and indumentum **E** cyme and infructescence **F** bracts **G** dissection of a flower **H** opened corolla, showing stamens and staminodes **I** pistils without corolla, showing stigma **J** young capsule **K** disc and calyx revolute of the young capsule. Scale bars: 2 cm (**A–C**); 1.5 cm (**D, E**); 3 mm (**F, I, K**), 1 cm (**G, H, J**).

to corolla 1–4 mm above base, included; filaments slender, the long two ca. 8 mm long, the short two ca. 6 mm long, sparsely glandular-pubescent, free, white to greenish; anthers yellow, basifixed, coherent in pairs, thecae divergent at base, oblong, ca. 0.5 mm long, 2-loculed, dehiscing longitudinally from arcuate slits, connective not projecting, glabrous; staminode 1, glabrous, 0.5–1.5 mm long, adnate to 1 mm above corolla tube base. *Disc* ring-like, 1–1.5 mm high, glabrous, entire or subentire, greenish-yellow. *Pistil* 1–1.2 cm; ovary narrowly oblong, 1-loculed, ca. 1 cm long; placentas 2, parietal, projecting inwards, 2-cleft, style 1–2 mm long, glabrous; stigma orbicular, emarginated, ca. 2 mm in diameter. *Capsules* oblong lanceolate to oblanceolate, straight, 2–4 cm long, dehiscing loculicidally to base; valves 2, glabrous. *Seeds* unknown.

Etymology. The specific epithet is derived from its greenish-yellow corolla.
Distribution and habitat. To date, *Oreocharis flavovirens* is only found at the type locality, Yuhe Provincial Nature Reserve, Gansu Province, which is located at the intersection of the Qinling Mountains and the Minshan Mountains. This species grows amongst moss on moist shady surfaces of stones near waterfalls, at an elevation of 950–1200 m a.s.l. The average temperature is 21°C, the average annual precipitation has been calculated as ca. 780 mm. The forest is a subtropical evergreen broad-leaved forest.

Notes. As is known, Oreocharis Benth. is a genus (more than 120 species) in the angiosperm family Gesneriaceae, which are mainly distributed in southern and southwestern China, at the same time with a few species extending into Vietnam, Myanmar, India, Bhutan, Japan and Thailand (Cai et al. 2017, Möller et al. 2016, 2018, Xu et al. 2017). SW China is rich in species diversity of the genus in China, especially on the north-facing shady slope nearby the summit of southern Yunnan Province and most species occur in relatively restricted and geographically isolated localities with very few widely distributed (Li and Wang 2005, Wei et al. 2010, Möller et al. 2011). Only three species of this genus were found in S. Gansu province before 2019, viz. Oreocharis farreri (W. G. Craib) M. Möller & A. Weber, O. glandulosa (Batalin) M. Möller & A. Weber and O. henryana Oliv. O. farreri was first published as Isometrum farreri base on the type specimens: Farrer et Purdom 262 [E, barcode no. 00135136, Fig. 3], which grows at low elevations on rather cool rocks or very steep banks of cool clammy soil that grows a fine film of moss in S. Gansu Province (Craib 1920). O. glandulosa was first described as Didissandra glandulosa by A.T. Batalin in 1892, based on the specimens [LE, barcode no. 01043081, Fig. 4] from G.N. Potanin's trip from 1884 to 1886, collected on the way from Songpan County, Tibetan Qiang Autonomous Prefecture of Ngawa, NE Sichang Province to Wenxian County, Longnan City, S. Gansu Province on 17 August 1885 (Batalin 1892, Bretschneider 1898). O. henryana was described and illustrated, based on the type specimens: A. Henry 8999 [K, barcode no. 000858129, Fig. 5], growing on shady and damp rocks in montane regions of Sichuan Province (Hooker 1890). No new species of Oreocharis were described from between the early 19th and late 20th Century in the regions, the new findings complementing the species richness of the genus in Central China. Due to the high endemism in the genus (Chen et al. 2017b, 2018), Table 1 details the differences between these species growing in the same regions.

Obviously, the genus is special for its remarkable floral diversity and it has made this genus to be one of the most taxonomy-difficult groups in the family. The new species, *Oreocharis flavovirens* which has a light-yellow cylindrical corolla with a distinct upturned tube, is a good example. The shape of the corolla tube, cylindrical and upturned, is a distinct character that not many species in *Oreocharis* possess. If we only consider the similarity of corolla tube shape, it is close to *Oreocharis tubiflora* K.Y. Pan and *O. argyreia* Chun ex K.Y. Pan, including constriction at the mouth but the latter two are lacking the upturned corolla tube. Given the corolla shape, the corolla of several species of former *Ancylostemon* Craib are similar too, except the tubes are straight or slightly turned down, but not up, though the flowers are predominantly yellow (rarely pink in *A. ronganensis* K. Y. Pan=*Oreocharis ronganensis* (K.Y.Pan) Mich.

HOLOTYPE of: Isometrum farreri Craib, Notes Roy. Bot. Gard. Edinburgh 11: 250, 1919. verif. & det. Anna L. Weitzman & Laurence E. Skog Jaometrum Farreri, Crails in Notes Roy. Bor. Gan Edin, Xi b. 250/19/9) No. 262____ HERB. HORT. REG. BOT. EDIN. PLANTS OF KANSU COLL. R. FARRER AND W. PURDOM Oreocharis Henryana, Oliver. q. 10. 19. 1944 Type Specimen F. 262 Situations of a clevate S. Konsu, in rather cool rocks, 2 Vanks of cool claim that grows a fine film of moss here sax at le them F. 261 (not Thracen Harrow sent which precisely resembles Jankova Heldreichi. with shaplike Boea hygrometrico (Bange) quer silver hairs & scapes of smaller Clac. Blue streptocarpus. Both species are only found at low slevations (up to 6500). Howe aug. 28. Seed ripe. Nov. 1.

Figure 3. Type of *Oreocharis farreri* (W. G. Craib) M. Möller & A. Weber, stored in Herbarium of Royal Botanic Garden Edinburgh, No. E 00135136.



Figure 4. Type of *O. glandulosa* (Batalin) M. Möller & A. Weber, stored in Herbarium of Komarov Botanical Institute, No. LE 01043081.



Figure 5. Type of *Oreocharis henryana* Oliv. stored in Herbarium of Royal Botanic Gardens, No. K 000858129.

Characters	Oreocharis viridifrons	O. glandulosa	O. humilis	O. farreri
Shape of leaf blade	ovate to obovate or	lanceolate-ovate	elliptic to lanceolate	rhombic-ovate to
	elliptic			obovate or elliptic
Indumentum of	adaxially sparsely rust-	adaxially densely	adaxially sparsely	gray pubescent
leaf blade	brown villous hairy	brownish villous	brown villous,	
			glabrescent	
Number of lateral	3-4	5–6	3–5	4–6
veins on each side				
of midrib				
Size of Bracts	3 mm	5 mm	2-4 mm	3.5–5 mm
Shape of tube	campanulate-tubular,	tubular to	tubular	campanulate-tubular
	laterally compressed at	subcampanulate		
	mouth			
Size of corolla	ca. 20 mm long	10–15 mm long	11–15 mm long	9–11 mm long
Color of corolla	greenish-yellow to	pale purple	yellow-white	purple-pink to orange-
	greenish			pink
Shape and size of	emarginate or rarely	emarginate or rarely	2-lobed, 2 mm	emarginate
adaxial lip	undivided, 2.5-3 mm	undivided, 4 mm		
size of abaxial lip	3–4 mm, longer than	2 mm, shorter than	3.5 mm, longer than	2 mm, shorter than
	to nearly equalling	to nearly equalling	to nearly equalling	abaxial lip
	abaxial lip	abaxial lip	abaxial lip	
Staminodes	adnate to 1 mm above	adnate to 0.5 mm	adnate to 3.5 mm	adnate to 1 mm above
	corolla tube base	above corolla tube base	above corolla tube base	corolla tube base
Ovary	10 mm	3-7 mm	6–8 mm	4 mm
Stigma	peltate, orbicular	emarginate	2-lobed	oblate

Table 1. Diagnostic character differences amongst *Oreocharis flavovirens* sp. nov., *O. glandulosa*, *O. humilis* and *O. farreri*.

Möller & A.Weber), but not greenish-yellow. On the other hand, the upturned tube is more reminiscent of former *Opithandra wentsaii* Z.Yu Li (*=Oreocharis wentsaii* (Z. Yu Li) M. Möller & A. Weber) and former *Opithandra pumila* (W.T.Wang) Wang (*=Oreocharis pumila* (W.T.Wang) Mich.Möller & A.Weber), only here the tubes of previous *Opithandra* are slightly more trumpet-shaped in dark pink or pink and have two fertile stamens rather than four (Wang et al. 1998, Li and Wang 2005, Wei et al. 2010). All in all, the upturned corolla tube combined with its greenish-yellow colour could be used alone to differentiate the new species from others in the genus.

Furthermore, although the genus *Oreocharis* was redefined to accommodate species with distinctive floral morphologies from ten other genera, based on molecular phylogenetic studies in the last two decades, the evolutionary trends of the floral characters have not yet been understood comprehensively. The major causes of the incongruence and conflict between classical taxonomy and molecular phylogenetic studies for *Oreocharis s.l.* remain largely unexplored. There are other similar examples in Gesneriaceae of Asian, for example, *Primulina s.l.* (Wang et al. 2011, Weber et al. 2011a) and *Petrocodon s.l.* (Weber et al. 2011b, Lu et al. 2017).

Additional specimens examined (paratypes). Gansu Province: Yuhe Provincial Nature Reserve, Longnan City, 24 September 2019, in fruit, Yun-Feng Gao et al.: *WF19092401* (AHU).

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RESEARCH ARTICLE



Oreocharis wumengensis, a new species of Gesneriaceae from northeastern Yunnan, China

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Abstract

A new species of Gesneriaceae, *Oreocharis wumengensis* Lei Cai & Z.L.Dao from Wumeng Mountain area, Yanjin County, Yunnan Province, China, is described. The new species is morphologically similar to *O. panzhouensis* Lei Cai, Y.Guo & F.Wen in the shape of corolla, number of stigma and stamens, but it can be easily distinguished from this species by the leaf shape and indumentum characters of leaf blade, calyx and stamens. Detailed descriptions with photographs of the plant and holotype, and comparisons with morphologically similar species, are also provided.

Keywords

flora of Yunnan, morphology, new taxon, Oreocharis, Wumeng Mountain

Introduction

Many genera and species in the family Gesneriaceae have been redefined in the past two decades based on new evidence following the development of molecular phylogenetics (Möller et al. 2011, 2016; Weber et al., 2011a, 2011b). *Oreocharis* Bentham was redefined in 2011 (Möller et al. 2011), and the vast majority associated species of the

other 10 genera were merged into the enlarged genera (Chen et al. 2014, Möller et al. 2014), including some new taxa described in recent years (Cai et al. 2015, 2017, 2019; Chen et al. 2017, 2018; Do et al. 2017; Guo et al. 2018; Han et al. 2017; Möller 2015; Möller et al. 2018; Wei et al. 2016; Yang et al. 2018), *Oreocharis s.l.* now comprises ca. 120 species, mainly with southern and southwestern Chinese distribution, but with a few species extending into Vietnam, Myanmar, India, Bhutan, Japan and Thailand (Cai et al. 2017, 2019; Chen 2016; Chen et al. 2017, 2018; Do et al. 2017; Guo and Wang 2014; Li and Wang 2004; Möller et al. 2018; Wang et al. 1990, 1998; Xu et al. 2017).

In April 2017, during field investigations in the Wumeng Mountain area (Yanjin County, northeastern Yunnan), an unknown species of Gesneriaceae without flowers was collected and then planted in Kunming Botanical Garden (KBG). In July 2018, we firstly observed flowering plants which were cultivated in KBG, also based on the floral characteristics; we confirmed that it is a member of *Oreocharis s.l.* Following a careful review of the relevant herbarium specimens and taxonomic publications of *Oreocharis* from Yunnan and the adjacent regions, we concluded that this species is new to science. *Oreocharis wumengensis* Lei Cai & Z.L.Dao is described for the first time below, and its morphological characters are compared with those of closely related species.

Material and methods

Samples of the new species were collected from living plants cultivated in KBG, originally introduced from Yanjin County, Yunnan. All available specimens of *Oreocharis s.l.* are stored in the herbaria (HITBC, IBK, KUN and PE) and Chinese Virtual Herbarium (http://www.cvh.ac.cn/) in China and Global Plants on JSTOR (https://plants.jstor.org/) were examined. We studied all morphological characters with dissecting microscopes, and described the morphological characters by using the terminology presented by Wang et al. (1990, 1998). The photographs were taken in the field and KBG by the first author.

Taxonomic treatment

Oreocharis wumengensis Lei Cai & Z.L.Dao, sp. nov. urn:lsid:ipni.org:names:77211188-1 Figures 1, 2

Diagnosis. Oreocharis wumengensis resembles O. panzhouensis in its floral characteristics, but can easily be distinguished from this species in the brown-pubescent, oblate petiole; the oblong, long elliptic to oblanceolate leaf blade; the glandular pubescent pedicel; the calyx 5-lobed to the base; and the apically coherent anthers.

Type. CHINA. Yunnan: Yanjin County, Miaoba Town, Liuchang Village, Houshanping, 27°52'N, 104°20'E, elev. ca. 1050 m, on moist rocks (cultivated in KBG), in flowering, 3 August 2018, *Lei Cai CL198* (holotype: KUN!, isotype: KUN!).



Figure I. Oreocharis wumengensis Lei Cai & Z.L.Dao, sp. nov. **A** Plant in the wild **B, C** plants in cultivation in KBG **D** adaxial and abaxial leaf surfaces **E** inflorescence **F, G** side and top view of flowers **H** front view of flower **I** opened corolla with pistil and calyx **J** pistil with disc and calyx **K** opened corolla showing stamens and staminode **L** adnate anthers, abaxial view **M** adnate anthers, adaxial view. Photographed by Lei Cai.

Description. Perennial herb, rhizome 4–12 mm long, 3–5 mm in diameter. Leaves 8–20, basal, petiole oblate, 0.8-5.5 cm long, brown pubescent, leaf blade oblong, long elliptic to oblanceolate, $3.0-8.5 \times 0.8-2.8$ cm, adaxially densely appressed pubescent, abaxially pubescent, densely brown pubescent along veins, lateral veins 3–5 on each side of midrib, adaxially inconspicuous, adaxially conspicuous, apex acute, base cuneate, margin serrated, upper half obvious. Cymes axillary 2–4, inflorescence 4–10-flowered; peduncle 6.5–12 cm long, brown pubescent; bracts 2, lanceolate to elliptic, $5-7 \times 1.5-3$ mm, both sides appressed pubescent, margin nearly entire to denticulate; pedicel 1.5–4.5 cm long, glandular pubescent. Calyx 4–6 mm



Figure 2. Holotype of *Oreocharis wumengensis* Lei Cai & Z.L.Dao, sp. nov. (KUN-1444080).

long, 5-lobed to the base, lobes unequal, linear or narrowly triangular, 4–6 mm long, ca. 1.5 mm wide, outside brown pubescent and glandular pubescent, inside glabrous. Corolla sigmoid, yellow, 2.2–2.6 cm long, outside extremely sparsely brown pubescent and densely glandular pubescent, inside glandular pubescent in the throat and on adaxial lobes, slightly contracted at the throat, 1.2-1.4 cm long, 4-7 mm in diameter; limb 2-lipped; adaxial lip 2-lobed to near base, semiorbicular, lobes $2-3 \times 2-3$ mm, abaxial lip 3-lobed to base, semiorbicular to oval, $6-8 \times 5-7$ mm. Stamens 4, 5–8 mm long, adnate to corolla 4–6 mm from base; filaments linear, glandular pubescent; anthers horseshoe shaped, coherent apically, 2-loculed, dehiscing longitudinally, connective glabrous; staminode 1, ca. 0.5 mm long, inserted ca. 1 mm from base. Disc ca. 1.5 mm high, yellow, margin undulate. Pistil 0.8–1 cm long; ovary long cylindrical, glabrous, 4-6 mm long; style 2–3 mm long, glandular pubescent; stigma bilobed, flabellate. Old Capsule linear, ca. 2 cm long.

Phenology. Flowering from July to August; fruiting unknown.

Etymology. The specific epithet '*wumengensis*' referring to the type locality where the new species was found, is located in the famous Wumeng Mountain area.

Vernacular name. The Chinese name for the new species is "Wū Méng Mǎ Líng Jù Tái" (乌蒙马铃苣苔).

Distribution and ecology. *Oreocharis wumengensis* is currently known only from one population of ca. 50 individuals at the type locality. The species could be endangered, but more data is needed to evaluate this reliably. The species was observed growing on moist rocks with other plants under forest cover in karst regions in Yanjin County, Yunnan.

Taxonomic affinities. Oreocharis wumengensis is morphologically unique with sigmoid corolla within Oreocharis s.l., however there are certain similarities with other species in this genus. O. wumengensis is similar to O. panzhouensis in the shape of corolla, however, it is obviously different from the latter species. The comparison of morphologically characters on related species are provided in Table 1.

Characters	O. wumengensis	O. panzhouensis
Petiole	oblate, brown pubescent	round, brown villous
Leaf blade	oblong, long elliptic to oblanceolate	ovate to suborbicular
Peduncle	brown pubescent	brown villous
Bract	lanceolate to elliptic	linear to subulate
Pedicel	glandular pubescent	brown villous
Calyx	5-lobed to the base, lobes linear or narrowly	5-lobed to the middle, lobes broadly triangular
	triangular	
Corolla	sigmoid, yellow, slightly contracted at the throat,	pale yellow, tube campanulate, outside pubescent
	outside sparsely brown pubescent and densely	and glandular-pubescent
	glandular pubescent	
Filament	linear, glandular pubescent	flattened, glabrous
Anthers	horseshoe shaped, coherent apically	broadly oblong, separated
Style	glandular pubescent	glabrous

Table 1. Morphological comparison between Oreocharis wumengensis and O. panzhouensis.

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RESEARCH ARTICLE



Oreocharis jasminina (Gesneriaceae), a new species from mountain tops of Hainan Island, South China

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Abstract

A new species of Gesneriaceae, *Oreocharis jasminina* S.J.Ling, F.Wen & M.X. Ren from Hainan Island, south China, is highlighted and described. The new species is distinguished by its actinomorphic corolla, narrow floral tube and ovate anthers hidden in the floral tube. The new species also showed clear geographic and altitudinal isolation from the three currently-recognised *Oreocharis* species on the Island. Molecular phylogenetic analysis, based on nuclear ITS1/2 and plastid *trnL-trnF* sequences, supported the delimitation of the new species, which forms a single lineage with all the other *Oreocharis* species from Hainan Island. The roles of geographic and floral isolation in the evolution of the new species and its affinities are discussed.

Keywords

Hainan Island, new taxon, Oreocharis

Introduction

The *Oreocharis* Bentham was recently re-circumscribed to a large genus by including ten more genera and over 135 species, based mainly on molecular phylogenetic studies (Möller et al. 2011, 2016; Xu et al. 2017; Möller 2019; Wen et al. 2019). The enlarged genus was predominantly distributed in China with some species in India, Myanmar, Thailand and Vietnam (e.g. Li and Wang 2005; Möller and Clark 2013; Möller et al. 2018). Regardless of the limited differences in habit and fruit structure, *Oreocharis* shows a strikingly-high diversity in floral syndromes (Li and Wang 2005; Wei 2010; Möller and Clark 2013).

As one of the globally-important biodiversity hotspots, Hainan Island harbours about 4000 seed-plant species, of which ca. 500 are endemics (Francisco-Ortega et al. 2010) and which are concentrated in the south-central mountains. Gesneriaceae, in Hainan Island especially, includes a high ratio of species endemism, eight out of the total of 24 species being endemic (Ling et al. 2017a). Currently, three taxa of *Oreocharis* are recorded on Hainan Island and all of them are Hainan-endemic and monophyletic, i.e. *O. dasyantha* Chun, *O. dasyantha* Chun var. *ferruginosa* Pan and *O. flavida* Merrill (Li and Wang 2005; Ling et al. 2020), while each of these species shows considerable variations in morphological traits (Wei 2010; Ling et al. 2020a).

During several fieldwork trips in the past three years, we found that some populations of *Oreocharis* on mountain tops in Hainan Island showed obvious differences in various morphological characters. After careful literature studies (Pan 1987; Li and Wang 2005; Wei 2010) and morphological and molecular examinations, we are convinced that populations from the mountain tops of Mt. Yingge and Mt. Limu represent a new species, which we report and describe here.

Materials and methods

Morphological observations

The field study and conservation on Gesneriaceae were undertaken by two of the authors (SJL and MXR) over a long period of time, especially focusing on the Hainan-endemic species (Ling et al. 2017a, b; Xing et al. 2018; Li et al. 2019). Morphological observations and measurements were carried out, based on living plants during fieldwork. All available specimens of *Oreocharis* species, stored in the herbaria in China (PE, KUN, IBK and IBSC), were examined. We also downloaded all *Oreocharis* specimens from JSTOR Global Plants (http://plants.jstor.org), and Chinese Virtual Herbarium (http://www.cvh.ac.cn) to compare detailed morphological traits between the proposed new species with the currently-accepted species of *Oreocharis*. Specifically, we compared morphological traits of the possible new species with all the three currently-recognised *Oreocharis* species from Hainan Island, i.e. *O. dasyantha*, *O. dasyantha* var. *ferruginosa* and *O. flavida*. The specimens of new species were collected over the past two years and deposited in the herbarium of Hainan University (HUTB) and Kunming Institute of Botany, Chinese Academy of Sciences (KUN).

Taxonomic sampling, DNA extraction and molecular markers

The leaf samples of *O. dasyantha*, *O. dasyantha* var. *ferruginosa*, *O. flavida* and the putative new species were collected in the field and dried in a vascular bag with silica gel. Total genomic DNA extraction was conducted using CTAB methods (Doyle and Doyle 1987). One nuclear ribosomal DNA (nrDNA) sequence, the ITS region comprising spacer 1, the 5.8S gene and spacer 2 (White et al. 1990) and one chloroplast DNA (cpDNA) intron-spacer region *trnL-trn*F (Taberlet et al. 1991) were used in this study. Laboratory procedures followed Ling et al. (2020) and newly-acquired sequences were deposited in GenBank (Table 2).

