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Predictive modelling of the distribution and evaluation of the conservation status with a taxonomic clarification of *Macromitrium fortunatii* Thér. (Orthotrichaceae, Bryophyta) in China and adjacent regions

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Abstract – *Macromitrium fortunatii* Thér. was included in the first Red List of Chinese endangered bryophytes, but later its inclusion was considered as uncertain because of taxonomic uncertainty and insufficient distribution data. Though the species was recently excluded from the latest threatened species list of China's higher plants, no detailed geographical records, voucher specimens or relevant references were included. From our morphological studies of type and other specimens, together with phylogenetic analyses based on ITS2, *trn*L and *trn*G sequences, we confirm its taxonomical identity and provide a detailed description and illustrations of the species. *Macromitrium fortunatii* var. *nigrescens* Tixier is reduced to synonymy with *M. fortunatii*. Since 2013 we have found 24 populations of *M. fortunatii* in Southwest and South China and North Vietnam. According to nine environmental variables and recent records of the species, we predict its potential distribution range with an aid of the maximum entropy algorithm modeling program (Maxent) and ArcGis 10.2. We find a high environmental suitability of the species in a wide region covering South and Southwest China, North Vietnam, North Myanmar, Nepal, and Northeast India. The predicted climate suitability for *M. fortunatii* is highest in areas where the annual precipitation is 1200 mm, the driest quarter receives 50 mm, and the wettest month 250 mm, the mean temperature of the coldest quarter is 10°C, and the altitude is about 1200 m. The exclusion of *M. fortunatii* from the threatened species list of China's higher plants is supported.

Bryophyte / Maxent / Prediction / Phylogenetic analysis / Synonym

INTRODUCTION

The numbers of species appearing in each category of threat in the IUCN Red List change with time. To monitor the status of biodiversity, it is important to reassess species periodically. This reassessment may result in species moving into a different Red List Category. Such situation was found in the saxicolous or epiphytic moss *Macromtrium fortunatii* Thér. (Orthotrichaceae, Bryophyta).

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Macromitrium fortunatii is listed in the TROPICOS (http://www.tropicos.org/Name/35160921) database as an insufficiently known species. The species was also not included in the updated Moss Flora of China (English version, vol. 5) (Jia *et al.*, 2011). In the International Symposium on conservation of Chinese bryophyte diversity in Shanghai in 2004, *M. fortunatii* was included in the first Red List of Chinese bryophytes because there had been no other records of the species except the original 1909 record in Pin-fa, Kouty Tcheou (Guizhou, Southwestern China) (Cao *et al.*, 2006). *Macromitrium fortunatii* was also included in the updated Red List of Chinese higher plants, but it was listed as a species whose endangered status was uncertain because of insufficient data (Ministry of Environmental Protection, P.R. China, 2013). More recently, the species was exclude from the latest threatened species list of China's higher plants, but no detailed geographical records, voucher specimens and relevant references were presented (Qin *et al.*, 2017). Taxonomy provides the basic foundation of conservation practice (Morrison *et al.*, 2009; Bancheva & Gorgorov, 2010). To clarify both the identity and conservation status of *M. furtunatii* in China, a reappraisal of its endangered status seems necessary.

Tixier (1966) described *M. fortunatii* var. *nigrescens* from Chapa, Vietnam on the basis of its blackish green, slightly wider branch leaves, thicker setae and slightly bulging capsules. However, with the discovery of more populations of *M. fortunatii*, we found that these differences were continuous among its known populations.

During 2012-2013 we examined *Macromitrium* specimens kept in MO and identified 14 specimens of *M. fortunatii* collected from three regions of Vietnam adjacent to China: Lao Cai, Ha Giang and Ha Tay. Although Ji (2008) did not record *M. fortunatii* in Guizhou, we found six specimens kept in GACP, which had been collected from different regions of Guizhou since 2002. We also collected two new specimens in 2017, from Guiyang (near its type locality) and Fanjingshan, Jiangkou County of Guizhou. There are now 23 known specimens collected from 22 different localities since 1999. From an analysis of the present known distribution and climate preference of *M. fortunatii*, there is little doubt the species has a wider distribution than presently recorded, possibly covering southwestern and southern China, and regions adjacent to China. To better reassess the endangered status of *M. fortunatii*, we used habitat modelling based on macroclimatic parameters to estimate its potential distribution range.

The objectives of this study are (1) to evaluate the taxonomical identity of *M. fortunatii* based on a morphological and molecular study, and (2) to evaluate the endangered status based on its recent geographical records, its potential distribution range and macroclimatic niche requirement with the help of Maxent modelling.

MATERIAL AND METHODS

Examination of specimens

Sections of branch leaves were made by hand. Microscopic examinations, measurements and photographs were obtained with an Olympus-BX51 light microscope and DP25 camera mounted on the microscope. Descriptions and illustrations of upper, medial and basal leaf cells were obtained from mid-stem and mid-branch leaves. Leaf width was measured at the widest part.

Taxon sampling and molecular protocols

Thirty-three samples were used for the analyses, covering 14 of the 17 Chinese *Macromitrium* species. *Schlotheimia grevilleana* Mitt. was used as an outgroup (Table 1).

Table 1. List of taxa for this study, including voucher information as well as GenBank accession numbers

