Systematic Botany (2005), 30(3): pp. 461–470

Preliminary Investigation of the Systematics of *Didymodon* (Pottiaceae, Musci) Based on nrITS Sequence Data

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Communicating Editor: Wendy B. Zomlefer

Abstract. A phylogenetic analysis of species of *Didymodon* from the Mediterranean area, Macaronesia, and Southwestern and Central Asia is presented. The ITS1, 5.8S rRNA gene, and ITS2 for 30 species have been sequenced, which represent about 25% of the recognized species in the genus. The molecular data confirm the monophyly of *Didymodon* as clearly separated from *Barbula*. The transfer of *Didymodon giganteus* to *Geheebia*, of *D. sinuosus* to *Oxystegus*, and *D. australasiae* to *Trichostomopsis* is not supported by the molecular data. The phylogenetic affinities of the species generally do not correspond with the infrageneric classification proposed for the genus based on morphological characteristics. The only monophyletic section is *Asteriscium*, but only after the inclusion of *D. bistratosus*.

Didymodon Hedw., a genus of 122 species, is taxonomically one of the most problematic groups in the moss family Pottiaceae. The genus is cosmopolitan and widely diversified in temperate and mountainous regions (Zander 1993) and found on a variety of substrates, mostly rocks or soil. The most recent treatments of Pottiaceae place *Didymodon* in different subfamilies but always in tribe Barbuleae. Chen (1941) included it in subfamily Barbuloideae, Saito (1975) and Corley et al. (1981) in the Pottioideae, and Zander (1993) in Merceyoideae. The view of Saito (1975) and Corley et al. (1981) is supported by molecular data of Werner et al. (2004b).

The definition of *Didymodon* has long been in dispute. The concept of the genus has changed a great deal since it was first described by Hedwig (1801), especially the morphological delimitation of *Didymodon* versus *Barbula* Hedw. Many authors have considered the peristome a key character to distinguish these genera: in *Barbula* comprising long and twisted teeth, and in *Didymodon*, short and straight teeth (Schimper 1876; Limpricht 1890; Brotherus 1923; Casares-Gil 1932; Lawton 1971). However, this criterion separates gametophytically very similar species into different genera—for example *D. fallax* and *D. spadiceus*, and *D. acutus* and *D. rigidulus*. Authors such as Dixon (1924), Mönkemeyer (1927), Hilpert (1933), Chen (1941), Nyholm (1989), Smith (1978), and Kürschner (2000) included all the species previously segregated into these two genera within *Barbula*. Hilpert (1933) justified *Barbula* s. l. by the presence of several shared characters: the leaf shape, the square to rounded, scarcely papillose upper laminal cells that gradually grade into the yellowish, rectangular, thick-walled basal cells, the typically homogeneous costa, and the almost undifferentiated perichaetial leaves.

Saito (1975) was the first author to use gametophytic characteristics to differentiate *Didymodon* and *Barbula*. He considered the axillary hairs of the leaves as the most important feature. The axillary hairs in *Didymodon* are formed of one or two short basal cells, always brown and containing several oil drops, quite unlike the upper hyaline cells. In *Barbula* the cells of the axillary hairs are uniformly hyaline throughout. Other characteristics used by Saito (1975) were the laminal cells of leaves well defined in surface view and the quadrate to short-oblong abaxial cells of the costa in *Didymodon*. In *Barbula* the laminal cells are mostly obscure in surface view and the abaxial cells of the costa are linear-oblong. Zander (1978) added other diagnostic gametophytic characteristics, such as the shape of the leaves, the differentiation of basal cells of leaves, and the number of cells in the gemmae. In *Didymodon* the leaves are mostly lanceolate to long-lanceolate, the basal cells are usually green, shortly rectangular and little differentiated, and the gemmae are formed of 1–10 cells. In *Barbula* the leaves are ovate to longly elliptical, the basal cells are hyaline, elongate and well differentiated, and the gemmae consist of 1–50 cells. These criteria have been accepted by most modern authors (Corley et al. 1981; Anderson et al. 1990; Li et al. 2001; Allen 2002; Smith 2004).

