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# Morphological and molecular evidence for a new species, *Mannia gradsteinii* sp. nov. (Aytoniaceae) from southwestern China

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## ABSTRACT

*Mannia* Corda (Aytoniaceae) is a small complex thalloid liverwort genus with 11 species, which show strongly disjunct and fragmented distributional patterns. China is one of the main centers of distribution for the genus with six species currently known. *Mannia gradsteinii* sp. nov., a new species from Hengduan Mountains in China, is described and illustrated. It is similar to *Mannia triandra* (Scop.) Grolle, but differing in the large epidermal pore (72-)136-204 µm in diameter, the smaller spores 47-57 µm with 3-6 irregular areolae across the spore distal surface, the large female receptacles 4.6-8.2 mm in diameter and the air chambers with 2-4 layers. Molecular phylogenetic analysis of the chloroplast gene *trnL*-F and the large 26S ribosomal DNA subunit confirmed that the new species is a member of *Mannia* subgenus *Neesiella*, and sister to *M. gracilis* (F.Weber) D.B.Schill & D.G.Long, which differs in the presence of a pseudoperianth and the absence of a trilete mark on the spore proximal view.

KEY WORDS  
China,  
Yunnan,  
liverworts,  
*Mannia*,  
new species.

MOTS CLÉS  
Chine,  
Yunnan,  
hépatiques,  
*Mannia*,  
espèce nouvelle.

## RÉSUMÉ

*Résultats morphologiques et moléculaires pour une espèce nouvelle, Mannia gradsteinii sp. nov. (Aytoniaceae) du Sud-Ouest de la Chine.*

*Mannia* Corda (Aytoniaceae) est un petit genre d'hépatique thalloïde complexe comptant 11 espèces, qui présentent des schémas de distribution fortement disjoints et fragmentés. La Chine est l'un des principaux centres de distribution du genre, avec six espèces actuellement connues. *Mannia gradsteinii* sp. nov., une nouvelle espèce des montagnes Hengduan en Chine, est décrite et illustrée. Elle est similaire à *Mannia triandra* (Scop.) Grolle, mais diffère par le grand pore épidermique de (72-)136-204 µm de diamètre, les spores plus petites de 47-57 µm avec 3-6 aréoles irrégulières sur la surface distale des spores, les grands réceptacles femelles de 4,6-8,2 mm de diamètre et les chambres à air de 2-4 couches. L'analyse phylogénétique moléculaire du gène chloroplastique *trnL*-F et de la grande sous-unité 26S de l'ADN ribosomique a confirmé que la nouvelle espèce est membre du sous-genre *Neesiella*, et sœur de *M. gracilis* (F.Weber) D.B.Schill & D.G.Long, qui diffère par la présence d'un pseudoperianthe et l'absence d'une marque trilet sur la vue proximale de la spore.

## INTRODUCTION

The genus *Mannia* Corda is the third largest genus of Aytoniaceae (Marchantiales) with 11 species accepted worldwide (Schill 2006; Söderström *et al.* 2016). It is generally distinguished from the other genera of Aytoniaceae by the cup-shaped involucres and the hemispherical to globose receptacles (Long 2006; Schill *et al.* 2010). *Mannia* occurs almost exclusively in the northern hemisphere with exceptions in Africa and South America (Schuster 1992; Bischler-Causse *et al.* 2005; Schill 2006; Schill *et al.* 2010). With the transfer of *Asterella gracilis* (F.Weber) Underw. to *Mannia* (Schill *et al.* 2010), the pseudoperianth, typical for the genus *Asterella*, is regarded as a plesiomorphic feature for the whole of the Aytoniaceae (Xiang *et al.* 2022a; Long & Zheng 2023).

Hengduan Mountains, located in the Indo-Burmese hotspot of biodiversity (Myers *et al.* 2000), is well known as a hotspot of bryophyte diversity in China (Xiang *et al.* 2016; Xiang & Zhu 2019; Bakalin *et al.* 2021a, b; Huang *et al.* 2023). During our recent expeditions to the western Hengduan Mountains, we encountered an interesting thalloid liverwort belonging to *Mannia* in Weixi County, northwestern Yunnan. This plant is not assignable to any currently known species of *Mannia*. Morphological studies and molecular phylogenetic analyses revealed that it represents an undescribed species.

## MATERIAL AND METHODS

### TAXON SAMPLING

Two fresh samples of the new species *Mannia gradsteinii* sp. nov. were collected from Weixi County, Yunnan, China. *Plagiochasma rupestre* (J.R.Forst. & G.Forst.) Steph., *Plagiochasma wrightii* Sull. and *Reboulia hemisphaerica* (L.) Raddi were selected as outgroups. The sequences of five *Mannia* samples and two *Reboulia hemisphaerica* were newly generated in the present study, and the remaining sequences were downloaded from GenBank (Table 1). Twenty-seven accessions of *Mannia* were included in the phylogenetic analyses. The taxa, voucher information, and GenBank accession numbers are presented in Table 1.

