



## Note

## A northernmost distribution record of *Cyathodium* (Cyathodiaceae), with validation of subg. *Metacyathodium*

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**ABSTRACT:** Based on our morphological review and phylogenetic analysis, *Cyathodium tuberosum* was recorded from Beijing City, representing the northernmost distribution record of the genus worldwide. Given the morphological differences in the genus *Cyathodium* and the phylogenetic results generated in this study, subg. *Metacyathodium* S.C.Srivast. & R.Dixit ex T.X.Zheng was formally validated.

**KEY WORDS:** Cyathodiaceae, *Cyathodium*, *Cyathodium foetidissimum*, *Cyathodium tuberosum*, distribution.

### INTRODUCTION

*Cyathodium* Kunze is a pantropical genus of complex thalloid liverworts, primarily distributed across the Indian subcontinent (Allen, 2001), comprising 13 accepted species worldwide (including one seriously doubt taxon; Bischler, 1998; Söderström *et al.*, 2016). Owing to its unique morphological characters (*e.g.*, translucent and delicate plants, thin and delicate thalli containing one dorsal and one ventral layer of cells separated by filament-absent air chambers, highly reduced ventral scales, and sessile or stalked antheridiophores), several attempts have been made to discuss its taxonomic placement (*e.g.*, Kashyap, 1929; Evans, 1939; Müller, 1940; Grolle, 1972). Currently, *Cyathodium* is classified under its own family, Cyathodiaceae (Crandall-Stotler and Stotler 2000), which is recognized as the only genus exhibiting RNA editing (*Cyathodium cavernarum* Kunze ex Lehm.; Shen *et al.*, 2024a) and few taxa showing rapid molecular evolution (Villarreal *et al.*, 2016; Zheng *et al.*, 2024) within Marchantiopsida.

In China, the specific treatment of *Cyathodium* warrants a brief discussion. Piippo (1990) provided a catalogue of Chinese Hepaticae and Anthocerotae and included three species of the genus based on previous literature. Srivastava and Dixit (1996) revised *Cyathodium* globally but did not appear to mention its occurrence in China. In 2006, Wu and Gao specifically reviewed the Chinese *Cyathodium* and recognized five species, namely *C. aureonitens* (Griff.) Mitt., *C. cavernarum* Kunze ex Lehm., *C. smaragdinum* Schiffn., *C. tuberculatum* Udar & D.K.Singh, and *C. tuberosum* Kashyap, which is widely accepted in subsequent studies (Gao and Wu, 2010; Jia and He, 2013; Wang *et al.*, 2018). According to Wu and Gao (2006), *C. tuberosum* is solely

found in Yunnan.

Through our long-term bryological investigation in Beijing City, north China, we found some samples that showed high resemblance to *Cyathodium* (Fig. 1). Consequently, morphological examination and molecular phylogenetic analysis were carried out on these specimens. Based on the findings of the present study and other molecular research involving *Cyathodium* taxa, we further discussed the taxonomy of the genus from an integrative perspective and formally validated its subgeneric classification.

### MATERIALS AND METHODS

#### Morphological examination

Two specimens of *Cyathodium tuberosum* collected from Longmenjian Scenic Area, Mentougou District, Beijing City in Sep. 2022, were examined. Voucher specimens are deposited in PE. Morphological examination was performed using a dissecting microscope (Nikon SMZ745T) and an optical microscope (Olympus BX43). Photos in the field were taken by a digital camera Sony ILCE-7RM4A.

#### Phylogenetic analysis

Three loci from the chloroplast genome were selected: *psbA*, *rbcL* and *rps4*. The protocol for DNA extraction, PCR amplification, and DNA sequencing followed Inoue and Aung (2023). The *psbA* gene was amplified using the primers: trnK2F and trnHR (Forrest and Crandall-Stotler, 2004), and cycle sequencing was performed using combinations of four primers: trnK2F, psbA501F, psbA576R and trnHR (Forrest and Crandall-Stotler, 2004). The *rbcL* gene was amplified using the primers: rbcL-53h and trnR24R (Inoue and Tsubota, 2014; Tsubota *et al.*,