Didymodon has been divided into two to five sections. In his revision of the family Pottiaceae, Zander (1993) recognized sections *Asteriscium*, *Didymodon*, *Fallaces*, *Rufidulus* and *Vineales*. In a phylogenetic analysis of the North American species, Zander (1998) recognized two sections: *Didymodon* and *Fallaces*. Zander (1999) later revived section *Rufidulus* to include *D. nevadensis* R. H. Zander.

This controversy concerning sections of *Didymodon* was discussed by Zander (1998), who recognized that the infrageneric classification of the genus is difficult to apply at a world-wide level. This is also the situation

in studies of the Mediterranean, Macaronesian, and Southwestern and Central Asian species (Jiménez 2003).

Section *Fallaces* is characterized by a combination of morphological characters; the most typical is the presence of elongate cells on the adaxial surface of the costa, although there are some exceptions, which include *D. asperifolius* and *D. tomaculosus*, in which the cells are quadrate or shortly elongate. Zander (1993) considered many other morphological characters present in these two species, when defining the section. However, *D. asperifolius* also has features considered distinctive for section *Rufidulus*.

Sections *Vineales*, *Didymodon*, and *Rufidulus* are difficult to characterize morphologically. Examples are *Didymodon bistratosus* and *D. sicculus*. *Didymodon bistratosus* is morphologically similar to *D. vinealis* and *D. sicculus* to *D. luridus*, both of which, according to Zander (1993), belong in section *Vineales*. However, *D. bistratosus* has bistratose leaf lamina, typical of section *Didymodon*. *Didymodon sicculus* sometimes has irregularly bistratose leaf lamina (typical of section *Asteriscium*) and shows a yellowish green color reaction with KOH (section *Didymodon*). Another difficult species to place is *D. glaucus* that has the hyaline basal cells typical of section *Asteriscium* but lacks the bistratose leaf margins also characteristic of this section.

Section *Asteriscium*, as established by Zander (1993), is also defined by a combination of morphological characters. Nevertheless, it is not possible to establish a list of morphological characters exclusive to this section, since most are also present in other sections.

Correlated with the infrageneric division is the transfer of *Didymodon* species into closely related genera. For example, some authors treat the species of section *Asteriscium* as *Trichostomopsis* Cardot (Magill 1981; Crum and Anderson 1981; Düll 1992; Frey et al. 1995; Kürschner 2000; Cortini-Pedrotti 2001; Allen 2002) and *Husnotiella* Cardot (Grout 1939; Bartram 1949; Crum and Anderson 1981; Allen 2002). Other genera closely related to *Didymodon* are *Geheebia* Schimp. and *Oxystegus* (Limpr.) Hilp. These four genera have the morphological features of *Didymodon* according to Saito (1975) and Zander (1978). The taxonomic treatments of Kučera (2000) and Jiménez (2003) have confirmed that the species included in these genera belong in *Didymodon*. Furthermore, a recent molecular study of Pottiaceae (Werner et al. 2004b) using the chloroplast gene *rps4* suggests that some of the species belonging to these genera [*Geheebia gigantea* (Funck) Boulay, *Oxystegus sinuosus* (Mitt.) Hilp., and *Trichostomopsis australasiae* (Hook. & Grev.) H. Rob.] are closely related to *D. rigidulus*, a typical representative of the genus *Didymodon*.

Using nrITS molecular markers sequenced in 35 *Didymodon* specimens, we investigated the phylogeny of *Didymodon*. The aim was to investigate (1) the monophyly of *Didymodon*, (2) the phylogenetic relationship of *Didymodon* with *Barbula*, and (3) the monophyly of *Didymodon* sections established by Zander (1993) and whether morphological similarities result from adaptive convergence.

