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Research article

Cyathodium tetrasporum (Cyathodiaceae, Marchantiopsida), a new species of liverworts with spores permanently united in tetrads

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Abstract. The genus *Cyathodium* (Marchantiophyta: Cyathodiaceae), a phylogenetically distinct lineage of cave-adapted liverworts, is characterized by a female involucre on the ventral surface of thalli and a distinctive green luminescence of thalli. Globally, 12 species of this genus are currently accepted, with a species diversity center in the Himalayas. During our taxonomic survey of the Chinese bryoflora, we found a remarkable undescribed species that exhibits spores permanently united in tetrads. Molecular evidence of chloroplast markers (*psb*A-*trn*H, *rbc*L and *rps*4) resolved it as sister to *Cyathodium tuberosum* Kashyap. Here, this plant is described as *Cyathodium tetrasporum* sp. nov. *Cyathodium* becomes the third genus of the class Marchantiopsida with species characterized by spores permanently united in tetrads, after *Riccia* and *Sphaerocarpos*. Detailed morphological descriptions, comparative analyses with congeners, SEM images of spores, and a provisional IUCN conservation assessment are provided.

Keywords. China, complex thalloid liverworts, cave-adapted liverworts, tetrad spore, endangered species, *p*-distances.

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Introduction

Cyathodium Kunze ex Lehm. (Cyathodiaceae Stotler & Crand.-Stotl., Marchantiales Limpr.), a complex thalloid liverwort genus comprising 12 globally recognized species (excluding one doubtful species, *Cyathodium spurium* (Dicks.) Lindb. ex Braithw.) (Söderström *et al.* 2016), exhibits a wide distribution extending from tropical to warm-temperate regions. It is a phylogenetically distinct lineage of cave-adapted liverworts characterized by a female involucre on the ventral surface of thalli and a distinctive green luminescence of thalli (Srivastava & Dixit 1996; Duckett & Ligrone 2006; Villarreal *et al.* 2016). The genus displays a marked center of diversity in the Himalayas (Srivastava & Dixit 1996), with disjunct extratropical populations reaching their northernmost limits at 39°56′39″ N in Beijing, China (Zheng *et al.* 2025) and 40°39′14″ N in Campania, Italy (Duckett & Ligrone 2006). Within Marchantiopsida, the genus *Cyathodium* is currently the only group known to exhibit RNA editing (Shen *et al.* 2024), a molecular uniqueness that makes it of particular interest in phylogenetic and evolutionary studies. Morphologically, this genus is also highly specialized, with unique morphological traits, including: (1) sporophytes ventral below the thallus apex; (2) simplified thallus architecture, e.g., unistratose epidermis, empty filament-absent air chambers, and (3) the frequent absence of a midrib. *Cyathodium* exhibits close morphological affinities to *Targionia* L. in thallus architecture and sporophyte positioning (Schiffner 1900; Evans 1939; Smith 1955; Udar 1964), and these two genera have been placed in a single family Targionicaeae Dumort. in the past. Recent molecular phylogenetic analyses demonstrated that *Cyathodium* has a closer relationship to the family Corsiniaceae Engl. and exhibits accelerated evolutionary rates in both organellar and nuclear markers compared to related marchantialean lineages (Villarreal *et al.* 2016; Xiang *et al.* 2025).

Species within *Cyathodium* were predominantly described during the 19th and 20th centuries, with the exception of *Cyathodium bischlerianum* N. Salazar described from Panama in 2001 (Salazar Allen 2001). During a taxonomic survey of the Chinese bryoflora, we encountered a peculiar *Cyathodium* population inhabiting shaded soil in Xianggelila County, Yunnan Province, China. Careful observations revealed its sporophytes producing mature spores united in tetrads, a trait unknown in the genus. Here, we describe this taxon as a new species, *Cyathodium tetrasporum* sp. nov., on the basis of morphological features, *p*-distances, and molecular evidence from chloroplast loci (*psb*A-*trn*H, *rbc*L, *rps*4). The systematic position of this taxon, emphasizing the evolutionary implications of persistent tetrads in bryophytes, is evaluated.

Material and methods

Taxon sampling

Two peculiar specimens of *Cyathodium* were collected from Xianggelila County, Yunnan Province, China, in 2016 and 2024. One specimen was selected for sequencing. To investigate the phylogenetic position of the species, *Corsinia coriandrina* (Sprengel) Lindberg, *Cronisia fimbriata* (Nees) Whittem. & Bischl., *C. weddellii* (Mont.) Grolle and *Exormotheca pustulosa* Mitt. were designated as outgroups, following established taxonomic relationships in previous studies (Villarreal *et al.* 2016; Xiang *et al.* 2022). The newly generated sequences of peculiar specimens of *Cyathodium* in this study have been deposited in GenBase under the accession numbers C_AA121236.1 (*rbc*L), C_AA121237.1 (*psb*A-*trn*H), and C_AA121238.1 (*rps*4). These data are publicly accessible at https://ngdc.cncb.ac.cn/genbase. Nucleotide sequences of the outgroup taxa and the remaining *Cyathodium* accessions were downloaded from GenBank (Fig. 1, Table 1). In total, 19 accessions of seven species representing the genus *Cyathodium* were incorporated into the phylogenetic analyses. Detailed information regarding the studied taxa, including voucher specimens and GenBank accession numbers, is provided in Table 1.

Morphological study

Field photographs were acquired with a Canon M6 digital camera. Morphological and anatomical characterization was done using an Olympus BX43 microscope coupled to a DP71 digital camera. Spore ultrastructure was analyzed using a Hitachi S4800 scanning electron microscope (SEM).

Table 1. Sequences used in the phylogenetic tree of *Cyathodium* Kunze ex Lehm., including taxa, vouchers, locations, and GenBank accession numbers. '–' missing data.

