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You-Liang XIANG, Xiao-Ying MA, Chao SHEN,
Shi-Hui LU, Wen-Zhuan HUANG, Gui-Quan TIAN &
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The Macaronesian liverwort *Riccia boumanii* Dirkse, Losada & M.Stech (Marchantiophyta: Ricciaceae) confirmed new to Asia by morphological and molecular evidence

You-Liang XIANG
Xiao-Ying MA
Chao SHEN
Shi-Hui LU
Wen-Zhuan HUANG

Bryology Laboratory, School of Life Sciences, East China Normal University,
500 Dongchuan Road, Shanghai 200241 (China)

Gui-Quan TIAN

College of Life Science and Technology, Inner Mongolia Normal University,
Hohhot 010022 (China)

Rui-Liang ZHU

Bryology Laboratory, School of Life Sciences, East China Normal University,
500 Dongchuan Road, Shanghai 200241 (China)
and Tiantong National Station of Forest Ecosystem, Shanghai Key Lab for Urban Ecological
Processes and Eco-Restoration, East China Normal University,
500 Dongchuan Road, Shanghai 200241 (China)
rlzhu@bio.ecnu.edu.cn (corresponding author)

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ABSTRACT

Riccia L. is the largest genus of complex thalloid liverworts with over 250 species currently accepted. *Riccia boumanii* Dirkse, Losada & M.Stech (Ricciaceae) has been considered to be endemic to the Canary Islands, Spain. Morphological evidence and molecular-phylogenetic analyses based on sequences of nuclear ribosomal ITS2, plastid *rbcL*, and plastid *trnL-F* confirm that this species occurs in Gansu and Sichuan, China. *Riccia boumanii* represents the first species of section *Pilifer* Volk of the genus in Asia. The low values of *p*-distances (0% for *trnL-F*-region, and 1.6% for ITS2-region) reveal that *R. boumanii* bears a low level of divergence. Micrographs, a distribution map and descriptions based on the Chinese plants are provided. The range extension of *Riccia boumanii* suggests that more taxa of *Riccia* may have a wider distribution.

KEY WORDS
China,
disjunct distribution,
Eurasian distribution,
liverworts,
Marchantiales,
p-distances.

RÉSUMÉ

L'hépatique macaronésienne Riccia boumanii Dirkse, Losada & M.Stech (Marchantiophyta : Ricciaceae) confirmée nouvelle en Asie par des preuves morphologiques et moléculaires.

Riccia L. est le plus grand genre d'hépatiques thalloïdes complexes avec plus de 250 espèces actuellement acceptées. *Riccia boumanii* Dirkse, Losada & M.Stech (Ricciaceae) a été considérée comme endémique des îles Canaries, en Espagne. Des preuves morphologiques et des analyses phylogénétiques moléculaires basées sur les séquences du ribosome nucléaire ITS2, du plastide *rbcL*, et du plastide *trnL-F* confirment que cette espèce est présente dans le Gansu et le Sichuan, en Chine. *Riccia boumanii* représente la première espèce de la section *Pilifer* Volk du genre en Asie. Les faibles valeurs des *p*-distances (0 % pour la région *trnL-F*, et 1.6 % pour la région ITS2) révèlent que *R. boumanii* présente un faible niveau de divergence. Des micrographies, une carte de distribution et des descriptions basées sur les plantes chinoises sont fournies. L'extension de l'aire de répartition de *Riccia boumanii* suggère que d'autres taxons de *Riccia* pourraient avoir une distribution plus large.

MOTS CLÉS
Chine,
répartition disjointe,
répartition eurasienne,
hépatiques,
Marchantiales,
p-distances.

INTRODUCTION

Riccia L. is the most species-rich genus in the complex thalloid liverworts (Marchantiopsida) with over 250 species currently accepted (Söderström *et al.* 2016; Xiang *et al.* 2022). Species diversity and distribution of the genus remain not well-known owing to the taxonomical difficulties and lack of revisions of the genus in most countries and regions (Na-Thalang 1980; Schuster 1992; Perold 1995; Bischler-Causse *et al.* 2005; Cargill *et al.* 2016; Cargill *et al.* 2021).

Riccia boumanii Dirkse, Losada & M.Stech was first described in 2016 based on the collection from the Canary Islands, Spain. It was thought to be a species endemic to the Canary Islands. The species was placed in *Riccia* subgenus *Riccia* section *Pilifer* Volk owing to the number of cells of the dorsal hyaline cell pillars, the shape of the epidermal cells, and the ornamentation of the distal spore face (Dirkse *et al.* 2016). Section *Pilifer* is a small section in *Riccia* with 18 species currently accepted (Dirkse *et al.* 2016; Söderström *et al.* 2016). It is mostly distributed in Africa, but with the only exception of *R. boumanii*, which is known in the Canary Islands (Perold 1999; Dirkse *et al.* 2016, 2018; Wigginton 2018; Hodgetts *et al.* 2020).

During our recent survey of complex thalloid liverworts in China, an interesting *Riccia* species similar to *Riccia albolimbata* S.W.Arnell with large hyaline scales extending beyond the margin of the thallus was discovered in southwestern and northwestern China. After a careful morphological and molecular study, we confirm that it is *Riccia boumanii*.