Alignments and phylogenetic analyses

According to Möller et al. (2011), Chen et al. (2014) and Ling et al. (2020), *Oreocharis sinohenryi* (Chun) Mich.Möller & A.Weber which had the closest phylogenetic relationships with the Hainan *Oreocharis* taxa was used as outgroup with sequences (Genbank with accession numbers HQ632913 and HQ633009). The original chromatograms from both directions of the ITS1/2 and *trnL-trn*F sequences were evaluated using Bioedit (Hall 1999) for base confirmation and contiguous sequences editing, then we manually aligned sequences, where necessary, using MEGA v.6.5 (Kumar et al. 2008) and ambiguous positions were excluded from the alignments. The ITS1/2 and *trnL-trn*F were concatenated to a single matrix after a congruency test by PAUP* 4.0a164 (Swofford 2003). Bayesian Inference (BI) analysis was conducted using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) and Maximum Likelihood (ML) analysis was performed using MEGA v.6.5 (Kumar et al. 2008). Both procedures followed the Ling et al. (2020), based on the combined ITS1/2 and *trnL-trn*F sequences.

Results

Phylogenetic reconstruction

The combined ITS1/2 and *trnL-trn*F datasets were 640 and 818 bp long, amongst which 64 and 17 were polymorphic sites and 27 and 6 were parsimony-informative sites, respectively. The aligned dataset was 1458 bp long and a total number of 81 polymorphic sites were measured, of which 33 were parsimony-informative sites. There was no significant incongruence, based on the incongruence length difference (ILD) test between the ITS1/2 and *trnL-trn*F (p > 0.05).

Molecular phylogeny recognised the individuals from different mountains and these were grouped as separate lineages. The putative new species from Mt. Limu and Mt. Yingge is accepted as a new species with PP (posterior probability) = 1 and BS (bootstrap value) = 100% (Fig. 1). All the *Oreocharis* species from Hainan Island form a single lineage with relatively-high support (Fig. 1).



Figure 1. Molecular phylogeny of Hainan *Oreocharis* taxa with outgroup *O. sinohenryi*, based on the combined nuclear ribosomal DNA (nrDNA) sequence ITS1/2 and chroloplast gene *trnL-trn*F data matrices. Posterior probability (PP) and Bootstrap value (BS) are showed above branches.

Taxonomic treatment

Oreocharis jasminina S.J.Ling, F.Wen & M.X. Ren, sp.nov.

urn:lsid:ipni.org:names:77211189-1 Figs 2, 3

Diagnosis. Oreocharis jasminina has the closest phylogenetic relationship with O. dasyantha, O. dasyantha var. ferruginosa and O. flavida with very high support values, all being Hainan-endemic and monophyletic. O. jasminina can be easily distinguished from them by having: (1) a long and narrow floral tube (both O. dasyantha and O. dasyantha var. ferruginosa have conical floral tubes, O. flavida has campanulate-tubular floral tube); (2) yellow and actinomorphic corolla (both O. dasyantha and O. dasyantha var. ferruginosa are zygomorphic with orange-red to yellow corolla, O. flavida is actinomorphic with orange corolla); (3) didynamous stamens with ovate anthers hidden in the floral tube (both O. dasyantha and O. dasyantha var. ferruginosa have exposed didynamous stamens with ovate anthers, O. flavida has four equivalent stamens with horseshoe-shaped anthers included in the floral tube) (Table 1, Fig. 4).

Type. CHINA. Hainan: Qiongzhong County (琼中县), Limu Mountain, 1350 m a.s.l., on moist rocks, 26 Nov 2018, *S.J.Ling 2018112601* (holotype: HUTB!; Iso-types: HUTB!, KUN!).



Figure 2. *Oreocharis jasminina* sp. nov. **A** Habitat **B** habit **C** face view of corolla **D** lateral view of corolla **E** opening flower showing stamens and staminode **F** stamens and staminode **G** pistil and sepals **H** fruit pods **I** adaxial leaf surface.

Description. Perennial herb, rhizomatous, leaves basal; $4.0-10.0 \text{ cm} \log$, 2-3 mm in diameter, densely pale brown villous or woolly; leaf blade ovate to broadly ovate, rarely elliptic or obovate, $6-11 \times 4-8$ cm, adaxially densely grey to brown pubescent, abaxially sparsely to densely grey or grey-brown pubescent, sparsely brown villous along veins



Figure 3. Oreocharis jasminina sp. nov. (all drawings based on the holotype *S.J.Ling 20181126–01* in HUTB, drawn by S.P. Guan). **A** Habit **B** face view of corolla **C** lateral view of corolla **D** opening corolla showing pistil and stamens **E** pistil **F** stamens and staminode.

which are adaxially sunken and abaxially ridged, lateral veins 6–7 on each side of midrib, base often cordate to rounded, margin nearly entire to shallowly crenate, apex rounded. Cymes axillary, 2–3, inflorescence 3–10-flowered; Peduncle 9–16 cm long, sparsely pale grey villous; bracts 2, linear to narrowly triangular, outside densely villous, apex acuminate, cuneate to triangular, margin entire; pedicel 1.5–2.2 cm long, densely pale brown villous to woolly. Calyx 5-lobed, divided to base, lobes green, narrowly lanceolate, 9–11 × ca. 2 mm, apex acuminate, margin entire, outside villous, inside glabrous. Corolla yellow, 1.7–2.2 cm long, outside pubescent; tube thin tubular, 1.8–2.1 cm × 3–4.5 mm, limb barely 2-lipped, adaxial lip shallowly 2-lobed from near base, abaxial 3-lobed slightly equal. Stamens 4, 8–9 mm long, included, adnate to corolla 4–5 mm from base; filaments slender, pubescent; anthers ovate, 2-loculed, dehiscing transversely; staminode 1, adnate to corolla 2–4 mm from base, ca. 2 mm. Disc ca. 1 mm high, entire. Pistil ca.

Characters	Oreocharis jasminina sp. nov.	0. dasyantha	0. dasyantha var. ferruginosa	0. flavida
Corolla colour	yellow	orange-red to yellow	orange-red to yellow	orange
Corolla tube	narrowly tubular,1.7–2.2 cm long 1.8–2.2 cm × 3–4.5 mm	conical, 1.6–2.4 cm long 0.9–2 cm × 6–7 mm	conical, ca.1.6 cm, tube 9–1.1 mm	campanulate-tubular,1.7–1.9 cm long 1.6–1.8 cm × 6–8 mm
Corolla symmetry	actinomorphic	zygomorphic	zygomorphic	actinomorphic
Leaf blade shape	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	cordate to rounded	oblique, cuneate to subrounded or cordate	sometimes oblique, cuneate to subcordate	oblique, subrounded
Leaf base margin	nearly entire to shallowly crenate, apex rounded	serrulate or crenate-serrate, apex acute to rounded	crenate-serrate	shallow crenate
Stamens	included, didynamous, staminode 1	exposed, didynamous, staminode absent	exposed, equivalent, staminode absent	included, equivalent, staminode 1
Anthers	ovate, 2-loculed, dehiscing transversely	broadly oblong, 2-loculed, dehiscing longitudinally	broadly oblong, 2-loculed, dehiscing longitudinally	horseshoe-shaped,1-loculed, dehiscing transversely
Filaments Pistil	pubescent ca. 9 mm long	pubescent ca. 22mm long	pubescent ca. 22mm long	glabrous ca.9 mm long
	2	2	2	s

Table 1. Diagnostic morphological characters of Oreocharis jasminina sp. nov. and all the three currently-recognised species in Hainan Island.



Figure 4. Three formerly-recognised *Oreocharis* taxa in Hainan Island. *Oreocharis dasyantha* (**A**, **B**), *Oreocharis dasyantha* var. *ferruginosa* (**C**, **D**) and *Oreocharis flavida* (**E**, **F**).

Species	trnL-trnF	ITS1/2	Voucher Number
O. dasyantha Chun (Mt. Bawang)-1	MK587993	MK587954	S.J.Ling & M.X. Ren 2015011803 (HUTB)
O. dasyantha Chun (Mt. Bawang)-2	MK587994	MK587954	S.J.Ling & M.X. Ren 2015011804 (HUTB)
O. dasyantha Chun (Mt. Jianfeng)-1	MK587995	MK587955	S.J.Ling 2015102201 (HUTB)
O. dasyantha Chun (Mt. Jianfeng)-2	MK587996	MK587955	S.J.Ling 2015102202 (HUTB)
O. dasyantha Chun var. ferruginosa	MK587954	MK587956	S.J.Ling 2015102203 (HUTB)
Pan (Mt. Jianfeng)-1			
O. dasyantha Chun var. ferruginosa	MK587954	MK587957	S.J.Ling 2015102204 (HUTB)
Pan (Mt. Jianfeng)-2			
O. flavida Merrill (Mt. Qixian)	MK587947	MK587990	S.J.Ling 2018112901 (HUTB)
O. flavida Merrill (Mt. Wuzhi)	MK587989	MK587943	S.J.Ling 2018112902 (HUTB)
O. jasminina (Mt. Yingge)-1	MK587987	MK587948	S.J.Ling 2018112601 (HUTB)
O. jasminina (Mt. Yingge)-2	MK587988	MK587950	S.J.Ling 2018112602 (HUTB)
O. jasminina (Mt. Limu)-1	MK587981	MK587949	S.J.Ling 2018112603 (HUTB)
O. jasminina (Mt. Limu)-2	MK587982	MK587953	S.J.Ling 2018112604 (HUTB)
O. sinohenryi (Chun) Mich.Möller &	HQ632913	HQ633009	M.Möller MMO 07-1150 (E)
A.Weber			

Table 2. List of Hainan *Oreocharis* taxa and outgroup *O. sinohenryi* used in the phylogenetic analysis, including respective Genbank accession and voucher numbers.

7 mm long; ovary cylindrical, ca. 5 mm long, glabrous. Stigma 2, equal, suborbicular. Capsula linear, 3–4 cm long, glabrous to sparsely puberulent.

Phenology. *Oreocharis jasminina* flowers from September to December and fruits from November to January.

Distribution and habitat. *Oreocharis jasminina* is currently only found in cloud forests on the mountain tops of Mt. Limu and Mt. Yingge, in the middle of Hainan Island. The habitat of *O. jasminina* is on the moss layer on wet rocks under cloud forests.

Etymology. The specific epithet refers to the yellow and narrowly tubular corolla of this new species.

Vernacular name. 迎春花马铃苣苔 (Yíng Chūn Huā Mǎ Líng Jù Tái) is the Chinese name for *Oreocharis jasminina*, the first three characters meaning 'winter jasmine', indicating its similar floral syndromes to *Jasminum nudiflorum* Lindl. The last four characters are the Chinese name for *Oreocharis*.

Conservation status. *Oreocharis jasminina* is, so far, known only from the two locations with about 800–1000 individuals. The populations are under threat due to the restricted and fragmented habitat. Therefore, we propose that *O. jasminina* should be considered as 'Vulnerable' (VU), according to the IUCN Red List Categories and Criteria (IUCN 2012).

Key to Oreocharis jasminina and its closely-related and sympatric species in Hainan Island

Discussion

Our former study showed the new species *O. jasminina* and the three other Hainanendemic taxa are homologous, indicating these species in Hainan Island had a common origin (Ling et al. 2020). The new species is only found on mountain tops higher than 1200 m in two mountains, Mt. Limu and Mt. Yingge, located at the middle of Hainan Island. These mountain tops likely formed island-like habitats because the deep and wide valleys interrupted gene flows, resulting in population differentiation and speciation (Shen et al. 2017; Ling et al. 2017a, b; Xing et al. 2018). Such 'sky islands' may be the main reason for the origin and maintenance of this Hainan-endemic alpine species (Robin et al. 2015; Ling et al. 2017a).

The new species also shows a clear geographic isolation from the three currently-recognised *Oreocharis* taxa on Hainan Island. The new species *O. jasminina* was only found in Mt. Limu and Mt. Yingge in the middle of the island, while *O. dasyantha* and *O. dasyantha* var. *ferruginosa* are restricted to the west side of the Island and *O. flavida* was only found in the east side (Fig. 5). They are isolated by a large river, the Changhua River (the second largest river on Hainan Island). Li et al. (2019) found that the geographic isolation by the Changhua River is a driving force for the great population differentiation in the two Hainan-endemic Gesneriaceae species, *Primulina heterotricha* (Merr.) Yan Liu and *Metapetrocosmea peltata* (Merr. et Chun) W. T. Wang. Thus, the geographic isolation by rivers or valleys may also play a key role in the evolution of *O. jasminina* and other Hainanendemic *Oreocharis* taxa. However, the relative contributions of such geographic isolation and altitudinal differentiation are still in need of further experimental examination.

Floral symmetry is widely recognised as a key trait in pollination and taxonomy. Normally, the zygomorphic corolla possesses higher pollen-transfer efficiency than the actinomorphic corolla (Sargent 2004). *Oreocharis jasminina* has yellow actinomorphic corolla with a long and narrow floral tube, differing from *O. dasyantha* and *O. dasyantha* var. *ferruginosa* (both have zygomorphic corolla). Although *O. flavida* has an actinomorphic flower, its campanulate corolla with four equivalent stamens and horseshoe-shaped anthers make it distinct from the new species *O. jasminina* (Table 1).

Floral shape was expected to be a vital factor in generating floral isolation and evolutionary shifts (Castellanos et al. 2004; Muchhala 2007). Generally, the floral



Figure 5. Geographic distribution of *Oreocharis jasminina* sp. nov. and the three congeners on Hainan Island. \star *O. jasminina* sp. nov. Δ *O. dasyantha* \Diamond *O. dasyantha* var. *ferruginosa* \Box *O. flavida*.

shape has a strong connection with the expected pollinators in Gesneriaceae, for example, bees or hummingbirds for tubular flowers, bats for campanulate flowers and subcampanulate flowers having generalised pollination systems (Martén-Rodríguez et al. 2009). *O. jasminina* has thin-tubular corolla (Fig. 1), differing from *O. dasyantha*, *O. dasyantha* var. *ferruginosa* (both are conical corolla) and *O. flavida* (campanulatetubular corolla), indicating a possible pollination mechanism associated with the longtongued butterflies and moths. Such distinctive morphological differences indicate different pollination adaptation and clear reproductive isolation amongst these taxa, suggesting *O. jasminina* should be treated as a new species.

Acknowledgements

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Appendix I

Voucher Number	Species	Voucher Number	Species
PE00030859	Oreocharis rhytidophylla	bm000041491	Oreocharis aurantiaca
IBSC0004917	Oreocharis henryana	gh00353683	Oreocharis dentata
A00353713	Oreocharis magnidens	k000858130	Oreocharis maximowiczii
bm000041734	Oreocharis benthamii	e00087519	Oreocharis tubicella
e00067455	Oreocharis cavaleriei	e00087520	Oreocharis nemoralis
a00025113	Oreocharis flavida	IBSC0004912	Oreocharis aurea
bm000041721	Oreocharis georgei	p04060117	Oreocharis forrestii
KUN1219176	Oreocharis cordato-ovata	e00135096	Oreocharis amabilis
e00087535	Oreocharis dasyantha	e00135074	Oreocharis bodinieri
bm000041708	Oreocharis cinnamomea	gh00353695	Oreocharis benthamii var.
			reticulata
PE00030854	Oreocharis tubiflora	p04060171	Oreocharis delavayi
IBSC0004920	Oreocharis xiangguiensis	CSFI028502	Oreocharis brachypodus
PE19401111	Oreocharis amabilis	PE02052999	Oreocharis heterandra
IBSC0550960	Oreocharis sericea	IBSC0550860	Oreocharis cordatula
IBSC0550891	Oreocharis georgei	IBSC0550875	Oreocharis elliptica
GZTM0075588	Oreocharis primuloides	JIU63907	Oreocharis speciosa
PE02052990	Oreocharis argyreia var. angustifolia	PE02053568	Oreocharis eximia
IBK00054784	Oreocahris auricula	PE01909883	Oreocharis mileensis
KUN1219104	Oreocharis hekouensis	WUK0494363	Oreocharis saxatilis
PE02053062	Oreocharis concava	PE01486523	Oreocharis rosthornii
PE02106072	Oreocharis begoniifolia	KUN1385365	Oreocharis nanchuanica
KUN1385575	Oreocharis urceolata	HITBC106680	Oreocharis longifolia
PE00030861	Oreocharis rotundifolia	IBSC0548683	Oreocharis chienii
PE02053433	Oreocharis acaulis	KUN1385156	Oreocharis bullata
PE02241281	Oreocharis burttii	PE02053072	Oreocharis cinerea
KUN1220227	Oreocharis convexa	IBK00054466	Oreocharis cotinifolia
PE00155697	Oreocharis craibii	WUK0350789	Oreocharis crenata
IBSC0550709	Oreocharis dalzielii	PE02106079	Oreocharis dinghushanensis
IBSC0551649	Oreocharis esquirolii	PE02052984	Oreocharis fargesii
IBSC0649611	Oreocharis flabellata	PE02053533	Oreocharis gamosepala
PE02052812	Oreocharis giraldii	PE02106025	Oreocharis glandulosa
PE02052995	Oreocharis humilis	PE01548041	Oreocharis jiangxiensis
PE02021009	Oreocharis lancifolia	FJSI004239	Oreocharis leiophylla
PE02053066	Oreocharis leucantha	IBSC0550069	Oreocharis lungshengensis
IBSC0551655	Oreocharis mairei	PE02053564	Oreocharis minor
WUK0160594	Oreocharis muscicola	IBSC0548476	Oreocharis notochlaena
PE02106041	Oreocharis obliqua	PE02052801	Oreocharis obliquifolia
PE02088092	Oreocharis obtusidentata	PE02053064	Oreocharis pankaiyuae
PE01270485	Oreocharis primuloides	WUK0213194	Oreocharis pumila
PE02053576	Oreocharis pinnatilobata	KUN1241303	Oreocharis primuliflora
PE02053532	Oreocharis rhombifolia	PE00030693	Oreocharis ronganensis
PE00030747	Oreocharis sichuanensis	IBSC0550081	Oreocharis sichuanica
IBK00054319	Oreocharis sinensis	IBK00207093	Oreocharis sinohenryi
PE02053579	Oreocharis stenosiphon	IBSC0548730	Oreocharis stewardii
PE02053570	Oreocharis trichantha	HEAC0016525	Oreocharis villosa
PE02053561	Oreocharis wangwentsaii	PE02053077	Oreocharis wanshanensis
Y. M. Shui et al.	Oreocharis synergia	Y.M.Shui et al. N699	Oreocharis ninglangensis
B2014-299 (KUN)		(KUN)	

The Oreocharis specimens we checked in this study.

Voucher Number	Species	Voucher Number	Species
PE-02114626	Oreocharis duyunensis	IBSC0825078	Oreocahris ovata
KUN1219115	Oreocharis acutiloba	PE00030682	Oreocharis agnesiae
PE00140281	Oreocharis billburttii	PE02025205	Oreocharis elegantissima
IBSC0649550	Oreocharis latisepala	PE00030685	Oreocharis parva
Z.K. Wu et	Oreocharis parvifolia	PE02025202	Oreocharis pinfaensis
al.C2016055 (KUN)			
IBSC0548691	Oreocharis shweliensis	PE01909893	Oreocharis tongtchouanensis
Y.M.Shui, Y.K.Sima & W.H.Chen	Oreocharis crispata	Y.M.Shui et al. 91309 (KUN)	Oreocharis jinpingensis
B2013-258 (KUN)			
Bo Pan & M. Q. Han HMQ859 (IBK)	Oreocharis purpurata	Yun-Hong Tan 3308 (HITBC)	Oreocharis tsaii
Averyanov, L., Hiep, N.T., Khang, N.S., Thang, N.D. & Qui, L.D. CPC 7019 (KUN)	Oreocharis blepharophylla	Jia-Mei Li and Yao-Guang Zhang 1606151 (HEAC)	Oreocharis zhenpingensis
Bo Pan & Jia-Jia Wei et al. GY002 (IBK)	Oreocharis curvituba	C.Z. Yang et al. 35042620140913001 (FNU)	Oreocharis striata
Y.M. Shui et al. B2013-551 (KUN)	Oreocharis longituba	Averyanov, L., Hiep, N.T., Khang, N.S., Thang, N.D. & Qui, L.D. CPC 7175 (KUN)	Oreocharis argyrophylla
Y.M. Shui et al. B2013-550	Oreocharis grandiflora	T.V. Do 57 (VNMN)	Oreocharis caobangensis
L.H. Yang et al. YLH197 (IBSC)	Oreocharis pilosopetiolata	Li-Hua Yang et al. YLH285 (IBSC)	Oreocharis uniflora
Ying Guo C2015005 (KUN)	Oreocharis panzhouensis	L. E. Yang 60 (KUN)	Oreocharis rubrostriata
Yan Liu and Wei-Bin Xu 08018 (IBK)	Oreocharis dayaoshanioides	Yun-Hong Tan 6925 (HITBC)	Oreocharis glandulosa
PE 02053063	Oreocharis farreri	Lin Qin-Wen et al. 0016 (FAFU)	Oreocharis baolianis
IBSC0548624	Oreocharis guileana	IBK00054993	Oreocharis dasyantha var. ferruginosa

RESEARCH ARTICLE



Petrocosmea nanchuanensis (Gesneriaceae), a new species from Chongqing, China

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Abstract

A new species, *Petrocosmea nanchuanensis* Z.Y. Liu, Z.Y. Li & Z.J. Qiu from Mt. Jinfo at Banhe valley of Nanchuan District in Chongqing Municipality (China) is described and illustrated for the first time. Even though this new species is similar to *Petrocosmea barbata*, it has several significant morphological differences, which includes smaller leaves, repand leaf margin, densely appressed longer pubescences on both surfaces of leaves, larger flower with length of its lower lips about three times longer than that of the upper lips, oblong lower lip lobes, shorter pistil, ovate anthers and styles that are shortly pubescent or approximately glabrous above the middle. The distinct features of *P. nanchuanensis* and four relative species namely, *P. barbata*, *P. longipedicellata*, *P. cavaleriei* and *P. xanthomaculata* were also represented in depth. However, *P. nanchuanensis* is most closely related to *P. barbata*, based on molecular studies.

Keywords

Gesneriaceae, new species, Petrocosmea nanchuanensis

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Introduction

The genus *Petrocosmea* Oliver, (1887) (Family: Gesneriaceae, Subfamily: Didymocarpoideae, Tribe: Trichosporeae) was established in 1887. At present, *Petrocosmea* genus consists of 50 species classified into five sections: sect. *Petrocosmea* Oliv., (1919), sect. *Anisochilus* Hemsl., (1899), sect. *Minor* Zhi J. Qiu, (2015), sect. *Barbata* Zhi J. Qiu, (2015) and sect. *Deinanthera* W.T. Wang, (1985) (Oliver 1887; Wang 1985; Wang et al. 1990, 1998; Burtt 1998, 2001; Li and Wang 2005; Wei and Wen 2009; Qiu et al. 2011, 2012, 2015). Herein, we report the discovery of a new species present on Mt. Jinfo in the Banhe valley, Nanchuan District, Chongqing Municipality of China. This new species has unconstructed anthers and an abaxial corolla lip that is approximately three times longer than the adaxial one with two yellow spots at the base of the lower lip's lobes. Hence, the new species, *P. nanchuanensis* belongs to sect. *Barbata*.