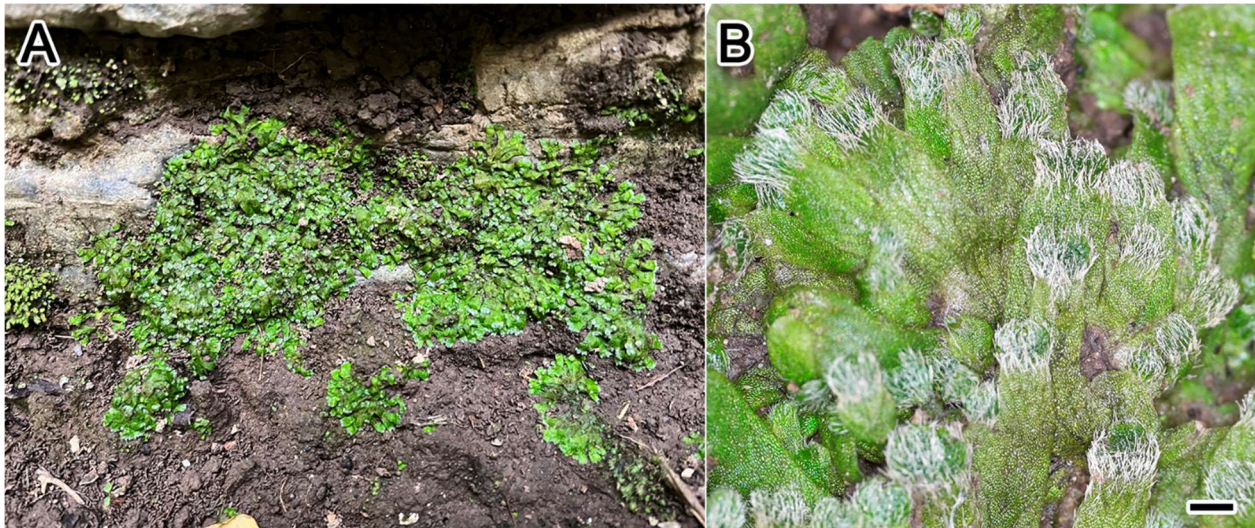


Fig. 1. *Cyathodium tuberosum* Kashyap in Beijing City. A. Population (*L. Luo* 772). B. Close view of thalli (*L. Luo* 769). Scale bar: 1 mm.

1999), and cycle sequencing was performed using combination of primers: *rbcL7*, *rbcL920Fmas*, *rbcL650Rmas* and *rbcL1301RL* (Tsubota *et al.*, 1999; Masuzaki *et al.*, 2010; Inoue *et al.*, 2011) The *rps4* gene was amplified using the primers: *trnT36R* and *trnS* (Inoue *et al.*, 2012; Souza-Chies *et al.*, 1997), and cycle sequencing was performed using combinations of two primers: *rps4\_19Fi* and *rps4\_578Ri* (Inoue and Tsubota, 2014). Sequences newly obtained in the present study have been submitted to the DNA Data Bank of Japan (DDBJ), a member of International Nucleotide Sequence Database Collaboration (INSDC).

Based on the BLAST research and the results of Villarreal *et al.* (2016), we selected 12 OTUs of *Cyathodium* as ingroup, and two OTUs of Corsiniaceae as outgroups (Table S1). The sequences of the three loci were separately aligned using MAFFT v.7.511 (Kato and Standley, 2013) with some manual adjustment on the sequence editor MEGA v.7.0.26 (Kumar *et al.*, 2016). Gaps were treated as missing data. The concatenated matrix of the two loci was used for maximum likelihood tree inference using IQ-TREE v.2.3.6 (Minh *et al.*, 2020). The optimal nucleotide substitution model and partitioning scheme were selected based on the Bayesian information criterion (BIC) using ModelFinder (Kalyaanamoorthy *et al.*, 2017) implemented in IQ-TREE (Table S2). Branch support was estimated using the value of the SH-like approximate likelihood ratio test (Guindon *et al.*, 2010), ultrafast bootstrapping (Hoang *et al.*, 2018), and fast local bootstrap probability (Adachi and Hasegawa, 1996), with 10,000 replicates, respectively.