Materials and Methods

Plant Material. The selection of species is based on the taxonomic treatment by Jiménez (2003) for the Mediterranean area, Macaronesia, and Southwestern and Central Asia. Two species were added (*Didymodon maximus* and *D. tomaculosus*) in order to include all species of Europe. Four species were not included in the study because material suitable for sequencing was not available: *Didymodon anserinocapitatus* (X. J. Li) R. H. Zander, *D. johansenii* (R. S. Williams) H. A. Crum, *D. maschalogena* (Renauld & Cardot) Broth. and *D. revolutus* (Cardot) R. S. Williams. Forty-five accessions were sequenced, 35 in *Didymodon*, representing 30 species, and as outgroup, 10 accessions (nine species). The outgroups were selected according to a previous study of a broader selection of Pottiaceae species using chloroplast *rps*4 gene sequences (Werner et al. 2004b) and unpublished data. These include *Leptophascum leptophyllum*, *Tortula inermis*, *Tortula muralis*, *Trichostomum unguiculatum*, *Triquetrella arapilensis*, and *Triquetrella tristicha*. As taxa considered closely related to *Didymodon*, *Barbula unguiculata*, *Pseudocrossidium hornschuchianum*, and *Bryoerythrophyllum recurvirostrum* were also selected. Molecular data show that *Barbula* is polyphyletic; of the European species with available sequences, only *B. unguiculata* is closely related to *Didymodon* (Werner et al. 2004b; own unpublished data). Details concerning voucher data and GenBank accession numbers are given in Appendix 1.

DNA Extraction and Sequencing. Total DNA was extracted by the NaOH method (Werner et al. 2002), in which 5 μl of the crude NaOH extract were 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. PCR reactions were performed in an Eppendorf Mastercycler using 4 μl of the DNA solution in 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, own unpublished data). Amplification started with 3 min 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 a 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 0.1. Using other gap penalties did not significantly change the topology of the phylogenetic trees (data not shown). BioEdit (Hall 1999) was used for minor manual adjustments of the alignment. A DNA data matrix with the aligned sequences is available at TreeBASE (study accession S1263, matrix accession M2206).

MEGA 2.1 (Kumar et al. 2001) was used for the Minimum Evolution (ME) analysis and PAUP* (Swofford 1998) with maximum parsimony (MP) as the optimality criterion. MrBayes 3.0 (Huelsenbeck and Ronquist 2001) was used for the Bayesian analysis. Gaps were excluded from all phylogenetic analyses.

Using distance-based methods to reconstruct phylogenetic trees does not necessarily produce better results (Nei and Kumar 2000).

Therefore, in the case of ME, the Kimura 2-parameter distance was used in order to reflect the transition/transversion ratio. The maximum number of trees retained at each step was set to 100, and the Close Neighbor Interchange (CNI) search level, to two.

In the case of MP, all characters were given equal weight, and the heuristic search used the following settings: steepest descent off, TBR branch swapping, MULTREES on, 100 random-sequence additions saving an unlimited number of trees per replicate. Bootstrap analyses (Felsenstein 1985) were carried out with 1,000 replicates and identical settings for both ME and MP.

For the Bayesian analysis, 500,000 generations were run sampling every 100th generation. Modeltest ver. 3.5 (Posada and 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). Based on empirical evaluation, burn-in (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)

We consider good bootstrap support ≥70%, moderate support <70% and ≥50%, and poor support <50%. In the case of Bayesian clade credibility values, good support is estimated as ≥90%, moderate support as <90% and ≥70% and poor support as <70%.

