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

Wen-Hong Chen¹³, Ya-Mei Zhang¹⁴, Shi-Wei Guo¹⁴, Zhi-Rong Zhang², Li Chen¹⁵, Yu-Min Shui¹³

¹ CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Kunming 650201, Yunnan Province, China ² Germplasm Bank of Wild Species, Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, 132 Lanhei Road, Kunming 650201, Yunnan Province, China ³ Tropical Mountain Forest Eco-station in Southeast Yunnan, Pingbian 551200, Yunnan Province, China ⁴ University of the Chinese Academy of Sciences, Beijing 100049, China ⁵ School of Life Sciences, Yunnan University, Kunming 650091, Yunnan Province, China

Corresponding author: Yu-Min Shui (ymshui@mail.kib.ac.cn)

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 *Oreocharis* 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 *trnL*-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 [≡ *Oreocharis 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 well-resolved 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*, *ndhB-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 bootstrap 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. B2015-255, 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 Oreocharis 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-ycf1, 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 espirolii, 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 2. The Bayes inference (BI) and Maximum likelihood (ML) tree inferred from six cp DNA markers (atpB-rbcL, ndhH-rps15-ycf1, rpl132, trnC-trnD, trnL-F, trnT-trnL) 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, ※ indicates < 50%.
Figure 3. The Bayes inference (BI) and Maximum likelihood (ML) tree inferred from six cp DNA markers (atpB-rbcL, ndhH-rps15-ycf1, rpl132, 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, ※ 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
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-
Bournea within Oreocharis

porate, aperture membrane granulum, exine verrucate, tectum verrucate, supratectal elements granulum (Fig. 4). The difference between them is polar axis 14–18 μm diam. in *B. sinensis*, 12–14 μm 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 marker 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 yellow-dominated 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 in *Bournea* and *Thamnocharis* would be synapomorphy and *Thamnocharis* autapomorphy. (Figs 2, 3, Suppl. material 2: Fig. S1, Suppl. material 3: Fig. S2). Therefore, it seems that floral actinomorphy could not be
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 verrucate 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.

**Acknowledgments**

We gratefully acknowledge Dr. Liang Zhang of the Kunming Institute of Botany, Chinese Academy of Sciences, and Dr. Michael Möller of Royal Botanic Gardens, Edinburgh, for providing constructive suggestions regarding the analysis and contents. We thank Prof, Wen-Bo Liao and Dr. Qiang Fan of Sun Yat-sen University, and Mr. Jian-Qin Wu of Tian-Bao-Yan National Nature Reserve in Fujian Province for providing help in surveys. Dr. Ming Kang and Dr. Li-Hua Yang provided some molecular sequences for our phylogenetic analysis. We would also like to thank Stephen Maciejewski, The Gesneriad Society, and Michael LoFurno, Adjunct Professor, Temple University, Philadelphia PA, USA, for their editorial assistance. This work was supported by the National Natural Science Foundation of China (Grant no. 31470306, 31000258).
References

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology 19(5): 455–477. https://doi.org/10.1089/cmb.2012.0021

Chen WH, Shui YM, Sima YK, Zhang RM, Wei ZD (2009) Pararuellia glomerata (Acanthaceae), a new species from Yunnan, China. Botanical Studies (Taipei, Taiwan) 50: 261–267.

Chen WH, Shui YM, Yang JB, Wang H, Nishii K, Wen F, Zhang ZR, Möller M (2014) Taxonomic status, phylogenetic affinities and genetic diversity of a presumed extinct genus, Paraisometrum W.T. Wang (Gesneriaceae) from the karst regions of Southwest China. PLoS One 9(9): E107967. https://doi.org/10.1371/journal.pone.0107967

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Guo YF, Wang YQ (2013) Supplemental study on pollen morphology of Epithandra (Gesneriaceae). Guihaia 33(4): 538–542.