MATERIAL AND METHODS

TAXON SAMPLING

Two specimens of *Riccia boumanii* were collected in Gansu province and Sichuan province, China, respectively. They were deposited in the herbarium of the East China Normal University (HSNU). *Ricciocarpos natans* Corda and *Oxymitra incrassata* (Brot.) Sérgio & Sim-Sim were selected as outgroups. Two chloroplast genes (*rbcL* and *trnL-F*) of

O. incrassata were from different papers and combined after identifying no incongruence. One sample of *R. boumanii* from Sichuan, China and two samples of *R. albolimbata* from Neimenggu, China were newly sequenced in the present study, and the remaining sequences (including *R. elongata* Perold and *R. furfuracea* Perold belonging to section *Pilifer*) were downloaded from GenBank (Table 1). Forty-one accessions of *Riccia* were included in the phylogenetic analyses. The taxa, voucher information, and GenBank accession numbers are presented in Table 1.

MORPHOLOGICAL STUDY

The field photos were taken with a Canon camera (M6). Morphological and anatomical characters were photographed by using an Olympus BX43 microscope equipped with a DP71 digital camera. Surface ornamentations of spores were scanned and photographed using a Hitachi S4800 Scanning Electron Microscope (SEM).

DNA EXTRACTION AND SEQUENCING

The isolation of plant tissues and extraction of total DNA followed protocols previously used in the group (Zhu *et al.* 2017; Xiang & Zhu 2019; Shu *et al.* 2022). Three DNA loci (ITS2, *rbcL* and *trnL-F*) were sequenced by Jie Li Biology Inc., China (<http://www.genebioseq.com>).

PHYLOGENETIC ANALYSES

All the sequences were initially aligned using MUSCLE (Edgar 2004) implemented in the MEGA 6.06 (Tamura *et al.* 2013) and then optimized manually in PhyDE version 0.9971 (Müller *et al.* 2010) (<http://www.phyde.de/>). Ambiguous alignment regions were trimmed manually. Absent data were coded as missing. Phylogenetic analyses were carried out using the maximum likelihood (ML) and Bayesian inference (BI) methods. Maximum likelihood (ML) analyses were performed in IQtree v2.0.6 (Minh *et al.* 2020) with the sampling repeated 1000 times. The best-fitting substitution model (TN+F+I+G4 for the ITS2-partition, TIM3+F+I+G4 for the *rbcL*-partition, and HKY+F+G4 for *trnL-F*-partition) was selected by ModelFinder (Chernomor

TABLE 1. — Sequences used in the study, including taxa, vouchers, and GenBank accession numbers.

