

Plagiochila xerophila (Plagiochilaceae, Marchantiophyta) – a highly xerophilous new species from the Tibetan Spur (China)

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Background and aims – The xeric landscapes of the Tibetan floristic province are adverse habitats for leafy liverworts. Here we report on the occurrence of a population of a species of the genus *Plagiochila* from exposed high-elevation cliffs in the Sichuan Province, China. We assessed its taxonomic distinctiveness and affinities within a morphological and phylogenetic framework.

Results and discussion – The population is accommodated in a new species, *Plagiochila xerophila* Bakalin & Vilnet – probably the most xerophilous taxon within the genus in Asia – and a new section (sect. *Xerophilae* Bakalin & Vilnet) based on integrative analyses of molecular and morphological traits. The species is characterized by a greenish colour, unexpected given the highly exposed habitat, rigid texture and stem paraphyllia. The closest morphological relatives from sect. *Poeltiae* are phylogenetically only distantly related, whereas members of its sister groups, namely of sect. *Trabeculatae* and sect. *Fruticosae* are morphologically conspicuously different.

Keywords – China; East Asia; liverworts; integrative research; *Plagiochila*; taxonomy.

INTRODUCTION

With an estimated 400 species (Frey & Stech 2009), of which many remain poorly studied, *Plagiochila* (Dumort.) Dumort. is currently the most speciose genus of liverworts. It is widely distributed in suboceanic areas, and most diverse in tropical montane forests. Söderström et al. (2016) listed 744 accepted names, about half of them (366) ‘incertae sedis’, and potentially belonging to other genera. The remaining taxa are distributed among 28 sections (Söderström et al. 2015). In total, 196 species are widely accepted, 379 are relatively poorly known (Söderström et al. 2016) and 167 are suspected to be doubtful, suggesting that even an estimate of the diversity of *Plagiochila* remains ambiguous. Renner et al. (2017), for example, suggest that the species diversity of

Australasian *Plagiochila* is 29% higher than currently recognized, whereas, Gradstein (2015) only retained 34 species of the 135 reported from Brazil.

The diversity of *Plagiochila* is noticeable high in East Asia not only in the areas adjacent to the Pacific Ocean and its insular part (e.g. in Japanese archipelago, cf. Inoue 1958a, 1958b), but also in mountainous highlands inland, relatively far from the Pacific Ocean coasts, but still moistened with wet air masses coming from the Pacific. A robust framework for the study of Asian species of *Plagiochila* is provided by the taxonomic revision of Chinese species by So (2001), who recognized 80 species, including the majority of taxa known from East Asia. The latter work was exclusively based on morphological features. Recently, Bakalin & Vilnet (2017) described a peculiar new species of *Plagiochila* from the

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Russian Far East based on integrative approach. Here, we describe a further highly unusual new species from xeric high-elevation cliffs in eastern Asia based on morphological and molecular evidence.

Exploring the liverwort flora of high elevations areas in the Sichuan Province of China, we unexpectedly uncovered an unidentified, green and vermicular plant resembling *Plagiochila*. The specimen was collected in an area where *Plagiochila* species are indeed hardly expected: a desert habitat at 4474 m a.s.l., of gentle slopes from the ridge with many well exposed and virtually most of the year dry rocky outcrops. These outcrops lack vascular plant vegetation, except for a few low *Rhododendron* and *Dasiphora* shrubs on fine gravel between rocks. The specimen of *Plagiochila* occurred in micro-crevices in the rocky outcrops in patches of two highly xerophilous liverworts, *Gymnomitrium sinense* Müll. Frib. and *G. crenatilobum* Grolle. It stands out from among other species of the *Plagiochila* by the transversely inserted and orbicular leaves. To assess whether its morphological distinctiveness is not merely reflecting a phenotypic response of a more widespread species in this extreme habitat, we reconstructed its phylogenetic relationship based on variation in three genetic loci, and demonstrate that the sample is best accommodated in a new species and even new section of *Plagiochila*.

MATERIAL AND METHODS

Studied area

The specimen was collected in Sichuan Province (China) near the Kangding airport (30°07'01.1"N, 101°46'41.7"E), at 4474 m a.s.l., in dry rock crevices in an open SW-facing gentle slope in an alpine environment. The climate in the area can only be characterized by data for the Xinduqiao Settlement (30°01'54"N, 101°30'48"E) located at 3400 m a.s.l., which is the nearest settlement, but while far lower than 4400 m a.s.l., reflects a similar environment. Based on Climate-data.org (<https://en.climate-data.org/asia/china/sichuan/xinduqiao-483085/>, accessed 10 Nov. 2018) the mean annual temperature at the Xinduqiao Settlement is 5.4°C and the annual precipitation is slightly above 800 mm with distinct summer maximum. The total precipitation from June to September is 595 mm, corresponding well to that of forestless landscapes in the plateau reported by Miede et al. (2001). Local inhabitants, however, mention a considerably colder climate near the Kangding airport, with negative mean monthly temperatures from November to March and a mean annual temperature near 0°C.

Overall, the studied area bears an 'alpine meadow' formation on permafrost soil (Wang et al. 2017). However, the vegetation of the landscape is more complex, composed of a mosaic of different communities ranging from very sparse plant cushions in gravelly barrens to dry alpine steppes and *Kobresia* meadows. In general, the mesophytic *Kobresia* communities are more abundant in the southeastern part of the Tibetan Plateau and reflect general trends in the climate variations across the plateau (Miede et al. 2011a). Besides, the edges of the plateau are the ecotone between forested areas and alpine steppe, each with their distinct climatic char-

acteristics, as recently discussed for the southern Tibetan Plateau (He et al. 2012). The series of xerophilous elements in the area where the specimen was collected is in high contrast with rather mesophytic *Picea-Abies* forest flora occurring as near as ca. 15 km eastward from the collecting locality near the city of Kangding in the Sichuan Province. These two nearby localities are divided by the Haizi Shan Range with elevations above 5000 m a.s.l. that may intercept wet air masses coming from the Pacific and thereby promote the formation of a dry and cold climate in the area of the collection.

