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A morphometric and molecular study in *Tortula subulata* complex (Pottiaceae, Bryophyta)

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Specimens belonging to *Tortula subulata* complex (*T. inermis*, *T. mucronifolia* and *T. subulata*) were analysed using a combination of morphometric methods based on quantitative characters [principal component analysis (PCA), discriminant analysis (DA); 76 samples)] and molecular methods (ITS1 – 5.8S rRNA gene and ITS2; 47 samples) to assess patterns of morphological and molecular differentiation within this complex of taxa. The study shows that four species can be recognized: *T. mucronifolia*, *T. subulata*, *T. inermis* and *T. subulata* var. *angustata* with bistratose border, which is elevated to the species rank as ***T. schimperi* nom. nov.** The most valuable quantitative characters for identification of these species are the strata number of the marginal laminal cells, the ratio of middle marginal laminal cell width/middle marginal laminal cell length, basal membrane of peristome length, middle laminal cell width and papillae number on the middle laminal cells. The internal transcribed spacer (ITS) data suggest that this group of taxa is not monophyletic, *T. mucronifolia* being close to *Protobryum bryoides* (= *Tortula protobryoides*). The remaining species seem to be a monophyletic group, *T. schimperi* being the sister group of the clade composed by *T. inermis* and *T. subulata*. *T. subulata* is considered to be of high morphological variability, for which ITS sequences did not resolve the internal relationships. *Botanical Journal of the Linnean Society*, 2005, **149**, 333–350.

ADDITIONAL KEYWORDS: bryophytes – ITS sequence – molecular systematics – taxonomy.

INTRODUCTION

The genus *Tortula* Hedw. represents one of the most complex and diverse genera in terms of morphological variation in the family Pottiaceae. Its taxonomic circumscriptions have been controversial during the last two centuries, and there has been no consensus about which species or even other genera should be included in it. Zander (1989, 1993), in his new classification of the genera of Pottiaceae, recognized genera such as *Chenia* R. H. Zander, *Dolotortula* R. H. Zander, *Hennediella* Paris, *Hilpertia* R. H. Zander, *Sagenotortula* R. H. Zander, *Stonea* R. H. Zander or *Syntrichia* Brid. as segregates of *Tortula* and included taxa that traditionally were placed in other genera such as some species of *Pottia* Ehrh. ex Fürnr., *Phascum* Hedw. (e.g. *Phascum cuspidatum* Hedw.) and the genus *Desmatodon* Brid. With the exception of the treatment of Brotherus (1924), the genus *Tortula* has not been monographed or critically revised for any wide region; the only floristic treatments are included in a diverse range of flora. Outstanding treatments are those of Steere (1937) for the flora of North America and north Mexico, Lawton (1971) for the Pacific north-west of North America, Nyholm (1989) for Scandinavia and Finland, Mishler (1994) for Mexico, and Smith (2004) for Great Britain and Ireland. According to the treatment by Zander (1993), the genus *Tortula* includes 141 species (Crosby *et al*., 1999).

From the morphological study carried out on *Tortula* for the *Flora Briofítica Ibérica* (Cano, 2004), we identified many taxonomic problems in the species related to *Tortula subulata*. These difficulties are the result of overlapping between the characters usually used and the inability to fit some forms found to any taxa described. This assemblage of taxa does not currently have any taxonomic status, but it shares a combination of morphological characters that differentiate it from the remaining of taxa of the genus *Tortula*. These include large leaves, (2.2)2.5–5.5 mm in length,

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without hair-point (generally mucronate or apiculate) and with a large capsule with twisted peristome from a high tessellated basal membrane similar to those present in the genus *Syntrichia*. According to our previous morphological study (Cano, 2004), this complex of species could be represented by three species: *Tortula inermis* (Brid.) Mont., *Tortula mucronifolia* Schwägr. and *T. subulata*, although numerous infraspecific taxa have been described of *T. subulata*. Thus, Zander (1993) includes in *T. subulata* two subspecies, 13 varieties and three forms, although the number according to van der Wijk, Margadant & Florschütz (1969) is close to 30. In recent floral descriptions (Nyholm, 1989; Cortini-Pedrotti, 2001; Smith, 2004), only three infraspecific taxa of *Tortula subulata* are usually recognized [*T. subulata* var. *angustata* (Schimp.) Kindb., *Tortula subulata* var. *graeffii* Warnst. and *T. subulata* var. *subinermis* (Bruch & Schimp.) Wilson]. Most authors separate *T. subulata* var. *angustata* from other varieties of *T. subulata* based on its lingulate-lanceolate leaves, crenulated near the apex, acuminate, strongly developed border extending almost to the apex and upper and middle cells 12–16 μm wide, with conspicuous papillae (Cortini-Pedrotti, 2001; Smith, 2004). Only Nyholm (1989) distinguished this taxon by its bistratose leaf border, which is also drawn in Dixon (1924). *Tortula subulata* var. *subinermis* is characterized by obtuse leaf apex, mucronate, poorly developed border ceasing at about mid-leaf, and upper and middle cells 12–16 μm wide, with conspicuous papillae, and *Tortula subulata* var. *graeffii* by its acute or acuminate leaf apex, apiculate, with poorly developed border from base to upper third, and upper and middle cells 16–28 μm, with inconspicuous papillae. *T. inermis* var. *submarginata* Schiffn., a variety with laminal marginal cells and leaf shape closer to *T. inermis* but with recurvature of the leaf margin and habit of the plant when dry closer to *T. subulata*, has been recorded in some Mediterranean countries (Düll, 1992; Cano, 2004).

All of these taxa show a Holarctic distribution, with a punctuated disjunction in New Zealand (*T. mucronifolia*).

Historically, the *Tortula subulata* complex has been included in different genera and suprageneric taxa. Some authors have included this complex with the species presently recognized in the genus *Syntrichia*, e.g. Schimper (1876) [*Barbula* subgen. *Syntrichia* (Brid.) Schimp., *Barbula* sect. *Subulatae* Bruch & Schimp.], or Mönkemeyer (1927) [*Syntrichia* sect. *Zygotrichia* (Brid.) Mönk.]. Others have included it in the genus *Tortula*, e.g. Brotherus (1924) [*Tortula* sect. *Zygotrichia* (Brid.) Mitt.] or more recently Corley *et al.* (1981) [*Tortula* sect. *Tortula* Broth.]. Warnstorf (1912) is the only author to have monographed part of this species group (he did not include *Tortula inermis*), in which he tried to classify all the variation observed. Consequently, he described new species, and accepted a great number of varieties and forms besides those previously described, recognizing three sections, namely section *Vulgatae* (including *T. subulata* with six varieties and one form plus *T. serrulata* Warnst., nom. illeg. = *T. crenulata* Warnst., with two varieties and two forms), section *Intermediae* (including *Tortula graeffii* with two varieties and one form plus *Tortula bürgeneri* Loeske) and sect. *Levifoliae* (which included *T. mucronifolia* with three varieties and six forms).

The current infrageneric classification of Zander (1993) of this complex of species is also rather confusing, because *Tortula inermis* is classified in the genus *Syntrichia* [*S. inermis* (Brid.) Bruch], *Tortula subulata* is included in *Tortula* sect. *Tortula*, and *T. mucronifolia* is included in *Tortula* sect. *Pottia* (Rchb.) Kindb., which Ochyra, Zarnowiec & Bednarek-Ochyra (2003) renamed as *Tortula* sect. *Cuneifoliae* (Schimp.) Ochyra, because *Pottia* does not seem to be the oldest available name for this group, *Barbula* sect. *Cuneifoliae* Schimp. being the priority.

Tortula inermis, placed in the genus *Syntrichia* by Zander (1993), was considered in *Tortula* by Gallego (2002) and Cano & Gallego (2003) after the study of the type material and numerous specimens from the Mediterranean basin. This was based on possession of a differentiated dorsal costal epidermis, a semicircular shape in the ventral stereid band and a yellow to orange KOH reaction of the upper laminal cells. In contrast, *Syntrichia* shows an undifferentiated dorsal costal epidermis, a lunate ventral stereid band and red KOH reaction. In addition, a molecular study, based on chloroplast gene *rps4* and the *rps4–trnS* spacer, showed the inclusion of *T. inermis* in the genus *Tortula*, and that *T. subulata* and *T. inermis* form a well-supported sister clade to the remaining *Tortula* species included in that study (Werner *et al.*, 2003).

The objectives of this paper are to evaluate (1) the best quantitative taxonomic characters, (2) the recognizable species within this group based both on quantitative and on qualitative characters, and (3) monophyly and proper circumscription of this group of species and the internal relationship between them based on internal transcribed spacer (ITS) sequences.

MATERIAL AND METHODS

Morphometric analysis

A total of 76 herbarium specimens (Appendix) were studied to span the morphological variability and geographical range of this complex of taxa as follows: *T. inermis* (11 specimens), *T. inermis* var. *submarginata* (9), *T. mucronifolia* (16), *T. subulata* (40) including

specimens below var. *subulata* (12), var. *graeffii* (7), var. *subinermis* (4) and var. *angustata* (17). Where possible, type specimens were included. We performed analyses using only quantitative characters. Preference was given to those which Smith (2004) and Cano (2004) considered important for taxa distinction. Further, potentially useful quantitative characters were also investigated. We also studied some qualitative characters which were used for identification of the species, but they were not used in statistical analyses. They included habit of the plant when dry, leaf shape, curvature of the leaf margins, leaf apex shape, shape and papillosity in laminal cells, and shape of marginal cells of the leaf.