Taxon	Voucher	ITS2	<i>rbcl</i>	<i>trnL-F</i>
<i>Oxymitra incrassata</i> (Brot.) Sérgio & Sim-Sim I	Italy, Long 35508 (E)	–	KJ590914	–
<i>Oxymitra incrassata</i> (Brot.) Sérgio & Sim-Sim II	United States, Wheeler 180 (OSC)	–	–	AF227673
<i>Riccia albida</i> Sull. ex Austin I	Australia, Palmer 684 (1) (CANB)	KX468505	KX468602	KX468730
<i>Riccia albida</i> Sull. ex Austin II	Australia, Palmer 684 (2) (CANB)	–	KX468603	KX468731
<i>Riccia albolimbata</i> S.W.Arnell I	China, Tian 16345 (HSNU)	ON616703	ON564419	ON564422
<i>Riccia albolimbata</i> S.W.Arnell II	China, Tian 15004A (HSNU)	ON616704	ON564420	ON564423
<i>Riccia albolimbata</i> S.W.Arnell III	South Africa, 28906 (Dirkse)	KT947042	–	KT947021
<i>Riccia beyrichiana</i> Hampe ex Lehm.	Netherlands, 0874113/0874114 (L)	KT947034	–	KT947016
<i>Riccia billardieri</i> Mont. & Nees I	Australia, Cargill 1275 (CANB)	KX468482	KX468578	KX468701
<i>Riccia billardieri</i> Mont. & Nees II	Australia, Wirf 795 (A) (CANB)	KX468495	KX468593	KX468715
<i>Riccia boumanii</i> Dirkse, Losada & M.Stech I	Spain, 27586 (Dirkse)	KT947037	–	KT947018
<i>Riccia boumanii</i> Dirkse, Losada & M.Stech II	Spain, 15018 (Dirkse)	KT947038	–	KT947019
<i>Riccia boumanii</i> Dirkse, Losada & M.Stech III	Spain, 26114 (Dirkse)	KT947039	–	KT947020
<i>Riccia boumanii</i> Dirkse, Losada & M.Stech IV	China, Zhu 20160816-14C (HSNU)	ON616702	ON564418	ON564421
<i>Riccia cavernosa</i> Hoffm.	Australia, Casanova sn (CANB)	KX468524	KX468625	KX468757
<i>Riccia ciliifera</i> Link ex Lindenb.	Switzerland, Schill 37 (CANB)	–	DQ286022	–
<i>Riccia crinita</i> Taylor I	Australia, Curnow 2756 (CANB)	KX468517	–	KX468746
<i>Riccia crinita</i> Taylor II	Australia, Purdie 8604 (1) (CANB)	KX468514	KX468612	KX468741
<i>Riccia crozalsii</i> Levier	Australia, Milne 291 (CANB)	KX468498	–	KX468721
<i>Riccia crystallina</i> L.	Australia, Purdie 8659 (CANB)	–	KX468596	KX468718
<i>Riccia elongata</i> Perold I	South Africa, 28895 (Dirkse)	KT947035	–	KT947017
<i>Riccia elongata</i> Perold II	South Africa, 28898 (Dirkse)	KT947036	–	–
<i>Riccia fluitans</i> L. I	United States, Cady s.n. (E)	–	DQ286023	–
<i>Riccia fluitans</i> L. II	China, Zhu 20191213-3 (HSNU)	–	OL771231	OL771243
<i>Riccia furfuracea</i> Perold	South Africa, 28899 (Dirkse)	KT947043	–	–
<i>Riccia gangetica</i> Ahmad ex L.Söderstr., A.Hagborg & von Konrat I	Australia, Cargill 1276 (CANB)	KX468483	KX468579	KX468702
<i>Riccia gangetica</i> Ahmad ex L.Söderstr., A.Hagborg & von Konrat II	Australia, Cargill 1318 (1) (CANB)	–	KX468607	KX468735
<i>Riccia glauca</i> L. I	Netherlands, 0874129 (L)	KT947033	–	KT947015
<i>Riccia glauca</i> L. II	Netherlands, 0874131 (L)	KT947032	–	KT947014
<i>Riccia gougetiana</i> Durieu & Mont.	United States, Wheeler 169 (OSC)	–	–	AF227656
<i>Riccia huebeneriana</i> Lindenb. I	China, Zhu & Promma 20160728-46 (HSNU)	–	OL771229	OL771241
<i>Riccia huebeneriana</i> Lindenb. II	Netherlands, 0874132 (L)	KT947028	–	KT947010
<i>Riccia junghuhniana</i> Nees & Lindenb. I	Australia, Cowie 13386 (CANB)	KX468493	KX468591	KX468713
<i>Riccia junghuhniana</i> Nees & Lindenb. II	Australia, Curnow 3126 (CANB)	KX468518	KX468617	KX468748
<i>Riccia lamellosa</i> Raddi I	Spain, 10558 (Dirkse)	KT947045	–	KT947023
<i>Riccia lamellosa</i> Raddi II	Portugal, 28912 (Dirkse)	KT947046	–	KT947024
<i>Riccia lamellosa</i> Raddi III	Spain, 26101 (Dirkse)	KT947044	–	KT947022
<i>Riccia multifida</i> (Steph.) Steph. I	Australia, Cargill 1316 (CANB)	KX468492	KX468590	KX468712
<i>Riccia multifida</i> (Steph.) Steph. II	Australia, Wirf 831 (CANB)	KX468507	KX468605	KX468733
<i>Riccia pullulans</i> Jovet-Ast I	Australia, Palmer 684 (3) (CANB)	KX468506	KX468604	KX468732
<i>Riccia pullulans</i> Jovet-Ast II	Australia, Franks 1305051 (CANB)	KX468522	KX468621	KX468752
<i>Riccia sorocarpa</i> Bisch. I	Australia, Cargill 926 (CANB)	KX468525	KX468626	KX468758
<i>Riccia sorocarpa</i> Bisch. II	Netherlands, 0874138 (L)	KT947030	–	KT947012
<i>Ricciocarpos natans</i> (L.) Corda I	Australia, Milne 349 (CANB)	KX468512	KX468611	KX468739
<i>Ricciocarpos natans</i> (L.) Corda II	China, Zhu <i>et al.</i> 20190414-31 (HSNU)	–	OL771232	OL771244

et al. 2016; Kalyaanamoorthy *et al.* 2017) according to the Bayesian Information Criterion (BIC). Bayesian inferences (BI) were conducted in MrBayes v3.2.6 (Ronquist *et al.* 2012) and were performed on the Cipres Science Gateway website (Müller *et al.* 2010). The best-fitting evolutionary model for each of the single partitions was selected based on the Akaike information criterion using the software jModelTest version 2.1.10 (Darriba *et al.* 2012). Nucleotide substitution models (GTR+G+I) is the best-fit model for the ITS2-partition, *rbcl*-partition and *trnL-F*-partition. The Markov Chain Monte Carlo (MCMC) analyses were run with one cold chain and three heated chains for five million generations and sampled every 1000 generations. The convergence of the MCMC chains was assumed when the average standard deviation of split frequencies (ASDFs) reached 0.01 or less. A 50% majority-rule consensus tree and

posterior probabilities (PP) were calculated after discarding 25% of trees as burn-in.

The value of infrageneric and infraspecific variability (*p*-distances) of DNA locus (*trnL-F* and ITS2) for all *Riccia* species was calculated in Mega 6.06 (Tamura *et al.* 2013), using the pairwise deletion option for counting gaps. The *p*-distances of the *rbcl*-region were not calculated because the *rbcl* sequences of *Riccia boumanii* from the Canary Islands were missing.

RESULTS

The combined dataset of 45 specimens included 2603 aligned nucleotides, 1820 are constant and 608 are parsimony informative. The *Riccia* clade is well supported as monophyletic

TABLE 2. — The value of intraspecific (INT) and infrageneric *p*-distances of *trnL*-region and ITS2-region (%) for *Riccia* L. species. n/a, missing data.

