

# *Gaolejeunea*, a new genus from China and new member of subtribe Echinolejeuneinae (Lejeuneaceae, Marchantiophyta)

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**ABSTRACT.** A narrowly endemic species known only from the type locality in Shangsi Co., Guangxi, as well as a peculiar member of the Chinese Lejeuneaceae, *Cheilolejeunea gaoi* has previously been treated as a species of *Cheilolejeunea*. However, the presence of *Allorgella*-type denticulations formed by two adjacent cells on leaf and underleaf margins, small ovate leaf lobules without distinct teeth, ental hyaline papilla, and finely segmented oil bodies all suggest that it belongs somewhere else. Maximum likelihood, maximum parsimony, and Bayesian analyses of a three marker dataset (nrITS, cpDNA *rbcl* and *trnL-F*) derived from 85 accessions of Lejeuneaceae and 15 outgroup species revealed that *C. gaoi* belongs to subtribe Echinolejeuneinae. Based on the results of phylogenetic analyses, combined with morphological data, we suggest here that *C. gaoi* should be established as a new genus, *Gaolejeunea*. We strongly recommend *in situ* or *ex situ* conservation activity for this species based on the IUCN Red List criteria.

**KEYWORDS.** China, conservation, endangered plant, *Haplolejeunea*, Hepaticae, oil bodies.



With more than 1800 species in about 70 genera (Zhu et al. 2017), Lejeuneaceae is the largest family of the liverworts. The phylogeny of Lejeuneaceae has been intensively studied in recent years. Gradstein (2013) proposed a revised classification of this family summarizing evidence from morphology and molecular phylogeny of Lejeuneaceae, recognized 68 genera, of which 49 were assigned to the subfamily Lejeuneoideae. Lejeuneoideae was further classified into three tribes, Brachiolejeuneae, Symbiezidieae and Lejeuneae. Gradstein (2013) accepted eight subtribes of Lejeuneae, but refrained from assigning seven genera to any subtribe due to the lack of molecular phylogenetic data. Since then, phylogenetic studies have introduced several changes into the classification of Gradstein (2013), especially on Lejeuneae. These treatments include the establishment of new subtribes Leiolejeuneinae (Schäfer-Verwimp et al. 2014), Leptolejeuneinae (Heinrichs et

al. 2014c), Pictolejeuneinae (Bechteler et al. 2016b), Pycnolejeuneinae and Xylolejeuneinae (Heinrichs et al. 2014b); the reinstatement of *Allorgella* Tixier (Bechteler et al. 2016a); the broader concept of *Cheilolejeunea* (Spruce) Steph. with *Aureolejeunea* R.M.Schust., *Cyrtolejeunea* A.Evans, *Cystolejeunea* A.Evans, *Evansiolejeunea* Vanden Berghen, *Omphalanthus* Lindenb. et Nees and *Leucolejeunea* A.Evans as its synonyms (Schäfer-Verwimp et al. 2014; Ye et al. 2015); the transfer of *Vitalianthus* R.M.Schust. et Giancotti from Drepanolejeuneinae to Lepidolejeuneinae (Bechteler et al. 2016a), *Otolejeunea* Grolle et Tixier from Cyclolejeuneinae to Lepidolejeuneinae (Heinrichs et al. 2014b) and *Bromeliophila* R.M.Schust. from Lejeuneinae to Cyclolejeuneinae (Heinrichs et al. 2014a). While reconstructing a comprehensive phylogeny of Cheilolejeuneinae (Ye et al. 2015), two accessions of *C. gaoi* were found to be well separated from Cheilolejeuneinae, forming a robust clade with *Anoplolejeunea* (Spruce) Schiffn., a member of subtribe Echinolejeuneinae. Bechteler et al. (2016b) further indicated that *C. gaoi* was

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**Table 1.** Sequences newly generated in the study, with missing sequences indicated by a dash (-).

Taxon	Voucher (all deposited in HSNU)	<i>rbcL</i>	<i>trnL</i> -F	nrITS
<i>Cheilolejeunea gaoi</i> R.L.Zhu, M.L.So et Grolle (I)	China, Ye & Wei 20090716-17	MG847529	-	-
<i>Cheilolejeunea gaoi</i> (II)	China, Ye & Wei 20090717-1	MG847530	-	-
<i>Cheilolejeunea gaoi</i> (III)	China, Zhu, Wei & He 20100822-15	MG847528	MG847537	MG847533
<i>Tuyamaella molischii</i> (Schiffn.) S.Hatt. (I)	China, Wei 20110214-13A	MG847531	MG847538	MG847534
<i>Tuyamaella molischii</i> (II)	China, Wei 20110214-19A	-	-	MG847535
<i>Tuyamaella molischii</i> (III)	China, Wei 20110217-2	MG847532	-	MG847536

resolved in Echinolejeuneinae, with a close relationship to *Haplolejeunea* Grolle. However, an investigation with extended sampling on the systematic position of *C. gaoi* was still needed. The subtribe Echinolejeuneinae represents a rather morphologically heterogeneous assemblage of genera that share bifid underleaves and pycnolejeuneoid gynoeical innovations (Bechteler et al. 2016a). The monospecific genus *Kymatolejeunea* Grolle has also been assigned to this subtribe based on morphological evidence (Gradstein 2013; Grolle 1984; Schuster 2001), which has recently been confirmed with molecular data with the establishment of a new genus *Thiersianthus* R.L.Zhu et L.Shu (subtribe Lepidolejeuneinae) from Borneo (Zhu et al. 2017). Most recently a new genus (*Reinerantha*) from the Western Cordillera of Ecuador has been added to subtribe Cololejeuneinae (Gradstein et al. 2018). Here, we investigate the systematic position of *C. gaoi* using extensive samplings in Lejeuneae, and propose a new genus *Gaolejeunea* based on our analyses.