Materials and methods

Measurements and observations of morphological characters of the new species, based on living individuals and specimens, were carried out in the field or greenhouse and at the herbarium. Hairs and glandular hairs and other tiny morphological characters were observed and measured by using a stereomicroscope (Nikon SMZ18). Morphological comparisons with related species were measured, based on living individuals in the greenhouse and specimens from PE, SZBG and KUN herbaria.

Taxonomy

Petrocosmea nanchuanensis Z.Y. Liu, Z.Y. Li & Z.J. Qiu, sp. nov. urn:lsid:ipni.org:names:77211190-1 Figs 1, 2

Diagnosis. *Petrocosmea nanchuanensis* is morphologically similar to *P. barbata* Craib, but is distinguished from the latter by smaller leaves, a repand leaf margin, densely appressed longer pubescences on both surfaces of its leaves, larger flower with the length of its lower lips three times longer than that of the upper lips, oblong lower lip lobes, shorter pistil, ovate anthers and styles that are shortly pubescent or approximately glabrous above the middle.

Type. CHINA. Chongqing Municipality: Nanchuan, Banhegou, Mt. Jinfo, 20 Sep 2002. *Z. Y. Li 2002016* (holotype, PE).

Description. Perennial herb, rosette-forming, with a short rhizome and crowded fibrous roots. Leaves 8 to 30, all basal, crowded, the inner leaves with short or absent petioles, the outer leaves with longer petioles; leaves orbicular-ovate, broad-ovate, cordate or almost circular, $0.5-2 \times 0.7-2$ cm, herbaceous, apex round, base cordate,



Figure 1. Drawing of *Petrocosmea nanchuanensis* Z.Y. Liu, Z.Y. Li & Z.J. Qiu, sp. nov. A habit B stamens C dissected corolla D calyx and pistil.

margin undulate teeth, both surfaces with densely villous, lateral veins 3 on each side, not distinct; petioles 0.5–6 cm, densely pilose. Cymes 5 to 15, 1–3 flowers per cyme; peduncle 3–10 cm, densely pilose; bracts 2 at upper- or middle-peduncle, lanceo-



Figure 2. *Petrocosmea nanchuanensis* Z.Y. Liu, Z.Y. Li & Z.J. Qiu **A** habitat **B** flowering plants **C** flower, front view **D** flower, side view **E** flower, front view, showing pistil and anthers **F** hairs on abaxial leaves **G** stamens **H** hairs on the pistil.

late, 0.5–1.2 cm, pubescent, pedicel 1–5 cm, densely hairy; sepals 5-divided to the base, narrow-lanceolate, ca. 4–5 mm, pubescent externally. Corolla light purple or white, outside and inside abaxial lip puberulent, inside adaxial lip and tube near mouth densely pubescent, 2 yellow spots inside abaxial lip base; tube ca. 3 mm, adaxial lip ca. 3–3.5 mm, bi-lobed near to base, lobes ovate, abaxial lip ca. 8–9 mm, deeply tri-lobed, lobes oblong; stamens 2, ca. 2.2 mm; filaments adnate to ca. 1 mm above the base of corolla tube, ca. 1.2 mm long, glabrous; anthers ovate, ca. 1 mm long, glabrous; staminodes 3, adnate to ca. 0.2–0.4 mm above the base of the corolla tube, ca. 0.3–0.8 mm long, glabrous; pistil ca. 4.5 mm; ovary densely villous, ovoid, oblique abaxially, ca. 1.5 mm long; style unfolded pilose and glandular hairs under the middle, shortly pubescent or approximately glabrous above the middle, ca. 3 mm long.

Habitat and distribution. *Petrocosmea nanchuanensis* grows on moist shady limestone cliffs or along forest edges at an altitude of 600–650 m on the Mt. Jinfo, Banhe valley, Nanchuan District, Chongqing Municipality, which is adjacent to Guizhou Province in south-western China.

International Union for Conservation of Nature (IUCN) Red List Category. The new species is determined to be Critically Endangered (CR A2c) according to the IUCN Red List of Threatened Species Categories and Criteria (IUCN 2001). About
850 individuals were found in two separate and restricted populations: 300 individuals were found growing in one location with ca. $4 \times 2 \text{ m}^2$ and 550 individuals were found at the other location with ca. $8 \times 3 \text{ m}^2$. *Petrocosmea nanchuanensis* is only known from the type locality, i.e. Nanchuan District and their habitat has been threatened and damaged by deforestation and over-exploitation due to native tourism despite its protection in the Mt. Jinfo National Nature Reserve.

Phenology. The new species was collected with flowers during September–November; fruits were not seen.

Additional specimens studied (paratypes). CHINA. Chongqing: Mt. Jinfo, Nanchuan, 3 Sep 2007, *R. H. Liang 2007010* (PE), *Z. J. Qiu* 2011020, QZJ-20131082 (SZG), *S.Z. Zhang* 20121077 (SZG), *Z. Y. Liu* 500119-1582ly, 500119-1595ly (IMC).

Discussion

In our previous study, molecular phylogenetic analysis of *Petrocosmea* genus which includes *P. nanchuanensis* has been studied using six chloroplast DNA regions (*atpI-atpH*, *matK*, *trnH-psbA*, *rps16* intron, *trnL-F* and *trnT-L*) and two nuclear DNA regions (*ITS* and *PeCYC1D*) (Qiu et al. 2015). The molecular phylogenetic study showed that *P. nanchuanensis* rests at the base of a clade containing three other species, *P. barbata* Craib, (1919), *P. longipedicellata* W.T. Wang, (1985) and *P. cavaleriei* Levl., (1911), as shown in Fig. 3. In the phylogenetic tree, this clade, which includes four species, *P. nanchuanensis*, *P. barbata*, *P. longipedicellata* and *P. cavaleriei*, has some morphological synapomorphies, such as two yellow spots at the base of the lower lip lobes, densely villous on the ovary, villous inside corolla tube and glabrous filaments. The phylogenetic tree revealed that *P. nanchuanensis* is most closely related to *P. barbata* that also belongs to sect. *Barbata*.



Figure 3. Drawing of the most parsimonious tree generated from six cpDNA and two nrDNA regions (partial & unpublished). Bootstrap values are shown above branches and Bayesian posterior probabilities are indicated below branches.

Features	P. nanchuanensis	P. barbata	P. longipedicellata	P. cavaleriei	P. xanthomaculata
Leaf shape	Ovate	Round-ovate	Sub circular	Broadly ovate	Broadly ovate
Length of mature leaves	1.8–2 mm	2.5–2.8 mm	4-4.5 mm	2–2.5 mm	2–2.3 mm
Leaf margin	Undulate teeth	Crenate	Shallow-serrate	Repand	Repand
Hairs on the leaf	Densely villous on both sides	Sparsely appressed with pubescence above and slightly densely pubescent below	Slightly densely appressed pubescent on both sides	Pubescent on both sides	Villous on both sides
Leaf base	Cordate	Cordate	Truncate	Subcordate	Subcordate
Hairs on the peduncle and pedicel	Unfolded pilose	Sparsely pubescent and glandular hairs	Unfolded pubescent	Pubescent and glandular hairs	Unfolded pilose and glandular hairs
Length of corolla	11–12 mm	7–8 mm	8–9 mm	8–9 mm	7–8 mm
Spots inside the corolla	There are 2 yellow spots at the base of lower lip lobes	There are 2 yellow bands at the base of lower lip lobes	There are 2 yellow spots at the base of lower lip lobes	There are 2 yellow spots at the base of lower lip lobes and 3 small yellow spots in the areas of staminodes	There are 2 yellow spots at the base of lower lip lobes and 3 small yellow spots in the areas of staminodes
The degree of upper lip cracking	Near base	Near the middle	Near the middle	Near base	Near base
Do the upper lip lobes press against each other	Yes	No	No	No	Yes
Shape of upper lip lobe	Ovate	Round-ovate	Rounded	Rounded	Rounded
The degree of lower lip cracking	More than the middle	Near the middle	Near the middle	Near base	Near base
Shape of lower lip lobe	Oblong	Broadly ovate	Ovate	Oblong	Long-ovate
The length ratio of upper to lower lip	- 1:3	~ 1:2	- 1:2	- 1:2	- 1:2
Length of stamen	2–2.5 mm	3–3.5 mm	4.5–4.7 mm	1.5–1.8 mm	1.6–1.8 mm
Shape of anther	Ovate	Round-ovate	Elliptic	Oblate	Round-ovate
Length of lateral staminodes	0.6–0.8 mm	0.2–0.3 mm	0.4–0.5 mm	0.4–0.5 mm	0.4–0.5 mm
Length of pistil	4.5–5 mm	7–8 mm	6–7 mm	6–7 mm	6–7 mm
Hairs on the styles	Unfolded pilose and glandular hairs under the middle, shortly pubescent or approximately glabrous above the middle	Unfolded villous and short glandular hairs under the top, short glandular hairs on the top	Unfolded pilose under the top	Unfolded pilose and glandular hairs under the middle	Unfolded villous under the top

Table 1. Distinguishing features of *P. nanchuanensis* in comparison with other related species.

Table 1 summarises the distinguishing features of *P. nanchuanensis* and its related species namely, *P. barbata, P. longipedicellata, P. cavaleriei* and *P. xanthomaculata* G.Q. Gou & X.Y. Wang (2010). Herein, both morphological and our previous molecular studies support *P. nanchuanensis* as a new species and being most similar to *P. barbata.* The foremost morphological differences between *P. nanchuanensis* and *P. barbata* include that *P. nanchuanensis* has smaller leaves than *P. barbata* and *P. nanchuanensis* leaves have an undulate-toothed margin, whereas, *P. barbata* has a crenate margin. Moreover, *P. nanchuanensis* shows a densely appressed villous on both surfaces of leaves instead of the adaxially puberulent and abaxial slightly and densely puberulent pattern of *P. barbata*.

Likewise, *P. nanchuanensis* has larger flowers than *P. barbata*, in which *P. nan*chuanensis has flowers about three times longer in lower than in upper lips instead of about two times longer in lower than in upper lips in *P. barbata*. Additionally, lower lip lobes are oblong in *P. nanchuanensis* instead of broadly ovate in *P. barbata*, while *P. nanchuanensis* has a shorter pistil than *P. barbata*. Besides, *P. nanchuanensis* has styles that are shortly pubescent or approximately glabrous above the middle instead of styles with unfolded villous and short glandular hairs under the top and short glandular hairs on the top in *P. barbata*. The *P. nanchuanensis* has ovate instead of round-ovate anthers in *P. barbata*.

We conclude that *Petrocosmea nanchuanensis* belongs to sect. *Barbata* due to its floral structure, particularly anthers that are not constricted near the apex, the length of its abaxial corolla lip being twice as long as the adaxial and two yellow spots at the base of the lower lips lobes. Even though, this new species is similar to *P. barbata* and mostly varies through smaller leaves, a repand leaf margin, densely appressed longer pubescences on both surfaces of its leaves, larger flowers with their lower lips about three times longer than the upper lips, oblong lower lip lobes, shorter pistil, ovate anthers and shortly pubescent styles or approximately glabrous above the middle.

Morphological similarity between *P. nanchuanensis* and *P. barbata* has been supported by our previous molecular phylogenetic data (Qiu et al. 2015). A phylogenetic tree, based on six cpDNA regions and two nrDNA regions, confirmed that *P. nan-chuanensis* is most closely related to *P. barbata* (Figure 3), which is distributed throughout Kunming County in the east-central portion of Yunnan Province, China.

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RESEARCH ARTICLE



Didymocarpus lobulatus (Gesneriaceae), a new species from Zhejiang Province, East China

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Abstract

Didymocarpus lobulatus, a new species endemic to Zhejiang province, eastern China, is described and illustrated with photographs. The new species is morphologically similar to *D. heucherifolius*, *D. cortusifolius* and *D. salviiflorus* in leaf morphology, but can be easily distinguished by a combination of characters, including the shape of bracts, calyx and calyx lobes.

Keywords

Flora of Zhejiang, new taxon, taxonomy, Didymocarpus sect. Heteroboea

Introduction

The delimitation of *Didymocarpus* has varied considerably over time with recent results from both molecular phylogenetic studies and morphological revisions (Weber et al. 2011, Möller et al. 2011, Möller and Clark 2013, Li et al. 2016). Now, prior to the new species being described here, there are approximately 70 species in the world (Weber et al. 2000) consisting of 34 species and four varieties in China (Cai et al. 2016).

Didymocarpus Wall. (Gesneriaceae) was once considered to consist of about 180 species (Wang et al. 1998). However, it was split into three genera: Didymocarpus s.s., Henckelia Spreng., and Hovanella A.Weber & B.L.Burtt (Weber and Burtt 1997). Thus, Didymocarpus was left with about 70 species after the removal of the Madagascan, southern Indian and Sri Lankan and most Malesian species (Nangngam and Maxwell 2013). Didymocarpus sensu stricto currently contains 70 species (Vitek et al. 2000, Weber et al. 2000, 2011). According to the treatment of *Didymocarpus* s.l. in Flora of China (Wang et al. 1990, 1998), this genus was recognised and divided into two sections: sect. Didymocarpus (herbs with stems (0.7-)3-62 cm long) and sect. Heteroboea (herbs stemless). The first section, Didymocarpus sect. Didymocarpus, is a natural unit with about 50 species distributed mainly in Bhutan, Burma, Southwest China, Malaya, Nepal, North & Northeast India, North Thailand and North Vietnam. Some new taxa belonging to this section were discovered and published recently, e.g. *Didymocar*pus puhoatensis Xin Hong & F. Wen, (2018), D. moellerii A.Joe, Hareesh & M.Sabu, (2016), D. anningensis Y.M. Shui, Lei Cai & J. Cai, (2016), D. tonghaiensis J.M. Li & F.S. Wang, (2015). The other section, sect. *Heteroboea*, includes seven species and one variety and is endemic to South and East China (Wang et al. 1990, 1998; Li and Wang 2004). In the past five years, two new taxa were discovered and published in eastern and southern China, namely Didymocarpus heucherifolius var. yinzhengii J.M. Li & S.J. Li (2014) from Hunan province and D. dissectus Fang Wen, Y.L. Qiu, Jie Huang & Y.G. Wei, (2013) from Fujian province.

In 2010, one of the authors (HX) found an unknown *Didymocarpus* species with the previous year's fruits during field investigations in Zhejiang Province, China. Soon afterwards, the same species was again collected by the other authors (XWY & ZJJ) during floristic surveys in 2014. Based on the recollected specimens of this uncertain species and from detailed inspection in 2016 and 2017, we confirmed it belongs to *Didymocarpus* because of its disc-like stigma (Wang et al. 1998) and it is a member of sect. *Heteroboea* as it is a stemless herb. We also concluded that this plant is new to science after thoroughly consulting the related literature (Wang et al. 1990, Wang et al. 1998, Li and Wang 2004). Here, the new species is described and illustrated and its morphological characters are compared with its morphologically similar congeners.

Material and methods

Measurements and morphological character assessments of the putative new species were undertaken and described using available specimens stored in the following herbaria in China, the United States and the United Kingdom: AHU, E, IBK, KUN, MO, PE and US. In addition, images of other type specimens were obtained from Tropicos (http://www.tropicos.org) and JSTOR Global Plants (http://plants.jstor.org). All morphological characters were studied under dissecting microscopes and are described using the terminology presented by Wang et al. (1998).

Taxonomic treatment

Didymocarpus lobulatus F. Wen, Xin Hong & W.Y. Xie, sp. nov. urn:lsid:ipni.org:names:77211191-1 Figures 1, 2

Diagnosis. Didymocarpus lobulatus is similar to D. heucherifolius Handel-Mazzetti and D. salviiflorus W.Y. Chun in having a similar zygomorphic corolla and pink to pinkish-purple funnel-shaped to tubular corolla tube, but can be distinguished from the former by its densely eglandular and glandular pubescent peduncle (vs. villous in D. *heucherifolius*), bracts subulate to subulate-triangular (vs. elliptic) and margin sparsely crenate from the middle (vs. entire), calyx shallowly 5-lobed to or lobed about twothirds of the calyx length from the base (vs. 5-lobed to the base). From D. salviiflorus, it differs by having subulate to subulate-triangular bracts (vs. semi-orbicular in D. salviiflorus), calyx lobes triangular but non-overlapping (vs. depressed oblong, overlapping at margin) and in size in ca. 5×2.5 mm (vs. $2-2.2 \times 4-4.5$ mm). It is also similar to D. cortusifolius in the shape and size of the leaves, but can be easily distinguished by having bracts subulate to subulate-triangular (vs. ovate to elliptic in *D. cortusifolius*), bract margin sparsely crenate from the middle (vs. entire), larger calyx lobes ca. 5×2.5 mm (vs. $1-3 \times ca. 2 \text{ mm}$) and lobes' margin nearly entire to entire (vs. denticulate), corolla white (vs. corolla pink to dark pink), fertile stamens adnate to corolla ca. 5 mm from base (vs. adnate to corolla 10-14 mm from base), filaments sparsely brownish glandular-puberulous (vs. glabrous).

Type. CHINA. Zhejiang Province: Shengzhou city, Chongren Town, Liwang village, 29°39'N, 120°39'E, 223 m a.s.l., 23 May 2014, flowering, Wen-Yuan Xie & Jia-Jun Zhou 140523-01 (holotype: IBK; isotype: AHU).

Description. Perennial acaulescent herb. Rhizome horizontal, 1–4 cm long, up to 1.5 cm in diameter, roots fibrous. Leaves 4-8 basal, clustered at the apex of the rhizome; petioles terete, 2–9 cm long, densely covered with spreading rust-brown villous and white puberulent indumentum; blades asymmetrically orbicular-ovate to orbicular triangular, 3–10 × 2.5–12.5 cm, apex rounded, base cordate, margin irregularly triangular denticulate, papery, upper surface densely covered with whitish short and long eglandular pubescent indumentum, green, lower surface sparsely covered with hairs as on upper surface confined to the veins, pale green, basic veins 3-6, obscure above, prominent beneath. Inflorescences axillary, cymes 1–2-branched, 3–8 (–12)-flowered; peduncle 3-16 cm long, densely covered with both eglandular and glandular pubescent hairs, pedicel 0.3-2 cm long, with indumentum as on the peduncle. Bracts 2, opposite, subulate to subulate-triangular, ca. 8 mm long, adaxially glabrous, abaxially puberulent, margin sparsely crenate from the middle; bracteoles 2, opposite, subulate, 3–3.5 mm long, indumentum same as bracts. Calyx actinomorphic, shallowly 5-lobed to about two-thirds of the calyx length from the base, symmetrical, 1.3–1.6 cm long, inside glabrous, outside densely puberulent, brownish-green; lobes equal, triangular,



Figure 1. *Didymocarpus lobulatus* **A** Habit **B** Flower in top view **C** Opened corolla, showing stamens and staminodes **D** Fertile stamens and anthers **E** Pistil, disc and stigma.

ca. 5×2.5 mm, apices obtuse, margin nearly entire to entire. Corolla zygomorphic, 2.5–3.2 cm long; outside sparsely puberulent to glabrescent, inside glabrous, pink to dark pink, becoming pinkish-white at the base, with brown lines inside. Tube funnel-shaped to tubular, 1.8–2.2 cm long, 0.8–1 cm in diameter at mouth; limb distinctly 2-lipped, adaxial lip 2-parted to near the middle, lobes ovate, ca. 6×5 mm, abaxial lip 3-lobed from the base, lobes oblong, ca. 8×4 mm, more or less equal. Fertile stamens 2, adnate to corolla ca. 0.5 cm from base; filaments slender, 6–10 mm long, geniculate near the base, sparsely brownish glandular-puberulous; anthers fused along their entire adaxial surfaces, reniform-oblong, 1.8–2.5 mm long, ca. 2 mm wide, pale yellow, bearded on the back; staminodes 3, reduced to capitate, 0.3–0.5 mm long, adnate to corolla 6-8 mm from base, brown. Disc cylindrical, ca. 2 mm long, margin irregular, glabrous. Pistils 2–3 cm long, densely puberulent with both glandular and eglandular hairs; ovary ca. 2.6 cm long, yellowish-green. Stigma 1, terminal, depressed-globose, undivided, translucent. Capsule 5.5–7 cm long, brownish, glabrous.

Etymology. The specific epithet is derived from the shallowly 5-lobed calyx.



Figure 2. *Didymocarpus lobulatus* F. Wen, Xin Hong & W.Y. Xie. A Habitat B Vegetative part of plants
C Habitat in flowering D Frontal view of corolla E Top view of cyme, showing actinomorphic calyx
F Opened corolla for showing stamens, pistil and calyx lobes G Adaxial surface view of leaf blade H Abaxial surface view of leaf blade.

Distribution and habitat. *Didymocarpus lobulatus* is locally abundant and endemic to a narrow area in eastern China, surrounding the type locality. This species grows on moist shady cliffs of sandy shale hills, at an elevation of 223 m a.s.l. in type locality. The average temperature is 16.4 °C, the average annual precipitation has been calculated as ca. 1,446.8 mm. The forest is a subtropical monsoon climate evergreen



Map 1. The distribution areas of *Didymocarpus lobulatus* and its congeners in Zhejiang prov.: ① *D. lobulatus* ② *D. cortusifolius* ③ *D. salviiflorus* ④ *D. heucherifolius* (in semi-elliptical region, the arrows point).

broad-leaved forest. Flowering in May. Another population growing in the Danxia landscape of Chuanyanshijiufeng, Xinchang County, Shaoxing city, not far away from the type locality, was discovered by the first author in 2016. The two places are about 40 kilometres apart.

Proposed conservation status. Based on the present field investigations, *Didymocarpus lobulatus* is currently only known from two sites around the type locality. The two places are about 40 kilometres apart. The type population, which grew close to a country road, is potentially threatened by human activities. Although no such habitat destruction is currently occurring, this population is likely to be threatened in the foreseeable future under influences of man-made factors, for example, by road widening. Fortunately, the second population has been found in the well-protected core zone of the scenic spot, Chuanyanshijiufeng. Furthermore, this population is healthy and locally abundant with many young plants and seedlings growing in the area. Thus,



Figure 3. A *Didymocarpus lobulatus* B *D. heucherifolius* C *D. cortusifolius* D *D. salviiflorus* I Habitats 2 The frontal view of corolla and cyme 3 Cyme and bracts 4 calyx lobes.

it seems that this species is not at serious risk now and it is proposed as 'Vulnerable' (VU D2) according to the IUCN Red List Categories and Criteria (IUCN 2016).