## RESULTS

### Phylogenetic analysis

The data matrix had a total length of 3,123 bp, of

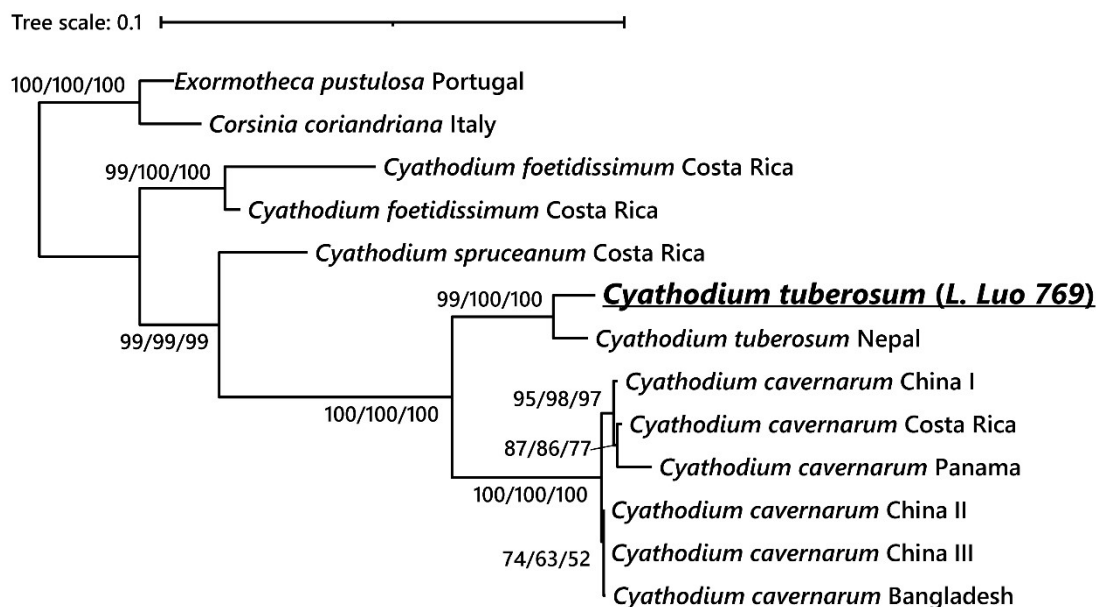
which 540 sites (17%) were variable, and 376 sites (69% of the variable sites) were parsimony informative. The sample collected from Beijing City (*L. Luo* 769) was resolved in the *Cyathodium tuberosum* clade with high supporting values of 99/100/100 (SH-aLRT / LBP / UFBoot in %, Fig. 2).

## DISCUSSION

In this study, we reported *Cyathodium tuberosum* from Beijing City, representing the northernmost distribution of the genus and the second record of the species in China (Srivastava and Dixit, 1996; Wu and Gao, 2006). A comparison of its morphological features with the Yunnan population have been omitted in the present study because the specimens from Yunnan were not available in the present study. However, it is worth noting that the habitat of *C. tuberosum* in Beijing is different from that in Yunnan. *Cyathodium tuberosum* in Beijing grows on semi-open and sand soil slope closely next to tourist trails, while it in Yunnan was only found on soil in the cave (Gao and Wu, 2010).

In this section, we primarily discuss the taxonomy of the genus *Cyathodium* from an integrative perspective. Since the Cyathodiaceae contains only one genus, we chose to use *Cyathodium* in the following text.

Currently, molecular-based inferences regarding the taxonomic placement of *Cyathodium* within Marchantiales remain controversial. Boisselier-Dubayle *et al.* (2002) estimated the phylogeny of Marchantiidae using morphological and nuclear rDNA approaches and found that the topology derived from molecular access resembled that from a coherent dataset, in which *Cyathodium* was sister to *Corsinia-Exormothea* (including *Stephensiella* Kashyap), and this combined group, along with Ricciaceae (including Oxymitracae), formed a monophyletic clade. These results were roughly



**Fig. 2.** Maximum likelihood tree based on concatenated *psbA*, *rbcL* and *rps4* sequences, depicted by IQ-TREE. Supporting values more than 50% are shown on each branch (SH-aLRT / LBP / UFBoot in %).