#### MATERIALS AND METHODS

**Taxon sampling, DNA extraction, PCR amplification, sequencing and alignment.** We included three accessions of *Cheilolejeunea gaoi*, three samples of *Tuyamaella* S.Hatt., as well as 266 sequences downloaded from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) containing two samples of *Kymatolejeunea* in this study to test the systematic position of *C. gaoi*. Total DNA was extracted from fresh collected material or dried herbarium specimens using DNeasy<sup>TM</sup> Plant Mini Kit (QIAGEN) after grind pre-treatment in a multi-sample tissue-lyser (Jinxin technology, China). Two chloroplast regions (*trnL*-F, *rbcL*) and the nuclear ribosomal internal transcribed spacer 1 and 2 with the intervening 5.8S ribosomal subunit (ITS1-5.8S-ITS2, collectively called nrITS) were amplified by

polymerase chain reaction (PCR), employing 35 cycles of 30 sec at 95°C, 45 sec at 50°C and 1 min at 72°C, preceded by an initial 1 min melting step at 95°C and followed by a final extension period of 7 min at 72°C. Amplification and sequencing primers followed those described in Shaw et al. (2003, *trnL*-F), Gradstein et al. (2006, *rbcL*), and Hartmann et al. (2006, nrITS). A total of 70 ITS, 58 *rbcL* and 62 *trnL*-F sequences were newly generated for this study.

Sequences were aligned manually using PhyDE v.1 (Müller et al. 2010). Positions that were ambiguously aligned in the dataset were excluded before any phylogenetic analysis; gaps were coded as missing data. The ingroup consisted of 85 accessions of Lejeuneae according to the analyses of Wilson et al. (2007). Fifteen representatives of Brachiolejeuneae and Ptychanthoideae were chosen as outgroups. Voucher information and Genbank accession numbers are listed in **Table 1** and **Supplementary Table S1**.

**Phylogenetic analysis.** Maximum parsimony (MP) analyses were performed with command files using the parsimony ratchet (Nixon 1999) generated from the program PRAP2 (Müller 2007) applying the default settings, and executed in PAUP\* 4.0b10 (Swofford 2000). Heuristic bootstrap searches under parsimony were performed with 10000 replicates. The three individual data sets were first analyzed separately (results not shown here) to check for possible incongruence. Strict consensus trees from these separate analyses were compared to identify conflicting nodes supported by at least 70% (Mason-Gamer & Kellogg 1996). The three datasets were combined into a single matrix due to no evidence of incongruence. jModelTest v.2.1.6 (Darriba et al. 2012) was used to select the best fit models of evolution for maximum likelihood (ML) analysis and Bayesian inference (BI) analysis. With the Bayesian information criterion (Posada & Buckley

2004), a Transversion Model (TVM) with gamma-distributed rate variation across sites (+G) and a proportion of invariable sites (+I) was chosen for the *rbcL* region; a Transitional Model with unequal base frequencies (TIM1), with gamma-distributed rate variation (+G) and invariable sites (+I) was chosen for the *trnL*-F region; and a General Time Reversible (GTR) model, with gamma-distributed rate variation (+G) and invariable sites (+I) was selected for the ITS1-5.8S-ITS2 region. The ML trees were generated using the program GARLI version 2.01 (Zwickl 2006). Best ML trees were found with partitioned analysis based on five independent searches. Bootstrap support (BS) was estimated based on 1000 bootstrap replicates.

Bayesian analyses were applied to the combined data in MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001). The data were partitioned by regions, employing the GTR+I+G model as suggested by jModelTest for each partition. Four independent Markov chain Monte Carlo (MCMC) simulations runs with four chains were run for 10 million generations, sampled every 1000 generations. The program Tracer v1.5 (Rambaut & Drummond 2009) was used to confirm the burn-in point and examine the log likelihoods. The outputs from the four runs were combined for final inference of posterior probabilities (PP) of both trees and model parameters after discarding the burn-in.

## RESULTS

The aligned matrix contained 3038 nucleotide characters, including 1407 in the ITS, 1189 in the *rbcL* and 442 in the *trnL*-F. Of the total character sites, 1435 were constant, 1311 were parsimony-informative and 292 were variable but parsimony uninformative.

The maximum parsimony (MP) analysis resulted in 47 maximally parsimonious trees of 10095 steps, with a consistency index (CI) of 0.306 and a retention index (RI) of 0.605, RC of 0.185, HI of 0.694. A single most likely tree ( $\ln L = -47821.8570$ ) was found in the maximum likelihood (ML) analysis. BI analyses resulted in highly similar topologies with the most likely tree. **Figure 1** shows the 50% majority-rule consensus tree from the analyses of the concatenated dataset obtained by the BI approach, with support values ( $>70$ ) from the ML and MP analyses plotted onto the Bayesian tree.