Notes. The geographical distributions of *Didymocarpus lobulatus* and its congeners are identified in Map 1. There are altogether 7 species and one variety of sect. *Heteroboea*, three species are endemic to East China (*Didymocarpus salviiflorus* and *D. cortusifolius* in Zhejiang prov. and *D. dissectus* in Fujian prov.) and two species and one variety (*D. yuenlingensis* W.T. Wang, *D. sinoprimulinus* W.T. Wang and *D. heucherifolius* var. *yinzhengii*) endemic to Hunan prov. of South China. Only one species, *Didymocarpus heucherifolius*, is widespread, being distributed from northern Guangdong prov. of South China. As more and more field investigations are undertaken, the biodiversity of sect. *Heteroboea* in East and South China will be better understood by researchers. The discovery of two new taxa, *Didymocarpus dissectus* Fang Wen, Y.L. Qiu, Jie Huang & Y.G. Wei (2013) and *D. lobulatus*, serve as two good examples. Differences between the new species and its morphologically related species in sect. *Heteroboea* in Zhejiang prov. are shown in the following identification table (Table 1) and Figure 3.

Character		D. lobulatus	D. heucherifolius	D. cortusifolius	D. salviiflorus
Bracts	shape	subulate to subulate- triangle	elliptic	ovate to elliptic	Semi-orbicular
	size	ca. $8 \times 2 - 3 \text{ mm}$	5-10 × 1.8–4.5 mm	3.5–7 × 1.5–3 mm	ca. 5 × 10 mm
	margin	sparsely crenate from the middle	entire	entire	sparsely crenate
Bracteoles	shape	subulate	usually lacking	ovate to oblong	usually lacking
Calyx	form	actinomorphic, shallowly 5-lobed to about two-thirds of the calyx length from the base	slightly zygomorphic, 5-lobed to the base	actinomorphic, 5-lobed to about half of the calyx length from the base	actinomorphic, 5-lobed to about half of the calyx length from the base
	lobes size	ca. 5 × 2.5 mm	$3-4 \times 1-2 \text{ mm}$	$1-3 \times ca. 2 \text{ mm}$	$2-2.2 \times 4-4.5 \text{ mm}$
	lobes margin	nearly entire to entire	sparsely denticulate	denticulate	denticulate to entire
Stamens	place indumentum	adnate to corolla ca. 5 mm from base sparsely brownish glandular-puberulous	adnate to corolla 10- 12 mm from base white glandular- puberulous	adnate to corolla 10- 14 mm from base nearly glabrous	adnate to corolla ca. 10 mm from base sparsely white puberulent

Table 1. Diagnostic character differences amongst *Didymocarpus lobulatus* sp. nov., *D. heucherifolius*, *D. cortusifolius* and *D. salviiflorus*.

Other specimen examined. CHINA. Zhejiang Province: Shaoxing city, Xinchang County, Chuanyanshijiufeng, Danxia landscape, secondary forests, 29°23'N, 120°48'E, 248 m a.s.l., 11 November 2018, in fruit, *WYG181111-01* (IBK!).

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RESEARCH ARTICLE



Two new species of Allocheilos (Gesneriaceae) from the karst regions in Yunnan, China

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Abstract

Allocheilos W.T.Wang in Gesneriaceae was described in 1983 and is characterized by its 4-lobed adaxial lip and undivided abaxial lip with acute apex. The genus is endemic to the karst regions in southwestern China and is classified as endangered due to habitat loss. During surveys of the karst areas in Yunnan of southwestern China in 2017, we collected two unknown species of the genus and later confirmed their novelty to science based on the detailed observation of their morphological characteristics, viz. *A. maguanensis* W.H.Chen & Y.M.Shui and *A. rubroglandulosus* W.H.Chen & Y.M.Shui. Their relationships with the similar species and provisional conservation status are discussed.

Keywords

Allocheilos, corolla, habitat, isolated distribution, staminodes

Introduction

The genus *Allocheilos* W.T.Wang in Gesneriaceae is endemic to the karst regions of China and restricted to a shady habitat of limestone hills. The genus shows a stable flower morphology and is characterized by its 4-lobed adaxial corolla lip and undivided

abaxial lip (Wang 1983; Wang et al. 1998), except *Oreocharis mileensis* (W.T.Wang) Mich.Möller & A.Weber, *Petrocodon coccineus* (C.Y.Wu ex H.W.Li) Yin Z.Wang and *Pet. viridescens* W.H.Chen & Y.M.Shui (Li 1982; Weitzman et al.1998; Möller et al. 2011; Wang et al. 2011; Chen et al. 2014a, b). However, the latter three exceptions are characterized by the long and narrow corolla tubes, which is different from morphology of flowers in *Allocheilos*. So far, there are only two recorded species recognized in *Allocheilos*, which show only weak morphological differences (Wei et al. 2010). They grow on shady rock surfaces of limestone hills in the Dian-Qian-Gui region, *viz*. Yunnan, Guizhou and Guangxi region in China (Fig. 1; Fang et al. 1995), and respectively have about 36 individuals of *Allocheilos cortusiflorus* W.T.Wang (30 in Xingyi, Guizhou and 6 in Luoping) and 40 individuals of *A. guangxiensis* H.Q.Wen, Y.G.Wei & S.H.Zhong in the field and thus acknowledged as critically endangered (Wang 1983; Wei et al. 2000; Wei et al. 2010).

During surveys in the karst regions in Yunnan in 2016 and 2017 we encountered two unknown species of Allocheilos. At first glance, they look like the species of Petrocodon without flowers in the adjacent region of southeastern Yunnan, such as Pet. viridescens W.H.Chen & Y.M.Shui (Chen et al. 2014a). However, they are characterized by white 4-lobed adaxial corolla lips and short corolla tubes, showing that they are a member of Allocheilos (Figs 2-5). Based on the examination of relevant publications and specimens of Allocheilos from E, IBK, K and P, we found that they are more similar to A. cortusiflorus with short ovary and long style than A. guangxiensis with long ovary and short style (Pellegrin 1930; Wang et al. 1990; Li and Wang 2005; Wei et al. 2010). Furthermore, after the detailed morphological and geographical comparison, we realized that they represent two different species (Table 1). Firstly, there are obvious morphological differences in bracts and staminodes. Secondly, there is a geographical isolation between them, which are respectively distributed in Maguan county and Mengzi county, separated by the Naxi River, SE Yunnan, China (Fig. 1). Thirdly, there are obvious different habitats between them, one of which grows near the summit of the limestone hills and another in the deep limestone sinkholes. So here we described the two species of Allocheilos unknown to science.

Materials and methods

The materials are from type specimens and other specimens kept in the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN). Photographs of the habitat, plants, leaf blade and flowers were taken in the field and greenhouse with camera (Nikon D700, Tokyo, Japan), and detailed morphological characteristics were observed and photographed with stereomicroscope (Leica S8 APO, Serial number 5683759, Shanghai, China). Table 1 is provided to explain the relationship among the two new species and their similar species of *Allocheilos*.



Figure 1. Geographical distribution of the genus *Allocheilos* W.T.Wang of Gesneriaceae in southwestern China. *Allocheilos maguanensis* W.H.Chen & Y.M.Shui, sp. nov. (▲), *A. rubroglandulosus* W.H.Chen & Y.M.Shui sp. nov. (■), *A. cortusiflorus* W.T.Wang (★), *A. guangxiensis* H.Q.Wen, Y.G.Wei & S.H.Zhong (●).

Characters	A. maguanensis	A. rubroglandulosus	A. cortusiflorus
Bracts	broadly ovate to rounded	elliptic	linear
Calyx lobes	elliptic, apex acute	triangular, apex awny	linear, apex acute
Corolla	glandular-villous outside	glandular-pubescence outside	glandular-villous outside
Adaxial lip of corolla	inner two lobes bigger than outer two lobes	inner two lobes bigger than outer two lobes	inner two lobes almost equal to outer two lobes
Abaxial lip of corolla	triangular, slightly reflexed, apex acute	triangular, extremely reflexed, apex acute	triangular, slightly reflexed, apex acute
Filaments	apex glabrescent	apex glabrous	apex glabrescent
Staminodes	3, major, 3–4 mm long	3, major, 3–4 mm long	2, minor, 0.4–0.5 mm long
Top of lateral staminodes	without red glands	with red glands	without red glands
Mature fruit linear, 1.0–1.1 cm		elliptic, 0.7–0.8 cm	linear, 1.5–1.8 cm
Habitat	near the summit of the limestone hills	deep limestone sinkholes	deep limestone sinkholes or valleys

Table 1. Morphological comparison of *Allocheilos maguanensis* sp. nov., *A. rubroglandulosus* sp. nov., and *A. cortusiflorus* in Gesneriaceae.

Taxonomic treatments

Allocheilos maguanensis W.H. Chen & Y.M. Shui, sp. nov.

urn:lsid:ipni.org:names:77211192-1 Figs 2, 3

Diagnosis. The new species is similar to *A. cortusiflorus* W.T.Wang in glandular-villous corolla on the adaxial surface and style longer than ovary, but differs in its broadly ovate bracts (*vs.* linear), calyx lobes elliptic (*vs.* linear), 11.1–11.3 mm long corolla (*vs.* 8.5–9.5 mm), inner two lobes bigger than outer two lobes (*vs.* equal between them), staminoides major 3–4 mm long (*vs.* minor 0.4–0.5 mm), and 1.0–1.1 cm longer mature fruit (*vs.* 1.5–1.8 cm).

Type. CHINA. Yunnan province, Maguan county, Bazhai town, 23°06'34"N, 104°03'18"E, on the cliffs of a limestone mountain, elev. ca. 1750 m, introduced on 7 April 2017 from the above locality to Kunming Botanic Garden (KBG), in flower on 11 July 2017 in KBG and prepared for specimens there, *Y.M. Shui, S.W. Guo et al. B2017-1343* (holotype, KUN).

Herbs perennial. Rhizome short, stem absent. Leaves basal, opposite; petiole 7-13 cm long, brown-red villous; blade herbaceous, slightly asymmetric, ovate to rounded, $7.5-10 \times 7-9.5$ cm, base cordate, apex obtuse, margin crenate, adaxially densely depressed white pubescent, abaxially white pubescent and brown-red villous along the nerves; venation palmate, lateral veins 5-7 on each side of midrib. Inflorescences axillary, cymes with many flowers; peduncle 11-12.5 cm, densely glandular brown-red villous; bracts 2, broadly ovate to rounded, $0.8-1 \times 0.7-0.8$ cm, adaxially strigose, abaxially densely villous, margin crenate, apex acute; bracteoles 2, broadly ovate, ca. 6×3 mm, adaxially strigose, abaxially densely villous; pedicel ca. 1-1.5 cm, glandular-villous. Calyx 5-parted to the base; segments elliptic, $4 \times 1-2$ mm, apex beaked, margin entire, adaxially sparsely glandular brown-red villous, abaxially densely pubescent. Corolla white, campanulate, zygomorphic, 1–1.3 cm long, ca. 0.4 cm wide at the throat, outside glandular-villous, inside glabrous, tube ca. 4 mm long; limb 2-lipped; adaxial lip ca. 9 mm long, 4-lobed to 1/3 of the lip, lobes triangular, ca. 3 mm long; abaxial lip lanceolate, undivided, ca. 9×3 mm. Stamens 2, adnate to the corolla ca. 2 mm above base; anthers 2, coherent laterally; filaments ca. 6 mm long, sparsely villous above middle and barbate near the anthers; staminodes 3, glabrous, adnate to the corolla tube ca. 2 mm above base. Disc ring-like, ca. 0.5 mm high. Pistil 1.5–1.8 cm long; ovary oblong, ca. 3 mm long, densely strigose and glandular strigose; style linear, ca. 1.5 cm long, densely strigose and glandular strigose; stigma 1, capitate. Capsule linear, 1.0–1.1 cm long.

Phenology. Flowering is from June to July (in the greenhouse).

Etymology. The epithet means the type locality of the new species.

Distribution and ecology. The new species is only distributed in the type locality, SE Yunnan, SW China. It grows in the limestone evergreen broad-leaved forests near the summit of the limestone hills. The main companying plants are *Sinosenecia oldhamianus* (Maxim.) B. Nord. in Compositae, *Petrocosmea minor* Hemsl. and



Figure 2. Vegetative morphology of *Allocheilos maguanensis* W.H. Chen & Y.M. Shui, sp. nov. (A, B, D-H from *Y.M. Shui, W.H. Chen, S.W. Guo, H.H. Xi et al. B2018-495* C from *Y.M. Shui, S.W. Guo et al. B2017-1343*) A-C habit and plants D adaxial surface of leaves E abaxial surface of leaves F close-up of petiole G indumenti on the adaxial surface of leaves H indumenti on the abaxial surface of leaves.



Figure 3. Flower morphology of *Allocheilos maguanensis* W.H. Chen & Y.M. Shui, sp. nov. (**A–C, E, F, H** from *Y.M. Shui, S.W. Guo et al. B2017-1343* **D, G, I, J** from *Y.M. Shui, W.H. Chen, S.W. Guo, H.H. Xi et al. B2018-495*) **A, B** inflorescence showing the wide bracts **C** face and lateral view of flowers **D, E** opened corolla showing stamens and staminodes **F, G** close-up of stamens coherent laterally **H** calyx showing ovate segments with beaked apex **I** face view of flower showing the white staminode inside **J** disk at the base of the ovary. Notes: **at** anther, **br** bract, **cx** calyx lobe, **dc** disc, **fm** filament, **gl** glandular, **sd** staminode, **sm** stigma.

Paraboea rufescens (Franch.) Burtt in Gesneriaceae, *Scutellaria sichourensis* C.Y.Wu et H.W.Li in Labiatae, *Eria coronaria* (Lindl.) Rchb. f., and *Bulbophyllum andersonii* (Hook.f.) J.J.Sm in Orchidaceae.

Provisional conservation status. Based on observations around the locality, this new species was found in two localities with two minor populations in Maguan Xian, Yunnan province, China. One population grows on limestone cliff around 10 mature individuals, and was easily disturbed by human activities. Another population grows among rocks of the limestone hills with around 20 mature individuals. Thus, further exploration should be conducted while an urgent conservational project is needed for this rare species with extremely small populations (Ma et al. 2013). So far, the species can be provisionally considered as Critically Endangered, CR: B1ab (iii) + 2ab (iii). (IUCN 2012; IUCN Standards and Petitions Subcommittee 2017).

Additional examined specimens. CHINA. Yunnan province, Maguan county, Bazhai town, 7 April 2017 introduced to KBG, *S.W. Guo et al. BZL04-012* (KUN). Maguan county, Pojiao town, 23°06'28"N, 104°18'30"E, 13 October 2017 introduced to KBG, *Y.M. Shui, W.H. Chen, S.W. Guo, H.H. Xi et al. B2018-495* (KUN). Maguan county, Pojiao town, *Y.M. Shui, W.H. Chen, S.W. Guo et al. PJL07-006* (KUN); ibid, 13 October 2017, *S.W. Guo, H.H. Xi PJL14-017* (KUN).

Discussion. The new species has obvious staminodes, which are usually difficult to observe on specimens in herbaria. So after the detailed observation in the field, the diversity of staminodes will provide us with an opportunity to explore its evolutionary implications for the genus in Gesneriaceae.

Allocheilos rubroglandulosus W.H. Chen & Y.M. Shui, sp. nov.

urn:lsid:ipni.org:names:77211201-1 Figs 4, 5

Diagnosis. The new species is similar to *A. maguanensis* W.H.Chen & Y.M.Shui with three major staminodes and style longer than ovary, but differs in its elliptic bracts (*vs.* broadly ovate to rounded), triangular calyx lobes awny at the apex (*vs.* elliptic and beaked at the apex), and lateral staminodes with red glands (*vs.* without red glands).

Type. CHINA. Yunnan province, Mengzi county, Shuitian community, 23°08'35"N, 103°21'46"E, on the cliffs of a limestone sink-hole, elev. ca. 1410 m, in flower, 6 November 2017, *Y. M. Shui et al. B2017-1287* (holotype KUN; isotype PE).

Herb perennial. Rhizome short, 6–7 mm in diam., stem absent. Leaves 8–12 in mature individuals, basal, rosette; petiole 9.4-11.6 cm long, densely brown-red villous; blade herbaceous, round or broadly ovate, $4.5-13 \times 3.6-9.8$ cm, base obliquely cordate, apex round or obtuse, margin shallowly crenulate, slightly reflected, adaxially densely depressed white pubescent, abaxially densely brown-red villous especially along the nerves; venation palmate, lateral veins 4–7 on each side of midrib, adaxially slightly depressed and abaxially prominent. Inflorescences axillary, 10–16-flowered cymes; peduncles 8–10 cm long, densely brown-red villous; bracts 2, opposite, elliptic, $5.6-6.4 \times 2.3-2.6$ mm, adaxially subglabrous, abaxially sparsely brownish hispid,



Figure 4. Vegetative morphology of *Allocheilos rubroglandulosus* W.H. Chen & Y.M. Shui, sp.nov. (From holotype, photographed by S. W. Guo and Y. M. Shui) **A** habitat and plants **B** adaxial surface of the round leave, arrow indicating the overlapped leaf blade at base **C** abaxial surface of the round leave, arrows indicating the reflected margin **D** adaxial surface of the broadly ovate leave with overlapped basal lobes of the leave annotated by arrow **E** abaxial surface of the round leaves, arrow indicating the reflected margin **F** hairs of petiole and main nerves on the abaxial surface of the round leaves **G** margin and hairs on the adaxial surface of the round leave, arrow indicating the reflected margin.

margin obscurely and irregularly crenulate and ciliate above the middle, apex acute; bracteoles 2, oblong-lanceolate, ca. 5 × 2 mm, adaxially subglabrous, abaxially densely brownish hispid; pedicel 0.6-1.8 cm long, brown-red hispid. Calyx actinomorphic, 5-parted to the base, segments lanceolate or triangular, $4.5-5.2 \times 2.1-2.2$ mm, apex acuminate or caudate, margin entire, abaxially densely white pubescent and brown-red villous, adaxially glabrous. Corolla white or pinkish at the base, broadly campanulate, zygomorphic, ca. 1.1 cm long, ca. 0.4 cm wide at the throat, outside white glandular pubescent, inside glabrous, tube 3.2-3.5 mm long; limb 2-lipped; adaxial lip 8.8-9.2 × 8.8-9.5 mm, slightly swollen at the base, ca. 6 mm high, ca. 7 mm wide, slightly longer than abaxial lip, 4-lobed, lobes triangular, 3.1-3.3 × 1.4–1.6 mm, apex obtuse, middle lobes 2 symmetric, lateral lobes 2 oblique; abaxial lip narrowly triangular, undivided, $8.1-8.5 \times 2.5-2.9$ mm, apex acute. Stamens 2, adnate ca. 2 mm above base; anthers 2, coherent laterally; filaments sparsely puberulent, base slightly swollen, 7-8 mm long; staminodes 3, lateral 2 red on the top, ca. 1.5 mm long, adnate to ca. 1.5 mm above base, middle white on the top, ca. 3.1 mm long. Disc ring-like, yellow, 0.7–0.8 mm high, margin entire. Pistil 1.4–1.6 cm long; ovary oblong, 3-4 mm long, sparsely pubescent; style 7-8 mm long, sparsely pubescent; stigma 1, capitate. Dry capsule straight, elliptic, $7-8 \times 2.1-2.3$ mm, dehiscing loculicidally, valves 2.

Phenology. Flowering is from October to November (June to July in the greenhouse).

Etymology. The epithet refers to the red glands on the top of lateral staminodes, which is an indicator to lead the pollinators to find the nectar. Hitherto, the character was first observed in Gesneriaceae (Weber 2004).

Distribution and ecology. The new species is only distributed in Mengzi county, Yunnan, southwestern China. It grows in deep limestone sinkholes. The adjacent habitat has been disturbed by local people for the purpose of planting corn. The main accompanying plants are *Begonia laminariae* Irmscher in Begoniaceae, *Impatiens apalophylla* J.D.Hooker in Balsaminaceae, *Pteris deltodon* Baker in Pteridiaceae, *Thalictrum ichangense* Lecoyer ex Oliver in Ranunculaceae.

Provisional conservation status. Based on observations around the locality, this new species was found only in one limestone sinkhole with around 30 mature individuals on the cliffs in three smaller populations. Thus, as the above new species, its further exploration should be conducted while an urgent project is needed for this rare species with extremely small populations (Ma et al. 2013). So far, the species can be provisionally considered as Critically Endangered, CR: B1b (v) + 2b (v). (IUCN 2012; IUCN Standards and Petitions Subcommittee 2017)

Additional examined specimens. CHINA. Yunnan province, Mengzi county, Shuitian community, elev. ca. 1410 m, fruits dry, 6 November 2017, *Y. M. Shui et al. B2017-1296* (KUN); ibid, *Y. M. Shui et al. B2017-1298* (KUN).

Discussion. At the background of the white flowers, two red glands at the top of staminodes are an important indicator for the pollinators to visit the plants. It is obvious that nectars are hidden at the back of the indicator (Fig. 5C). The discovery will provide us an opportunity to explore the diversity and evolution of staminodes of the genus in Gesneriaceae and the other groups endemic to the karst regions in China and the adjacent countries (Laos, Myanmar, Thailand and Vietnam).



Figure 5. Flower morphology of *Allocheilos rubroglandulosus* W.H. Chen & Y.M. Shui, sp. nov. (From holotype, photographed by S. W. Guo and Y. M. Shui) **A** inflorescence **B** right part of the inflorescence **C** left part of the inflorescence **D** lateral view of the leaf part of the inflorescence **E** face view of the flower **F** face view indicating 3 staminodes, middle white and lateral 2 red on the top **G** lateral view of flower indicating stamens **H** opened corolla indicating the stamens and staminodes and the swollen nodes at the base of filaments. Notes: **at** anther, **br** bract; **cx** calyx lobe, **dc** disc, **fm** filament, **gl** glandular, **sd** staminode, **sl** style, **sm** stigma, **sn** swollen nodes at base of filament.

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RESEARCH ARTICLE



Petrocodon luteoflorus (Gesneriaceae), a new species from karst region in Guizhou, China

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Abstract

A new species of Gesneriaceae, *Petrocodon luteoflorus* Lei Cai & F.Wen was first described and illustrated from Maolan National Nature Reserve, Libo County, Guizhou Province, China. The diagnostic characters and notes of this species between its most morphologically similar species, *P. dealbatus* Hance, a detailed description, colour photographs, etymology, as well as distribution and habitat, are also provided in this paper.

