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Species boundaries in European and Macaronesian *Porella* L. (Jungermanniales, Porellaceae) [1]

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Abstract – The genus *Porella* is known for high phenotypic plasticity and rather ill defined species boundaries. Genetic and morphological data are analyzed for a broad sample of European and Macaronesian species (328 colonies collected in about 150 localities) in order to i) separate genetic entities and ii) identify the morphological characters that best define these entities. Nine species are recognized, including *P. platyphylloidea* from Canada, included for comparison. Many *Porella* colonies are multiclonal, attested by diversity found within colonies with intraspecific and interspecific polymorphisms, but also by male and female individuals found growing intermixed. At the intraspecific level, high levels of genetic variation are found, especially for *P. canariensis, P. obtusata* and *P. platyphylla*. Finally a revised key of the species is proposed.

***Porella* / isozymes / morphology / species delimitation / allopolyploidy / Europe / Macaronesia**

Résumé – Il est difficile de reconnaître les espèces du genre *Porella*, connu pour sa grande plasticité phénotypique. Des analyses génétiques et morphologiques ont été réalisées sur un grand échantillonnage d'espèces européennes et macaronésiennes (328 colonies collectées dans 150 localités environ) afin de i) définir les entités génétiques et ii) trouver les meilleurs caractères permettant leur identification. Neuf espèces sont ainsi reconnues, dont *P. platyphylloidea* avec des colonies du Canada incluses pour comparaison. La plupart des colonies de *Porella* sont multiclonales ; elles présentent du polymorphisme intra- et interspécifique et des mélanges de thalles mâles et femelles. Au niveau intraspécifique, le niveau de variabilité génétique observé est élevé, spécialement chez *P. canariensis, P. obtusata* et *P. platyphylla*. Enfin, une clé révisée des espèces est donnée.

***Porella* / isoenzymes / morphologie / délimitation d'espèces / allopolyploïdie / Europe / Macaronésie**

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[1] This manuscript was mainly written by Helene in 2004, with a last version dated on May 2004, ten months before she died. She performed all the statistical treatments and drew illustrations.

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INTRODUCTION

Species of *Porella* L. (Jungermanniales, Porellaceae) are common on trees and rocks in Europe and are a part of most forest floras in all areas with at least 600 mm annual precipitation. They are most common in northern and central Europe, the Atlantic coast and islands, but may occur scattered also in the Mediterranean area. Optimal growth conditions are found in the laurel forest of Madeira Island where nearly every tree and every rock surface harbour colonies of *Porella*.

The genus *Porella* is known for high phenotypic plasticity and rather ill defined species boundaries. At least 100 species, centered mainly in eastern Asia, have been described (Hattori, 1978; Schuster, 1980). Many show significant morphological intergradations. In a previous treatment of a limited number of European and Canadian colonies, Boisselier-Dubayle and Bischler (1994) used morphological, isozyme and Randomly Amplified Polymorphic DNA (RAPD) markers to define eight groups of individuals of which seven corresponded to described species: *P. arboris-vitae* (With.) Grolle, *P. canariensis* (Web.) Underw., *P. cordaeana* (Hüb.) Moore, *P. obtusata* (Tayl.) Trev., *P. pinnata* L., *P. platyphylla* (L.) Pfeiff. from Europe and *P. platyphylloidea* (Schwein.) Lindb. from Canada. The eighth group, represented by a single colony, could not be assigned to any known taxon. Subsequent analyses including additional colonies corresponding to this group and to its close relatives *P. platyphylla* and *P. cordaeana*, revealed that this genetic entity was an allopolyploid hybrid between *P. platyphylla* and *P. cordaeana* that corresponds to *P. x baueri* (Schiffn.) C. Jens (Boisselier-Dubayle *et al.*, 1998).

The present study aims at testing previous hypotheses of species delimitation based on critical analyses of genetic and morphological data for a broad sample of colonies of European and Macaronesian species (including the endemic *P. inaequalis* (Gottsche ex Steph.) Perss.). Specifically, 1) species boundaries will be drawn from genetic data, 2) quantitative and qualitative morphological features that best characterize the defined genetic entities will be identified, 3) the intraspecific phenotypic and genotypic variation will be outlined for the species and 4) a revised key to the species will be proposed.

MATERIALS AND METHODS

Sampling

Our analysis included European and Macaronesian *Porella* species. A total of 328 colonies were sampled between 1991 and 1996 from about 150 localities in France (188), Germany (2), Switzerland (12), England (4), Spain (3), Portugal (19), the Canary Islands (9), Madeira (80), and, for comparison, Canada (Quebec, 9). The colonies are defined as continuous carpets ranging from 10 cm^2 to 20 cm^2 in cover, each comprising several tens of stems. A minimum distance of 100 m separated two collection sites (*i.e.* colonies) in anyone locality. Voucher specimens are deposited in PC and MADJ.

The colonies were either analyzed directly from the field, or cultivated as indicated in Boisselier-Dubayle and Bischler (1994). Three to 30 stems were taken

at random from the different colonies for isozyme assays (3185 stems for 321 colonies) and one to 14 other stems were used for morphological analyses (1693 stems for 328 colonies). Both analyses could not be performed on the same stem because of their small size.

Isozyme analyses

Electrophoretic techniques were as described in Boisselier-Dubayle *et al.* (1995a, b). Plant extracts were generally obtained from single gametophytic stems.

Six enzyme systems were surveyed and 11 loci could be scored for most of the extracts: 2 for esterase (*est-1, est-2*), glutamate-oxaloacetate transaminase (*got-1, got-2*), peroxidase (*per-1, per-2*) and isocitrate dehydrogenase (*idh-1, idh-2*), 1 for glutamate dehydrogenase (*gdh*), malic enzyme (*me*), and finally phosphoglucomutase (*pgm*) only scored for 735 extracts.

Letters were assigned to the different alleles found at these 11 loci in decreasing order of mobility. Allele frequencies were then tabulated according to our results and analyzed using PopGene version 1.32 package (Yeh *et al.*, 1997). Mean number of alleles per locus and percent of loci polymorphic per population were calculated, and the genetic identities between species were determined using Nei's (1972) coefficient, except within the polyploid *P. baueri*.

Morphological analyses

Porella is characterized by deeply conduplicate-bilobed, incubous leaves with large dorsal lobes and small ventral lobules with very short keels, large unlobed underleaves, and gametangia developing on short lateral branches. The sporophytes are ephemeral and therefore rarely seen; the spores are small (35-50 μm diam.). Leaf or shoot fragments function as asexual propagules.

Twenty qualitative and 20 quantitative morphological characters were selected as in our previous studies (Boisselier-Dubayle and Bischler, 1994; Boisselier-Dubayle *et al.*, 1998a). The characters were coded and character-states (see Table 1) for the qualitative and quantitative characters scored separately on two sheets for 1693 stems.

Porella species are dioecious. The stems with reproductive structures were either male or female, and many were sterile. The eight qualitative characters describing sex organs (male: bm, bms, female: bft, bfa, bfla, bfm, bflm, pe, Table 1) were on average absent in half of the individuals, introducing missing data in the data sheet of qualitative characters.

Statistical treatments

Multivariate analyses of the data sets were performed with ADE–4 (Thioulouse *et al.*, 2000). Dioecy combined with the overall rarity of reproductive organs account for 10% of the data to be missing. Thus, the variables dealing with the male sex organs (bms, bms, Table 1), are particularly scanty, and had to be discarded. For the other morphological characters, the same character state was then attributed to all the analyzed stems of the colony when exhibited by several ones, in order to minimize the missing data.

Qualitative characters were analyzed with multiple correspondence analyses (MCA), and quantitative characters with principal component analyses (PCA). The qualitative and quantitative data sheets shared the same individuals in the same order allowing us to summarize the data with Hill & Smith correspondence analyses. These analyses yielded the correlation ratios of the individuals

Table 1. Codes of qualitative and quantitative morphological characters used for the morphological analyse of European and Macaronesian *Porella* species.