	INT	1	2	3	4	5	6	7	8	9	10	11
1. <i>R. albida</i>	0.4/n/a	–	–	–	–	–	–	–	–	–	–	–
2. <i>R. albolimbata</i>	0.2/2.7	4.2/24.1	–	–	–	–	–	–	–	–	–	–
3. <i>R. beyrichiana</i>	n/a	5.7/28.9	4.7/31.3	–	–	–	–	–	–	–	–	–
4. <i>R. billardieri</i>	1.1/6.1	5.8/22.0	6.2/29.4	8.7/34.3	–	–	–	–	–	–	–	–
5. <i>R. boumanii</i> China	n/a	3.6/21.4	1.0/9.6	5.3/28.9	6.0/26.5	–	–	–	–	–	–	–
6. <i>R. boumanii</i> Spain	0/0.1	4.0/25.7	1.0/11.2	5.3/29.5	6.7/30.6	0/1.6	–	–	–	–	–	–
7. <i>R. cavernosa</i>	n/a	4.5/23.8	4.9/20.3	6.7/28.2	7.0/26.5	4.7/17.7	5.3/20.2	–	–	–	–	–
8. <i>R. ciliifera</i>	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	–	–	–	–
9. <i>R. crinita</i>	0.8/2.8	5.0/26.7	5.1/28.7	2.9/23.3	8.6/31.3	5.0/26.9	5.7/30.5	6.0/26.0	n/a	–	–	–
10. <i>R. crozalsii</i>	n/a	4.6/26.9	3.9/28.0	0.8/13.0	7.7/32.0	4.2/26.0	4.7/28.1	5.6/26.4	n/a	2.8/22.3	–	–
11. <i>R. crystallina</i>	n/a	4.5/n/a	4.8/n/a	6.7/n/a	7.7/n/a	4.5/n/a	5.1/n/a	2.5/n/a	n/a	6.1/30.8	5.6/n/a	–
12. <i>R. elongata</i>	n/a/0.4	5.2/26.7	2.4/10.5	6.2/29.1	7.6/29.8	2.0/3.5	2.0/5.3	6.8/20.6	n/a	6.1/n/a	6.1/27.3	6.6/n/a
13. <i>R. fluitans</i>	n/a	5.7/n/a	5.7/n/a	7.0/n/a	6.7/n/a	5.4/n/a	6.1/n/a	6.0/n/a	n/a	6.6/n/a	6.0/n/a	6.2/n/a
14. <i>R. furfuracea</i>	n/a	n/a/20.4	n/a/7.3	n/a/19.0	n/a/23.7	n/a/5.8	n/a/6.1	n/a/8.7	n/a	n/a/20.8	n/a/19.6	n/a
15. <i>R. gangetica</i>	0.2/n/a	5.6/22.7	6.2/32.7	8.7/37.2	5.6/21.0	5.8/29.6	6.4/33.5	7.4/29.7	n/a	7.9/34.4	7.6/33.3	8.0/n/a
16. <i>R. glauca</i>	0/0	5.2/25.9	4.0/29.7	0.6/8.0	8.3/34.0	4.7/29.1	4.7/30.0	6.0/28.6	n/a	2.9/21.6	0.4/11.7	6.0/n/a
17. <i>R. gougetiana</i>	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
18. <i>R. huebeneriana</i>	0/n/a	7.1/26.8	7.1/29.0	7.3/27.4	9.5/29.7	7.1/26.2	7.3/27.3	7.3/24.2	n/a	6.8/28.0	7.0/27.4	7.5/n/a
19. <i>R. junghuhniana</i>	0.2/0.7	5.9/25.8	5.9/27.5	6.9/33.1	8.8/29.4	5.9/24.4	6.7/27.9	6.9/23.6	n/a	6.4/28.1	5.8/26.4	6.5/n/a
20. <i>R. lamellosa</i>	0/2.0	4.5/20.1	1.0/8.3	5.3/16.1	6.3/23.1	1.3/8.7	1.3/9.0	5.3/9.4	n/a	5.6/17.8	4.7/16.9	5.1/n/a
21. <i>R. multifida</i>	0.6/0	6.6/23.5	6.6/25.2	6.5/28.6	8.9/25.7	6.4/22.2	7.0/26.4	6.6/22.2	n/a	6.2/25.5	5.7/25.0	6.9/n/a
22. <i>R. pullulans</i>	0.4/2.0	5.3/24.8	5.3/28.5	5.7/31.2	8.4/29.9	5.4/25.8	5.8/29.2	6.5/25.0	n/a	5.7/27.7	5.3/27.0	6.1/n/a
23. <i>R. sorocarpa</i>	0.4/2.0	5.6/24.2	5.0/19.6	3.6/11.5	8.4/26.9	5.2/18.6	5.6/19.9	6.5/15.1	n/a	3.4/15.0	3.7/10.1	6.3/n/a