Morphological and molecular study

The morphological traits of the specimen (*V.A. Bakalin & K.G. Klimova China-46-2-17*) were studied at VBGI, and the molecular investigation conducted at KPABG based on a duplicate of the collection (herbarium codes following Thiers continuously updated).

DNA was extracted from dried liverwort tissue using the NucleoSpin Plant Kit (Macherey-Nagel, Germany). Amplification and sequencing were performed using primers given by White et al. (1990) for ITS1–2, Taberlet et al. (1991) for *trnL*-F and Kress & Erickson (2007) for *rbcL*.

PCR was carried out in 20 µL volumes with the following amplification cycles: 3 min at 94°C, 30 cycles (30 s at 94°C, 40 s at 56°C, 60 s at 72°C) and 2 min of final extension time at 72°C. Amplified fragments were visualized on 1% agarose TAE gels by ethidium bromide staining, purified using the GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences, USA), and then used as templates for sequencing reactions with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) following the standard protocol provided for 3100 Avant Genetic Analyzer (Applied Biosystems, USA).

Phylogenetic analyses

ITS1-2 nrDNA and *trnL*-F cpDNA were initially targeted to assess based on BLAST outcomes (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to clarify its morphological identification sequence similarity to those of other species of *Plagiochila*. To determine the phylogenetic affinities of the potential new species, relationships were inferred from analyses of partial *rbcL* cpDNA and ITS1-2 sequences based on datasets published by Renner et al. (2017), minus the *rps4* cpDNA sequences.

The implemented phylogenetic estimation of 160 *Plagiochilaceae* and related taxa by the Bayesian method (BA) with MrBayes v. 3.2.1 (Ronquist et al. 2012) estimated the Chinese specimen to be related to a clade composed by representatives from sections *Trabeculatae* S.Hatt. ex Inoue and *Fruticosae* Inoue of *Plagiochila*. Later the entire ITS1-2 and *rbcL* sequence data were downloaded from GenBank for 27 and 24 *Plagiochila* specimens, respectively, distributed among six sections (Patzak et al. 2016; Jamy et al. 2016; Renner et al. 2017): *Plagiochila*, *Trabeculatae*, *Fruticosae*, *Peculiares* Schiffn., *Vagae* Lindenb. and *Poeltiae* Inoue. The recently described *Plagiochila sikhotensis* (Bakalin & Vilnet 2017) was also included, and *P. andina* was chosen as the outgroup (table 1).

Table 1 – List of taxa, voucher specimens and GenBank accession numbers.

Accessions obtained in this study are in bold.

Taxon	Specimen voucher	GenBank accession numbers	
		ITS1-2 nrDNA	<i>rbcL</i> cpDNA
<i>Plagiochila abietina</i> (Nees) Nees & Mont.	Fiji, Renner <i>et al.</i> 5464 (NSW)	KX090150	KX090162
<i>Plagiochila andina</i> Steph.	Bolivia, Heinrichs & Müller 4046 (GOET)	DQ194028	DQ194111
<i>Plagiochila asplenioides</i> (L.) Dumort.	Italy, Schaefer-Verwimp & Verwimp 35859 (M)	KT992544	KT992617
<i>Plagiochila austinii</i> A. Evans	USA, Kentucky, Risk 10849 (DUKE)	AJ748130	DQ439699
<i>Plagiochila britannica</i> Paton	United Kingdom, Rycroft 00015, B.E.G.P. 199 (GOET)	AY275162	DQ194119
<i>Plagiochila carringtonii</i> subsp. <i>lobuchensis</i> Grolle	Bhutan, Long 28857 (GOET)	AJ414631	DQ194121
<i>Plagiochila elegans</i> Mitt.	China, Long 33675 (GOET)	AM180599	No data
<i>Plagiochila flexuosa</i> Mitt.	Japan, Kurita 147 (HIRO)	AY550138	DQ194140
<i>Plagiochila frondescens</i> (Nees) Lindenb.	Indonesia, Schaefer-Verwimp 20704 (GOET)	AY438237	DQ194141
<i>Plagiochila fruticosa</i> Mitt.	India, Long 23002 (GOET)	AY438235	No data
<i>Plagiochila hakkodensis</i> Steph.	Japan, Yamaguchi 12271 (HIRO)	AY275164	DQ194149
<i>Plagiochila korthalsiana</i> Molck. ex Sande Lac.	Indonesia, Mt. Gedeh, Java, Gradstein 10258 (GOET)	DQ194049	DQ194156
<i>Plagiochila magna</i> Inoue	Japan, Kyushu, Kurita 258 (HIRO)	AY275167	DQ194162
<i>Plagiochila orbicularis</i> (S.Hatt.) S.Hatt.	Japan, Kurita 132 (HIRO)	AY275168	DQ194167
<i>Plagiochila ovalifolia</i> Mitt.	Japan, Ohnishi 5723 (HIRO)	AY275169	No data
<i>Plagiochila peculiaris</i> Schiffn.	Bhutan, Long 28832 (GOET)	AY550141	DQ194176
	Australia, Renner 880483 (NSW)	KY051315	KY050816
<i>Plagiochila poeltii</i> Inoue & Grolle	India, Long 22802 (GOET)	AY550142	DQ194177
<i>Plagiochila porelloides</i> (Torr. ex Nees) Lindenb.	Portugal, Schaefer-Verwimp 31246 (M)	KT992543	KT992616
<i>Plagiochila pulcherrima</i> Horik.	Japan, Kyushu, Ohnishi 5771 (HIRO)	AY438239	DQ194179
<i>Plagiochila renitens</i> (Nees) Lindenb.	Malaysia, Schaefer-Verwimp 18736/A (GOET)	AY569441	DQ194184
<i>Plagiochila satoi</i> S.Hatt.	Japan, Ohnishi 5720 (HIRO)	AY550144	DQ194192
<i>Plagiochila semidecurrens</i> (Lehm. & Lindenb.) Lehm. & Lindenb.	Nepal, Long 21348 (GOET)	AY275172	DQ194194
<i>Plagiochila sichotensis</i> Bakalin & Vilnet	Russia, Primorsky Territory, Bakalin 1-25-13 (VBGI), 118477 (KPABG)	MF947695	MF947696
	Australia, Renner 855503 (NSW)	KY051508	KY051015
<i>Plagiochila</i> sp.	Fiji, Renner <i>et al.</i> 890205 (NSW)	KY051517	KY051026
<i>Plagiochila trabeculata</i> Steph.	Japan, Kyushu, Kurita 257 (HIRO)	AY550146	DQ194204
<i>Plagiochila trapezoidea</i> Lindenb.	Australia, Renner 897031 (NSW)	KY051545	KY051055
<i>Plagiochila xerophila</i> Bakalin & Vilnet	China, Sichuan Prov., V.A. Bakalin & K.G. Klimova China-46-2-17 (VBGI)	MK121889	MK123265 (<i>trnL-F</i> cpDNA: MK123266)