QUALITATIVE CHARACTERS AND CHARACTER STATES

code	*character*	*character state*
fs	lobe, apex	1=broadly rounded to rounded-truncate 2=obtuse-rounded 3=apiculate 4=from apiculate to truncate
fd	lobe, dorsal margin	1=entire 2=repand
fv	lobe, ventral margin	1=forming an auricle 2=not forming an auricle
fb	lobe, ventral margin	1=not wavy 2=wavy and crisped
ft	lobe cells, trigones	1=small 2=medium sized 3=large, bulging
lm	lobule, margin	1=entire 2=paucispinose 3=spinose
lv	lobule, margin	1=flat 2=ventrally reflexed 3=ventrally reflexed and twisted
la	lobule, apex	1=acute and tapering towards apex 2=obtuse, not tapering towards apex 3=rounded 4=broadly rounded, as wide at apex as at base
lb	lobule, basal portion	1=entire 2=toothed and crispate 3=lobulate 4=spinose
am	underleaf, margin	1=entire 2=paucispinose 3=spinose
av	underleaf, margin	1=flat 2=reflexed
ab	underleaf, basal portion	1=hardly decurrent, entire 2=sinuose-toothed 3=lobulate, not spinose 4=spinose, lobulate or not lobulate
bm	male bracts and bracteole	1=nearly free 2= coalescent
bms	male bracts, apex	1=rounded 2=obtuse or acute
bft	female bracts, size	1=smaller than normal leaves 2=nearly the same size than normal leaves
bfa	female bracts, lobe apex	1=rounded 2=obtuse or acute 3=apiculate

code	*character*	*character state*
bfla	female bracts, lobule apex	1=rounded-obtuse 2=acute 3=apiculate
bfm	female bracts, lobe margin	1=entire 2=spaced teeth or papillae 3=paucispinose (at least one of the bracts) 4=densely toothed
bflm	female bracts, lobule margin	1=entire 2=spaced teeth or papillae 3=spinose 4=densely toothed
pe	perianth mouth	1=crenulate-sinuose 2=ciliate, cilia crowded 3=cilia or teeth irregular, distant 4=spinose lobes
QUANTITATIVE CHARACTERS (in μm)		
tl	stem width	
flo	lobe length	
fla	lobe width	
fc	lobe cells, diameter	
llo	lobule length (decurrent part included)	
lla	lobule width	
ld	lobule, length of decurrence	
alo	underleaf length (decurrent part included)	
ala	underleaf width	
ad	underleaf, length of decurrence	
RATIOS		
clw	lobe width / lobe cell diam.	fla / fc
rf	lobe length / lobe width	flo / fla
rlt	lobule width / stem width	lla / tl
rat	underleaf width / stem width	ala / tl
rla	underleaf width / lobule width	ala / lla
rlf	lobe width / lobule width	fla / lla
rld	lobule length / lobule, length of decurrent part	llo / ld
ald	underleaf length / underleaf, length of decurrent part	alo / ad
rlb	lobule length / lobule width (decurrent part not included)	llo - ld / lla
rl	lobule length / lobule width (decurrent part included)	llo / lla

or the variables to the axes of each analysis. The factorial maps of variables and of individuals can be superimposed to show their relationships.

Groups of taxonomical entities overlapping in the global analysis performed on the totality of stems and each species individually were then treated separately with the same method.

RESULTS

Isozymes

No modifications in allelic composition were observed between cultivated material and colonies analyzed directly from the field and no differences were observed in allelic composition at studied isozyme loci between male and female individuals. Results are in accordance with our previous delimitation of the eight species (Boisselier-Dubayle and Bischler, 1994). Moreover, *P. inaequalis* can easily be identified because it is genetically clearly defined by a diagnostic allele on three loci (*est-1*, *per-1* and *idh-2*). Except for the allopolyploid *P. baueri* (*P. platyphylla* X *P. cordaeana*), each species is characterized by diagnostic alleles.

On this basis, 23 colonies appear to contain 2 (and once 3) species growing intermixed (7% of colonies). *Porella inaequalis*, *P. pinnata* as well as *P. platyphylloidea* were never found growing intermixed with other species. *Porella canariensis* was found intermixed only with *P. obtusata*, whereas *P. platyphylla, P. baueri, P. cordaeana, P. arboris-vitae* and *P. obtusata* may grow together in the same colony (Table 2). At the locality level, several species (generally 2, rarely 3 or 4) were collected in 32 localities (20% of localities). *Porella inaequalis* and *P. platyphylloidea* never occurred with another species at any of the sites. *Porella pinnata* was collected twice with another species. *Porella canariensis* was only found with *P. obtusata*, whereas the other species may grow in the same habitats, especially *P. platyphylla*, *P. baueri* and *P. cordeana* (Table 2). In our sampling, *P. canariensis* was only found in Madeira, continental Portugal and Canary Islands.

Allele frequencies calculated at the 11 loci, mean numbers of alleles observed by locus and percentages of polymorphic loci for the nine taxonomic entities are shown in Table 3. A total of 67 alleles were present in our sample: 10 for *per-1*, 8 for *est-1* and *got-1*, 7 for *got-2* and *per-2*, 6 for *est-2*, 5 for *gdh* and *idh-2*, 4 for *pgm* and *me* and 3 for *idh-1*. Except for *P. arboris-vitae* and *P. inaequalis* all species exhibited allelic diversity. The polyploid *Porella baueri* excepted, the highest mean number of alleles observed per locus and percentage of polymorphic loci were scored in *P. canariensis,* followed by *P. obtusata* and *P. platyphylla* (Table 3). *Porella canariensis* and *P. obtusata* exhibited seven and three, respec-

Table 2. Interspecific polymorphism found at the colony level (below) and combinations of species found at the locality level (above) in European and Macaronesian *Porella*.

	P. plat.	*P. bau.*	*P. cord.*	*P. arb.*	*P. can.*	*P. obt.*	*P. pinn.*
P. platyphylla	-	8 (+2)$	5(+3)$	2(+3)$		1(+3)$	1
P. baueri	5	-	2(+2)$	2(+1)$		1(+1)$	
P. cordaeana	1	1	-	(+2)$		1(+2)$	1
P. arboris-vitae	1 (+1)*	2		-		(+2)$	
P. canariensis					-	8	
P. obtusata	3 (+1)*			1	8	-	
P. pinnata							-

* indicate the unique colony where 3 species were growing intermixed.
$ indicate the number of localities where this combination was found, when more than 2 species were collected.

Table 3. Allele frequencies observed on 11 enzymatic loci for European and Macaronesian Porella species. Diagnostic alleles for species (*P. baueri* excepted) are underlined. * indicate the alleles only found in colonies sampled in Madeira. A = mean number of alleles observed per locus; P = percentage of polymorphic loci.

		P. plat.	*P. baueri*	*P. cord.*	*P. arb.*	*P. can.*	*P obt.*	*P. pinn.*	*P. inaeq.*	*P. plad.*
est-1	*N*	*1253*	*237*	*215*	*211*	*584*	*349*	*94*	*47*	*78*
	A					100				37.2
	B								100	
	C	63.1	75.5	100	100					
	D	0.8								
	E	36.1	24.5							
	F									62.8
	G						100			
	H							100		
est-2	*N*	*1253*	*237*	*215*	*211*	*584*	*349*	*94*	*47*	*78*
	A				100		100	100	100	
	B									37.2
	C	100	100							
	D			100						
	E									62.8
	F					100				
got-1	*N*	*1232*	*235*	*212*	*211*	*661*	*359*	*94*	*49*	*78*
	A					51.7*				
	B				100					
	C					48.3	96.4			
	D						3.6*			
	E							100	100	
	F		50.0	100						100
	G	8.4								
	H	91.6	50.0							
got-2	*N*	*1232*	*235*	*212*	*211*	*661*	*359*	*94*	*49*	*78*
	A						2.2*			
	B						97.8			
	C					100				
	D		27.7	100	100					
	E									100
	F	100	72.3						100	
	G							100		
per-1	*N*	*1239*	*238*	*216*	*211*	*664*	*359*	*94*	*49*	*78*
	A					14.0*				100
	B	0.2	55.9							
	C	1.6								
	D	98.2								
	E		44.1	100						
	F							100		
	G								100	
	H					42.8*				
	I					43.2				
	J				100		100			
per-2	*N*	*1239*	*238*	*216*	*211*	*664*	*359*	*94*	*49*	*78*
	A					86.0				
	B	99.2	55.9					100		100
	C		4.2	100						
	D		39.9							
	E	0.8								
	F				100		100			
	G					14.0*			100	

		P. plat.	*P. baueri*	*P. cord.*	*P. arb.*	*P. can.*	*P obt.*	*P. pinn.*	*P. inaeq.*	*P. plad.*
gdh	*N*	*1100*	*235*	*207*	*173*	*572*	*335*	*67*	*48*	*70*
	A									14.3
	B	100	100	100				1.5		77.1
	C					1.8			100	
	D				100	98.2	100	1.5		
	E							97.0		8.6
pgm	*N*	*31*	*2*	*31*	*0*	*461*	*99*	*27*	*46*	*38*
	A	100	50.0			39.0		100		
	B						54.6			
	C			3.2		61.0*	45.4*		100	100
	D		50.0	96.8						
idh-1	*N*	*1073*	*175*	*187*	*201*	*623*	*324*	*106*	*44*	*61*
	A	100	100	100		55.0*	99.4		100	
	B							100		
	C				100	45.0	0.6			100
idh-2	*N*	*1073*	*175*	*187*	*201*	*623*	*324*	*106*	*44*	*61*
	A							100	100	
	B				100		0.6			
	C						99.4			100
	D	100	100	100		55.0*				
	E					45.0				
me	*N*	*1096*	*193*	*164*	*192*	*590*	*326*	*87*	*49*	*65*
	A				100		100			
	B								100	
	C	100	100	100		100		100		
	D									100
A		1.55	1.64	1.09	1.00	1.82	1.45	1.18	1.00	1.36
P		36.36	54.55	9.09	0	63.64	45.45	9.09	0	27.27

tively, distinctive alleles in Madeira. By contrast, two alleles of *P. obtusata* were found only on the continent. No genetic differentiation between the continent and Madeira was detected in *P. cordaeana*, the third species present in Madeira.