	12	13	14	15	16	17	18	19	20	21	22	23
1. <i>R. albida</i>	–	–	–	–	–	–	–	–	–	–	–	–
2. <i>R. albolimbata</i>	–	–	–	–	–	–	–	–	–	–	–	–
3. <i>R. beyrichiana</i>	–	–	–	–	–	–	–	–	–	–	–	–
4. <i>R. billardieri</i>	–	–	–	–	–	–	–	–	–	–	–	–
5. <i>R. boumanii</i> China	–	–	–	–	–	–	–	–	–	–	–	–
6. <i>R. boumanii</i> Spain	–	–	–	–	–	–	–	–	–	–	–	–
7. <i>R. cavernosa</i>	–	–	–	–	–	–	–	–	–	–	–	–
8. <i>R. ciliifera</i>	–	–	–	–	–	–	–	–	–	–	–	–
9. <i>R. crinita</i>	–	–	–	–	–	–	–	–	–	–	–	–
10. <i>R. crozalsii</i>	–	–	–	–	–	–	–	–	–	–	–	–
11. <i>R. crystallina</i>	–	–	–	–	–	–	–	–	–	–	–	–
12. <i>R. elongata</i>	–	–	–	–	–	–	–	–	–	–	–	–
13. <i>R. fluitans</i>	7.0/n/a	–	–	–	–	–	–	–	–	–	–	–
14. <i>R. furfuracea</i>	n/a/4.4	n/a	–	–	–	–	–	–	–	n/a	–	–
15. <i>R. gangetica</i>	6.2/33.9	7.9/n/a	n/a/26.2	–	–	–	–	–	–	–	–	–
16. <i>R. glauca</i>	6.0/29.5	6.7/n/a	n/a/20.3	8.5/34.5	–	–	–	–	–	–	–	–
17. <i>R. gougetiana</i>	n/a	n/a	n/a	n/a	n/a	–	–	–	–	–	–	–
18. <i>R. huebeneriana</i>	7.8/26.0	8.3/n/a	n/a/18.4	8.9/32.0	7.1/26.6	n/a	–	–	–	–	–	–
19. <i>R. junghuhniana</i>	7.4/25.9	7.2/n/a	n/a/18.1	7.6/31.1	6.7/26.4	n/a	3.2/10.5	–	–	–	–	–
20. <i>R. lamellosa</i>	5.9/7.1	5.9/n/a	n/a/7.5	6.4/23.4	4.7/16.5	n/a	7.3/17.9	6.7/16.6	–	–	–	–
21. <i>R. multifida</i>	7.7/24.4	7.5/n/a	n/a/17.9	8.3/30.0	6.3/26.3	n/a	1.7/6.7	2.7/11.3	7.0/17.8	–	–	–
22. <i>R. pullulans</i>	6.5/28.2	6.8/n/a	n/a/17.3	7.2/31.2	5.5/27.1	n/a	2.8/11.7	1.1/8.2	5.8/15.6	2.6/11.4	–	–
23. <i>R. sorocarpa</i>	6.0/18.2	6.8/n/a	n/a/19.5	8.1/27.3	3.4/11.9	n/a	7.3/20.3	6.6/19.7	5.6/16.4	6.3/19.1	5.7/18.1	–

(MLBS = 96; PP = 1). Within *Riccia*, four samples of *Riccia boumanii* from the Canary Islands (type locality) and China form a well-supported clade (MLBS = 96; PP = 1), which is sister to *Riccia elongata* (MLBS = 96; PP = 1) (Fig. 1).

The interspecific *p*-distance in the *trnL*-F-region and ITS2-region within the *Riccia* species varies from 0.4% to 9.5%, and from 3.2% to 37.2%, respectively (Table 2). The observed intraspecific *p*-distances in our analysis vary from 0% to 1.1% in *trnL*-F-region, and from 0% to 6.1% in ITS2-region (Table 2). *Riccia billardierei* Mont. & Nees is highly divergent with the highest intraspecific *p*-distances (1.1% in *trnL*-F-region and 6.1% in ITS2-region) (Table 2). *Riccia boumanii* from China and the Canary Islands are characterized by a low level of divergence, the value of

p-distances calculated for *trnL*-F-region is 0% and ITS2-region is 1.6% (Table 2).

Family RICCIACEAE Rchb.
Genus *Riccia* L.

Riccia boumanii Dirkse, Losada & M.Stech
(Fig. 2)

Journal of Bryology 38 (2): 96 (Dirkse *et al.* 2016). — Type: Spain. Prov. de Santa Cruz de Tenerife, La Gomera, La Fortaleza de Chipude, N slopes and plateau, 28°5'47.05"N, 17°17'24.31"W, 1200-1243 m a.s.l., 16.III.1988, G.M. Dirkse (holo-, RIN[RIN-015035]).