consistent with those of Forrest *et al.* (2006) and Flores *et al.* (2020). Villarreal *et al.* (2016) presented a complete generic-level phylogeny of complex thalloid liverworts based on extended molecular sequences and resolved a similar relationship between *Cyathodium* and *Corsinia-Exormothecha*, but placing them as sisters to Monosoleniaceae. Xiang *et al.* (2022) inferred that *Corsinia-Cyathodium-Exormothecha* was sister to Aitshiconiellaceae-Cleveaceae using chloroplast genome data, which was supported by Shen *et al.* (2024b). Bechteler *et al.* (2023) presented a bryophyte time-tree based on 228 nuclear genes, considering *Cyathodium* the second branching node within Marchantiales, following the Marchantiaceae. Li *et al.* (2024) employed plastid data to establish the phylogeny and inferred that *Cyathodium* was sister to Wiesnerellaceae. Zheng *et al.* (2024) analyzed 22 liverwort taxa using 88 protein-coding sequences from chloroplast genomes and resolved *Cyathodium* forming a monophyletic clade with *Monosolenium tenerum-Wiesnerella denudata*. However, as Aitshiconiellaceae, Cleveaceae, and Corsiniaceae were not included in Li *et al.* (2024) and Zheng *et al.* (2024), it cannot be asserted that their findings differ from those of the aforementioned studies. In summary, the sister relationship between *Cyathodium* and Corsiniaceae is generally accepted. However, the connections between this combined group and other taxa within Marchantiales requires further investigation.

Additionally, the infrageneric classification of *Cyathodium* has not yet been determined. Srivastava and Dixit (1996) initially divided this genus into two subgenera: subg. *Cyathodium* and subg. *Metacyathodium* S.C.Srivast. & R.Dixit, based on the mid rib and storage

zone of the thalli. The former (subg. *Cyathodium*) was originally marked as “Subgen. Nov.” but is, in fact, no more than an autonym created from the genus (Art. 22.1; Turland *et al.*, 2018). The latter subgenus, supposedly comprising *C. denticulatum* Udar & S.C.Srivast, *C. foetidissimum* Schiffn., and *C. steerei* Hässel, was erected without a designated type, and should thus be considered nomenclaturally invalid (Art. 30.1; Turland *et al.*, 2018), which has not been formally validated to date (Söderström *et al.*, 2016). According to the present analysis and existing molecular studies involving several *Cyathodium* taxa (Allen and Korpelainen, 2006; Villarreal *et al.*, 2016; Xiang *et al.*, 2022), *C. foetidissimum* (assigned to subg. *Metacyathodium* by Srivastava and Dixit (1996)) was consistently resolved as independent from the clade containing the taxa of subg. *Cyathodium* (*C. cavernarum*, *C. smaragdinum*, *C. spruceanum* Prosk., and *C. tuberosum*). This finding probably indicates that the subgeneric classification of *Cyathodium* suggested by Srivastava and Dixit (1996) is also phylogenetically informative, even though it was based on a small number of subset of species within the genus (5 of 13).

In addition to the distinctions in vegetative characters and molecular phylogeny, spore morphology, an effective approach in the classification of complex thalloid liverworts (Bischler, 1998; Bischler-Causse *et al.*, 2005; Long, 2006a; Schill, 2006; Rubasinghe, 2011; Cargill *et al.*, 2021; Long and Zheng 2023), further supports the independence of subg. *Metacyathodium*. In subg. *Metacyathodium*, spore ornamentation is verrucose (*C. denticulatum* and *C. foetidissimum*) or lamellate (*Cyathodium steerei*), which is spiny, baculate,



tuberculate, granulose, muricate and reticulate in remaining species of the genus (Srivastava and Dixit, 1996; Allen, 2001). Based on the above morphological (spores, mid rib and storage zone of the thalli) and phylogenetic evidences, we here propose the validation of subg. *Metacyathodium* on the basis of *C. foetidissimum*.

Recently, within the Marchantiopsida, phylogenetic distinction and morphological differences in both gametophytes and sporophytes have become essential criteria for establishing novel generic or higher taxa (Long, 2006b; Long and Zheng, 2023, Zheng and Long, 2024; Xiang *et al.*, 2022). As discussed above, these aspects also apply to distinguishing the two subgenera from each other, making subg. *Metacyathodium* eligible for elevation to the rank of an independent genus. Additionally, while the subgeneric treatment of *Cyathodium* was validated in the present study, the spores still showed a high diversity between the two subgenera (Srivastava and Dixit 1996; Allen, 2001). To resolve these taxonomic uncertainties, it is necessary to introduce more molecular samples and refer to the results of phylogenetic analysis.

## TAXONOMIC TREATMENT

1. *Cyathodium* Kunze ex Lehm., Nov. Stirp. Pug. 6: 17. 1834.