The genetic identities between species (except *P. baueri*) determined using Nei's (1972) coefficient are shown in Table 4. Higher identities are found between *P. obtusata* and *P. arboris-vitae* followed by *P. platyphylla* and *P. cordaeana*. Low

Table 4. Nei's genetic identity (1972) calculated on 11 enzymatic loci between European and Macaronesian *Porella* species (*P. baueri* excepted).

	P. plat.	*P. cord.*	*P. arb.*	*P. can.*	*P obt.*	*P. pinn.*	*P. inaeq.*	*P. plad.*
P. plat.		0.436	0.062	0.272	0.096	0.283	0.188	0.176
P. cord.			0.191	0.225	0.095	0.093	0.094	0.175
P. arb.				0.158	0.493	0.098	0.096	0.102
P. can.					0.248	0.149	0.139	0.176
P obt.						0.095	0.229	0.145
P. pinn.							0.274	0.106
P. inaeq.								0.097
P. plad.								

values mainly include the two species *P. pinnata* and *P. inaequalis* whereas the lowest one is between *P. platyphylla* and *P.arboris-vitae* (0.062).

At the intraspecific level, allelic polymorphism was found within some colonies, especially within *P. platyphylla* for which 23.08% of colonies exhibited variation at the *est-1* and/or *got-1* loci. In *P. cordaeana*, *P. plaphylloidea*, *P. arboris-vitae*, *P. pinnata* and *P. inaequalis*, no intra-colony genetic polymorphism was present. The percentages of intraspecific polymorphic colonies were 5.6% in *P. canariensis*, 6.7% in *P. baueri* and 7.1% in *P. obtusata*. The polymorphic colonies of *P. obtusata* and *P. canariensis* were only found in Madeira.

Morphology

Analysis of the total sampling — The analysis partitioned the 1693 stems in three groups along the two first axes (45.7% of the total variance): the first comprised *P. platyphylla*, *P. baueri, P. cordaeana* and *P. platyphylloidea*, that are overlapping in large parts, the second included *P. obtusata, P. arboris-vitae* and *P. canariensis*, that are less overlapping, and the third is composed of two clearly separated entities, *P. pinnata* and *P. inaequalis* (Fig. 1). The groups and the character states of the qualitative characters that best characterize each of them are indicated. Fifteen qualitative and only four quantitative characters showed the highest contributions

Fig. 1. Projection of the analysed colonies (small squares) of *Porella* and of the morphological variables (abbreviations of variables as in Table 1) showing the highest contributions on the first and second axes of the factorial correspondence analysis. Specific identification according to genetic data (AR = *P. arboris-vitae*, CA = *P. canariensis*, CO = *P. cordaeana*, HY = *P. baueri*, IN = *P. inaequalis*, OB = *P. obtusata*, P = *P. platyphylla*, PI = *P. pinnata*, PL = *P. platyphylloidea*).

to the first axis, indicating that it was mainly an axis of shape, whereas the 11 higher contributions to the second axis were quantitative characters, showing that it was an axis of cell size.

Stems of a single colony (635) and one stem of the colony 776 (776-6), from Madeira, appear as morphologically different from the bulk. Isozymes electrophoresis performed on other shoots of the same colonies gave an allelic structure corresponding to *P. canariensis* for 635 and *P. inaequalis* for 776.

Porella pinnata, characterized by fs1, lb1, and *P. inaequalis*, identified by fs4, ab1, were mainly individualized on the scatter diagram of the first two axes while the other species were set apart on the scatters of the following axes. *Porella arboris-vitae* (with lm3, lb4, am3 and ab4) and *P. cordaeana* (with lv3, la1, pe1) were separated on the scatter diagram of axes 1/3. *Porella canariensis* (with fs2, lm1, am1) and *P. obtusata* (with fs3, lm2, am2) were set apart on the scatter diagram of axes 4/6. *Porella platyphylloidea* (characterized by fs1, fb2, bfm1, pe2), *P. baueri* (identified by fv1, pe 2) and *P. platyphylla* (with fv2, pe3) were individualized on the scatter diagrams of subsequent axes.

Analyses of the two main groups of species — The group *P. platyphylla-P. baueri-P. cordaeana* was analyzed in our previous paper (Boisselier-Dubayle *et al.*, 1998a), without *P. platyphylloidea*. The present analysis included 812 samples and the first two axes accounted for 38.8% of the total variance. *Porella cordaeana* was nearly individualized on axes 1/2 (lv, la, av, ft, pe). The western individuals of *P. baueri* overlapped partially with *P. cordaeana*, partially with *P. platyphylla*, whereas the eastern samples clustered with *P. platyphylla* alone, demonstrating their relationships with the parental species (Boisselier-Dubayle *et al.*, 1998a). The hybrid was not individualized on any combination of axes. *Porella platyphylloidea* was set apart from all other species on axes 2/5 and 3/5 (fs, fb).

The analysis of the group *P. obtusata-P. canariensis-P. arboris-vitae* included 709 stems. The first two axes accounted for 37.8% of the total variance. The first axis was defined by ten qualitative characters (fs, fv, lm, am, av, bfa, bfla, bfm, bflm), and three quantitative (ad, rlb, rl) characters, the second axis by four quantitative ones (fla, llo, lla, alo), and the third axis by a single qualitative one (ab). The significance of the first two axes was close to that of the analysis of the totality of individuals. The best separation of the three species was reached on axes 1/3, seen on Figure 2, together with the character states discriminating them. Superimposing the geographical origin of the individuals showed that the continental populations of the three species are clearly distinct (continuous lines), whereas the Madeiran samples of *P. obtusata* and *P. canariensis* overlapped partially (dotted lines). Morphological variation of both species is higher on Madeira than on the continent.

Analyses of each species — The stems of each species analyzed separately showed that measurements had high contributions on the first axes (98.5%), and ratios (the relations between parts of the plant, 65%) on the second. Size defined the first axis for all studied species, with robust individuals on the positive, small on the negative side. Qualitative morphological characters hardly contributed to differentiate the individuals (3.9%). Except colony n° 635, no morphologically distinct groups within the species were observed in our sampling.

