SYSTEMATICS AND PHYLOGENY

Environmental variation obscures species diversity in southern European populations of the moss genus *Ceratodon*

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Abstract A major problem in taxonomy is to determine if morphological variation in field-collected specimens is caused by genetic differentiation, and therefore corresponds to evolutionary distinct units, or is caused by environmental variation acting on a single interbreeding population. To evaluate the effect of environmental variation on the taxonomy of the moss genus *Ceratodon*, we compared biometric analyses based on 22 morphological characters on both field-collected plants and cultivated plants to a clustering based on DNA sequence and genome size data published previously. We sampled *Ceratodon* species from mountainous areas of the Mediterranean region, and other mountain regions and lowlands, mostly from southern Europe. We found that the expression of several gametophytic traits changed between field and laboratory conditions, confirming that environmental variability complicates taxonomic inferences, and suggesting that some characters should be used with caution in distinguishing among species. However, consistent with the genetic and flow cytometry data, we found a clear biometric discontinuity between some plants collected from southern Spain, and those from other parts of the world. Samples considered of hybrid origin, based on genetic data, were morphologically indistinguishable from plants from the southern Spanish mountains. Integrative taxonomy based on genetic, genome size and morphological data unambiguously support the recognition of a new species, *Ceratodon amazonum*. These data also suggest that the previously recognized *C. conicus* is a recombinant between *C. amazonum* and *C. purpureus* and is considered here to be a nothospecies, for which an epitype is designated because the lectotype is demonstrably ambiguous.

Keywords *Ceratodon amazonum*; *Ceratodon* ×*conicus*; *in vitro* cultures; integrative taxonomy; morphometric analysis; Spanish Sierra Nevada

Supplementary Material The Electronic Supplement (Tables S1 & S2, Fig. S1) is available from https://doi.org/10.12705/674.1.S

INTRODUCTION

Phenotypic variation within species may be caused by genotypic differences (Såstad & al., 1999; Savolainen & al., 2013) or by differential gene expression induced by environmental variability (Price & al., 2003; Pigliucci & al., 2006). Assessing the adaptive significance of morphological trait variation is a fundamental issue in evolutionary ecology (Såstad & al., 1999; Yousefi & al., 2017) and in systematics (Shaw & Bartow, 1992; Såstad, 1999), avoiding the overestimation of biodiversity. On the other hand, relying on morphological methods alone may miss cryptic species and therefore underestimate species diversity (Bickford & al., 2007). Cases of cryptic speciation have been widely documented in bryophytes (Shaw, 2001; McDaniel & Shaw, 2003; Hedenäs & Eldenäs, 2007; Kreier & al., 2010; Heinrichs & al., 2010, 2011; Ramaiya & al., 2010; Hutsemékers & al., 2012; Medina & al., 2012; Caparrós & al., 2016; Patiño & al., 2017), in which substantial genetic divergence within morphologically complex groups was observed. Genetic adaptation to habitats with relatively minor differences in ecological conditions was demonstrated in mosses as well (Såstad & al., 1999). Controlled experiments are the only way to determine functional correspondence between morphological, genotypic and environmental variability (Såstad, 1999; Såstad & al., 1999; Yousefi & al., 2017).

The delimitation of species in organisms with high dispersal potential by spores across variable environments and with considerable morphological variation remains a great challenge, such that any single methodology may not be sufficient to discriminate between species (Medina & al., 2012, 2013; Renner & al., 2013; Hedenäs & al., 2014; Draper & al., 2015; Caparrós & al., 2016; Gama & al., 2017; Patiño & al., 2017). In such cases, an integrative taxonomic approach (combining morphology, phylogeny, biogeography, ecological niche or genome size studies) is necessary.

The genus *Ceratodon* Brid. is distinguishable from other members of the family Ditrichaceae by molecular data (Cox & al., 2010; Stech & al., 2012; Fedosov & al., 2016) and morphological characters (Magill, 1981; Allen, 1994; Chien & al., 1999; Frey & al., 2006; McIntosh, 2007). Nevertheless, species within the genus are highly polymorphic (Dixon & Jameson, 1896; Watson, 1968; Crum & Anderson, 1981). The most abundant species, *C. purpureus* (Hedw.) Brid. occurs on every continent (Crum, 1973). Wijk & al. (1959, 1969) listed 22 *Ceratodon*

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species, and within *C. purpureus* alone two subspecies and 31 varieties. In a global survey of the genus, Burley & Pritchard (1990) recognized four species. *Ceratodon antarcticus* Cardot grows on bare soil, rock crevices and ledges in the Antarctic region. *Ceratodon conicus* (Hampe) Lindb. is found on calcareous substrates of arid habitats in North America, Europe and Africa. *Ceratodon heterophyllus* Kindb. is known from arctic bare soils in North America, Europe and Asia. Finally, *C. purpureus* is frequent on a wide range of substrata (acid-calcareous), bare disturbed ground, soil-covered rock ledges, sand dunes or on rotten wood. This latter species is common from natural and well-conserved to contaminated sites (Shaw & al., 1991), and is a frequent colonizer of recently burned places (Duncan & Dalton, 1982; Foster, 1985; Clément & Touffet, 1990). Burley & Pritchard (1990) recognized three infraspecific taxa for *C. purpureus*: subsp. *purpureus* and subsp. *convolutus* (Reichardt) Burley, both found in temperate areas, and subsp. *stenocarpus* (Bruch & Schimp.) Dixon, found in tropical regions. *Ceratodon purpureus* subsp. *purpureus* and *C. purpureus* subsp. *stenocarpus* are fairly well differentiated based on morphological characteristics, largely due to sporophyte features, and they would be recognized with the rank of species if it were not for the existence of plants with intermediate characteristics between both of them, which they called *C. purpureus* subsp. *convolutus*.

The taxonomic treatment of Burley & Pritchard (1990) has been challenged by numerous authors based on the heterogeneous distribution of morphological variation within the range of the taxa and on the apparent gradation in all the diagnostic characters. For example, Ochyra (1998) considered both *C. heterophyllus* and *C. antarcticus* to be synonyms of *C. purpureus*; O'Shea (2006) questioned the value of *C. purpureus* subsp. *purpureus* and *C. purpureus* subsp. *convolutus*; and McIntosh (2007) considered *C. conicus* a subspecies within the *C. purpureus* complex based on the apparent gradation and reduction of all the characters that Burley & Pritchard (1990) used in their treatment. Phylogenetic analyses of DNA sequences generally have not strongly supported recognizing these taxa as distinct. For example, several authors found no evidence that the Antarctic specimens formed distinct genetic clusters relative to Australasian and Subantarctic isolates, in spite of finding high levels of genetic variability (Clarke & al., 2009). Additionally, McDaniel & Shaw (2005) performed population genetic analyses of DNA sequence data from three unlinked loci to examine biogeographical patterns in a global sampling of *Ceratodon*. They found limited population structure across the global distribution, suggesting that long-distance migration is common, at least within the Northern Hemisphere and Australasian regions, although migration among equatorial populations was not frequent.

In parallel to the morphometric study presented here, we carried out a genetic and genome size study, including identifying the sex of not fructified individuals (Nieto-Lugilde & al., 2018a). In that paper we describe a phylogenetic analyses based on five nuclear introns and a single chloroplast locus of *Ceratodon* specimens from Mediterranean mountainous areas, other European mountainous systems and western and central European and South African lowlands. We could clearly distinguish two groups of specimens, corresponding to the cosmopolitan *C. purpureus* and a newly discovered species, the latter only known at present from the Sierra Nevada and the southeastern mountains of Spain. Coalescent simulations showed that the divergence between *C. purpureus* and the newly discovered species was too great to be explained by coalescent stochasticity. Several isolates had sequences from both species, which we interpreted as recombinants produced by meiosis from a hybrid sporophyte. We also examined the genome sizes of these taxa using flow cytometry. We discovered that specimens of *C. purpureus* had the smallest genome, while the new species possessed a genome 25% larger than *C. purpureus*, and the recombinants had the genome size either of the new species or the sum of the genome sizes of *C. purpureus* and the new species, the latter group potentially arising by allopolyploidy. Curiously, we found no male individuals in samples of the new species, either scanning for antheridia using a dissection microscope or using the PCR-based approach proposed by Norrell & al. (2014).

Here we use a common garden experiment to test the role of environmental variance in shaping the phenotype in the field of these distinct genetic entities. This is generally significant because we have a relatively poor understanding of the potential for the environment to shape our taxonomic concepts of bryophyte species (Vanderpoorten & al., 2002). In the present study we carried out a morphometric study, both from field-collected and *in vitro* cultivated plants (laboratory isolates grown from field plants), on a subset of the plants used previously in the phylogenetic and genome size study (Nieto-Lugilde & al., 2018a). We had three main goals: (i) to determine what the relevance of environmental variance in morphological variation in the genus *Ceratodon* is (i.e., to determine which proportion of the morphological variation has a genetic basis, and which proportion is due to environment); (ii) to search for relevant characters to discriminate molecularly defined species in the genetically induced morphological variation, and (iii) to make a taxonomical proposal according to the results obtained.

■ MATERIALS AND METHODS

Plant material. — We performed a morphological analysis of 64 samples of the genus *Ceratodon* (voucher information is listed in Appendix 1). We collected 43 specimens (67.2% of the total) from Mediterranean mountain areas (28 from Spanish Sierra Nevada; 10 from the Spanish Sistema Central, 3 from the Spanish southeastern mountains [eastern sierras de Segura and Sierra de Alcaraz], and 2 from Sicilian Mount Etna). Of the remaining 21 samples, 9 (14.1%) were from other European mountainous systems (6 from the Alps, 3 from the Pyrenees) and 12 specimens (18.7%) were from lowlands (3 from Czech Republic, 3 from Germany [including the type of *C. conicus*], 2 from Sweden, 2 from U.K., 2 from South Africa). Most of the studied samples (55) were collected by the authors and are deposited at MUB, and the rest (9) were loaned from several herbaria: BOL, CBFS, GOET and S, and 2 samples were donated by Laura Forrest (at Royal Botanic Garden Edinburgh, U.K.).

***In vitro* cultures. —** We generated *in vitro* cultures for 32 out of the 64 specimens. Apart from the type specimen that could not be used for axenic culture, 31 field specimens did not grow successfully *in vitro* (Appendix 1). As most of the samples did not produce sporophytes (only 11 presented mature capsules), the cultures were prepared from gametophytic material for all the specimens. First, several gametophore fragments from field-collected plants were put on polystyrene ∅ 55 × 14 mm petri dishes with moistened quartz sand containing a nutrient solution (Murashige and Skoog basal salt solution 0.4×) and kept in a growth chamber with 22 ± 3°C and 16/8 h of light to darkness supplied by cool-white fluorescent tubes (Lumilux, Osram Germany) at a photon fluency rate of 33.5–55.0 µmol m^{-2} s^{-1}. To reduce the risk of death of the samples by contamination of fungi and algae when the fragments began to develop protonemata, they were sterilized with 0.10% sodium dichloroisocyanurate ($C_3Cl_2N_3NaO_3$) for 10 s. We then washed the samples with sterile distilled water and dried them on absorbent paper to remove any residual of sodium dichloroisocyanurate, and put them on polystyrene ∅ 55 × 14 mm petri dishes containing Gelzan with MS (half-strength Murashige and Skoog basal medium) with cellophane overlays, and maintained in the growth chamber with the same conditions as above. When the protonemata developed rhizoids, caulidia and several phyllidia (80–278 days), they were used for biometric study as it was done with the field-collected plants. During the period in growth chamber, plates were changed from position in the chamber, two times per week, to reduce chamber effect (Measures & al., 1973; Porter & al., 2015).

Biometric study. — We selected 22 descriptive morphological characters, following the morphological results of Burley & Pritchard (1990) and our own observations (Table 1). Of these, 9 were qualitative and 13 quantitative characters. Terms used in the present work are based on *Glossarium polyglottum*

Table 1. Descriptive morphological characters included in the biometric study and HCl soil reaction of the substrate of the *Ceratodon* Brid. samples, with indication of the number of items studied, abbreviations used, type (QL = qualitatitive, QT = quantitative) and status characters.