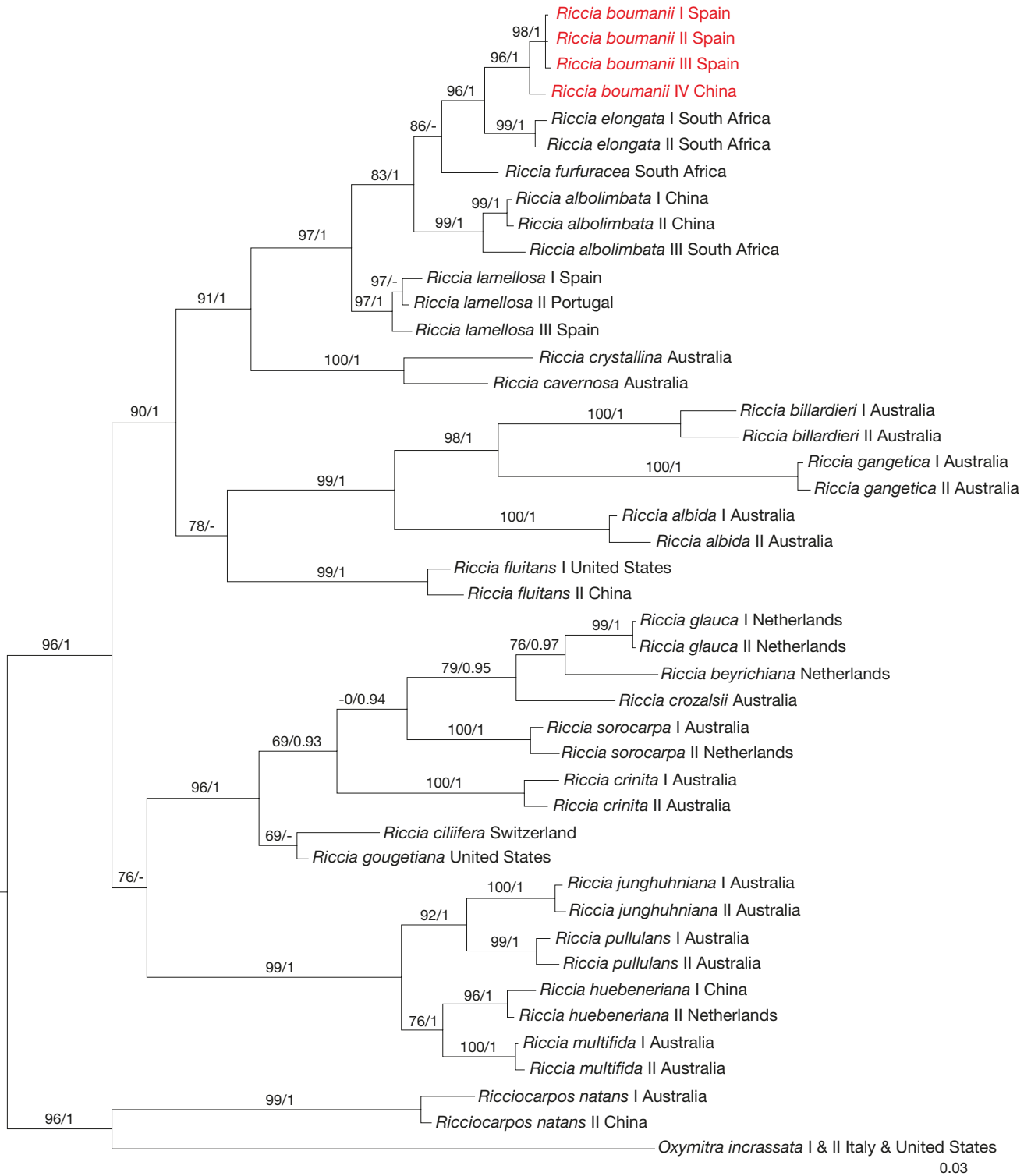


FIG. 1. — Phylogeny of *Riccia* L. illustrating position of *Riccia boumanii* Dirkse, Losada & M. Stech, inferred from combined dataset (*rbcL*, *trnL-F* and ITS2) and topology displayed as majority rule consensus tree of trees recovered in stationary phase of Bayesian search. ML bootstrap values BS \geq 65 and Bayesian posterior probabilities values PP \geq 0.90 are shown at left and at right, respectively.

SPECIMENS EXAMINED. — **China.** Gansu, Maqu County, slope near road G345, 33°51'28.14"N, 101°53'28.14"E, 3744 m a.s.l., on soil, C. Shen et al. 20200827-53 (HSNU); Sichuan, Xiaojin County, Siguniang Mountains Nature Reserve, Haizigou, Huahaizi, 31°02'23.66"N, 102°55'52.76"E, 3828 m a.s.l., on soil, R.L. Zhu 20160816-14C (HSNU).

DISTRIBUTION AND HABITAT. — Known from Europe (the Canary Islands) and Asia (China) (Fig. 3). In China, it grows on moist and shady soil, from 3744 m to 3828 m (Fig. 4). The elevation of the populations in China is much higher than that of the type locality, which is from 100 m to 1640 m.