The data matrix contained 76 specimens × 55 characters (available upon request). Different analyses were carried out on the matrix. The range of each character was compared for all species with box-plots. A principal components analysis (PCA) based on the correlation matrix and using individual plants as operational units (OTUs) was performed. This method was used to detect primary patterns among all the OTUs, because this method requires no a priori knowledge of the origins of the OTUs and will reveal any groups. The most discriminatory descriptors were inferred from the box-plots. Eight of them are quantitative continuous, two are ratios and two are quantitative discrete. When descriptors are ratios the use of any correlated descriptor was avoided. To identify the existence of different specimen groups a discriminant analysis (DA) was conducted. It is the best statistical method to distinguish subgroups within a pool of OTUs if an a priori classification is possible. The same discriminatory descriptors were used, except for the quantitative discrete characters which were excluded. The morphometric study was conducted using the STATISTICA 6.0 package (http://www.statsoft.com).

Molecular analysis

Plant material

A total of 47 accessions were used from the material included in the morphometric study of the *Tortula subulata* complex. These represent the eight subgroups previously observed and as outgroup six accessions (six species). The outgroup species were selected from previous results in *Tortula* using chloroplast *rps4* gene sequences (Werner *et al*., 2002a, 2003, 2004). These include *Leptophascum leptophyllum* J. Guerra & M.J. Cano, *Desmatodon latifolius* (Hedw.) Brid. [*Tortula hoppeana* (Schultz) Ochyra], *T. canescens* Mont., *T. cuneifolia* (Dicks.) Turner, *Tortula muralis* Hedw. and *Protobryum bryoides* (Dicks.) J. Guerra & M.J. Cano [*Pottia bryoides* (Dick.) Mitt.; *Tortula protobryoides* R.H. Zander]. Details concerning voucher data and GenBank accession numbers are given in the Appendix.

DNA extraction

Total DNA was extracted by the NaOH method (Werner, Ros & Guerra, 2002b), in which 5 μL of the crude NaOH extract was diluted by the addition of 45 μL of 100 mM Tris –1 mM EDTA (pH 8.3) and stored frozen at –18 °C until the PCR reaction was carried out.

DNA sequencing

PCR reactions were performed in an Eppendorf Mastercycler using 4 μL of the DNA solution in a 50-μL final volume. The reaction mix contained the primers 18S (5′-GGAGAAGTCGTAACAAGGTTTCCG-3′), designed by Spagnuolo *et al*. (1999) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′; White *et al*., 1990), at a final concentration of 400 μM, in the presence of 200 μM of each dNTP, 2 mM $MgCl_2$, 2 units *Taq* polymerase (Oncor Appligene), 1 μL BLOTTO (10% skimmed milk powder, 0.2% NaN_3 in water) and the buffer provided by the supplier of the enzyme. BLOTTO attenuates the PCR inhibition by plant compounds (De Boer *et al*., 1995; our unpubl. data). Amplification started with 3 min of denaturation at 94 °C, followed by 35 cycles of 15 s at 94 °C, 30 s at 50 °C and 1 min at 72 °C. A final extension step of 7 min at 72 °C completed the PCR. Finally, 5 μL of the amplification products were visualized on a 6% polyacrylamide gel and successful amplifications were cleaned with the QIAquick purification kit (Qiagen). The amplification primers were used in the sequencing reactions with the Big Dye sequencing kit and separated on an ABI-Prism 3700 sequencing machine using standard protocols.

The sequences were aligned using CLUSTALX (Thompson *et al*., 1997) with the gap open penalty set to 10 and the gap extension penalty set to 1. BioEdit (Hall, 1999) was used for minor manual adjustments of the alignment. A DNA data matrix with the aligned sequences is available on request.

MEGA 2.1 (Kumar *et al*., 2001) was used for neighbour-joining (NJ; Saitou & Nei, 1987) and with maximum parsimony (MP) as the optimality criterion. MrBayes 3.0 (Huelsenbeck & Ronquist, 2001) was used for the Bayesian analysis. Gaps were treated in two ways: regions with gaps were eliminated from the matrix in treatment I, and were treated as binary characters in indel treatment II.

The NJ analyses used the number of differences as distance measure.

With MP, all characters were given equal weight, and the heuristic search used the following settings: close-neighbour interchange (CNI) with search level 3; random addition trees = 10 replications.

Bootstrap analyses (Felsenstein, 1985) were carried out with 1000 replicates and identical settings for both NJ and MP.

For the Bayesian analysis, 500 000 generations were run sampling every 100th generation. MODELTEST ver. 3.5 (Posada & Crandall, 1998) was used to estimate the likelihood parameters and using the following settings: Nst = 6, rates = invgamma (general model of DNA substitution with gamma-distributed rate variation across sites and invariant sites). Recodified gaps were treated as standard data. Based on empirical evaluation, burning (the number of starting generations discarded from further analysis) was set at 100 000 generations. A 50% majority rule tree was constructed using the 'sumt' command of MrBayes. The tree was edited using TreeView version 1.6.6 (Page, 1996).

For the purpose of our discussion, good bootstrap support is represented by values of ≥ 70%, moderate support by <70 to ≥50% and poor support by <50%. In the case of the Bayesian clade credibility values, good support is estimated as ≥90%, moderate support as < 90 to ≥70% and poor support as <70%.

RESULTS

Morphometric analysis

PCA reveals that 70.1% of the observed variation is explained by three factors (38.57, 22.26 and 9.29%, respectively). The descriptors used for this analysis were: leaf length, ratio of upper marginal laminal cell width/upper marginal laminal cell length, ratio of middle marginal laminal cell width/middle marginal laminal cell length, basal marginal laminal cell length, strata number of marginal laminal cells, mucro length, upper laminal cell width, middle laminal cell length, middle laminal cell width, papillae number on middle laminal cells, and peristome basal membrane length (Figs 1, 2). The characters that contribute considerably to the three first PC axes are as follows: Factor 1: ratio of middle marginal laminal cell width/middle marginal laminal cell length, leaf length, basal marginal laminal cell length, mucro length, ratio of upper marginal laminal cell width/upper marginal laminal cell length and upper laminal cell width; Factor 2: strata number of marginal laminal cells and peristome basal membrane; Factor 3: papillae number on middle laminal cells.

The scatterplot of the 76 specimens for the first and second PCA (Fig. 3A) reveals four groups. The first group contains the specimens determined as *T. inermis*, mixed with some specimens of *T. subulata* (including also some specimens of *T. inermis* var. *submarginata*), the second group includes the OTUs identified as *T. subulata s.l.* (var. *subulata*, var. *graeffii*, var. *subinermis* and var. *angustata* with unistratose marginal cells) and the remaining specimens identified as *T. inermis* var. *submarginata*, the third group includes the specimens of *T. mucronifolia*, and the fourth group comprises *T. subulata* var. *angustata* with bistratose marginal cells, which is more clearly separated in the scatterplot for the second to third PCA (Fig. 3B). Therefore, two morphotypes can be distinguished in the specimens identified as *T. subulata* var. *angustata* in the studied herbaria, one morphotype with bi- or more rarely tristratose marginal cells, which is clearly separated by PCA analysis (hereafter *Tortula schimperi*) and the other with unistratose marginal cells, which is included in the complex of *Tortula subulata* in the analysis.

From the previous PCA results, we performed a DA to study the separation between the four groups (Fig. 4). The same descriptors used in PCA were used, except the quantitative discrete characters (strata number of marginal cells and papillae number of middle laminal cells). The DA results in 86.84% of the specimens being correctly classified. The first discriminant function is most highly correlated with the ratio of middle marginal laminal cell width/middle marginal laminal cell length and basal membrane of peristome length, whereas the second is contributed to mostly by middle laminal cell width, and the third by mucro length and upper laminal cell width. The plot of root 1 against root 2 shows the separation of the four groups recognized in the PCA, although in each group some specimens are classified differently by the discriminant function compared with the a priori classification; an exception is the *T. inermis* group, in which no specimen of *T. inermis* is allocated to another species (Table 1).

For further distinction between the species investigated, the observed qualitative characters were compared. The variables which most contribute to the discrimination of the species were:

Habit when dry. *T. inermis* is the only species that shows regularly twisted leaves when dry. The remaining specimens are individually twisted or crispate when dry.

Shape of marginal upper and middle cells of the leaf. *T. inermis* usually has oblate upper and middle cells. In the remaining taxa of this complex, the marginal upper and middle cells are elongate, short rectangular or quadrate, although some specimens of *Tortula subulata* exceptionally have some upper and middle cells that are oblate.

Papillosity of laminal cells. *Tortula schimperi*, *T. inermis* and most specimens of *T. subulata* have conspicuous and branched papillae. *T. mucronifolia* and more