	Abbreviation	Character	Type and status character
Substrate	SR	HCl soil reaction	QL: Acidic (0); Basic (1)
Caulidium (2 caulidia per collection)	PP	Phyllidium posture in the caulidium when moist	QL: Straight (1); Curved (2)
	CT	Comal tuft in the caulidium apex	QL: Absent (0); Present (1)
	CL	Caulidium length	QT (mm)
Phyllidium (10 phyllidia: 5 from each caulidium)	LL	Lamina length	QT (µm)
	LLW	Lamina length from apex to widest part	QT (µm)
	LW	Lamina width at widest part	QT (µm)
	AT	Teeth at apical part of lamina	QL: Absent (0); Dentate (1); Serrate (2)
	PM	Phyllidium margins	QL: Plane at middle part of lamina (0); Recurved at middle part of lamina (1); Recurved at middle and apical part of lamina (2)
	PA	Phyllidium apex	QL: Acute (1); Obtuse (2)
	EN	Excurrent nerve	QL: Absent (0); Present (1)
	ENL	Excurrent nerve length	QT (µm)
	NW	Nerve width at base of lamina	QT (µm)
	NC	Nerve color	QL: Reddish (1); Greenish (2)
	CS	Middle laminal cells shape	QL: Rounded (0); Quadrate (1)
	LL/LLW	Lamina length/lamina length from apex to widest part ratio	QT
	LL/LW	Lamina length/lamina width in widest part ratio	QT
	LL/NW	Lamina length/nerve width at base of lamina ratio	QT
	ENL/LL	Excurrent nerve length/lamina length ratio	QT
	NW/LW	Nerve width at base of lamina/lamina width at widest part ratio	QT
Middle laminal cells (12 cells: 3 from each 2 phyllidia coming from 2 caulidia)	CW	Cells width	QT (µm)
	CWT	Cells wall thickness	QL: ≤2 µm (1); >2 µm (2)
Phyllidium cross sections (6 sections: 3 from each phyllidium coming from 2 caulidia)	NGC	Number of guide cells	QT (number of cells)

bryologiae, a multilingual glossary for bryology (Magill, 1990). No sporophytic characters were considered for the biometric study because of the absence of capsules in the field-collected specimens and because the plants obtained *in vitro* never developed sporophytes. When they were available, sporophyte traits were only studied to confirm the identity of the specimens. We also estimated the pH of the substrate on which the moss was growing by placing a few drops of hydrochloric acid (HCl) in soil present in each sample. HCl reacts with carbonated minerals (calcite, $CaCO_3$, is the most commonly encountered) such that carbon dioxide bubbles (effervescence) are produced if the soil presented basic characteristics, and no effervescence if the soil was acidic.

In each collection, the HCl soil reaction (SR) was tested and two gametophore shoots were taken, in which the next characters were observed: phyllidium posture in the caulidium when moist (PP), presence/absence of a comal tuft in the caulidium apex (CT) and caulidium length (CL). Five phyllidia of the middle part of each caulidium were dissected, in which the following characters were studied (Fig. 1): lamina length (LL), lamina length from apex to widest part (LLW), lamina width at widest part (LW), presence/absence of teeth at apical part of lamina (AT), phyllidium margins as seen in section (PM), phyllidium apex (PA), presence/absence of excurrent nerve (EN), excurrent nerve length (ENL), nerve width at base of lamina (NW), nerve color (NC), middle laminal cells shape (CS), lamina length/lamina length from apex to widest part ratio (LL/LLW), lamina length/lamina width in widest part ratio (LL/LW), lamina length/nerve width at base of lamina ratio (LL/NW), excurrent nerve length/lamina length ratio (ENL/LL), and nerve width at base of lamina/lamina width at widest part ratio (NW/LW). Two phyllidia from each of the two caulidia were selected, in each of them three measures were taken of the middle laminal cells width (CW) and their wall thickness (CWT). After selection of one phyllidium coming from each caulidium, at least three cross sections were made at the basal part near the middle of each phyllidium for observation of the number of guide cells (NGC). We measured the morphological parameters using a light microscope (Olympus BH2) with a micrometer inserted in an ocular and in a stereomicroscope (Leica A8APO) with a video camera connected (Leica camera: DFC295) in order to transfer the images to a computer. The Leica Application Suite, v.4.1.0 was used for image analysis.

Statistical analyses. — All characters were entered in a LibreOffice spreadsheet (Calc program, The Document Foundation) and then imported in R (R Core Team, 2016). For the evaluation of morphological characters and multivariate analyses, the specimens were assigned to one of three groups based on the phylogenetic analyses of the specimens carried out by Nieto-Lugilde & al. (2018a): (1) Sierra Nevada (SN) group: formed by 14 Sierra Nevada samples and 1 of the Spanish southeastern mountains, included in a highly supported clade by all the six loci studied (SN clade); (2) Worldwide (Ww) group, that consistently included 31 specimens mostly coming from the rest of the sampled areas, but also including a low number from SN and Spanish southeastern mountains, also in a highly supported clade by all the six loci studied (Ww clade); and (3) Recombinant group: formed by 17 samples mainly coming from Sierra Nevada, but also from Spanish Sistema Central, Alps and United Kingdom; that were strongly resolved in either the SN clade or the Ww clade, depending on the studied locus. Each group was subdivided into two subgroups: field-collected plants and cultivated plants. All samples from SN group and Ww group for which the genome size could be determined previously were haploid (13 and 20 respectively);

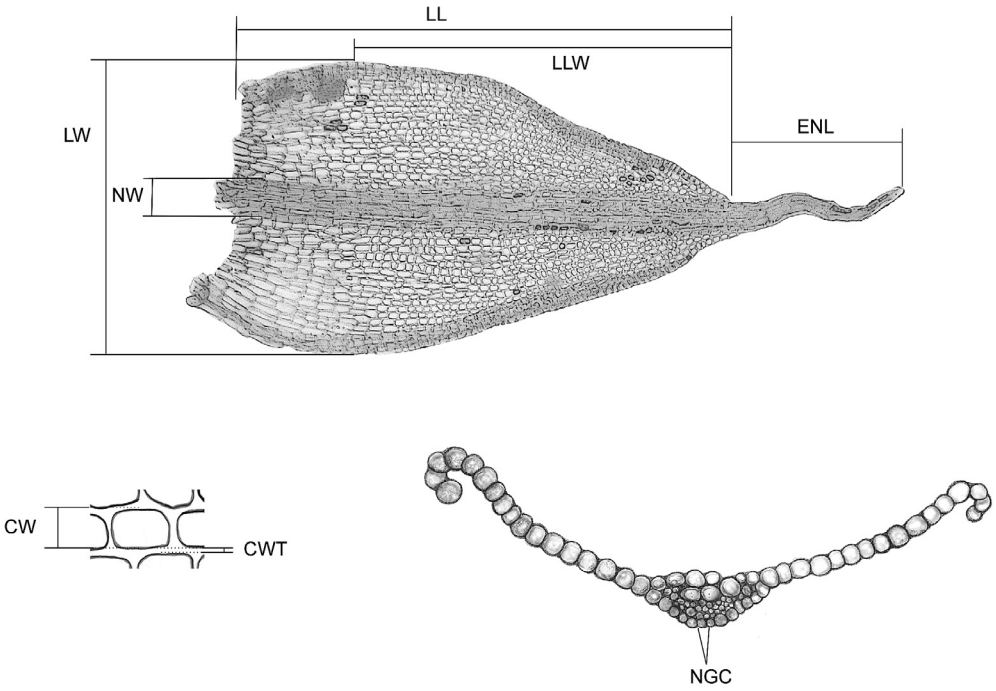

Fig. 1. Diagrammatic definition of the quantitative morphological characters included in the biometric study of *Ceratodon*. LL, lamina length; LLW, lamina length from apex to widest part; ENL, excurrent nerve length; LW, lamina width; NW, nerve width at base of lamina; CW, middle laminal cells width; CWT, middle laminal cells wall thickness; NGC, number of guide cells in phyllidium cross section.

the recombinant samples contained four putative allopolyploids and nine haploids (Appendix 1). The type of *C. conicus* was studied morphologically and considered a recombinant based on its morphological characteristics, because neither the genotype nor the genome size could be determined.

We used Wilcoxon test (non-parametric test; Wilcoxon, 1945) to examine the significance of the differentiation between different subgroups, and we adjusted the *p*-value to test the significance level using the Benjamini-Hochberg correction (Benjamini & Hochberg, 1995). We plotted the reaction norms for each quantitative morphological character for the cultivated plants and the corresponding field-collected plants for each genetic group. The set of R functions for the morphometric analysis was based on Koutecký (2015). The transformations performed to improve their distribution were: Log_{10} (CL), Log_{10} (LL/LLW), Log_{10} (ENL + 1) and Log_{10} (ENL/LL × 100 + 1). The characters AT and PM were split in two binary characters: AT d (dentate), AT s (serrate), PM p (plane) and PM r2 (recurved at middle and apical part of lamina). We performed two-way ANOVAs on each quantitative morphological character to study the effect of environmental conditions and the genotype, and the interaction term between them. The environment where the plants were grown was employed as variation factor with two levels (field-collected plants and cultivated plants) and the genetic groups obtained in Nieto-Lugilde & al. (2018a) were used as variation factor with three levels (SN group, Ww group, Recombinant group). Finally we performed three multivariate morphometric analyses: principal component analysis (PCA), linear discriminant analysis (LDA) and classificatory discriminant analysis (CDA). PCA converts a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components (PC). The essential part of LDA is a dimensionality reduction, which allows replacing the original variables-classifiers by the linear discriminants (LD), a smaller number of derivate classifiers. LDA finds the line that best separates the classes, on the contrary to PCA that is not optimal for classification. In addition we performed two types of significance tests of individual characters, first the marginal effects (i.e., when a character is alone in the model) and second the unique contributions of the characters (i.e., the addition of each character into the model with all other characters). The CDA quantifies the proportion of specimens correctly assigned to a species by the best combination of morphological characters. We performed a LDA using the real morphological character as variables in the discriminant analysis; moreover we tested the use of the PC as variables in LDA (PCA-LDA). To avoid using highly correlated characters ($r > |0.95|$) in the discriminant analysis (Koutecký, 2015) we calculated a Spearman's matrix of the correlation coefficients of the characters. Only the characters LL and LLW showed high correlation between them ($r = 0.989$). For this reason, LL was omitted for discriminant analysis. The PCs with eigenvalues >1 were selected as optimal number of variables (Kaiser, 1960), moreover the amount of explained variance was 70%–80%. The most useful characters for separating the three groups of samples both field-collected plants and cultivated plants were determined by a higher absolute value of contribution of characters to individual axes (both LD1 and LD2). These values were standardized by within-group variance and reflected better the relative importance of the characters (Lepš & Šmilauer, 2003).

■ RESULTS

Environmental variance in morphological variation. — The statistical results of qualitative and quantitative characters included in the biometric study are shown in Table 2, and the results of analyses of the variance (two-way ANOVAs) of the quantitative morphological characters are given in Table 3.

Most of the analyzed traits (92%) showed significant ($\alpha = 0.05$) effects of environment (except LL/NW) and genotype (except NW/LW) on ANOVAs. Only Log_{10} (ENL + 1) and Log_{10} (ENL/LL × 100 + 1) did not show significant effects due to the interaction between genotype and the environment (G × E). The *p*-value for some characters are greater for the genotype factor than for the environment. The environment factor explains more than 50% of the observed phenotypic variation for Log_{10} (CL), LW, Log_{10} (ENL + 1) and NW. The genetic factor explains more than 50% of the variation in LL, LLW, LL/LW, LL/NW, Log_{10} (ENL/LL × 100 + 1) and NGC. Finally the interaction between the two factors explains more than 50% of the variation in Log_{10} (LL/LLW) and NW/LW, but in these cases the environment factor is more important than the genetic factor. Furthermore, the character CW does not show a clear explanation by only one factor (E = G × E = 40.24%). In general, the morphological variation within the genus *Ceratodon* is more influenced by environmental variation in seven characters and by genetic variation in six characters. Reaction norm plots for each quantitative morphological trait (except NGC) were shown in Fig. S1 (Electr. Suppl.). All morphological characters presented environmental variance for all or at least some of the genotypes. The magnitude of environmental variance was quantified by the slope of the line which varies enormously between genotypes. It is usually observed that the characters vary in the same sense although each genotype varies with a different magnitude, the exceptions were LL/LLW, LL/LW, LL/NW, NW/LW, and CW. Wilcoxon's test also confirmed clear differences between field-collected plants and cultivated plants in most of the studied characters (Table 4); in the samples from SN (Sierra Nevada) and Ww (Worldwide) groups 81.8% of the characters studied were different, but the recombinants presented a little more variation between different environments (86.4%). We could not detect a significant environmental response in samples from SN group in the characters CL, NC, CS and CW ($p > 0.05$), and LLW and PA characters were marginally significant ($\alpha = 0.01–0.05$). In the samples from the Ww group, the CT, PA, LL/LW and NW/LW characters were not different. In the recombinant samples the CS, LL/LLW and LL/NW characters were not different, and again the PA character was marginally significant ($\alpha = 0.01–0.05$). We have highlighted differences between field-collected plants and cultivated plants in the boxplots (Figs. 2–3), in which the data obtained for 12 of the 13 quantitative characters studied were shown (with the exception

Table 2. Statistical results of qualitative and quantitative characters of *Ceratodon* Brid. specimens included in the biometric study.