Taxa	*Voucher information*	*GenBank Accession*		
		trn*L-F*	trn*G*	*nrITS2*
Macromitrium angustifolium	Malaysia; Klazenga *et al.* 1946 (MO)	MH730450	MH730483	MH730516
	Vietnam; He & Nguyen 42907 (MO)	MH730451	MH730484	MH730517
Macromitrium blumei	Vietnam; He & Nguyen 42935 (MO)	MH730452	MH730485	MH730518
Macromitrium cavaleriei	China, Taiwan; Guo & Cao 323 (SHTU)	MH730453	MH730486	MH730519
	Vietnam; He & Nguyen 42846 (MO)	MH730454	MH730487	MH730520
Macromitrium cuspidatum	Malaysia; Schäfer-Verwimp 18535 (MO)	MH730455	MH730488	MH730521
	Malaysia; Klazenga 1174 (MO)	MH730456	MH730489	MH730522
Macromitrium fortunatii	China, Guizhou; Anonym LB20151102046 (GACP)	MH730457	MH730490	MH730523
	China, Guizhou; Anonym LL20141129122 (GACP)	MH730458	MH730491	MH730524
	China, Guizhou; Anonym DJ2016061017 (GACP)	MH730459	MH730492	MH730525
	China, Guizhou; Guo & Li 20171109045 (SHTU)	MH730460	MH730493	MH730526
	Vietnam; He & Nguyen 41699 (MO)	MH730461	MH730494	MH730527
	Vietnam; He & Nguyen 41853 (MO)	MH730462	MH730495	MH730528
	Vietnam; He & Nguyen 42205 (MO)	MH730463	MH730496	MH730529
	Vietnam; He & Nguyen 42320 (MO)	MH730464	MH730497	MH730530
Macromitrium gymnostomum	China, Sichuan; Guo & Cao 130805005 (SHTU)	MH730465	MH730498	MH730531
	China, Taiwan; Guo & Cao 546 (SHTU)	MH730466	MH730499	MH730532
Macromitrium japonicum	Laos; He 44258 (MO)	MH730467	MH730500	MH730533
	China, Taiwan; Guo & Cao 517 (SHTU)	MH730468	MH730501	MH730534
Macromitrium microstomum	China, Taiwan; Guo & Cao 316 (SHTU)	MH730469	MH730502	MH730535
	Vietnam; He & Nguyen 43021 (MO)	MH730470	MH730503	MH730536
Macromitrium nepalense	Laos; He 43782 (MO)	MH730471	MH730504	MH730537
	Vietnam; He & Nguyen 41590 (MO)	MH730472	MH730505	MH730538
Macromitrium ousiense	China, Guangxi; Guo & Yu 49 (SHTU)	MH730473	MH730506	MH730539
Macromitrium rhacomitrioides	China, Taiwan; Guo & Cao 156 (SHTU)	MH730474	MH730507	MH730540
	China, Taiwan; Guo & Cao 313 (SHTU)	MH730475	MH730508	MH730541
Macromitrium tosae	China, Taiwan; Guo & Cao 242 (SHTU)	MH730476	MH730509	MH730542
	China, Yunnan; Ma 08-617 (MO)	MH730477	MH730510	MH730543
Macromitrium turgidum	Vietnam; Averyanov NTH B 024 (MO)	MH730478	MH730511	MH730544
	China, Yunnan; Crosby 15120 (MO)	MH730479	MH730512	MH730545
Macromitrium uraiense	China, Taiwan; Guo & Cao 509 (SHTU)	MH730480	MH730513	MH730546
	China, Taiwan; Guo & Cao 527 (SHTU)	MH730481	MH730514	MH730547
Schlotheimia grevilleana	China, Zhejiang; Guo 110619117 (SHTU)	MH730482	MH730515	MH730548

Table 2. Primers of amplification and the related references

Genes	*Primer name*	*Direction*	*Primer sequenee (5'-3')*	*References*
*trn*L-F	*trn*C	forward	CGAAATCGGTAGACGCTACG	Taberlet *et al.*, 1991
	*trn*F	reverse	ATTTGAACTGGTGACACGAG	
*Trn*G	*trn*GF	forward	GGCTAAGGGTTATAGTCGGC	Werner *et al.*, 2009
	*trn*GR	reverse	CGGGTATAGTTTAGTGG	Pacak *et al.*, 2000
ITS2	5.8SF	forward	GACTCTCAGCAACGGATA	Hartmann *et al.*, 2006
	26SR	reverse	AGATTTTCAAGCTGGGCT	

One nuclear and two plastid markers were chosen: nuclear ribosomal internal transcribed spacer region ITS1-5.8S-ITS2 (hereafter, ITS2), tRNA (Gly) (UCC) (hereafter, *trn*G), and *trn*L-*trn*F intergenic spacer (hereafter, *trn*L-F), all widely used in phylogenetic analyses of pleurocarpous mosses (Draper & Hedenäs, 2009; Hedenäs, 2012; Li, 2014).

DNA was extracted from fresh samples or herbarium specimens using the CTAB method (Hou *et al.*, 2003) and quantified on 1.0% agarose gels. DNA was stored at -20°C. The primers in Table 2 were used to amplify the three makers. PCR amplifications were performed in a total volume of 30μL, containing 10× PCR buffer 3μL, 2.5mmol/L dNTP 1.2μL, 20μmol/L primer, 0.2 U Taq polymerase and 2μL DNA template, using a program of 30 cycles of 94°C for 30 s; 58°C for 30s; 72°C for 1 min and finished with 72°C for 15 min. PCR products were separated on 1.5% agarose gels, observing the strip's brightness. PCR products were purified and bidirectionally sequenced by BGI (www.genomics.cn) using the amplification primers. Species names, voucher information, and GenBank accession numbers for all sequences are presented in Table 1.

Distribution data and environmental variables

Twenty five occurrences of *M. fortunatii* were recorded based on our field collection and examination of the specimens kept in herbaria (MO, PC, GACP and SHTU) (Table 3, Fig. S1). Twenty three of the collections had been made since 1999.

Nineteen world bioclimatic and altitude variables were downloaded from the WorldClim database at a spatial resolution of 2.5 arc-minutes (≈ 22 km2 cells) (Fick & Hijmans, 2017) and percent tree cover from http://www.iscgm.org/ (Geospatial Information Authority of Japan, Chiba University and collaborating organizations).

Data analysis

Phylogenetic tree construction

Sequence chromatograms were compiled using Seqman II (DNASTAR Inc., Madison, WI, USA), and then aligned automatically in PhyDE 0.9971 (Müller *et al.*, 2010). Regions of partially incomplete data at the beginning and end of sequences were excluded from subsequent analyses. Gaps were treated as missing data.