			GenBank accession		
Taxon	**Voucher information**	**Localities**	***psb*A-*trn*H**	***rbc*L**	***rps*4**
Corsinia coriandrina I	*Schill 9* (E)	Portugal	DQ265750	DQ286006	DQ220680
C. coriandrina II	*Long 35445* (E)	Italy	KT793462	KT793560	KT793710
Cronisia fimbriata I	*Peralta 10276* (E)	Brazil	KT793463	KT793561	–
C. fimbriata II	*Peralta 10272* (E)	Brazil	KT793464	KT793562	–
C. weddellii	*Peralta 10213* (E)	Brazil	KT793465	KT793563	–
Exormotheca pustulosa	*Schill 8* (E)	Portugal	DQ265754	DQ286010	DQ220684
Cyathodium aureonitens I	*Xiang et al. 20160709-98* (HSNU)	China, Sichuan	PQ768093	PQ768071	PQ768082
C. aureonitens II	*Xiang et al. 20161103-20* (HSNU)	China, Sichuan	PQ768094	PQ768072	PQ768083
C. aureonitens III	*Xiang et al. 20161109-39* (HSNU)	China, Yunnan	PQ768095	PQ768073	PQ768084
C. aureonitens IV	*Zhu et al. 20150824-24* (HSNU)	China, Yunnan	PQ768096	PQ768074	PQ768085
C. cavernarum I	*Salazar Allen 20698* (PMA)	Costa Rica	KT793467	KT793565	KT793712
C. cavernarum II	*Cuadra, Pacheco & Montenegro* (PMA)	Panama	KT793468	KT793566	KT793713
C. cavernarum III	*Xiang et al. 20161109-46* (HSNU)	China, Yunnan	PQ768097	PQ768075	PQ768086
C. cavernarum IV	*Long 28255* (E)	Bangladesh	KT793466	KT793564	KT793711
C. foetidissimum I	*Salazar Allen 20635* (PMA)	Costa Rica	KJ590868	KJ590911	KJ590954
C. foetidissimum II	*Salazar Allen et al. 20627* (PMA)	Costa Rica	KT793469	KT793567	KT793714
C. indicum I	*Xiang et al. 20161107-35* (HSNU)	China, Yunnan	PQ768098	PQ768076	PQ768087
C. indicum II	*Xiang 20160917-12* (HSNU)	China, Yunnan	–	PQ768077	PQ768088
C. spruceanum	*Salazar Allen & Lepiz 20916* (PMA)	Costa Rica	KT356963	KT356973	KT356983
C. tuberosum I	*Xiang 20160922-31* (HSNU)	China, Sichuan	PQ768099	PQ768078	PQ768089
C. tuberosum II	*Wei & Shu 20151012-4* (HSNU)	China, Sichuan	PQ768100	PQ768079	PQ768090
C. tuberosum III	*Long 30558* (E)	Nepal	DQ265752	DQ286008	DQ220682
C. tuberosum IV	*Yin et al. 20160712-51* (HSNU)	China, Sichuan	PQ768101	PQ768080	PQ768091
C. tuberosum V	*Xiang et al. 20161107-36* (HSNU)	China, Yunnan	PQ768102	PQ768081	PQ768092

DNA extraction and sequencing

Total genomic DNA was isolated from approximately 20 mg of fresh leaf tissue using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany), following the protocols described by Xiang & Zhu (2019, 2023). Tissue was homogenized in liquid nitrogen using a sterile mortar and pestle prior to extraction. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Three chloroplast DNA regions—*psb*A-*trn*H, *rbc*L, and *rps*4—were amplified by PCR using gene-specific primers and sequenced by the commercial sequencing service provider Jie Li Biology Inc., China (http://www.genebioseq.com). The PCR primers and amplification protocols were based on previously published methods: *psb*A-*trn*H followed Forrest & Crandall-Stotler (2004), *rbc*L followed Gradstein *et al.* (2006) and Wilson *et al.* (2004), and *rps*4 followed Cox *et al.* (2000). PCR products were verified by electrophoresis on 1.5% agarose gels prior to purification and sequencing. All regions were sequenced bidirectionally using the amplification primers.

Phylogenetic analyses

Sequence assembly was performed using Geneious Prime v2022.2.2 (https://www.geneious.com). Multiple sequence alignment was conducted using MAFFT (Katoh & Standley 2013) within PhyloSuite. Ambiguous alignment regions were manually trimmed. The aligned CDS sequences were subsequently