Abbreviation of characters studied	SN Field (N = 15)	Ww Field (N = 31)	Recombinant Field (N = 17)	SN Cultivated (N = 9)	Ww Cultivated (N = 14)	Recombinant Cultivated (N = 9)
SR	**50.00% Acidic**	96.60% Acidic	82.30% Acidic			
PP	93.75% Straight	**67.20% Straight**	97.00% Straight	100.00% Curved	100.00% Curved	100.00% Curved
CT	59.30% Absent	**96.90% Absent**	50.00% Absent	100.00% Absent	100.00% Absent	100.00% Absent
CL	4.46 ± 2.30 [2.05–9.45]	**10.65 ± 5.54 [2.81–29.26]**	5.17 ± 2.24 [2.26–10.01]	3.65 ± 1.93 [1.30–7.50]	2.53 ± 1.13 [0.60–4.93]	2.20 ± 0.50 [1.52–2.94]
LL	**594.10 ± 162.30 [350.00–1150.00]**	**1142 ± 357.48 [362.70–2586.00]**	**717.20 ± 192.16 [354.60–1350.00]**	512.90 ± 129.09 [266.00–806.00]	**811.10 ± 295.84 [338.50–1656.00]**	558.90 ± 130.26 [322.40–846.30]
LLW	**462.30 ± 151.81 [241.80–950.00]**	**946.30 ± 334.26 [241.80–2182.00]**	**569.30 ± 182.68 [241.80–1200.00]**	411.20 ± 127.04 [161.20–750.00]	**637.30 ± 289.15 [241.80–1515.00]**	441.90 ± 125.66 [225.70–725.40]
LW	378.00 ± 80.71 [200.00–556.10]	387.80 ± 72.76 [230.00–612.60]	384.00 ± 102.39 [193.40–710.00]	235.60 ± 40.78 [129.00–346.60]	**297.50 ± 64.28 [177.30–483.60]**	226.90 ± 46.96 [137.00–346.60]
AT	98.1% Absent 1.9% Dentate	**91.9% Absent** **8.1% Dentate**	98.8% Absent 1.2% Dentate	56.0% Absent 44.0% Dentate	49.3% Absent 50.0% Dentate 0.7% Serrate	66.7% Absent 33.3% Dentate
PM	**89.4% (2)** **6.9% (0):** **3.7% (1)**	97.8% (2) 2.2% (1)	97.6% (2) 1.2% (0) 1.2% (1)	44.0% (2) 29.0% (0) 27.0% (1)	38.0% (0) 31.3% (1) 30.7% (2)	36.6% (2) 32.2% (1) 31.2% (0)
PA	90.0% Acute	**98.8% Acute**	94.1% Acute	98.0% Acute	97.3% Acute	100.0% Acute
EN	**75.0% Present**	**54.4% Present**	**94.1% Present**	59.0% Absent	**76.0% Absent**	58.9% Present
ENL	**128.60 ± 69.45 [40.00–322.40]**	**107.40 ± 58.60 [24.18–350.00]**	**159.20 ± 91.37 [40.00–600.00]**	58.47 ± 35.22 [16.12–200.00]	61.12 ± 30.61 [16.12–137.00]	70.72 ± 45.46 [16.12–225.70]
NW	64.22 ± 17.43 [25.00–114.00]	**79.13 ± 17.36 [38.00–140.00]**	67.48 ± 19.19 [25.00–140.00]	48.38 ± 13.13 [20.00–72.00]	**57.84 ± 17.16 [26.00–108.00]**	50.02 ± 12.24 [25.00–90.00]
NC	74.4% Greenish	66.9% Greenish	**87.6% Greenish**	84.0% Greenish	92.7% Greenish	74.4% Greenish
CS	**92.5% Quadrate**	**62.2% Quadrate**	**99.4% Quadrate**	97.0% Quadrate	98.0% Quadrate	100.0% Quadrate
LL/LLW	**1.30 ± 0.14 [1.07–1.80]**	**1.23 ± 0.14 [1.03–2.20]**	**1.29 ± 0.16 [1.00–2.00]**	1.27 ± 0.15 [0.92–2.00]	1.33 ± 0.19 [1.05–2.12]	1.29 ± 0.14 [1.06–1.66]
LL/LW	**1.58 ± 0.32 [0.85–2.78]**	**2.96 ± 0.83 [1.06–5.83]**	**1.91 ± 0.42 [1.07–3.18]**	**2.18 ± 0.44 [1.25–3.18]**	**2.78 ± 1.06 [1.33–6.52]**	**2.52 ± 0.65 [1.39–4.16]**
LL/NW	**9.56 ± 2.30 [5.40–18.00]**	**14.51 ± 3.96 [5.79–34.26]**	**10.99 ± 2.65 [5.33–21.25]**	11.06 ± 2.83 [6.16–21.49]	**14.33 ± 4.06 [7.31–32.83]**	11.65 ± 3.26 [6.24–22.00]
ENL/LL	0.22 ± 0.12 [0.05–0.74]	**0.09 ± 0.05 [0.02–0.29]**	0.22 ± 0.11 [0.05–0.60]	0.11 ± 0.06 [0.03–0.29]	**0.06 ± 0.03 [0.02–0.14]**	0.13 ± 0.09 [0.03–0.47]
NW/LW	0.17 ± 0.04 [0.08–0.30]	**0.21 ± 0.04 [0.10–0.30]**	0.18 ± 0.04 [0.10–0.28]	0.21 ± 0.05 [0.10–0.30]	0.20 ± 0.06 [0.09–0.36]	0.23 ± 0.06 [0.10–0.35]
CW	8.17 ± 2.01 [4.00–15.00]	7.75 ± 1.70 [3.00–12.50]	8.44 ± 1.96 [4.00–16.00]	8.26 ± 1.44 [6.00–12.00]	**10.22 ± 3.00 [4.00–18.00]**	8.96 ± 2.64 [4.00–16.00]
CWT	77.22% ≤2	77.30% ≤2	75.56% ≤2	96.60% ≤2	95.83% ≤2	100.00% ≤2
NGC	**0.98 ± 1.02 [0.00–3.00]**	**3.28 ± 1.32 [0.00–6.00]**	**1.37 ± 1.35 [0.00–5.00]**	0.00 ± 0.00 [0.00–0.00]	**1.45 ± 1.68 [0.00–4.00]**	0.00 ± 0.00 [0.00–0.00]

Character abbreviations follow those given in Table 1.

Specimens were grouped according to the three genetic groups obtained from phylogenetic analyses (Nieto-Lugilde & al., 2018a) and each of them was subdivided in two subgroups: the field-collected plants and the *in vitro* cultivated plants (SN Field: Sierra Nevada group samples from field, Ww Field: Worldwide group samples from field, Recombinant Field: Recombinant group samples from field, SN Cultivated: Sierra Nevada group samples from *in vitro* cultures, Ww Cultivated: Worldwide group samples from *in vitro* cultures, Recombinant Cultivated: Recombinant group samples from *in vitro* cultures). Number (N) of specimens examined for each group is given. Descriptive statistics (mean ± SD [range]) for quantitative characters are presented.

All measurements are given in µm, except for CL in mm.

Values with statistically significant difference ($\alpha = 0.05$) are written in bold.

Table 3. Analyses of the variance (ANOVAs, two way factorial design) of the quantitative morphological characters.

Characters studied	Variation factors	Df	Sum of Squares	Mean Squares	*F* value	*P* value (>*F*)	Variance explained
CL	E	1	5.856	5.856	155.68	**<2e-16**	66.95%
	G	2	0.985	0.492	13.09	**7.07e-06**	11.26%
	G × E	2	1.906	0.953	25.34	**6.27e-10**	21.79%
	Residuals	122	4.590	0.038			
LL	E	1	13098465	13098465	216.3	**<2e-16**	25.89%
	G	2	33017908	16508954	272.6	**<2e-16**	65.27%
	G × E	2	4469967	2234984	36.9	**6.93e-16**	8.84%
	Residuals	634	38400805	60569			
LLW	E	1	10536668	10536668	193.90	**<2e-16**	26.40%
	G	2	24834829	12417414	228.51	**<2e-16**	62.22%
	G × E	2	4544316	2272158	41.81	**<2e-16**	11.38%
	Residuals	634	34451281	54340			
LW	E	1	3078582	3078582	567.966	**<2e-16**	88.19%
	G	2	345789	172894	31.897	**6.32e-14**	9.91%
	G × E	2	66654	33327	6.149	**0.00227**	1.90%
	Residuals	634	3436510	5420			
ENL	E	1	7.324	7.324	136.387	**<2e-16**	89.73%
	G	2	0.665	0.332	6.190	**0.00229**	8.15%
	G × E	2	0.173	0.086	1.608	0.20185	2.12%
	Residuals	336	18.043	0.054			
NW	E	1	78322	78322	271.023	**<2e-16**	74.05%
	G	2	23613	11807	40.855	**<2e-16**	22.32%
	G × E	2	3841	1920	6.645	**0.00139**	3.63%
	Residuals	634	183219	289			
LL/LLW	E	1	0.0476	0.04756	21.968	**3.40e-06**	34.07%
	G	2	0.0203	0.01016	4.693	**0.00948**	14.53%
	G × E	2	0.0718	0.03592	16.590	**9.49e-08**	51.40%
	Residuals	634	1.3726	0.00217			
LL/LW	E	1	4.6	4.63	8.787	**0.00315**	2.45%
	G	2	148.4	74.20	140.897	**<2e-16**	79.15%
	G × E	2	34.5	17.27	32.792	**2.81e-14**	18.40%
	Residuals	634	333.9	0.53			
LL/NW	E	1	1	0.60	0.047	0.8283	0.03%
	G	2	3104	1552.20	127.384	**<2e-16**	94.55%
	G × E	2	178	89.20	7.318	**0.000721**	5.42%
	Residuals	634	7725	12.20			
ENL/LL	E	1	2.122	2.1223	48.071	**2.11e-11**	24.73%
	G	2	6.235	3.1176	70.614	**<2e-16**	72.66%
	G × E	2	0.224	0.1121	2.539	0.0805	2.61%
	Residuals	336	14.834	0.0441			
NW/LW	E	1	0.0543	0.05428	25.737	**5.15e-07**	29.88%
	G	2	0.0127	0.00634	3.007	0.0502	6.99%
	G × E	2	0.1147	0.05737	27.202	**4.63e-12**	63.13%
	Residuals	634	1.3372	0.00211			
CW	E	1	231	230.78	50.27	**3.05e-12**	40.24%
	G	2	112	55.90	12.18	**6.22e-06**	19.52%
	G × E	2	231	115.56	25.17	**2.59e-11**	40.24%
	Residuals	762	3498	4.59			
NGC	E	1	254.2	254.20	188.72	**<2e-16**	42.01%
	G	2	318.7	159.33	118.28	**<2e-16**	52.67%
	G × E	2	32.2	16.09	11.95	**9.49e-06**	5.32%
	Residuals	357	480.9	1.35			

The environment of growing (E) was employed as variation factor with two levels: field-collected plants and cultivated plants under laboratory conditions; the genetic species (G; obtained in Nieto-Lugilde & al., 2018a) was used as variation factor with three levels: SN = Sierra Nevada group samples, Ww = Worldwide group samples, and Recombinant group samples. The residuals tell us about the variation within each level. Character abbreviations follow those given in Table 1; the transformations performed to improve their distribution were: Log_{10} (CL), Log_{10} (LL/LLW), Log_{10} (ENL + 1) and Log_{10} (ENL/LL × 100 + 1). Df = Degree of freedom.
Values with statistically significant difference ($\alpha = 0.05$) are written in bold.

of CW). Most quantitative plant characters presented low mean values for cultivated plants in comparison with field-collected plants, as observed in LL, LLW, LW, ENL, NW, ENL/LL or NGC (Table 2).