Bayesian phylogenetic analysis was performed. According to the Akaike information criterion (AIC), we firstly ran the MrModeltest v.2.3 (Nylander, 2009) in conjunction with PAUP 4.0b10 (Swofford, 2003) to select the best-fit models of

Table 3. Records of *Macromitrium fortunatii.* 1-15 from Vietnam, 16-25 from China; * geographic coordinates were obtained by using google map

No.	*Localities*	*Altitude (m)*	*Longitude (Decimal)*	*Latitude (Decimal)*	*Collection time*	*Collector and number*	*Herbaria*
1	Ha Giang	1064	104.993	23.154	2001.2.10	Daniel *et al.* 6055	MO
2	Ha Giang	1150	104.998	23.151	2001.2.12	Daniel *et al.* 6239	MO
3	Ha Giang	11001200	104.983	23.150	2000.4.5	Daniel *et al.* 5154	MO
4	Ha Giang	1310	105.013	23.136	2000.4.6	Daniel *et al.* 5295	MO
5	Ha Giang	1400-1500	105.133	23.117	1999.10.9	Averyanov NTH B 034	MO
6	Ha Giang	1250-1300	105.250	23.183	1999.10.6	Averyanov NTH B 044	MO
7	Ha Giang	300–1050	104.959	22.868	2001.2.16	Daniel & Averyanov 6458	MO
8	Ha Giang	1224	104.974	23.079	2008.10.16	He & Nguyen 41809	MO
9	Ha Giang	1224	104.957	23.079	2008.10.16	He & Nguyen 41853	MO
10	Ha Giang	438-915	105.009	23.055	2008.10.15	He & Nguyen 41699	MO
11	Ha Giang	438-915	105.009	23.055	2008.10.15	He & Nguyen 41683	MO
12	Ha Tay	1118-1234	105.218	21.030	2008.11.6	He & Nguyen 42320	MO
13	Lao Cai	700-1113	103.976	22.042	2001.2.26	Daniel 6957	MO
14	Ninh Binh	360-646	105.359	20.209	2008.10.29	He & Nguyen 42205	MO
15	Ha Tay	1118-1234	105.216	21.030	2008.11.6	He & Nguyen 6165718	MO
*16	Guizhou, Guiding	1067	107.082	26.213	1909.4.5	Fortunat 1749	PC
17	Guizhou, Guiyang	1410	106.802	26.354	2017.11.12	Guo & Li 20171109045	SHTU
18	Guizhou, Jiangkou	2230	108.794	27.911	2017.11.17	Guo & Li 20171117080	SHTU
19	Guizhou, Libo	913	107.919	25.348	2015.11.2	Anonym LB20151102046	GACP
20	Guizhou, Dejiang	825	107.892	28.264	2016.6.1	Anonym DJ20160601017	GACP
21	Guizhou, Qiannan	1240	106.959	26.418	2014.11.29	Anonym LL20141129122	GACP
22	Guizhou, Xingyi	1250	105.083	25.200	2002.11	Wang XY0211001	GACP
23	Guizhou, Zunyi	550	108.167	28.700	2009.11.27	Anonym WC09112714	GACP
24	Guizhou, Wanmo	1520	106.489	25.323	2009.5.2	Anonym MS09050203	GACP
25	Guangdong, Jiaolin	234	116.097	24.548	1957.5.23	Deng 4834	IBSC

nucleotide substitution for each gene. The relevant parameters were set accordingly for each compartment. A phylogenetic tree combined with three genes was constructed under a Bayesian Markov Chain Monte Carlo approach using MrBayes v.3.2.6 (Ronquist *et al.*, 2012). Four parallel runs, each with four chains, were run for 1000 000 generations, with trees being sampled every 1000 generations. Posterior probabilities (PP) were calculated after discarding the first 25% trees (Huelsenbeck & Ronquist, 2001). Trees were visualized and annotated in TreeGraph 2 (Stöver & Müller, 2010).

Distribution prediction

The 19 bioclimatic variables are a set of climate layers derived from monthly temperature and precipitation records taken worldwide (Hijmans & Graham, 2006). To avoid problems of multicollinearity, we conducted a principal component analysis (PCA) on the 19 bioclimatic variables to reduce those that exhibited little spatial variability across the world. The first two PCA components accounted for 76.39% of the total variation, mainly for temperature and precipitation variations. From this we selected seven variables (with largest absolute loadings), which are also biologically informative and uncorrelated variables with Pearson correlation coefficients between 0.7 and -0.7 (Kruijer *et al.*, 2010). These seven bioclimatic variables included bio1 (annual mean temperature), bio5 (max temperature of warmest month), bio7 (temperature annual range), bio11 (mean temperature of coldest quarter), bio12 (annual precipitation), bio13 (precipitation of wettest month) and 17 (precipitation of driest quarter). Altitude and percent tree cover were also included in our prediction (Table S1).

We applied Maxent 3.3.2 (Phillips *et al.*, 2006, http://www.cs.princeton.edu/ yschapire/maxent/ maxent-submit.cgi) to predict the potential distribution region of *M. fortunatii* in the world based on presence-only data and nine environmental variables. In model prediction, we only used 23 recent distribution data (collected since 1999). We divided the data into 17 training data (75%) and 6 testing data (25%) (Phillips *et al.*, 2006). Other procedures of the prediction followed Lou *et al.* (2014). We selected the minimum training presence logistic threshold (Young *et al.*, 2011). Continuous model outputs (corresponding to logistic probability values) were transformed into maps to show the climate suitability for *M. forunatii* above a threshold. There were six classes above the logistic threshold: I(0.386-0.481), II(0.482-0.578), III(0.579-0.674), IV(0.675-0.770), V(0.771-0.866) and VI (0.867-0.963). To better understand the rationale of the prediction, we calculated an integrated environmental suitability index (*IESI*) above the threshold for each focal region with the potential distribution range of *M. fortunatii* as follows:

$$IESI = \sum_{i=1}^{6} L_i \times AP_i$$

Where L_i is the average value of the logistic probability in Class i, and AP_i is the area percentage in Class i of the region.

RESULTS

Phylogenetic analysis

After deletion of incomplete regions at the beginning and end of the alignments, the total number of aligned sites from the three genes is 1860, 240 sites

Table 4. Numbers of sites in each locus based on sequence length (bp), polymorphic variable (p.v.), parsimony informative sites (p.i.), and the models selected for Bayesian analysis

Gene	*Length (bp)*	*p.v. sites*	*p. i. sites*	*Models*
ITS2	807	119	93	GTR+I+G
*trn*G	580	73	51	HKY+I
*trn*L-F	473	48	28	GTR+G
Total	1860	240	172	

are variable characters and 172 are parsimony-informative. The numbers of sites in each locus based on sequence length, parsimony variable sites, parsimony informative sites, and the optimal substitution models selected for Bayesian analysis are given in Table 4.