Considering the limited sampling of *trnL*-F data for species of *Plagiochila* on GenBank we did pursue using this locus to assess the affinities of the Chinese specimen. The combined ITS1-2+*rbcL* alignment (supplementary file 1) for 29 specimens of *Plagiochila* was analysed using the criteria of maximum parsimony (MP) with TNT v. 1.5 (Goloboff & Catalano 2016), and maximum likelihood method (ML) with PhyML v. 3.0 (Guindon et al. 2010), and also using a Bayesian approach. The parsimony analysis involved a New Technology Search with search for the minimal length tree by five iterations and 1000 bootstrap replicates, the default settings were used for other parameters, indels were taken into account by a modified complex coding algorithm in SeqState (Müller 2005). The GTR+I+G model was selected for the ML analyses as the best-fit evolutionary model of nucleotide substitutions for alignment using the ModelGenerator software (Keane et al. 2004). For the ML analysis, the rate heterogeneity among sites was modelled using a gamma distribution with four rate categories. Bootstrap support (BS) for individual nodes was assessed using a resampling procedure with 500 replicates. Based on the stopping criterion (Pattengale et al. 2010) 200 replicates would in fact suffice enough to reach BS convergence with Pearson average $\rho_{100} = 0.994058$ as estimated by RAxML v. 7.2.6 (Stamatakis 2006).

For the Bayesian analysis, each of the partitions of combined alignment (ITS1-2, *rbcL*) was separately assigned the GTR+I+G model, and gamma distributions were approximated using four categories. Two independent runs of the Metropolis-coupled μ MC were used to sample parameter values in proportion to their posterior probability. Each run included three heated chains and one unheated, and two starting trees were chosen randomly. The number of generations was one million, and trees were saved once every 10^4 generation. The average standard deviation of split frequencies between two runs was 0.008395. The software tool Tracer (Rambaut & Drummond 2007) revealed effective sample size (ESS) as 4134.3033 and auto-correlation time (ACT) as 435.3865 for our data. The first 10000 trees as determined by Tracer were discarded in each run, and 180000 trees from both runs were sampled after burning. Bayesian posterior probabilities (PP) were calculated as branch support values from trees sampled after burn-in.

The infrageneric variability of ITS1-2 and *rbcL* sequences for selected *Plagiochila* species was estimated as the value of the *p*-distances, as calculated in Mega v. 5.1 (Tamura et al. 2011) using the pairwise deletion option for counting gaps.

RESULTS

The combined ITS1-2+*rbcL* alignment for 29 specimens of *Plagiochila* consists of 1416 characters (i.e., 857 for ITS1-2 and 559 for *rbcL*). The number of invariable sites in the ITS1-2 and *rbcL* are 564 (65.81%) and 508 (90.88%), respectively, variable positions are 268 (31.27%) and 51 (9.12%), parsimony informative positions are 136 (15.87%) and 24 (4.29%).

The MP analysis with TNT yielded two equally parsimonious trees with a length of 997 steps, a Consistency Index of 0.754941 and Retention Index of 0.762452 calculated

in Mega 5.1. The ML calculation resulted in a single tree, the arithmetic means of Log likelihood was -5037.316145. Arithmetic means of Log likelihoods in BA analysis for both runs sampled were -4906.88 and -4906.52.

The tree topologies achieved by the three methods are highly congruent and also consistent with those published in Patzak et al. (2016) and Renner et al. (2017). The most likely topology is presented in fig. 1 with MP and ML bootstrap support values and Bayesian posterior probabilities. All species were distributed among clades corresponding to the six sampled sections currently accepted in *Plagiochila*. Our unusual Chinese specimen was weakly resolved as sharing a unique common ancestor with two robust sister clades, i.e., section *Trabeculatae* and *Fruticosae*. The ITS1-2 and *rbcL* sequences of the new specimen diverged from those of species of sections *Trabeculatae* and *Fruticosae* by 6.3–8.8% and 0.7–1.6%, respectively, and thus a degree similar to that between species of these two sections (4.2–6.2% in ITS1-2, 1.1–1.8% in *rbcL*, table 2).

DISCUSSION

A new section and a new species

Based on an integrative taxonomic approach based on molecular, morphological, ecological and geographical data we accommodate the population from the arid region of the Kangding area in a new species for science, *Plagiochila xerophila*, described in the Taxonomic Treatment below.

The phylogenetic position of the new species and its morphological similarity to *P. aspericaulis* (see below, Relationships) show that this species pair should be placed in a new section named here *Xerophilae*, described and typified in the Taxonomic Treatment.