The three accessions of *Cheilolejeunea gaoi* are recovered in a robust sister clade of *Haplolejeunea* (MLBS=100, MPBS=100, PP=1.00). The *C. gaoi*/*Haplolejeunea* clade is placed sister to a well-supported clade (MLBS=94, MPBS=86.51, PP=1.00) of *Anoplolejeunea conferta*. The type species of the monospecific genus *Kymatolejeunea*, *K. bartlettii* is found sister to a clade formed by *Echinolejeunea* R.M.Schust. and *Allorgella* Tixier. All the represented species from subtribe Echinolejeuneinae form a highly supported monophyletic clade (MLBS=100, MPBS=100, PP=1.00) (**Fig. 1**).

## DISCUSSION

The present study with 33 genera of Lejeuneaceae, including all the present members of Echinolejeuneinae, is by far the most comprehensive generic sampling of this tribe. The molecular phylogeny reveals *Cheilolejeunea gaoi* as a member of Echinolejeuneinae supported by bootstrap percentages and Bayesian posterior probabilities (**Fig. 1**). The results also support the former treatments (Bechteler et al. 2016a; Gradstein 2013; Zhu et al. 2017) classifying *Allorgella*, *Anoplolejeunea*, *Echinolejeunea*, *Haplolejeunea* and *Kymatolejeunea* in Echinolejeuneinae.

*Cheilolejeunea gaoi* was originally placed within *Cheilolejeunea* (Zhu et al. 2000). However, researchers were puzzled by its (1) delicate plants, (2) marginal leaf denticulations of *Allorgella*-type, (3) small, ovate leaf lobules without distinct teeth, (4) ental hyaline papilla, (5) finely segmented oil bodies, and (6) absence of gynoecial innovations (Zhu et al. 2000; Zhu & So 2003), which cannot be easily fitted into any existing Chinese Lejeuneaceae genus. Furthermore, a recent revised phylogeny and classification of subtribe Cheilolejeuneinae already indicated that *C. gaoi* is not a member of *Cheilolejeunea* (Bechteler et al. 2016b; Ye et al. 2015; Zhu et al. 2017), but much closer to *Anoplolejeunea* (subtribe Echinolejeuneinae).

Morphologically, *Cheilolejeunea gaoi* has an affinity with members of *Pictolejeunea* Grolle by the lack of gynoecial innovation; thin-walled leaf cells without distinct trigones, reduced first tooth and indistinct second tooth of leaf apex (Zhu et al. 2000). Yet *Pictolejeunea* is distinguishable by having numerous deeply pigmented ocelli in leaves, underleaves, bracts and perianths (Grolle & Reiner-Drehwald 2005). The latest phylogenetic analyses with 30 genera of Lejeuneaceae favored *Pictolejeunea* as