Keywords

Didymocarpoideae, limestone area, Petrocodon s. l., new taxon, flora of Guizhou

Introduction

Since *Petrocodon longistylus* Kraenzl., 1928 was merged into *P. dealbatus* Hance, 1883, *Petrocodon s. str.* remained as a monotypic genus for over a century. However, two new species, *P. ferrugineus* Y.G. Wei, 2007 and *P. multiflorus* Fang Wen & Y.S. Jiang, 2011,

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were discovered and published after the beginning of the 21st century. They have similar small white bell-shaped flowers. Thus, this genus became a small one and three species and one variety (P. dealbatus var. denticulatus (W.T. Wang) W.T. Wang) (Wang 1975, Wang et al. 1990, 1998, Wei et al. 2010) exist at that time. Soon afterwards, the genus was redefined based on molecular phylogenetic studies. The small Chinese genus Petrocodon has been recently enlarged to include four former monotypic genera (Calcareoboea C.Y. Wu ex H.W. Li, Dolicholoma D. Fang & W.T. Wang, Paralagarosolen Y.G. Wei & Tengia Chun), all species of Lagarosolen W.T. Wang, a few species of Didymocarpus Wall., one species of Wentsaiboea D. Fang & D.H. Qin (Weber et al. 2011) and one species of Primulina Hance (Xu et al. 2014). Thus, Petrocodon s.l. consists of at least 35 species and one variety, including 14 species newly described after 2011 (Lu et al. 2017a, Xu et al. 2017), e.g. P. asterocalyx F. Wen, Y.G. Wei & R.L. Zhang, 2018, P. pulchriflorus Y.B. Lu & Q. Zhang, 2017a, P. urceolatus F.Wen, H.F. Cen & L.F. Fu, 2017, P. retroflexus Q. Zhang & J. Guo, 2016, and so on. Obviously, the genus is so special on account of its remarkable and highly variable floral structures that it also becomes one of the most taxonomically difficult groups in Gesneriaceae (Möller et al. 2016, Lu et al. 2017b). For example, P. guangxiensis (Yan Liu & W.B. Xu) W.B. Xu & K.F. Chung was mistakenly identified as a member of Primulina Hance, 1883, P. guangxiensis Yan Liu & W. B. Xu (Liu et al. 2011, Xu et al. 2014) while it was published. On the other hand, similar characters of leaves sometimes affect our judgement of some Petrocodon congeners.

During field investigations in the karst region of Guizhou province in 2017, an interesting species of Gesneriaceae attracted our attention. Previously, the corresponding author (FW) also collected specimens of the same species without flowers from Jiudongtian, Dongtang town, Libo County, Guizhou. After we checked the flowering plants which were being cultivated in Guilin Botanical Garden and Guizhou Botanical Garden, we confirmed that it is a member of *Petrocodon* because the morphology of plants and flowers is similar to *P. dealbatus*. Subsequently, we re-collected the flowering specimens from Maolan National Nature Reserve, Libo County, Guizhou province in 2018. After careful review of the relevant specimens and literature of *Petrocodon*, we concluded that this unknown species represents a species new to botany and science which we describe and illustrate here and its morphological characters are compared with the closely related species *P. dealbatus*.

Material and methods

All available specimens of *Petrocodon s.l.* stored in the herbaria (IBK, KUN and PE) in China were examined. The photographs were taken in the field by the authors. Morphological observations, measurements and description of the new species were carried out based on living plants, dry specimens and preserved materials. All morphological characters were studied with dissecting microscopes and are described using the terminology presented by Wang et al. (1990, 1998), Li and Wang (2004).

Taxonomy treatments

Petrocodon luteoflorus Lei Cai & F.Wen, sp. nov.

urn:lsid:ipni.org:names:77211193-1 Fig. 1

Diagnosis. *Petrocodon luteoflorus* most resembles *P. dealbatus* in plant type and floral size, but can be easily distinguished from the latter by the following diagnostic characters: longer calyx lobes 6–8 mm long (vs. 2–5 mm long); larger corolla 9–11 mm long, pale yellow to yellow (vs. 5.5–8 mm long, white); corolla lobes in equal shape and size, triangular and ca. 2 mm long (vs. in different shape and size: adaxial lobes 0.8–2 mm long, triangular; abaxial lobes 1.8–3 mm long, triangular to ovate).

Type. CHINA. Guizhou: Libo County, Limingguan Town, Yaolan, 25°17'N, 107°55'E, 735 m a.s.l., on moist rocks under forest, in flower, 23 August 2018, *Lei Cai* et al. *CL193* (holotype: KUN!, isotypes: KUN! & IBK!).

Description. Perennial herb, stemless. Rhizome 8-12 cm long, 5-12 mm in diameter. Leaves 8-16, basal; petiole 3.5-6.5 cm long, densely pruinate; leaf blade narrow elliptic or oblanceolate, $8-20 \times 2.5-8$ cm, coriaceous, adaxially green, appressed pubescent, abaxially appressed pubescent along veins, whitish-green, pruinate, base cuneate, gradually tapered to petiole, margin nearly entire to denticulate or crenulate, apex acuminate; lateral veins 4-6 on each side of midrib. Cymes axillary, 1-5 on a plant, flowers numerous, 8-25-flowered or more; peduncle 8-20 cm long, densely puberulent; bracts 2, lanceolate, $8-10 \times 1.5-2$ mm, both sides puberulent, margin entire, apex obtuse. Pedicel 0.8–2.2 cm long, pubescent. Calyx 6–8 mm long, 5-lobed to the base; lobes equal, linear, $6-8 \times 1.5-2.0$ mm, outside pubescent, inside glabrous, margin entire to denticulate, apex acuminate. Corolla slightly or inconspicuously 2-lipped, pale yellow to yellow, 9-11 mm long, 4-6 mm in diameter, outside densely puberulent, inside glabrous; tube cannulate, $7-9 \times 4-6$ mm; adaxial lobes 2, abaxial lobes 3, all lobes triangular, in nearly equal size, ca. 2 mm long, 2.5 mm wide at the base. Filaments ca. 7 mm long, S-shaped, glabrous, inserted ca. 2 mm from base; anthers dorsifixed, ca. 3 mm long, reniform or water-chestnut shaped, apexes acute; staminodes 3, ca. 0.5 mm long, linear, glabrous, inserted ca. 1 mm from base. Disc ca. 1.5 mm high, margin asymmetrical, one side absent, on the other side horned. Pistil ca. 1.5 cm long, glabrous; ovary linear, ca. 6 mm long, style linear, ca. 9 mm long; Stigma discoid, small, 0.3–0.5 mm in diameter. Capsule linear, glabrous, 2–3 cm long.

Phenology. Flowering from August to September; fruiting from September to November.

Etymology. The specific epithet '*luteoflorus*' derives from the Latin prefix, *luteo*-, yellow and the Latin suffix, '*-florus*', of flower, referring to its small and yellow flowers of the new species. The Chinese name is "Xiǎo Huáng Huā Shí Shān Jù Tái" (小黄花石山苣苔).

Distribution and habitat. *Petrocodon luteoflorus* is currently known only from the type locality and might be endangered but more data are needed to evaluate that reliably. The species only grows on the surface of moist rocks under the forest.



Figure 1. *Petrocodon luteoflorus* Lei Cai & F.Wen, sp. nov. **A** Habitat **B**, **C** plants with flowers **D** petiole and adaxial leaf surface **E** petiole and abaxial leaf surface **F**, **G** gymes **H** side view of flower **I** opened corolla and pistil with calyx **J** pistil, disc and calyx **K** opened corolla showing stamens and staminodes **L** adnate anthers, adaxial view **M** adnate anthers, abaxial view. Scale bars: 20 cm (**A**); 10 cm (**B**, **C**); 5 cm (**D**, **E**); 1 cm (**F**, **G**); 5 mm (**H**, **I**, **J**, **K**), 3 mm (**L**, **M**). Photographed by Lei Cai and Fang Wen (**D**, **E**).

Conservation status. Current information for this new species is known from only a few collections and details on the size of the population are known in Maolan National Nature Reserve, where the plants' protected status is guaranteed. Based on some careful field investigations in recent years, this species appears to be locally abundant. Thus, it is assessed temporarily as Least Concern (LC) according to the IUCN Red List Categories and Criteria (IUCN 2017).



Figure 2. *Petrocodon dealbatus* Hance. **A** Habitat **B** cyme **C** the lateral view corolla and calyx lobes **D** the frontal view of corolla. Photographed by Fang Wen.

Additional specimens examined. Guizhou: Libo County, Dongtang Town, Raosuo village, Jiudongtian, 25°17'N, 103°03'E, 795 m a.s.l., on moist rocks at the entrance of limestone caves, 11 September 2017, *Fang Wen WF160113-01* (IBK!).

Notes. At first glance, this new taxon and the type species, *Petrocodon dealbatus* (Fig. 2), could easily have been confused because of its similar leaves if the pruinate abaxial leaf surfaces escaped collectors' attention or were not in flower. However, once the flowers of the two congeners are compared to each other, they are easy to distinguish. The new species can be easily distinguished from the latter in the shape and

length of bracts (lanceolate, 8–10 mm long in *P. luteoflorus* vs. linear, 3–7 mm long in *P. dealbatus*); the length of calyx lobes (6–8 mm long vs. 2–5 mm long); the size and colour of corolla (9–11 mm long, pale yellow to yellow vs. 5.5–8 mm long, white); the shape and size of corolla lobes in equal shape and size: triangular and ca. 2 mm long vs. in different shape and size: adaxial lobes 0.8–2 mm long, triangular; abaxial lobes 1.8–3 mm long, triangular to ovate); indumentum of corolla outside (densely puberulent vs. upper half of corolla puberulent to glabrescent); filaments (S-shaped vs. straight); shape of anthers (reniform or water-chestnut shaped vs. elliptic); the shape and height of disc (ca. 1.5 mm high, asymmetrical, one side absent, on the other side horned vs. ca. 1 mm high, symmetrical and annular).

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RESEARCH ARTICLE



Petrocodon rubiginosus, a new species of Gesneriaceae from Guangxi, China

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Abstract

Petrocodon rubiginosus Y.G. Wei & R.L. Zhang, **sp. nov.**, from Guangxi of South China, is described and illustrated with photographs. The new species is morphologically similar to *Pet. hechiensis*, but can be easily distinguished by a combination of characters, especially in its petioles, peduncles and pedicels covered with densely ferruginous pilose hairs.

Keywords

Jingxi, Karst, Limestone flora, New taxa, Sino-Vietnamese border area

Introduction

Although the newly delimitation *Petrocodon* Hance is not the most speciose genus in Gesneriaceae of China, the highly variable corolla and leaf morphology impels us to continuously study and understand the species biodiversity of this genus (Lu et al. 2017, Xu et al. 2017). Now, approximately 35 species and one variety range from China to northern Thailand and northern Vietnam (Weber et al. 2011, Chen et al. 2014, Xu et al. 2014, Middleton et al. 2015, Möller et al. 2011, 2013, 2016).

During a floristic expedition to Guangxi, China in 2015, the authors observed a population of an interesting Gesneriaceae in Yuexu Town, Jingxi city, Guangxi. We confirmed that it is a member of the genus *Petrocodon* because it looks like *Pet. hechiensis* (Y.G.Wei, Yan Liu & F.Wen) Y.G.Wei & Mich.Möller (Wei et al. 2008, Weber et al. 2011). Over the past three years, the living plants were monitored in the conservation nursery of the Gesneriad Conservation Centre of China (GCCC) in Guilin Botanical Garden and in the field, where an ecological survey was conducted.

After thorough comparisons of diagnostic morphological and anatomical features of similar taxa from China, Vietnam and Thailand (Wang et al. 1990, 1998, Li and Wang 2005, Phuong 2005, Wei et al. 2010, Middleton et al. 2015), it has been revealed that its morphological characters do not fit any known species, therefore, we conclude that it is a new species to science and accordingly describe it herein. Its morphological characters are compared with the closely related species, *Petrocodon hechiensis*.

Material and methods

Measurements and morphological character assessments of the new species were performed and described using specimens obtained by the current authors, living material observed in the field and also cultivated at GCCC. All available *Petrocodon* specimens of China, Thailand and Vietnam, stored in the following herbaria, were examined: E, GH, HN, IBK, K, KUN, MO, PE, PH, US and VNMN. At the same time, the specimen's images and name list of the above-mentioned species were obtained and checked from Tropicos (http://www.tropicos.org), JSTOR Global Plants (http:// plants.jstor.org), The Plant List (http://www.plantlist.org/) and the International Plant Names Index (http://www.ipni.org). All morphological characters were studied under dissecting microscopes and are described using the terminology presented by Wang et al. (1990, 1998).

Taxonomic Treatment

Petrocodon rubiginosus Y.G.Wei & R.L.Zhang, sp. nov. urn:lsid:ipni.org:names:77211194-1 Figure 1

Diagnosis. It is morphologically close to *Pet. hechiensis*, but can be distinguished by its petioles, peduncles and pedicels densely curly rubiginous to ferruginous villous, bracts lanceolate to subulate and both surfaces densely rubiginous to dark brown pubescent, corolla lobes oblong to nearly rounded and margin entire, calyx lobes densely rubiginous to ferruginous pubescent.

Type. CHINA. Guangxi Zhuangzu Autonomous Region: Jingxi city, Yuexu Town, Siming village, 22°56'N, 106°37'E, alt. 470 m, 12 November 2015, flowering, *Wen Fang et al. WF151112-01* (holotype: IBK; isotype: IBK).


Figure 1. *Petrocodon rubiginosus* Y.G.Wei & R.L.Zhang, sp. nov. **A** flowering plant in natural habitat (Jingxi, Guangxi, China) **B** flowering plant cultivated in GCCC **C** cyme **D** corolla in front view **E** corolla in lateral view and bud on cyme **F** indumentum of peduncle **G** flower in top view **H** flower in lateral view **I** flower in upward view **J** resolved flower for showing corolla, calyx lobes, pistil and pedicel **K** pistil and calyx lobes (adaxial & abaxial surfaces) **L** style and stigma. Photographs by authors.

Description. Perennial herb, stemless. Rhizome subterete, 3-8 cm long, 5-7 mm in diam. Leaves 8-16, all basal; leaf blades herbaceous, green to yellowish-green, zygomorphic to slightly unequal in each pair, broadly ovate to nearly rounded, 3.5-20 × 3.8-15 cm, apex obtuse to rounded, base cordate or obliquely cordate and bases on both sides usually overlapping, margin obviously crenate to undulate, pubescent on both surfaces, 5-6 nerves on each side, adaxially impressed, abaxially prominent, densely ferruginous and erect villous along abaxial main vein and lateral veins; petioles 15–25 cm or longer, densely ferruginous and erect villous. *Cymes* 4–8, 20–30 cm long, usually curved or squiggly,1-2-branched, 8-12-flowered; peduncle 15-20 cm long, ca. 2 mm in diam., densely curly rubiginous to ferruginous villous; bracts 2, opposite, lanceolate to subulate, $5-6 \times 1-2$ mm, entire, both sides densely rubiginous to dark brown pubescent; bracteoles 2, opposite, linear-lanceolate, ca. 5 ×0.8 mm, both sides densely rubiginous to dark brown pubescent; pedicel 4-7 mm long, 0.8-1 mm in diam., densely rubiginous to dark brown pubescent. Calyx 5-parted to the base, sepals lanceolate-linear to subulate, ca. 10 mm long, 0.6-0.7 mm at base, both sides densely rubiginous to dark brown pubescent. Corolla bilabiate, pale yellow, throat with two brightly yellow longitudinal stripes and dark yellow glands on stripe surface, ca. 3 cm long, outside white pubescent; tube slender, 2-2.2 cm long, 6.5-7 mm in diam. at middle, slightly constricted at ca. 6 mm above base of corolla base; adaxial lip 7-8 mm long, 2-partite nearly to base, lobes oblong to nearly rounded, apex acuminate, abaxial lip 2–2.5 cm long, 3-partite nearly to base, lobes nearly rounded, apex obtuse. Stamens 2, sparsely glandular-puberulent, adnate to 1.1-1.2 cm above the base of corolla tube; filaments pale vellow to white, 5-5.5 mm long; anthers 1.2-1.5 mm long, elliptical, 1.8–2 mm long, 1–1.5 mm in diam. Staminodes 3, glabrous, lateral ones 6–7 mm long, adnate to ca. 10 mm above the base of corolla tube, median ca. 1.5 mm long, adnate to ca. 7 mm above the base of corolla tube. Disc glabrous, ca. 1.2 mm high, margin entire. *Pistil* ca. 2 cm long; *ovary* ovoid, ca. 5 mm long, covered pale rubiginous or white pubescent, style ca. 1.5 cm long, densely pubescent; stigmas inapparent triangular, 2, each one semicircular, ca. 0.8 mm long. Capsule ovoid, valvular dehiscence, pubescent.

Phenology. Flowering occurs in November and fruiting from December to January of next year.

Etymology. The specific epithet is derived from the conspicuous indumentum of petioles, peduncles and pedicels, which are covered with densely long ferruginous or rubiginous hairs.

Vernacular name. Xìu Géěng Shí Shān Jù Tái (Chinese pronunciation); 锈梗石 山苣苔 (Chinese name).

Distribution and habitat. *Petrocodon rubiginosus* is hitherto only known from two close localities at elevational ranges from 450–500 m in Jingxi city, Guangxi, China, growing on shaded and moist rock surface with no more than 50 individuals at each of the localities. Two localities are about three kilometres apart. The species grows in sub-tropical broad-leaved evergreen monsoon forest with sufficient seasonal run-off water.

Preliminary conservation assessment. Population information of *Petrocodon rubiginosus* is still unclear, which makes it difficult to determine an assessment of the extinction risk faced by this new taxon. At present, two distribution points of the species



Figure 2. *Petrocodon rubiginosus* Y.G.Wei & R.L.Zhang, sp. nov. (1) and its congener, *Pet. hechiensis* (Y.G.Wei, Yan Liu & F.Wen) Y.G.Wei & Mich.Möller (2) A adaxial surfaces of leaf blades and petioles of two species B abaxial surfaces of leaf blades and petioles of two species C indumentum of petioles D indumentum of main nerves on adaxial surface E cymes and flowers F indumentum of peduncles G indumentum of bracts and pedicels H abaxial and adaxial surfaces of bracts I corolla in frontal views J corolla in top views K corolla in lateral views L indumentum of abaxial surfaces of calyx lobes M stigma. Photographs by authors.

are known and its estimated area of occupancy is less than 10 km². The main threat now comes from environmental damage caused by grazing and there is a risk of poaching in the future because its distribution is not far away from the villages. Furthermore, prolonged droughts and illegal logging in the area, including nearby potential habitat, should be considered as potential risks to the persistence of *Pet. rubiginosus*. Thus, following the IUCN Red List Categories and Criteria (IUCN 2017), it is assessed temporarily as endangered [EN B2ab (ii, iii)].

Notes. It is morphologically close to *Petrocodon hechiensis* because two congeners share some similarities, for example, they have look-alike leaf shape and size and a pale-yellow corolla (Wei et al. 2008, 2010). As a result, it is sometimes mistaken for *Pet. hechiensis*. However, one of the largest differences between *Pet. rubiginosus* and *Pet. hechiensis* is the indumentum of petioles, peduncles and pedicels. There are absolutely no glandular-hairs on petioles, peduncles and pedicels of *Pet. rubiginosus*, but in *Pet. hechiensis*, all are covered by densely short and sticky glandular-hairs and pubescenthairs (Figure 3). The other major differences between the species are outlined in Table 1.



Figure 3. The difference of indumentum between *Petrocodon rubiginosus* Y.G.Wei & R.L.Zhang, sp. nov. (**A**) and *Pet. hechiensis* (Y.G.Wei, Yan Liu & F.Wen) Y.G.Wei & Mich.Möller (**B**): densely curly rubiginous to ferruginous villous on surface of petiole (**A–1**), pedicel (**A–2**) and calyx lobes (**C–1**) and densely short glandular-pubescent and pubescent on surface of petiole (**B–1**), pedicel (**B–2**) and calyx lobes (**C–2**).

Table	١.	Diagnostic	character	differences	between	Petrocodon	rubiginosus	sp.	nov.	and	Pet.	hechiensis
(Figure	2)											

Characters	Pet. rubiginosus	Pet. hechiensis
Indumentum of petioles,	densely curly rubiginous to ferruginous	densely short glandular-pubescent and
peduncles and pedicels	villous (C-1, F-1)	pubescent (C-2, F-2)
Bracts	lanceolate to subulate, 5–6 × 1–2 mm,	linear-lanceolate, $8-16 \times 1-2$ mm, both sides
	both sides densely rubiginous to dark	glandular-pubescent to hispid (G-2, H-2)
	brown pubescent (G-1, H-1)	
Corolla lobes	oblong to nearly rounded, margin entire	lanceolate-triangular, margin dentate (I-2)
	(I-1)	
Calyx lobes	densely rubiginous to ferruginous	densely white glandular-pubescent and
	pubescent (L-1)	pubescent (L-2)
Stigma	inapparent triangular, 2, each one	slightly curved oblong, 2, each one oblong to
	semicircular (M-1)	rounded (M-2)
Florescence	November	September to October

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RESEARCH ARTICLE



Petrocodon wenshanensis, a new species of Gesneriaceae from southwestern China

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Abstract

A new species of *Petrocodon*, *P. wenshanensis* from Yunnan province of southwestern China, is described and illustrated here. *P. wenshanensis* morphologically closely resembles *P. jingxiensis* and *P. lithophilus*, but differs in vegetative and generative characters. Differences between the new species and others *Petrocodon* species occurring in Yunnan Province are also shown in the identification key.

Keywords

Didymocarpoideae, limestone flora, new taxa, Yunnan Province

Introduction

Petrocodon Hance has recently been much expanded and is now one of the most morphologically variable genera in Asian Gesneriaceae (Weber et al. 2011), with a wide

These authors contributed equally to this work.

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range of corolla and leaf morphology (Lu et al. 2017a). Yunnan is one of the world's 34 most species-rich regions, with the highest biodiversity resources in China (Huang et al. 2011). However partly for natural and geographic reasons associated with Yunnan, there are still enormous species waiting to be discovered and further revised. Recently, four new Gesneriaceae species were reported here (Chen et al. 2014; Chen et al. 2019), so there are now a total of 6 species of this genus found in Yunnan Province: *Petrocodon ainsliifolius, P. lithophilus, P. viridescens, P. tenuitubus, P. coccineus* and *P. hispidus*.