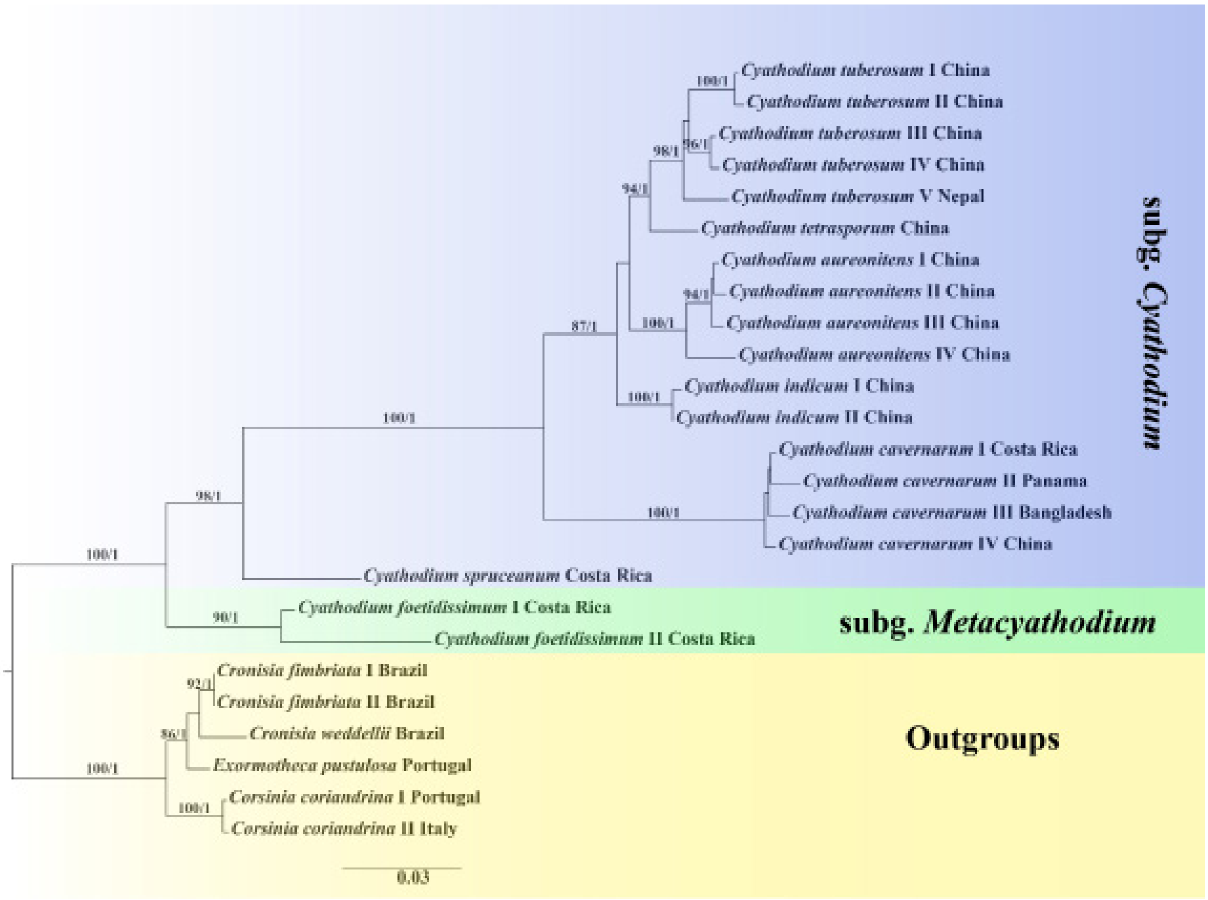

Fig. 1. Phylogeny of *Cyathodium* Kunze ex Lehm. inferred from the combined dataset (*psb*A-*trn*H, *rbc*L, *rps*4) and derived from the optimal maximum likelihood (ML) tree obtained with IQ-TREE. ML ultrafast bootstrap support values (BS≥80) and Bayesian posterior probabilities (PP≥0.95) are indicated on the left and right, respectively.

concatenated using the "Concatenate Sequence" function in PhyloSuite (Zhang *et al.* 2020). Phylogenetic analyses were performed using both Maximum Likelihood (ML) and Bayesian Inference (BI). ML analysis was executed with IQ-TREE v2.0.6 (Minh *et al.* 2020), employing 1000 ultrafast bootstrap replicates. The optimal nucleotide substitution models for each partition (*psb*A-*trn*H and *rbc*L: TPM3+F+I; *rps*4: HKY+F+G4) were selected using ModelFinder (Kalyaanamoorthy *et al.* 2017) based on the Bayesian Information Criterion (BIC). BI analysis was performed using MrBayes v3.2.6 (Ronquist *et al.* 2012) via the CIPRES Science Gateway (Miller *et al.* 2010). Partition-specific substitution models (*psb*A-*trn*H and *rbc*L: GTR+G+I; *rps*4: GTR+G) were selected using the Akaike Information Criterion corrected (AICc). Two independent Markov chain Monte Carlo (MCMC) analyses were run for 2 million generations, sampling every 1000 generations from random starting trees. Convergence was assessed by monitoring the average standard deviation of split frequencies (ASDSF), halting the analysis once it fell below 0.01. After discarding the initial 25% of sampled trees as burn-in, a majority-rule consensus tree (50% majority-rule) was constructed to summarize the posterior distribution of trees.

***p*-distances**

Genetic variability (*p*-distances) for each DNA locus (*psb*A-*trn*H, *rbc*L, *rps*4) among all species of *Cyathodium* was calculated using MEGA6 (Tamura *et al.* 2013), with gaps handled via pairwise deletion.

Preliminary conservation status assessments

Preliminary conservation assessments for peculiar species of *Cyathodium* were conducted following the IUCN Red List Categories and Criteria (IUCN 2012), specifically focusing on Criterion B (geographic range size measured as Extent of Occurrence (EOO) and/or Area of Occupancy (AOO)). EOO and AOO were calculated using the GeoCAT tool (Bachman *et al.* 2011).

Results

The phylogenetic trees inferred from individual loci were incongruent; therefore, we conducted a combined analysis of all three loci. The combined dataset of 25 accessions representing 10 species included 2984 aligned nucleotides, 2333 are constant and 503 were parsimony informative, while 148 are parsimony-uninformative. The clade of *Cyathodium* is well supported as monophyletic (MLBS=100; PP=1). Within *Cyathodium*, the previously recognised subgenera, *Cyathodium* and *Metacyathodium* S.C.Srivast. & R.Dixit ex T.X.Zheng, are both recovered as strongly supported monophyletic groups in all phylogenetic analyses (Fig. 1).The peculiar species of *Cyathodium* is sister to *C. tuberosum* Kashyap (MLBS=94; PP=1) (Fig. 1). Morphological examination show that the peculiar species of *Cyathodium* is well characterized by: (1) dorsal pores absent or rare, found only on the posterior part of the thallus with indistinct concentric rings; (2) midrib absent; (3) air chambers in one layer, without filament; (4) dioecious condition; (5) ventral surface of involucres without hairs (Fig. 3A–B); and (6) spores permanently united in tetrads, brown, tetrads 62–68 µm in diameter with irregular tuberculate projections all over the surface (Figs 3C–D, 4A–C).

The interspecific *p*-distances in *psb*A-*trn*H, *rbc*L, and *rps*4 within the species of *Cyathodium* vary from 1.3%–7.0%, 0.4%–9.1%, and 4.1%–16.7%, respectively (Table 2). The intraspecific *p*-distances in our analysis vary from 0%–1.3% in the *psb*A-*trn*H-region, 0.3%–1.4% (5.7%) in the *rbc*L-region, and 0.1%–1.6% in the *rps*4-region (Table 2). The value of *p*-distances calculated for the peculiar species of *Cyathodium* and the other species of *Cyathodium* in *psb*A-*trn*H, *rbc*L, and *rps*4 vary from 1.3%–5.7%, 1.8%–7.9%, and 4.1%–16.5%, respectively (Table 2).

Table 2. Infraspecific (INT, bold) and interspecific *p*-distances of *psb*A-*trn*H-region, *rbc*L-region, and *rps*4-region (%) of species of *Cyathodium* Kunze ex Lehm..

No. and taxon	INT	1	2	3	4	5	6
1. *C. aureonitens*	**1.1/1.1/1.6**	–	–	–	–	–	–
2. *C. cavernarum*	**0.8/0.7/0.8**	5.1/5.9/11.4	–	–	–	–	–
3. *C. foetidissimum*	**0/5.7/0.3**	6.2/8.7/16.7	6.9/9.1/16.6	–	–	–	–
4. *C. indicum*	**nc/0.3/0.1**	2.4/2.7/6.1	3.9/5.8/11.7	5.1/8.1/16.6	–	–	–
5. *C. spruceanum*	**nc/nc/nc**	6.5/5.6/13.3	7.0/6.7/13.6	3.0/0.4/10.5	5.4/5.3/12.4	–	–
6. *C. tetraspora*	**nc/nc/nc**	1.5/2.5/6.3	4.2/5.5/11.2	5.6/7.9/16.5	1.3/1.8/5.6	5.7/4.7/12.7	–
7. *C. tuberosum*	**1.3/1.4/1.6**	2.9/2.9/5.4	5.5/6.1/10.0	5.9/8.5/16.5	2.4/2.4/4.9	6.3/5.5/13.2	1.4/2.0/4.1

Taxonomy

Class Marchantiopsida Cronquist, Takht. & W.Zimm.
Order Marchantiales Limpr.
Family Cyathodiaceae Stotler & Crand.-Stotl.
Genus *Cyathodium* Kunze ex Lehm.