Morphology and geographical distributions — A north-south trend in size was observed in some species. For instance, in *P. arboris-vitae*, samples from southern France were robust whereas those from the central and eastern parts of the country are smaller. In *P. baueri*, the western stems were smaller and morphologically closer to *P. cordaeana*, whereas the eastern were robust and closer to *P. platyphylla*. In *P. obtusata*, Spanish and most French specimens were smaller than those

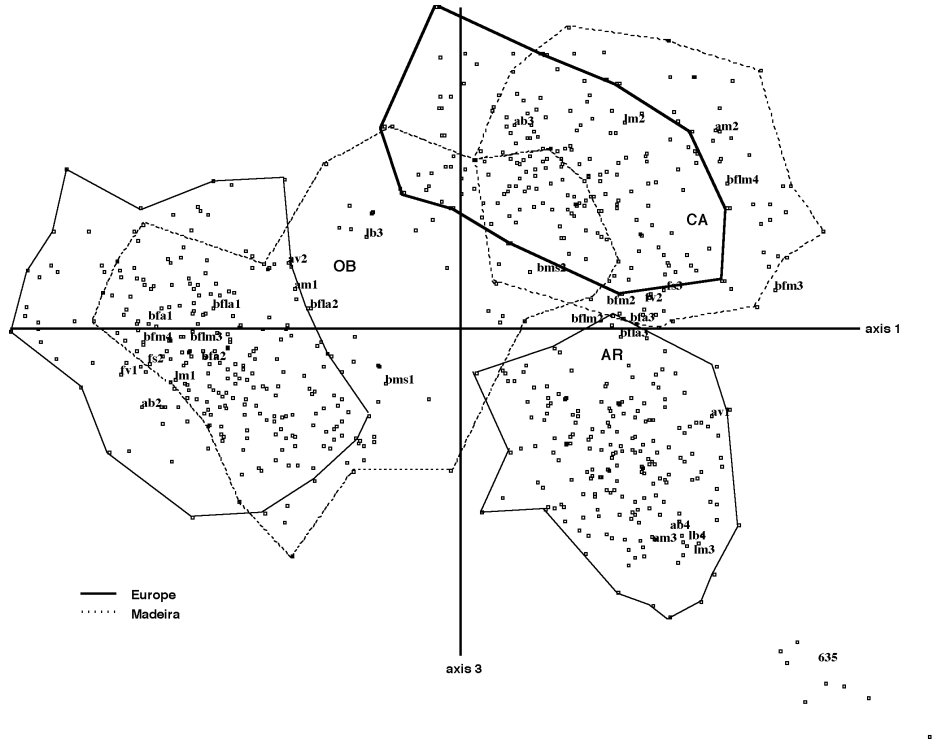

Fig. 2. Projection of the *Porella* colonies (small squares) of the group *P. arboris-vitae (*AR), *P. canariensis* (CA), and *P. obtusata* (OB), and of the morphological variables (abbreviations of variables as in Table 1) exhibiting the highest contributions on the first and third axes of the factorial correspondence analysis. For each species, the colonies coming from Europe (continuous lines) and Madeira (dotted lines) are delimited.

from Madeira, and finally, in *P. pinnata*, the individuals from southern England and Quebec were less robust than most of those from France.

Morphological variation was not linked to geographical origin in *P. canariensis*, *P. cordaeana*, *P. inaequalis, P. platyphylla,* and *P. platyphylloidea*. However, the sample of *P. platyphylloidea* came from a very small area in the geographical range of the species, and *P. inaequalis* was only found in Madeira Island.

Morphological variation was higher on Madeira than on the continent in *P. obtusata* and *P. canariensis* (Fig. 2), whereas the stems from the few colonies of *P. cordaeana* collected in Madeira did not show any morphological difference with the continental samples (data not shown).

Morphology and gender — Female individuals were relatively frequent (49.3% of shoots, Table 5), whereas males were scanty (10.1% of shoots). No males were found in *P. arboris-vitae* (they exist in herbarium specimens), *P. baueri,* and *P. inaequalis*. Nevertheless, with the exception of *P. inaequalis* (a single female stem is known, Fontinha, 2000), perianths were present (5.6-58.2% of the female shoots). Since perianth development is a post-fertilization phenomenon in *Porella* (Schuster, 1966), fertilization had to occur in these species. No size differences or differences in geographical distribution were observed between male and female individuals.

Table 5. Frequency of female stems found with European and Macaronesian Porella species.

Species	*total indiv.*	*female*	*%*	*female with perianths*	*% of females with perianth*
P. arboris-vitae	194	123	63.4	25	20.3
P. baueri	214	118	55.1	29	24.5
P. canariensis	665	287	43.2	167	58.2
P. cordaeana	212	116	54.7	63	54.3
P. inaequalis	80	0	0	0	0
P. obtusata	400	188	47	52	27.7
P. pinnata	92	36	39.1	2	5.6
P. platyphylla	346	212	61.3	120	56.6
P. platyphylloidea	70	40	57.1	18	45
Total	2273	1120	49.3	476	42.5

Male and female stems have been found intermixed in 16 colonies, for five species, implying that those colonies were founded with more than one spore.

DISCUSSION

Genetic diversity among the European and Macaronesian species of *Porella* can be partititoned among nine groups, and each group is differentiated by morphological characteristics. The nine groups correspond, as suggested earlier by Boisselier-Dubayle and Bischler (1994), to nine species, including one allopolyploid taxon. Two species, well defined by genetic markers, are only subtly distinct: *P. platyphylla* and *P. platyphylloidea*. Few qualitative morphological characters separated *P. platyphylla* and *P. platyphylloidea*: leaf lobe shape and perianth mouth. However, only mature perianths show the irregular, pluricellular and distant teeth characteristic for *P. platyphylla*; immature ones often have distant cilia 1-2 cells long (as those shown in Therrien *et al.*, 1998: Fig. 18, A, B). Figure 17 in Therrien *et al.* (1998), from a European sample, fit the morphological definition of *P. platyphylla*, whereas figure 16, and the figures in Evans (1916) and Schuster (1980), that of *P. platyphylloidea*. Instead, the species appear well defined with regard to allelic structure (Nei's genetic identity between *P. platyphylla* and *P. platyphylloidea* = 0.176) and the species appear to be more clearly defined by their allelic patterns than by morphology, a situation observed in other liverworts, e.g. in *Conocephalum conicum* (Odrzykoski and Szweykowski, 1991), *Riccia dictyospora* (Dewey, 1989), the subspecies *of Marchantia polymorpha* (Boisselier-Dubayle *et al.*, 1995b), *Reboulia hemisphaerica* (Boisselier-Dubayle *et al.*, 1998b).

Therrien *et al.* (1998) found no significant morphological difference between *P. platyphylla* and *P. platyphylloidea* in North America with morphometric studies based on quantitative characters. Despite differences in allelic structure, they concluded: "since there are no morphological characters that can be used to distinguish among the genotypes, they are recognized as "cryptic species"

within a single morphologically defined taxon", and treated *P. platyphylla* and *P. platyphylloidea* as synonymous. More recently, Wyatt *et al.* (2005) did not detect any discordant isozymic patterns of variation that would suggest the presence of two species in their geographically limited samples from the southeastern United States. However, on the basis of the differences found between our samples of *P. platyphylla* from Europe and from *P. platyphylloidea* from Canada, we suggest recognizing the two species despite the presence of few morphological distinctive characters.

With regard to the other taxa, taxonomic varieties have been described in several species (e.g. by Müller (1958) in *P. arboris-vitae* and *P. cordaeana*, by Schuster (1980) in *P. pinnata* and *P. platyphylloidea*). Nevertheless, no distinct groups of individuals that could correspond to these taxonomic units have been encountered in our sampling.

Species structure

At the intraspecific level, morphological variation was linked to size parameters, not to qualitative characters. It was sometimes geographically correlated, as in *P. arboris-vitae*, with robust individuals in the south, or in *P. canariensis* and *P. obtusata* in which overall morphological variation was more important on Madeira than on the continent. In the first case, no genetic differences existed, whereas in the second, additional alleles were present. However, even in *P. canariensis* and *P. obtusata*, morphological variation was not linked to allelic variation. They were connected only in the exceptional case of the allopolyploid *P. baueri*.

Male individuals were less numerous than female, as observed in many other liverworts (e.g., *Marchantia*, Bischler, 1993) and mosses (e.g., Cronberg *et al.*, 2003) but showed no morphological or genetic differences. The species that commonly reproduce sexually presented only slightly higher levels of genetic polymorphism. Spore dispersal distances may be short and efficient, mainly within the colonies, as supposed by Freitas and Brehm (2001).

Wide distributions are not associated with level of genetic polymorphism. *Porella platyphylla* and *P. cordaeana* both have wide ranges in the Northern Hemisphere, from Europe to eastern Asia. The first showed important genetic polymorphism in Europe, whereas the second was found to be nearly monomorphic in Europe and on Madeira. Absence of correlation between geographic and genetic distance have been recorded in many liverwort species (e.g., Boisselier-Dubayle and Bischler, 1997; Freitas and Brehm, 2001), including in *Porella* (Therrien *et al.*, 1998).

Transcontinental distribution is known in *P. pinnata* and *P. cordaeana*. No North American sample from the latter species was analyzed, whereas in *P. pinnata*, allelic structure was identical in colonies from Europe and North America. However, the species was collected mainly on stones in rivers and ponds in Europe, whereas in North America, it seems to colonize bark also. Other liverworts with transcontinental distributions were found to be genetically similar over several continents with isozyme studies, e.g. *Preissia quadrata* (Boisselier-Dubayle and Bischler, 1997), *Lunularia cruciata* (Boissselier-Dubayle *et al.*, 1995a), *Reboulia hemisphaerica* (Boisselier-Dubayle *et al.*, 1998b). Other species showed few or no morphological differences but strong genetic differentiation, as between *P. platyphylla* and *P. platyphylloidea*: *Targionia hypophylla* (Boisselier-Dubayle *et al.*, 1999), and *Corsinia coriandrina* (Boisselier-Dubayle and Bischler, 1998). More recently, phylogeographic structure was also revealed by sequence data in

morphologically undifferentiated species of mosses across continents (McDaniel and Shaw, 2003; Shaw *et al.*, 2003).