Results

The combined length of the 18S rRNA gene (partial sequence)-ITS1–5.8S rRNA-ITS2–26S rRNA gene (partial sequence) region was 717 to 1044 base pairs (bp). The shortest sequence was of *Trichostomum unguiculatum*, and the longest sequence, of *Didymodon bistratosus*. Within *Didymodon*, the shortest sequence was of *D. sinuosus* (735 bp). The partial 18S rRNA gene had a uniform length of 26 bp. The ITS1 region was highly variable with multiple indels and ranged from 233 bp in *Trichostomum unguiculatum* to 551 bp in *Didymodon bistratosus*. Within *Didymodon*, *D. sinuosus* showed the shortest ITS1 region with 244 bp. The 5.8S rRNA gene had a constant length of 159 bp in all accessions. The ITS2 region was less variable in length in comparison with the ITS1 region measuring between 264 bp (*Trichostomum unguiculatum*) and 305 bp (*Pseudocrossidium hornschuchianum*). *Didymodon trivialis* had the longest ITS2 region within this genus with 293 bp, and *Didymodon tomaculosus*, the shortest (265 bp). The alignment had a total length of 1,444 bases.

Of the aligned sequences, 936 positions were gapped sites and excluded from further analysis. Of the remaining sites, 380 were constant, 39 variable but parsimony-uninformative, and 91 parsimony-informative.

The MP search revealed 93 most parsimonious trees of 265 steps, CI = 0.487, RI = 0.785 (considering only parsimony-informative sites) (Fig. 1). The ME criterion and the Bayesian inference resulted in very similar trees with an almost identical topology (Figs. 2, 3). The *Didymodon* clade was in both cases well supported with bootstrap values of 98% (MP) and 93% (ME) and a clade credibility value of 100% under Bayesian inference, thereby supporting the monophyly of *Didymodon*. A clade recognized with moderate to good support (MP 64% and ME 56% bootstrap support and Bayesian inference 97% clade credibility) is formed by a group of species between *D. subandreaeoides* and *D. tectorum* (Figs. 1–3). A well supported clade is formed by *D. umbrosus*, *D. bistratosus*, *D. trivialis*, *D. aaronis* and *D. australasiae* (MP 99% and ME 100% bootstrap support and Bayesian inference 100% clade credibility). The relative position of the remaining species of *Didymodon* is less clear but some monophyletic clades appear in all of our analyses. The first of these clades comprises *D. tophaceus*, *D. erosus*, and *D. sicculus*; the second clade, *D. spadiceus*, *D. maximus*, and *D. luridus*; and the third, *D. lamyanus*, *D. nicholsonii*, *D. insulanus*, and *D. vinealis*. *Didymodon giganteus* is embedded within *Didymodon*.

Discussion

Our molecular data support the monophyly of *Didymodon*, and its distinction from *Barbula*, as was proposed by Saito (1975) and followed by most current bryologists. Our data also support inclusion of *Didymodon giganteus* in *Didymodon*, which was formerly segregated by some authors into the monotypic genus *Geheebia*. This genus was segregated from *Didymodon* because of characters intermediate between *Trichostomum* Bruch and *Grimmia* Hedw., such as the large size of plants and the sinuous walls of the laminal cells.

Didymodon sinuosus has been often placed in *Oxystegus*, along with other species that have now mostly been transferred to *Trichostomum*. Norris and Koponen (1989) justified the segregation of *Oxystegus* on the basis of sharp papillae peripheral to the lumens in the leaf lamina, the long (more than 5:1), very frequently fragile leaves, and the usually thin-walled cells of the shoulders. These characteristics are only partially present in *D. sinuosus*. Moreover, this species has axillary hairs with brown basal cells, a character that is not usual in *Oxystegus* but present in all *Didymodon* species. The morphological similarity to *Didymodon* is confirmed by the molecular data. *Didymodon sinuosus* was included by Zander (1993) in section *Vineales*, where it shares some morphological features with other species, such as *D. insulanus*: the posture of the leaves, crisped when dry and erect-patent to spreading when moist, the linear-lanceolate leaf shape, the margins recurved in the lower half of the leaf, the presence of a hyaline area in the adaxial upper part of the costa, and the costal transverse section without adaxial stereids or with a layer of substereids (Jiménez 2003). Nevertheless, the species presents other characters that are absent from other *Didymodon* species studied, such as the fragile leaves with dentate margins in the upper part. The presence of dentate margins is an apomorphy for the species.