### MORPHOLOGICAL STUDY

The field pictures were taken using a digital camera (Canon M6). Morphological and anatomical characters were measured and photographed by using an Olympus BX43 microscope equipped with a DP71 digital camera. Spores were scanned and photographed using a Hitachi S4800 SEM as in Xiang *et al.* (2022b).

### DNA EXTRACTION AND SEQUENCING

The isolation of plant tissues and extraction of total DNA followed protocols previously described by Xiang *et al.* (2016). The total genomic DNA was extracted by using the DNAeasy plant mini kits (Qiagen, Hilden, Germany). Two DNA fragments, the chloroplast gene *trnL*-F and the large 26S ribosomal DNA subunit were sequenced by Jie Li Biology Inc., China (<http://www.genebioseq.com>).

### PHYLOGENETIC ANALYSES

All obtained sequences were assembled and checked for improper base-calling using Geneious Prime v2022.2.2 (<https://www.geneious.com>) and aligned using MAFFT v7.311 (Katoh & Standley 2013). Ambiguous alignment regions were trimmed manually. Data that were lacking were coded as missing. The individual genetic locus and the combined dataset were first analyzed separately and compared by eye to check for possible incongruence in the topology. As the trees showed no conflicting nodes, the datasets were combined. Phylogenetic analyses were carried out using the Maximum likelihood (ML) and Bayesian inference (BI) methods. Maximum likelihood analyses were performed in IQtree v2.0.6 (Minh *et al.* 2020) with the sampling repeated 1000 times. The best-fitting substitution model (K3Pu + F + G4 for *trnL*-F partition; TIM2 + F + I + G4 for the 26S-partition) was selected by ModelFinder (Chernomor *et al.* 2016; Kalyaanamoorthy *et al.* 2017) according to the Bayesian Information Criterion (BIC). Bayesian inferences were conducted in MrBayes v3.2.6 (Ronquist *et al.* 2012) and were performed on the Cipres Science Gateway website (Miller *et al.* 2010). Nucleotide substitution models are the best-fit model for the *trnL*-F-partition (GTR + G), and for the 26S-partition (GTR + I + G) according to the Akaike Information Criterion (AIC). Bayesian operation settings refer to Xiang *et al.* (2022b).

### P-DISTANCES

The value of infrageneric and infraspecific variability (*p-distances*) of each DNA locus (*trnL*-F and 26S) for all *Mannia* species was calculated in Mega 6.06 (Tamura *et al.* 2013), using the pairwise deletion option for counting gaps.

## RESULTS

The combined dataset of 36 taxa included 1784 aligned nucleotides; 1522 were constant and 195 were parsimony informative, while 67 were parsimony-uninformative. The *Mannia* clade is well supported as monophyletic (MLBS = 100; PP = 1). Two well-supported clades are recognized for *Mannia* (Fig. 1; clades I-II). The new species, *Mannia gradsteinii* sp. nov. is further recovered in clade II. Two samples of *M. gradsteinii* sp. nov. form a well-supported clade (MLBS = 100; PP = 1), which is sister to *Mannia gracilis* (F.Weber) D.B.Schill & D.G.Long (MLBS = 92; PP = 1) (Fig. 1).

The observed interspecific *p-distances* in *trnL*-F-region and 26S-region, varied from 1.5%-7.6% and 0.8%-4.0%, respectively (Table 3). The observed intraspecific *p-distances* in our analysis varied from 0.2%-1.2% in *trnL*-F region and 0.1%-1.3% in the 26S-region. The value of *p-distances* calculated for *Mannia gradsteinii* sp. nov. and *M. gracilis* in *trnL*-F-region and 26S-region is 2.8% and 1.4%, respectively (Table 3). They are higher than the minimum interspecific *p-distances* in *Mannia* (1.5% in *trnL*-F-region and 0.8% in 26S-region).