The tree topology of the Bayesian phylogenetic inference based on the combined dataset is shown in Fig. 1, together with posterior probability (PP) values. All 14 species of *Macromitrium* formed a robust clade (PP=1) with *Schlotheimia grevilleana* as an outgroup. The genus *Macromitrium* was resolved as a monophyletic supported by Bayesian posterior probabilities (PP=1). Most acceptable species form independent clades or subclades. The *M. blumei* and *M. turgidum* clade was resolved monophyletically (PP=1), sister to the other five clades, *M. uraiense* clade (PP=1), *M. angustifolium*, *M. cuspidatum* and *M. microstomum* clade (PP=0.5), *M. gymnostomum* clade (PP=1), *M. nepalense*, *M. ousiense*, *M. rhacomitrioides*, *M. cavaleriei* and *M. japonicum* clade (PP=1), *M. tosae* and *M. fortunatii* clade (PP=1).

All samples of *M. fortunatii* form a monophyletic group together with maximal support (PP=1), which is sister to *M. tosae*. Therefore, *M. fortunatii* is a distinct species, well supported by the phylogenetic analyses.

Taxonomic treatment

Macromitrium fortunatii Thér., *Bull. Acad. Int. Géogr. Bot.* 19: 19. 1909 ["i"]. Type: "Chine, Kouy Tcheou, Pin-fa, sur rochers, *Leg. Fortunat, 1749,* 5, April 1904" (Lectotype designated by Guo *et al.* (2013): PC0083654!; isolectotype: PC0083657!, PC0719719!) ....**Fig. 2**

= *Macromitrium fortunatii* var. *nigrescens* Tixier, *Rev. Bryol. Lichénol.* 34: 140. f. 8. 1966. ***syn. nov.*** Type: "Chapa, sur arbuste 1500 m, lisière de la forêt, Février 1929 (*Pételot, P.A. no. 141*)" (Lectotype designated here: PC 0083660!, isolecotypes: PC 0721001!, PC 0719722!, PC 0137695!, S B115583!)

Guo *et al.* (2013) described *M. fortunatii* in detail based on its type specimens. The species is characterized by a combination of the following characters: 1) plants forming dense, brownish mats, dark-brownish below, brownish or yellow-brownish above; 2) stems long creeping, densely reddish tomentose below; 3) branch leaves densely arranged, in spiral ranks, giving the shoots a rope-like appearance when dry, oblong-lanceolate to oblong-ligulate, acute, acuminate-mucronate, shortly cuspidate to broadly acuminate; 4) upper and medial laminal cells quadrate to subquadrate, clear, strongly conic-bulging, unipapillose; lower laminal cells little different from medial cells; basal laminal cells short to long-rectangular, inflated,