It is difficult to identify synapomorphies for sections of *Didymodon*, because usually only gametophytic

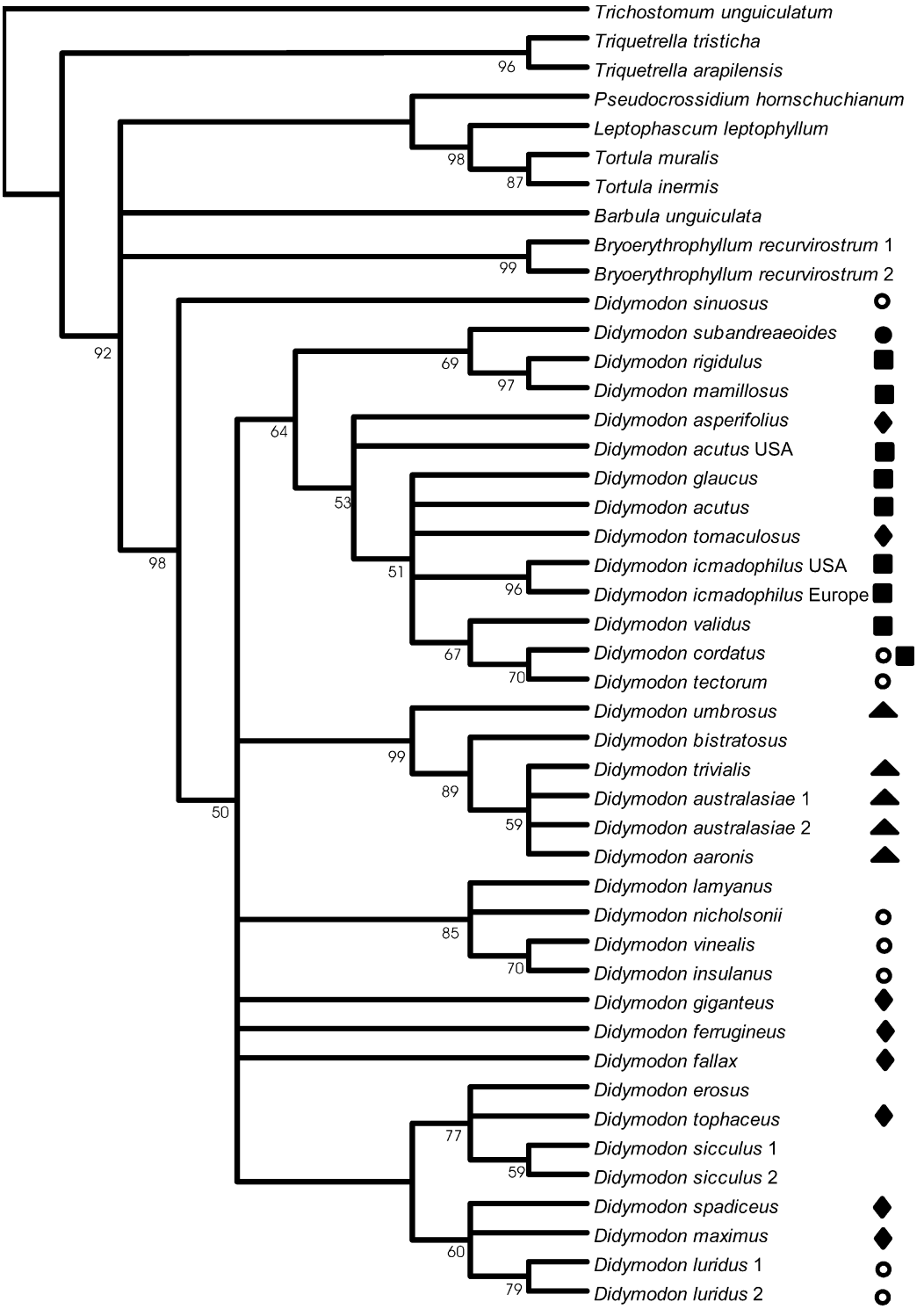

FIG. 1. Strict consensus of 93 most parsimonious trees with tree length of 265 steps (RI = 0.785665, CI = 0.487, considering only parsimony-informative sites), based on ITS sequences. Bootstrap values above 50% (1,000 replicates) are given below the branches. The traditional sections of *Didymodon* are indicated as: *Asteriscium* = solid triangles; *Didymodon* = solid squares; *Fallaces* = solid diamonds; *Rufidulus* = solid circles; *Vineales* = open circles. *Didymodon cordatus* has been placed in the sections *Didymodon* or *Vineales*. Species without section designation have not placed in any section to date.