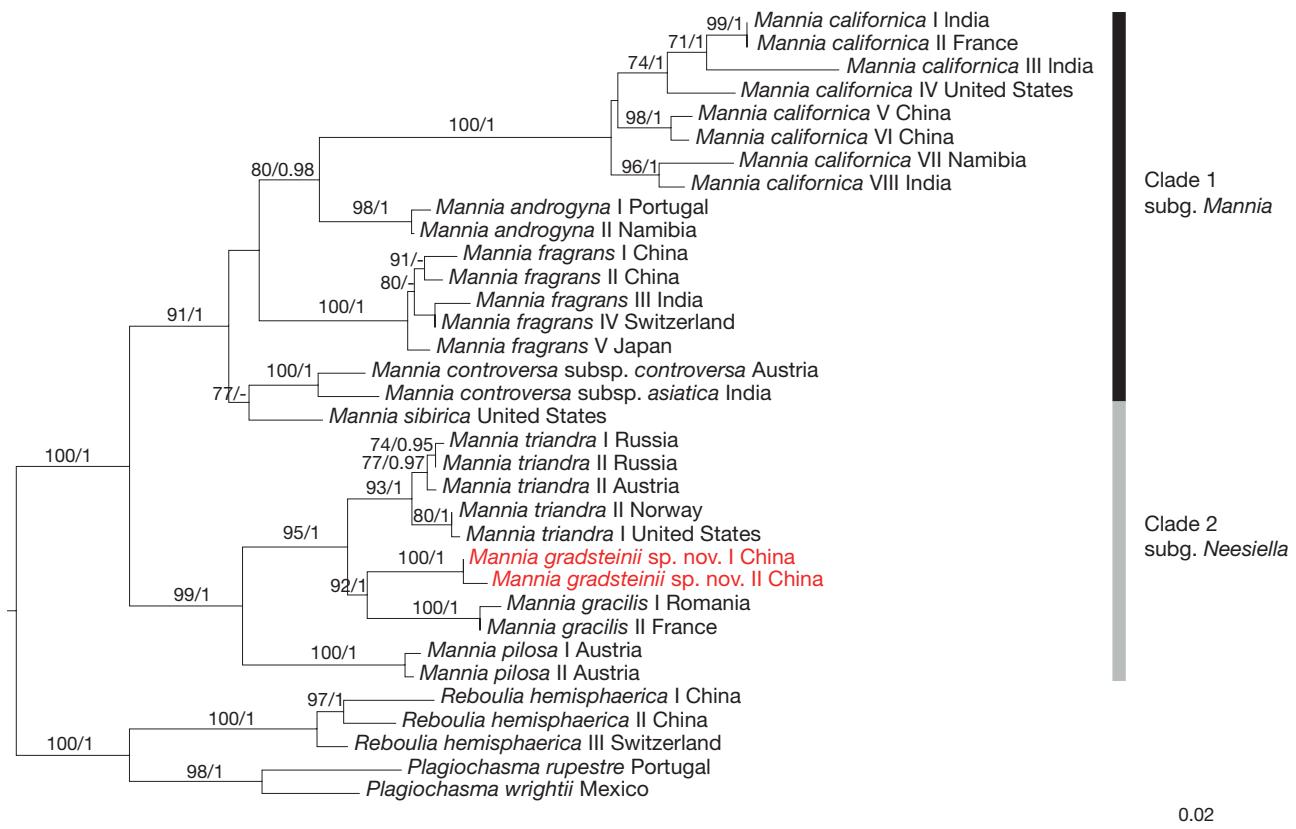


FIG. 1. — Phylogeny of *Mannia* Corda illustrating the position of *Mannia gradsteinii* sp. nov., inferred from combined dataset (*trnL-F* and *26S*) and topology displayed as the best-scoring ML tree in IQtree. ML bootstrap values BS $\geq$ 65 and Bayesian posterior probabilities values PP $\geq$ 0.90 are shown at left and at right, respectively.

### *Mannia gradsteinii* sp. nov. (Figs 2-4)

Similar to *Mannia triandra* (Scop.) Grolle but differing in larger epidermal pores (72)136-204  $\mu\text{m}$  in diameter, smaller spores 47-57  $\mu\text{m}$ , 3-6 areolae across the spore distal surface, air chambers with 2-4 layers and ventral scales not reaching beyond thallus edge.

**HOLOTYPE.** — **China.** Yunnan, Weixi County, Tacheng Town, near Duonage Village, 27°20'39.40"N, 99°19'01.22"E, 2270 m, on soil, 28.IV.2017, Y.L. Xiang & P.F. Wang 20170428-12 (holo-, HSNU!).

**ADDITIONAL SPECIMEN EXAMINED.** — **China.** Yunnan, Weixi County, Biluoxueshan, 28°08'18.59"N, 98°47'48.26"E, 3084 m, on soil, 28.IV.2017, Y.L. Xiang & P.F. Wang 20170428-3 (HSNU).

**ETYMOLOGY.** — *Mannia gradsteinii* sp. nov. is named in honor of the remarkable bryologist, Prof. Stephan Robbert Gradstein on the occasion of his 80<sup>th</sup> birthday.

**ECOLOGY AND DISTRIBUTION.** — *Mannia gradsteinii* sp. nov. occurs at altitudes of 2270-3084 m, growing on soil or on rock with a thin layer of soil under shrubs. Until now, it is known only from Weixi County, northwestern Yunnan, China.

### DESCRIPTION

Plants thalloid, light green on dorsal surface, thin and delicate, sub-dichotomously branched, lobes 8-19 mm long, 2.8-4.6 mm wide; dorsal surface flat to slightly concave, with faint reticulation mark due to the underlying chamber

walls; margins thin, not crenate; dorsal epidermal cells of thallus, 24-71  $\times$  22-42  $\mu\text{m}$ , thin-walled, trigones often absent; oil-cells scattered in epidermis, yellowish-brown; epidermal pores simple, (72)136-204  $\mu\text{m}$  in diameter, surrounded by 2-3 concentric rings, each formed by 6-9 cells, cells thin-walled, radial walls not thickened; assimilatory tissue loose, with large air chambers in 2-4 layers, occupying c. 2/3-3/4 of the thallus height in the middle and completely filling the wings, without photosynthetic filaments, seldom with inconspicuous yellowish brown oil bodies in assimilation and storage tissue; rhizoids of two kinds: smooth and pegged; ventral scales in two rows, not reaching beyond thallus edge, hyaline to pale purplish, semicircular to oblong semicircular or triangular, marginal slime-papillae one-celled, hyaline, abundant, oil cells numerous; appendages 1(-2) per scale, subulate, acuminate, purplish; appendage 324-441  $\mu\text{m}$  long and 73-193  $\mu\text{m}$  wide, without oil cells or sometimes with a single oil cell.