Many *Porella* colonies are multiclonal, attested by diversity found within some colonies with intraspecific and interspecific polymorphisms, but also by male and female individuals found growing intermixed. The intraspecific polymorphism within colonies varied from none to 23% in *P. platyphylla*. At the intraspecific level, the species that often reproduce sexually presented in part only higher levels of genetic polymorphism, e.g. *P. platyphylla*. Instead, low intraspecific polymorphism within colonies and a high number of females with perianths characterized *P. cordaeana* and *P. canariensis.*

Our data underline the high levels of variation found within the European and Macaronesian *Porella,* especially *P. canariensis, P. obtusata* and *P. platyphylla*. Wyatt *et al.* (2005) reported also high levels of polymorphism for populations of *Porella* from the southeastern United States where they recognized only one species: *P. platyphylla*. Compared to thalloid liverworts, the highest intracolony polymorphism we observed was 31.1% in *Preissia* (Boisselier-Dubayle and Bischler, 1997), but most thalloid species showed much lower levels.

Porella canariensis and *P. obtusata* both showed higher levels of polymorphism in Madeira and seven and three specific alleles, respectively, that were not found elsewhere. No polymorphism was found among the European and Canary Islands samples in *P. canariensis*. Freitas and Brehm (2001) observed, with RAPD markers, 39.6% of polymorphic colonies in this species, and less genetic variation in the samples from the continent, the Azores and the Canary Islands than in those from Madeira. They assumed a formerly widespread population having found refuge on this island, essentially untouched by climatic changes. The Tertiary laurel forest called Laurisilva survives well-preserved on Madeira Island, harboring a rich, relict and endemic biodiversity.

Relationships of species

The analysis of morphological characters subdivided the species into four groups*:* 1 - *P. platyphylla-P. cordaeana-P. baueri-P. platyphylloidea,* 2 - *P. obtusata-P. arboris-vitae-P. canariensis*, 3 - *P. pinnata*, 4 - *P. inaequalis.* Morphological and genetic relationships did not always overlap. Allelic structure showed *P. platyphylloidea* to be as distant to *P. platyphylla* as to *P. canariensis*. *Porella canariensis* and *P. pinnata* appeared remotely related to *P. arboris-vitae* and *P. inaequalis*, respectively. Following Schuster's (1980) systematic concepts, the European and Macaronesian species of *Porella* should fall into two groups, corresponding to sect. *Porella* (with *P. inaequalis* and *P. pinnata*) and sect. Platyphyllae (Schust., *Fl. N. Amer.* 4: 690. 1980, type *P. platyphylla*) for the remainder of the species. Inferences from genetic and morphologic characters suggest that *P. pinnata* and *P. inaequalis* are not closely related and hence do not support the sectional concepts of Schuster.

Key characters

The morphological characters that emerge as diagnostic from our analyses, are used to construct a key. Ecology and geographical distribution were screened to test if they could contribute to for species distinction.

The geographical distribution of the species seems to be limited mainly by climatic factors*: P. inaequalis* is endemic to Madeira Island. *Porella canariensis* has mainly an Atlantic distribution and is not found North of the Iberian Peninsula. Both species need equilibrated climates with cool summers and mild

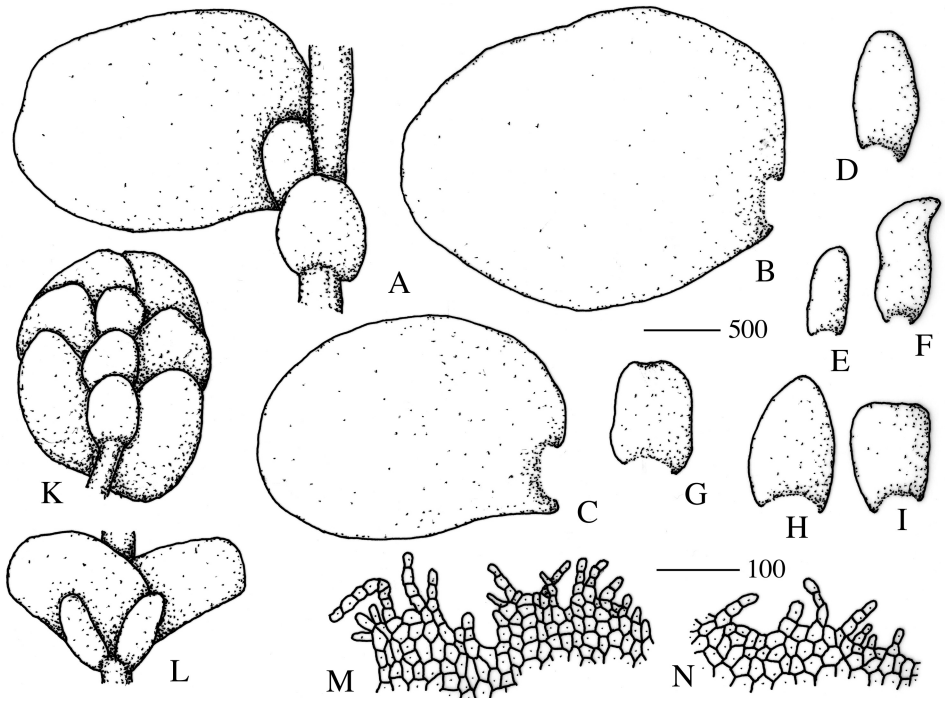

Fig. 3. *Porella pinnata.* **A**, stem, ventral view; **B-C**, dorsal lobes; **D-F**, lobules; **G-I**, underleaves; **K**, male bracts; **L**, female bracts; **M-N**, perianth mouth (A-B, E, I: England; C, K: Canada, Quebec; D, F-H, L-N, France).

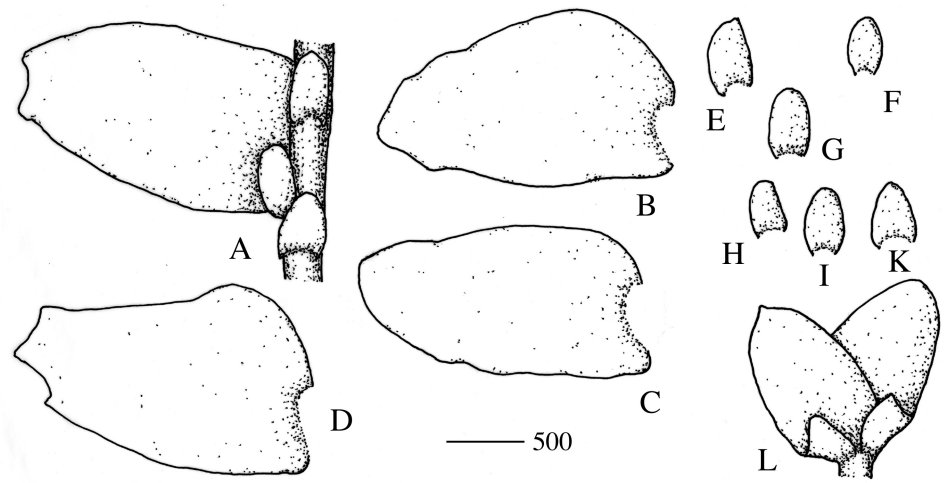

Fig. 4. *Porella inaequalis*. **A**, stem, ventral view; **B-D**, dorsal lobes; **E-G**, lobules; **H-K**, underleaves; **L**, female bracts (all from Madeira; L from Fontinha, 2000).