Plagiochila habitats

Unlike in South America, where many species of *Plagiochila* occur above 4000 m a.s.l. (Gradstein 2016), not many species exhibit an altitudinal range exceeding 4000 m a.s.l. in East Asia. The following species were listed by So (2001): *P. aspericaulis* Grolle & M.L.So (above 4760 m), *P. biondiana* C.Massal. (to 4300 m), *P. delavayi* Steph. (to 5000 m), *P. duthiana* Steph. (to 5000 m), *P. carringtonii* (Balf. ex Carrington) Grolle (to 5100 m), *P. poeltii* Inoue & Grolle (to 5100 m), *P. pseudopoeltii* Inoue (to 4030 m), *P. recurvata* (W.E.Nicholson) Grolle (to 4800 m), *P. retusa* Mitt. (to 4200 m), and *P. semidecurrrens* (Lehm. & Lindenb.) Lindenb. (to 4500 m). Most of the taxa are mesophytic and occur in forests, and only a few are characterized by densely-leaved shoots and occurrence in arid habitats (see table 3).

The distinctly xerophilous habitat deviates from the ecological preference of the overwhelming majority of *Plagiochila* taxa. The most 'xeric' taxa of the genus from East Asia are found in sect. *Poeltiae* and sect. *Peculiares* (including the former sect. *Zonatae*). The morphologically similar taxa from both sections are nevertheless more water-dependent than *P. xerophila* and more brightly coloured with well-developed secondary brown pigmentation. The greenish coloration of *P. xerophila* is indeed unexpected, as in the

Table 2 – *P*-distances ITS1-2 /*rbcL*, % for the species from sections *Trabeculatae*, *Fruticosae* and the new species.
 - : non calculated value due to lack of DNA for specific locus.

Section	Species	<i>Trabeculatae</i>				<i>Fruticosae</i>		<i>Xerophilae</i>
		<i>P. trabeculata</i>	<i>P. flexuosa</i>	<i>P. austinii</i>	<i>P. pulcherrima</i>	<i>P. fruticosa</i>	<i>P. frondescens</i>	<i>P. xerophila</i>
<i>Trabeculatae</i>	<i>P. trabeculata</i>							
	<i>P. flexuosa</i>	0.5/0.2						
	<i>P. austinii</i>	5.7/0.7	5.8/0.5					
	<i>P. pulcherrima</i>	4.2/1.8	4.3/1.6	6.1/1.8				
<i>Fruticosae</i>	<i>P. fruticosa</i>	4.2/-	4.3/-	6.1/-	0.3/-			
	<i>P. frondescens</i>	4.5/1.1	4.7/1.3	6.2/1.4	2.3/1.4	2.3/-		
<i>Xerophilae</i>	<i>P. xerophila</i>	6.5/0.9	6.6/0.7	8.8/0.9	6.3/1.6	6.3/-	7.0/1.3	

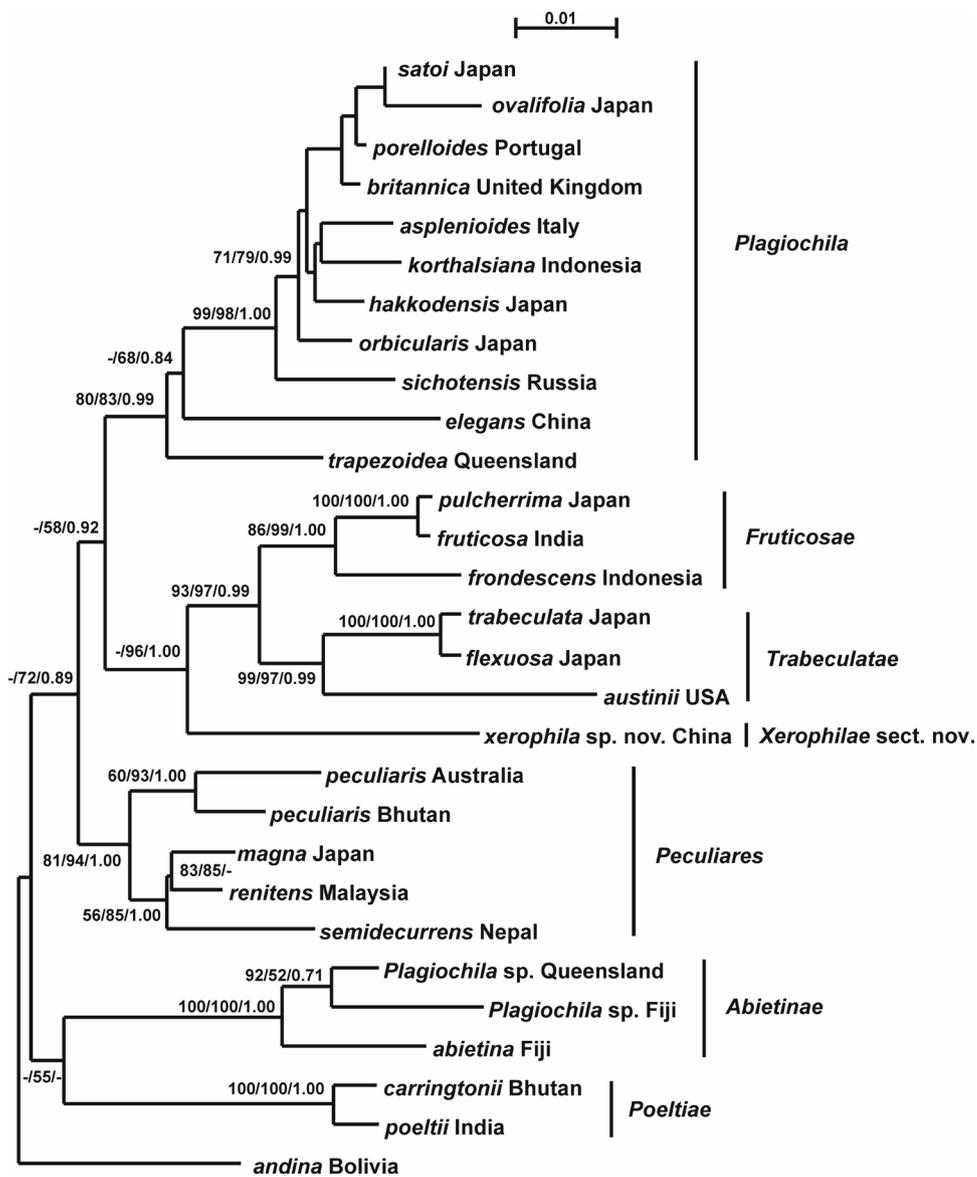


Figure 1 – Phylogram obtained in a maximum likelihood calculation for the selected sections of the genus *Plagiochila* based on combined nucleotide sequences dataset of ITS1-2 nrDNA and *rbcL* cpDNA. Bootstrap support values under maximum parsimony, maximum likelihood, and Bayesian posterior probabilities > 50% (0.50) are indicated.