Monoicous. Archegoniophore arising from apices of main branches; androecia close to archegoniophore bases; androecia sessile, usually several, slightly upraised in poorly defined slightly raised rounded clusters or depressions; female receptacles hemispherical, without pseudoperianth, 4.6-8.2 mm in diameter; receptacle usually bearing four sporophytes; stalk 8.1-12.3 mm long, 0.4-0.6 mm in diameter, with single rhizoidal furrow; spores yellow to brown, 47-57  $\mu\text{m}$  in diameter (SEM), trilete, with similar sculpturing on proximal

TABLE 1. — List of taxa used for this study, including vouchers, localities and GenBank accession numbers.

Taxon	Voucher information	Localities	GenBank accession	
			trnL-F	nr 26S
<i>Mannia androgyna</i> (L.) A.Evans I	Schill & Lobos 32-2 (E)	Portugal, Madeira	GQ910679	GQ910718
<i>M. androgyna</i> II	Schill & Clarke 124 (E)	Namibia, Otozondjupa	GQ910680	GQ910719
<i>M. californica</i> (Gottsche ex Underw.) L.C.Wheeler I	Schill & Clarke 165 (E)	India, Uttaranchal	GQ910682	GQ910721
<i>M. californica</i> II	Hugonnnot s.n. (E)	France, Ardèche	GQ910688	GQ910727
<i>M. californica</i> III	Schill & Clarke 164 (E)	India, Uttaranchal	GQ910681	GQ910720
<i>M. californica</i> IV	Schill & Clarke 102 (E)	United States, California	GQ910686	GQ910725
<i>M. californica</i> V	Xiang et al. 20160418-21 (HSNU)	China, Yunnan	OR689868	OR700027
<i>M. californica</i> VI	Long 35035 (E)	China, Yunnan	GQ910687	GQ910726
<i>M. californica</i> VII	Schill & Clarke 131 (E)	Namibia, Otozondjupa	GQ910684	GQ910723
<i>M. californica</i> VIII	Schill & Clarke 144 (E)	India, Tamil Nadu	GQ910685	GQ910724
<i>M. controversa</i> subsp. <i>controversa</i> (Meyl.) D.B.Schill	Köckinger s.n. (E)	Austria, Carinthia	GQ910689	GQ910728
<i>M. controversa</i> subsp. <i>asiatica</i> D.B.Schill & D.G.Long	Schill & Clarke 163 (E)	India, Uttaranchal	GQ910696	GQ910735
<i>M. fragrans</i> (Balb.) Frye & L.Clark I	Xiang 20160925-8 (HSNU)	China, Sichuan	OR689869	OR700028
<i>M. fragrans</i> II	Tian 15080 (HSNU)	China, Neimenggu	OR689870	—
<i>M. fragrans</i> III	Schill & Clarke 162 (E)	India, Uttaranchal	GQ910690	GQ910729
<i>M. fragrans</i> IV	Schill et al. 51 (E)	Switzerland, Valais	GQ910692	GQ910731
<i>M. fragrans</i> V	Itouga s.n. (E)	Japan, Honshu	GQ910691	GQ910730
<i>M. gracilis</i> (F.Weber) D.B.Schill & D.G.Long I	Stefanut B3497 (E)	Romania, Bucegi	GQ910668	GQ910707
<i>M. gracilis</i> II	Hugonnnot s.n. (E)	France, Haute-Loire	GQ910669	GQ910708
<i>M. gradsteinii</i> sp. nov. I	Xiang & Wang 20170428-12 (HSNU)	China, Yunnan	OR689871	OR700029
<i>M. gradsteinii</i> sp. nov. II	Xiang & Wang 20170428-3 (HSNU)	China, Yunnan	OR689872	OR700030
<i>M. pilosa</i> (Hornem.) Frye & L.Clark I	Schill et al. 84 (E)	Austria, Carinthia	GQ910693	GQ910732
<i>M. pilosa</i> II	Schill et al. 85 (E)	Austria, Carinthia	GQ910694	GQ910733
<i>M. sibirica</i> (K.Mull.) Frye & Clark	Long & Schill 35669 (E)	United States, Minnesota	GQ910695	GQ910734
<i>M. triandra</i> (Scop.) Grolle I	Konstantinova 82-1-00 (KPABG)	Russia, Kemerovo	KR024197	KR024171
<i>M. triandra</i> II	Polozova s.n. (LE, KPABG)	Russia, Chukotka	KR024196	—
<i>M. triandra</i> III	Schill et al. 87 (E)	Austria, Carinthia	GQ910698	GQ910736
<i>M. triandra</i> IV	Borovichev BE171-5-09 (KPABG)	Norway, Svalbard	KR024195	KR024170
<i>M. triandra</i> V	Long & Schill 35668 (E)	United States, Minnesota	GQ910697	GQ910737
<i>Plagiochasma rupestre</i> (J.R.Forst. & G.Forst.) Steph.	Schill 5 (E)	Portugal, Madeira	GQ910700	GQ910739
<i>P. wrightii</i> Sull.	Long 29636 (E)	Mexico, Veracruz	GQ910701	GQ910740
<i>Reboulia hemisphaerica</i> (L.) Raddi I	Zhang et al. 20150830-182 (HSNU)	China, Fujian	OR689873	OR700031
<i>R. hemisphaerica</i> II	Xiang 20160218-1 (HSNU)	China, Hunan	OR689874	OR700032
<i>R. hemisphaerica</i> III	Schill et al. 46 (E)	Switzerland, Valais	GQ910702	GQ910741