1A. subg. *Cyathodium*

*Cyathodium tuberosum* Kashyap, New Phytol. 13: 210. 1914. (Fig. 1)

**Description:** The following description is based on the specimens collected from Beijing City. *Plants* luminescent, growing in mat-shaped. *Thallus* green or greyish to darkish green, delicate, once to twice dichotomous, 0.7–1.1 mm wide; dorsal and ventral epidermis one cell-layered; margin entire to crenulate; dorsal surface with distinct reticulate blackish line. *Epidermal cells* nearly circular to pentagonal, sometimes long ovate, 52–83 × 24–49 μm, thin-walled. *Midribs* absent. *Air-chambers* 3–5 in each cross section of thalli, chlorophyllose filament-free, separated by uniseriate cells, in 2–4 cells height. *Tubers* dark green, present in the apex of thalli, densely covered by whitish and straight hairs. *Rhizoid* smooth. *Ventral scales* highly reduced in structure, unistratose, nearly linear, in 5–6 cells height and 2–3 cells wide.

**Ecology and habitat:** On soil in a mixed forest, along the road.

**Distribution:** India (Vayalil *et al.*, 2023), Myanmar (Srivastava and Dixit, 1996), China (Beijing, this study; Yunnan (Wu and Gao, 2006))

**Specimen examined:** China. Beijing City, Mentougou District, Longmenjian Scenic Area, 115.633981°E, 39.944326°N, 470 m alt., 19 Sep. 2022, *L. Luo* 769 (PE 02151648), *L. Luo* 772 (PE 02151651).

1B. *Cyathodium* subg. *Metacyathodium* S.C.Srivast.

& R.Dixit ex T.X.Zheng, *subg. nov.* ≡ *Cyathodium* Kunze subg. *Metacyathodium* S.C.Srivast. & R.Dixit, J. Hattori Bot. Lab. 80: 195. 1996. *nom. inval.* (ICN 40.1, without indicating a type).

**Type:** *Cyathodium foetidissimum* Schiffn., Denkschr. Kaiserl. Akad. Wiss., Wien Math.-Naturwiss. Kl. 67: 154. 1898.

**Diagnosis:** see Srivastava and Dixit (1996, p. 195).

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## LITERATURE CITED

- Adachi, J., Hasegawa, M. 1996 MOLPHY version 2.3: programs for molecular phylogenetics based on maximum likelihood.
- Allen, N.S. 2001 *Cyathodium bischlerianum*, *sp. nov.* (Marchantiales) a new species from the Neotropics. *Bryologist* **104**(1): 141–145.
- Allen, N.S., Korpelainen, H. 2006 Notes on neotropical *Cyathodium*. *Cryptogam. Bryol.* **27**: 85–96.
- Bechteler, J., Peñalosa-Bojacá, G., Bell, D., Burleigh, J.G., McDanile, S.F., Davis, E.C., Sessa, E.B., Bippus, A., Cargill, D.C., Chantanoarrapint, S., Draper, I., Endara, L., Forrest, L.L., Garilleti, R., Graham, S.W., Huttunen, S., Lazo, J.J., Lara, F., Larrain, J., Lewis, L.R., Long, D.G., Quandt, D., Renzaglia, K., Schäfer-Verwimp, A., Lee, G.E., Sierra, A.M., von Konrat, M., Zartman, C.E., Pereira, M.R., Goffinet, B., Villarreal J.C.A. 2023 Comprehensive phylogenomic time tree of bryophytes reveals deep relationships and uncovers gene incongruences in the last 500 million years of diversification. *Am. J. Bot.* **110**(11): e16249.
- Bischler, H. 1998 Systematics and evolution of the genera of the Marchantiales. *Bryophyt. Biblioth.* **51**: 1–201.
- Bischler-Causse, H., Gradstein, S.R., Jovet-Ast S., Long D.G., Allen N.S. 2005 Marchantiidae. *Fl. Neotrop. Monogr.* **97**: 1–267.
- Boisselier-Dubayle, M.C., Lambourdière, J., Bischler, H. 2002 Molecular phylogenies support multiple morphological reductions in the liverwort subclass Marchantiidae (Bryophyta). *Mol. Phylogenet. Evol.* **24**(1): 66–77.
- Crandall-Stotler, B., Stotler, R.E. 2000 Morphology and Classification of the Marchantiophyta. In: Shaw, A.J., Goffinet, B. (eds.), *Bryophyte Biology*. 21–70. Cambridge University Press, Cambridge, United Kindom.
- Cargill, D.C., Beckmann, K., Seppelt, R. 2021 Taxonomic revision of *Riccia* (Ricciaceae, Marchantiophyta) in the monsoon tropics of the Northern Territory, Australia. *Aust. Syst. Bot.* **34**(4): 336–430.
- Evans, A.W. 1939 The classification of Hepaticae. *Bot. Rev.* **5**(1): 49–96.
- Flores, J.R., Bippus, A.C., Suárez, G.M., Hyvönen, J. 2020 Defying death: incorporating fossils into the phylogeny of