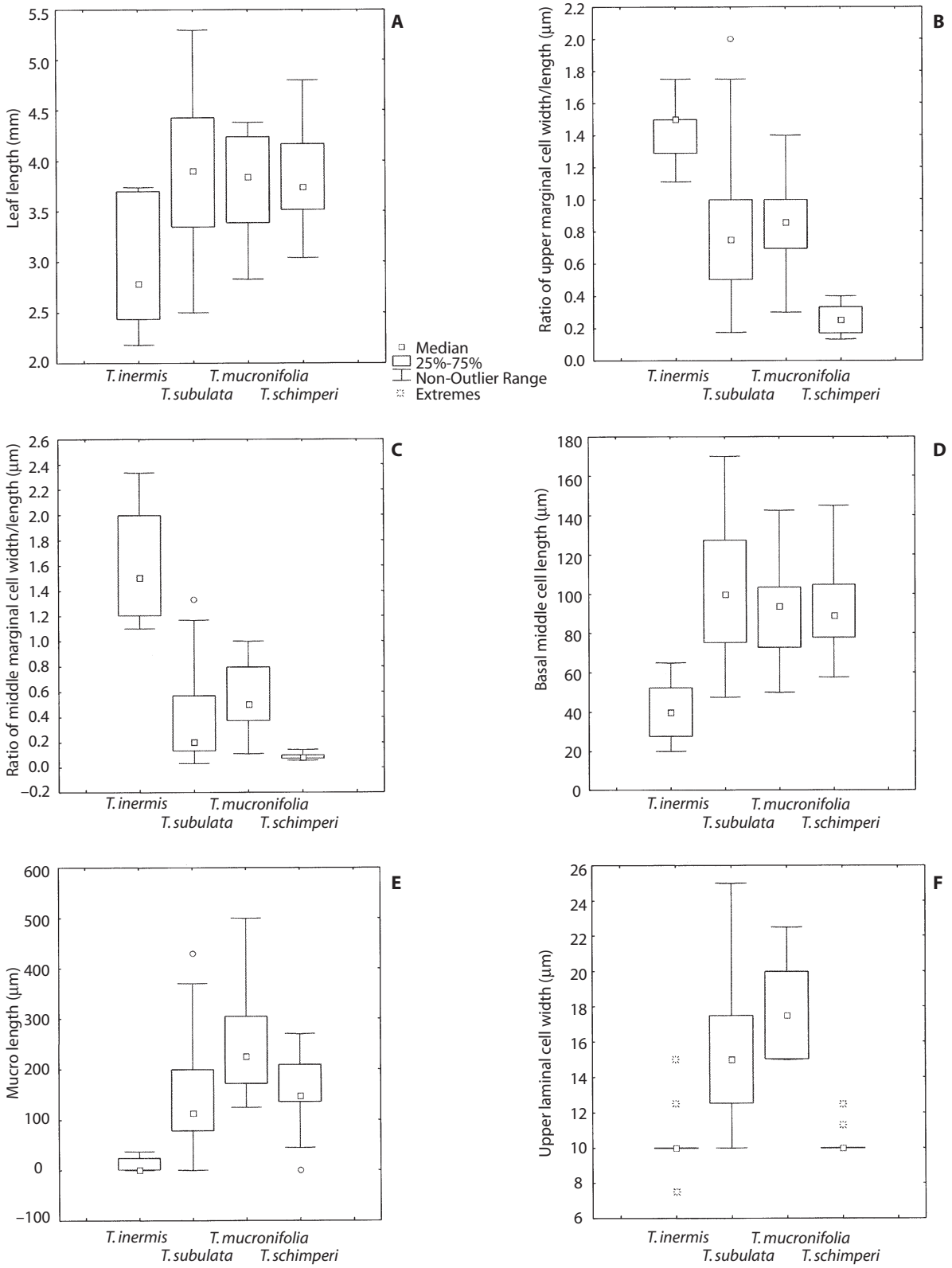

Figure 1. Box plot of: A, leaf length; B, ratio of upper marginal leaf cell width/marginal leaf length; C, ratio of marginal middle leaf cell width/marginal middle leaf cell length; D, marginal basal leaf cell length; E, mucro length; F, upper leaf cell width.

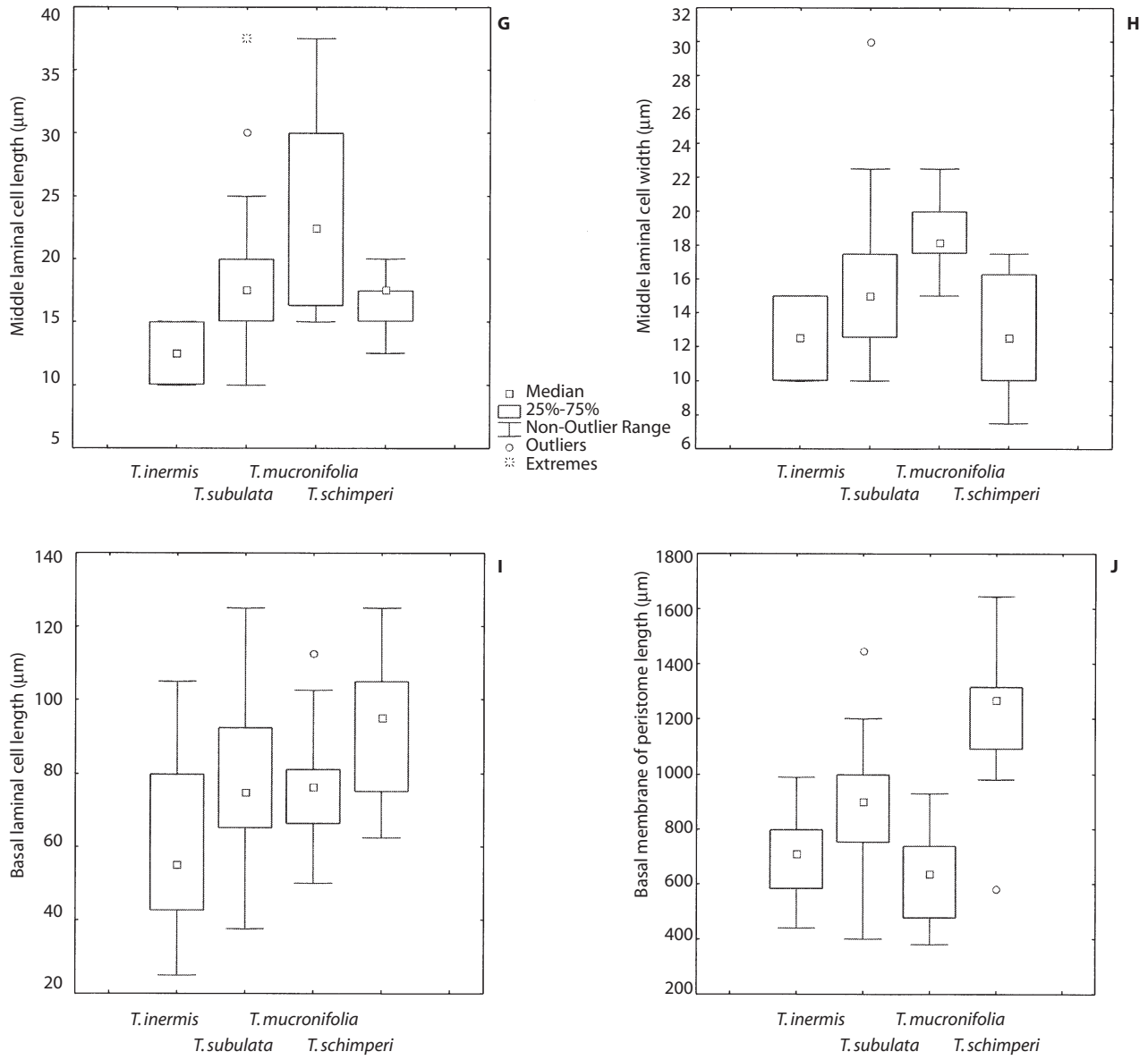

Figure 2. Box plot of: G, middle laminal cell length; H, middle laminal cell width; I, basal laminal cell length; J, peristome basal membrane length.

Table 1. Classification matrix for the discriminant analysis into the *Tortula subulata* complex. Rows: observed classifications; columns: predicted classifications

	Per cent	*T. inermis*	*T. subulata*	*T. mucronifolia*	***T. schimperi***
T. inermis	100.0000	11	0	0	0
T. subulata	82.0513	2	32	3	2
T. mucronifolia	87.5000	0	2	14	0
T. schimperi	90.0000	0	1	0	9
Total	86.8421	13	35	17	11

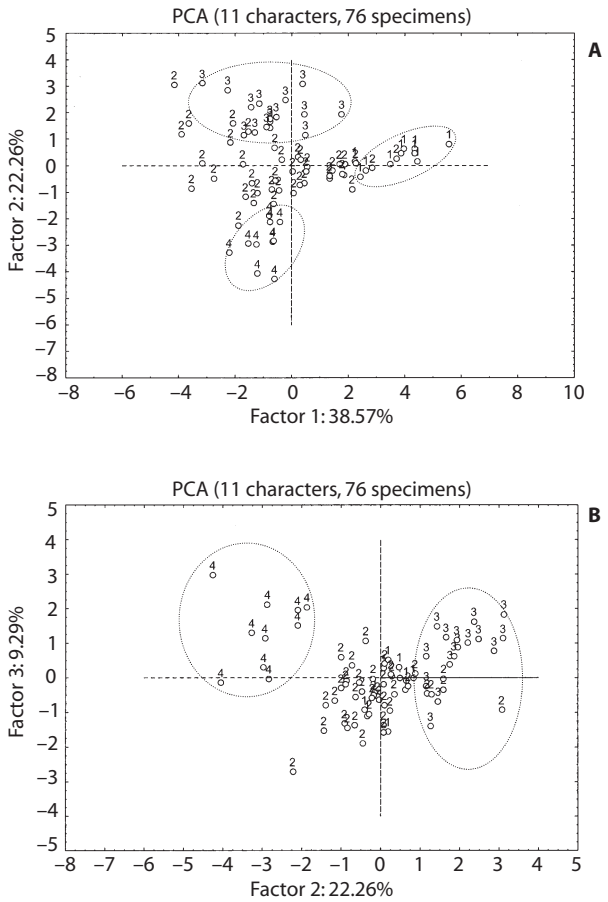

Figure 3. A, scatterplot of the first two axes of the principal component analysis (PCA) into *Tortula subulata* complex. B, scatterplot of the second and third axes of the PCA into the *T. subulata* complex. 1, *T. inermis*; 2, *T. subulata*; 3, *T. mucronifolia*; 4, ***T. schimperi***.