TABLE 2. — Morphological comparisons of *Mannia gradsteinii* sp. nov., *M. gracilis* (F.Weber) D.B.Schill & D.G.Long and *M. triandra* (Scop.) Grolle.

	<i>Mannia gradsteinii</i> sp. nov.	<i>M. gracilis</i> (F.Weber) D.B.Schill & D.G.Long	<i>M. triandra</i> (Scop.) Grolle
No. of air chamber layers	2-4 layers	1 layer	2-3 layers
Air pore (diameter)	(72)136-204 µm	20-35 µm	8.7-46.4 µm
Ventral scale color	hyaline to pale purplish	red-purple	purple with sometimes paler margins
Ventral scales	not reaching beyond thallus edge	not reaching beyond thallus edge	reaching beyond thallus edge
Pseudoperianth	absent	present	absent
Spore (SEM)	47-57 µm	55-70 µm	55-75 µm
Spore shape	trilete	alete	trilete
Spore distal surface	3-6 areolae across diameter	3-6 irregular incomplete areolae across diameter	4-7 areolae across diameter

TABLE 3. — The value of infraspecific (INT) and infrageneric *p*-distances of *trnL*-region and 26S-region (%) for species of *Mannia* Corda.

No.	Taxon	INT	1	2	3	4	5	6	7	8	9
1	<i>M. androgyna</i>	0.2/0.2	—	—	—	—	—	—	—	—	—
2	<i>M. californica</i>	1.2/1.3	3.4/2.7	—	—	—	—	—	—	—	—
3	<i>M. controversa</i>	0.4/1.0	1.5/2.7	3.6/3.2	—	—	—	—	—	—	—
4	<i>M. fragrans</i>	0.6/0.5	2.6/2.3	5.0/3.4	3.2/1.8	—	—	—	—	—	—
5	<i>M. gracilis</i>	0.2/0.2	5.2/2.4	7.2/3.6	4.9/2.1	6.6/2.1	—	—	—	—	—
6	<i>M. gradsteinii</i> sp. nov.	0.2/0.3	5.6/2.6	7.6/4.0	5.3/2.9	7.0/2.3	2.8/1.4	—	—	—	—
7	<i>M. pilosa</i>	0.6/0	3.8/2.9	5.8/3.4	3.0/2.8	4.8/2.7	4.7/2.0	4.6/2.1	—	—	—
8	<i>M. sibirica</i>	—/—	2.3/1.8	4.4/2.7	1.6/1.3	3.8/1.1	5.7/2.2	5.8/2.0	3.7/2.2	—	—
9	<i>M. triandra</i>	1.0/0.1	4.6/2.4	6.6/3.5	4.4/2.0	6.2/1.8	3.3/0.8	3.0/1.1	3.9/1.4	5.1/1.8	—