Table 3 – *Plagiochila* taxa morphologically similar to *P. xerophila*.

Species	Section	Colour	Hyaloderm in stem	Paraphyllia-like structures	'Vitta' cells in the leaf base	Leaf dentation	Leaf position	Ecology
<i>P. caulimammillosa</i> Grolle & M.L.So	sect. <i>Peculiares</i> Schiffn.	pale brown to dark brown	present	absent	present	present	laterally spreading	mesophyte, epiphytic
<i>P. biondiana</i> C.Massal.	sect. <i>Poeltiae</i> Inoue	dark brown	absent	absent	absent	present, rather coarse	laterally appressed	mesophyte, epiphytic
<i>P. carringtonii</i> (Balf. ex Carrington) Grolle	sect. <i>Poeltiae</i> Inoue	reddish brown	absent	absent	absent	absent or very few	laterally appressed	meso-hygrophyte, epilithic, damp mossy mats
<i>P. dulhiana</i> Steph.	sect. <i>Poeltiae</i> Inoue	yellowish brown	absent	absent	absent	absent or inconspicuous	laterally spreading	mesophyte, epilithic to epigeric and epiphytic
<i>P. poeltii</i> Inoue & Grolle	sect. <i>Poeltiae</i> Inoue	dull brown	absent	present, lamelliform, entire	absent	present, coarse	laterally appressed	mesophyte epixylic
<i>P. recurvata</i> (W.E.Nicholson) Grolle	sect. <i>Poeltiae</i> Inoue	reddish brown	absent	present lamelliform, entire	absent	absent or inconspicuous	laterally appressed to somewhat spreading	meso-hygrophyte, epilithic to epigeric
<i>P. delavayi</i> Steph.	sect. <i>Plagiochila</i>	brown	absent	absent	absent	present	laterally spreading	mesophyte
<i>P. aspericaulis</i> Grolle & M.L.So	sect. <i>Xerophilae</i> Bakalin & Vilnet	brown to dark brown	absent, but overgrowth (paraphyllia) very think-walled in cross section	present, inconsistently lamelliform, dentate	present	present, coarse	appressed to somewhat spreading	mesophyte, forest floor
<i>P. xerophila</i> Bakalin & Vilnet	sect. <i>Xerophilae</i> Bakalin & Vilnet	yellowish greenish to greenish brownish	absent	present, inconsistently lamelliform, dentate	absent	present, sparse	laterally appressed	xerophyte, epilithic

majority of liverworts the occurrence in well exposed places is commonly associated with a brown, red or purple pigmentation (Post 1990; Waterman 2018).

Relationships

The inferred shared ancestry of *Plagiochila* sect. *Xerophilae* with sect. *Trabeculatae* and sect. *Fruticosae* is also difficult to expect (and explain) from the morphological point of view. The representatives of the two sections, commonly characterized by distinctly longer than wide, somewhat plane and distanced to slightly contiguous leaves (So 2001), are in striking contrast with the densely-leaved shoots with suborbicular leaves in *P. xerophila*. Members of sect. *Poeltiae* are morphologically somewhat similar to *P. xerophila*. All superficially similar taxa of sect. *Poeltiae* differ by the more distinct secondary pigmentation and other traits (see table 3). Two species of this section, *P. poeltii* and *P. recurvata*, resemble *P. xerophila* by their paraphyllia. *Plagiochila poeltii* is perhaps the most likely to be confused with *P. xerophila* due to its dull brown colour. They differ, however, in several traits: 1) nodulose trigones in the leaf cells of *P. poeltii* versus concave trigones in *P. xerophila*, 2) smooth leaf cuticle versus commonly finely asperulose, 3) well developed lamelliform paraphyllia with entire margins versus paraphyllia inconsistent with dentate to denticulate margins. The distance is also shown by the phylogenetic analysis (fig. 1).

Plagiochila xerophila is morphologically most similar to *P. caulimammillosa* and especially to *P. aspericaulis* from subsect. *Caulimammillosae* Grolle & So of sect. *Peculiares* (see fig. 3). *Plagiochila xerophila* differs from *P. caulimammillosa* in plant size (less than 1.5 mm versus to 4.5 mm wide), in leaf areolation (very poorly developed vitta-like area in the basal part of the leaf versus vitta-like area well developed), and, especially, in the hyalodermis (absent, although epidermal stem cells have walls somewhat thinner than inward, but never so thin to be eroded, versus well-developed and composed of thin-walled cells that are commonly eroded in older part of the shoot). *Plagiochila caulimammillosa* is a peculiar species of the genus in this latter trait (cf. So 2001).

Plagiochila aspericaulis is another taxon morphologically most similar to *P. xerophila*, but differs by its brown to dark brown color (versus greenish to greenish brownish and dirty green coloration of *P. xerophila*), its ovate leaves that distinctly longer than wide and prominently dentate (versus rounded and sparsely and obtusely dentate in *P. xerophila*), its stem diameter reaching 270 µm in width (versus less than 200 µm in *P. xerophila* (despite copious and well developed material of *P. xerophila* in our collection contrary to scanty plants of *P. aspericaulis* in iso- and paratypes), and stem overgrowths commonly reaching two or three cells in height on both the dorsal and ventral side in *P. aspericaulis* (versus outgrowth on dorsal side only in *P. xerophila*). Furthermore, the two taxa differ in their ecology: *P. aspericaulis* grows in mesophytic habitats on the forest floor whereas *P. xerophila* occurs on fully sun-exposed cliffs. The intricate problem is the distribution pattern differences between *P. xerophila* and *P. aspericaulis*. As currently estimated, *P. aspericaulis* is confined to the forested areas of Tibetan Plateau edges.