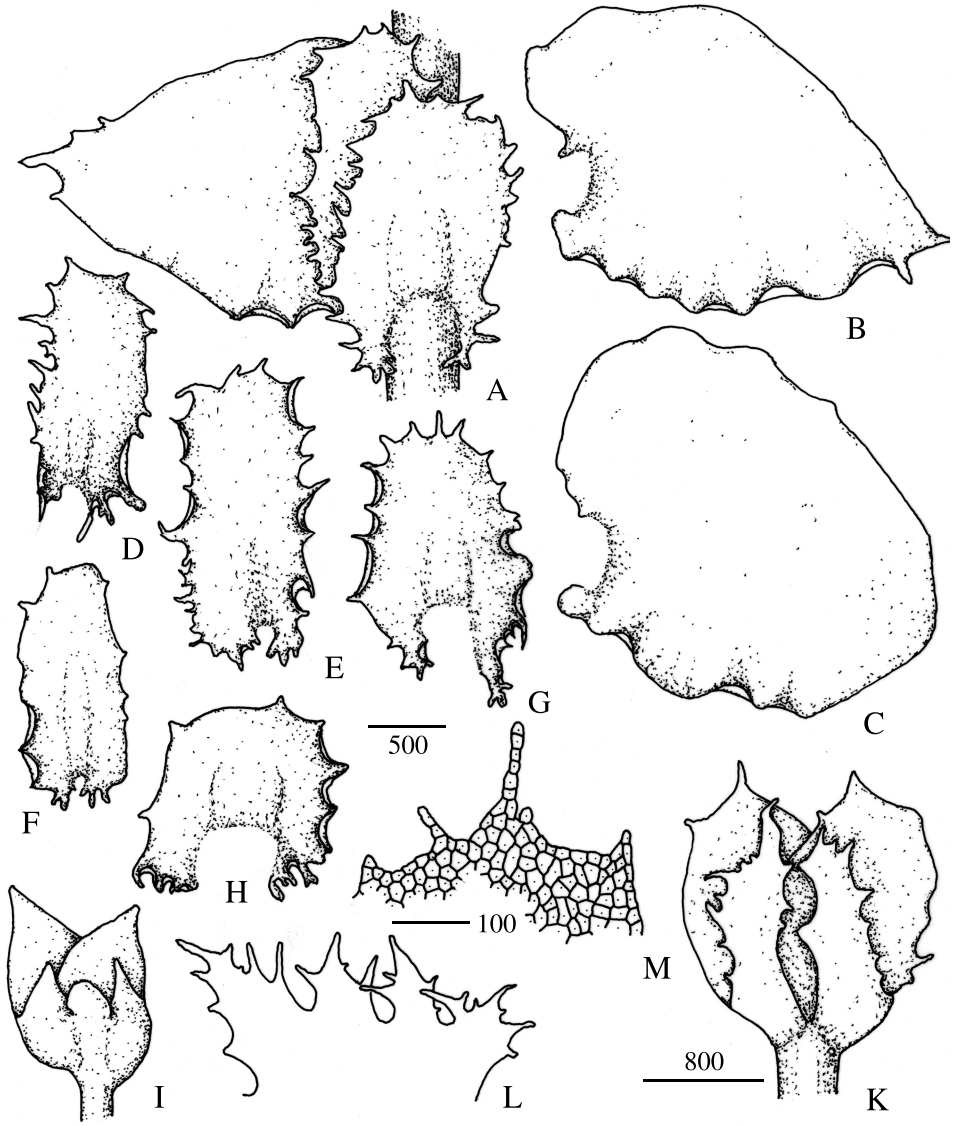

Figure 5. *Porella arboris-vitae*. **A**, stem, ventral view; **B-C**, dorsal lobes; **D-F**, lobules; **G-H**, underleaves; **I**, male bracts; **K**, female bracts; **L-M**, perianth mouth (all from France; I from herbarium material).

winters, and substrata with low pH. In contrast, *P. arboris-vitae* is restricted to southern continental areas and, as *P. obtusata*, withstands high summer temperature and grows in the lowlands, whereas *P. cordaeana* hardly supports high temperature and prefers high mountains in the southern part of its range. *Porella pinnata*, more sensitive to ecological conditions, at least in western and central Europe, is known from flooded stones in rivers in the lowland, but seems to occur on bark in southern US (Schuster, 1980).

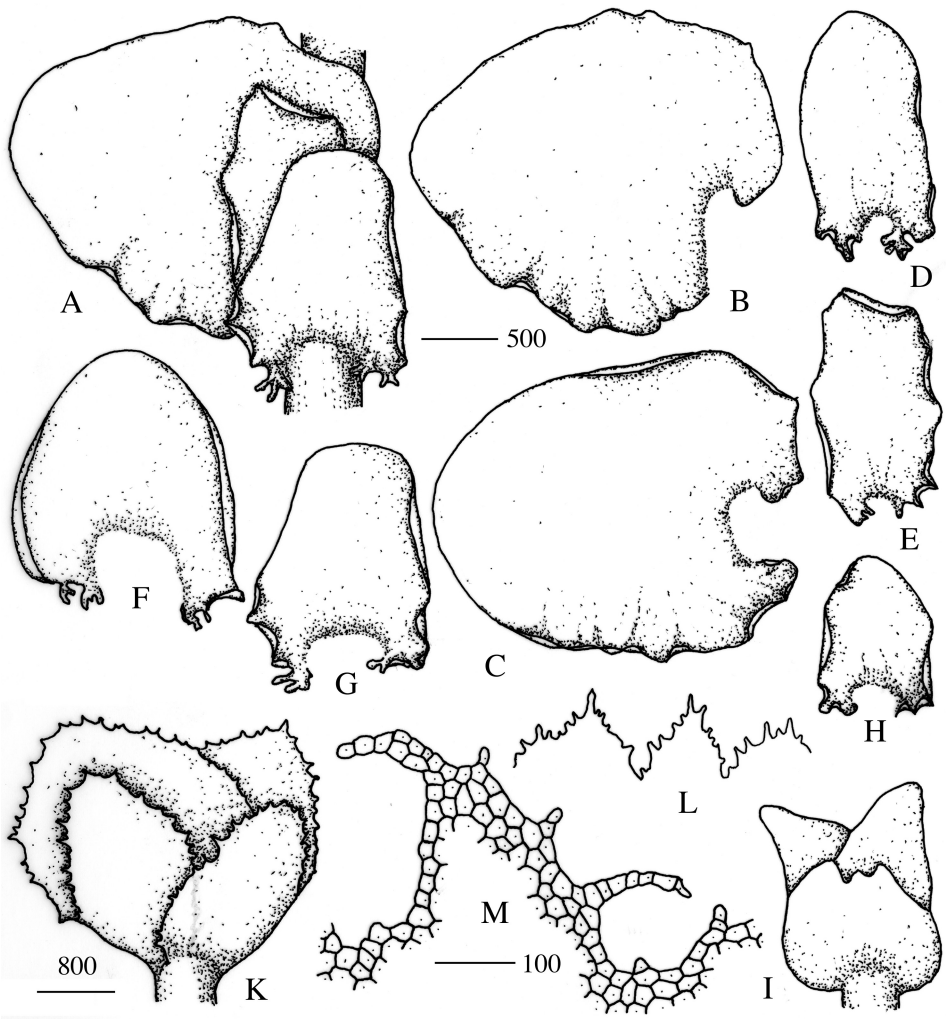

Fig. 6. *Porella obtusata*. **A**, stem, ventral view; **B-C**, dorsal lobes; **D-E**, lobules; **F-H**, underleaves; **I**, male bracts; **K**, female bracts; **L-M**, perianth mouth (A-B, E-G, I-M: Portugal; C-D: France; H: Madeira).

Porella arboris-vitae, P. obtusata, P. platyphylla and *P. cordaeana*, have wide ranges in Europe and Asia. They have been found, as well as *P. baueri*, to grow intermixed in the same colony, or in the same locality. Their ecological requirements are overlapping. Accordingly, few ecological or distributional data can be used for species distinction.