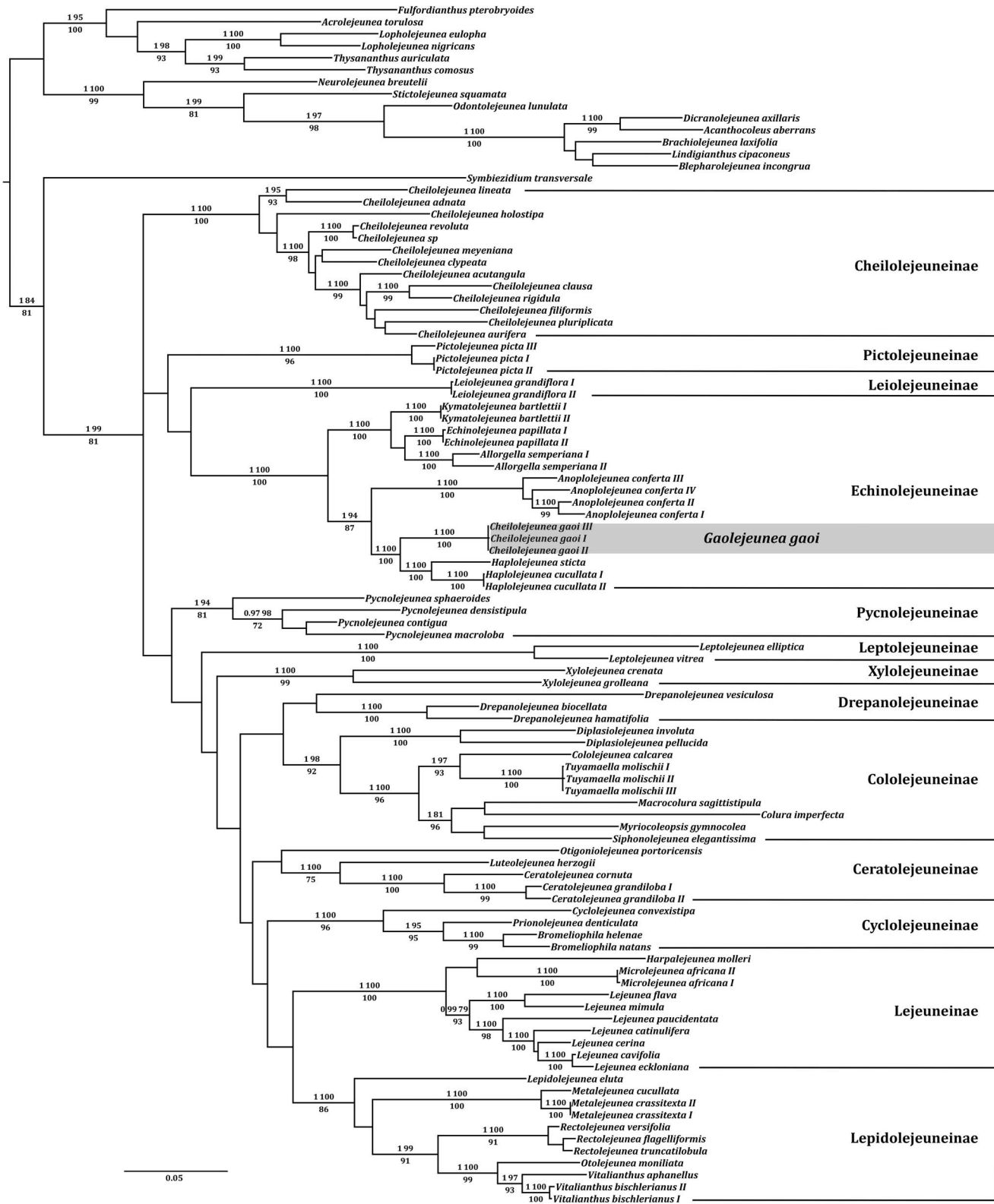


Figure 1. A 50% majority-rule phylogram derived from Bayesian inference of the combined nrITS, *rbcL* and *trnL-F*. Support values with Bayesian posterior probabilities (PP) above 0.9, maximum likelihood bootstrap support values (MLBS) above 70, and maximum parsimony bootstrap support values (MPBS) above 70 simultaneously are indicated on the branches as follows, PP left above the branch, MLBS right above the branch, and MPBS below the branch.

a separated subtribe Pictolejeuneinae (Bechteler et al. 2016b), with no close affinity with *C. gaoi*, which is supported in this study.

The marginal leaf denticulations of *Allorgella*-type in *C. gaoi* also occur in *Allorgella*, *Haplolejeunea*, *Kymatolejeunea*, *Tuyamaella*, and some species of *Cololejeunea* (Zhu et al. 2000; Zhu & So 2001). The latter two genera resulted in a well-supported clade (PP=1, MLBS=97, MPBS=93) in our analyses. Together with *Colura*, *Diplasiolejeunea*, *Macrocolura*, *Myriocoleopsis* and *Siphonolejeunea*, they formed a strongly supported Cololejeuneinae, far from *C. gaoi* (Fig. 1).

In our analyses *Cheilolejeunea gaoi* was resolved in the subtribe Echinolejeuneinae clade. The subtribe Echinolejeuneinae as circumscribed by Gradstein (2013) is a small subtribe characterized by the ball-shaped lobule with strongly involute free margin and hyaline papilla distal to lobule apex. It was originally established with three monospecific genera, *Anoplolejeunea*, *Echinolejeunea* and *Kymatolejeunea*. Phylogenetic work (Bechteler et al. 2016a) later reinstated the genus *Allorgella*, and expanded Echinolejeuneinae with two more genera, *Allorgella* and *Haplolejeunea*. Our results reveal a close relationship between *C. gaoi* and *Haplolejeunea*. *Haplolejeunea* is a small Afro-American genus with only two species (Söderström et al. 2016) and a possible new species (*H. sp.*) from French Guiana (Gradstein & Ilkiu-Borges 2009), while *C. gaoi* is endemic to China. Morphologically, *Haplolejeunea* and *C. gaoi* share several characters including the minute size of plants, small leaf cells without intermediate thickenings, usually swollen leaf lobules without a distinct tooth at apex, ental lobular hyaline papilla, small, distant and bilobed underleaves, smooth keels of perianths, and lack of vegetative reproductive organs. *Haplolejeunea*, however, clearly differs from *C. gaoi* in the glistening green plants, presence of scattered ocelli in leaf lobes and underleaves, pycnolejeuneoid gynoecial innovations, male bracteoles present only at the base of androecium, and fewer oil bodies (only 1–3) per leaf cell). *Haplolejeunea* is readily separated from other members of Echinolejeuneinae by the presence of scattered ocelli that appear not only in leaf lobes, but also in the underleaves, bracts, and perianths. The occurrence of ocelli is common in Lejeuneaceae and Frullaniaceae (Gradstein et al. 2003). The existences and patterns of ocelli often serve as a useful character in species and

generic delimitations. The separation of *Lejeunea*, *Harpalejeunea* and *Microlejeunea* (Czumay et al. 2013; Dong et al. 2013; Heinrichs et al. 2013) was supported by the presence of ocelli in the latter two genera. Based on the presence of ocelli and pycnolejeuneoid innovations, *Lejeunea huctumalensis* was transferred to *Physantholejeunea* R.M.Schust., a younger name of *Otigoniolejeunea* (Spruce) Schiffn. (Wei et al. 2014). Most recently, *Thiersianthus*, a new genus endemic to Borneo, has been separated from the pantropical *Metalejeunea* Grolle mainly by the presence of scattered ocelli in leaf lobes (Zhu et al. 2017). Therefore, morphological data clearly indicate that *C. gaoi* does not fit in any of the five known genera of Echinolejeuneinae. *Cheilolejeunea gaoi* merits the recognition as a new genus, proposed in this paper as *Gaolejeunea*. *Gaolejeunea* is characterized and recognized by the delicate, green to yellowish green plants, remote bilobed underleaves, small leaf cells without intermediate thickenings, strongly inflated, usually ball-shaped leaf lobules with involute free margins, ental lobular hyaline papilla, marginal leaf denticulations of *Allorgella*-type, lack of ocelli in the plant, smooth keels of perianths, truncate apex of the leaf lobule without any distinct teeth, usually 3–5 oil bodies per leaf cell (Fig. 2), male bracteoles present throughout the androecium, and lack of vegetative reproductive organs.