Cyathodium tetrasporum R.L.Zhu & You L.Xiang sp. nov.

Figs 2–4

Diagnosis

Similar to *Cyathodium tuberosum* but differing in the ventral surface of female involucres without hairs, and spores permanently united in tetrads, with irregular, tuberculate projections all over the surface.

Etymology

The specific epithet, consisting of 'tetra' and 'sporum', means that the spores are permanently united in tetrads.

Type material

Holotype

CHINA – **Yunnan Province** • Xianggelila County, from Habaxueshan to Luoji Town; 27°34′17.00″ N, 100°00′57.34″ E; 2997 m a.s.l.; 17 Sep. 2016; *Y.L. Xiang 20160917-11B*; on soil; HSNU.

Other material examined

CHINA – **Yunnan Province** • Xianggelila County, from Habaxueshan to Luoji Town; 27°34′4.79″ N, 100°00′57.30″ E; 2970 m a.s.l.; 29 Aug. 2022; *R.L. Zhu et al. 20220829-5*; on soil; HSNU.

Description

Male plants not seen. Female plants thalloid, light green, 1–2× furcate, 3–5 mm long and 1–2 mm broad, yellowish green. Dorsal pores absent, or rare, only on posterior part of the thallus, simple; dorsal epidermal cells subquadrate to polygonal, thin-walled, 42–72 µm long, 22–48 µm wide. Midrib absent. Air chambers in one row, without filaments. Rhizoids numerous on ventral surface, hyaline and smooth. Ventral scales inconspicuous, sometimes uni- or biseriate, without appendages, uniseriate to multiseriate, 2–7 cells long, 2–3 cells wide at base. Tubers and ventral branches absent. Involucres bivalved, large, ovoid, not projecting beyond the thallus margin, mouth narrow, ventral surface of involucres without hairs. Sporophytes usually one in each involucre, seta remaining short. Capsules ovoid-globose, 0.4–0.6 mm in diameter, blackish-brown; capsule wall single-layered. Spores permanently united in tetrads, brown, tetrads 62–78 µm in diameter, with irregular, tuberculate projections all over the surface. Elaters rare, 302–351 µm long, 14.4–15.6 µm wide, usually trispiral.

Preliminary conservation status assessment

This species is currently known only from Xianggelila County, where it inhabits a minuscule area (0.0002 km^2) of damp soil beneath shrubs within stream valleys. As mentioned below, the species has only been found at a single location, in spite of extensive bryophyte inventorying across southwestern China. Being restricted to a single location and exhibiting an Area of Occupancy (AOO) of only 0.0002 km^2, it persists in a highly fragmented landscape that has undergone significant decline in both extent and quality. Consequently, we recommend its classification as Critically Endangered under IUCN criterion CR B2ab(iii) (IUCN 2012).

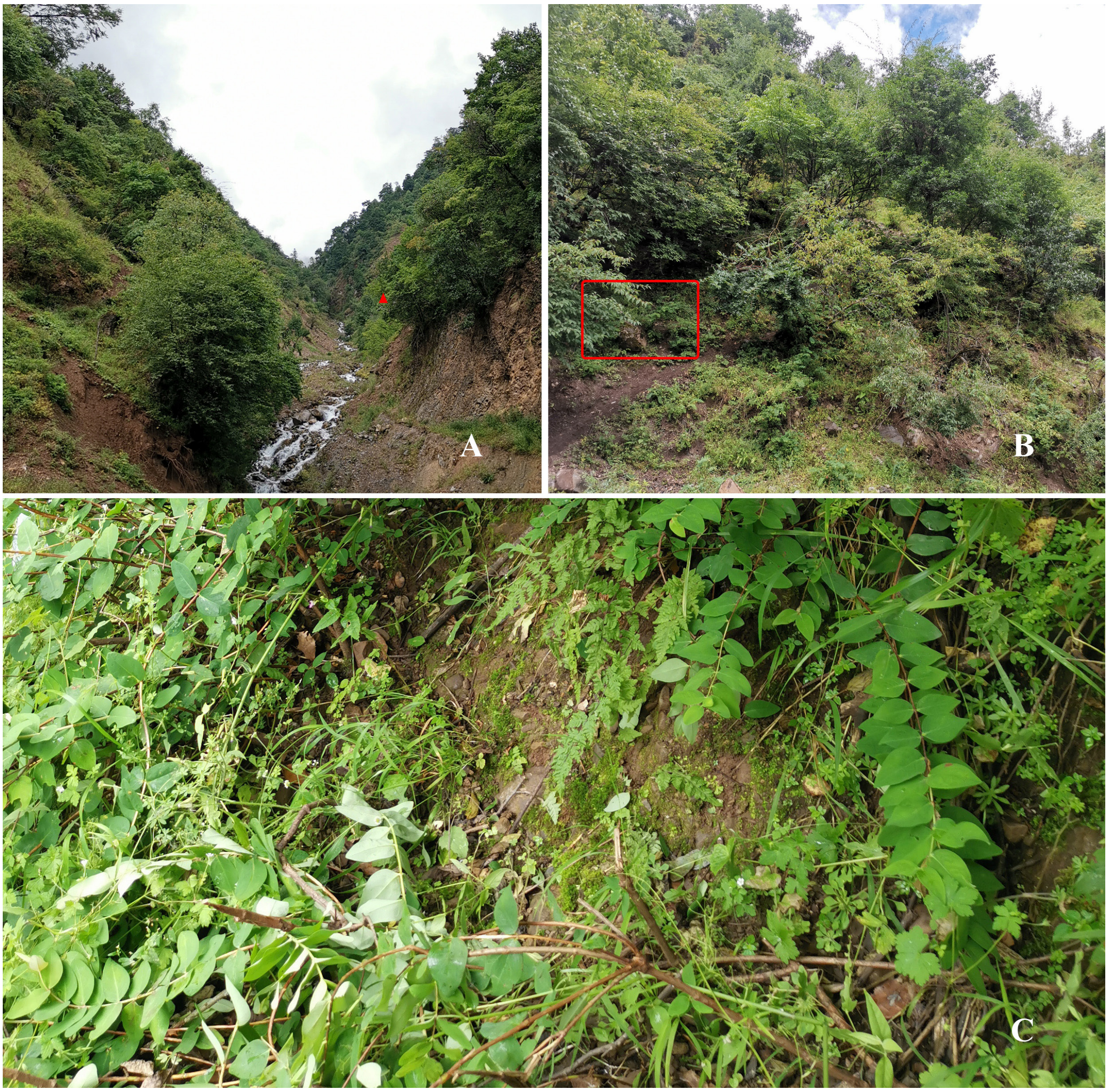

Fig. 2. A–B. Locality of *Cyathodium tetrasporum* R.L.Zhu & You L.Xiang sp. nov. (red triangle and red rectangular box). **C**. Habitat of *C. tetrasporum*.