- the complex thalloid liverworts (Marchantiidae, Marchantiophyta) confirms high order clades but reveals discrepancies in family-level relationships. *Cladistics* **37**(3): 231–247.
- Forrest, L.L., Crandall-Stotler, B.J.** 2004 A phylogeny of the simple thalloid liverworts (Jungermanniopsida, subclass Metzgeriidae) as inferred from five chloroplast genes. *Monogr. Syst. Bot. Missouri Bot. Gard.* **98**: 119–140.
- Forrest, L.L., Davis, E.C., David, D.G., Crandall-Stotler, B.J., Clark, A., Hollingsworth, M.L.** 2006 Unraveling the evolutionary history of the liverworts (Marchantiophyta): multiple taxa, genomes and analyses. *Bryologist* **109**(3): 303–334.
- Gao, C., Wu, Y.-H.** 2010 *Genera Hepaticopsida et Anthocerotopsida Sinicorum*, Science Press, Beijing. 636 pp.
- Grolle, R.** 1972. Die Namen der Familien und unterfamilien der Lebermoose (Hepaticopsida) *J. Bryol.* **7**(2): 201–236.
- Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O.** 2010 New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst. Biol.* **59**(3): 307–321.
- Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q., Vinh, L.S.** 2018 UFBoot2: improving the ultrafast bootstrap approximation. *Bol. Biol. Evol.* **35**(2): 518–522.
- Inoue, Y., Aung, M.M.** 2023 *Reimersia inconspicua* (Griff.) P.C.Chen (Pottiaceae, Bryophyta) new to Myanmar, and its phylogenetic position. *Bull. Natl. Mus. Nat. Sci., Ser. B, Bot.* **49**: 17–23.
- Inoue, Y., Tsubota, H.** 2014 On the systematic position of the genus *Timmiella* (Dicranidae, Bryopsida) and its allied genera, with the description of a new family Timmiellaceae. *Phytotaxa* **181**(3): 151–162.
- Inoue, Y., Tsubota, H., Kubo, H., Uchida, S., Mukai, S., Shimamura, M., Deguchi, H.** 2011 A note on *Pottia intermedia* (Turner) Fűrnr. (Pottiaceae, Bryopsida) with special reference to its phylogeny and new localities in SW Japan. *Hikobia* **16**: 67–78.
- Inoue, Y., Tsubota, H., Sato, H., Yamaguchi, T.** 2012 Phylogenetic note on *Pachyneuroopsis miyagii* T.Yamag. (Pottiaceae, Bryophyta). *Hikobia* **16**: 221–228.
- Jia, Y., He, S.** 2013 *Species Catalogue of China. Volume 1 Plants. Bryophytes*, Science Press, Beijing. 525 pp.
- Kalyanamoorthy, S., Minh, B.Q., Wong, T.K., Von Haeseler, A., Jermini, L.S.** 2017 ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* **14**(6): 587–589.
- Kashyap, S. R.** 1929 *Liverworts of the Western Himalayas and the Panjab Plain (Illustrated). Part I*, The University of the Panjab, Lahore, Lahore, India. 133 pp.
- Katoh, K., Standley, D.M.** 2013 MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* **30**(4): 772–780.
- Kumar, S., Stecher, G., Tamura, K.** 2016 MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **33**(7): 1870–1874.
- Li, Y.-F., Luo, L., Liu, Y., He, Q., Yu, N.-N., Gaowa, N., Yi, Z.-Q., Wang, J.-J., Han, W., Peng, T., Ho, B.-C., He, X., Zhang, L., Chen, Z.-D., Jia, Y., Wang, Q.-H.** 2024 The Bryophyte Phylogeny Group: A revised familial classification system based on plastid phylogenomic data. *J. Syst. Evol.* **62**(4): 577–588.
- Long, D.G.** 2006a Revision of the genus *Asterella* P. Beauv. in Eurasia. *Bryophyt. Biblioth.* **63**: 1–299.
- Long, D.G.** 2006b New higher taxa of complex thalloid liverworts (Marchantiophyta-Marchantiopsida). *Eding. J. Bot.* **63**(2-3): 257–262.
- Long, D.G., Zheng, T.-X.** 2023 A new subfamily Calasterelloideae and new genus *Calasterella* for a phylogenetically and morphologically distinct member of the Aytoniaceae. *Phytotaxa* **606**(3): 225–230.
- Masuzaki, H., Shimamura, M., Furuki, T., Tsubota, H., Yamaguchi, T., Majid, H.M.A., Deguchi, H.** 2010 Systematic position of the enigmatic liverwort *Mizutania* (Mizutaniaceae, Marchantiophyta) inferred from molecular phylogenetic analyses. *Taxon* **59**(2): 448–458.
- Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A., Lanfear, R.** 2020 IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* **37**(8): 1530–1534.
- Müller, K.** 1940 Beiträge zur Systematik der Lebermoose. *Hedwigia* **79**: 72–80.
- Piippo, S.** 1990 Annotated catalogue of Chinese Hepaticae and Anthocerotae. *J. Hattori. Bot. Lab.* **68**: 1–192.
- Rubasinghe, S.C.K.** 2011 *Phylogeny and Taxonomy of the Complex Thalloid Liverwort family Cleveaceae Cavers*. PhD thesis. Royal Botanic Garden, Edinburgh.
- Schill, D.B.** 2006 *Taxonomy and Phylogeny of the Liverwort Genus Mannia* (Aytoniaceae, Marchantiales). PhD thesis. University of Edinburgh and Royal Botanic Garden Edinburgh, Edinburgh.
- Shen, C., Xu, H., Huang, W.-Z., Zhao, Q., Zhu, R.-L.** 2024a Is RNA editing truly absent in the complex thalloid liverworts (Marchantiopsida)? Evidence of extensive RNA editing from *Cyathodium cavernarum*. *New Phytol.* **242**(6): 2817–2831.
- Shen, C., Li, H., Shu, L., Huang, W.-Z., Zhu, R.-L.** 2024b Ancient large-scale gene duplications and diversification in bryophytes illuminate the plant terrestrialization. *New Phytol.* **245**(5): 2292–2308.
- Söderström, L., Hagborg, A., von Konrat, M., Bartholomew-Began, S., Bell, D., Briscoe, L., Brown, E., Cargill, D.C., Costa, D.P., Crandall-Stotler, B.J., Cooper, E.D., Dauphin, G., Engel, J.J., Feldberg, K., Glenny, D., Gradstein, S.R., He, X., Heinrichs, J., Hentschel, J., Ilkiu-Borges, A.L., Katagiri, T., Konstantinova, N.A., Larrain, J., Long, D.G., Nebel, M., Pócs, T., Puche, F., Reiner-Drehwald, E., Renner, M.A.M., Sass-Gyarmati, A., Schäfer-Verwimp, A., Moragues, J.G.S., Stotler, R.E., Sukkharak, P., Thiers, B.M., Uribe, J., Váña, J., Villarreal, J.C., Wigginton, M., Zhang, L. & Zhu, R.-L.** 2016 World checklist of hornworts and liverworts. *PhytoKeys* **59**: 1–828.
- Souza-Chies, T.T., Bittar, G., Nadot, S., Carter, L., Besin, E., Lejeune, B.** 1997 Phylogenetic analysis of Iridaceae with parsimony and distance methods using the plastid gene *rps4*. *Plant Syst. Evol.* **204**(1-2): 109–123.
- Srivastava, S.C., Dixit, R.** 1996 The genus *Cyathodium* Kunze. *J. Hattori Bot. Lab.* **80**: 149–215.
- Tsubota, H., Nakao, N., Arikawa, T., Yamaguchi, T., Higuchi, M., Deguchi, H., Seki, T.** 1999 A preliminary phylogeny of Hypnales (Musci) as inferred from chloroplast *rbcL* sequence data. *Bryol. Res.* **7**: 233–248.





- Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Kusber, W.H., Li, D.-Z., Marhold, K., May, T.W., McNeill, J., Monro, A. M., Prado, J., Price, M. J., Smith, G.F. 2018 International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile* 159. Koeltz Botanical Books, Glashütten.
- Vayalil, S.R.V., Syamala, S.S., Sarayu, M.G. 2023 Atlas of *Cyathodium* Kunze Species from India. *Asian J. Biol. Sci.* **12**(2): 326–331.
- Villarreal, A.J.C., Crandall-Stotler, B.J., Hart, M.L., Long, D.G., Forrest, L.L. 2016 Divergence times and the evolution of morphological complexity in an early land plant lineage (Marchantiopsida) with a slow molecular rate. *New Phytol.* **209**(4): 1734–1746.
- Wang, L., Jia, Y., Zhang, X., Qin, H. 2018 Species Catalogue of China, Volume 1 Plants, A Synoptic Checklist (I), Science Press, Beijing. 141 pp.
- Wu, Y.-H., Gao, C. 2006 Notes on Chinese *Cyathodium* Kunze. *Bull. Bot. Res.* **26**: 522–526.
- Xiang, Y.-L., Jin, X.-J., Shen, C., Cheng, X.-F., Shu, L., Zhu, R.-L. 2022 New insights into the phylogeny of the complex thalroid liverworts (Marchantiopsida) based on chloroplast genomes. *Cladistics* **38**(6): 649–662.
- Zheng, T.-X., Inoue, Y., Shimamura, M. 2024 Complete chloroplast genome of *Marchantia emarginata* subsp. *cuneiloba* (Marchantiaceae, Marchantiophyta): towards organellar phylogenomics of a model liverwort, *Marchantia*. *Bry. Div. Evo.* **48**: 1–11.
- Zheng, T.-X., Long, D.G. 2024 Validation of the family Aitchisoniellaceae (Marchantiales, Marchantiophyta) for the monotypic genus *Aitchisoniella* Kashyap. *Acta Phytotax. Geobot.* **75**: 19–24.