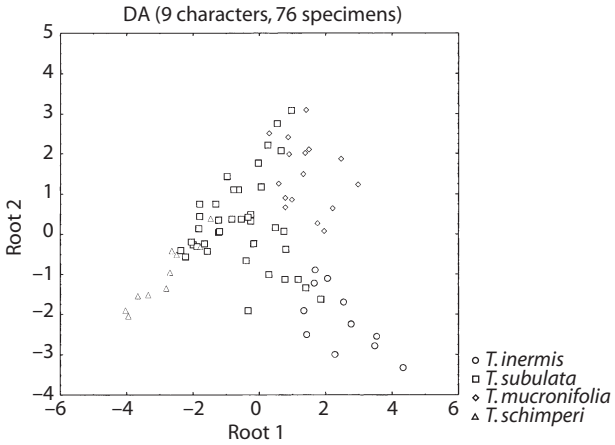

Figure 4. Scatterplot of the first two axes of the discriminant analysis (DA) into the *Tortula subulata* complex.

rarely some samples of *T. subulata* have inconspicuous and simple papillae when present.

Recurved leaf margins. Only *T. inermis* has regularly recurved leaf margins from the apex to near the base. In the rest of the species they are more variable or are planar. In some specimens of *T. subulata* it is possible to find some leaves with recurved margins from base to apex, but in the same plant other leaves usually appear with planar or irregularly recurved margins.

Molecular analysis

The total length of the alignment is 937 bp. The individual sequences vary between 649 bp for one sample of *Tortula mucronifolia* [*Hakelier s.n.* (S B72991)] and 734 bp for three samples of *Tortula schimperi* [*Heras & Infante 776/98* (VIT 22294); *Norris 90243* (UC); *Ros & Werner s.n.* (MUB 14040)]. Lengths of the ITS1 sequences lay in the range of 209 bp for *Protobryum bryoides* and 285 bp for two samples of *T. schimperi* [*Heras & Infante 776/98* (VIT 22294), *Ros & Werner s.n.* (MUB 14040)]. The 5.8S rRNA gene has a length of 159 bp in all cases and the sequences are identical. The length of the ITS2 region varies between 263 bp for *T. mucronifolia* [*Cano 993* (MUB 13630)] and 300 bp for *T. cuneifolia*.

Calculating the pairwise distances between all samples (excluding gapped sites), *Leptophascum leptophyllum* shows the highest number. The mean value for this species is 30.827 whereas the average number for all samples is 12.232. *Tortula mucronifolia* is separated from the other three species studied here by relatively high mean values in the range 17.167 (with *T. schimperi*) to 24.008 (with *T. subulata*) while the mean values between the other three species are always below ten pairwise differences. The number of differences within the species is between 0.667 in the case of *T. schimperi* and 3.623 in the case of *T. subulata*. The distance data are given in Table 2.

Using indel treatment I, 402 most parsimonious trees (length = 115 steps; CI = 0.53; RI = 0.899) were generated. All the analyses (NJ, MP and the Bayesian inference) resulted in trees with very similar topology (Figs 5, 6). In all cases, the monophyly of *Tortula subulata* complex (including *T. inermis*, *T. subulata s.l.* and *T. mucronifolia*) was not supported by ITS sequences, showing two clearly separated groups: a clade formed by *T. mucronifolia* and *Protobryum bryoides* (= *Tortula protobryoides*) recognized with a bootstrap support of 71% (MP) and 69% (NJ) and Bayesian inference of 100%, and another clade formed by *Tortula subulata s.l.* (including *T. schimperi*) and *T. inermis* supported by bootstrap values of 88%

Table 2. The mean number of nucleotide differences between and within the four species of *Tortula* studied. Gapped sites are excluded from the calculations. *T. mucronifolia* is clearly the species with most differences in the comparison between species. *T. subulata* has the highest within-species value. The main differences between the two clades of *T. mucronifolia* are based on indels. Therefore, the number of differences is low when the calculation is based on a matrix with excluded gapped sites

	T. subulata	*T. inermis*	***T. schimperi***	*T. mucronifolia*
T. subulata	3.623			
T. inermis	5.148	0.964		
T. schimperi	7.924	8.958	0.667	
T. mucronifolia	24.008	25.625	17.167	1.091

(MP) and 98% (NJ) and a clade credibility value of 99% under Bayesian inference (Figs 5, 6). In the *T. mucronifolia–Protobryum bryoides* clade, *P. bryoides* is not resolved under Bayesian inference, with *T. canescens* the sister group. However, under MP and NJ, *P. bryoides* is the sister group of the two subclades of *T. mucronifolia*. In the *Tortula subulata s.l.* and *T. inermis* group a well-supported clade is formed by *Tortula schimperi* (MP 90% and NJ 94% bootstrap support, Bayesian inference 100% clade credibility), which is the sister group of the remaining species. In addition, a third moderately supported clade is formed by *T. inermis* (MP 58% and NJ 64% bootstrap support, Bayesian inference 98% clade credibility), which is embedded within the *Tortula subulata s.l.* clade. Within *T. subulata*, the relationships are generally not well resolved. The exceptions are a clade formed by two samples [*Cano s.n.* (MUB 17231); *Norris 88731* (UC)] on a well-supported clade (71% NJ, 70% MP, 99% MrBayes) and a clade formed by eight samples of *T. subulata* that is only poorly supported by MP (41%), moderately by NJ (54%), but receives good support by MrBayes (100%).

When regions with deletions were coded as a binary character (present/absent), 82 most parsimonious trees (length = 497 steps; CI = 0.440; RI = 0.861) were generated. The strict consensus trees and the phylogram based on the Bayesian approach were better resolved, but very similar to those shown in Figures 5 and 6 (Fig. 7). However, in all the analyses, *Protobryum bryoides* was sister to one of the subclades of *T. mucronifolia* with high bootstrap support. Some interesting observations that can be made concern gaps. *Protobryum bryoides* and all specimens of *T. mucronifolia* share a major deletion at positions 121–215 of the aligned sequences. Within the specimens identified as *T. mucronifolia* based on morphological considerations, there are two subclades that receive only poor to moderate support in the analyses, when gaps are excluded, but these two subclades are characterized by a high number of synapomorphic smaller gaps. When using *Protobryum bryoides* as a reference, there are five synapomorphic indels for *T. mucronifolia* clade I (three in ITS1 and two in ITS2) and 12 synapomorphic indels for clade II (three in ITS 1 and nine in ITS2). Because there are more deletions in the ITS2 region of clade II, this region is about 20 bp shorter in clade II than in clade I. As a consequence of this high number of indels shared by members of the two clades, support for these two clades rises considerably when gaps are included in the analyses and the clades are well supported under these conditions, with values of 99–100%. The inclusion of gaps in the molecular analyses even leads to a situation in which *P. bryoides* is nested within the *T. mucronifolia* clade. Another interesting fact is that all specimens of *T. subulata* but none of the other specimens studied here share a deletion at positions 588–595, which might be interpreted as a synapomorphy of this taxon. Some other species do have gaps in this region, but never with the same limits. The inclusion of indels changes the results so three analyses made never assign *T. inermis* to a position nested within the *T. subulata* clade. The relationships in this case remain unresolved for the NJ and MP analyses while the Bayesian analysis favours a position of the samples of *T. inermis* on a sister clade of *T. subulata.* Within the complex of *T. subulata*, the inclusion of indels does not lead to major changes in tree topology, but improves the resolution in some cases. The most interesting case is that four of the five samples identified as *T. graeffii* are clustered together under these circumstances with good support. The cluster of four samples of *T. graeffii* have identical sequences while the fifth sequence [*Cano 949* (MUB 13601)] differs by four base changes and four indels.

DISCUSSION

The monophyly of this complex of taxa, which was hypothesized from our previous morphological studies, has not been supported by the ITS sequences. *Tortula mucronifolia* could correspond to a basal line in *Tortula* characterized by the absence or weak