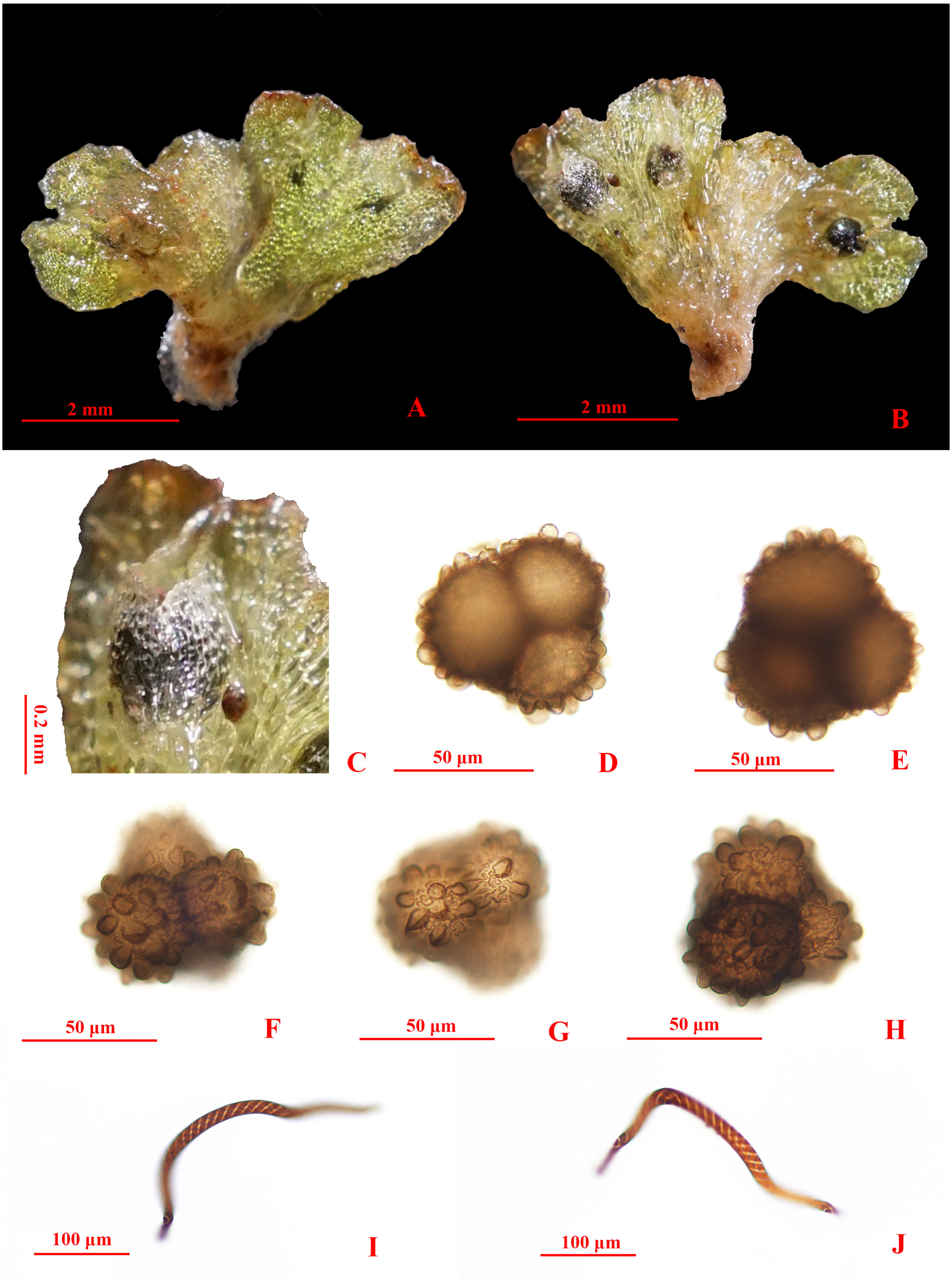

Fig 3. *Cyathodium tetrasporum* R.L.Zhu & You L.Xiang sp. nov. **A**. Female plant, dorsal view. **B**. Female plant, ventral view. **C**. Involucre. **D**–**J**. Light microscopy micrographs. **D**–**H**. Spore tetrads. **I**–**J**. Elaters. All from *Y.L. Xiang 20160917-11B* (holotype, HSNU).

Distribution and habitat

The new species is known only from Xianggelila County, northwestern Yunnan, China. It grows on damp soils under shrubs in stream valleys (Fig. 2), associated with *Cryptomitrium himalayense* Kashyap, *Cyathodium indicum* Udar & S.K.Singh, and *Targionia hypophylla* L.