## TAXONOMY

***Gaolejeunea*** R.L.Zhu et W.Ye, gen. nov.

TYPE: *Gaolejeunea gaoi* (R.L.Zhu, M.L.So et Grolle)

R.L.Zhu et W.Ye ( $\equiv$  *Cheilolejeunea gaoi* R.L.Zhu, M.L.So et Grolle, The Bryologist 103: 499. 2000).

*Autoicous*. Plant pale green to light yellowish green.

*Leaf insertion short*. Leaf surface smooth, marginal denticulations of *Allorgella*-type, which are formed by the projecting corners of two adjacent cells, instead of one single cell. Leaf cells without or with very small trigones. Oil bodies small, finely segmented, (2–)3–5 per cell. Ocelli absent. Lobules small, ovate, with strongly incurved free margin; first lobule tooth reduced; second tooth indistinct; hyaline papilla ental, occasionally hanging distally at the apex of the lobule. Underleaves bifid, underleaf lobes slightly diverging. Gynoecial innovations absent. Perianth inflated, with 4–5 keels.

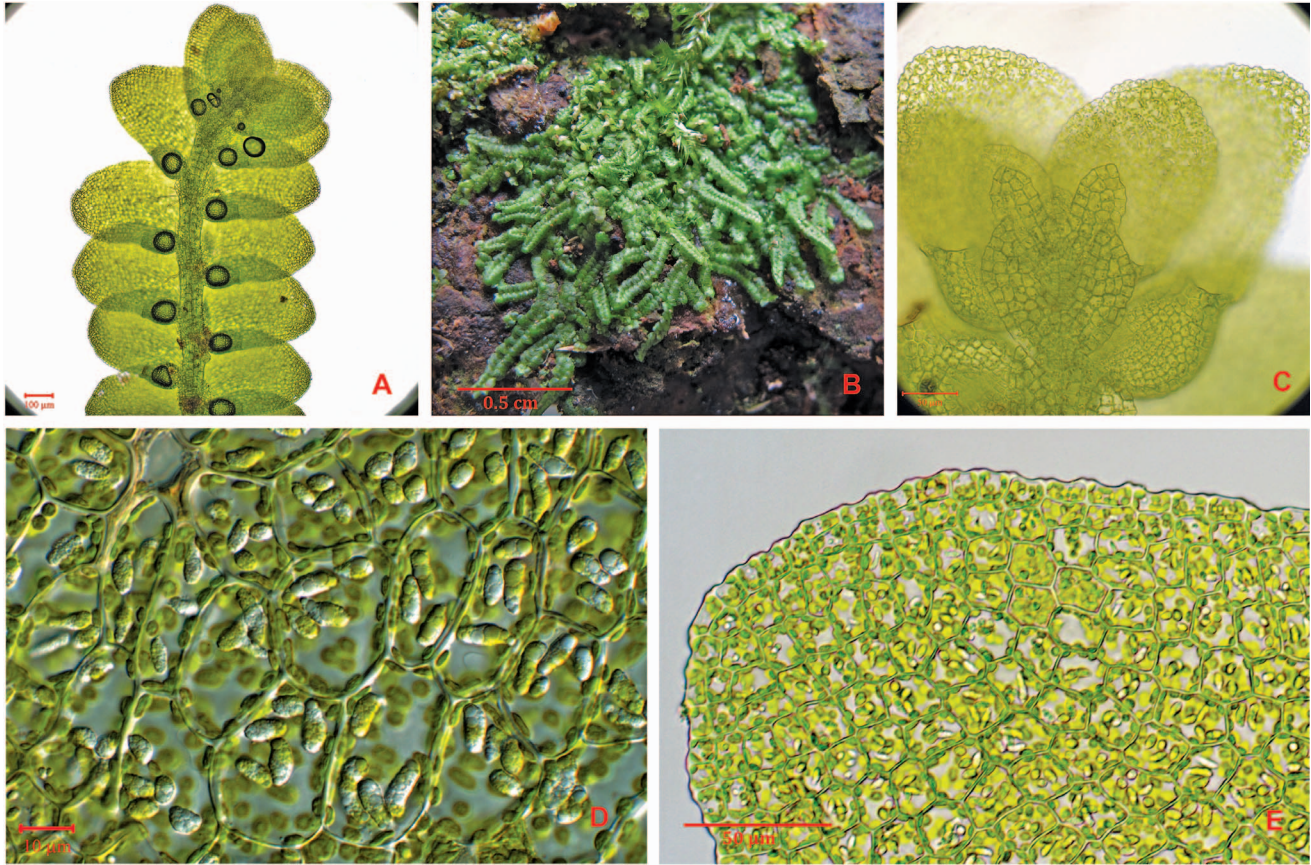


Figure 2. *Gaolejeunea gaoi*. A. Plant (ventral view). B. Habitat. C. Tip of a branch showing the shape of the underleaves. D. Oil bodies in leaf cells. E. Marginal leaf denticulations of *Allorgella*-type. C from Ye & Wei 20090715-45A (HSNU), the others from Zhu et al. 20100822-15 (HSNU).

*Androecia* terminal or proliferating vegetatively from apex, with bracteoles present throughout.