Relevant characters to discriminate molecularly defined species. — Studying the three genetic groups of field-collected plants separately, approximately one-third (36.4%) of the means of the measured morphological characters differed between the SN group samples and Recombinant group samples from Ww group samples (PP, CT, CL, AT, PA, NW, ENL/LL and NW/LW; Tables 2–4). Slightly higher proportion of the characters (40.9%) separated the three genetic groups (LL, LLW, EN, ENL, CS, LL/LLW, LL/LW, LL/NW and NGC; Tables 2–4). In these characters, the differences between the mean values in the Ww group and the other two groups were always larger (2–5.5 times) than the difference between the SN group and the Recombinant group. Two characters were homogeneous among the three groups (LW and CWT; Table 4). The SN group samples were indifferent to the type of soil (50% acid), while the Ww group and Recombinant group samples showed a preference for acid soils, 96.6% and 82.3% respectively (Table 2).

In the PCA, 46.08% of the total variance was explained with the first two PCs (32.35% PC1, 13.73% PC2), and 14 PCs explained 96.51% of the total variance. PC1 separated the Ww samples from the SN and recombinant samples (Fig. 4A), while the other PCs did not clearly separate any group. Six PCs had an eigenvalue higher than 1 (73.77% of the variance); the contributions of morphological characters are shown in Table S1 (Electr. Suppl.). We performed a LDA using the morphological variables (Fig. 4B). The LD1 (74.21%) separated two clear groups: Ww group with positive score values and SN group together with Recombinant group with negative score values. The LD1 was influenced primarily by LL/LW and LLW, and the next most important characters were NGC, and Log_{10} (ENL/LL × 100 + 1) (Electr. Suppl.: Table S2). The LD2 (25.79%) slightly separated SN group and Recombinant group, and the Ww group samples show value ranges as broad as the other two groups together; the LL/LW loaded heavily also on LD2, together with PM p, PM r2 and LL/NW (Electr. Suppl.: Table S2). The first significance test (marginal effects) highlighted all characters with $p < 0.05$, except LW and CWT. On the other hand, the second significance test (unique contributions) showed only LL/NW and Log_{10} (ENL/LL × 100 + 1) with $p < 0.05$. The CDA showed the correctness of classification. For the Ww group it was 90.32%, while for the SN group and Recombinant group it was 63.33% and 63.88% respectively.

Table 4. *P*-values of the morphological characters included in the biometric study and HCl soil reaction of the substrate of the *Ceratodon* Brid. samples (Wilcoxon test) between genetic groups from field and *in vitro* cultivated plants.

	Groups compared	SR	PP	CT	CL	LL	LLW	LW	AT	PM	PA	EN	ENL
Field vs. Cultures	1–2		**0.0000**	**0.0012**	0.2251	**0.0001**	**0.0161***	**0.0000**	**0.0000**	**0.0000**	**0.0137***	**0.0000**	**0.0000**
	3–4		**0.0000**	0.3384	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.2696	**0.0000**	**0.0000**
	5–6		**0.0000**	**0.0003**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0193***	**0.0000**	**0.0000**
Field	1–3	**0.0002**	**0.0043**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.1208	**0.0068**	**0.0000**	**0.0068**	**0.0000**	**0.0081**
	1–5	**0.02583***	0.5338	0.4525	0.1453	**0.0000**	**0.0000**	0.8093	0.6061	**0.0021**	0.6061	**0.0000**	**0.0000**
	3–5	0.2464	**0.0008**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.1563	**0.0016**	0.8930	**0.0016**	**0.0000**	**0.0000**
Cultures	2–4		–	–	0.0670	**0.0000**	**0.0000**	**0.0000**	0.2828	0.0392	0.7389	**0.0044**	0.5516
	2–6		–	–	0.0217	0.0156	0.0749	0.0703	0.1336	0.4191	0.1810	0.0141	0.0086
	4–6		–	–	0.2918	**0.0000**	**0.0000**	**0.0000**	**0.0083***	0.2429	0.1200	**0.0000**	0.5345

		NW	NC	CS	LL/LLW	LL/LW	LL/NW	ENL/LL	NW/LW	CW	CWT	NGC
Field vs. Cultures	1–2	**0.0000**	0.0687	0.1313	**0.0069**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.7660	**0.0000**	**0.0000**
	3–4	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.4499	**0.0002**	**0.0001**	0.0662	**0.0000**	**0.0000**	**0.0000**
	5–6	**0.0000**	**0.0070**	0.4718	0.6254	**0.0000**	0.1695	**0.0000**	**0.0000**	**0.0017**	**0.0000**	**0.0000**
Field	1–3	**0.0000**	0.0933	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0004**	0.2688	**0.0000**
	1–5	0.2102	**0.0021**	**0.0013**	**0.0206***	**0.0000**	**0.0000**	0.8891	0.1416	0.0435	0.8959	**0.0158***
	3–5	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.2612	0.3307	**0.0000**
Cultures	2–4	**0.0000**	0.0310	0.6159	0.0379	**0.0000**	**0.0000**	**0.0000**	0.2459	**0.0000**	1.0000	**0.0000**
	2–6	0.6298	0.1048	0.0998	0.3039	**0.0005**	0.2070	0.4080	0.0066	0.0621	0.0567	–
	4–6	**0.0005**	**0.0001**	0.1795	0.2774	**0.0000**	**0.0000**	**0.0000**	**0.0011**	**0.0001**	0.0560	**0.0000**

Character abbreviations follow those given in Table 1.
Significant *p*-values <0.01 are written in bold after being corrected with Benjamini-Hochberg test; * means 0.01 < *p*-value < 0.05.
Groups obtained from phylogenetic analyses (Nieto-Lugilde & al., 2018a): 1 = Sierra Nevada group samples from field, 2 = Sierra Nevada group samples from *in vitro* cultures, 3 = Worldwide group samples from field, 4 = Worldwide group samples from *in vitro* cultures, 5 = recombinant samples from field, and 6 = recombinant samples from *in vitro* cultures.

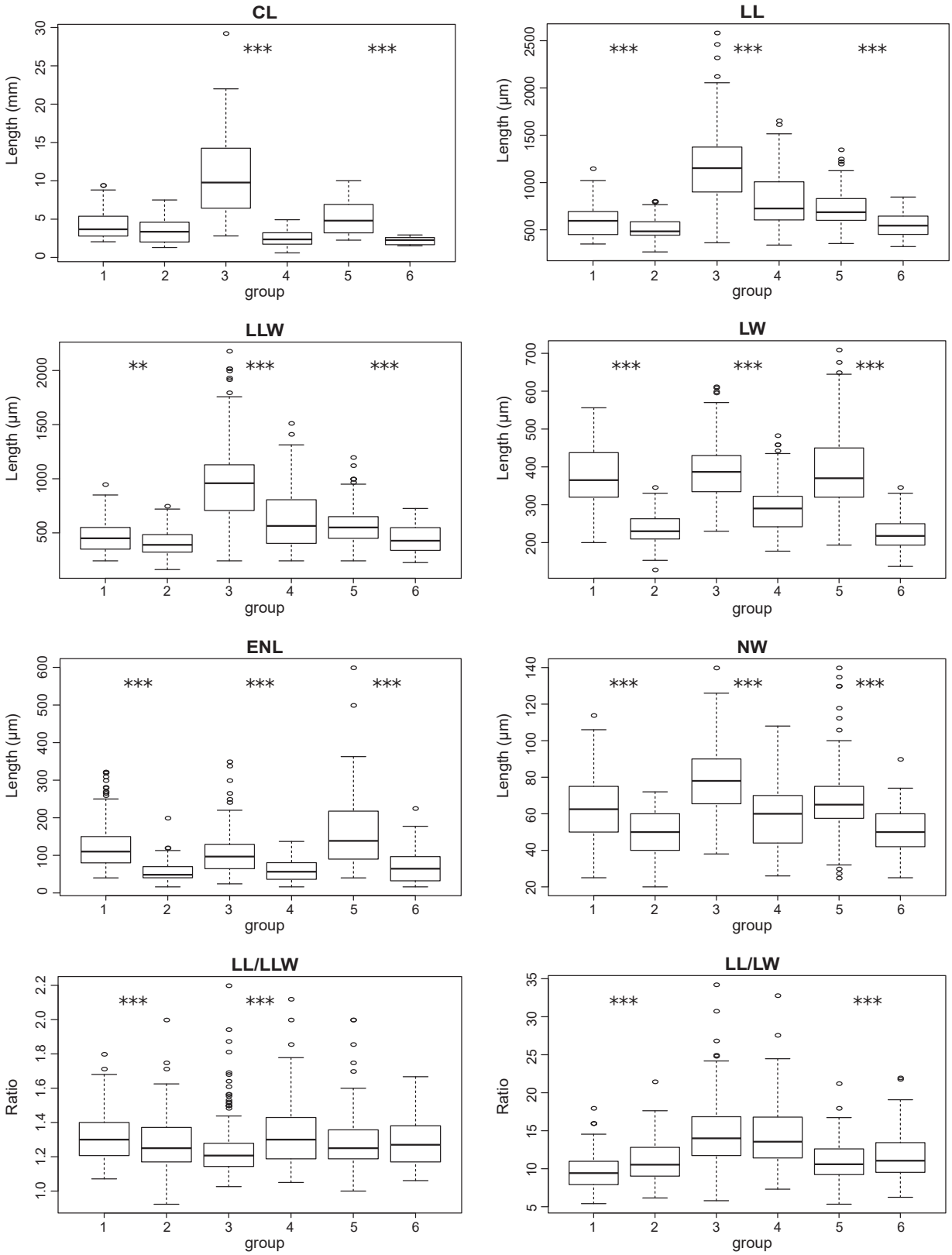

Fig. 2. Box-plots of some of the principal morphological characters studied: caulidium length (CL), lamina length (LL), lamina length from apex to widest part (LLW), lamina width at widest part (LW), excurrent nerve length (ENL), nerve width at base of lamina (NW), lamina length/lamina length from apex to widest part ratio (LL/LLW) and lamina length/lamina width in widest part ratio (LL/LW). Number of groups as in Table 4. Significant p-value < 0.01 and 0.01 < p-value < 0.05 after being corrected with Benjamini-Hochberg test are indicated with *** and **, respectively.

Two caulidia of the lectotype of *Ceratodon conicus* were included in multivariate analyses. In the PCA analysis they are together with the Recombinant group and SN group, but in LDA and PCA-LDA analysis they are grouped with the recombinants and separated from the SN group (Fig. 4A, B). One sample from Austria without sporophytes (CBFS 6162, included in this study) showed some morphological characteristics typical of *C. heterophyllus*, such as broadly ovate, concave, almost cucullate phyllidia, and nerves ending shortly below the obtuse apex. Nevertheless, despite these morphological characteristics, the Austrian sample belongs to the Ww group based on genetic data. Our PCA results slightly discriminate this sample from most of the Ww group samples, but LDA results indicated that this sample could not be separated by morphological gametophytic characters from *C. purpureus* (Fig. 4A, B). Two samples with sporophytes from South Africa studied here (BOL 46302, BOL 46303) presented the morphological diagnostic characters of *C. purpureus* subsp. s*tenocarpus*: erect to sub-erect, smooth to slightly sulcate when dry, ± lacking struma capsules, and peristome teeth usually narrowly bordered, with 0–5 trabeculae (*C. purpureus* subsp. *purpureus*, in contrast, presents inclined to horizontal, deeply sulcate when dry, strumose capsules, and peristome teeth with broad and prominent border, and with (5)7–9(13) trabeculae). Based on genetic data, these two African samples also belonged to the Ww group. According with that, our morphological analyses did not differentiate them from the rest of samples from Ww group.