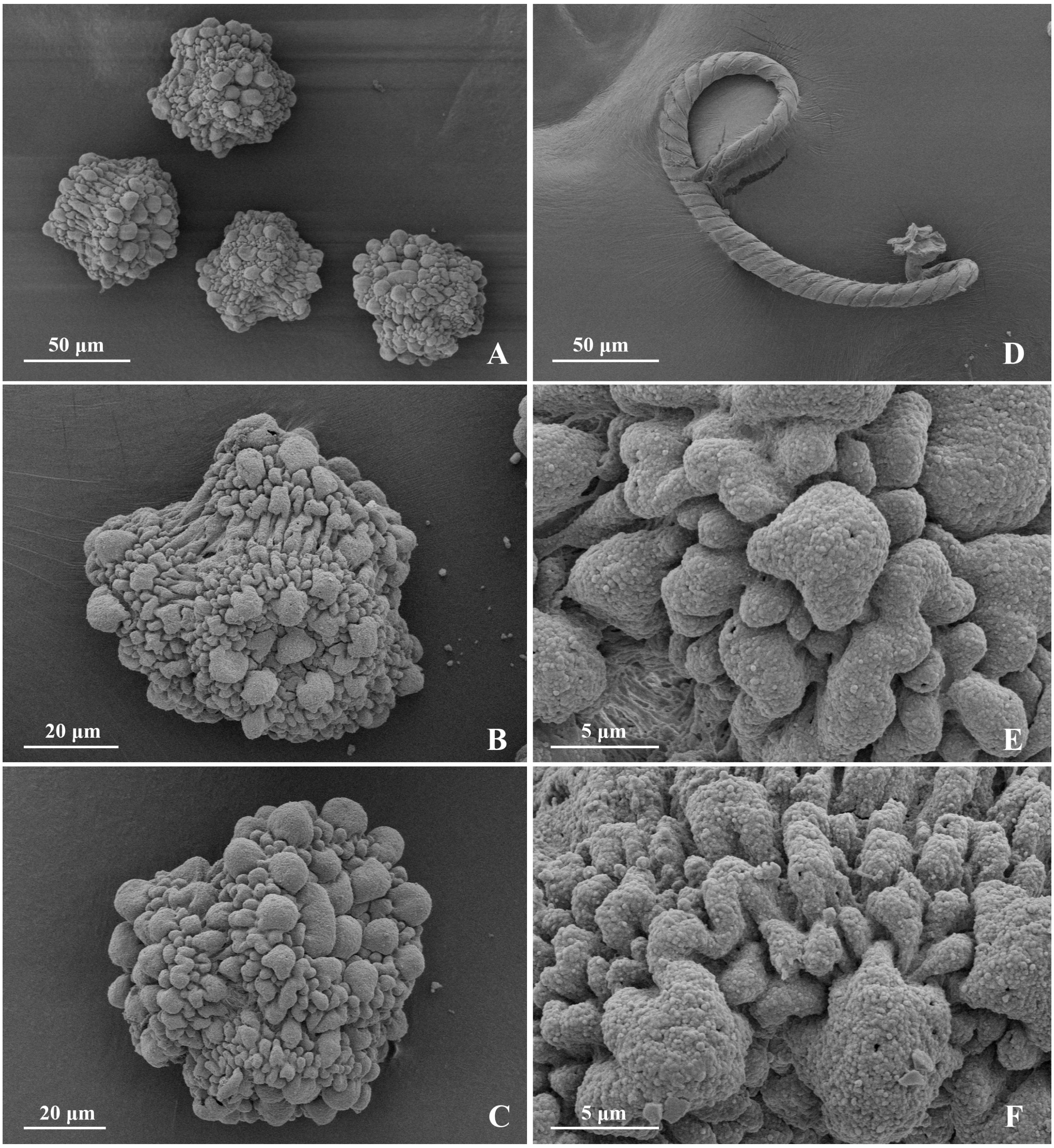

Fig. 4. *Cyathodium tetrasporum* R.L.Zhu & You L.Xiang sp. nov. **A**–**F**. SEM micrographs. **A**–**C**. Spores. **D**. Elaters. **E**–**F**. Surface ornamentation of spores. All from *Y.L. Xiang 20160917-11B* (holotype, HSNU).

Discussion

Phylogenetic analyses resolve the *Cyathodium tetrasporum* sp. nov. as sister to *C. tuberosum*. The interspecific *p*-distances between *C. tetrasporum* and *C. tuberosum* exceed the maximum intraspecific *p*-distances observed among all other species of *Cyathodium* examined, across most loci. An exception was observed for the *rbc*L region, where the interspecific distance between *C. tetrasporum* and *C. tuberosum* did not exceed the maximum intraspecific distance (5.7%). This anomaly is likely attributable to suboptimal *rbc*L sequence quality (e.g., potential ambiguities or missing data) in the *C. foetidissimum* specimens that yielded the highest intraspecific variation for this marker. Morphologically, *Cyathodium tetrasporum* clearly differs from *C. tuberosum* in having involucres with a ventral surface lacking hairs, spores permanently united in tetrads, and spores with irregular tuberculate projections covering the surface. In addition, *Cyathodium tetrasporum* occurs on damp soil beneath shrubs in a single stream valley. Despite extensive surveys across southwestern China, the species has thus far been found only at the type locality (Fig. 2). In contrast, *Cyathodium tuberosum* is a very common species in China, and is often found on moist, shady sites and dark caves (Srivastava & Dixit 1996; Wu & Gao 2006). The differences in morphology, habitat, and genetic divergence between these two species support the establishment of the new species, *Cyathodium tetrasporum*. The recent observation of *Cyathodium indicum* in China (Xiang *et al.* 2025) and *Cyathodium tetrasporum* in this study has increased diversity of China's *Cyathodium* to seven species, representing 58% of the genus' global diversity. The species diversity of *Cyathodium* in China exceeds that of Africa (4 species), the Neotropics (4 species), and Southeast Asia (4 species), but is surpassed only by India, which harbors 9 species (Salazar Allen 2005; Salazar Allen & Korpelainen 2006; Wang *et al.* 2018; Dixit & Kerketta 2020). The discovery not only expands the known diversity of *Cyathodium*, but also shows that China is an important diversity center for this genus.

Cyathodium was historically divided into two subgenera: *Cyathodium* subgen. *Cyathodium* and *Cyathodium* subgen. *Metacyathodium*, as proposed by Srivastava & Dixit (1996) and later validated by Zheng *et al.* (2025). *Cyathodium tetrasporum* sp. nov. clearly belongs to the subgenus *Cyathodium* owing to its lack of a midrib, smooth rhizoids, a single layer of air chambers, and the absence of a storage zone, and as supported by the phylogenetic analyses (Fig. 1). Within this subgenus, *Cyathodium tetrasporum* resembles *C. cavernarum* Kunze ex Lehm., *C. smaragdinum* Schiffn. and *C. spruceanum* Prosk. in having non-hairy involucres. *Cyathodium tetrasporum*, however, can be distinguished by the spores permanently united in tetrads and rarely having dorsal pores on the posterior part of the thallus. *Cyathodium tetrasporum* is also similar to *C. indicum* in its dioecious condition, the sparse and reduced dorsal pores, and the single-layered air chambers. It, however, differs in the ventral surface of involucres with hairs and spores typically united in diads with prominent tubercles interspersed with baculae (Udar & Singh 1978). When sterile, *Cyathodium tetrasporum* can be readily distinguished from *C. aureonitens* (Griff.) Mitt., *C. tuberculatum* Udar & D.K.Singh, and *C. mehranum* D.K.Singh by its rare, highly reduced dorsal pores with ill-defined concentric rings.