Whether it penetrates the alpine steppes and, therefore could be a potential competitor with *P. xerophila* is unknown.

As previously shown by Söderström et al. (2015) and Renner et al. (2017) members of *Plagiochila* sect. *Zonatae* subsect. *Zonatae* belong to *Plagiochila* sect. *Peculiares* Schiffn. (cf. Renner et al. 2017: Fig. 1). This ‘*Peculiares-Zonatae*’ complex is clearly different from the newly described species. Moreover, we estimate that if even we will erect subsect. *Caulimammillosae* (type species *P. caulimammillosa*) to the section level, the pair *P. aspericaulis*-*P. xerophila* should hardly be referred to that section due to the morphological differentiations. Unfortunately, three attempts to extract DNA for *P. caulimammillosa* and *P. aspericaulis* failed. Therefore, we prefer to retain *P. caulimammillosa* in sect. *Zonatae* subsect. *Caulimammillosae* and to separate *P. aspericaulis* and *P. xerophila* in a new section.

The phylogenetic hypothesis (fig. 1) whereby the morphologically similar sect. *Xerophilae* and *Poeltiae* are rather distantly related suggest parallel evolution of features such as densely foliated shoots, and rounded leaves with recurved margin or paraphyllia.

Biogeography

The studied area marks the transition between the Tibetan Province of the Irano-Turanian floristic region and the East Asian floristic province (cf. Chang 1981; Takhtajan 1986). Chang (1981) and He et al. (2012) noted the proximity of two different vegetation complexes in the edges of the plateau. The area where *Plagiochila xerophila* occurs shows drastically contrasting environments that may promote speciation. The same was observed in the Tibetan Plateau in general (Wen et al. 2014) and results in a large proportion of endemic taxa as it was noted for vascular plants in the alpine steppe in other parts of the plateau (Miehe et al. 2011b). Indeed, *P. xerophila* was collected in the dry alpine steppe, whereas as near as 10–15 km eastward (although behind of range of over 5000 m a.s.l.) more mesophytic communities are abundant and provide the suitable habitats for taxa with Sino-Himalayan distribution. These two vegetation complexes are markedly different in water requirements, with dry steppe plants commonly drought tolerant, and forest taxa, distributed also in the wetter edges of spurs of Tibetan Plateau, being mostly mesophytic and hydro-mesophytic.

Takhtajan (1986) characterized the Tibetan Province as relatively poor in species and relatively young, a consequence of its modern ‘vegetation history’ following the last glaciation. He argued that its vegetation probably originated from the transformation of Central Asian and (in broader view) ancient Mediterranean (~circum Paleo-Tethyan) ancestors. Spicer (2017) argued for a Paleogene origin of the diversity of southern Asia, including the Proto-Tibetan Highland at the northern extreme of the latter (uplifted before final Paleo-Tethys Ocean closure). However, he also confirmed (Spicer 2017) that the East Asian Monsoon, which governs patterns of precipitations, is a Neogene (ca. 22 Ma) phenomenon. The monsoon might be associated with Himalaya uplift, cooling in Central Asia and therefore additional decreasing of precipitation in the Tibetan Highland than it was before the splitting of single Asian monsoon into East

Asian and South-East Asian ones. The latter events drastically changed ecological conditions (for plant growth) in the Tibetan Highlands. These sudden changes in climate resulted in vegetation community movements and changes and could promote speciation in contrasting environments (Herzschuh et al. 2011; Wen et al. 2014).

It is difficult to predict the distribution of *Plagiochila xerophila*. It may be more widely distributed across Tibetan Plateau, at least in its eastern part, because of presence of virtually suitable habitats.

Liverworts provide many examples of mesophytic elements of meta-Himalayan distribution (cf. the term in Bakalin et al. 2018a). Outside of the eastern part of the Himalayas these taxa occur southeastwards along the large spur that geomorphologically belongs to Tibetan Plateau. Some taxa of the mesophytic vegetation complex (cf. Bakalin et al. 2018b) occur within 10–15 km from the locality of *Plagiochila xerophila*.

Liverworts, related to the xerophilous Tibetan complex are likely rare. *Plagiochila xerophila* may be one of the few rare cases. *Gymnomitrium sinense* and *G. crenatilobum* which are associated with *P. xerophila* may also belong to the xerophilous Tibetan complex. The representatives of the two complexes – mesophytic and xerophytic – probably originated in different areas at a different time. By now, the two complexes, however, penetrate one another. The penetration may be especially simple for the taxa of xerophytic nature, since even in generally moist regions (e.g. even along Pacific Ocean Coast), there are dry substrates that may house taxa distinctly alien to the general flora characteristics. Such evidence was found, e.g. in the distribution of xerophilous *Plagiochasma* in Japan (Bischler 1978) and the distribution of primarily paleo-Tethyan xerophytic *Riccia* in relict habitats of coastal areas in North-Eastern part of the East Asian mainland (Borovichev & Bakalin 2016).

TAXONOMIC TREATMENT

Plagiochila (Dumort.) Dumort.

Sect. *Xerophilae* Bakalin & Vilnet, **sect. nov.**

Type species – *Plagiochila xerophila* Bakalin & Vilnet (present paper).

Description – Plants of xerophytic habitats, yellowish greenish to greenish brownish, densely leaved; leaves suborbicular, paraphyllia present.

Plagiochila aspericaulis Grolle & M.L.So (Grolle & So 1999: 30).

Type – China, Xizang Province, Yadong (27°30'N 89°E), 4760 m, in rock crevice, 1975, *M. Zhang 713* (holotype: KUN; isotypes: HKBU, JE!).

Description – So (2001): 30, fig. 8.

Distribution – Xizang and Yunnan Provinces of China and Nepal.

Habitat – “On forest floor; above 4760 m.” (So 2001:31).