Hong X, Ma W, Xu SS, Lin DZ, Zhou SB, Wen F (2015) The supplementary study on pollen morphology of Gesneriaceae from southern China. Journal of Anhui Normal University 38(5): 446–473. [Natural Science]

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics (Oxford, England) 17(8): 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Katoh K, Kuma K, Toh H, Miyata T (2015) MAFFT version 5: Improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33(2): 511–518. https://doi.org/10.1093/nar/gki198

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics (Oxford, England) 28(12): 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Li ZY, Wang YZ (2004) Plants of Gesneriaceae in China. Henan Science and Technology Publishing House, Zhengzhou. [in Chinese]

Möller M, Middleton D, Nishii K, Wei YG, Sontag S, Weber A (2011) A new delineation for Oreocharis incorporating an additional ten genera of Chinese Gesneriaceae. Phytotaxa 23(1): 1–36. https://doi.org/10.11646/phytotaxa.23.1.1

Oliver D (1893) Hooker’s icones plantarum; or figures, with descriptive characters and remarks of new and rare plants, ser.IV, vol.III, part. III. Dulau & Co., London, 2251–2257.

Pan KY (1987) Taxonomy of the genus Oreocharis (Gesneriaceae). Acta Phytotaxonomica Sinica 25: 264–293.
Punt W, Hoen PP, Blackmore S, Thoms AL (2007) Glossary of pollen and spore terminology. Review of Palaeobotany and Palynology 143(1–2): 1–81. https://doi.org/10.1016/j.revpalbo.2006.06.008

Shui YM, Chen WH (2018) Bournea Oliver In: Li DZ, Chen ZR, Wang H, Lu AM (Eds) A Dictionary of the Families and Genera of Chinese Vascular Plants. Science Press, Beijing, 70 pp.

Shui YM, Chen WH (2020) Gesneriaceae Richard & Jussieu In: Li DZ, Chen ZR, Wang H, Lu AM, Luo Y, Yu WB (Eds) The Families and Genera of Chinese Vascular Plants. Science Press, Beijing, 1911–1940.

Silvestro D, Michalak I (2012) raxmlGUI: A graphical front-end for RAxML. Organisms, Diversity & Evolution 12(4): 335–337. https://doi.org/10.1007/s13127-011-0056-0

Stamatakis A (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics (Oxford, England) 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Tan Y, Hu GW, Long CL, Motley T, Wang Z, Sui XY (2011) The systematic placement of the monotypic genus Paraisometrum (Gesneriaceae) based on molecular and cytological data. Plant Diversity and Resources 33(5): 465–476.

Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: Concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27(2): 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Wang WT, Pan KY, Li ZY (1990) Gesneriaceae. In: Wang WT (Ed.) Flora Reipublicae Popularis Sinicae (Vol. 69). Science Press, Beijing.

Wang WT, Pan KY, Li ZY, Weitzman AL, Skog LE (1998 onw.) Gesneriaceae. In: Wu ZY, Raven PH (Eds) Flora of China. 18. Scrophulariaceae through Gesneriaceae. Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, 244–401.

Wang YZ, Liang RH, Wang BH, Li JM, Qiu ZJ, Li ZY, Weber A (2010) Origin and phylogenetic relationships of the Old World Gesneriaceae with actinomorphic flowers inferred from ITS and trnL-trnF sequences. Taxon 59(4): 1044–1052. https://doi.org/10.1002/tax.594005

Weber A (2004) Gesneriaceae. In: Kubitzki K, Kadereit J (Eds) The Families and Genera of Vascular plants. Vol. 7. Dicotyledons. Lamiales (except Acanthaceae including Avicenniaceae). Springer, Berlin/Heidelberg, 63–158. https://doi.org/10.1007/978-3-642-18617-2_8

Weber A, Skog LE (2007 onw.) The genera of Gesneriaceae. Basic information with illustrations of selected species. Ed. 2. http://www.genera-gesneriaceae.at

Weber A, Middleton DJ, Forrest A, Kiew R, Lim CL, Rafidah AR, Sontag S, Triboun P, Wei YG, Yao TL, Möller M (2011a) Molecular systematics and remodeling of Chirita and associated genera (Gesneriaceae). Taxon 60(3): 767–790. https://doi.org/10.1002/tax.603012