FIG. 2. Minimum Evolution tree of the ITS sequences. Bootstrap values above 50% (1,000 replicates) are given below the branches. Symbols as in Fig. 1.

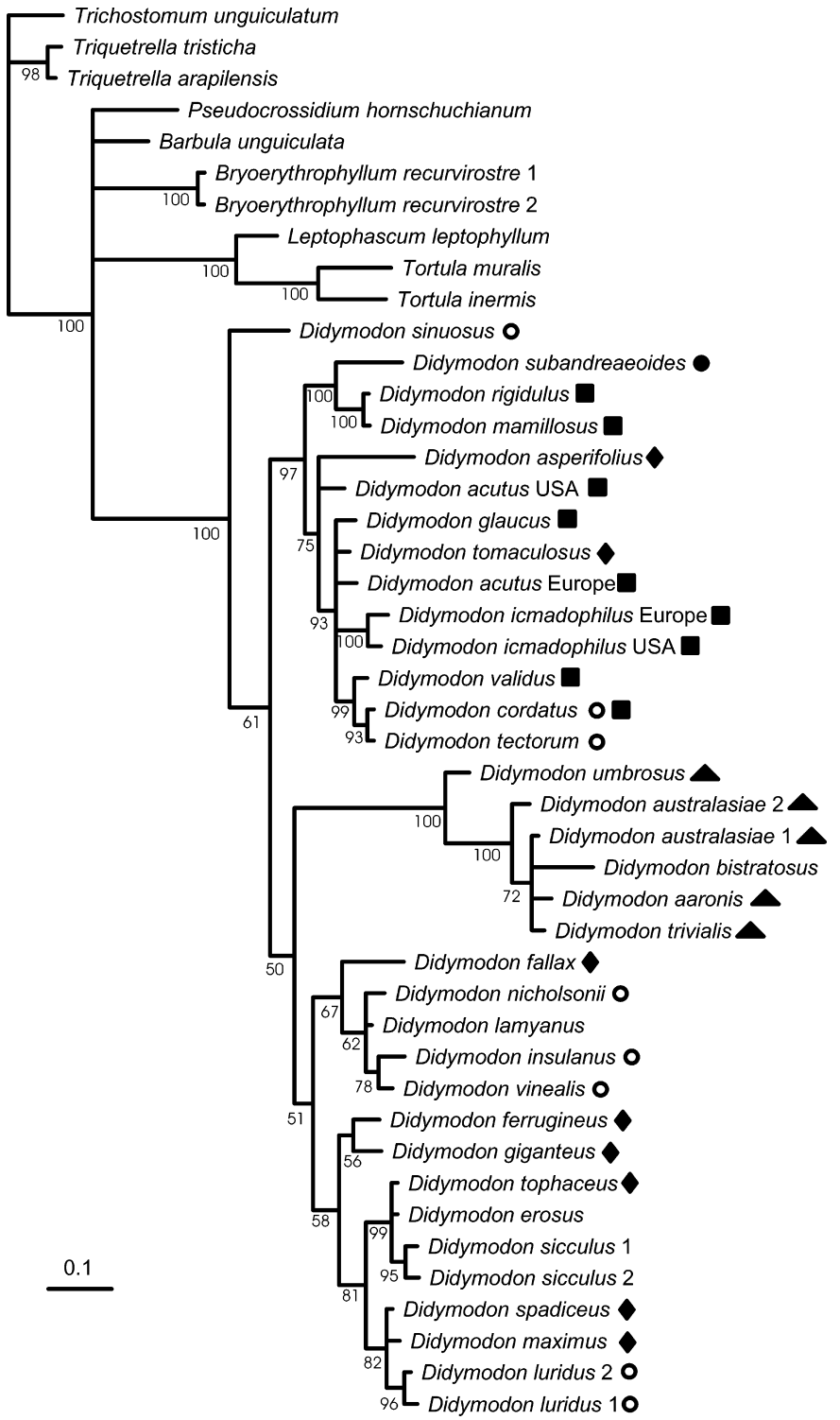

FIG. 3. Phylogram based on the Bayesian approach for the genus *Didymodon* with ITS sequence data. Clade credibility values above 50% are given below the branches. Values above 50% are shown. Symbols as in Fig. 1.

specimens are available. In addition, gametophytic characters are often homoplastic.

The cladograms obtained by Zander (1998) show a different topology from our results, partly due to the low number of species common to both studies (12 out of the 22 used by Zander and 30 used in our study). The only exception is the clade including *D. australasiae* and *D. umbrosus*, which are grouped together in both analyses.

The controversy concerning the segregation of some species of *Didymodon* section *Asteriscium* into *Trichostomopsis* has a long history (Jiménez et al. 2005). This genus is characterized by very lax hyaline basal cells, bistratose upper leaf margins, a section of the costa without adaxial stereids but with one layer of abaxial stereids, and peristome teeth scarcely twisted (Robinson 1970). These characters are present in section *Asteriscium*, although when the section was defined (Zander 1981; Zander 1993) other characters were included: the stem occasionally with a hyalodermis, the non-decurrent leaf margins, and the usually yellow or yellowish orange KOH color reaction.

According to Werner et al. (2004a), another species in the *Asteriscium* clade and characterized by the typical morphological features of this section is *Didymodon paramicola* (H. Rob.) O. Werner, J. A. Jiménez & Ros, considered previously as a member of the Dicranaceae (*Kingiobryum paramicola* H. Rob.). A species placed in the *Asteriscium* clade but morphologically different is *Didymodon bistratosus*. Our data suggest that this species is most closely related to species in section *Asteriscium*.