Key

1. Lobule flat, small (length 331-644 μm, width 137-312 μm), hardly decurrent (8-59 μm); underleaves flat, small (length 289-718 μm, width 174-499 μm); female bracts smaller than normal leaves, with entire margins. 2

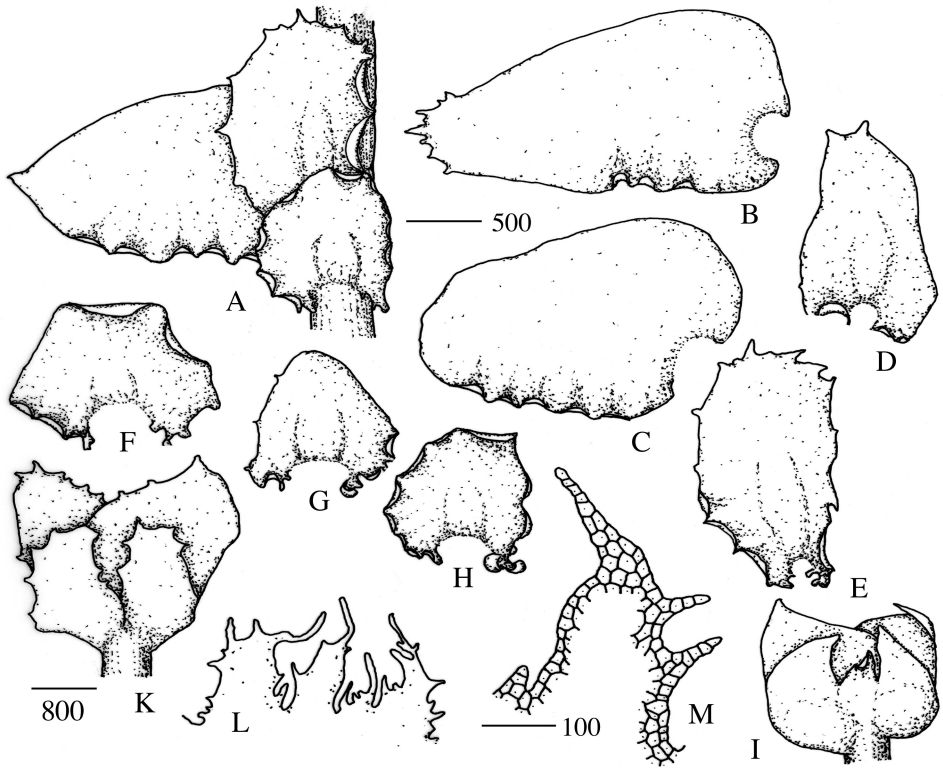

Fig. 7. *Porella canariensis*. **A**, stem, ventral view; **B-C**, dorsal lobes; **D-E**, lobules; **F-H**, underleaves; **I**, male bracts; **K**, female bracts; **L-M**, perianth mouth (A-C, E, I-M: Madeira; D, F-H: Portugal).

1. Lobule large (length 786-1526 µm, width 266-810 µm), margins often partly revolute, more or less decurrent (102-557 µm); underleaves large (length 803-1378 µm, width 539-1066 µm), margins often partly revolute; female bracts smaller or larger than normal leaves, with entire or toothed or spinose margins 3
2. Lobe ratio length: width 1.3-1.6:1, width 1190-1751 µm, apex broadly rounded to rounded-truncate, dorsal margin not undulate; lobule flat, slightly decurrent (33-59 µm), ratio length: decurrency 8.7-15.7:1; underleaves flat, slightly decurrent (42-98 µm), ratio length: decurrency 6.6-11:1; ratio lobe width lobe cell size 41.4-59.7: 1; bract lobes rounded apically, margins entire; growing usually immersed . . . . . *P. pinnata* (Fig. 3)
2. Lobe ratio length width 1.7-2.3:1, width 768-1046 µm, apex from apiculate to truncate, dorsal margin undulate; lobule hardly decurrent (8-17 µm), ratio length decurrency 23.8-56.9:1; underleaves hardly decurrent (8-17 µm), ratio length decurrency 20.7-57.1; ratio lobe width: lobe cell size 24.2-33.2:1; bract lobes obtuse to acute apically, margins undulate (Fontinha, 2000); usually on slopes, endemic (Madeira Island). . . . . *P. inaequalis* (Fig. 4)

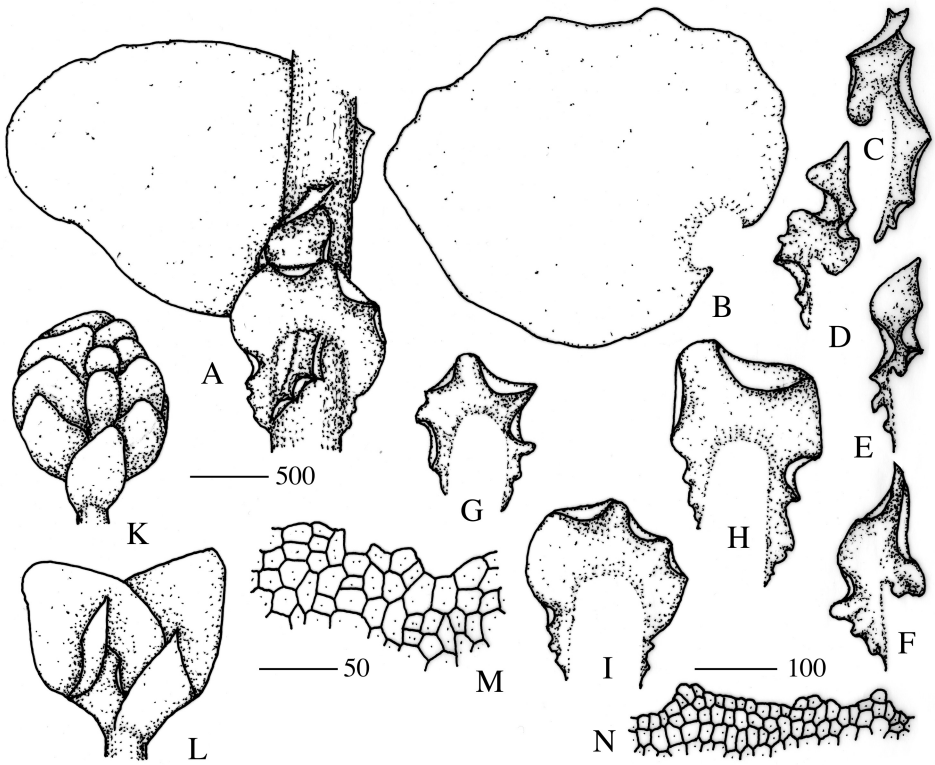

Fig. 8. *Porella cordaeana*. **A**, stem, ventral view; **B**, dorsal lobe; **C-F**, lobules; **G-I**, underleaves; **K**, male bracts; **L**, female bracts; **M-N**, perianth mouth (all from France).

3. Basal portion of lobule lobulate or spinose, not decurrent along the stem below level of keel (decurrency 102-194 μm), ratio lobule length: decurrency 6.4-12.8: 1; underleaves hardly decurrent (102-194 μm), ratio length: decurrency 2.5-6.1; female bracts larger than normal leaves, spinose or densely toothed; perianth mouth with spinose lobes; male bracts and bracteole connate 4

3. Basal portion of lobule toothed and/or curled, decurrent along the stem below level of keel (decurrency 230-557 μm), ratio lobule length: decurrency 1.9-4.1:1; underleaves decurrent (230-557 μm), ratio length decurrency 1.8-2.3; female bracts smaller than normal leaves, margins entire or with unicellular teeth or papillae; perianth mouth crenulate-sinuose, or ciliate, or with spaced, coarse teeth; male bracts nearly free (absent in *P. baueri*) 6

4. Lobule margins and basal portion coarsely spinose; underleaf margins spinose, basal portion spinose and lobulate . . . . . *P. arboris-vitae* (Fig. 5)

4. Lobule margins entire or paucispinose, basal portion lobulate; underleaf margins entire or paucispinose, basal portion lobulate 5

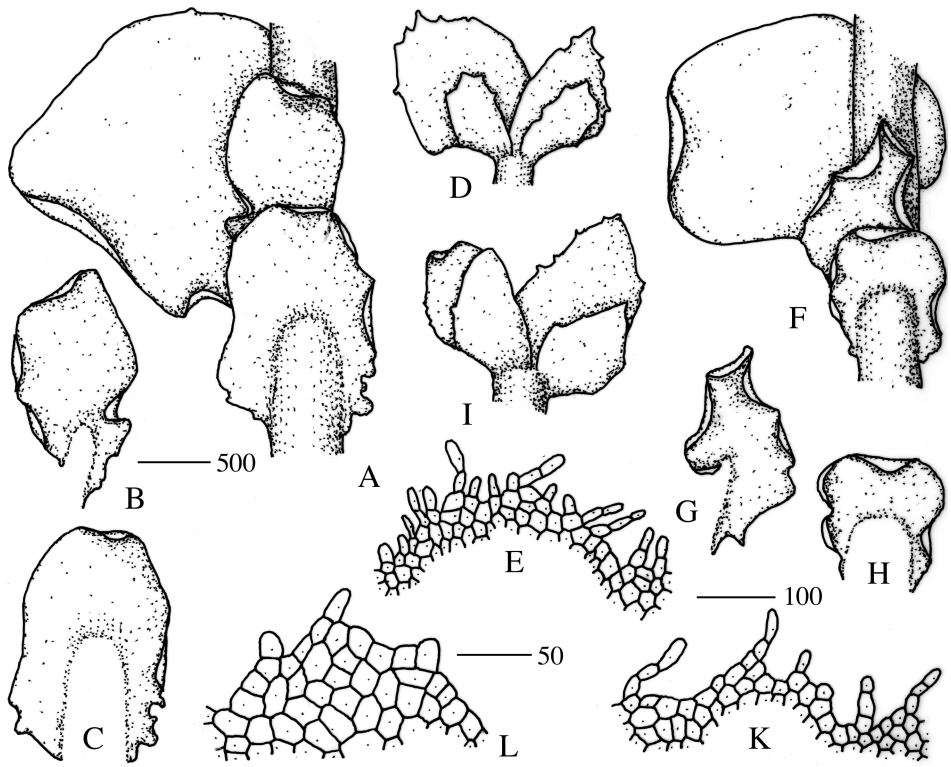

Fig. 9. *Porella baueri*. **A, F**, stem, ventral view; **B, G**, lobules; **C, H**, underleaves; **D, I**, female bracts; **E**, **K**, perianth mouth (A-D: Germny, type locality; E: eastern France; F-K: western France).