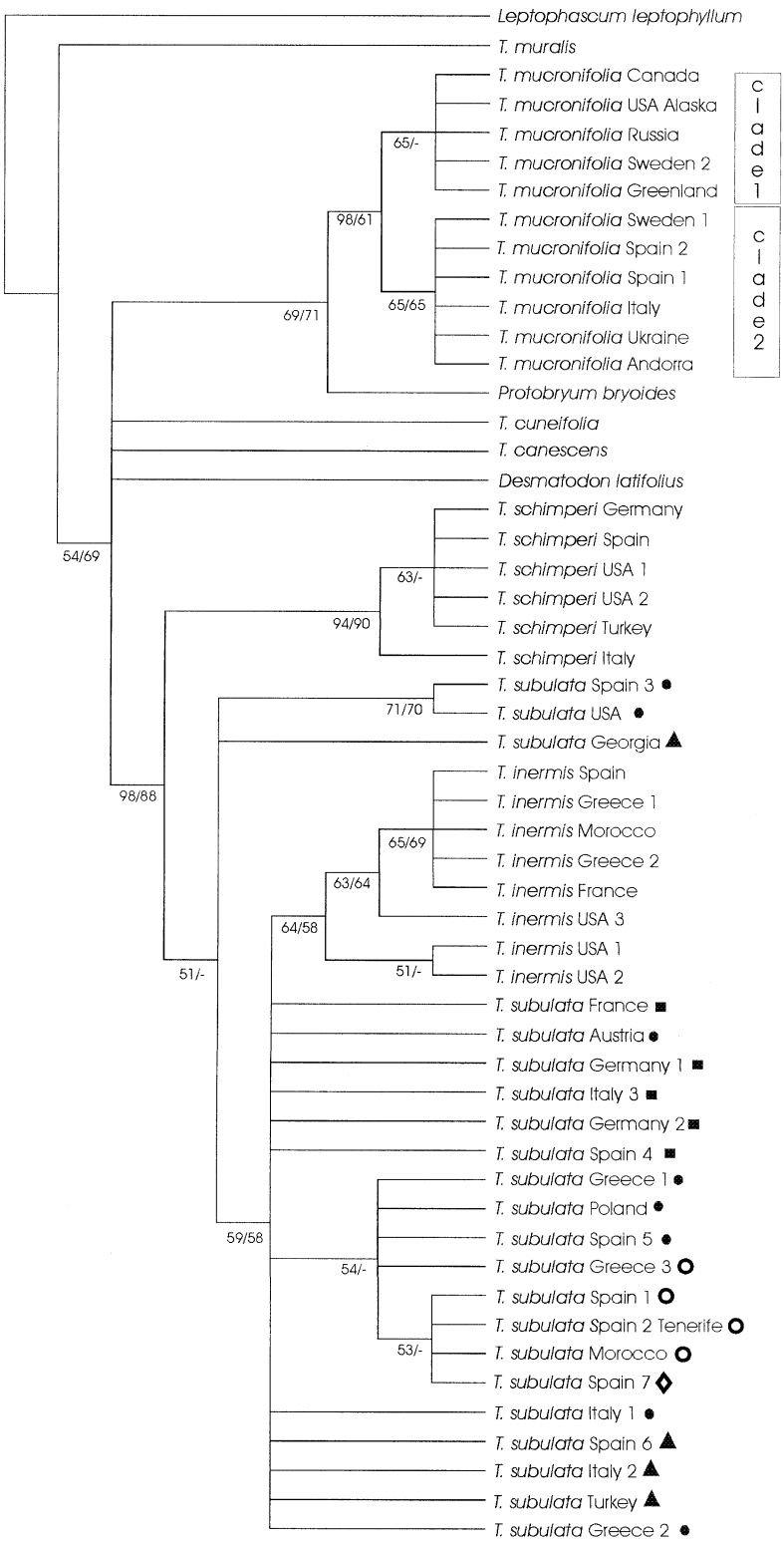

Figure 5. Strict consensus of 402 most parsimonious trees with tree length of 115 steps (RI = 0.899, CI = 0.53, considering only parsimony-informative sites), based on internal transcribed spacer (ITS) sequences. Gaps were eliminated from the analysis. Bootstrap values above 50% are given below the clades using maximum parsimony/neighbour-joining. The tree was rooted with *Leptophascum leptophyllum* as outgroup. The varieties are indicated as: var. *subulata*, solid circles; var. *submarginata*, open circles; var. *graeffii*, solid squares; var. *angustata*, solid triangles; var. *subinermis*, open diamonds.

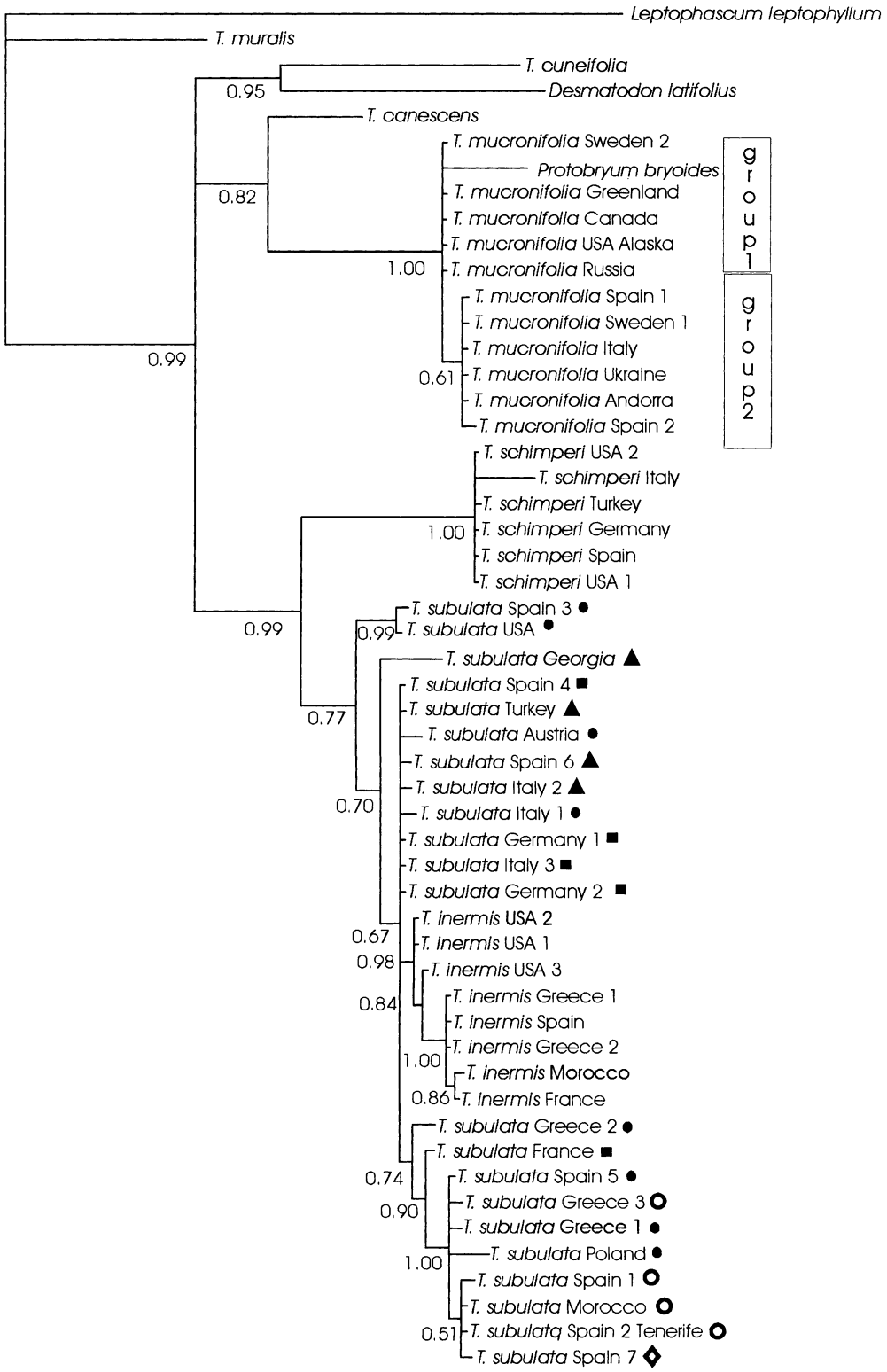

Figure 6. Phylogram based on the Bayesian approach for the *Tortula subulata* complex with internal transcribed spacer (ITS) sequence data. Gaps were eliminated from the analysis. Numbers indicate the clade credibility values of the nodes. Values above 50% are shown. The tree was rooted with *Leptophascum leptophyllum* as outgroup. The varieties are indicated as: var. *subulata*, solid circles; var. *submarginata*, open circles; var. *graeffii*, solid squares; var. *angustata*, solid triangles; var. *subinermis*, open diamonds.

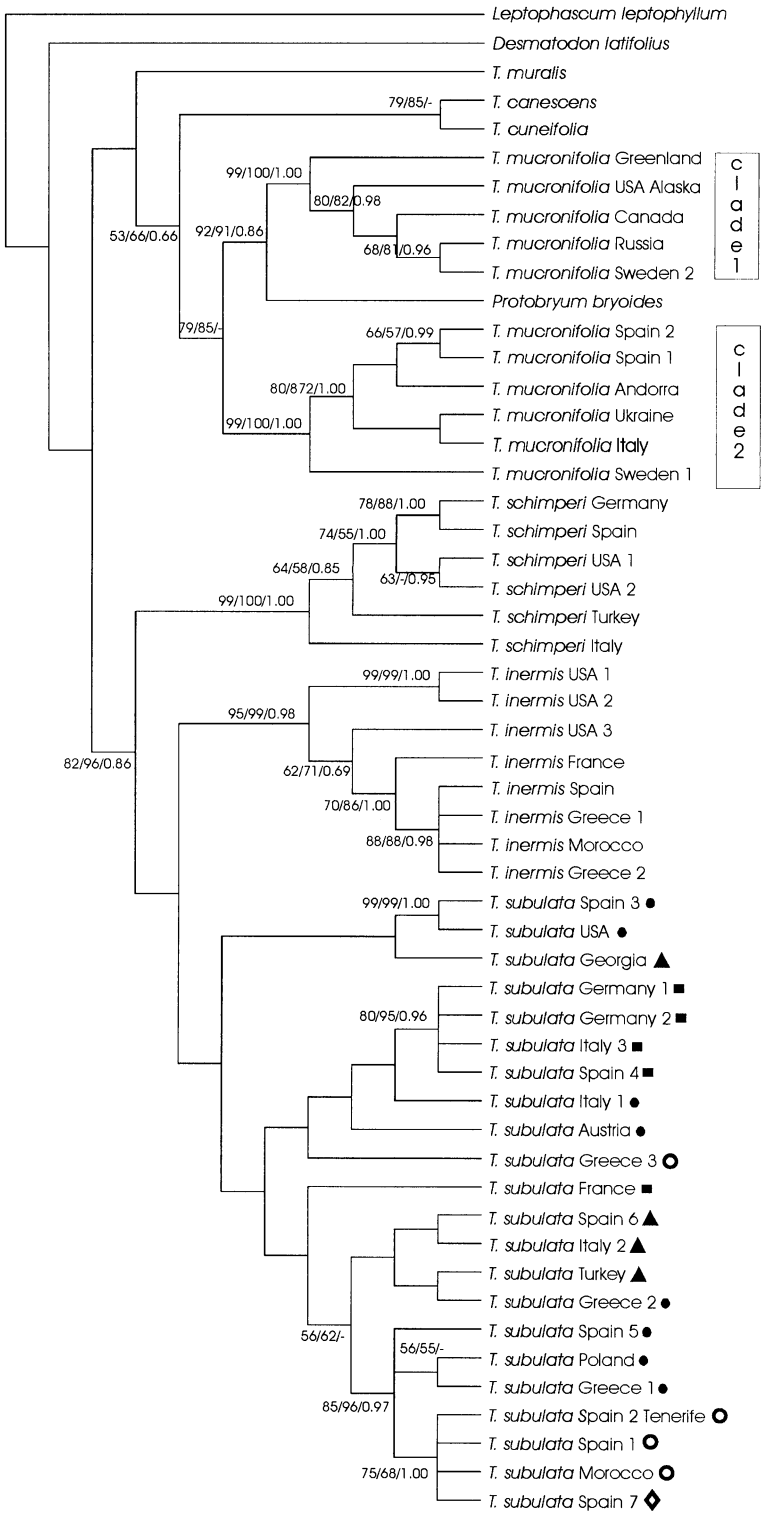

Figure 7. Strict consensus of 82 most parsimonious trees with tree length of 497 steps (RI = 0.861, CI = 0.440, considering only parsimony-informative sites), based on internal transcribed spacer (ITS) sequences. Indels were treated as an additional binary character added to the data matrix. Bootstrap values above 50% are given in the clades using maximum parsimony/neighbour-joining/Bayesian approach. The tree was rooted with *Leptophascum leptophyllum* as outgroup. The varieties are indicated as: var. *subulata*, solid circles; var. *submarginata*, open circles; var. *graeffii*, solid squares; var. *angustata*, solid triangles; var. *subinermis*, open diamonds.