Spores permanently coherent in tetrads refer to a specific biological condition in which the four spores produced from a single spore mother cell through meiotic division remain permanently united and do not separate into individual spores. They persist as cohesive, four-spored units (tetrads) throughout maturation and dispersal. In bryophytes, this phenomenon is relatively rare, occurring only in a few liverwort genera, including *Sphaerocarpos* Boehm., *Riccia* L., *Aneura* Dumort., and *Haplomitrium* Nees. Prominent examples of species with spores united in tetrads include *Aneura mirabilis* (Malmb.) Wickett & Goffinet, *Riccia curtisii* (Austin) Austin, and *Sphaerocarpos donnellii* Austin (Schuster 1992a, 1992b; Paton 1999).

These permanent spore tetrads exhibit significantly larger volume and mass than individual spores. This increased size poses a considerable challenge for long-distance wind dispersal, suggesting that these tetrads rely more heavily on water for dispersal (Zanten & Gradstein 1988; Sundberg 2013). Notably, several typical species with permanent spore tetrads are dioecious, which display marked sexual dimorphism in their gametophytes. The presence of permanent spore tetrads may facilitate the growth of male and female thalli in close proximity through a 'clustering' strategy, thereby enhancing fertilization opportunities (Schuster 1984). This 'clustering' strategy may also improve early establishment success by increasing resilience to environmental stresses, such as desiccation, shading, and competition. Consequently, it promotes the rapid formation of localized populations within microhabitats (Glime 2017). Nevertheless, the conspicuous absence of male plants in our collections raises questions regarding sex distribution patterns in these colonies. This may be explained by several non-exclusive factors, a primary one being that male gametophytes in species of *Cyathodium* are often smaller, shorter-lived, and significantly scarcer than their female counterparts (Srivastava & Dixit 1996).

Key to the species of *Cyathodium* in China

1. Plants possessing non-hairy involucres 2
– Plants possessing hairy involucres 4

2. Mature spores permanently united in tetrads .... ***C. tetrasporum*** sp. nov.
– Mature spores not permanently united in tetrads 3

3. Spores 40–83 µm in diameter with baculate ornamentations; thalli up to 4 mm wide; elaters 8–11 per capsule .... ***C. cavernarum*** Kunze ex Lehm.
– Spores 36–52 µm in diameter with small granulose ornamentation; thalli 0.5–2.6 mm wide; elaters 4–8 per capsule .... ***C. smaragdinum*** Schiffn.

4. Mature spores singly or in diads; spore surface with baculate-tuberculate ornamentation .... ***C. indicum*** Udar & D.K.Singh
– Mature spores occurring singly; spore surface with baculate-muricate ornamentation 5

5. Thallus with ventral pores; spores with spinate ornamentations, spines with rounded apex .... ***C. tuberosum*** Kashyap
– Thallus without ventral pores; spores with tuberculate or spinate ornamentations, spines with pointed apex 6

6. Spores with spinate ornamentation, spines 5–7 µm long .... ***C. aureonitens*** (Griff.) Mitt.
– Spores with tuberculate ornamentation, tubercles 2.5–3.0 µm long .... ***C. tuberculatum*** Udar & D.K.Singh

Conclusion

Cyathodium tetrasporum sp. nov. is uniquely distinguished by its mature spores that remain permanently united in tetrads. It represents the first recorded species within the family Cyathodiaceae with spores persistently coherent in tetrads. Among the Marchantiopsida, the formation of persistent spore tetrads is an uncommon characteristic, previously documented only in *Sphaerocarpos* and *Riccia* (Schuster 1992a, 1992b). With its tetrads of permanently united spores, *Cyathodium* thus represents the third genus in the class known to exhibit this feature, following *Riccia* and *Sphaerocarpos*. *Cyathodium tetrasporum* is proposed to be assessed as Critically Endangered (CR), highlighting the urgent need for conservation of the species and its habitat.

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References

Bachman S., Moat J., Hill A.W., de la Torre J. & Scott B. 2011. Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. *ZooKeys* 150: 117–126. https://doi.org/10.3897/zookeys.150.2109

Cox C.J., Goffinet B., Newton A.E., Shaw A.J. & Hedderson T.A.J. 2000. Phylogenetic relationships among the diplolepideous-alternate mosses (Bryidae) inferred from nuclear and chloroplast DNA sequences. *The Bryologist* 103: 224–241. https://doi.org/10.1639/0007-2745(2000)103[0224:PRATDA]2.0.CO;2

Dixit A.K. & Kerketta M.A. 2020. *Cyathodium denticulatum* Udar et Srivastava: A rare liverwort new to Chhattisgarh, Central India. *Current Botany* 12: 171–174. https://doi.org/10.25081/CB.2020.V11.5882

Duckett J. & Ligrone R. 2006. *Cyathodium* Kunze (Cyathodiaceae: Marchantiales), a tropical liverwort genus and family new to Europe, in Southern Italy. *Journal of Bryology* 28: 88–96. https://doi.org/10.1179/174328206X105353

Evans A.W. 1939. The classification of Hepaticae. *Botanical Review* 5: 49–96.

Forrest L.L. & Crandall-Stotler B.J. 2004. A phylogeny of the simple thalloid liverworts (Jungermanniopsida, Metzgeriidae) as inferred from five chloroplast genes. *Monographs in Systematic Botany from the Missouri Botanical Garden* 98: 119–140.

Glime J.M. 2007. *Bryophyte Ecology. Vol. 1: Physiological Ecology*. Michigan Technological University and the International Association of Bryologists, Houghton.

Gradstein S.R., Wilson R., Ilkiu-Borges A.L. & Heinrichs J. 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. https://doi.org/10.1111/j.1095-8339.2006.00531.x

Kalyaanamoorthy S., Minh B.Q., Wong T.K.F., von Haeseler A. & Jermiin L.S. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589. https://doi.org/10.1038/nmeth.4285

Katoh K. & Standley D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780. https://doi.org/10.1093/molbev/mst010

IUCN 2012. *The IUCN Red List Categories and Criteria, Version 3.1*. Second edition. IUCN Red List Unit, Cambridge. Available from https://www.iucnredlist.org [accessed 15 Mar. 2025].

Miller M.A., Pfeiffer W. & Schwartz T. 2010. *Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. 2010 Gateway Computing Environments Workshop (GCE), 14 Nov. 2010*. New Orleans: 1–8. https://doi.org/10.1109/GCE.2010.5676129

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. *Molecular Biology and Evolution* 37: 1530–1534. https://doi.org/10.1093/molbev/msaa015

Paton J.A. 1999. *The Liverwort Flora of the British Isles*. Harley Books, Colchester.