5. Lobe apex rounded, median lobe cells 26.1-33.5 µm; lobule margins entire; underleaf margins entire, base slightly decurrent (268-483 µm); length: decurrency 2.5-4: 1 ; apex of male bracts rounded; distribution continental and Atlantic . . . . . *P. obtusata* (Fig. 6)
5. Lobe apex often apiculate, or with 2-3 coarse teeth, rarely rounded, lobe cell 21.8-26.8 µm; lobule margin paucispinose, rarely rounded; underleaf margin entire or with some coarse teeth; base hardly decurrent (149-288 µm), ratio length: decurrency 4-6.1 1; apex of male bracts usually acute; distribution Atlantic . . . . . *P. canariensis* (Fig. 7)
6. Lobule ventrally reflexed and twisted, apex acute, tapering apically, decurrency 347-557 µm; bract lobe and lobule with entire margins, lobule usually acute apically; perianth mouth crenulate-sinuose . . . . . *P. cordaeana* (Fig. 8)
6. Lobule margin ventrally reflexed but not twisted, apex rounded or obtuse, not tapering apically, decurrency 230-467 µm; bract lobe and lobule with entire or faintly toothed-papillose margins, lobule usually rounded apically; perianth mouth ciliate, cilia either crowded and mostly 1 cell wide basally, or irregular, distant teeth 2-6 cells wide basally 7
7. Median lobe cell width 36.5-41.2 µm; margins of female bracts with unicellular teeth; perianth mouth ciliate, cilia crowded; chromosome number n=16. . . . . *P. baueri* (Fig. 9)

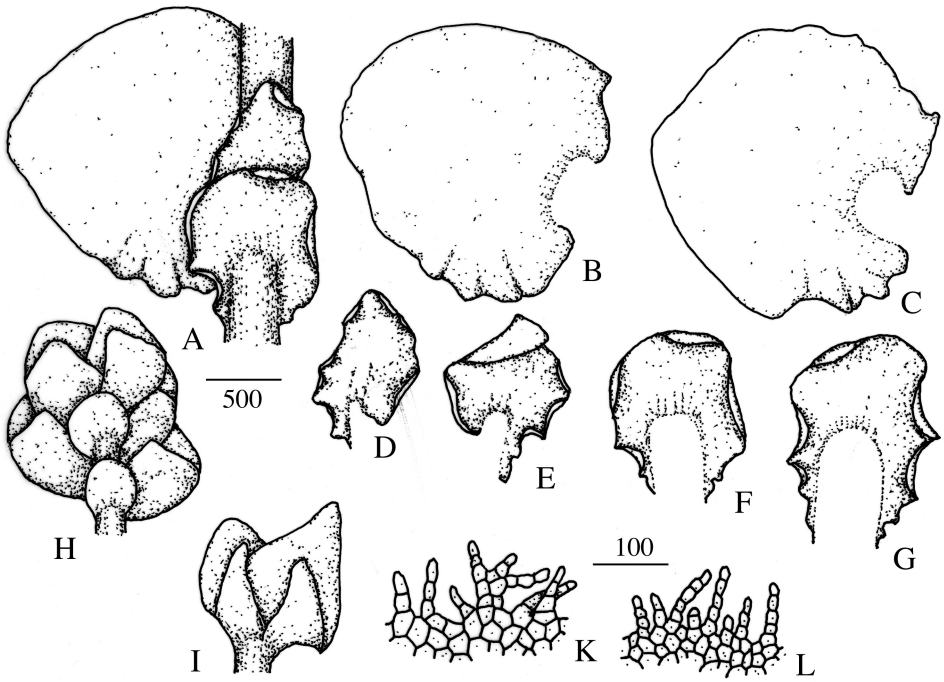

Fig. 10. *Porella platyphylloidea*. **A**, stem, ventral view; **B-C**, dorsal lobes; **D-E**, lobules; **F-G**, underleaves; **H**, male bracts; **I**, female bracts; **K-L**, perianth mouth (all from Canada, Quebec).

7. Median lobe cell width 28.5-36.1 µm; margins of female bracts usually entire; perianth mouth ciliate, or with irregular, distant teeth 2-6 cells wide basally; chromosome numbers n=8 (or 9) 8
8. Lobe apex broadly rounded, ventral margin wavy, often with some plicae; perianth mouth with crowded cilia, to 5 cells long, mostly 1 cells wide basally, sometimes forked; distribution North and Central American . . . . . *P. platyphylloidea* (Fig. 10)
8. Lobe apex obtuse-rounded, ventral margin not wavy or plicate; perianth mouth when fully grown with irregular, distant teeth 2-6 cells wide basally; distribution European and Asiatic (North American?) . . . . . *P. platyphylla* (Fig. 11)

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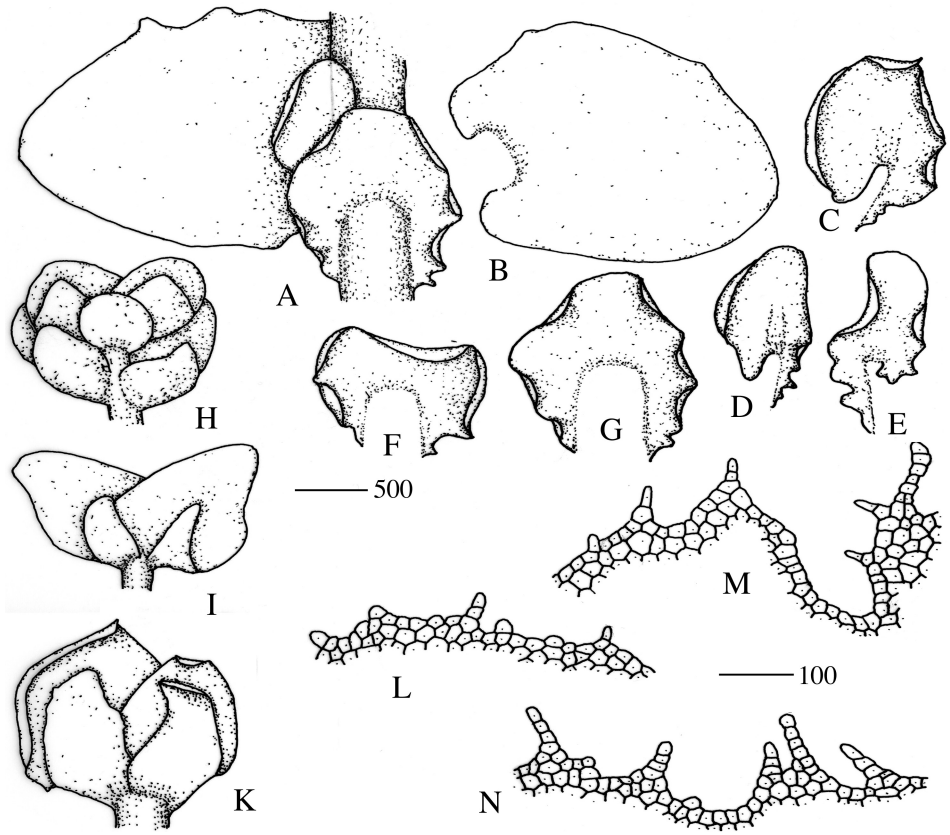

Fig. 11. *Porella platyphylla*. **A**, stem, ventral view; **B**, dorsal lobe; **C-E**, lobules; **F-G**, underleaves; **H**, male bracts; **I-K**, female bracts; **L**, young perianth mouth; **M-N**, mature perianth mouth (A-H, K-N: France; I: Switzerland).

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