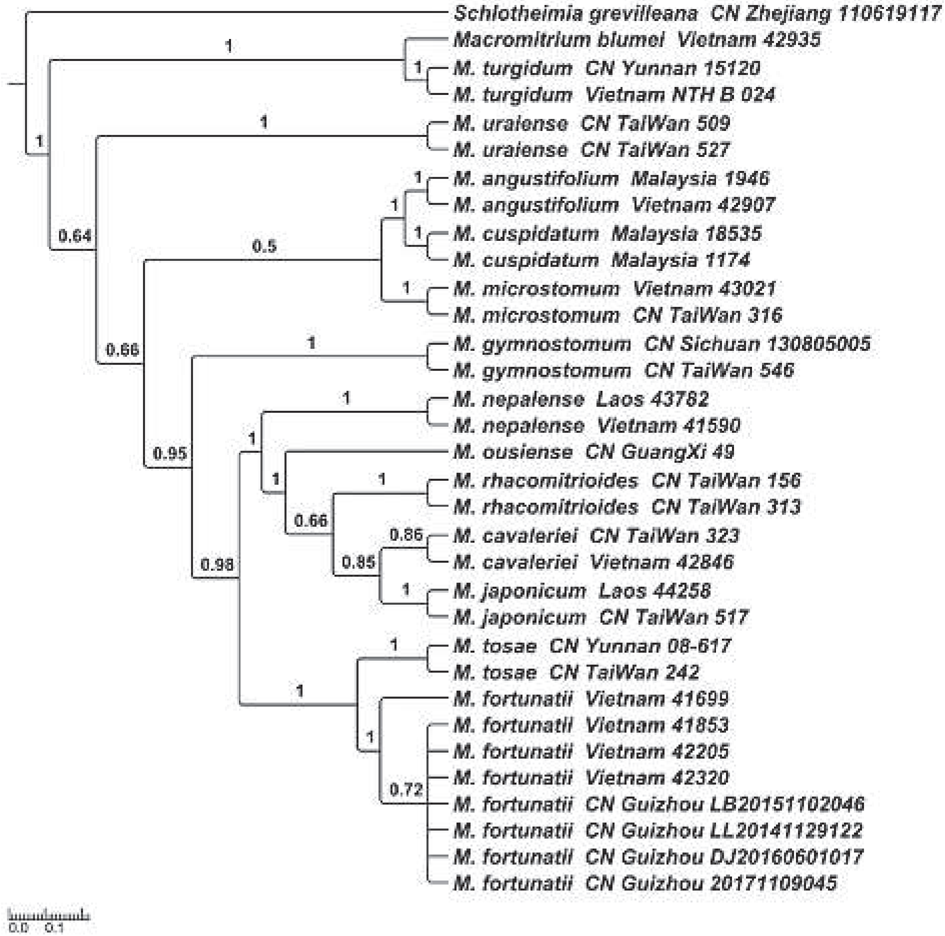


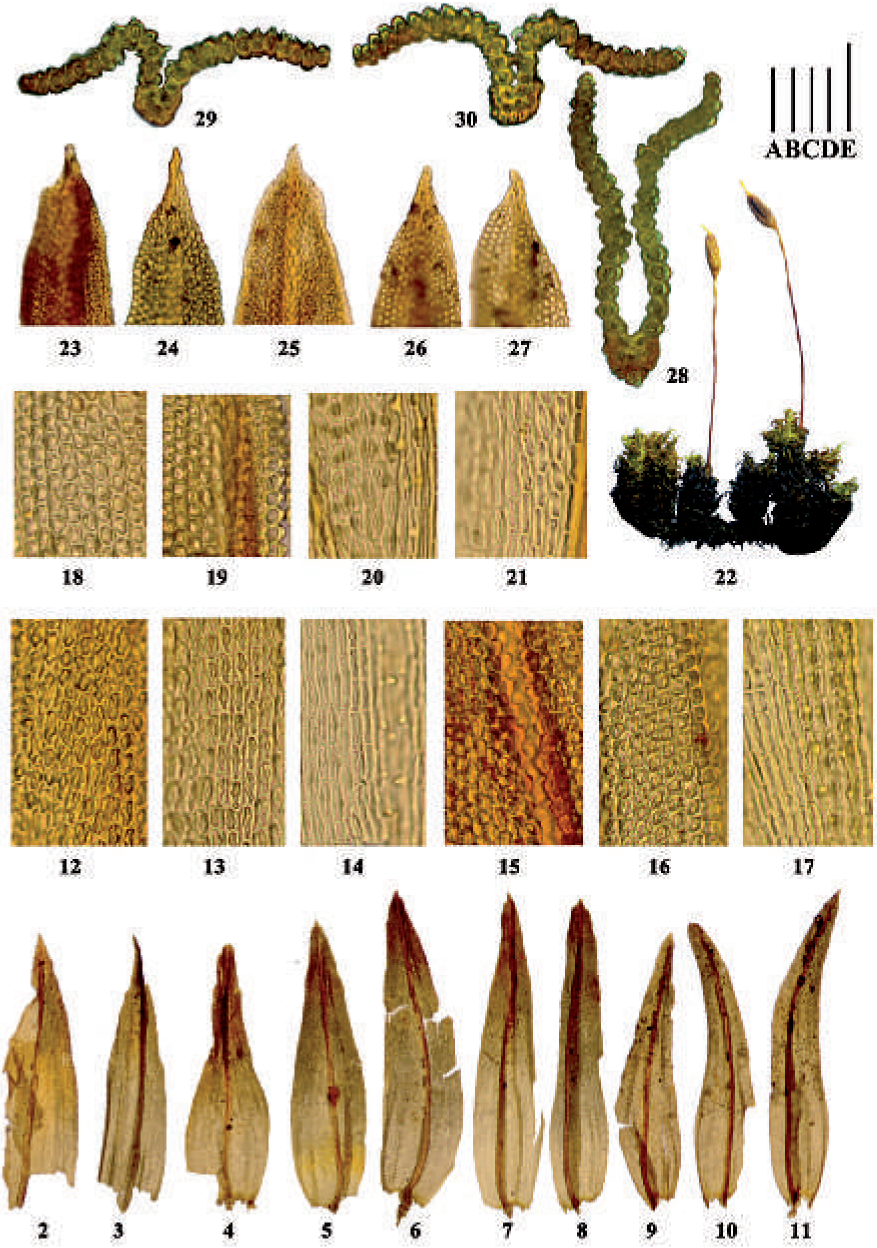
Fig. 1. Bayesian majority consensus tree calculated from the results of Bayesian analysis of the combined datasets of *trn*L-F, *trn*G and ITS2. The Bayesian posterior probabilities are labeled above the branches.

distinctly unipapillose; 5) inner perichaetial leaves oblong-lanceolate, acuminate, all laminal cells longer than wide, incrassate and porous; 6) setae smooth, varying from 4.0 mm to 20.0 mm; 7) capsules not contracted under mouth, with exostome; and 8) calyptrae sparsely hairy.

Predicted distribution of *Macromitrium fortunatii*

Application of 17 training and 6 test presence records in Maxent yielded an average AUC of 0.996 for the test data (Fig. S2), suggesting a high predictive power of the model (Phillips *et al.*, 2006).

We found a higher environmental suitability (IESI) for *Macromitrium fortunatii* in South, and Southwest China including Chongqing (IESI, 0.657), Southern Xizang (0.653), Guizhou (0.626), Guangdong (0.605), Southwestern Taiwan (0.593), Guangxi (0.518), Eastern Sichuan (0.494), Southeastern



Figs 2-30. *Macromitrium fortunatii* Thér. **2-3.** Perichaetial leaves. **4-11.** Branch leaves. **12.** Upper cells of perichaetial leaf. **13.** Medial cells of perichaetial leaf. **14.** Basal cells of perichaetial leaf. **15.** Upper cells of branch leaf. **16, 18-19.** Medial cells of branch leaf. **17, 20-21.** Basal cells of branch leaf. **23-27.** Apices of branch leaves. **28.** Lower transects of branch leaf. **29-30.** Upper transect of branch leaf. **22.** Habits with capsules. (2-8, 12-17, 23-25, 28-30 from lectotype of *M. fortunatii* PC 0083654; 9-11, 18-21, 26-27 from lectotype of *M. fortunatii* var. *nigrescens* in PC 0083660. 22 from Longli, Guizhou, *Cui LL20141129122* (GACP). Scale bars: A=0.2 mm (2-11), B=20 µm (12-21), C=50 µm (23-27), D=40 µm (29-30); E=4 mm (22).

Table 5. Environmental suitability indices corresponding to different classes (_L*AP_) and integrated indices (∑_L*AP_) of *Macromitrium fortunatii* in 14 regions

Localities / area (km^2)	*Suitability classes (above threshold)*						*IESI*
	I (0.386 -0.481)	*II (0.482 -0.578)*	*III (0.579 -0.674)*	*IV (0.675 -0.770)*	*V (0.771 -0.866)*	*VI (0.867 -0.963)*	
Northern Burma/7.41	0.009	0.046	0.075	0.167	0.262	0.149	0.708
Northern Vietnam/11.99	0.021	0.047	0.127	0.243	0.193	0.074	0.705
China, Chongqing/8.23	0.015	0.045	0.133	0.464	0.000	0.000	0.657
China, Southern Xizang/6.8*	0.018	0.022	0.036	0.100	0.275	0.202	0.653
China, Guizhou/17.6	0.023	0.057	0.178	0.258	0.110	0.000	0.626
China, Guangdong/18.0	0.042	0.108	0.224	0.149	0.081	0.001	0.605
China, Southwestern Taiwan/1.62*	0.011	0.076	0.127	0.180	0.191	0.008	0.593
Northeastern India/18.72*	0.045	0.081	0.134	0.173	0.097	0.061	0.591
China, Guangxi/23.6	0.061	0.088	0.154	0.167	0.048	0.000	0.518
China, Eastern Sichuan/20.71*	0.036	0.043	0.134	0.221	0.043	0.017	0.494
China, Southeastern Fujian/8.59*	0.074	0.064	0.090	0.103	0.017	0.000	0.348
Nepal/1.47	0.042	0.064	0.072	0.052	0.074	0.058	0.362
China, Yunnan/38.33	0.106	0.110	0.065	0.040	0.020	0.004	0.345
China, Hainan/3.4	0.080	0.044	0.032	0.021	0.003	0.000	0.180

* not administrative regions

Fujian (0.348), Yunnan (0.345) and Hainan (0.180), and some Chinese adjacent regions including Northern Myanmar (0.708), Northern Vietnam (0.705), Northeastern India (0.591) and Nepal (0.362) (Table 5, Fig. 3).