During an expedition to prepare a National forest resources inventory in April 2016, one of the authors, De-Ming He discovered some plants without flowers in Funing County, Wenshan Zhuang and Miao Autonomous Prefecture, Yunnan Province, south-western China. Afterwards, the other authors collected specimens from the same cave when they were undertaking the field works in ecologically functional zones of Yunnan Province. On the basis of its habit (leaves in basal rosette), corolla shape (limb 2-lipped), number of fertile stamens (two), anthers (dorsifixed, coherent apically) and capsule dehiscence (loculicidal), it was identified as belonging to Petrocodon s.l. (Wang 1990, 1998; Weber et al. 2011). At the time, they improperly identified it as P. jingxiensis (Y. Liu, H.S. Gao & W.B. Xu) A. Weber & Mich. Möller (Weber et al. 2011) due to the similar characters of leaf morphologies. In order to study it thoroughly, particularly the floral morphology, it was cultivated during the past three years in the greenhouse of the Gesneriad Conservation Center of China, which is located in the Guangxi Institute of Botany. The specimens of the new species were deposited in IBK and ANU and living individuals were cultivated at the Gesneriad Conservation Center of China. All morphological characters were studied under dissecting microscopes and were described using the terminology proposed by Wang (1990), Wang et al. (1998). After consulting the monographs (Li and Wang 2005, Wei et al. 2010) and comparing the species with all other congeners described (i.e. Chen et al. 2014, Chen et al. 2019, Xu et al. 2014, Hong et al. 2014, Guo et al. 2016, Cen et al. 2017, Lu et al. 2017b, Zhang et al. 2018, Li et al. 2019) and specimens of Gesneriaceae deposited at IBSC, IBK, KUN, PE, US and VMN. We confirmed that it is a new species and hence we describe and illustrate it below as such. A morphological comparison between *P. wenshanensis* and its congeners is provided in Table 1 and the Key.

Character	P. wenshanensis	P. lithophilus	P. jingxiensis
Leaf blade	apex acute	apex rounded	apex obtuse or rounded
Bracts	glabrous inside	pubescent on both sides	pubescent on both sides
Calyx	glabrous inside	pubescent inside	puberulent inside
Corolla	purple	light greenish yellow	purple
Corolla tube	gradually dilated and bent towards the throat	thin tubular	slender
Corolla lobes	apex acute, margin erosulate near the apex	apex acute, margin entire	apex round, margin entire
Filaments	strongly geniculate at the middle, glabrous	straight, glabrous	straight, puberulent

Table 1. Diagnostic character differences between *Petrocodon wenshanensis* and its close relatives: *P. jin-gxiensis, P. lithophilus.*

Key to the species of the *Petrocodon* (Gesneriaceae) occurring in Yunnan Province, China

1	Adaxial corolla lip 4-fided, the abaxial integrate	2
_	Adaxial corolla lip 2-fided, the abaxial 3-fided	3
2	Flower red; leaf blades elliptic	P. coccineus
_	Flower greenish; leaf blade round	P. viridescens
3	Flowers greenish	P. lithophilus
_	Flowers purple	4
4	bracts 3, corolla tubes curved	P. tenuitubus
_	bracts 2, corolla tubes strict	5
5	Corolla lobes acuminate, not reflexed	P. wenshansnsis
_	Corolla lobes caudate, reflexed	6
6	Leaf margin shallowly crenulate, apex rounded	P. hispidus
_	Leaf margin entire, apex acute	P. ainsliifolius

Taxonomic treatment

Petrocodon wenshanensis Xin Hong, W.H. Qin & F. Wen, sp. nov.

urn:lsid:ipni.org:names:77211195-1 Figure 1

Diagnosis. The new species is vegetatively similar to *P. jingxiensis*, it differs from the latter in having acute apex of leaf blade (*vs.* obtuse or rounded), bracts and calyx glabrous inside (*vs.* pubescent), corolla tubes gradually dilated and bent towards the throat (*vs.* slender tubular), and lobes margin erosulate (*vs.* entire), filaments strongly geniculate at the middle, glabrous (*vs.* straight, puberulent). And it is also morphologically similar to *P. lithophilus*, but differs by its apex of leaf blade acute (*vs.* rounded), bracts and calyx glabrous inside (*vs.* pubescent), corolla purple (*vs.* light greenish yellow), tubes gradually dilated and bent towards the throat (*vs.* thin tubular) and lobes margin erosulate (*vs.* entire), filaments strongly geniculate

Type. CHINA. Guangxi Province, cultivated in the nursery of Gesneriad Conservation Center of China (GCCC), introduced from Yunnan Province: Muyang Town, Funing County, Wenshan Zhuang and Miao Autonomous Prefecture, 23°33'N, 105°28'E, 1,360 m a.s.l., growing in rocky crevices at the mouth of a karst cave. 14 June 2019, flowering, WF170807-06 (holotype: IBK; isotype: AHU).

Description. Perennial herbs. Rhizomatous stem subterete, 30-75 mm long, 6-10 mm in diameter. Leaves basal, opposite and congested at rhizome apex; petiole 1.5-4.5 cm long, densely pubescent. Leaf blade chartaceous, ovate, $3-6 \times 2-4$ cm, apex acute, base broadly cuneiform or shallowly cordate, slightly oblique, margin crenulate, densely pubescent on both surfaces, lateral veins ca. 4 pairs on each side of midrib, concave adaxially, prominent abaxially. Cymes 1-3, axillary, 1-2-branched, 1-3(-6)-flow-



Figure I. *Petrocodon wenshanensis* Xin Hong, W.H. Qin & D.M. He. **A** Habitat **B** habit when in flower **C** lateral view of corolla (arrow indicates the bent of the throat) **D** leaf blade **E** opened corolla (arrow indicates margin of corolla lobes erosulate near the apex) **F** stamens **G** pistil without corolla, showing calyx segments, inside glabrous (above), outside strigose (below) **H** stamens.

ered; peduncles 4–6 cm long, densely glandular puberulent and sparsely strigillose; bracts 2, opposite, linear, ca. 5×0.8 mm, outside densely strigose, inside glabrous. Pedicels 2–8 mm long, glandular and eglandular pubescent. Calyx 5-lobed from base, segments narrowly lanceolate-linear, equal, ca. 5×0.9 mm, outside strigose, inside glabrous. Corolla zygomorphic, purple, 2.5–3.0 cm long, glandular and eglandular pubescent on both sides; corolla tubes infundibula-form, slender, gradually dilated and bent towards the throat; 17–19 mm long, 4–6 mm in diameter at the mouth, 2–2.5 mm in diameter



Figure 2. Distribution of *Petrocodon wenshanensis* (\blacktriangle) and its similar species: *P. jingxiensis* (\bigstar), *P. lithophilus* (\bullet), and the location of the Wenshan National Nature Reserve.

at the base; limb distinctly 2-lipped; adaxial lip 2-lobed to the base, lobes ca. 8 mm long, orbicular triangular, apex acute, margin erosulate only near the apex; abaxial lip 3-lobed to the base, lobes 8–9 mm long, subequal, oblong, apex acute, margin erosulate only near the apex to nearly entire. Stamens 2, adnate ca. 10 mm above the corolla base, glabrous; filaments 2–3.5 mm long, strongly geniculate at the middle; anthers yellow, nearly reniform, ca. 1 mm, fused by their entire adaxial surfaces, dehiscing longitudinally; staminodes 3, linear, 0.5–0.7 mm long, adnate ca. 8 mm above the corolla base. Disc ring-like, ca. 0.7 mm high, with repand margin. Pistil 1.3–1.5 cm long; ovary ca. 2 × 1 mm, pilose; style 11–13 mm long, densely pilose, stigmas 2, ovate, ca. 0.6 mm long, lobes ca. 0.4 mm long. Capsule narrowly elliptic, dehiscing loculicidally into 2 valves.

Etymology. The specific epithet is derived from the type locality, Wenshan Zhuang and Miao Autonomous Prefecture, Yunnan Province, China.

Vernacular name. Wén Shān Shí Shān Jù Tái (Chinese pronunciation); 文山石山 苣苔 (Chinese name).

Distribution and habitat. The new species has so far been found only in the type locality, Muyang Town, Funing County, Wenshan Zhuang and Miao Autonomous Prefecture, Yunnan Province, China. The geographical distributions of *Petrocodon wenshanensis* and its similar species are identified in Figure 2. The forest

where *P. wenshanensis* occurs is monsoon evergreen broad-leaved forest. The average temperature of Funing County is about 19.8 °C and the average annual precipitation is over 1103 mm. Flowering is from June to August.

Provisional conservation status. The landform of the type locality is karst topography, and the new species seems locally abundant in the limestone cave, the type locality. But all individuals grow on moist and shaded rocky faces on the cliff at the mouth of a karst cave. After carefully estimating and counting, the type population consists of approx. 8000 mature individuals. Although this type of cave is near the Wenshan Nature Reserve, it is also very close to the local village, so is excluded from the range of the reserve and is not protected by the reserve's law. Hence it is easily disturbed by human activities (i.e reclamation and quarrying activities). Based on five careful field investigations over recent years, there has been no significant change in the number of individuals Thus, following the IUCN Red List Categories and Criteria (IUCN 2019), it is temporarily assessed as Critically Endangered [CR B2ab (iii)].

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RESEARCH ARTICLE



The taxonomic identity of Didymostigma trichanthera (Gesneriaceae)

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Abstract

Based on consulting original literature, the examination of specimens, and field investigations, *Didymostigma trichanthera* is shown to be conspecific with *Henckelia anachoreta*. Therefore, *Didymostigma trichanthera* is formally treated as a synonym of *Henckelia anachoreta* here.

Keywords

China, Guangdong, Didymostigma trichanthera, Henckelia anachoreta, taxonomy

Introduction

The genus *Didymostigma* W.T. Wang (1984) was once considered to be a monotypic genus, having only one species, *D. obtusum* (C.B. Clarke) W.T. Wang (1984). Subsequently, two new taxa, *D. leiophyllum* D. Fang & X.H. Lu (Fang et al. 1994) and *D. trichanthera* C.X. Ye & X.G. Shi (2005) were discovered and described. The type species of this genus, *D. obtusum*, is widely distributed from eastern Guangdong to southern Fujian, China (Wang et al. 1998; Li and Wang 2004; Wei et al. 2010). The

other two species, however, are regarded as narrowly endemic species and have only been found at their type localities (Fang et al. 1994; Ye and Shi 2005).

Didymostigma trichanthera was simply described based on a single collection (*Chuang-Xing Ye 5960*) from Nankunshan, Guangdong Province, China. Ye and Shi (2005) thought that *D. trichanthera* mainly differs from *D. obtusum* by its lanose fertile anthers, hairy filaments, and the unhidden pistil to the corolla tube. The species status of *D. trichanthera* was once doubted by Wei et al. (2010). They find the calyx of *D. trichanthera* is completely different from the type species *D. obtusum*, but they did not carry out further study on this questionable species. After consulting original literature about this genus, type species, and this so-called new species (Wang 1984; Ye and Shi 2005), carrying out field investigations in Nankunshan, and conducting examinations of type materials of *D. trichanthera*, we are convinced that *D. trichanthera* is not a species belonging to the genus *Didymostigma*. Our detailed morphological comparisons find that this so-called *Didymostigma species* is actually conspecific with *Henckelia anachoreta* (H.F. Hance) D.J. Middleton & Mich. Möller (Weber et al. 2011). Consequently, it is essential to reduce *Didymostigma trichathera* to a synonym of *Henckelia anachoreta*.

Material and methods

We performed detailed comparisons of type materials between *Didymostigma trichanthera* and the type species of *Didymostigma* (*D. obtusum*), and also between *D. trichanthera* and *Henckelia anachoreta*. The study of specimens was conducted in IBK, IBSC and SYS. We also checked high-resolution digital images of the specimens in A (https://huh.harvard.edu/), BM (http://data.nhm.ac.uk/), E (http://www.rbge. org.uk/), K (https://www.kew.org/), P (https://science.mnhn.fr/institution/mnhn/search), TI (http://umdb.um.u-tokyo.ac.jp/Dshokubu/Tshokubu.htm) and WU (http://herbarium.univie.ac.at/index.htm) by their web service, as well as via online databases, such as the Chinese Virtual Herbarium (http://www.cvh.ac.cn/), JSTOR Global Plants (http://plants.jstor.org/) and Specimens Database of Native Plants in Taiwan (http://www.hast.biodiv.tw/Specimens/specimenQueryC.aspx). Moreover, detailed morphological studies of both *H. anachoreta* and *Didymostigma obtusum* were undertaken based on plants from natural populations at Nankunshan. Some other field observations of these two species were carried out in Guangxi, Guangdong, Fujian of China.

Results and discussion

We did not find any other *Didymostigma* species in Nankunshan in our field work, except for *D. obtusum* (Fig. 1B). However, *Henckelia anachoreta*, a species which is similar to *Didymostigma obtusum* in its vegetative characteristics to some extent, can



Figure 1. Living plants of *Henckelia anachoreta* (**A**, **C**, **D**) and *Didymostigma obtusum* (**B**, **E**) at Nankunshan. **A**, **B** habitat **C** side view of flower **D**, **E** opened corolla showing pistil and stamens. Scale bar: 1 cm. Arrow indicates calyx and stigma.

be easily found here (Fig. 1A). In fact, Nankunshan has been intensively botanized in recent years, because of its high biodiversity (Chen et al. 2017; Xu et al. 2017), and these studies also did not find *D. trichanthera*. As we know, the natural environment of Nankunshan has been well protected since it was listed as a national forestry park in 1993. Nevertheless, *D. trichanthera* has not been found or collected again since it was described in 2005. It is difficult to believe that *D. trichanthera*, if indeed a well characterized species, is represented only by its type collection.

Unfortunately, the holotype of *D. trichanthera* cannot be found in SYS. According to the isotype (Fig. 2B) preserved in A and the description made by Ye and Shi (2005), we can clearly find that the calyx of *D. trichanthera* is 5-lobed to near middle, with an obvious calyx tube, and the lobes are triangular (Fig. 2B). In contrast, the representative calyx of *Didymostigma* is 5-parted to near base, and the



Figure 2. A Holotype of *Henckelia anachoreta (F.H. Hance 11377,* BM-000041739!) and B isotype of *Didymostigma trichanthera (Chuang-Xing Ye 5960,* A-00135544!). Arrow indicates calyx.

lobes are lanceolate-linear (Fig. 1E; Wang 1984). Additionally, the typical stigma of *Didymostigma* is only ca. 1 mm long (Fig. 1E; Wang 1984). Therefore, although the detailed characteristics of the stigma of *D. trichanthera* have not been clearly observed in the isotype, the description (3–4 mm long) of it in the protologue (Ye and Shi 2005) indicates that it is not a typical character of *Didymostigma*. However, it seems that these two important characters of *D. trichanthera* are well matched with *Henckelia* Spreng. Our detailed comparisons find that there is no obvious difference between the isotype of *Didymostigma trichanthera* (Fig. 2B) and the holotype of *Henckelia anachoreta* (Fig. 2A), and between the images of *Didymostigma trichanthera* in the protologue, especially the diagnosis characters (lanose fertile anthers, hairy filaments and the unhidden pistil to the corolla tube), are exactly matched with *Henckelia anachoreta*.

All the reasons mentioned above have prompted us to carefully consider that *Didymostigma trichanthera* is conspecific with *Henckelia anachoreta*. As a result, the taxonomic treatment of *Didymostigma trichanthera* needs to be made here.

Taxonomic treatment

Henckelia anachoreta (H.F. Hance) D.J. Middleton & Mich. Möller in Weber et al. (2011: 774)

- *≡Chirita anachoreta* H.F. Hance (1866: 231). *Roettlera anachoreta* (H.F. Hance) O. Kuntze (1891: 476). *Didymocarpus anachoretus* (H.F. Hance) H. Lév. (1906: 427).
- =Chirita minutiserrulata B. Hayata (1915: 133). Didymocarpus minutiserrulatus (B. Hayata) Y. Yamamoto (1936: 72). Type: China. Taiwan: Boho, July 1911, Inaba s.n. (TI, not seen).
- =Didymostigma trichathera C.X. Ye & X.G. Shi (2005: 447), syn. nov. Type: China. Guangdong: Longmen County, Nankunshan National Forest Park, 17 August 2003, Chuang-Xing Ye 5960 (Holotype SYS; Isotype A-00135544!).

Type. China. Guangdong: Qingyuan City, North River, 27 July 1864, F.H. Hance 11377 (Holotype BM-000041739!; Isotype K-000858355!).

Distribution and habitat. *Henckelia anachoreta* is a common species with a wide distribution in China (Guangdong, Guangxi, Hunan, Taiwan, Xizang, Yunnan), India (Sikkim), Laos, Myanmar, northern Thailand and northern Vietnam (Weber et al. 2011). Plants often grow on moist rocks or ground surfaces in forest or near valley stream sides.

Additional specimens examined. China. Guangdong: Haifeng County, 15 August 1935, W.T. Tsang 25492 (IBSC); Longmen County, 27 October 1981, G.C. Zhang 280 (HGAS); Maomin County, 2 August 1956, L. Deng 1751 (HITBC); Qingyuan County, 15 September 1936, K.Z. Hou 74155 (IBK); Qujiang County, 13 August 1956, Z. Huang 41855 (IBSC); Ruyuan County, 8 July 2014, J.M. Li 7840, 7763 (HEAC); Wengyuan County, 16 August 1933, X.Q. Liu 2053 (IBSC); Yangshan County, 5 July 1956, L. Deng 1691 (IBSC). Guangxi: Fangchenggang County, 14 July 1908, Anonymous s.n. (PE); ibid. 7 July 1912, K.K. Chung (IBSC); ibid. 7 August 1933, J.L. Zuo 23588 (IBSC); ibid. 25 August 1936, W.T. Tsang 26748 (IBSC); ibid. 10 September 1936, W.T. Tsang 26826 (IBSC); ibid. 9 July 2010, Shiwandashan team 2619, 2656 (IBK); Gongcheng County, 14 August 1957, Gongcheng team 195 (IBK); Hengxian County, 15 October 2007, Y.Q. Su 15915 (GXMG); ibid. 7 September 2008, Ching-I Peng 21784 (HAST); Jinxiu County, 8 September 1981, Dayaoshan team 10146 (IBSC); ibid. 19 September 1981, Dayaoshan team 10317 (IBSC); ibid. 12 September 1981, Dayaoshan team 10488 (IBK); ibid. 1 November 1981, Dayaoshan team 12255 (IBK); Jingxi County, 17 September 2010, Y.S. Huang & L. Wu LYJX0509 (IBK); Lingle County, 15 August 1928, R.C. Ching 6928 (IBSC); Luocheng County, 15 July 1931, S.S. Sin 22411 (IBSC); Ningming County, 19 August 2010, W.B. Xu & W.H. Wu NM396 (IBK); Pingxiang County, 27 August 1986, Beijing team 0973 (PE); Shanglin County, 6 August 1973, Y. Wang et al. 67046 (PE); ibid. 19 October 2011, L. Wu & J.C. Yang D3372 (IBK); Wuming County, 5 August 2010,

L. Wu & R.H. Jiang D0235 (IBK); Xing'an County, 24 September 2014, Xing'an team 450325140924027LY (GXMG); Yongfu County, 21 July 1956, H.F. Qin 700342 (IBK); Zhaoping County, 11 August 1957, C.Z. Jiang & M.S. Xia 4069 (IBK). Hunan: Guidong County, 19 September 1977, B.G. Li 5533 (IBSC); Jiangyong County, 8 July 1959, P.X. Tan 62211 (IBK); 12 July 1959, P.X. Tan 63671 (FJSI). Taiwan: Kaohsiung hsien, 19 September 1991, C.C. Wang 588 (HAST); ibid. 13 September 1997, W.L. Chiou and K.C. Yang s.n. (WU); ibid. 16 September 2000, C.I. Peng 18073 (HAST); ibid. 12 August 2008, C.I. Huang 3463 (HAST); ibid. 6 November 1991, C.I. Peng 14739, 14770 (HAST); Pingtung hsien, 20 September 1990, W.P. Leu 551 (HAST); ibid. 23 August 2006, C.I. Huang 2831 (HAST); ibid. 8 October 2011, P.F. Lu 22985 (HAST). Xizang: Jilong County, 14 September 2008, L.M. Gao et al. GLM-081579 (KUN). Yunnan: Cangyuan County, 27 August 2013, J.M. Li 9485 (HEAC); Hekou County, 27 November 1992, Y.Z. Wang 92065 (PE); ibid. 18 August 1993, Y.M. Shui 003411 (PE); ibid. 2 October 2003, J.M. Li 1022 (PE); ibid. 9 October 2011, M.T. Liu LMT2011025 (PE); ibid. 17 August 2013, Z.J. Qiu et al. QZJ-0957 (PE); Jinping County, 12 August 1951, P.Y. Mao 314 (PE); ibid. 21 September 2006, L.M. Gao GLM-06283, GLM-06287 (KUN); ibid. 8 September 2012, Jinping team 5325300650 (IMDY); Luchun County, 30 September 1973, D.D. Tao 635 (KUN); ibid. 18 October 2000, Y.M. Shui & W.H. Chen 13123 (KUN); ibid. 23 October 2000, Y.M. Shui & W.H. Chen 13747 (KUN); ibid. 25 October 2000, Y.M. Shui & W.H. Chen 13905 (KUN); Maguan County, 19 August 2013, Z.J. Qiu et al. QZJ-0962 (PE); ibid. 18 September 2013, P.W. Li LPW2013144, LPW2013143 (PE); Malipo County, 10 August 2004, J.M. Li LJM-2004-54 (PE); ibid. 28 August 2012, P.W. Li LPW2012016 (PE); Menghai County, 24 August 2011, J.M. Li 82412 (HEAC); Menglian County, 6 August 1973, Menglian team 9967 (KUN); ibid. 14 August 1973, Menglian Team 10172 (KUN); Mengla County, 23 October 1959, X.W. Li 13540 (KUN); Pingbian County, 9 July 1934, H.T. Tsai 62481 (PE); ibid. 18 September 1939, Q. W. Wang 81896 (KUN); ibid. 20 September 1939, Q. W. Wang 81981 (KUN, PE); ibid. 28 September 1954, K.M. Feng 4697 (PE); ibid. 18 September 2012, Pingbian team (IMDY); Wenshan County, 14 August 1947 K.M. Feng 11242 (PE, IBSC); ibid. 20 August 1947, K.M. Feng 11376 (PE); Xichou County, 29 August 1947, K.M. Feng 11450 (PE, IBSC); Yanshan County, 19 October 1939, Q. W. Wang 84483 (PE). Vietnam. Hà Tây: Mont-Bavi, 22 July 1886, Anonymous s.n. (P); ibid. 4 September 1886, Anonymous s.n. (P). Ha Giang: Vi Xuyen Dist., 7 September 2000, Harder, D.K., Hieu, N.Q., Du, N.V. 5302 (E). Thanh Hoa: Ba Thuoc Dist., 9 October 2003, Averyanov, L.; Loc, P.K.; Doan, D.T.; Vinh, N.T. HAL4197 (E). Tonkin: Sai Wong Mo Shan, 18 July-9 Sepetember 1940, W.T. Tsang 30389 (E). Laos. Khammouan: Kaeng Meaung landing on Nakai Nam Theun, 21 October 2005, Newman, M F; Thomas, P I; Armstrong, K E; Sengdala, Khamphone & Lamxay, Vichith LAO 385 (E). Myanmar. Haungrys: 15 August 1919, Kingdon-Ward, F. 3536 (E). Thailand. Nakhon Ratchasima: Khao Yai Nat. Park, 22 October 1969, C.F. van Beusekom, C. Charoenpol 1833 (P); Siam: September 1910, Q.J.G. Kew 1417 (P).