**Etymology.** Named for the late Professor Chien Gao, Institute of Applied Ecology, Chinese Academy of Sciences. His work has contributed greatly to our knowledge of Chinese bryology. Prof. Gao also collected the type specimen of *Cheilolejeunea gaoi* in 1974.

***Gaolejeunea gaoi*** (R.L.Zhu, M.L.So et Grolle) R.L.Zhu et W.Ye, comb. nov. **Fig. 2**  
 ≡ *Cheilolejeunea gaoi* R.L.Zhu, M.L.So et Grolle, The Bryologist 103: 499. 2000. Type: CHINA. GUANGXI: Shangsi Co., Shiwandashan National Forest Park, Hongqilinchang, ca. 21°44'N, 108°16'E, 25 Sept. 1974. *Chien Gao 1815* (holotype: HSNU!; isotypes: IFP!, JE!).

**Description and illustrations.** See Zhu et al. (2000 as *Cheilolejeunea gaoi*).

**Selected specimens examined.** CHINA. GUANGXI: Shangsi Co., Shiwandashan National Forest Park, 21°53.630'N, 107°54.628'E, ca. 418 m, Ye & Wei 20090716-17 (HSNU), 20090717-1 (HSNU); Ibid., 21°53.511'N, 107°54.568'N, 406 m, Zhu et al. 20100822-15 (HSNU); Huangpaoshan, along the rivulet of a dam, 21°57'18.11"N, 108°02'26.05"E, 285 m, Zhu et al. 20140414-29 (HSNU), 20140414-28B (HSNU).

**Habitats.** On tree trunks, sometimes on wet rocks, tree roots and tree roots in moist lowland forests at altitudes of 193–691 m. For detailed habitats, see **Supplementary Table S2**.

With this addition Echinolejeuneinae contains six genera that can be separated in the following key.

**KEY TO GENERA OF ECHINOLEJEUNEINAE**

1. Scattered ocelli present in leaf lobes and underleaves..... *Haplolejeunea*
1. Scattered ocelli absent in leaf lobes and underleaves ..... 2
2. Apex of underleaves undivided..... *Anoplolejeunea*
2. Apex of underleaves bifid..... 3

3. Leaf lobe apex obtuse; perianths obcordate, with 2 broad lateral keels expanded above into distinct auricles..... *Allorgella*
3. Leaf lobe apex rounded; perianth not obcordate, with 4–5 keels ..... 4
4. Leaf lobule apex truncate; hyaline papilla ental, occasionally hanging distally at the apex of the lobule; innovations absent; endemic to China ..... *Gaolejeunea*
4. Leaf lobule apex with a small notch; hyaline papilla inserted in the notch; innovations pycnolejeuneoid; endemic to New Zealand ... 5
5. Leaf surface spinose-toothed with sharply pointed papilla; leaf margin somewhat uniformly convex, without strong curving..... *Echinolejeunea*
5. Leaf surface smooth, without cellular spines; leaf margin wavily curled ..... *Kymatolejeunea*

### CONSERVATION BIOLOGY

As a remarkable member of Lejeuneaceae, *Gaolejeunea gaoi* is surprisingly narrowly distributed in Shangsi Co., Guangxi, China. This usually epiphytic species is restricted to heavily and critically threatened lowland forests. The first national red list of Chinese endangered bryophytes classified this species as critically endangered (CR) based on its small population and habitat, which has been threatened by human activities (Cao et al. 2006; Zhu & So 2003). Its status as CR is reconfirmed (criteria A3c+3d, B1ab(iii), IUCN Red List Categories and Criteria Version 3.1, Second edition, 2012) in the up to date threatened species list of China's higher plants (Qin et al. 2017). During recent years, intensive and thorough surveys have been conducted in Guangxi and adjoining regions, as well as in the Indochinese Peninsula (Shu et al. 2017). Among these collections, 18 specimens are identified as *G. gaoi*, including the type. Specimens collected and examined are listed in **Supplementary Table S2**. Despite these efforts, there are still no records of *G. gaoi* outside the range of its type locality, Shangsi Co. Results show that the occurrence of *G. gaoi* is restricted to two locations, one inside the Shiwandashan National Forest, the other at a nearby resort called Huangpaoshan, both deeply affected by human activities. According to our data, the extent of occurrence of *G. gaoi* is less than 100 km<sup>2</sup>, severely fragmented, and the extent and quality of habitat is continuing to decline, which meets the IUCN red list criteria B1ab(iii). The primary threat to *G. gaoi* has been habitat loss in the past owing to deforestation, and the few remaining subpopulations continue to be exposed to this threat. The second area outside Shiwadashan National Forest where this species was recently found is at a local resort, along the riverside of a rivulet of a dam, with no protected status known that would prevent further loss of habitat in that area.

Therefore we strongly recommend *in situ* or *ex situ* conservation activity for this species.