### Supplementary materials

**Table S1.** List of taxa used in phylogenetic analyses with accession numbers (*psbA/rbcL/rps4*). Newly obtained sequences are shown in bold.

		<i>psbA</i>	<i>rbcL</i>	<i>rps4</i>
<b>Ingroup:</b>				
<i>Cyathodium cavernarum</i> Kunze ex Lehm.	Bangladesh	KT793466	KT793564	KT793711
<i>Cyathodium cavernarum</i> Kunze ex Lehm.	Panama	KT793468	KT793566	KT793713
<i>Cyathodium cavernarum</i> Kunze ex Lehm.	Costa Rica	KT793467	KT793565	KT793712
<i>Cyathodium cavernarum</i> Kunze ex Lehm.	China I	PP078733	PP078733	PP078733
<i>Cyathodium cavernarum</i> Kunze ex Lehm.	China II	<b>LC858736</b>	<b>LC858739</b>	<b>LC858742</b>
<i>Cyathodium cavernarum</i> Kunze ex Lehm.	China II	<b>LC858737</b>	<b>LC858740</b>	<b>LC858743</b>
<i>Cyathodium foetidissimum</i> Schiffn.	Costa Rica	KJ590868	KJ590911	KJ590954
<i>Cyathodium foetidissimum</i> Schiffn.	Costa Rica	KT793469	KT793567	KT793714
<i>Cyathodium spruceanum</i> Prosk.	Costa Rica	KT356963	KT3569733	KT35698
<i>Cyathodium tuberculatum</i> Udar & D.K.Singh	Nepal	DQ265752	DQ286008	DQ220682
<i>Cyathodium tuberculatum</i> Udar & D.K.Singh	China: <i>L. Luo</i> 769	<b>LC858735</b>	<b>LC858738</b>	<b>LC858741</b>
<b>Outgroup</b>				
<i>Corsinia coriandrina</i> (Spreng.) Lindb.	Italy	KT793462	KT793560	KT793710
<i>Exormotheca pustulosa</i> Mitt.	Portugal	DQ265754	DQ286010	DQ220684

**Table S2.** List of best-fit models per partition selected by ModelFinder.

Partition no.	Model	Partitioning scheme
1	TIM3+F+G4	rps4_1st + rps4_2nd + psbA_1st + psbA_2nd + rbcL_1st + rbcL_2nd
2	K3Pu+F+I	rps4_3rd + rbcL_3rd
3	TPM3u+F	psbA_3rd