Environmental variables which mostly contributed to the model were annual precipitation, precipitation of the driest quarter, precipitation of the wettest month, and mean temperature of the coldest quarter, with respectively 25.0%, 25.0%, 21.6% and 18.2% contributions to the model (Table S2).

The jackknife test showed that the variables producing the greater gain in the model when used in isolation were annual precipitation, precipitation of the wettest month and of the driest quarter, and annual temperature range (Fig. S3). Precipitation variables were more important than temperature variables in explaining the distribution of *M. fortunatii*.

Based on the 10-times cross-validation in the Maxent model, response curves in relation to nine environmental variables were generated (Fig. S4). The predicted environmental suitability for *M. fortunatii* was higher in regions where the following parameters prevail: annual precipitation was about 1200 mm (Fig. S4A); precipitation of the driest quarter about 50 mm (Fig. S4B); precipitation of the wettest month 260 mm (Fig. S4C); mean temperature of the coldest quarter about 10°C (Fig. S4); annual mean temperature was 16-18°C (Fig. S4G); annual temperature annual about 2°C (Fig. S4H); maximum temperature of the warmest month 28-29°C (Fig. S4I); altitude about 1100 m (Fig. S4E); and tree cover about 20% (Fig. S4F).

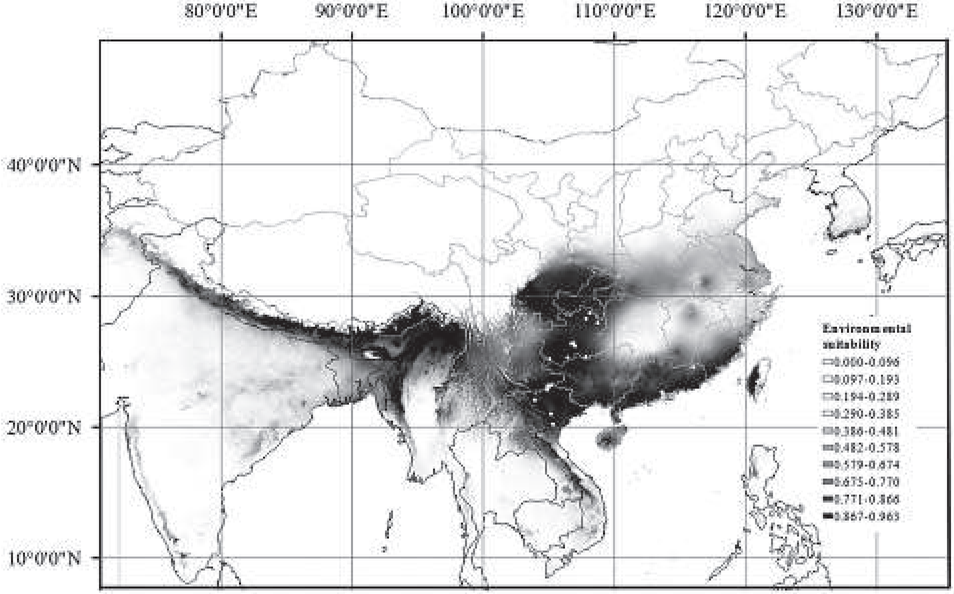


Fig. 31. Predicted distribution regions of *Macromitrium fortunatii* (white dots represent actual records).

DISCUSSION

Based on Fortunat's collections from Pin-fa (a small village of Yunwu town, Guiding County), Kouty Tcheou (Guizhou, China), Thériot (1909) described *Macromitrium fortunatii* as a new species giving only a rather brief morphological description of the species, with type information "Pin-fa, sur rochers; leg. Fortunat". In PC are several specimens which had been collected from Pin-fa, by Fortunat, or by Fortunat and Cardot, and identified as *M. fortunatii* by Thériot before 1909. From the protologue, the authority for *M.* fortunatii is clearly Thériot, and not Cardot et Thér. in Thér, as stated in the TROPICOS database.

Thériot (1909) thought that *M. fortunatii* was similar to *M. nepalense* (Hook. & Grev.) Schwägr., but differed by its longer branches and setae, and slightly or not papillose laminal cells. In the field, *M. fortunatii* is easily confused with *M. nepalense* by its branch leaf shape and the rope-like appearance when dry. However, *M. nepalense* can be distinguished from *M. fortunatii* by its irregular 1-3-stratose proliferation with pluripapillose cells on both adaxial and abaxial lamina surfaces. *M. fortunatii* is also somewhat similar to *M. tosae* Besch. in the branch leaves when moist, but could be separated from the latter species by: 1) the rope-like arrangement of branch leaves when dry; 2) rather longer setae; 3) upper and median laminal cells strongly conic-bulging and unipapillose.

Bryophytes are small in size and have a limited number of morphological traits useful in species identification. Bryologists often misidentify bryophyte specimens of a given taxon with high morphological variation if they are not taxonomical experts of the focal taxon. Numerous papers refer to "bad" taxonomy hindering conservation (Gittleman & Pimm, 1991; Funk *et al.*, 2002; McNeely, 2002; Mace, 2004; Russello *et al.*, 2005; Khuroo *et al.*, 2007). Bryophyte floristic information is also insufficient for numerous regions due to lack of bryologists. Therefore, the endangered status of many bryophytes remains uncertain, especially for taxonomically problematic taxa.

Macromitrium fortunatii was excluded from the latest threatened species list of China's higher plants, although no explanatory reasoning was given (Qin *et al.*, 2017). For the selection of endangered bryophytes in China, a critically endangered species is designated if it is rare and distributed in less than three localities, and its populations are rather small, with extremely threatened habitats. Since 1999, *M. fortunatii* has been collected from 23 different localities covering Guizhou (China), and northern Vietnam. On the basis of our studies of herbarium and recent collections concur with the decision of Qin *et al.*, (2017) to remove the species from the Chinese Red List of higher plants.