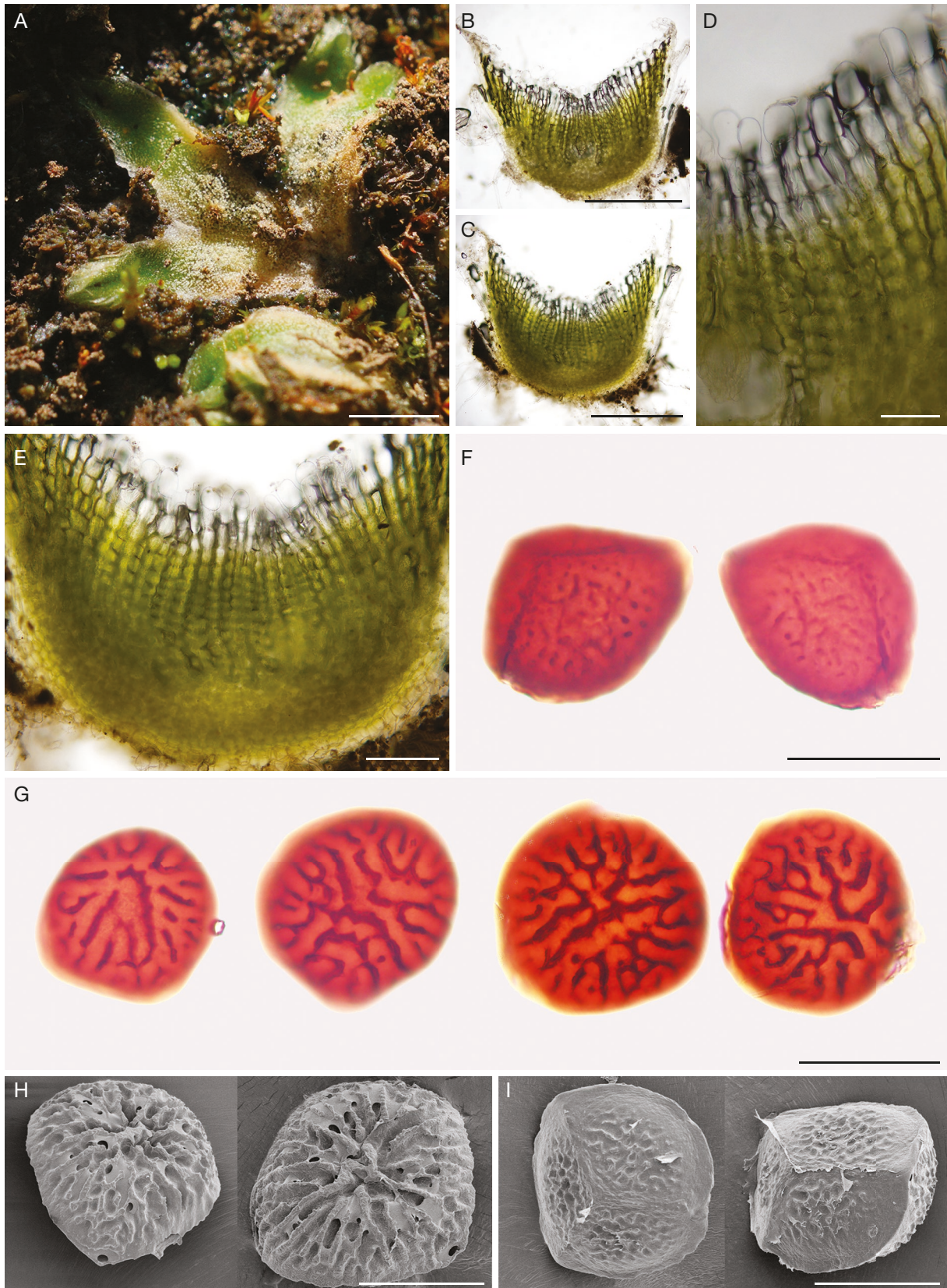


FIG. 2. — *Riccia boumanii* Dirkse, Losada & M.Stech: **A**, habit in the field; **B-G**, light microscopy micrographs; **B-E**, transverse section of segments; **D**, showing the dorsal hyaline epithelial cells; **F**, proximal face of spore; **G**, distal face of spore; **H, I**, SEM micrographs of spores; **H**, distal face; **I**, proximal face. All from R.L. Zhu 20160816-14C (HSNU). Scale bars: A, 2 mm; B, C, 500 μ m; D, 100 μ m; E, 200 μ m; F-I, 50 μ m.

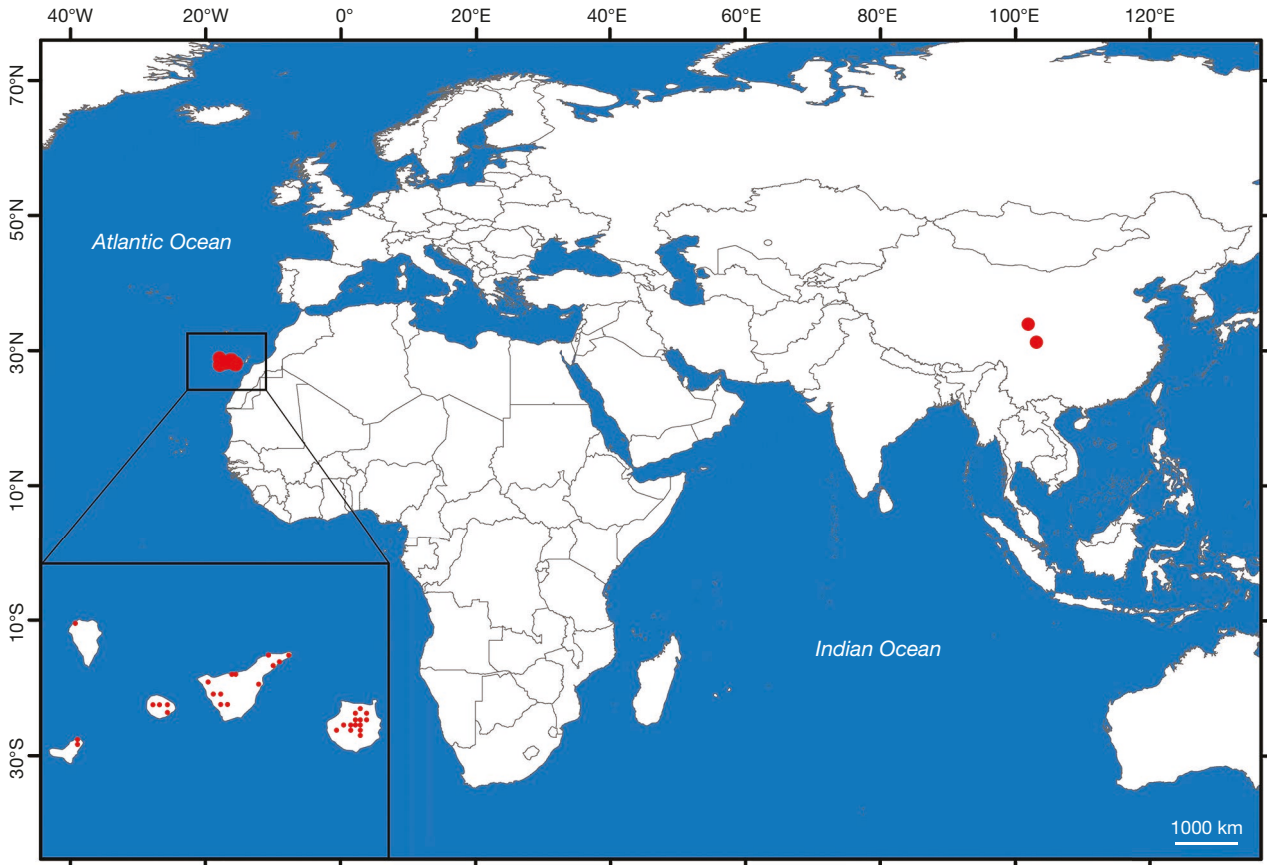


FIG. 3. — Distribution of *Riccia boumanii* Dirkse, Losada & M.Stech (red dots), based on data from Dirkse *et al.* (2016) and this study. The distribution in the Canary Islands is shown in the black rectangle.