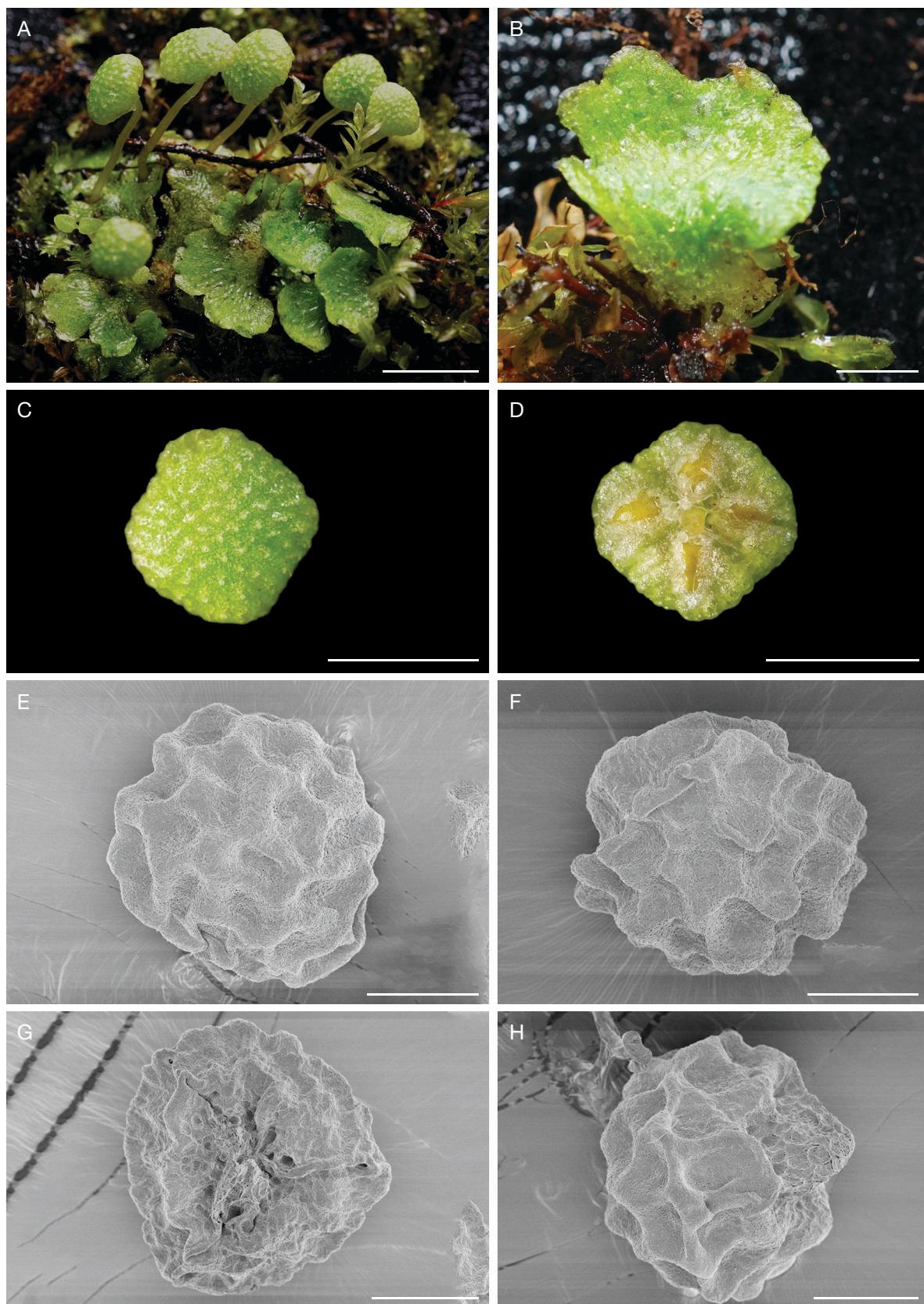


FIG. 2. — *Mannia gradsteinii* sp. nov.: **A**, habit in the field; **B**, dorsal surface of thallus; **C, D**, female receptacle, dorsal and ventral view respectively; **E-H**, SEM micrographs of spores; **E, F**, distal view; **G**, proximal view; **H**, side view. All from Y.L. Xiang & P.F. Wang 20170428-12 (holo-, HSNU). Scale bars: A, 1 cm; B, 2 mm; C, D, 5 mm; E-H, 20  $\mu$ m.