The predicted potential distribution range of *Macromitrium fortunatii* covers southern, southwest China, northern Vietnam, northern Myanmar, Nepal, and northeastern India. Eastern Sichuan and Guangdong (China) are included within the potential distribution region of *M. fortunatii*. Though we did not collect the species from these provinces, we found two specimens in collections in S and IBSC. In S, a specimen (S: B118852: China, Setschwan, oberhalb Doloho nahe Yungning im Bezirk von Muli, *Handel-Mazzetti 7201*) identified as *M. fortunatii* var. *brevisetum* Thér. (*nom. nud.*) because of its short setae. Considering the high variation of seta in length in populations of *M. fortunatii*, we refer this specimen to *M. fortunatii*. We found a specimen of *M. fortunatii* (Guandong, Jiaolin Co., *Deng L., 4834*) in IBSC. The above two specimens verifies the prediction of the species distribution in eastern Sichuan and Guangdong.

Precipitation plays a role more important than temperature in determining the macroclimatic niche of *Macromitrium fortunatii*. There is an optimal precipitation for *M. fortunatii*. In Guizhou, the species were recorded from Guiyang, Guiding, Dejiang, Zhuanyi and Longli. The annual precipitation of these five regions is around 1000 mm (Xu *et al.*, 2015). *M. fortunatii* was also recorded from Libo and Xinyi of Guizhou. Though their annual precipitation is over 1200 mm, these two localities are a typical Karst landform, with a relatively dry climate. In Guizhou, all specimens of *M. fortunatii* were recorded from saxicolous substrates in sparsely-shaded habitats, indicating a drought preference or tolerance of the species. Such habitats are rather common in southwest and southern China.

Geographical background (namely the extent of the study region, GB) has a substantial influence on the prediction of the distribution of a target species (Acevedo *et al.*, 2012). Lou *et al.* (2014) found that the predicted distribution of *Macromitrium cavaleriei* (from geographical records within China) by using the whole world as GB was much more realistic than by using small GBs. We similarly used the whole world as GB in predicting the potential distribution of *M. fortunatii*.

The highest ESI value indicates the possibility of a species occurrence in a region, while the IESI value provides more information on how widely a species may be distribute in a focal region. Though Guizou does not have the highest ESI (Class VI), the province has a higher IESI value leading to its wide occurrence in the province.

Additional specimens studied:

Macromitrium fortunatii Thér., China. Guizhou: (Kouy Tcheou), Pin-fa: sur rochers, *Cavalerie & Fortunati, s.n* (PC 0083652); *Fortunati & Cavalerie 1552* (PC 0083653); *P. Cavalerie* (PC 0083655); *P. Cavalerie 1992* (PC 0083656); *J. h. Erquirol 3141* (PC

0083658-59); *Fortunat 1552* (PC0719719); *Fortunat s.n.* (S: B163497, B163498); Gan Chouen Fou: *R. P. Cavalerie*, 1910 (S B115581); *R. P. Cavalerie*, 1912 (PC 0719720); Kouy-Tcheou, Tong Tcheou, Oct. 1904 *Fortunati* (H-BR 2581004). Guangdong: Jiaolin Co., *L. Deng 4834, 09517.* Vietnam, Tonkin, Chapa, *Pételot, P.A. 138* (IBSC).

Macromitrium nepalense (Hook. & Grev.) Schwägr., Nepal, *Wallich s.n.* (lectotype: BM000982533, isolectotype: E). Bhutan, *Griffith s.n* (BM000876994). China, Yunnan: Jinghong Co., *Crosby 14832, 15013, 15029* (all in MO). Laos, *He 43782* (MO). Nepal, *Wallich, s.n.* (BM000982523). India, Sikkim: *Kurz 2187* (H-BR).

Macromitrium tosae Besch., China, Fujian: Fuzhou, *Chung B159, B288, B311, B6158* (all in FH); Guangdong: Lofu Mountain, *Magill et al. 8148* (MO); Guangxi: Longzhou Co., *He 40511* (MO); Guizhou: Pinfa, *Cavalerie 9691* (PC); Hainan: Chim Fung, *Lau 5374*; Jianfengling (Mt.), *Chen et al. 869b, 869c* (all in MO). India. Madra [Madras], Madura [Madurai] district, *G. Foreau 1925.* Japan. Shikoku: *Faurie 11190* (isotype H-BR 2581002!). The Philippines, *Cuming s.n.* (BM 000982524). Thailand, Payap, granitic massive Doi (Mt.) Inthanon, *A. Touw, 10277* (MO). Vietnam, Ninh Binh, *He & Khang 42123* (MO).

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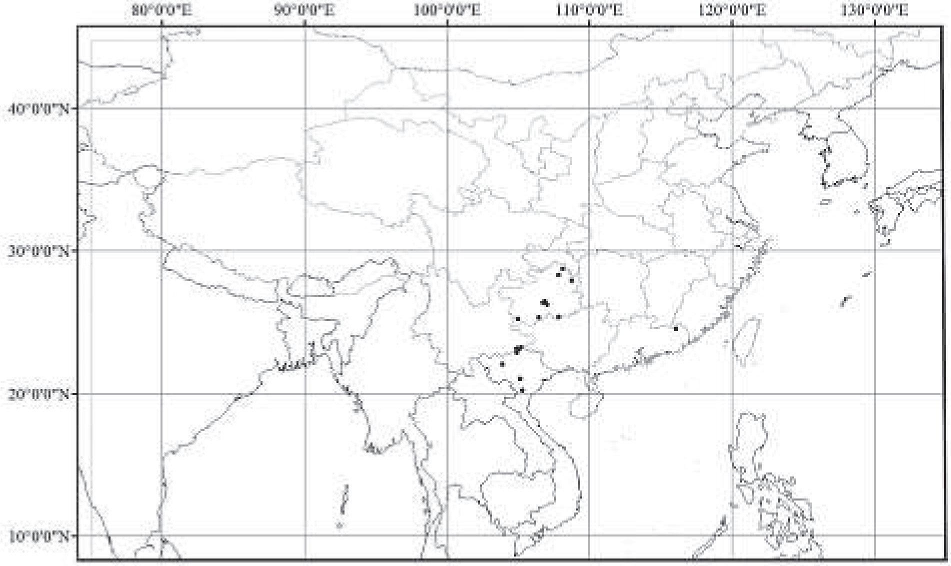