Comparing each genotype from cultivated plants, we observed that 9 out of 22 morphological characters separate the SN group samples and Recombinant group samples from Ww group samples (LL, LLW, LW, EN, NW, CW, NGC, LL/NW, ENL/LL). Only one character (LL/LW) differed among the three genetic groups in culture, and nine characters did not differ among groups (PP, CT, CL, PM, PA, ENL, CS, CWT, LL/LLW). The other three characters (AT, NC, NW/LW) separate the Ww group samples and Recombinant group samples. The groups were barely intermingled with each other in the PCA, but no clear-cut discontinuity was detected among them (Fig. 4C). Six PCs had an eigenvalue higher than 1 (77.06% of the variance), the contribution of morphological characters is shown in Table S1 (Electr. Suppl.). In LDA analyses we obtained results similar to field-collected plants, but LD1 discriminated 62.09% and LD2 37.91% (Fig. 4D). Looking at the contribution of characters (Electr. Suppl.: Table S2), it was observed that LW, Log_{10} (ENL/LL × 100 + 1), together with LL/NW were the characters with the highest influence on LD1, while on LD2 were Log_{10} (CL), NW/LW, NC and Log_{10} (ENL/LL × 100 + 1). The first significance test (marginal effects) showed less amount of significant characters than in field-collected plants; Log_{10} (CL), LLW, LW, Log_{10} (ENL + 1), NW, LL/LW, LL/NW, NW/LW, Log_{10} (ENL/LL × 100 + 1), CW and NGC presented $p < 0.05$. The second significance test (unique contributions) showed only Log_{10} (CL) and CW with $p < 0.05$. The CDA showed here less capacity to discriminate groups than for field-collected plants. For the Ww group it was 89.29%, while for SN group and Recombinant group it was 62.50% and 58.82% respectively.

In addition, for field-collected plants as well as cultivated plants we performed a LDA analysis employing the six most

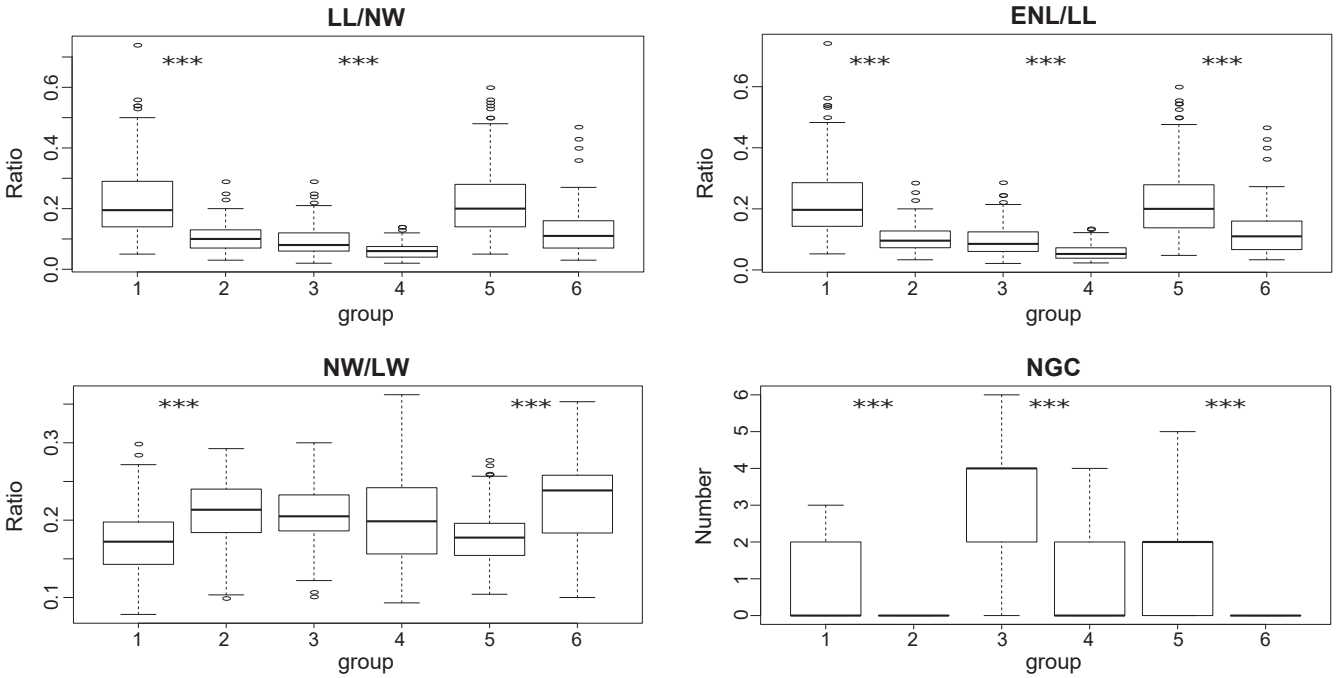

Fig. 3. Box-plots of some of the principal morphological characters studied: lamina length/nerve width at base of lamina ratio (LL/NW), excurrent nerve length/lamina length ratio (ENL/LL), nerve width at base of lamina/lamina width at widest part ratio (NW/LW) and number of guide cells (NGC). Number of groups as in Table 4. Significant p-value < 0.01 after being corrected with Benjamini-Hochberg test is indicated with asterisks.

important PC as variables, PCA-LDA and a CDA (data not shown). The results were very similar but with less capacity of discrimination between conflicting groups (Recombinant group and SN group).

We also conducted the same analyses using genome size data obtained by flow cytometry as a clustering criterion. Although only four allopolyploid individuals were found, we obtained biometric data of all, from field-collected plants and cultivated plants. The morphological characters CW and NGC showed significant *p*-values between 0.01 and 0.05 after being corrected with Benjamini-Hochberg test, and LL, LLW, LW, ENL, NW presented significant *p*-value <0.01 from field-collected plants (Table 5). From cultivated plants only ENL, ENL/LL and NC showed significant *p*-values. Although the Wilcoxon test showed these differences when we performed the LDA, the allopolyploid recombinants were mixed with the haploid recombinants, hindering their signaling in the graphs (data not shown). We obtained four LD from field-collected plants and, only LD3 (15.03%) showed the allopolyploid recombinants in an intermediate zone between the group of SN

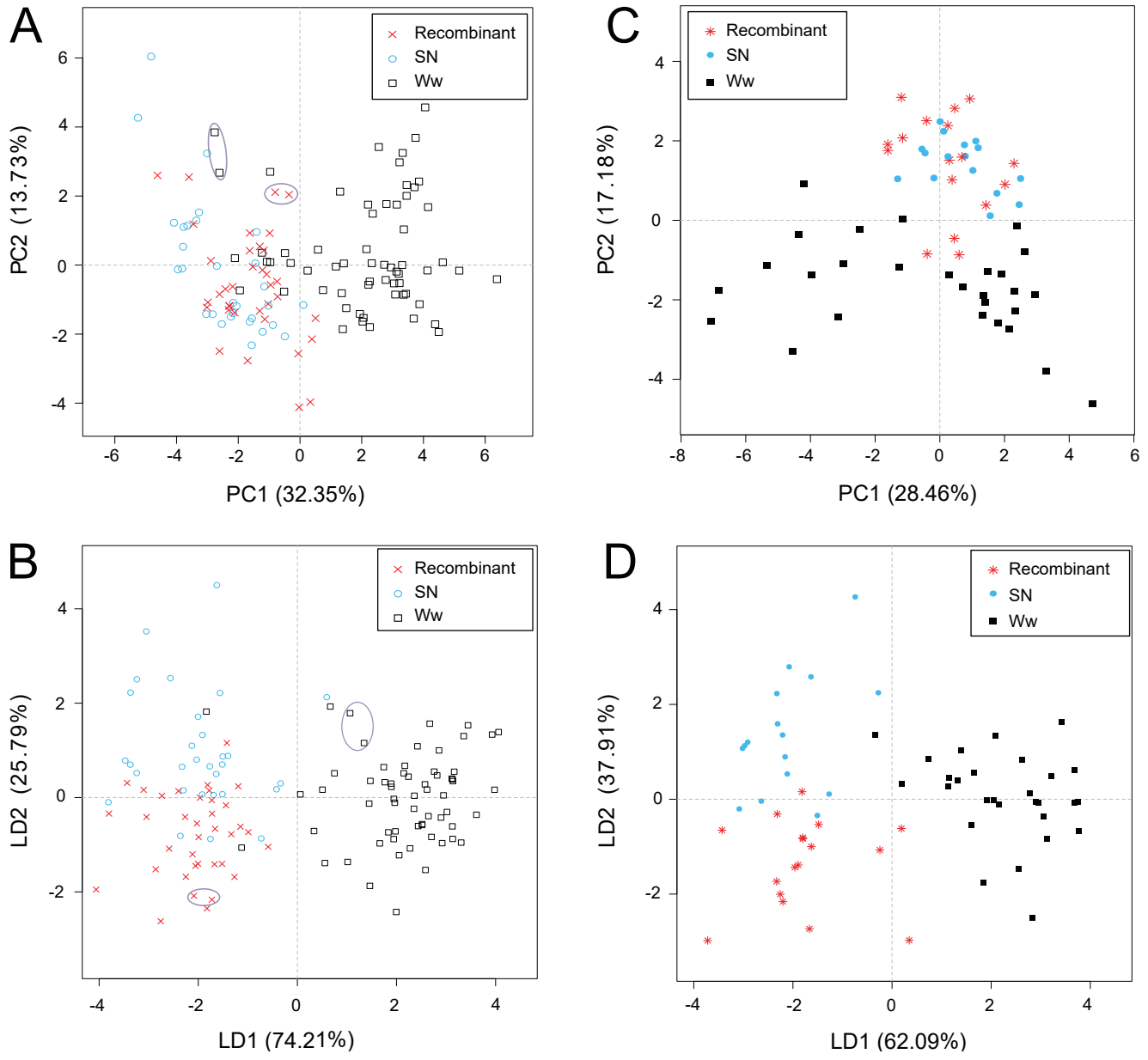

Fig. 4. Principal component analysis (PCA) and linear discriminant analysis (LDA) plots for *Ceratodon* samples showing separation of morphotypes. Grouping of samples according to Nieto-Lugilde & al. (2018a): SN = Sierra Nevada group samples, Ww = Worldwide group samples, Recombinant = Recombinant group samples. The grouped samples from Ww group correspond with *C. heterophyllus* sample and the two grouped recombinants are the type of *C. conicus*. **A,** PCA plot analyzing only field-collected samples; **B,** LDA plot analyzing only field-collected samples; **C,** PCA plot analyzing only *in vitro* cultivated samples; **D,** LDA plot analyzing only *in vitro* cultivated samples.

(haploid) and the haploid recombinants, but without any clear separation. The CDA showed 37.50% of the correctness of classification for the allopolyploid recombinants, the SN group and haploid recombinants were 56.66% and 57.69% respectively, and the Ww group was 88.70%. On other hand, the LDA from cultivated plants showed three LD, but any of them showed clear separation between allopolyploids and haploids (both SN group or Recombinant group). Finally the CDA highlighted similar results for allopolyploid recombinants, SN group and Ww group (37.50%, 56.25% and 85.71%, respectively), but haploid recombinants presented minor correctness of classification (22.22%).

■ DISCUSSION

A central challenge in taxonomy is to identify morphological variants that distinguish separate species but remain homogeneous among groups of interbreeding populations. This is particularly difficult in widespread species with distributions that span multiple environmental gradients. Our most important finding is that a higher proportion of morphological characters in *Ceratodon* varies stronger due to environmental factors than to genetic factors compared to vice versa. This was observed for the three cytotypes and for the genetic groups found previously (SN group, Ww group, Recombinant group).

Table 5. Statistical results of qualitative and quantitative characters of recombinants samples included in the biometric study.