differentiation of papillae and large upper and middle leaf cells. This line would be closer to species of *Pottia* included in *Tortula* by Zander (1993), such as *Protobryum bryoides* (*Tortula protobryoides*) in our study. Chen (1941) and Zander (1993) commented on the similar morphology of many *Pottia* species and *T. mucronifolia*, emphasizing the absence of papillae, and the latter author included this species in *Tortula* sect. *Pottia*, in spite of its apparent similarity to *T. subulata* (Zander, 1993). However, with the present data we consider that it is impossible to provide more information about the phylogeny of *Tortula* or its infrageneric classification, because of the complexity of this genus, and this has already been observed in a study with *rps4* sequences (Werner *et al*., 2002a). However, our molecular data clearly support the monophyly of *T. subulata s.l.–T. inermis*, which was also confirmed by chloroplast *rps 4* sequences (Werner *et al*., 2003).

The results obtained from PCA and DA, largely supported by the ITS sequences, suggest that it is possible to recognize four different entities, which are accepted by many authors: *Tortula inermis*, *T. mucronifolia*, *T. schimperi* and *T. subulata*.

Although some authors have treated *T. mucronifolia* as synonymous with or a variety of *T. subulata* (Röhling, 1813; Lindberg, 1864) most authors considered both as different species, although some intermediate specimens have been found (Steere, 1940; Nyholm, 1989; Mishler, 1994; Cano, 2004). The main descriptors for this species are the peristome basal membrane length, number of papillae on middle laminal cells and middle laminal cell width. The PCA and DA show overlapping between some specimens of *T. mucronifolia* and *T. subulata*, specifically with specimens identified as *T. subulata* var. *graeffii*, because morphological similarity between both species exists. Thus, they share broad upper laminal cells and inconspicuous papillae cells (low and simple), which are absent in most of the *T. mucronifolia* specimens studied. Specimens of both taxa with inconspicuous papillae can be distinguished by a higher basal membrane of the peristome and more elongate middle marginal leaf cells in *T. subulata* var. *graeffii.* In contrast, the molecular study places the specimens identified as var. *graeffii* in the *T. subulata s.l.–T. inermis* clade, and *T. mucronifolia* in a clearly separate clade close to *Protobryum bryoides* (even embedded in it when indels are included in the analysis) with a clade credibility of 100% with Bayesian inference, and a bootstrap support of 61% (MP) and 98% (NJ). Two subclades are clearly formed in the specimens of *T. mucronifolia* which might correspond to two different geographical areas: the first includes specimens from northern Europe and America (Canada, Greenland, Russia, USA), and the other from southern Europe (Andorra, Italy, Spain, Ukraine), although geographically close specimens from Sweden are found in both subclades. According to Steere (1937), the natural suspicion that the North American moss might be a species different from the European one is not substantiated by a comparison of specimens. In addition, our morphometric study did not find significant differences between both groups of species identified as *T. mucronifolia*, and therefore they are not recognized at any taxonomic level. Nevertheless, the high number of 17 indels that separates the two clades of *T. mucronifolia* might indicate that it is a case of cryptic speciation where two morphologically indistinct taxa are separated by genetic differences. Evidence for cryptic speciation has been observed in other bryophytes (Shaw, 2001). In this case, the separation might be due to geographical separation along a north–south gradient, one of the cryptic species adapted to more arctic climates, the other to more temperate zones, although overlap between both geographical ranges is observed. Werner & Guerra (2004) observed that one of the chloroplast *rps4* gene haplotypes they found in *Tortula muralis* was restricted to specimens from samples collected outside human settlements. This species is very frequent on walls and other man-made structures. A cryptic speciation might therefore have occurred a number of times in *Tortula*.

T. subulata var. *angustata* is currently treated as a variety of *T. subulata* (Nyholm, 1989; Cortini-Pedrotti, 2001; Smith, 2004) or it has been included in the *T. subulata* concept (Steere, 1940; Lawton, 1971; Corley *et al*., 1981). Warnstorf (1912) described *Tortula serrulata* Warnst., which was subsequently renamed as *T. crenulata* Warnst., because there was another taxon previously described with the same epithet (Warnstorf, 1913). Under *T. crenulata*, Warnstorf included the specimens both with dentate-crenulate apex and a border of elongate cells from the apex to the base. However, Warnstorf did not mention the bistratose margins as a differentiating character. Dixon (1924) commented that *T. angustata* was very different from typical *T. subulata*, especially 'in the form of the fruit', and it certainly merited a higher rank than a mere variety. However, it seemed best to subordinate it to that species rather than to give it a separate specific position, especially as intermediate forms occurred. In addition, Steere (1940) did not recognize this taxon at any taxonomic level owing to the existence of many intermediate forms. From the specimens identified as *T. subulata* var. *angustata* in the herbaria studied, two morphotypes have been distinguished by DA and PCA, one with bistratose leaf margin cells and width of middle laminal cells from (7.5)10 to 16.3(17.5) μm (*T. schimperi*) and the second with unistratose leaf

margins and width of middle laminal cells from (13.75)15 to 20 μm. The main descriptors for *T. schimperi* are the strata number in marginal laminal cells and peristome basal membrane length, which is usually very high [(580)1090–1316(1644) μm]. Also, the morphotype with unistratose margins is included in the PCA and DA in the variability shown by *T. subulata*. The ITS analyses show that all the species sequenced identified as *Tortula schimperi* are included in one of the separate clades recognized by the molecular analysis with a bootstrap support of 90% (MP), 94% (NJ) and 100% clade credibility with Bayesian inference. However, the specimens of *Tortula subulata* var. *angustata* with unistratose margins are included in the *T. subulata s.l.* clade, and they are not discriminated by the ITS sequence data (Figs 5, 6).

Tortula inermis is mainly discriminated by the ratio of middle marginal cell width/middle marginal cell length, and middle marginal cell width. The DA and, above all, PCA show an overlapping between *T. inermis* and some specimens of *T. subulata*, especially with specimens identified as *T. subulata* var. *subinermis* and, particularly, with specimens of *T. inermis* var. *submarginata*, because these taxa lack or have a small mucro as in *T. inermis* (*T. subulata* usually has a long mucro) and also lack elongate cells in the middle marginal cells (in *T. subulata* they are usually rectangular or elongate). Also, some forms of *T. inermis* var. *submarginata* have oblate upper and middle marginal cells, similar to those present in *T. inermis*. However, it must be stressed that in PCA and DA we neglected important qualitative characters. Thus, in *T. inermis* the plants when dry are regularly twisted and the leaf margins are recurved from near the apex to the base. In *T. inermis* var. *submarginata* and *T. subulata* var. *subinermis* the habit when dry is irregularly or individually twisted, similar to the habit when dry exhibited by *T. subulata*. The leaf margins are planar or only recurved from the base to the middle in *T. subulata* var. *subinermis*. They are planar or recurved up to the middle or upper third, but more rarely are recurved near the apex, and then generally only one margin or only one leaf in the plant is recurved in *T. inermis* var. *submarginata.* The ITS sequence data places the specimens of *T. inermis* in a clade together with the specimens of *Tortula subulata s.l.* The specimens belonging to *Tortula inermis* have similar sequences and are situated in the same clade and supported by moderate bootstrap values (64% NJ; 58% MP) and highly supported based on the Bayesian analysis (98%). The basal part of the *T. subulata–T. inermis* clade is not well resolved in the analyses of the ITS sequences. Exclusion of information from the indels seems to favour a situation that would leave *T. subulata* paraphyletic with *T. inermis* as a monophyletic group nested inside this clade. Nevertheless, inclusion of the indels suggests that *T. inermis* forms a sister clade to *T. subulata.* In this case, *T. subulata* would be defined by a deletion of positions 588–595 of the aligned sequences. Together with the morphological data, a recognition of *T. inermis* at the species level seems to be justified based on the information available at present. The molecular analysis with ITS also indicates that *T. inermis* var. *submarginata* are not included in the *T. inermis* clade. However, it should be stressed that all the specimens identified with the morphotype *T. inermis* var. *submarginata* show similar ITS sequences.