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### LITERATURE CITED

- Bechteler, J., G. E. Lee, A. Schäfer-Verwimp, T. Pócs, D. F. Peralta, M. A. Renner, H. Schneider & J. Heinrichs. 2016a [2015]. Towards a monophyletic classification of Lejeuneaceae IV: reinstatement of *Allorgella*, transfer of *Microlejeunea aphanella* to *Vitalianthus* and refinements of the subtribal classification. *Plant Systematics and Evolution* 302: 187–201.
- Bechteler, J., G. E. Lee, A. Schäfer-Verwimp, M. A. M. Renner, D. F. Peralta & J. Heinrichs. 2016b. Towards a monophyletic classification of Lejeuneaceae V: The systematic position of *Pictolejeunea*. *Phytotaxa* 280: 259–270.
- Cao, T., R.-L. Zhu, B. C. Tan, S. L. Guo, P. C. Wu & X. J. Li. 2006. A report of the first national red list of Chinese endangered bryophytes. *Journal of the Hattori Botanical Laboratory* 99: 275–295.
- Czumaj, A., S. Dong, A. Scheben, A. Schäfer-Verwimp, K. Feldberg & J. Heinrichs. 2013. Transfer of *Lejeunea huctumalcensis* to *Physantholejeunea* (Lejeuneaceae, Porellales). *Australian Systematic Botany* 26: 386–392.
- Darriba, D., G. L. Taboada, R. Doallo & D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature methods* 9: 772–772.
- Dong, S., A. Schäfer-Verwimp, T. Pócs, K. Feldberg, A. Czumaj, A. R. Schmidt, H. Schneider & J. Heinrichs. 2013. Size doesn't matter – recircumscription of *Microlejeunea* (Lejeuneaceae, Porellales) based on molecular and morphological evidence. *Phytotaxa* 85: 41–55.
- Gradstein, S. R. 2013. A classification of Lejeuneaceae (Marchantiophyta) based on molecular and morphological evidence. *Phytotaxa* 100: 6–20.
- Gradstein, S. R. & A. L. Ilkiu-Borges. 2009. Guide to the plants of Central French Guiana. Part 4. Liverworts and hornworts. *Memoirs of the New York Botanical Garden* 76: 1–140.
- Gradstein, S. R., R. Wilson, A. L. Ilkiu-Borges & J. Heinrichs. 2006. Phylogenetic relationships and neotenic evolution of *Metzgeriopsis* (Lejeuneaceae) based on chloroplast DNA sequences and morphology. *Botanical Journal of the Linnean Society* 151: 293–308.
- Gradstein, S. R., M. E. Reiner-Drehwald & H. Schneider. 2003. A phylogenetic analysis of the genera of Lejeuneaceae (Hepaticae). *Botanical Journal of the Linnean Society* 143: 391–410.
- Gradstein, S. R., R.-L. Zhu, L. Shu & A. J. Pérez. 2018. *Reinerantha foliicola*, a new genus and species of Lejeuneaceae subtribe Cololejeuneinae (Marchantiophyta) from Ecuador. *Journal of Systematics and Evolution* 56: 67–75.
- Grolle, R. 1984. *Kymatolejeunea* Grolle - eine neue Gattung der Lejeuneoideae aus Neuseeland. *Wissenschaftliche Zeitschrift der*