Characters studied	Field-collected plants		Cultivated plants	
	Haploids (N = 9)	Diploids (N = 4)	Haploids (N = 3)	Diploids (N = 4)
SR	77.77% Acidic	100.00% Acidic		
PP	94.44% Straight	100.00% Straight	100.00% Curved	100.00% Curved
CT	61.11% Present	62.50% Present	100.00% Absent	100.00% Absent
CL	5.58 ± 2.43 [2.94–10.01]	4.16 ± 1.70 [2.26–6.92]	2.00 ± 0.42 [1.52–2.54]	2.36 ± 0.55 [1.60–2.94]
LL	**781.50 ± 210.22 [400.00–1350.00]**	**581.00 ± 121.69 [354.60–830.00]**	514.50 ± 127.19 [322.40–800.00]	569.40 ± 135.96 [370.80–846.30]
LLW	**621.30 ± 210.90 [270.00–1200.00]**	**457.50 ± 112.41 [241.80–700.00]**	395.40 ± 111.32 [241.80–700.00]	444.70 ± 123.19 [225.70–685.10]
LW	**401.90 ± 94.00 [230.00–710.00]**	**321.90 ± 86.36 [193.40–510.00]**	215.50 ± 34.07 [137.00–270.00]*	247.00 ± 55.07 [153.10–346.60]*
AT	100% Absent	100% Absent	70.00% Absent: 30.00% Dentate	55.00% Absent: 45.00% Dentate
PM	98.88% (2) 1.11% (0)	95.00% (0) 2.50% (1) 2.50% (2)	46.67% (2) 40.00% (0) 13.33% (1)	40.00% (0) 32.50% (2) 27.50% (1)
PA	94.44% Acute	95.00% Acute	100.00% Acute	100.00% Acute
EN	92.22% Present	95.00% Present	50.00% Present	75.00% Present
ENL	**185.50 ± 106.16 [40.00–600.00]**	**121.10 ± 65.13 [40.00–310.00]**	**107.48 ± 57.72 [16.12–225.68]**	**48.09 ± 26.64 [24.18–128.96]**
NW	**69.30 ± 16.05 [25.00–135.00]**	**55.99 ± 15.70 [30.00–85.00]**	46.27 ± 13.70 [25.00–74.00]	52.85 ± 11.83 [30.00–90.00]
NC	86.66% Greenish	80.00% Greenish	**53.33% Reddish**	**87.50% Greenish**
CS	98.88% Quadrate	100.00% Quadrate	100.00% Quadrate	100.00% Quadrate
LL/LLW	1.29 ± 0.18 [1.00–2.00]	1.29 ± 0.12 [1.10–1.57]	1.32 ± 0.14 [1.09–1.67]	1.30 ± 0.13 [1.07–1.66]
LL/LW	1.96 ± 0.40 [1.25–2.93]	1.88 ± 0.49 [1.34–3.18]	2.41 ± 0.58 [1.55–4.00]	2.36 ± 0.60 [1.39–3.86]
LL/NW	11.51 ± 2.76 [5.33–21.25]	10.77 ± 2.15 [6.26–15.52]	11.98 ± 4.32 [6.33–22.00]	11.05 ± 2.54 [6.24–15.58]
ENL/LL	0.24 ± 0.13 [0.05–0.60]	0.20 ± 0.09 [0.05–0.54]	**0.22 ± 0.13 [0.03–0.46]**	**0.09 ± 0.04 [0.04–0.17]**
NW/LW	0.17 ± 0.03 [0.10–0.28]	0.17 ± 0.03 [0.12–0.26]	0.22 ± 0.06 [0.10–0.35]	0.22 ± 0.05 [0.11–0.32]
CW	**7.88 ± 1.94 [4.00–12.50]***	**8.84 ± 1.67 [5.00–12.50]***	9.54 ± 2.37 [6.00–15.00]	9.62 ± 2.69 [6.00–16.00]
CWT	74.07% ≤2	70.83% ≤2	100.00% ≤2	100.00% ≤2
NGC	**1.519 ± 1.37 [0.00–5.00]***	**0.50 ± 0.88 [0.00–2.00]***	0.00 ± 0.00 [0.00–0.00]	0.00 ± 0.00 [0.00–0.00]

Character abbreviations follow those given in Table 1.
The field-collected plants and *in vitro* cultivated plants were subdivided according to the cytotypes obtained from flow cytometry analyses (Nieto-Lugilde & al., 2018a).
Number (N) of specimens examined for each group is given. Descriptive statistics (mean ± SD [range]) for quantitative characters are presented. All measurements are given in µm, except for CL in mm.
Significant p-values <0.01 are written in bold after being corrected with Benjamini-Hochberg test; * means $0.01 < p\text{-value} < 0.05$.

Furthermore, the genetic factor has a very important role, too. We found that in spite of the great variability in phyllidia and sporophytes within the Ww group of *C. purpureus*, as well as the demonstrable importance of environmentally induced variation in some characters, other morphological traits allowed us to clearly distinguish the Ww group from the SN group and Recombinant group. Similarly, in agreement with Burley & Pritchard (1990), we could not separate *C. heterophyllus* (CBFS 6162 sample) from members of the Ww group of *C. purpureus* by morphological analysis of gametophytic characters, nor could we morphologically differentiate (with gametophytic characters) the African samples (BOL 46302, BOL 46303) from the Ww group (although these samples presented sporophytes typical of *C. purpureus* subsp. *stenocarpus*). Together these data highlight the complexity of the morphological variation within the Ww group of *C. purpureus*.

The fact that some morphological characters were influenced by environmental factors is shown in the SN and Ww groups by the gametophytic characters PP, CT, CL, AT, PM, PA, ENL, CS, LL/LLW and NW/LW in field-collected plants, which were clearly different, but these differences disappeared when comparison was made in culture plants. This is not surprising because the field-collected plants studied here grew in locations with different climates (mainly from Mediterranean mountains, but also from Alpine mountains and Atlantic lowlands). Nevertheless, these data suggest that such characters should be used with caution when delimiting species based on morphology.

However, some characters differing between species appear to be regulated by genetic factors independent of the environment, such as NW, LL/LW and ENL/LL because they vary the same way both in field and in cultures. An additional factor that cannot be discounted is that variation observed under controlled growth conditions would not be expressed in the field, due to G × E. Our data show a high proportion of characters clearly influenced by this interaction. Some studies performed a reciprocal transplant experiment (Såstad, 1999; Såstad & al., 1999; Hassel & al., 2005; Yousefi & al., 2017) to advise on the adaptive capacity of morphological characters. Ecological gradients have been studied extensively in *Sphagnum* L. species from the same natural area, where variations in pH or water level are easily observable (Såstad, 1999; Såstad & al., 1999). In the case of *Ceratodon*, these key ecological gradients have not yet been detected, but the genus seems to have a widespread ecological distribution. We should caution that relying exclusively on cultivated plants could lead to an under-estimation of the range of variation that could be displayed along an environmental gradient (Såstad, 1999). Whereas characters that do not differ much between field-collected plants and cultivated plants may be useful for identification, characters that are expressed in the specific natural habitats may be even more indicative (shown by the fact that the field-collected plants were more frequently placed in the correct genotype group by the CDA). Such diagnostic characters may not be expressed under axenic culture (Anderson & al., 1992).

These data show that plants of the Ww group can be distinguished morphologically from the SN group and Recombinant group samples using careful, multivariate biometrical study of caulidia and phyllidia, although the differences are quantitative rather than qualitative: longer caulidia without a comal tuft, phyllidia straight or curved when moist, with margins sometimes dentate, acute apex, wider nerve at base of lamina, excurrent nerve length/lamina length ratio smaller, and nerve width/lamina width ratio bigger are characteristic of *C. purpureus*. On the contrary, plants of the SN group and Recombinant group samples often present shorter caulidia, sometimes with a comal tuft, phyllidia straight when moist, with margins mostly entire, apex generally acute but sometimes obtuse, narrower nerve at base of lamina, excurrent nerve length/lamina length ratio bigger, and nerve width/lamina width ratio smaller. The Ww and SN groups are also clearly distinguishable by flow cytometry (Nieto-Lugilde & al., 2018a), as the Ww group had a DNA mean content of 0.37 pg versus 0.46 pg in the SN group.

Based on our morphological results in field-collected samples it was not possible to distinguish clearly between the SN group and Recombinant group. The recombinants did manifest a higher percentage of phyllidia showing excurrent nerves, and the average length of the awn was greater than in the pure SN genotype, although these differences were based on statistical tests, the distributions between the species are broadly overlapping. Depending upon the genetic architecture of the group differences, recombinants may be morphologically intermediate between the two parental species or virtually indistinguishable from one of the parental species. The few available studies reveal that the viable recombinant progeny tend to resemble one of the parental species (bibliographic review in bryophytes by Natcheva & Cronberg, 2004), probably because incompatibility between the genomes makes complete mixtures unviable (Cronberg & Natcheva, 2002; Natcheva & Cronberg, 2007, McDaniel & al., 2007, 2008). We also observed that the Recombinant group had two different cytotypes (Nieto-Lugilde & al., 2018a), one the size of the SN species (0.46 pg) and another the sum of the genome sizes of the SN species and *C. purpureus* (0.82 pg). We observed no clear morphological differences between haploid recombinants and allopolyploids, although we cannot rule out that this is due to the low number (four) of putative allopolyploids studied. However, observable morphological differences are rarely recorded between haploids and diploids of bryophyte taxa in nature (Anderson, 1980; Uniyal, 1998; Ricca & al., 2008).

It is possible that the lack of males in our sample of SN individuals could elevate the morphological and genetic differences between the Ww and SN groups. Since *C. purpureus* is strongly sexually dimorphic (Shaw & Beer, 1999; McDaniel, 2005; Slate & al., 2017), the trait distributions for the SN group could be narrower and shifted toward the female value. While different sex ratios in the samples from the SN and Ww groups may play a role, we do not believe that sexual dimorphism can explain the strong differences that we found between these groups for two main reasons. First, we could not morphologically distinguish between the SN group, in which we detected no males nor any signs of sexual reproduction, and recombinants, which has sporophytes and genetic signatures of recombination, indicating that males must be present. Thus, the recombinant

males are likely to be similar to the SN females. Additionally, in the Ww group the male and female trait distributions are statistically different but broadly overlapping (Shaw & Beer, 1999; McDaniel, 2005). Slate & al. (2017) observed higher levels of variation among females within and among populations than among males. Therefore, we conclude that the morphological gaps observed between species in our data are greater than those that may exist between males and females within the same species.

These morphological analyses, combined with DNA sequence and genome size data, indicate that two clearly separated lineages exist in the studied samples of *Ceratodon*. Moreover, we find evidence for hybridization between them. One lineage was only found in southern Spanish mountains, mainly in Sierra Nevada. The other one has a worldwide distribution. The SN genotype is very abundant locally, but seems to decline rapidly in frequency northwards in the Iberian Peninsula, as it has not been found in Spanish Sistema Central or even in sites with similar Mediterranean climatic conditions, like the Sicilian Mount Etna, which is situated at almost the same latitude and has a very similar altitude as Sierra Nevada. We think that the potential distribution of the SN genotype in southern Spain is broader than presently known, as many other recorded samples from neighboring areas have been previously reported as *C. purpureus* or *C. conicus* (Martínez Sánchez & al., 1991; García-Zamora & al., 1998; Cano & al., 2010; Rams & al., 2014), but show the morphology of SN and Recombinant group samples, as observed after revision of MUB and GDA/GDAC herbaria samples. Outside southern Spanish mountains, only the Ww genotype and recombinants were found. Therefore, if the SN genotype is present outside southeastern Spain, it is probably rare and seems to be less frequent than recombinants, at least in central Europe where the type of *C. conicus* comes from.

Our morphological multivariate analyses situated the lectotype of *C. conicus* together with the recombinant samples including two samples identified as *C. conicus* from United Kingdom lowlands (MUB 52185, MUB 52186), where *C. conicus* has been found several times but is not common (Smith, 2004; Porley, 2013; Martin, 2014). We must highlight that *C. conicus* has never been found again in the type locality (Flegessen, Lower Saxony, Germany) (Nieto-Lugilde & al., 2018b), despite the efforts of many bryologists (Meinunger & Schröder, 2007). Moreover, we identified a sample from the Alps (MUB 49604) that was a recombinant based on sequence data, with a genome size equivalent to the SN pure genotype (Nieto-Lugilde & al., 2018a), and sporophytic and gametophytic characters similar to the lectotype of *Ceratodon conicus* (GOET011795). The samples identified as *C. conicus* from Europe that we have sequenced are also clearly morphologically similar to the recombinants. The broad distribution of recombinants across Europe suggests that they originate not simply through regional introgression where the two species are in contact. The genotype of the type of *C. conicus* is unknown. Therefore, there will always be a doubt related to its genetic composition. *Ceratodon conicus* is scarce outside of Spain and we suspect that we have not fully examined the variability of the species. Nevertheless, although a higher number of samples with morphology similar to *C. conicus* from outside southeastern Spain would have been desirable, the basic outline of our results seems to be reliable.