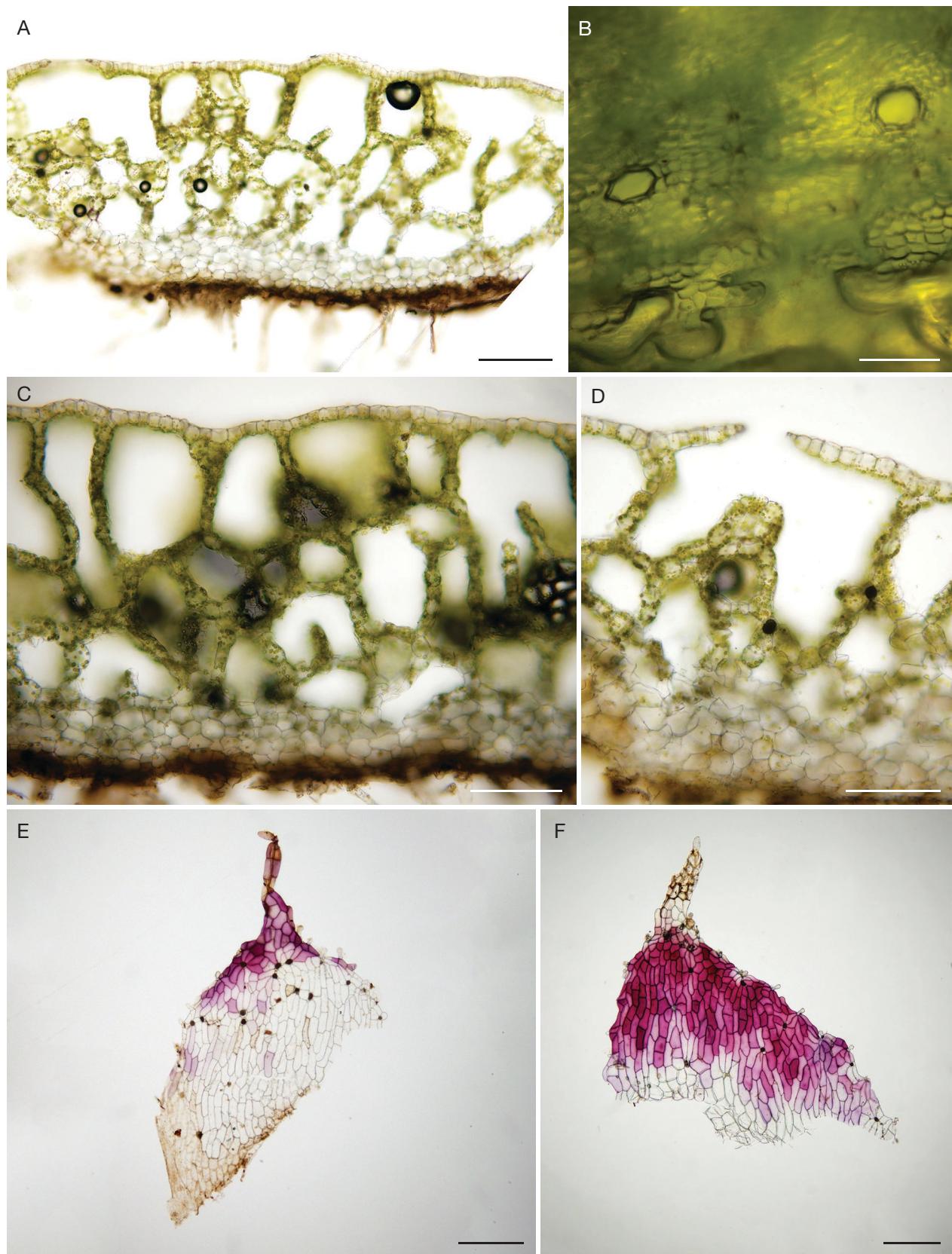


FIG. 3. — *Mannia gradsteinii* sp. nov.: A, C, D, transverse section of segments; B, epidermal pores; E, F, ventral scales with appendages. All from Y.L. Xiang & P.F. Wang 20170428-12 (holo-, HSNU). Scale bars: A, E, F, 250 µm; B-D, 200 µm.