Plagiochila xerophila Bakalin & Vilnet, **sp. nov.**

Figs 2, 3

Type – China, Sichuan Province, Kangding airport area (30°07'01.1"N 101°46'41.7"E), 4474 m, SW-facing gentle slope of the range, cliffs in alpine environments, open, dry crevice in the cliff, 14 Oct. 2017, *V.A. Bakalin & K.G. Klimova China-46-2-17* (holotype: VGBI; isotype: KPABG) (Genbank accession numbers: MK121889; MK123265, MK123266).

Description – **Plants** rigid, pale green to yellowish greenish to greenish brownish, erect in dense patches, 0.8–1.5 mm wide and 3–6 mm long. **Rhizoids** in erect spreading fascicles, grayish. **Stem** greenish-brownish, sparsely laterally intercalary branched, cross section transversely elliptic, ca. 225 × 175 μm, with mammilose epidermal cell walls or with overgrowths 1 or 2(–5) cells high (forming somewhat dentate, lamelliform outgrowths (= paraphyllia) on the dorsal side of the stem), epidermis cells 15–22 μm in diameter, more or less thin-walled, with external wall thick, inward of epidermis cell walls become thicker and forming a 1 or 2 layers of thick-walled cells, further inward to the stem middle cell walls gradually become thin, the innermost cells 17–25 μm in diameter, with small, concave trigones. **Leaves** transversely inserted, decurrent for 0.5–1.0 of stem width on both sides, strongly ventral, at the first glance looking as incubous, suborbicular when flattened, 0.5–1.0 mm in diameter, dorsal side with strongly recurved margin, apical margin and upper half of ventral leaf margin sparsely and shortly dentate, with wide-based triangular teeth, teeth 1–3 cells long. **Midleaf cells** 12–25 × 12–20 μm, thin-walled, with moderately-sized concave trigones, in larger cells sometimes with intermediate thickenings, cuticle very finely and at times obscurely asperulous, cells near leaf base 30–75 × 12–22 μm, thin-walled with small trigones, bistratose in lower 120–170 μm from base; marginal cells 10–16 μm along leaf margin, thin-walled to with slightly thickened walls, with moderate to small trigones. **Generative structures** unknown.

Distribution – Unknown, perhaps widely distributed across the Tibetan Plateau, at least in its eastern part.

Habitat – Poorly known, collected in rocky outcrops in alpine environment.

SUPPLEMENTARY FILE

One supplementary file is associated to this paper:

Combined ITS1-2 nrDNA+ *rbcL* cpDNA alignment for 29 specimens (Nexus file):

<https://doi.org/10.5091/plecevo.2020.1560.2051>

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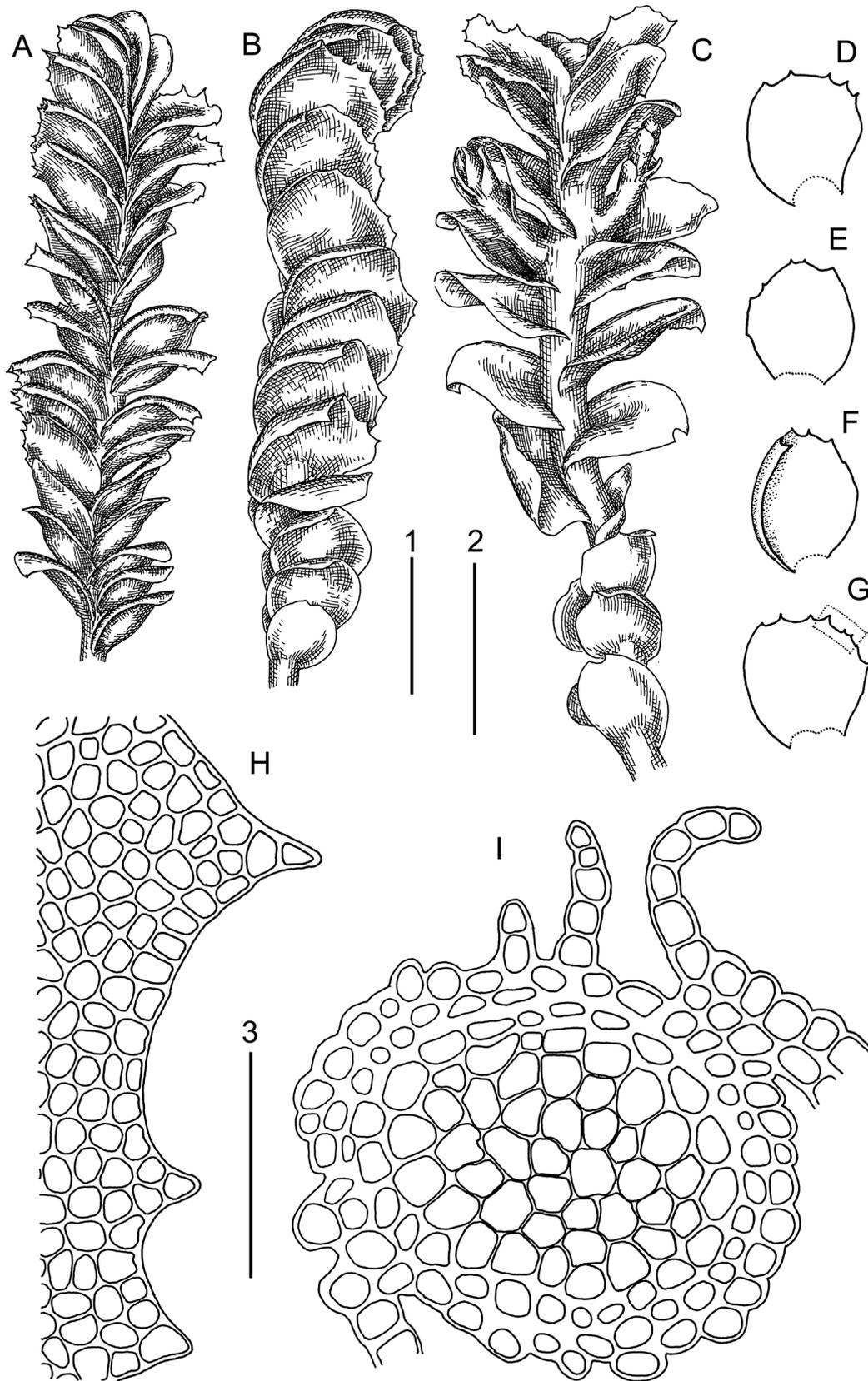