The most parsimonious conclusion from these data is that there are three entities: the pure SN genotype represents one species, the Ww genotype corresponds to another species, and a swarm of recombinants (hybrids) exists. There is no doubt that the Ww genotype is *C. purpureus*. Whether the type of *C. conicus* is a recombinant or a pure SN type is not known at this moment, but the genetic, biogeographic, and reproductive data strongly favor the recombinant status. Therefore, we formally propose that *C. conicus* is a nothospecies, and the parents are the cosmopolitan *C. purpureus* and the SN species. The latter represents a new species of the genus *Ceratodon*, here described, for which we propose the name *Ceratodon amazonum*. We consider that recombinants and the new genotype must not be considered the same species, as the *ICN* (Turland & al., 2018) allows describing nothospecies based on their morphology. There are some cases of moss species described as hybrids (Williams, 1966; Anderson & Lemmon, 1972; Guerra & al., 1994; Ros & al., 1994; Werner & al., 2014). In our case, the nothospecies (*C. conicus*) and one parent (*C. purpureus*) were first described, and later we have discovered the other parent. Therefore, we consider that the temporal sequence in the description of species or nothospecies should not be taken as an argument for not giving a name already described to a hybrid. We cannot exclude the possibility that some of the samples identified as pure SN based on our genetic data are indeed recombinants that lack the Ww-type allele at the marker genes we employed in our genetic analysis. In contrast to vascular plants, where the F1 generation of hybrids is long-lived, bryophyte F1 sporophytes are quite ephemeral, meaning that the persistent recombinant gametophytes produced from F1 meiosis will not be heterozygous across the genome but rather will show different patterns of interspecies haplotypes. However, it is well known from other studies that the assignment of individuals to pure species or hybrid lines is not always perfect, especially in homoploid hybrids where backcrosses are possible (Nielsen & al., 2006; Gramlich & al., 2016).

In Sierra Nevada metamorphic and carbonate rocks predominate and soils are very diverse, some of them having acidic and other basic characteristics (Molero Mesa & al., 1992). Most of our Sierra Nevada samples were observed to grow on acid substrates, although some samples were found on basic soils. Burley & Pritchard (1990) and many Floras (e.g., Smith, 2004; Frey & al., 2006) described *C. conicus* as strictly calcicolous. Perhaps for this reason previous authors identified samples collected in Sierra Nevada as xeric forms of *C. purpureus* and discarded the presence of *C. conicus* in this area (Höhnel, 1895; Rams & al., 2014; Brugués & Ruiz, 2015). Unfortunately, Burley & Pritchard (1990) did not describe the methodology employed to elucidate the type of substrate on which the 18 representative specimens they studied grew (from Canada, England, Norway, Scotland and the United States). It cannot be discarded that the substrate of these specimens and maybe others (probably excluded because they do not present sporophytes) would have given an acid reaction according to the

method applied by us. The recombinant samples found outside Sierra Nevada grew mainly on basic substrate, except MUB 49568 from Spanish Sistema Central. Our representatives of *C. conicus* are limited as we have previously stated, but our results indicate that both recombinants and *C. amazonum* sp. nov. can be found on basic and acid substrates. The type of substrate does not seem to be a limiting factor in the distribution of *Ceratodon* species here studied.

■ TAXONOMIC CONCLUSIONS

Species description

Ceratodon amazonum Nieto-Lugilde, O.Werner, S.F.McDaniel & Ros, **sp. nov.** – Holotype: SPAIN, Granada Province, Sierra Nevada, ascent to El Dornajo by the road A4025, km 3, 37°07′46.7″ N, 03°25′34.9″ W, bare soil between spiny pillow bushes, 1850 m a.s.l. (sample 6/16), 21 Jul 2012, *R.M. Ros & O. Werner s.n.* (MUB No. 49413; isotype: FLAS barcode FLAS B66910).

See Fig. 5 for images of the holotype and other specimens.

Specific diagnosis. – Plants in compact turfs. Caulidia 2.05–6.76(9.45) mm high. Phyllidia sometimes forming a comal tuft, phyllidia of the middle part of caulidia plane or concave, ovate or longly ovate, rarely lanceolate, (0.35)0.43–0.76(1.15) × (0.20)0.30–0.46(0.56) mm; nerves (25.0)46.8–86.7(114.0) µm wide at base of lamina, generally excurrent in a smooth awn, (40.0)60.0–198.0(322.0) µm long, excurrent nerve length/lamina length ratio between (0.05)0.10–0.34(0.74), rarely percurrent, lamina length/nerve width ratio (5.4)7.0–12.0(18.0); middle phyllidia cross-section crescent-shaped, with 0–3 guide cells. Propagules absent. Gametangia and sporophyte unknown. It differs from *C. purpureus* by having shorter caulidia, comal tuft often present, phyllidia of the middle part of caulidia mostly ovate or longly ovate, nerve narrower at base of lamina, and generally excurrent in a relatively long awn.

Description. – Plants in compact turfs, usually yellow-green or yellowish. *Caulidia* 2.05–6.76(9.45) mm, often branched, 110–160 µm diameter in cross-section, with central strand 15–25 µm wide, central cylinder and cortex with 1(2) layers of cells sometimes differentiated as sclerodermis. *Rhizoids* orange, smooth or slightly papillose. *Phyllidia* straight to slightly twisted when dry, sometimes forming a comal tuft, erecto-patent to spreading when wet, phyllidia of the middle part of caulidia plane or concave, ovate or longly ovate, rarely lanceolate, (0.35)0.43–0.76(1.15) × (0.20)0.30–0.46(0.56) mm; margins recurved to near apex, rarely plane, usually entire; apices mostly acute, rarely obtuse; *nerves* mostly greenish, rarely reddish, (25.0)46.8–86.7(114.0) µm wide at base of lamina, generally excurrent in a smooth awn, (40.0)60.0–198.0(322.0) µm long, excurrent nerve length/lamina length ratio (0.05)0.10–0.34(0.74), rarely percurrent, lamina length/nerve width ratio (5.4)7.0–12.0(18.0), superficial cells elongate ventrally, rectangular dorsally; middle phyllidia cross-section crescent-shaped, with 0–3 guide cells, stereid dorsal band well developed, ventral stereid band absent, and ventral epidermal cells well developed, often so big as the guide cells; *middle laminal cells* quadrate, rarely rounded, smooth, (4.0)6.2–10.2(15.0) µm wide, cell walls rarely thicker than 2 µm. Propagules absent. Gametangia and sporophyte unknown.

Distribution. – Southern Spain: Sierra Nevada (Andalusia) and mountains in the northwest of the Region of Murcia.

Habitat. – Acid or basic soils, very frequently without plant cover in open areas, sometimes in more developed soils and somehow protected under herbaceous plants or small shrubs, at 1290–2870 m a.s.l. altitude.

Etymology. – The specific epithet refers to the Amazons, a name given in Greek mythology to a tribe formed and governed entirely by women warriors, given the absence of males in the present known populations of the species.

Paratypes. – SPAIN, Murcia Province, mountains in the NW, El Sabinar, 38°13′41.2″ N, 02°02′46.6″ W, 1290 m a.s.l., 13 Nov 2011, *R.M. Ros & O. Werner s.n.* (MUB No. 43730). Granada Province, Sierra Nevada, ski resort "Borreguiles", 37°04′21.3″ N, 03°23′06.5″ W, 2690 m a.s.l., 20 Jul 2012, *R.M. Ros & O. Werner s.n.* (MUB No. 49306). *Ibidem*, down to Barranco de San Juan, 37°05′12.4″ N, 03°22′44.4″ W, 2540 m a.s.l., 20 Jul 2012, *R.M. Ros & O. Werner s.n.* (MUB Nos. 49327 & 49329). *Ibidem*, Hoya de la Mora, 37°05′36.7″ N, 03°23′11.3″ W, 2510 m a.s.l., 20 Jul 2012, *R.M. Ros & O. Werner s.n.* (MUB No. 49342). *Ibidem*, "Albergue militar de montaña General Oñate", 37°06′47.9″ N, 03°25′10.0″ W, 2205 m a.s.l., 20 Jul 2012, *R.M. Ros & O. Werner s.n.* (MUB No. 49356). *Ibidem*, "Centro de visitantes El Dornajo", 37°07′57.9″ N, 03°26′06.2″ W, 1667 m a.s.l., 21 Jul 2012, *R.M. Ros & O. Werner s.n.* (MUB Nos. 49366 & 49370). *Ibidem*, road GR-3200 near to "Casas Rurales Telecabina Las Catifas", 37°08′24.8″ N, 03°25′34.4″ W, 1539 m a.s.l., 21 Jul 2012, *R.M. Ros & O. Werner s.n.* (MUB No. 49382). *Ibidem*, road GR-3200, 1 km after the junction with A4025 going from "Centro de visitantes El Dornajo", 37°08′44.8″ N, 03°25′30.2″ W, 1431 m a.s.l., 21 Jul 2012, *R.M. Ros & O. Werner s.n.* (MUB No. 49399). *Ibidem*, ascent to El Dornajo by the road A4025, km 3, 37°07′46.7″ N, 03°25′34.9″ W, 1850 m a.s.l., 21 Jul 2012, *R.M. Ros & O. Werner s.n.* (MUB No. 49408). *Ibidem*, ascent to El Dornajo by the road A4025, km 5, 37°07′09.1″ N, 03°26′23.4″ W, 2020 m a.s.l., 21 Jul 2012, *R.M. Ros & O. Werner s.n.* (MUB Nos. 49426 & 49427). *Ibidem*, ascent to El Dornajo by the road A4025, km 7, 37°07′28.8″ N, 03°25′52.2″ W, 2093 m a.s.l., 21 Jul 2012, *R.M. Ros & O. Werner s.n.* (MUB No. 49442).

Key to species

In order to facilitate the distinction of *C. amazonum* from *C. purpureus* a key is presented based on morphological characteristics of field plants studied in this work. As in many individuals it is impossible to assign them to either *C. amazonum* or the nothospecies *Ceratodon* ×*conicus* based on morphological data, they are keyed together. Nevertheless, based on results of the statistical analyses of the biometric study presented here, the most easily observable differences that allow identifying samples at the extremes of the variability are indicated for each of them.