Ronquist F., Teslenko M., van der Mark P., Ayres D.L., Darling A., Höhna S., Larget B., Liu L., Suchard M.A. & Huelsenbeck J.P. 2012. MrBAYES 3.2: efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology* 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Salazar Allen N. 2001. *Cyathodium bischlerianum*, sp. nov. (Marchantiales) a new species from the Neotropics. *The Bryologist* 104 (1): 141–145. https://doi.org/10.1639/0007-2745(2001)104[0141:CBSNMA]2.0.CO;2

Salazar Allen N. 2005. *Cyathodium*. *In*: Bischler-Causse H., Gradstein S.R., Jovet Ast S., Long D.G. & Salazar Allen N. (eds) *Marchantiidae*. *Flora Neotropica Monographs* 97: 1–267.

Salazar Allen N. & Korpelainen H. 2006. Notes on neotropical *Cyathodium*. *Cryptogamie, Bryologie* 27 (1): 86–95.

Schiffner V. 1900. *Die Hepaticae der Flora Von Buitenzorg I*. Band. E. J. Brill, Leiden.

Schuster R.M. 1984. Evolution, phylogeny and classification of the Hepaticae. *In*: Schuster R.M. (ed.) *New Manual of Bryology. Vol. 2*: 982–1070. Hattori Botanical Laboratory, Nichinan.

Schuster R.M. 1992a. *The Hepaticae and Anthocerotae of North America. V*. Columbia University Press, New York.

Schuster R.M. 1992b. *The Hepaticae and Anthocerotae of North America. VI*. Columbia University Press, New York.

Shen C., Xu H., Huang W.Z., Zhao Q. & Zhu R.L. 2024. Is RNA editing truly absent in the complex thalloid liverworts (Marchantiopsida)? Evidence of extensive RNA editing from *Cyathodium cavernarum*. *New Phytologist* 242: 2817–2831. https://doi.org/10.1111/nph.19750

Smith G.M. 1955. *Cryptogamic Botany. Vol. 2*. Second edition. McGraw Hill, New York.

Söderström L., Hagborg A., von Konrat M., Bartholomew-Began S., Bell D., Briscoe L., Brown E., Cargill D.C., Cooper E.D., Costa D.P., Crandall-Stotler B.J., Dauphin G., Engel J.J., Feldberg K., Glenny D., Gradstein S.R., He X., Ilkiu-Borges A.L., Heinrichs J., Hentschel J., 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., Segarra Moragues J.G., Stotler R.E., Sukkharak P., Thiers B.M., Uribe J., Váňa J., Villareal J.C., Wigginton M., Zhang L. & Zhu R.L. 2016. World checklist of hornworts and liverworts. *PhytoKeys* 59: 1–828. https://doi.org/10.3897/phytokeys.59.6261

Srivastava S.C. & Dixit R. 1996. The genus *Cyathodium* Kunze. *Journal of the Hattori Botanical Laboratory* 80: 149–215.

Sundberg S. 2013. Spore rain in relation to regional sources and beyond. *Ecography* 36: 364–373. https://doi.org/10.1111/j.1600-0587.2012.07664.x

Tamura K., Stecher G., Peterson D., Filipski A. & Kumar S. 2013. MEGA6: Molecular evolutionary genetics analysis Version 6.0. *Molecular Biology and Evolution* 30 (12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Udar R. 1964. Palynology of Bryophytes. *In*: Nair P.K.K. (ed.) *Advances in Palynology*: 79–100. National Botanical Gardens, Lucknow.

Udar R. & Singh D.K. 1978. *Cyathodium indicum* Udar et Singh, sp. nov. from western Himalayas India. *Journal of Bryology* 10 (2): 139–142.

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 Phytologist* 209: 1734–1746. https://doi.org/10.1111/nph.13716

Wang L.S., Jia Y., Zhang X.C. & Qin H.N. 2018. *Species Catalogue of China. Vol. 1: Plants: A Synoptic Checklist (I)*. Science Press, Beijing. [In Chinese.]

Wilson R., Gradstein S., Heinrichs J., Groth H., Ilkiu–Borges A. & Hartmann F. 2004. Phylogeny of Lejeuneaceae: a cladistic analysis of chloroplast gene *rbc*L sequences and morphology with preliminary comments on the mitochondrial *nad*4-2 spacer region. *Monographs in Systematic Botany from the Missouri Botanical Garden* 98: 189–202.

Wu Y.H. & Gao C. 2006. Notes on Chinese *Cyathodium* Kunze. *Bulletin of Botanical Research* 26 (5): 522–526. [In Chinese.]

Xiang Y.L. & Zhu R.L. 2019. Sphaerocarpales (Marchantiophyta) new to China, with special references to a new species of *Sphaerocarpos* from Hengduan Mountains. *The Bryologist* 122 (4): 586–596. https://doi.org/10.1639/0007-2745-122.4.586

Xiang Y.L. & Zhu R.L. 2023. Morphological and molecular evidence for a new species, *Mannia gradsteinii* sp. nov. (Aytoniaceae) from southwestern China. *Cryptogamie, Bryologie* 44: 237–245. https://doi.org/10.5252/cryptogamiebryologie2023v44a12

Xiang Y.L. & Zhu R.L. 2025. Phylogenetic position of *Cyathodium indicum* (Cyathodiaceae, Marchantiophyta), a rare Indian liverwort newly found in China. *Journal of Bryology* 47 (2): 118–126. https://doi.org/10.1080/03736687.2025.2493477

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 thalloid liverworts (Marchantiopsida) based on chloroplast genomes. *Cladistics* 38 (6): 649–662. https://doi.org/10.1111/cla.12513

Zanten B.O. van & Gradstein S.R. 1988. Experimental dispersal geography of Neotropical liverworts. *Beihefte zur Nova Hedwigia* 90: 41–94.

Zhang D., Gao F., Jakovlić I., Zou H., Zhang J., Li W.X. & Wang G.T. 2020. PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. *Molecular Ecology Resources* 20 (1): 348–355. https://doi.org/10.1111/1755-0998.13096.

Zheng T.X., Inoue Y., Luo L., He Q., Wang P. & Jia Y. 2025. A northernmost distribution record of *Cyathodium* (Cyathodiaceae), with validation of subg. *Metacyathodium*. *Taiwania* 70 (2): 371–376. https://doi.org/10.6165/tai.2025.70.371

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