Weber A, Wei YG, Puglisi C, Wen F, Mayer V, Möller M (2011b) A new definition of the genus Petrocodon (Gesneriaceae). Phytotaxa 23(1): 49–67. https://doi.org/10.11646/phytotaxa.23.1.3

Weir YG, Wen F, Möller M, Monro A, Zhang Q, Gao Q, Mou HF, Zhong SH, Cui C (2010). Gesneriaceae of South China. Guangxi Science and Technology Publishing House, Guilin, 1–777.
Bournea within Oreocharis

Xi YZ (1987) Pollen morphology of Epithandra Burtt (Gesneriaceae). Bulletin of Botanical Research 7(2): 17–26.
Yan ZJ, Li ZY, Wang FH (1997) Pollen morphology of tribe Trichosporeae (Gesneriaceae) in China and its systematic significance. Harvard Papers in Botany 10: 113–120.
Yang X, Pang HB, Liu BL, Qiu ZJ, Gao Q, Wei L, Dong Y, Wang YZ (2012) Evolution of double-positive autoregulatory feedback loops in CYCLOIDEA2 clade genes is associated with the origin of floral zygomorphy. The Plant Cell 24(5): 1834–1847. https://doi.org/10.1105/tpc.112.099457
Yang JB, Li DZ, Li HT (2014) Highly effective sequencing whole chloroplast genomes of angiosperms by nine novel universal primer pairs. Molecular Ecology Resources 14: 1024–1031. https://doi.org/10.1111/1755-0998.12251
Yang L-H, Wen F, Kong H-H, Sun Z-X, Su L-Y, Kang M (2020) Two new combinations in Oreocharis (Gesneriaceae) based on morphological, molecular and cytological evidence. In: Shui Y-M, Chen W-H, Ren M-X, Wen F, Hong X, Qiu Z-J, Wei Y-G, Kang M (Eds) Taxonomy of Gesneriaceae in China and Vietnam. PhytoKeys 157: 43–58. https://doi.org/10.3897/phytokeys.157.32609
Ying TS, Zhang YL, Boufford DE (1993) The Endemic Genera of Seed Plants of China. Science Press, Beijing.
Zhang YM (2018) Systematic taxonomy of Oreocharis sensu lato (Gesneriaceae) and its pollen morphology. M. S. thesis, Kunming Institute of Botany, Chinese Academy of Sciences, China.
Zhou XR, Wang YZ, Smith FJ, Chen RJ (2008) Altered expression patterns of TCP and MYB genes relating to the floral developmental transition from initial zygomorphy to actinomorphy in Bournea (Gesneriaceae). The New Phytologist 178(3): 532–543. https://doi.org/10.1111/j.1469-8137.2008.02384.x

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
Explanation note: The Bayes inference (BI) and Maximum likelihood (ML) tree inferred from 5 cp DNA markers (atpB-rbcL, ndhH-rps15-ycf1, rpl132, trnL-F, trnT-trnL) 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, ※ indicates < 50%.
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.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, rpl132, 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, ※ indicates < 50%.
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.suppl3
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 Oreocharis 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/76c56fd0ff6fca26485
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 atpB-rbcL from 52 samples and 48 species of Oreocharis in Gesneriaceae. https://figshare.com/s/b177b05c6c08d22d9c3
Data 7 DNA sequences of rpl132 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 Oreocharis in Gesneriaceae. https://figshare.com/s/8ed14b5f7de0552107cc
Data 9 DNA sequences of trnC-trnD from 52 samples and 48 species of Oreocharis in Gesneriaceae. https://figshare.com/s/93609c5db11bc52b7d80
Data 10 DNA sequences of trnL-F from 52 samples and 48 species of Oreocharis in Gesneriaceae. https://figshare.com/s/ee47f235d59d00f3e89a
Data 11 DNA sequences of trnT-trnL from 52 samples and 48 species of Oreocharis in Gesneriaceae. https://figshare.com/s/b55363c06cfd1235e435.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odb/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.suppl4