Fig. S1. Record locality of *Macromitrium fortunatii*

Table S1. Nine environmental variables used in the distribution prediction of *M. fortunatii*

No.	*Environmental variables*
1	Altitude (m)
2	Tree cover percentage
3	Bio1: Annual Mean Temperature (°C)
4	Bio5: Max temperature of warmest month (°C)
5	Bio7: Temperature Annual Range (°C)
6	Bio11: Mean Temperature of Coldest Quarter (°C)
7	Bio12: Annual Precipitation (mm)
8	Bio13: Precipitation of wettest month (mm)
9	Bio17: Precipitation of Driest quarter (mm)

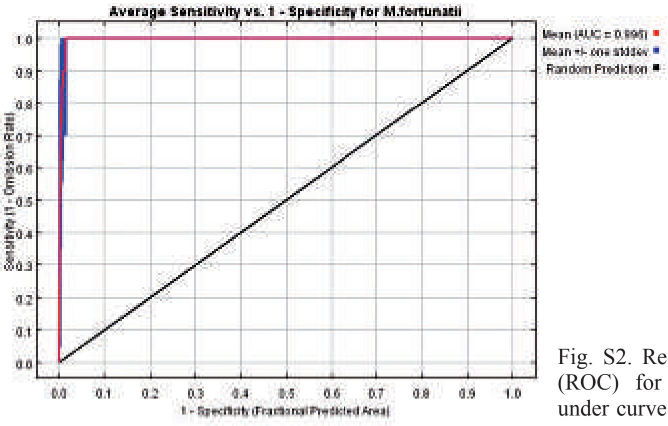

Fig. S2. Receiver Operator Characteristic (ROC) for Maxent model. AUC: area under curve.

Table S2. The percent contribution of environmental variables in predicting the distribution model of *M. fortunatii*

Variables	*Contribution (%)*
Annual Precipitation (mm)	25.0
Precipitation of Driest Quarter (mm)	25.0
Precipitation of Wettest Month (mm)	21.6
Mean Temperature of Coldest Quarter (°C)	18.2
Altitude (m)	6.3
Global vegetation (%)	1.8
Annual Mean Temperature (°C)	1.0
Temperature Annual Range (°C)	0.9
Max Temperature of Warmest Month (°C)	0.4

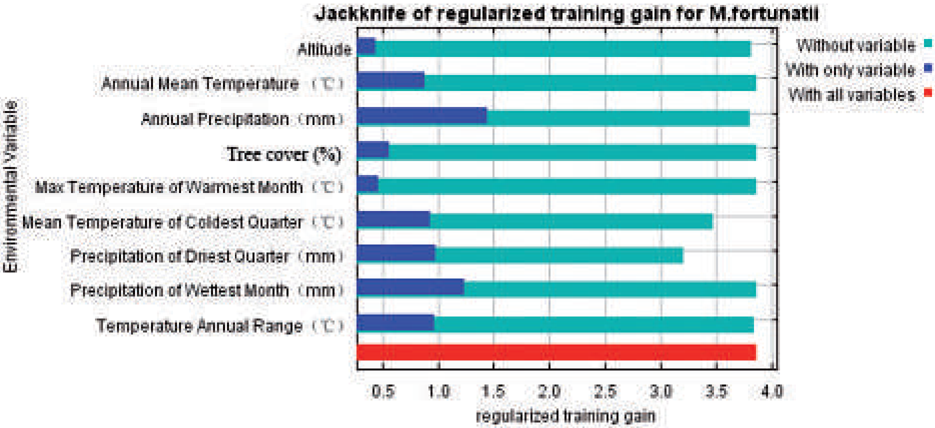

Fig. S3. Gains of the variables in the Maxent model (jackknife test). Torques bars: model gain without the corresponding variable; blue bars: model gain with only the corresponding variable; red bars: total gain using all the variables.

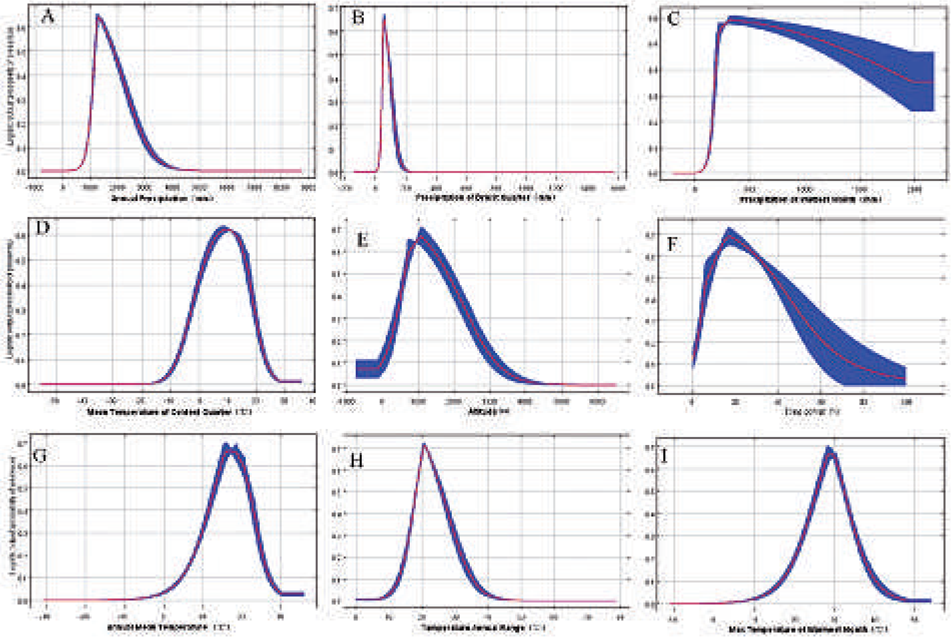

Fig. S4. Marginal response curves of the predicted probability of *Macromitrium fortunatii* occurrence to nine environmental variables. The value shown on the y-axis of the response curves (0 - 1) is predicted probability of suitable conditions. The highest predicted probability values in each response curve show the most suitable environmental conditions. Red lines indicate mean values of 10 replicates of the mdoel with standard deviation in blue shading.