Figure 2 – A–H. *Plagiochila xerophila*, from Bakalin & Klimova China-46-2-17 (VBGI). A. Plant habit, dorsal view. B. Plant habit, lateral view. C. Shoot fragment, ventral view. D–G. Leaves (rectangular area at G is increased at H). H. Leaf margin. I. Stem cross section. Scales: 1 (for A, B, D–G) = 1 mm; 2 (for C) = 1 mm; 3 (for H, I) = 100 μ m. Drawn by Matvei Bakalin.

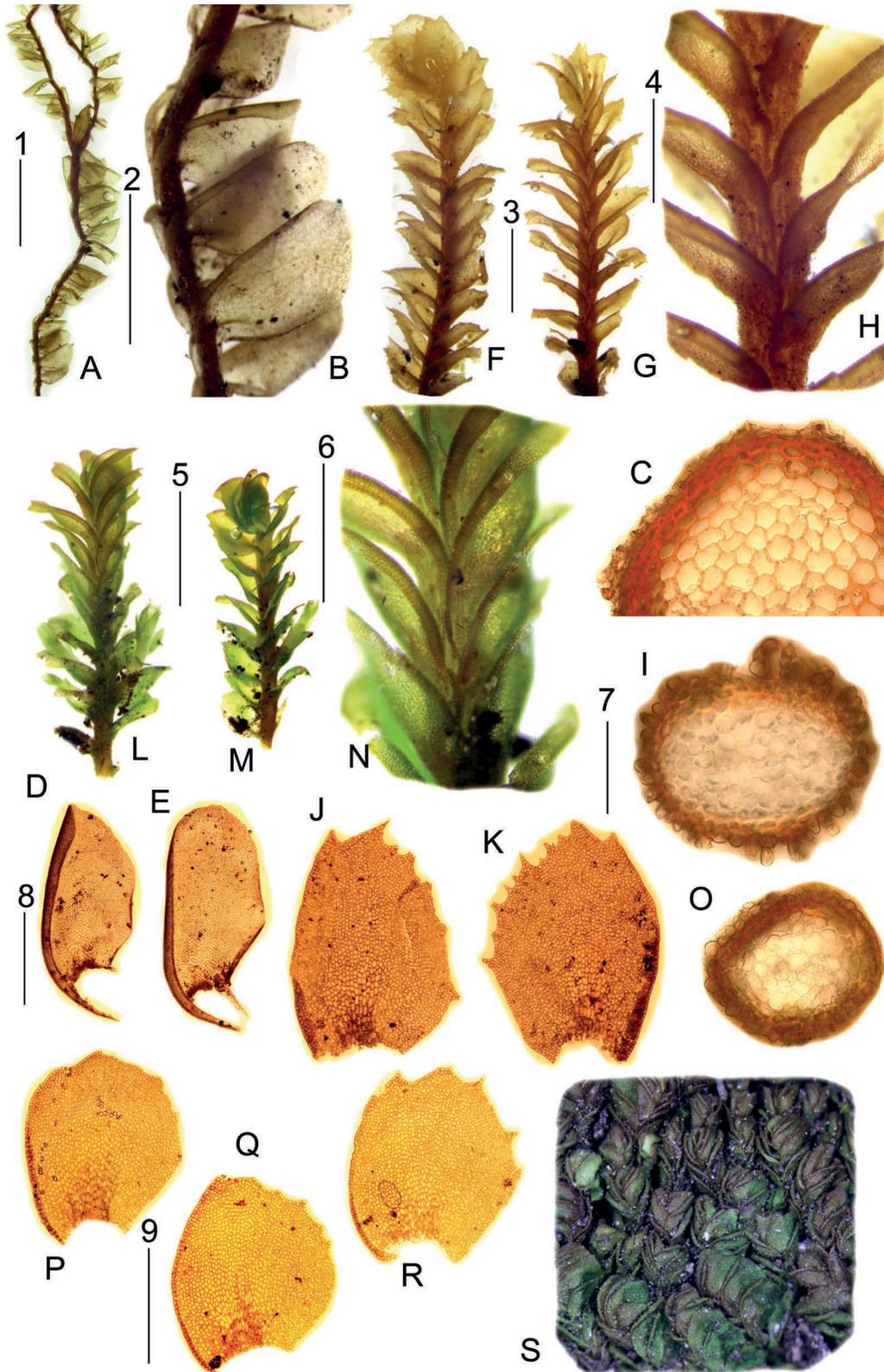


Figure 3 – A–E. *Plagiochila caulimammillosa*, from Long 24304 (JE). A. Dorsal view, perianthous branch. B. Shoot lateral view, fragment. C. Stem cross section, fragment. D, E. Leaves. F–K. *Plagiochila aspericaulis*, from Zhang Mu 713 (JE). F. Plant habit ventral view. G. Plant habit dorsal view. H. Dorsal view shoot, fragment. I. Stem cross section. J, K. Leaves. L–S. *Plagiochila xerophila*, from Bakalin & Klimova China-46-2-17 (VBGI). L. Plant, dorsal view. M. Plant, ventral view. N. Plant dorsal view, fragment. O. Stem cross section. P–R. Leaves. S. Cushion. Scales: 1 (for A) = 5 mm; 2 (for B) = 3 mm; 3 (for F, G) = 1 mm; 4 (for H) 500 μ m; 5 (for L, M) = 1 mm; 6 (for N) = 500 μ m; 6 (for C, I, O) = 100 μ m; 8 (for D, E) = 1 mm; 9 (for J–R) = 500 μ m.

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