Tortula subulata has a very high level of morphological variation, as shown in the high number of infraspecific taxa described for this taxon. The varieties described for this species and included in this study could not be discriminated, with the exception of *T. subulata* var. *angustata* with bistratose margins (*T. schimperi*). *Tortula subulata* can be mainly distinguished by its leaves with unistratose and elongate marginal cells from the base to the middle, upper third or apex, which form a conspicuous border (*T. inermis* has oblate marginal cells and *T. mucronifolia* usually quadrate to short rectangular marginal cells, and in both cases they lack a conspicuous differentiated border). Most of the floras identify *T. subulata* var. *subinermis* with specimens with shorter mucro than the typical variety and the border of nearly inconspicuous marginal cells, because most of the marginal cells in the upper half are short rectangular. When the middle laminal cells are broader and with inconspicuous papillae instead of densely papillose as in the typical variety, they are determined as *T. subulata* var. *graeffii.* If the border of marginal cells was very conspicuous extending from base to the apex, it was determined as *T. subulata* var. *angustata*, which has been commented upon previously. According to Smith (2004), var. *graeffii* retains its character in cultivation and appears to have a genetic basis. The combination of characters previously cited for the varieties can be observed in the samples studied. However, we have observed many specimens, which have not been included in the morphometric analysis, with intermediate characteristics resulting in great difficulty in classifying. *T. subulata* is the most variable species not only morphologically but also genetically. The mean genetic distance within the four species treated here is more than three times as high as in any other species when gaps are excluded. As observed with the morphological characters, the ITS sequences of this clade reveal a number of intermediate forms that unite the extreme values. As a result, only one clade formed by two specimens [*Cano s.n.* (MUB 17231) and *Norris 88731* (UC)] is well supported, although with the cur-

KEY TO THE SPECIES

1. Middle marginal cells of the leaf in 2(3) strata . . . . . ***T. schimperi***
1. Middle marginal cells of the leaf in 1-stratum 2
2. Leaf margins regularly recurved from near the base to the apex; middle marginal cells of the leaf oblate, more rarely quadrate or short-rectangular [ratio of middle marginal cell width/middle marginal cell length (1.10)1.20–2.0(2.33)]; leaves regularly twisted when dry . . . . . *T. inermis*
2. Leaf margins recurved from near the base to the middle or upper third, or plane, more rarely irregularly recurved to the apex and then only in some leaves of the plant; middle marginal cells of the leaf quadrate, short-rectangular or elongate, rarely oblate [ratio of middle marginal cell width/middle marginal cell length (0.03)0.11–0.79(1.33)]; leaves individually twisted or crispate when dry 3
3. Upper and middle laminal cells smooth, more rarely with inconspicuous papillae; middle marginal cells of the leaf quadrate or short-rectangular, rarely elongate [ratio of middle marginal cell width/middle marginal cell length (0.11)0.37–0.79(1.0)]; peristome basal membrane (380)475–740(930) μm long . . . . . *T. mucronifolia*
3. Upper and middle laminal cells with (4)6–9(14) papillae, more rarely with inconspicuous papillae; middle marginal cells of the leaf rectangular to elongate, more rarely quadrate or short-rectangular [ratio of middle marginal cell width/middle marginal cell length (0.03)0.13–0.57(1.3)]; peristome basal membrane (400)750–1000(1445) μm long . . . . . *T. subulata*

rent data no morphological characters can be found for this group. The inclusion of information contained in the indels leads to partially better resolved trees, but typically morphological and genetic characters do not match in all the cases. For example, four of the five samples that were initially classified as *T. graeffii* are found on a well-supported clade when indels are taken into consideration, but the fifth sequence of *T. graeffii* is clearly different. The situation may be summarized as follows. The *T. subulata* species complex is variable both morphologically and genetically. On a morphological basis there are intermediate forms that connect the extreme forms and do not allow a clear separation of existing species. Genetically the pattern is similar and the existing variability is only poorly structured, and when there are signals, these only partly correspond with morphological characters. It is well known that recently separated species are not mutually monophyletic at the sequence level (Avise, 2000). The relative high morphological and genetic variability within the *T. subulata* complex might therefore indicate that the speciation process has taken place in recent times or is even still ongoing. *T. subulata* could possibly provide a useful model for the observation of speciation processes in mosses. For this reason, we prefer to use a more pragmatic concept of species, and consider only a highly morphologically variable taxon at species level.

TAXONOMIC CONCLUSIONS

Based on comparative morphometric and molecular data, we propose to recognize at species level *T. subulata* var. *angustata* with bistratose margins under *T. schimperi.* By contrast, *T. inermis* var. *submarginata* is included in the high morphological variation of *T. subulata*. A determination key of this group of species is proposed above.

***Tortula schimperi* nom. nov.**
Tortula angustata Lindb., nom. illeg, Öfvers. Förh. Kongl. Svenska Vetensk.-Akad. 21: 243. 1864, non *Tortula angustata* Mitt., J. Proc. Linn. Soc., Bot., Suppl. 1: 28. 1859.

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APPENDIX

List of specimens measured for morphometric study and those used for the ITS sequence analysis (the GenBank accession number is given in square brackets)

Tortula inermis (Brid.) Mont.
ARGELIA: Sahara-Atlas, E kais an der Strasse Batna-Khonchela, *J.-P. Frahm 844488* (MUB 12601). FRANCE: Languedoc-Rosellón, Lozère, Gorge de la Jonte, La Caze, 26.iv.1994, *G. Een s.n.* (S B72229) [AY934548]. GREECE: Biviavn, 28.vii.1999, *M.J. Cano s.n.* (MUB 14047) [AY934549]; Stereá Ellás, Kalesménon, 28.viii.1999, *M.J. Cano s.n.* (MUB 14049) [AY437133]. MOROCCO: Alto Atlas, Oukaïmeden, 19.iv.1989, *R.M. Ros s.n.* (MUB 13106). Anti Atlas, Prov. Ouazarzate, Jbel Siroua, above Tachacoucht, 18.iv.1993, *R.R. Brooks et al. s.n.* (MUB 11758) [AY934547]. SPAIN: Almería, Sierra de Alhamilla, 'Los Manueles', Turrillas, *P. García-Zamora & R.M. Ros* (MUB 8069). Murcia, Bullas, Sierra de la Lavia, *M.J. Cano s.n.* (MUB 10045) [AY934550]. USA: Arizona, Pima County, Helvetia area, 4.xi.1985, *L.E. Anderson & J. Shaw 24571* (NY) [AY934553]. California, Kern Co, BLM Owens Peak Wildernees, Short Canyon Trail, eastern base of Owens Peak west of L.A. aqueduct and California highway 14, 26.iii.1998, *J.R. Shevock & C. Newberry 16961* (NY) [AY934551]. New Mexico, Dona Ana County, at Aguirre Springs Park, Organ Mountains, 6.iv.1985, *D. Kizirian s.n.* (NY) [AY934552].

Tortula mucronifolia Schwägr.
ANDORRA: Santa Coloma, 17.vii.2001, *M.J. Cano 993* (MUB 13630) [AY934582]. CANADA: Ontario, Leeds and Grenville United Co, Mill Pond Conservation Area, 4 km west of old Frontenac Road., on county rd. 38, S. Burguess Twp., 4.vi.1988, *L.M. Ley 834* (NY) [AY934589]. DENMARK: Greenland, Greenland Disko, Unartoq, north coast of Disko Island, 14.viii.1977, *G.S. Mogensen & G.R. Brassard s.n.* (NY) [AY934590]. ITALY: Trentino-Alto Adige, Südtirol, Katherinenberg-Schnalstal, 6.viii.2002, *R.M. Ros & O. Werner s.n.* (MUB 14041) [AY934584]. NEW ZEALAND: Central Otago, Arrow river, 11/2–2 miles upstream from Arrowton, Lakes County, 18.xii.1964, *J.T. Linzey 3257* (CHR, NY). RUSSIA: Moscow, Odintzovo, Nikolina Gora, outcrop on slope of the Moscow River valley, 20.vi.1988, *M. Ignatov s.n.* (NY) [AY934587]. SPAIN: Huesca, Valle de Bujaruelo, 13.vii.1998, *M. J. Cano et al. s.n.* (MUB 8486) [AY934580]. Murcia, Moratalla, Macizo de Revolcadores, pr. Pico El Obispo, 7.iii.2004, *M.J. Cano 1433b* (MUB 17402) [AY934581]. SWEDEN: Närke, Hammar, N om Vänneviken, 10.vi.1990, *N. Hakelier s.n.* (S B72991) [AY934585]. Västmanland, Norberg, Klackberg, 20.v.1991, *N. Hakelier s.n.* (S B11383) [AY934586]. Härjedalen, Tännäs, Fröstsjöberget, 7.viii.1986, *N. Hakelier* (S B11385). UKRAINE: The Crimea, *c.* 15 km SW of Yalta; Mt. St. Peter's in Fagus/carpinus forest just below 'Yaila', 25.v.1981, *T. Elias et al. 5573* (NY) [AY934583]. USA: Alaska, north side of mountain NW of Umiatr, 30.vi.1974, *W.C. Steere & Z. Iwatsuki s.n.* (NY). New York, Erie Co., village of Akron, Murder Creek, Akron Falls, 21.v.1981, *R. Duell & R.H. Zander s.n.* (NY) [AY934588]. North Dakota, Cavalier County, 9 km west of Walhalla, on county road 55, 5.vii.1981, *S.P. Churchill 11770* (NY). Lectotype of *Tortula mucronifolia* (G).

***Tortula schimperi* nom. nov.**
AUSTRIA: Steiermark, zwischen den Bergen 'Gleichenberger Kogel' und 'Birkblösse' bei Gleichenberg, 16.ix.1927, *A. Boros s.n.* [Musci Europ. & Amer. Exsic. n.º 2018] (S B72719). CZECH REPUBLIC: Brno, Solamov, in convalle Josephé vs. Kitiny, 18.iii.1934, *J. Podpera s.n.* (S). GERMANY: Baden-Württemberg, Kaiserstsuhl, Schelinger Höhe, *R.M. Ros & O. Werner s.n.* (MUB 14040) [AY934577]. GREAT BRITAIN: Bronmsberrow, W. Gloster, 9.v.1910, *H.H. Knight s.n.* (S B72707). ITALY: Trentino-Alto Adige, Südtirol, de Schlaneid a Satherhüte, 30.vii.2002, *R.M. Ros & O. Werner s.n.* (MUB 14042) [AY934579]. SPAIN: Huesca, Canfranc, Canal Royal, 25.viii.1998, *P. Heras & M. Infante 776/98* (VIT 22294) [AY934576]. Logroño, Valdezcaray, 19.vii.2003, *J. Guerra s.n.* (MUB 15605). TURKEY: Bolu, Abant, *E. Nyholm* (S B54919) [AY934578]. USA: California, Madera Co., on Willow Creek north of Soquel Campground, north-east of Oakhurst, Sierra National forest, 10.vii.2000, *D.H. Norris 99859* (UC) [AY934575]. Washington, Jefferson Co., at Sunnybrook Meadows at top of watershed of Sunny Brook on trail to Constance Pass, Olympic National Park, 19.ix.1997, *D.H. Norris 90243* (UC) [AY934574].