DESCRIPTION

Plants in rosettes, irregular patches or gregarious; terrestrial. Thalli small to medium-sized, 0.8–2.1 mm wide, 5–8 mm long; (1)–2–3-times furcated, shortly to deeply divided. Dorsal surface glabrous, glistening greyish-green when wet, dull, scurfy when dry; median furrow distinct at apex, but immediately become shallow and disappears posteriorly. Dorsal epidermis 2–5-stratose, cells spherical or ovoid, hyaline, in loose cell pillars. Thallus sections of lobes 0.82–1.2 mm high and 1.5–2 times as wide; air chambers absent, but consist of a series of photosynthetic filaments, 8–10 cells high. Storage tissue cells irregularly rounded, thin-walled. Ventral scales prominent, hyaline white, never purplish or violet, wavy, quadrate to hexagonal, mostly going beyond thallus margin, thin-walled. Cilia absent. Rhizoids smooth and pegged. Monoicous. Antheridia scarce, conspicuous near median furrow. Spores tetrahedral, 83–100 μm in diameter, dark brown or blackish; distal surface largely lacunose, low radiating ridges run over distal face, gradually forming small areolae, 3–10 μm in diameter; proximal surface with straight or fragmented trilete mark, distinct, areolae incomplete with papillae or short tubercles at the corners; wing distinct, 7–9 μm wide.

DISCUSSION

The remote populations of *Riccia boumanii* from the Canary Islands (Spain) and China are characterized by a low level of divergence (Table 2). In morphology, the Chinese plants are also almost identical with the Spanish ones. Compared with the Spanish samples, the Chinese plants, however, exhibit a minor variation in spore patterning with fewer papillae on the proximal surface. The low level of genetic divergence together with morphological similarities, allows them to be assigned to a single species. This is thus far its first report outside the Canary Islands. In addition, the section *Pilifer* is newly reported for Asia. The values of infraspecific *p*-distances in *R. billardierei* (1.1% in *trnL-F*-region and 6.1% in ITS2-region) suggest a divergence between populations. Further study may result in the recognition of separate taxa in *R. billardierei* as in *Riccia crinita* Taylor (Xiang *et al.* 2022).

Riccia boumanii is a recently described species from the Canary Islands, a geographic area of islands in the North Atlantic Ocean off the coasts of Europe and Africa. As commented by Dirkse *et al.* (2016), the species is characterized and easily recognized by: 1) medium-sized thallus up to 2.5 mm wide; 2) glistening grey-green dorsal surface when



FIG. 4. — Habitat of *Riccia boumanii* Dirkse, Losada & M.Stech in Huahaizi, Haizigou, Siguniang Mountains Nature Reserve, Sichuan, China. The population was found around the lake.

fresh and whitish scurfy when dry; 3) dorsal pillars made up of multiple hyaline cells not tapering; 4) large hyaline-white ventral scales projecting well beyond the thallus margins; and 5) distal spore faces with radiating walls and few central areolae. *Riccia boumanii* is morphologically close to *R. elongata* and *R. concava* Bisch. also in section *Pilifer*. *Riccia boumanii* may possibly be confused with *R. elongata*, both of which have medium-sized thalli, similar multiple epidermal cell layer and spore size. *Riccia elongata*, however, differs in the narrow spore wings only 3-5 μm wide, and a thallus with red or purple flanks due to the basal parts of scales (Dirkse *et al.* 2016). *Riccia boumanii* is also similar to *R. concava* restricted to the Cape region of South Africa (Perold 1999; Dirkse *et al.* 2016). They share several important characters including the photosynthetic tissue consisting of tightly packed vertical columns of cells, the large hyaline scales and the dorsal epithelium consisting of free cell pillars. Unfortunately, no molecular data of *R. concava* are available for testing their relationship. In China, *Riccia boumanii* is easily confused with *R. albolimbata*. Both of them are autoicous, with large hyaline scales extending beyond the margin of the thallus and with photosynthetic tissue consisting of tightly packed vertical columns of cells. *Riccia albolimbata*, however, can be separated by the unistratose dorsal epidermis, the distal surface of the spore with 10-12 fine areolae across the diameter, and a narrow spore wing, 3-5 μm wide.

The geographical locations of *R. boumanii* in China are a long distance from the Canary Islands. Its distribution pattern

is obviously disjunct. More populations may be discovered in Asia and Europe after an intensive survey is made. Compared with other genera of complex thalloid liverworts, molecular data of *Riccia* are available only in some 42 species. It is still a tiny proportion of such a large cosmopolitan genus with over 250 species. The species diversity and distribution as well as the subgeneric classification need to be deeply investigated using integrative methods.

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