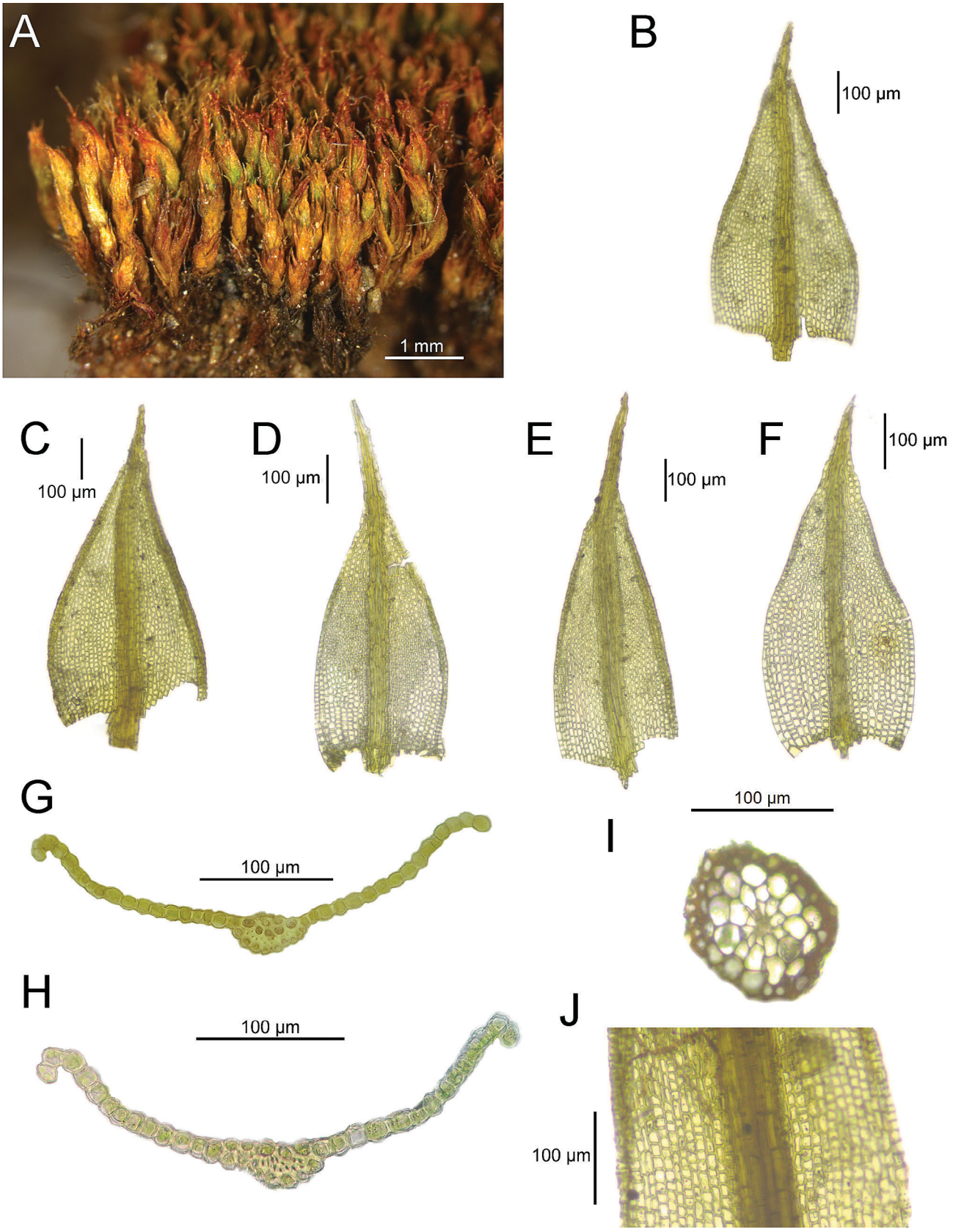

Fig. 5. *Ceratodon amazonum*. **A,** habit; **B–F,** phyllidia of the middle part of caulidium; **G–H,** middle phyllidia cross-sections; **I,** caulidium cross-section; **J,** middle laminal cells. A, D–F and J, MUB 49413 (holotype); B and C, MUB 49427; G, MUB 49306; H and I, MUB 43730.

1. Caulidia length (2.8)5.1–16.2(29.0) mm, comal tuft usually absent; phyllidia of the middle part of caulidia ovate, ovate-lanceolate, lanceolate or triangular-lanceolate, (0.4)0.8–1.5(2.6) mm long; lamina length/lamina width in widest part ratio (1.06)2.13–3.79(5.83); nerve (38.0)61.8–96.5(140.0) µm wide at base of lamina, percurrent or excurrent in a smooth awn 24–350 µm long; excurrent nerve length/lamina length ratio (0.02)0.04–0.14 (0.30); lamina length/nerve width ratio (5.8)10.5–18.5(34.0) ….. …… ***Ceratodon purpureus***
1. Caulidia length (2.0)2.5–7.1(10.0) mm, comal tuft usually present; phyllidia of the middle part of caulidia ovate or longly ovate, rarely lanceolate, 0.4–0.9(1.4) mm long; lamina length/lamina width in widest part ratio (0.85)1.26–2.33(3.18); nerve (25.0)46.8–86.7(140.0) µm wide at base of lamina, rarely percurrent, usually excurrent in a smooth awn 40–600 µm long; excurrent nerve length/lamina length ratio (0.05)0.11–0.34(0.74); lamina length/nerve width ratio (5.3)7.0–13.6(21.25) …… **2**
2. Phyllidia of the middle part of caulidia ovate or longly ovate, rarely lanceolate; lamina length/lamina width in widest part ratio (0.85)1.26–1.90 (2.78); nerve (25.0)46.8–86.7(114.0) µm wide at base of lamina, generally excurrent in a smooth awn, 40–322 µm long; lamina length/nerve width ratio (5.4)7.0–12.0(18.0) … ***Ceratodon amazonum***
2. Phyllidia of the middle part of caulidia ovate, longly ovate or ovate-lanceolate; lamina length/lamina width in widest part ratio (1.07)1.49–2.33(3.18); nerve (25.0)48.3–86.7(140.0) µm wide at base of lamina, generally excurrent in a smooth awn, 40–600 µm long; lamina length/nerve width ratio (5.3)8.3–13.6(21.25) …. ***Ceratodon ×conicus***

Typification of the name *Ceratodon ×conicus*

Ceratodon ×conicus (Hampe ex Müll.Hal.) Lindb. (*Ceratodon amazonum* × *Ceratodon purpureus*), Musc. Scand.: 27. 1879, non *Ceratodon conicus* (Lindb.) Müll.Hal. in Hedwigia 38: 98. 1899, nom. illeg. ≡ *Trichostomum conicum* Hampe ex Müll.Hal., Syn. Musc. Frond. 1: 575. 1849 ≡ *Ceratodon purpureus* var. *conicus* (Hampe ex Müll.Hal.) Husn., Muscol. Gall.: 60. 1884 ≡ *Ceratodon purpureus* subsp. *conicus* (Hampe ex Müll.Hal.) Dixon, Stud. Handb. Brit. Mosses: 68. 1896 – Lectotype (designated by Burley & Pritchard in Harvard Pap. Bot. 2: 60. 1990): [Germany, Niedersachsen] Auf Mauern bei Flegesen … [unreadable] Hameln bei Hohnsen, Mai coll. *Schlotheuber* pastor eccl. *784*, Hampe misit 15/2 48, C. Müller det. (GOET barcode GOET011795!). **Epitype (designated here):** ITALY, Südtirol, Stilfser Joch, 46°31′43.7″ N, 10°27′09.7″ E, on accumulated earth at the base of an artificial wall in anthropized area, frequently covered by snow, 2763 m a.s.l., 14 Sep 2012, *R.M. Ros & O. Werner s.n.* (MUB No. 49604!).

More data on the taxonomy and nomenclature of this species are given in Nieto-Lugilde & al. (2018b).

It was considered necessary to select an epitype of *Ceratodon ×conicus* to serve as an interpretative type because the lectotype is demonstrably ambiguous with regards to its genotype and amount of nuclear DNA and cannot be critically identified for purposes of the precise application of the name to the nothospecies (Art. 9.9 of *ICN*, Turland & al., 2018). The MUB 49604 sample was designated as epitype as it presents similar morphology and diagnostic characteristics (gametophytic and sporophytic) to those showed by the lectotype of *C. ×conicus*, it was sequenced showing a recombinant genotype and its DNA amount was measured, being 0.48 pg.

■ AUTHOR CONTRIBUTIONS

OW and RMR conceived the experimental strategy, collected the plant material and supervised the work. MN-L generated *in vitro* cultures and performed the biometrical study. MN-L and OW analyzed data. RMR and MN-L elaborated the taxonomic and nomenclatural part. MN-L wrote the paper. SFM aided in interpreting the results. All authors discussed the results and commented on the manuscript.

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Appendix 1. Voucher information of the specimens studied.

For each sample, information is given as follows: herbarium code; geographic origin; MC letters in the case that *in vitro* cultivated plants were included in the biometric study; genomic group based on phylogenetic study of Nieto-Lugilde & al. (2018a); genome size determined by flow cytometry technology if known (H for haploid sample, A for allopolyploid sample) from Nieto-Lugilde & al. (2018a); sex if known (F = female, M = male) from Nieto-Lugilde & al. (2018a); presence of sporophyte if appropriate (indicated by an asterisk). For the type specimen, also collection data are given.

MEDITERRANEAN MOUNTAIN AREAS: MUB 43730: Spanish southeastern mountains (eastern Sierra del Segura), SN group, H, F. **MUB 49306:** Spanish Sierra Nevada (hereafter Sierra Nevada), SN group, F. **MUB 49323:** Sierra Nevada, MC, Recombinant group, H, F. **MUB 49327:** Sierra Nevada, SN group, F. **MUB 49329:** Sierra Nevada, MC, SN group, H, F. **MUB 49339:** Sierra Nevada, MC, Recombinant group, A, F. **MUB 49342:** Sierra Nevada, MC, SN group, H, F. **MUB 49356:** Sierra Nevada, SN group, H. **MUB 49366:** Sierra Nevada, MC, SN group, H, F. **MUB 49370:** Sierra Nevada, SN group, H. **MUB 49382:** Sierra Nevada, SN group, H, F. **MUB 49399:** Sierra Nevada, MC, SN group, H, F. **MUB 49408:** Sierra Nevada, MC, SN group, H, F. **MUB 49413:** Sierra Nevada, MC, SN group, H, F. **MUB 49426:** Sierra Nevada, MC, SN group, H, F. **MUB 49427:** Sierra Nevada, MC, SN group, H, F. **MUB 49442:** Sierra Nevada, MC, SN group, H, F. **MUB 49451:** Sierra Nevada, Recombinant group, H, F. **MUB 49461:** Sierra Nevada, MC, Recombinant group, H, F. **MUB 49471:** Sierra Nevada, MC, Ww group, H, M. **MUB 49473:** Sierra Nevada, MC, Recombinant group, A, F. **MUB 49480:** Sierra Nevada, Recombinant group, H, F. **MUB 49485:** Sierra Nevada, MC, Recombinant group, A, F. **MUB 49492:** Sierra Nevada, MC, Recombinant group, H, F. **MUB 49501:** Sierra Nevada, MC, Recombinant group, F. **MUB 49504:** Sierra Nevada, MC, Recombinant group, A, F. **MUB 49505:** Sierra Nevada, Recombinant group, F. **MUB 49518:** Sierra Nevada, Recombinant group, H, F. **MUB 49528:** Sierra Nevada, Recombinant group, H, F. **MUB 49538:** Spanish Sistema Central, MC, Ww group, H, F. **MUB 49545:** Spanish Sistema Central, Ww group, H. **MUB 49554:** Spanish Sistema Central, Ww group, H, F. **MUB 49560:** Spanish Sistema Central, MC, Ww group, H, F*. **MUB 49562:** Spanish Sistema Central, Ww group, H. **MUB 49564:** Spanish Sistema Central, Ww group, H. **MUB 49566:** Spanish Sistema Central, MC, Ww group, H, F. **MUB 49567:** Spanish Sistema Central, Ww group, F. **MUB 49568:** Spanish Sistema Central, MC, Recombinant group, F. **MUB 49569:** Spanish Sistema Central, Ww group, F. **MUB 49570:** Sicilian Mount Etna, Ww group, H, F. **MUB 49593:** Sicilian Mount Etna, Ww group, H, F. **MUB 49600:** Spanish south eastern mountains (Sierra de Alcaraz), MC, Ww group, F*. **MUB 49602:** Spanish south eastern mountains (Sierra de Alcaraz), MC, Ww group, F. — **OTHER EUROPEAN MOUNTAINOUS SYSTEMS: CBFS 6159:** Alps, Ww group. **CBFS 6162:** Alps, Ww group, F. **MUB 49604:** Alps, Recombinant group, H, F*. **MUB 49613:** Alps, MC, Ww group, H, F*. **MUB 49617:** Alps, Ww group, H, F*. **MUB 49619:** Alps, Ww group, H, M. **MUB 49624:** Pyrenees, MC, Ww group, H, F*. **MUB 49629:** Pyrenees, MC, Ww group, F. **MUB 49650:** Pyrenees, MC, Ww group, H, F*. — **LOWLANDS: BOL 46302:** South Africa, Ww group, F*. **BOL 46303:** South Africa, Ww group, F*. **GOET 011795:** Germany, *, *Trichostomum conicum* Hampe ex Müll.Hal. lectotype, *Schlotheuber 784*, 15 Feb 1848. **MUB 49652:** Germany, MC, Ww group, H, F*.**MUB 49653:** Germany, Ww group, H, F*. **MUB 49654:** Czech Republic, MC, Ww group, H, F. **MUB 49655:** Czech Republic, MC, Ww group, H, F. **MUB 49659:** Czech Republic, MC, Ww group, H, F. **MUB 52185:** United Kingdom, Recombinant group. **MUB 52186:** United Kingdom, Recombinant group, H. **S B201182:** Sweden, Ww group, F. **S B201183:** Sweden, Ww group, F.