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RESEARCH ARTICLE



Deinostigma fasciculatum, a new species of Gesneriaceae in Yunnan, China

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Abstract

A new species of *Deinostigma* (Gesneriaceae) from Yunnan, China, *Deinostigma fasciculatum* W.H.Chen & Y.M.Shui, sp. nov., has been discovered and described. In the genus, the new species is similar to *D. cicatricosum* (W.T. Wang) D.J. Middleton & Mich. Möller and *D. cyrtocarpum* (D. Fang & L. Zeng) Mich. Möller & H.J. Atkins in dark purple flowers and falcate fruit, but differs from them mainly in the inflorescences with fasciculate flowers, calyx lobes (reflexed, narrowly lanceolate and 1.2–1.3 cm long), corolla tubes (sharply contracted below middle and white outside and below throat). The above three species grow nearby non-limestone wet cliffs and geographically isolated with different distributions (the new species in Southeast Yunnan, *D. cicatricosum* in Eastern Guangxi and *D. cyrtocarpum* in Southern Guangxi and Guangdong, China).

Keywords

Deinostigma cicatricosum, Deinostigma cyrtocarpum, new species, Sino-Vietnamese border, Yunnan

Introduction

The genus Deinostigma W.T. Wang & Z.Y. Li (Gesneriaceae) was established in 1992, based on the type species D. poilanei (Pellegr.) W.T. Wang & Z.Y. Li which was transferred from Hemiboea Clarke, from South of Vietnam (Wang and Li 1992). Möller et al. (2016) enlarged this genus to 7 species, including some species in Deinostigma and previously in Primulina in South of China and Vietnam, based on molecular (ITS and trnL-F regions), morphological and cytological characters. Five Vietnamese species are in the genus and all distributed in Central Vietnam and South Vietnam, far from the border with China, viz. Deinostigma cycnostylum (B.L.Burtt) D.J.Middleton & H.J.Atkins, D. eberhardtii (Pellegr.) D.J.Middleton & H.J.Atkins, D. minutihamatum (D.Wood) D.J.Middleton & H.J.Atkins, D. poilanei (Pellegr.) W.T.Wang & Z.Y.Li, D. tamiana (B.L.Burtt) D.J.Middleton & H.J.Atkins. Up to now, two Chinese species, viz. Deinostigma cyrtocarpum (D. Fang & L. Zeng) Mich. Möller & H.J. Atkins and D. cicatricosum (W.T.Wang) D.J. Middleton & Mich. Möller are recognised as members of the genus (Wang 1981; Fang et al. 1993). Although D. cicatricosum, formerly Chirita cicatricosa W.T. Wang, was regarded as a synonym of Chirita minutihamata D. Wood from Vietnam (Wang et al. 1990, 1998; Li and Wang 2005), Möller et al. (2016) and Wen et al. (2019) still recognised D. cicatricosum in the genus.

Previous orthography of species epithets in *Deinostigma* has used the feminine ending (i.e., D. "cycnostyla", see Möller et al. 2016). The generic name *Deinostigma* is



Figure 1. The distribution of *Deinostigma fasciculatum* W.H.Chen & Y.M.Shui, sp. nov. (■), with *D. cic-atricosum* (W.T.Wang) D.J.Middleton & Mich.Möller (●) and *D. cyrtocarpum* (D.Fang & L.Zeng) Mich. Möller & H.J.Atkins (▲).

Characters	Deinostigma fasciculatum	D. cicatricosum	D. cyrtocarpum		
Leaf base	often slightly peltate	seldom peltate	often slightly peltate		
Inflorescences	with fasciculate flowers	with remote flowers	with remote flowers		
Calyx lobes	narrowly lanceolate, 12–13 × ca. 2	narrowly oblong, 8–10 × 1.8–2.5	narrowly oblong, $5-8 \times 1-2$ mm,		
	mm, inside sparsely glandular villous	mm, inside nearly glabrous	inside nearly glabrous		
Calyx margin	margin reflexed	margin compacted	margin compacted		
Corolla tube	outside white, sharply contracted	outside purple, slightly contracted	outside purple, gradually		
	below throat		contracted		
Capsule	narrowly oblong	linear	narrowly oblong		
Locality	Southeast Yunnan, China	Southern Guangxi and Guangdong,	Eastern Guangxi, China		
		China			
Altitude	500–850 m	300–737 m	100–200 m		

Table 1. Morphological comparison between *Deinostigma fasciculatum* sp. nov., *D. cicatricosum* and *D. cyrtocarpum* in China.

neuter however, and so all epithets have been corrected here (e.g., to *D. cycnostylum*) to comply with Article 62.2(c) of the ICN.

After the surveys in the Sino-Vietnamese border (Fig. 1), a new species of *Deinostig-ma* from Jinping county, Yunnan province, China, has been confirmed and described. Careful examination of the type specimens and related publications reveals that the new species is more similar to *D. cicatricosum* (W.T. Wang) D.J. Middleton & Mich. Möller and *D. cyrtocarpum* (D. Fang & L. Zeng) Mich. Möller & H.J. Atkins than the other five Vietnamese species in fruit morphology (Wang et al. 1998; Wei et al. 2010; Möller et al. 2016; Wen et al. 2019). Although the above three Chinese species are similar to each other in habit and falcate fruit, the floral morphology and geographical distribution provide evidence to identify them respectively (Figs 1–3, Table 1).

Materials and method

We observed the morphology of the species and took photographs of the habitat and macro-morphological characters, both during the fieldwork in Jinping County, Southeastern Yunnan, China and at Kunming Botanical Garden. We also examined the specimens of *Deinostigma* in the herbaria (E, KUN, P & PE). All micro-morphological characters were observed and photographed with a Leica S8 APO stereomicroscope (Shanghai, China) and a Nikon D700 microscope camera (Tokyo, Japan).

Taxonomy

Deinostigma fasciculatum W.H.Chen & Y.M.Shui, sp. nov. urn:lsid:ipni.org:names:77211196-1 Figure 2

Type. CHINA. Yunnan province, Jinping County, Ma-an-di town, 22°58'33"N, 104°50'32"E, 11 August 2018, collected from the living plants at Kunming Botanical Garden, *Y.M. Shui & S.W. Guo B2018-493* (holotype, KUN!).

Diagnosis. The new species is similar to *D. cicatricosum* and *D. cyrtocarpum* in dark purple flowers and falcate fruit, but differs from the latter two species in the inflorescences with fasciculate flowers (*vs.* with remote flowers), calyx lobes reflexed (*vs.* compacted), corolla tubes white outside and below throat (*vs.* purple) (Figs 2C, 3). The new species differs from *D. cicatricosum* in calyx lobes narrowly lanceolate (*vs.* narrowly oblong) and 1.2–1.3 cm long (*vs.* 0.8–1.0 cm), corolla tube sharply contracted below middle (*vs.* slightly contracted), capsule narrowly oblong (*vs.* linear) 2–2.5 cm long (*vs.* 3–4 cm long). It differs from *D. cyrtocarpum* in calyx lobes 1.2–1.3 cm long (*vs.* 0.5–0.8 cm long), corolla tube sharply contracted (*vs.* gradually contracted).

Herbs perennial. Stems pendulous, 30-60 cm long, densely glandular villous. Leaves alternate near stem apex; petiole 2-3.5 cm long, densely glandular villous; leaf blade herbaceous, ovate, elliptic or cordate, $3-9 \times 2.5-4$ cm, base oblique, often slightly peltate, cuneate, cordate or round, apex acuminate, margin serrate, adaxially densely glandular villous, abaxially densely glandular villous; venation penninerved, lateral veins 3-5 on each side of mid-rib. Cymes axillary near stem apex, fasciculate; peduncle 1.5–11.5 cm long, densely glandular villous; bracts 2, ovate, caducous, $0.8-1.2 \times ca$. 0.6 cm, adaxially sparsely glandular villous, abaxially densely glandular villous; bracteoles 2, lanceolate, caducous, ca. 0.6×0.2 cm, adaxially sparsely glandular villous, abaxially densely glandular villous; pedicel ca. 0.5 cm long, densely glandular villous. Calyx 5-parted to the base, segments lanceolate, 1.2–1.3 × ca. 0.2 cm, apex acute, margin entire, outside densely glandular villous, inside sparsely glandular villous. Corolla funnelform, zygomorphic, 3.5-4 cm long, ca. 1 cm wide at the throat, outside dark purple, densely glandular pubescent, inside dark purple, glabrous; tube ca. 2.5 cm long; limb 2-lipped, adaxial lip 2-lobed, lobes semi-circular, ca. 0.8 cm long, 0.5 cm in diam. at base; abaxial lip ca. 1.5 cm long, 3-lobed, middle lobes orbicular, ca. 0.5×0.5 cm, lateral lobes orbicular, ca. 0.5×0.6 cm. Stamens 2, adnate to corolla tube ca. 1.5 cm from base, coherent; anthers densely villous; filaments densely villous, ca. 1.2 cm long; staminode 3, lateral 2, villous, slightly coherent with the anthers, adnate to corolla tube ca. 1.5 cm from base, ca. 0.8 cm long; middle 1, adnate to corolla tube ca. 1.5 cm from base, ca. 1 mm long. Disc ring-like, ca. 1 mm high. Pistil ca. 3.5 cm long; ovary linear, densely glandular pubescent, ca. 0.8 cm long; style linear, ca. 2.7 cm long; stigmas obtrapeziform, emarginate. Capsule obliquely narrowly oblong, 2–2.5 cm long, curved.

Phenology. Flowering is from May to August and fruiting from July to September.

Etymology. The name refers to the flowers, which are fasciculate on inflorescences of the new species.

Vernacular name. Cù Huā Qí Zhù Jù Tái (Chinese pronunciation); 簇花奇柱苣苔 (Chinese name).

Distribution and habitat. The new species only grows on the wet cliff in the valley and only occurs at the type locality, Jinping County, Yunnan province, China.

Additional examined specimens. CHINA. Yunnan province: Jinping county, Ma-an-di town, 22°58'33"N, 104°50'32"E, in valleys, alt. 500 m a.s.l., with fruits, 22 January 2016, *Y.M. Shui & W.H. Chen B2016-084* (KUN!). The same county, Ma-an-di town, Maguaitang village, on wet cliff, alt. 520–850 m a.s.l., with buds, 1 May 2019, *Z.Y. Yu B2019-001*(KUN!).



Figure 2. *Deinostigma fasciculatum* W.H.Chen & Y.M.Shui, sp. nov. **A** habit **B** mature fruits **C** frontal view of flower **D** leaf abaxial side **E** top and back view of flowers **F** top view of opened corolla showing the interior surface of corolla tube, stamens and staminodes **G** pistil and calyx, arrow showing the calyx **H** ovary, calyx and bract. (b = bract, c = calyx, d = disc).



Figure 3. Photographs of *Deinostigma cicatricosum* (W.T. Wang) D.J. Middleton & Mich. Möller (**A–H**) and *D. cyrtocarpum* (D. Fang & L. Zeng) Mich. Möller & H.J. Atkins (I & J) **A** habit **B** adaxial surface of leaf **C** frontal view of flower **D** top view of flower **E** opened corolla showing the interior surface of corolla tube, stamens and staminodes, arrows showing the staminodes **F** stamens and staminodes, arrows showing the staminodes **G** pistil and calyx **H** bract and young flower **I** inflorescence of *D. cyrtocarpum* **J** lateral view of inflorescence (b = bract, c = calyx, d = disc).

Conservation state. The new species has been only observed from the type locality in the nature reserve, with ca. 30, 000 m² area (300 m \times 100 m) and ca. 160 mature individuals on the cliff. The type locality is located in a deep valley with a small power station. Occasionally, local people go there to camp. Additionally, due to the building

of a road, some of the slopes may become unstable and fall, resulting in some individuals being destroyed in the future. So, we hereby assessed the new species as "Critically Endangered (CR)" (C2+a+ii or B2+b+iii). (IUCN 2012, 2017).

Note. *Deinostigma cyrtocarpum* is easily distinguished from *D. cicatricosum* and *D. fasciculatum* by its short calyx (Figs 1, 3J). Secondly, in *D. cicatricosum* and *D. fasciculatum*, corolla tubes are obviously contracted at the middle. As to the L/U ratio (width of lower part/width of upper part of corolla tube), the L/U ratio of *D. cicatricosum* is about 1/2.5 and lightly contracted, while the L/U ratio of *D. fasciculatum* is about 1/5 and sharply contracted (Figs 2, 3). Besides, after the careful examination of the type specimens, *Deinostigma minutihamatum* is distributed in Central Vietnam with 2300 m elevation and characterised by almost straight capsules instead of falcate capsules and so considerably different from the Chinese species of the genus with falcate capsules (Fig. 2B; Wang et al. 1998, Wei et al. 2010).

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RESEARCH ARTICLE



Two new species of *Paraboea* (Gesneriaceae) in *Caryota obtusa* forests in Southwest China, with compound and simple dichasia, respectively

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Abstract

Two new species of Gesneriaceae, *Paraboea myriantha* **sp. nov.** and *P. brevipedunculata* **sp. nov.** are described and illustrated with photos. They grow in the *Caryota obtusa* forests from Yunnan Province of China. *P. myriantha* is closely related to *P. glutinosa* (Hand.-Mazz.) K.Y.Pan, but differs mainly in corolla outside glandular-puberulent, adaxial corolla lobes semicordate, corolla tube obliquely campanulate, and filaments glandular-puberulent. *P. brevipedunculata* is closely related to *P. crassifolia* (Hemsley) B. L. Burtt, but different mainly in simple dichasia with 1 and 2 flowers, peduncles 0.5–2 cm long and capsules slightly twisted. The geographical relationship between the two new species and their similar species has been discussed.

Keywords

Dichasia, Karst regions, new species, Paraboea brevipedunculata, Paraboea myriantha

Introduction

The genus *Paraboea* (Clarke) Ridl. (Gesneriaceae), including about 90 species, mainly occurs in Bhutan, China, Indonesia, Malaysia, Myanmar, Philippines, Thailand and Vietnam (Xu et al. 2008). Recently, several new species have been published (Chen et al. 2008, 2012; Kiew 2010; Xu et al. 2012; Wen et al. 2013; He et al. 2018). Most of them are distributed in the karst regions in South China and Indo-China (Li 1987; Wang 1990; Xu 1993; Fang et al. 1995; Zhu et al. 2003; Li and Wang 2004; Shui and Chen 2006; Zhu 2007; Shui et al. 2017). The genus is easily recognised by the thick hairs on the adaxial surface and lax hairs on the abaxial surface of the leaves in the karst regions, especially in *Caryota obtusa* forest in southwest China (Chen et al. 2017). The forest is a special vegetation subtype in the karst regions and harboured numerous endemic species, such as *Paraboea hekouensis* Y.M. Shui & W.H. Chen and *P. manhaoensis* Y.M. Shui & W.H. Chen in Gesneriaceae (Chen et al. 2012; Chen et al. 2019).

Long-term surveys of *Caryota* forests revealed some new findings in the karst regions in Southwest China. From 2001 to 2005, during our botanical exploration to *Caryota* forests in karst areas in the southeast of Yunnan Province, China, we collected some species of the genus *Paraboea* in Gesneriaceae in Hekou County of SE Yunnan, China (Figure 1). Amongst them, one species with up to 0.9 m tall habit, produces a compound dichasium with hundreds of flowers (Shui and Chen 2006; Chen et al. 2008). With



Figure 1. The geographical distribution of *Paraboea brevipedunculata* W.H. Chen & Y.M. Shui, sp. nov. (square) and *P. myriantha* Y.M. Shui & W.H. Chen, sp. nov. (star).

further observation, it is similar to *P. glanduliflora* Barnett in glandular hairs outside the corollas and different in the basal leaves (Wang et al. 2012). After careful comparison with the other species of *Paraboea* in China (Li 1987; Wang 1990; Fang et al. 1995; Li and Wang 2004; Chen et al. 2008, 2012, 2017; He et al. 2018) and bordering countries (Thúọngtiền 2000; Xu et al. 2008), we confirmed that the species represents an undescribed species of *Paraboea* in Gesneriaceae. After a complete examination to the main worldwide herbaria, we confirmed several additional specimens collected in the adjacent karst regions in China during the 2001–2018 period.

In June 2013, on the other hand, we collected one small doubtful species of *Paraboea* with fruits in Malipo county in the southeast of Yunnan Province (Figure 1). In the field, it grows on cliffs, as well as in the *Caryota* forest at the border with Vietnam in Malipo county, Yunnan, China. However, we missed the flowering period in 2014 and 2015. In May 2016, we collected the plants with flowers and confirmed that it belonged to the genus *Paraboea*. After an examination of literatures and related specimens, we determined that it is unique in ca. 5 cm high habit and simple dichasia and should be an undescribed species in the genus. It is possible that it may be collected in Vietnam in the future (Figure 1).

Materials and methods

We confirmed two new species after examination of the specimens preserved in worldwide herbaria (E, IBSC, K, KUN, P, PE). We took photographs of the habit and macro-morphological characters in the field. Subsequently, we carried out morphological observations and measurements of the two new species, based on living plants in the field and Kunming Botanical Garden, together with additional specimens in KUN. All micro-morphological characters were observed and photographed with a Leica S8 APO stereomicroscope and a Nikon D700 microscope camera.

Taxonomy

Paraboea myriantha Y.M. Shui & W.H. Chen, sp. nov. urn:lsid:ipni.org:names:77211197-1 Figure 2

Type. CHINA. Yunnan Province: Hekou County, Nanxi Community, 22°38'18.44"N, 104°00'28.93"E, in the limestone forests, alt. 900 m, 26 August 2005, in flowers, *Y.M. Shui et al. 44536* (holotype KUN).

Diagnosis. The new species is similar to *P. glutinosa* (Hand.-Mazz.) K.Y.Pan in winged petioles, leaf-like bracts and compound dichasia, but distinguished by adaxial corolla lobes semicordate (vs. nearly rounded), tube obliquely campanulate (vs. urceolate) outside glandular-puberulent (vs. glabrous or rarely pubescent) and laterally

uneven (vs. even), and glandular-puberulent filament (vs. covered by a beard of multicellular hairs); and similar to *P. thorelii* (Pellegr.) B.L.Burtt in winged petioles and compound dichasia, but distinguished by corolla tubes 9–10 mm long (vs. 3–4 mm long) outside glandular-puberulent (vs. glabrous) and staminodes 2 (vs. inconspicuous).

Description. Herbs, up to 90 cm tall, stems 10-20 cm. Leaves opposite, clustered at stem apex; petiole 5–8 (-15) cm long and winged, wings (1-) 5–20 mm wide each side; leaf blade obovate, 12-30 (-48) × 5-13 (-16) cm, chartaceous, adaxially sparsely pubescent to glabrous, abaxially densely matted arachnoid, base cuneate to wing-like on petiole, margin repand-crenate and involute, apex mucronate to rounded; midrib depressed adaxially, protuberant abaxially; lateral veins 8-13 (-16) pairs, obscure adaxially and distinct abaxially. Dichasium terminal paniculate, with hundreds of flowers; peduncles up to 70 cm, densely arachnoid when young, sparsely puberulent to glabrous when mature; bracts (the lowermost fertile leaves) 2, leaf-like, ca. 7×3 cm, densely arachnoid abaxially; bracteoles 2, linear, ca. 2 × 0.5 mm; pedicels 5-8 mm long. Calyx 5-sect from base; segments linear, ca. 1 mm long, glabrous. Corolla purple adaxially and white abaxially (corollas purple when young), outside glandular-puberulent; tube obliquely campanulate, ca. 10 mm long, outside glandular-puberulent; adaxial lip 2-lobed, lobes semicordate ca. 3×6 mm; abaxial lip 3-lobed, lobes ca. 5×6 mm. Stamens 2, included; filaments lateral-fixed, ca. 8 mm long, glandular-puberulent from middle to top; anthers ca. 5 mm long; staminodes 2, 3-5 mm long. Pistil glabrous; ovary oblong, ca. 6 mm long; style linear, 4–5 mm long; stigma capitate. Capsule obviously twisted, 4.5–6.7 cm long, glabrous, with persistent calyx. Seeds ellipsoid, 0.6–0.7 × 0.15–0.2 mm.

Etymology. The new species is named after its numerous flowers per an individual.

Distribution and habitat. The new species only grows in *Caryota obtusa* forests of limestone areas in Hekou and Maguan counties of Yunnan, China (Figure 1).

Phenology. Flowering from June to August; fruiting from July to November.

Additional specimens examined (paratype). CHINA. Yunnan Province: Hekou County, in dense forests on the limestone hillsides, alt. 700–950 m, 21 October 2001, *Y.M. Shui et al. 15105* (KUN); at the same county, in the limestone seasonal forests, alt. 1000–1200 m, with fruits of last year, 28 March 2002, *Y.M. Shui et al. 20595* (KUN); at the same county, in dense forests on limestone hillsides, alt. 1000 m, with young dichasia, 28 March 2002, *Y.M. Shui et al. 20946* (KUN); at the same county, Nanxi Zhen, 22°40'8"N, 104°01'16"E, in forests, alt. 900 m. 6 September 2013, *Y.M. Shui, B. Xiao, J. Wang et al. B2013-528* (KUN). Maguan County, Gulinqing Community, 22°43'N, 103°59'E, in the evergreen broad-leaved forests on limestone hillsides, alt. 1000 m altitude, 3 October 2002, *Y.M. Shui et al. 30261* (KUN); at the same county, in the limestone evergreen broad-leaved forests, alt. 794 m, 11 November 2006, *Y.M. Shui et al. 16118* (KUN); at the same county, Jiahanqing Community, Moshizhai Village, 9 August 2018, *Y.M. Shui et al. B2018-183* (KUN).