- Friedrich-Schiller-Universität Jena, Mathematisch-naturwissenschaftliche Reihe 32: 1005–1012.
- Grolle, R. & M. E. Reiner-Drehwald. 2005. *Pictolejeunea levis*, a new species of Lejeuneaceae from Cuba. *Journal of Bryology* 27: 281–283.
- Hartmann, F. A., R. Wilson, S. R. Gradstein, H. Schneider & J. Heinrichs. 2006. Testing hypotheses on species delimitations and disjunctions in the liverwort *Bryopteris* (Jungermanniopsida: Lejeuneaceae). *International Journal of Plant Sciences* 167: 1205–1214.
- Heinrichs, J., A. Czumaj, S. Dong, A. Scheben, A. Schäfer-Verwimp, D. F. Peralta, K. Feldberg, A. R. Schmidt & H. Schneider. 2014a. The Bromeliaceae tank dweller *Bromeliophila* (Lejeuneaceae, Porellales) is a member of the *Cyclolejeunea-Prionolejeunea* clade. *Plant Systematics and Evolution* 300: 63–73.
- Heinrichs, J., S. Dong, A. Schäfer-Verwimp, D. F. Peralta, K. Feldberg, A. R. Schmidt, H. Schneider. 2014b. Towards a monophyletic classification of Lejeuneaceae II: subtribes *Pycnolejeuneinae* and *Xylolejeuneinae* subtr. nov., transfer of *Otolejeunea* to *Lepidolejeuneinae*, and generic refinements. *Phytotaxa* 163: 61–76.
- Heinrichs, J., A. Schäfer-Verwimp, A. Czumaj, S. Dong, A. Scheben, K. Feldberg & H. Schneider. 2014c. Towards a monophyletic classification of Lejeuneaceae I: subtribe *Leptolejeuneinae* subtr. nov. *Phytotaxa* 156: 165–174.
- Heinrichs, J., S. Dong, A. Schäfer-Verwimp, T. Pócs, K. Feldberg, A. Czumaj, A. R. Schmidt, J. Reitner, M. A. M. Renner, J. Hentschel, M. Stech & H. Schneider. 2013. Molecular phylogeny of the leafy liverwort *Lejeunea* (Porellales): evidence for a Neotropical origin, uneven distribution of sexual systems and insufficient taxonomy. *PLoS ONE* 8: e82547.
- Huelsenbeck, J. P. & F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.
- IUCN. 2012. IUCN Red list Categories and Criteria: Version 3.1. Second edition. IUCN Species Survival Commission, Gland and Cambridge.
- Mason-Gamer, R. J. & E. A. Kellogg. 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Systematic Biology* 45: 524–545.
- Müller, K., D. Quandt, J. Müller & C. Neinhuis. 2010. PhyDE® 0.997: Phylogenetic Data Editor. <http://www.phyde.de>.
- Müller, K. 2007. PRAP2 — Parsimony and likelihood ratchet analyses with PAUP, v.0.9. <http://systevol.nees.uni-bonn.de/software/PRAP2>.
- Nixon, K. C. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15: 407–414.
- Posada, D. & T. R. Buckley. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* 53: 793–808.
- Qin, H. N., Y. Yang, S. Y. Dong, Q. He, Y. Jia, L. N. Zhao, S. X. Yu, H. Y. Liu, B. Liu, Y. H. Yan & J. Y. Xiang. 2017. Threatened Species List of China's Higher Plants. *Biodiversity Science* 25: 696–744.
- Rambaut, A. & A. J. Drummond. 2009. Tracer v1.5. <http://beast.bio.ed.ac.uk/Tracer>.
- Schäfer-Verwimp, A., K. Feldberg, S. Dong, H. Van Melick, D. F. Peralta, A. R. Schmidt, H. Schneider & J. Heinrichs. 2014. Towards a monophyletic classification of Lejeuneaceae III: the systematic position of *Leiolejeunea*. *Phytotaxa* 170: 187–193.
- Schuster, R. M. 2001. Studies on Lejeuneaceae, IV. On the circumscription and subdivision of the subfamily Lejeuneoideae. *Journal of the Hattori Botanical Laboratory* 91: 137–172.
- Shaw, A. J., C. J. Cox & S. Boles. 2003. Polarity of peatmoss (*Sphagnum*) evolution: who says bryophytes have no roots? *American Journal of Botany* 90: 1777–1787.
- Shu, L., Y.-L. Xiang, X.-F. Cheng, Y.-M. Wei, J. Wang, L.-N. Zhang, W. Li, X.-B. Yin, W.-P. Zhang, C.-X. Zhao, T. Peng, T. V. Do, T. N. Lu & R.-L. Zhu. 2017. New liverwort and hornwort records for Vietnam. *Cryptogamie Bryologie* 38: 411–445.
- Söderström, L., A. Hagborg, M. von Konrat (eds.), S. Bartholomew-Began, D. Bell, L. Briscoe, E. Brown, D. C. Cargill, E. D. Cooper, D. P. Costa, B. J. Crandall-Stotler, G. Dauphin, J. J. Engel, K. Feldberg, D. Glenny, S. R. Gradstein, X. He, A. L. Ilkiu-Borges, J. Heinrichs, J. Hentschel, T. Katagiri, N. A. Konstantinova, J. Larrain, D. G. Long, M. Nebel, T. Pócs, F. Puche, E. Reiner-Drehwald, M. A. M. Renner, A. Sass-Gyarmati, A. Schäfer-Verwimp, J. G. Segarra Moragues, R. E. Stotler, P. Sukkharak, B. M. Thiers, J. Uribe, J. Váña, J. C. Villarreal, M. Wigginton, L. Zhang & R.-L. Zhu. 2016. World checklist of hornworts and liverworts. *PhytoKeys* 59: 1–828.
- Swofford, D. L. 2000. PAUP\*, phylogenetic analyses using parsimony (\* and other methods), version 4.01b10. Sinauer Associates, Sunderland, Massachusetts.
- Wei Y.-M., R.-L. Zhu & S. R. Gradstein. 2014. Notes on Early Land Plants Today. 49. On *Lejeunea huctumalcensis* and the resurrection of *Otigoniolejeunea*, an older name for *Physantholejeunea* (Marchantiophyta, Lejeuneaceae). *Phytotaxa* 162: 236–238.
- Wilson, R., S. R. Gradstein, H. Schneider & J. Heinrichs. 2007. Unravelling the phylogeny of Lejeuneaceae (Jungermanniopsida): evidence for four main lineages. *Molecular Phylogenetics and Evolution* 43: 270–282.
- Ye, W., S. R. Gradstein, A. J. Shaw, B. Shaw, B. C. Ho, A. Schäfer-Verwimp, T. Pócs, J. Heinrichs & R.-L. Zhu. 2015. Phylogeny and classification of Lejeuneaceae subtribe Cheilolejeuneinae (Marchantiophyta) based on nuclear and plastid molecular markers. *Cryptogamie Bryologie* 36: 313–333.
- Zhu, R.-L. & M. L. So. 2001. Epiphyllous liverworts of China. *Nova Hedwigia Beiheft* 121: 1–418.
- Zhu, R.-L. & M. L. So. 2003. Liverworts and hornworts of Shansi County of Guangxi (Kwangsi), with an updated checklist of the hepatic flora of Guangxi Province of China. *Cryptogamie Bryologie*, 24: 319–334.
- Zhu, R.-L., M. L. So & R. Grolle. 2000. *Cheilolejeunea gaoi* (Hepaticae, Lejeuneaceae), a new species from Guangxi, China. *The Bryologist* 103: 499–502.
- Zhu, R.-L., L. Shu, A. M. Mustapeng & M. Suleiman. 2017. *Thiersianthus* (Marchantiophyta: Lejeuneaceae), a new genus from lowland rainforests in Borneo. *The Bryologist* 120: 511–520.
- Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. GARLI version 2.0 available online at <https://code.google.com/p/garli/>.

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### Supplementary documents online:

**Supplementary Table S1.** Voucher information with GenBank accession numbers. Missing sequences are indicated by a dash (–).

**Supplementary Table S2.** List of specimens (all deposited in HSNU) collected and examined since the discovery of *Gaolejeunea gaoi*.