Tortula subulata Hedw. [the varieties were mainly identified according to Smith's (2004) key, and they are given in parentheses]
AUSTRIA: Vorarlberg, Marul, Laguz A. to NE Hanflender, 16.vii.1992, *L. Hedenäs s.n.* (S B51994) [AY934570] (*T. subulata* var. *subulata*). FRANCE:

entre le col du Lautaret, et le coteau de la Verzilla, vallon du torrent du Col, 16.vii.1981, *J.J. De Sloover 34793* (NY) (*T. subulata* var. *angustata*). Midi-Pyrénées, Ariége, castillo de Montsegur, 15.viii.2002, *M.J. Cano 949* (MUB 13601) [AY934572] (*T. subulata* var. *graeffii*). GEORGIA: Abjasia, Caucasus occidentalis, distr. Sukhumi, in valle rivi Klich, 28.vi.1986, *V. Vasâk s.n.* (NY) [AY934573] (*T. subulata* var. *angustata*). GERMANY: Baden-Württemberg, Schwäbische Alb, Hayingen, 14.viii.2002, *R.M. Ros & O. Werner s.n.* (MUB 14044). [AY934557] (*T. subulata* var. *graeffii*). Baden-Württemberg, Lichtenstein, Station Lichtenstein, 14.viii.2002, *R.M. Ros & O. Werner s.n.* (MUB 17232). [AY934555] (*T. subulata* var. *graeffii*). Bavaria, Lenggries, path from Brauneck-Bahn, 1.vi.1989, *B.J. O'Shea s.n.* (NY) (*T. subulata* var. *angustata*). GREAT BRITAIN: Wales, near Newton, Montgomery, 4.iv.1975, *A.J.E. Smith s.n.* (Herb. A.J.E. Smith) (*T. subulata* var. *subinermis*). GREECE: Monte Olimpo, *M.J. Cano s.n.* (MUB 13022) [AY934565] (*T. subulata* var. *subulata*). Entre Brallos e Itis, 27.viii.1999, *M.J. Cano s.n.* (MUB 14046) [AY934567] (*T. subulata* var. *subulata*). Oros Parnitha, 14.iii.1999, *M.J. Cano et al. s.n.* (MUB 12171) [AY934566] (*T. inermis* var. *submarginata*). Phokis, Parnass, Hochfläche 'Livadi', 26.iv.1911, *V. Schiffner 84* (FH) (*T. inermis* var. *submarginata*). ICELAND: Laugarvatn, Arnessysla, 3.vii.1983, *L. Hedenäs s.n.* (S B72989) (*T. subulata* var. *subulata*). ITALY: Sicily, Parco de'll Etna, 2 km del Refugio Sapienza, 19.ix.2001, *M.J. Cano et al. s.n.* (MUB 14022) (*T. subulata* var. *subulata*). Sicily, Parco delle Madonie, Piano Zucci, 18.ix.2001, *M.J. Cano et al. s.n.* (MUB 14023) [AY934558] (*T. subulata* var. *subulata*). Trentino-Alto Adige, Südtirol, Schnalstal, Pfossenbachtal Vorderkaaser, 6.viii.2002, *R.M. Ros & O. Werner s.n.* (MUB 14043). [AY934568] (*T. subulata* var. *angustata*). Trentino-Alto Adige, Südtirol, Katherinenberg-Schnalstal, 6.viii.2002, *R.M. Ros & O. Werner s.n.* (MUB 17230) [AY934556] (*T. subulata* var. *graeffii*). MOROCCO: Bab-Taza, Jbel Bouhalla, 16.vi.1997, *M.J. Cano & R.M. Ros s.n.* (MUB 10951) (*T. subulata* var. *subulata*). Alto Atlas, Toubkal, subida desde Arndt hacia el refugio de Nelther, 2400 m, 19.vi.1998, *M.J. Cano et al. s.n.* (MUB 8365) (*T. inermis* var. *submarginata*). Hoher Atlas, zwischen Tadmant und Asni, suedl Marrakech, 18.iii.1986, *J.-P. Frahm s.n.* (MUB12584) [AY934562] (*T. inermis* var. *submarginata*). Tazzeka, SW of Taza, Jbel Tazekka National Park, near summit of J. Tazekka, 2100 m, 30.x.1993, *S.L. Jury & T.M. Upson 13092* (MUB 11791) (*T. inermis* var. *submarginata*). POLAND: Kraków-Częstochowa Upland, Olsztyn, 12 km SE of Częstochowa, 2.v.1987, *H. Bednarek-Ochyra & R. Ochyra 41/87* (S B72807) [AY934564] (*T. subulata* var. *subulata*). SPAIN: Albacete, Sierra del Calar del Mundo, umbría de la fuente de las Raigadas, 10.iv.1984, *M.N. Jiménez & R.M. Ros s.n.* (MUB 1640) (*T. inermis* var. *submarginata*). Albacete, Sierra del Calar del Mundo, Cañada de los Mojones, 26.iv.1984, *M.N. Jiménez s.n.* (MUB 1641) (*T. inermis* var. *subinermis*). Albacete, Sierra de Alcaraz, bajada del Puerto de las Crucetas, 8.v.1987, *R.M. Ros s.n.* (MUB 2223) (*T. inermis* var. *submarginata*). Almería, Sierra de Filabres, Gérgal, 'Piedras del Deseo', 26.v.1990, *P. García-Zamora & R.M. Ros s.n.* (MUB 8063) [AY934559] (*T. inermis* var. *submarginata*). Canary Islands, Tenerife, El Portillo, Las Cañadas, 5.iv.1977, *D.G. Long 5875* (E) [AY934561] (*T. inermis* var. *submarginata*). Granada, Sierra Nevada, Peñones de San Francisco, 21.iv.2002, *M.J. Cano s.n.* (MUB 17231) [AY934545] (*T. subulata* var. *subulata*). Huesca, pr. Pineta, Llanos de la Larri, *M.J. Cano 923a* (MUB 13579) [AY934554] (*T. subulata* var. *graeffii*). Huesca, Llanos del Hospital del Obispo hacia la Renclusa, Valle Álto del Ésera, 14.vii.1998, *M.J. Cano et al. s.n.* (MUB 8487) [AY934563] (*T. subulata* var. *subulata*). Huesca, Álto del Ésera, Camping los Baños de Benasque, 14.vii.1998, *M.J. Cano et al. s.n.* (MUB 8490) [AY934569] (*T. subulata* var. *angustata*). Madrid, Sierra de Guadarrama, subida al Puerto de Navafría, *M.J. Cano s.n.* (MUB 11595) (*T. subulata* var. *subinermis*). Zaragoza, Berrueco, Sierra de Santa Cruz-Valdelacasa, 15.iv.1999, *P. Heras 546/99* (VIT 24059) [AY934560] (*T. subulata* var. *subinermis*). SWEDEN: Närke, Glanshammar, *c.* 300 m NO om kyrkan, 31.v.1980, *N. Hakelier s.n.* (S B72997) (*T. subulata* var. *subulata*). 1 km north of Ottenby, 23.vii.1959, *E. Nyholm & A.C. Crundwell s.n.* (Herbarium A.J.E. Smith) (*T. subulata* var. *graeffii*). SWITZERLAND: Graubünden, bei Pontresina, vii.1883, *Graeffe s.n.* (JE) (*T. subulata* var. *graeffii*). Weissenstein (Jura), *W. Schimper s.n.* (STR) (*T. subulata* var. *angustata*). TURKEY: Ovit dagi Gecidi, 28.vi.2001, *C. Aedo s.n.* (MUB 13424). [AY934571] (*T. subulata* var. *angustata*). USA: Washington, Clallam Co., along Dungeness Trail from US Forest Service Road 2860 to boundary of Olimpic National Park along Royal Creek, Olympic National Forest, 8.x.1996, *D.H. Norris 88731* (UC) [AY934546] (*T. subulata* var. *subulata*).

Outgroups

Desmatodon latifolius (Hedw.) Brid.

SPAIN: Granada, Sierra Nevada, *Ros et al. s.n.* (MUB 11476) [AY934544].

Leptophascum leptophyllum (Müll. Hal.) J. Guerra & M. J. Cano

SPAIN: Murcia, Jardín La Seda, *S. Rams & Imbernon* (MUB 10427) [AY437134].

Protobryum bryoides J. Guerra & M.J. Cano

SPAIN: Murcia, Molina de Segura, 5.i.2004, *O. Werner s.n.* (MUB 18088) [AY934591].
Tortula canescens Mont.
SPAIN: Albacete, Sierra del Relumbrar, 6.iv.2001, *Ros s.n.* (MUB 11381) [AY934542].
Tortula cuneifolia (Dicks.) Turner
SPAIN: Cádiz, Sierra de Grazalema, pr. Villaluenga del Rosario, 2.i.2004, *M.J. Cano 1396* (MUB 15946) [AY934543].
Tortula muralis Hedw.
YUGOSLAVIA: Serbia, Belgrado, *M. Sabovljevic s.n.* (MUB 13827) [AY437132].