Note. The new species appeared as *Paraboea auriculata* Y.M. Shui & W.H. Chen (nom. nud.) because of its winged petioles in Shui and Chen (2006). However, we de-



Figure 2. *Paraboea myriantha* Y.M. Shui & W.H. Chen, sp. nov. (**A**, **C–G**, **J**) and its similar species *P. glutinosa* (**B**, **H**, **I**) **A**, **B** habitat **C** abaxial surface of leaf **D** flower bud **E** lateral view of flower **F** bird view of flower **G**, **H** front view of flower **I** lateral view of flower **J** flower and fruits (**A** and **C** photographed by Gui-Liang Zhang and all the others by Yu-Min Shui).

cided to name it as *Paraboea myriantha*, after careful comparison of references and type specimens with similar species, *P. glutinosa* (Hand.-Mazz.) K.Y.Pan and *P. thorelii* (Pellegr.) B.L.Burtt. The new species is similar to the above two species on winged leaves and compound dichasia, but distinguished by corolla lobes (shape) and tubes (shape and indumenti) and glandular filament, which are described in diagnostics (Figure 2; Xu et al. 2008; Vu et al. 2011). Furthermore, *P. glutinosa* is distributed in South China, *P. thorelii* in South Laos (type locality) and North Vietnam, but the new species we proposed seems geographically distributed between the above two similar species. So, the future molecular work in the context of the whole genus may reveal if the above three species become a species complex with obvious geographical replacement.

Paraboea brevipedunculata W.H. Chen & Y.M. Shui, sp. nov.

urn:lsid:ipni.org:names:77211198-1 Figure 3

Type. CHINA. Yunnan province: Malipo County, Tianbao community, 22°58'33.31"N, 104°50'32.92"E, in limestone forests, alt. 900 m, 30 April 2017, *Y.M. Shui & W.H. Chen B2017-1342* (holotype, KUN).

Diagnosis. Paraboea brevipedunculata is similar to *P. crassifolia* (Hemsley) B. L. Burtt in morphology and indumenti of the leaves, but different in simple dichasia with 1–2 flowers (vs. compound dichasia with many flowers), peduncle 0.5–2 cm (vs. 8–12 cm), 4–5 mm calyx segments (vs. 2–3 mm), capsules slightly twisted (vs. multi-twisted) and 0.6–0.7 mm long when mature (2–2.5 cm long). The new species is also similar to *P. velutina* (W.T.Wang & C.Z.Gao) B.L.Burtt. in the small plant, short peduncle and simple dichasia, but distinguished by purple corolla (vs. white), calyx 4–5 mm long (vs. ca. 1 mm), lobes of adaxial lip ca. 3 × 5 mm (vs. ca. 1.5 × 1 mm), lobes of abaxial lip ca. 5 × 7 mm (vs. ca. 1.5 × 2.3 mm) and capsule slightly twisted (vs. not twisted).

Description. Herbs 4–5 cm high and without stems. Leaves clustered; petiole very short, 0.2–1 cm, densely arachnoid; leaf blade obovate, 2.6–6 × 1–3 cm, thick papery, adaxially pubescent when young and subglabrous when mature, abaxially densely matted arachnoid, base cuneate, margin subentire to shallowly repand-crenate, apex rounded; midrib depressed adaxially, protuberant abaxially; lateral veins 4–7 pairs, obscure adaxially and distinct abaxially. Dichasium terminal axillary, with 1–4 flowers; peduncle 0.5–2 cm, densely arachnoid; bracts 2, 0.5–0.6 × ca. 0.1 cm, sparsely pubescent abaxially; bracteoles 2 (sometimes absent), linear, ca. 2 × 0.5 mm; pedicel 0.3–1.2 cm long, densely arachnoid. Calyx 5-sect from base; segments linear, 4–5 × 1–2 mm, glabrous. Corolla purple, glabrous; tube 5–7 mm; adaxial lip 2-lobed, lobes ca. 3 × 5 mm; abaxial lip 3-lobed, lobes ca. 5× 7 mm. Stamens 2, included; filaments curved, ca. 4 mm long, glabrous; anthers ca. 2 mm long; staminodes 3, the lateral two ca. 1 mm long and the middle one ca. 0.5 mm long. Pistil glabrous; ovary oblong, ca. 2 mm long; style linear, 6–7 mm long; stigma capitate. Capsule 0.6–0.7 mm long when mature, slightly twisted, glabrous, with persistent calyx. Seeds ellipsoid, 5–7 × 0.2–0.3 mm.

Etymology. The new species is named after its short peduncle per dichasium.


Figure 3. *Paraboea brevipedunculata* W.H. Chen & Y.M. Shui, sp. nov. **A** habit **B** adaxial surface of leaf **C** abaxial side of leaf **D** adaxial surface of leaf, showing indumentum **E**, **F** abaxial surface of leaves, showing indumenti **G** dichasia **H** front view of flower **I** vertical view of flower, showing bracteoles (br) and calyx lobe (ca) **J** opened corolla, showing staminodes (sta) and pistil **K** pistil with calyx lobes (All photographed by Yu-Min Shui).

Distribution and habitat. The new species only grows in *Caryota obtusa* forests of limestone areas in Malipo County of Yunnan, China (Figure 1).

Phenology. Flowering from April to May; fruiting from June to July.

Additional specimens examined (paratype). CHINA. Yunnan Province: Malipo County, 22°58'33.31"N, 104°50'32.92"E, in limestone forests, alt. 900 m, 24 June 2013, *Y.M. Shui & W.H. Chen B2013-094* (KUN); Malipo County, Tianbao, 22°58'33.31"N, 104°50'32.92"E, in limestone forests, alt. 900 m, 30 April 2017, *Y.M. Shui & W.H. Chen B2017-1342* (KUN); the same place, 14 September 2018, in fruits, *Y.M. Shui & W.H. Chen B2018-021* (KUN).

Note. The new species is more similar to *P. crassifolia* than *P. neurophylla* (Collett & Hems1.) B.L. Burtt in its linear bracts (Wang et al. 1998; Li and Wang 2004). *P. crassifolia* is distributed in W Huibei, SE Chongqing, Guizhou, Guangxi and SE Yunnan in China, while *P. neurophylla* is distributed in China (Central and West Yunnan) and Myanmar. The new species is distributed in SE Yunnan and shares the similar distribution with *P. crassifolia*, which is considered as the similar species to the new species. Besides, as to the small habit and fruits, it is somewhat similar to *P. velutina* in West Guangxi, which is next to SE Yunnan, but distinguished by corolla colour, size of calyx and corolla lobes, and twisted capsules (see the above diagnosis).

Vu et al. (2011) reported *Paraboea neurophylla* as a new record in Vietnam. The voucher specimens are collected from Ba Be National Park, Bac Kan province, Vietnam. However, the figure (based on *HLF 608* in HN) reveals that it seems to be conspecific with the new species we proposed here. Additionally, the description and geographical distribution of the new record in Vietnam roughly match that of the new species (Vu et al. 2011). Although we are still waiting for further confirmation from the detailed surveys, it is possible that the new species will also occur in North Vietnam. In fact, *P. neurophylla* grows at ca. 2000 m elevation in China (Yunnan, e.g. *B.Y. Qiu 55121* in PE, *P. I Mao 1322* in PE, *S. E. Liu 831, 13970, 14087, 19713 and 20886* in PE, *K.M. Feng 10115* in PE, *K.Y. Pan 1* in PE, *J. Wu WJ2015010* in PE, *Z.J. Qiu QZJ-0936* in PE, *C.J. Chen 38* in PE, *J.S. Xin 51404* in IBSC) and Myanmar (Shan hills, *Collett 804*, holotype K and isotype in E), but its habitat is very different from the habitat of the new species at less than 1000 m elevation (Wang 1990; Wang et al. 1998; Xu et al. 2008).

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RESEARCH ARTICLE



Two new taxa of Gesneriaceae in the karst regions in North Vietnam

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Abstract

One new species and one new variety of Gesneriaceae in Vietnam are described, viz. *Paraboea sinensis* var. *glabrissima* var. nov. and *Primulina xuansonensis* sp. nov. These two new taxa grow in limestone regions in North Vietnam. The former new variety differs from *Paraboea sinensis* (Oliv.) Burtt var. *sinensis* in its young leaf abaxially, stem and peduncle sparsely and thin pannose, acute top of leaves, pistil glandular-pubescent or pubescent. The latter new species differs in its bracts $1.6-2.5 \times 1.3-1.5$ cm, corolla 3-3.5 cm long, corolla tube slightly curved near the base and inflated on the adaxial surface near the corolla lobes, and corolla abaxial lip lobes rounded. The two new taxa grow at Xuan Son National Natural Reserve, North Vietnam and remain undisturbed with low risk of extinction.

Keywords

Didymocarpoideae, Flora of Vietnam, limestone, natural park, new taxa, protection

Introduction

Vietnam is one of the regions with the highest species biodiversity in Asia (Myers et al. 2000). It is estimated that there are about 70 species of Gesneriaceae in Vietnam (Ho 2000). However, biodiversity studies are scarce in Vietnam. For example, the discoveries in Gesneriaceae only focused on certain genera: *Oreocharis* in North Vietnam (Do et al. 2016, 2017; Chen et al. 2017, 2018a; Möller et al. 2018), *Billolivia* in Central Vietnam (Middleton et al. 2014a, 2014b; Luu et al. 2015, 2018; Vu et al. 2015; Nguyen et al. 2016; Lý 2017) and other genera (*Deinostigma*: Möller et al. 2016; *Didymocarpus*: Hong et al. 2018; *Raphiocarpus*: Vu et al. 2012; *Hemiboea*: Chen et al. 2018b; *Paraboea*: Middleton 2018; *Aeschynanthus*: Middleton and Atkins 2018; and so on).

Many species of Gesneriaceae are distributed both in South China and in North Vietnam. China is a significant centre of diversity of the family Gesneriaceae, with the majority of taxa found in the South and Southwest China (Wang et al. 1990, 1998; Li 1991, 2005; Wei et al. 2010; Möller et al. 2016). After long-term collaborative surveys between China and Vietnam, we not only confirmed the common species and records from two countries, but also published some new species endemic to Vietnam, especially in the adjacent region to South China (Chen et al. 2017, 2018a, 2018b). Here, we describe two new taxa of Gesneriaceae in the karst regions at Xuan Son National Park in North Vietnam (Fig. 1), viz. *Paraboea sinensis* var. *glabrissima* W.H.Chen & Y.M.Shui, var. nov. and *Primulina xuansonensis* W.H.Chen & Y.M.Shui, sp. nov.



Figure 1. The distribution of *Paraboea sinensis* var. *glabrissima* W.H.Chen & Y.M.Shui var. nov. (triangle) and *Primulina xuansonensis* W.H. Chen & Y.M. Shui sp. nov. (square).

Materials and method

After reviewing preserved herbarium specimens worldwide (BM, E, HNU, K, KUN, P, PE), two new taxa were confirmed. Habitat photographs and images of macromorphological characters were taken in the field, in greenhouses and from the fixed (FAA) material. Morphological observations and measurements of the new taxa, based on living and dry plant specimens (from KUN) and preserved materials, were carried out. All morphological characters were observed and photographed with a Leica S8 APO stereomicroscope and a Nikon D700 microscope camera.

Taxonomy

Paraboea sinensis var. *glabrissima* W.H.Chen & Y.M.Shui, var. nov. urn:lsid:ipni.org:names:77211199-1 Figure 2

Type. VIENTAM, Pho Tho Province: Xuan Son County, Xuan Son National Park, 21°07'49.3"N, 104°57'09"E, 463 m a.s.l., 8 April 2016, *Y. M. Shui, W.H. Chen, C. Liu, H.Q. Nguyen, H.T. Nguyen, N.Q. Chuong CK909* (holotype, KUN!; isotype, CPC!=Herbarium of Center for Plant Conservation of Vietnam).

Diagnosis. The new variety is similar to *Paraboea sinensis* (Oliv.) Burtt var. *sinensis* in its morphology of habit, calyx, corolla and fruits, but differs in its young leaf abaxially, stem and peduncle sparsely and thin pannose (vs. dense and thick pannose), acute top of leaves (vs. acuminate), pistil glandular-pubescent or pubescent (vs. glabrous).

Subshrubs. Stem erect, 50-80 cm tall, ca. 0.3 cm in diam., with many branches, pannose when young. Leaves opposite, equal to subequal in pairs on the stem; petiole 1-10 cm long, pannose when young; blade herbaceous, slightly asymmetric, oblong to obovate, $9-19 \times 3.5-8$ cm, base cuneate, apex acute, margin denticulate from the base, adaxially glabrous, abaxially sparsely pannose when young; venation penninerved, lateral veins 7–12 on each side of the midrib. Cymes axillary near branch apices; peduncle 1.5-4.5 cm long, sparsely pannose; bracts caducous; pedicel 1.4-2 cm long, sparsely pannose; bracteoles caducous. Calyx zygomorphic, 2-lipped, adaxial calyx ca. 1 cm long, 3-lobed to the middle, lobes rounded ca. 0.5×0.5 cm, abaxial calyx 2-lobed to the base, lobes obovate, ca. 1.2×0.6 cm, apex round, margin entire, outside glabrous, inside glabrous. Corolla campanulate, zygomorphic, 2-2.3 cm long, ca. 1.3 cm wide at the throat, both sides glabrous, tube 1–1.4 cm long; limb 2-lipped; adaxial lip 2-lobed, lobes broadly ovate, ca. 0.9×1.5 cm; abaxial lip 3-lobed, lobes broadly ovate, middle lobe ca. 0.7×1.2 cm. Stamens 2, adnate to the corolla base; anthers glabrous; filaments ca. 1 cm long, pubescent, staminodes 3, glandular-pubescent, lateral ones 2.5-3.0 mm long, adnate to the corolla tube base, the middle one ca. 1 mm long, adnate to the corolla tube ca. 2 mm above the base. Disc absent. Pistil ca. 1.2 cm long; ovary linear, ca. 0.8 cm long, sparsely glandular-pubescent or pubescent; style linear, ca. 0.4 cm long, glandularpubescent; stigma 1, capitate. Capsule linear, spirally twisted, 2.5–3.7 cm long, glabrous.



Figure 2. *Paraboea sinensis* var. *glabrissima* W.H.Chen & Y.M.Shui, var. nov. (**A**–**F**) and *P. sinensis* (Oliv.) Burtt var. *sinensis* (**G & H**) **A** habit, arrows indicate the young leaves with thin brown pannose **B** inflorescence with twisted fruits, arrows showing the indumentum of abaxial surface of leaf (pe = peduncle, abl = abaxial leaf) **C** face view of flower **D** dorsal view of the calyx, the arrow showing the coherent position **E** birds-eye view of opened corolla **F** stamens and staminodes, arrows showing the staminodes **G** habitat **H** inflorescence.

Characters	P. sinensis var. glabrissima	P. sinensis var. sinensis
Indumenti	Young leaf abaxially, stem and peduncle	Young and mature leaf abaxially, stem and
	sparsely and thin pannose, mature glabrous	peduncle dense and thick pannose
Base of leaves	Cuneate	Broadly cuneate to round
Top of leaves	Acute	Acuminate
Pistil	Glandular pubescent or pubescent	Glabrous

Table 1. Morphological comparison of *Paraboea sinensis* var. *glabrissima* W.H. Chen & Y.M. Shui and *P. sinensis* var. *sinensis*.

Phenology. Flowering from March to April, fruiting from April to May.

Etymology. The word "*glabrissima*" indicates the glabrous surface across the whole plant except the young leaves (Fig. 2A), which become glabrous as soon as the leaves become mature. In this manner, it is different from the original variety of *Paraboea sinensis* var. *sinensis*.

Distribution and habitat. The new variety only occurs in Xuan Son County, Pho Tho Province and grows in soil, on rocks in limestone forests.

Provisional conservation status. Based on field surveys and detailed discussions with Vietnamese colleagues, including Hieu Quang Nguyen, this new variety has only been observed in the Xuan Son National Park. There were only a total of 30 mature individuals, so we provisionally considered it as Critically Endangered (CR): B1b (v) + 2b (v). (IUCN 2012; IUCN Standards and Petitions Subcommittee 2017).

Note. This new variety is very similar to the original variety of *Paraboea sinensis* in subshrub habit, obovate calyces and twisted fruits, but different in the almost glabrous habit (Table 1; Wang et al. 1998; Xu et al. 2008). Furthermore, the new variety is covered by pannose indumenti on the young leaves (Fig. 1A), but quickly becomes glabrous on the mature ones, while pannose indumenti consistently covers the original variety on both young and mature leaves (Figs 1G, H). Additionally, the top of the leaf is acute rather than acuminate in the original variety. It is necessary to explore the morphology diversity and genetic differentiation of *Paraboea sinensis* in the future, a wide-distributed species in the genus.

Primulina xuansonensis W.H.Chen & Y.M.Shui, sp. nov.

urn:lsid:ipni.org:names:77211200-1 Figure 3

Type. VIENTAM. Phu Tho Province: Xuan Son County, Xuan Son National Park, 21°07'01"N, 104°57'29"E, 438 m a.s.l., without flowers and fruits, 8 April 2016, introduced at Kunming Botanical Garden, in flower from October to December 2017, *Y. M. Shui, W.H. Chen, C. Liu, H.Q. Nguyen, H.T. Nguyen, N.Q. Chuong B2017-1341* (holotype, KUN!; isotype, CPC!).

Diagnosis. The new species is similar to *Primulina lungzhouensis* (W.T. Wang) Mich.Möller & A.Weber in having prominent bracts, with a corolla, the outside of



Figure 3. *Primulina xuansonensis* W.H.Chen & Y.M.Shui, sp. nov. (**A–H**) and its similar species *P. lungzhouensis* (W.T.Wang) Mich.Möller & A.Weber (**I–J**) **A** habit **B** view of adaxial leaf **C** inflorescence **D** bracts **E** face view of flower **F** lateral view of flower **G** birds-eye view of the opened corolla, arrow showing the staminodes (sta.) **H** pistil and calyx, arrow showing the disc ring **I** habitat of *P. lungzhouensis* **J** face view of flower and bracts.

which is white. However, it differs in its bracts $1.6-2.5 \times 1.3-1.5$ cm (vs. bracts $2.5-3.8 \times 2.2-2.8$), corolla 3-3.5 cm long (vs. corolla 4.5-5 cm long), corolla tube slightly curved near the base (vs. not curved), inflated on the adaxial surface near the corolla lobes (vs. not inflated) and corolla abaxial lip lobes rounded (vs. triangular).

Herbs perennial. Stem absent. Leaves basal; petiole 2-5 cm long, strigose; blade carnose and papyraceous when dry, ovate to rounded, $11-18 \times 8.5-11$ cm, base cuneate, apex round, margin crenate from the base, adaxially sparsely strigose, abaxially sparsely strigose; venation penninerved, lateral veins 5-7 on each side of the midrib. Cymes umbel-like, axillary, ca. 9-flowered; peduncle 6.5–8.5 cm long, densely strigose; bracts 2, triangular-ovate, $1.6-2.5 \times 1.3-1.5$ cm, both sides densely strigose, margin with ca. 2-minute dentes near apex; pedicel 1–1.2 cm long, glandular-pubescent; bracteoles 2, narrowly ovate, ca. 1.2×0.4 cm, both sides densely strigose. Calyx 5-parted to the base, segments lanceolate, 0.8-1 × ca. 0.3 cm; apex acute, margin entire, outside glandular-pubescent, inside strigose. Corolla funnelform, zygomorphic, 3–3.5 cm long, ca. 0.8 cm wide at the throat, outside white, densely glandular-pubescent, inside white with two yellow stripes along the abaxial lip, glabrous; tube 2-2.3 cm, slightly curved near the base; limb 2-lipped; adaxial lip 2-lobed, lobes broadly ovate, ca. $0.5 \times$ 0.8 cm; abaxial lip ca. 1.2×1.7 cm, 3-lobed to the middle, lobes rounded, middle lobe ca. 0.8×0.7 cm, lateral lobes ca. 0.6×0.7 cm. Stamens 2, adnate to the abaxial side of corolla tube ca. 1.4 cm above base; anthers glabrous; filaments glabrous, ca. 0.5 cm long; staminodes 3, ca. 1 mm long, glabrous, lateral two adnate to ca. 1 cm above the corolla base, central staminode adnate to ca. 0.6 cm above the corolla base. Disc ringlike, 1–2 mm high. Pistil glandular-pubescent, ca. 1.6 cm long; ovary linear, ca. 1 cm long; style linear, ca. 0.6 cm long; stigma cuneate, 2-lobed. Capsule unknown.

Phenology. Flowering from October to December, fruiting from December to January from cultivated plants.

Etymology. The name refers to the type locality.

Distribution and habitat. The new species only grows in the limestone forests' rock crevices and distributes in the type locality, Xuan Son National Park of Pho Tho Province, North Vietnam.

Provisional conservation status. We observed it in the field with very few individuals (about ten individuals). Due to the similarity of plants without flowers in the genus *Primulina*, we did not pay attention to the species in the field, so the number of mature individuals remains unknown. Provisionally, it is best to consider it as Data Deficient (DD) (IUCN 2012; IUCN Standards and Petitions Subcommittee 2017).

Note. Within the genus *Primulina*, there is a morphological species complex with prominent shell-like bracts, such as *P. beiliuensis* B. Pan & S. X.Huang, *P. eburnea* Hance, *P. lunglinensis* (W.T. Wang) Mich. Möller & A.Weber, *P. lungzhouensis*, *P. maguanensis* (Z.Yu Li, H.Jiang & H.Xu) Mich. Möller & A.Weber, *P. minutimaculata* (D. Fang & W. T. Wang) Yin Z.Wang, *P. obtusidentata* (W.T. Wang) Mich. Möller & A.Weber (e.g. Wang et al. 1998; Wei et al. 2010; Pan et al. 2013; Shui et al. 2017; Wen et al. 2019). Within the above complex, the new species is different from all the others in its white throat out-

Characters	Primulina xuansonensis	P. lungzhouensis
Bracts	1.6–2.5 × 1.3–1.5 cm	2.5–3.8 × 2.2–2.8 cm
Corolla	3–3.5 cm long, ca. 0.8 cm wide at the	4.5–5 cm long, ca. 1.4 cm wide at the
	throat	throat
Corolla tube	Slightly curved near the base, inflated on	Not curved, not inflated on the adaxial
	the adaxial surface near the corolla lobes	surface near the corolla lobes
Abaxial lip lobes of corolla	Rounded	Triangular

Table 2. Morphological comparison of *Primulina xuansonensis* W.H. Chen & Y. M. Shui and *P. lungzho-uensis* (W.T. Wang) Mich.Möller & A.Weber.

side the corolla, except for *P. lungzhouensis*. Furthermore, the new species is different from *P. lungzhouensis* in its bracts $1.6-2.5 \times 1.3-1.5$ cm, corolla 3-3.5 cm long, corolla tube slightly curved near the base, inflated on the adaxial surface near the corolla lobes and corolla abaxial lip lobes rounded. *P. lungzhouensis*, however, has bracts $2.5-3.8 \times 2.2-2.8$ cm, corolla 4.5-5 cm long, corolla tube not curved, not inflated on the adaxial surface near the corolla lobes and corolla abaxial lip lobes and corolla abaxial lip lobes triangular. In a word, the new species is unique in its narrow and curved corolla tube amongst the above complex with prominent bracts (Table 2).

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