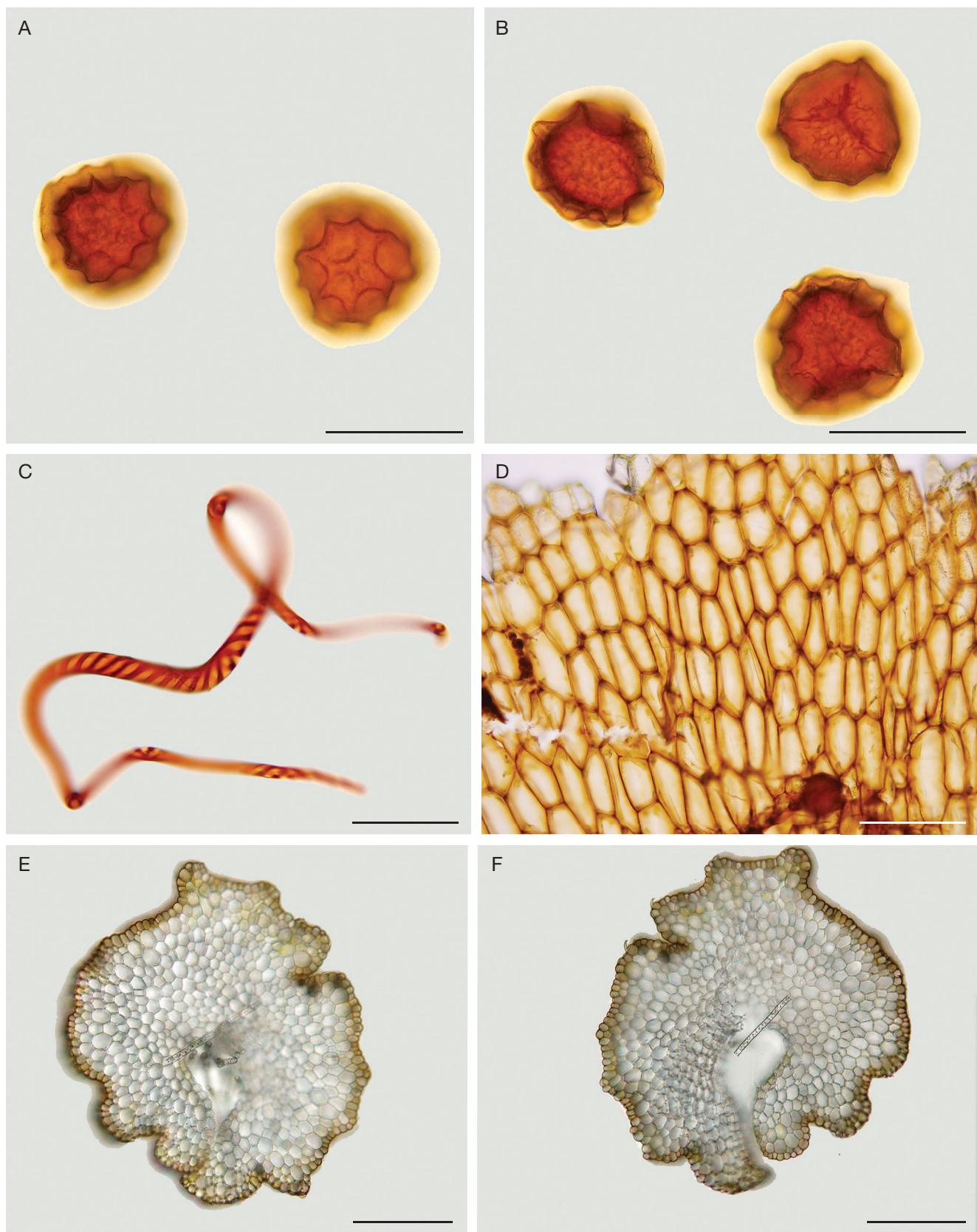


FIG. 4. — *Mannia gradsteinii* sp. nov.: **A, B**, light microscopy micrographs of spores; **A**, distal view; **B**, proximal view; **C**, elater; **D**, capsule wall; **E, F**, transverse section of archegoniophore stalks. All from Y.L. Xiang & P.F. Wang 20170428-12 (holo-, HSNU). Scale bars: A-D, 50 µm; E, F, 200 µm.

and distal surfaces, 3-6 areolae across the spore distal surface and with middle depression on spore proximal face; proximal surface incompletely areolate; proximal disc absent; equatorial apertures rather inconspicuous, usually three; equatorial rim inconspicuous; elaters yellowish brownish, 359-560 µm long, 8.7-11 µm broad at middle, 2-3-spiral.

## DISCUSSION

Consistent with previous molecular phylogenetic studies (Schill *et al.* 2010; Borovichev *et al.* 2015), two subgenera (subg. *Mannia* and subg. *Neesiella* (Schiffn.) D.B.Schill & D.G.Long) are recognized based on available molecular data and several morphological characters, including the morphology of the female receptacles and spores. *Mannia gradsteinii* sp. nov. is resolved as sister to *Mannia gracilis* and is morphologically compatible with other taxa in this subgenus and is therefore placed in that subgenus.

*Mannia gradsteinii* sp. nov. is well characterized by: 1) light green thallus, with faint reticulation marks due to the underlying chamber walls (Fig. 2B); 2) large epidermal pores, (72)136-204 µm in diameter (Fig. 3B); 3) assimilatory tissue with big air chambers, 2-4 layers, occupying c. 2/3-3/4 of the thallus height (Fig. 3A, C, D); 4) ventral scales not reaching beyond thallus edge, hyaline to pale purplish; 5) monoicous, archegoniophore arising from apices of main branches, androecia close to archegoniophore bases; 6) female receptacles hemispherical, without pseudoperianth, 4.6-8.2 mm in diameter (Fig. 2C, D); and 7) spores 47-57 µm in diameter (SEM), trilete, 3-6 areolae across the spore distal surface (Fig. 2E, F). The morphology of *M. gradsteinii* sp. nov. clearly indicates its classification within the subgenus *Neesiella* owing to the presence of spores exhibiting similar sculpturing on both proximal and distal surfaces, the absence of a proximal disc, and the inconspicuous equatorial pores contribute to its characterization.

*Mannia gradsteinii* sp. nov. bears some resemblances to *Mannia triandra*, which is known as a short-lived plant. *Mannia gradsteinii* sp. nov. can be distinguished from the latter as follows: 1) 2-4 layers air chambers vs 2-3 layers air chambers in *M. triandra*; 2) large epidermal pores (72)136-204 µm in diameter vs small epidermal pores 8.7-46.4 µm in *M. triandra*; 3) ventral scales not reaching beyond thallus edge vs ventral scales reaching beyond thallus edge in *M. triandra*; 4) spore distal surface 3-6 areolae across diameter vs spore distal surface 4-7 areolae across diameter in *M. triandra*; 5) female receptacles 4.6-8.2 mm in diameter vs female receptacles 0.8-2.1 mm in diameter in *M. triandra*; and 6) female receptacles usually bearing 4 sporophytes vs female receptacles bearing 2-3 sporophytes in *M. triandra* (Table 2). *Mannia gradsteinii* sp. nov. may be confused with *M. gracilis*. Both taxa have hemispherical female receptacles and the par-autoicous sexual condition. *Mannia gracilis*, however, can be differentiated by the small epidermal pores, 20-35 µm in diameter, the red-purple ventral scales, air chambers in a single layer, the presence of a pseudoperianth and alete spore (Table 2).

*Mannia gradsteinii* sp. nov. may be also similar to *M. pilosa* (Hornem.) Frye & L.Clark. They share several important characters including the hemispherical female receptacles, and air chambers in 2-4 layers. *Mannia pilosa*, however, can be easily distinguished by: 1) androecia typically borne on reduced ventral branches of the main thallus; 2) oil cells often absent or 0-3(4) in ventral scales; 3) small epidermal pores 16.5-14.5(17.4) µm in diameter; and 4) large spores 63-85.7 µm in diameter (the spore size was measured under scanning electron microscope).

Our results also indicate that the *p-distances* calculated for *Mannia gracilis* and the samples of *Mannia gradsteinii* sp. nov. (Table 3) exceed the minimum interspecific *p-distances*, fall below the maximum interspecific *p-distances* of *Mannia*. Therefore, morphological and molecular evidence all support the setting of the new species, *Mannia gradsteinii* sp. nov.

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