According to our sequence data, section *Asteriscium* is within *Didymodon* and does not merit recognition as a separate genus. This section needs to be re-evaluated morphologically, in order to include at least *D. bistratosus* and *D. paramicola*. In the light of the molecular data, section *Asteriscium* should include species with an adaxial stereid band in the costa and a red color reaction of the lamina with KOH.

The other sections of *Didymodon* are polyphyletic. For section *Fallaces* the Bayesian analysis (Fig. 3) indicates that species with elongate cells on the adaxial side of the costa (*D. fallax*, *D. ferrugineus*, *D. giganteus*, *D. maximus*, *D. spadiceus*, and *D. tophaceus*) are more closely related to each other than to the species with quadrate or shortly rectangular cells on the adaxial side of the costa (*D. asperifolius* and *D. tomaculosus*), although neither group is monophyletic.

In section *Vineales*, *Didymodon cordatus* and *D. tectorum* are separated by only three mutation steps, while the more similar sequence corresponds to *D. validus*, with 11 differences in the case of *D. cordatus* and 12 in the case of *D. tectorum*. This supports the close relationship of *D. cordatus* and *D. tectorum*, indicated by morphology. The synapormophic features of *D. cordatus* and *D. tectorum* are the deltoid, oblong or ovate-lanceolate leaves, the margins revolute up to the apex, and a stout costa, shortly excurrent in a mucro. In MP and Bayesian inference, these two species are a sister group to *D. validus*, traditionally included in section *Didymodon*. Three characters are likely synapomorphic in all three species: the stout and excurrent costa and the presence of adaxial stereids.

Didymodon insulanus, *D. nicholsonii*, and *D. vinealis* are closely related according to morphological and molecular data. *Didymodon lamyanus*, a species with unclear affinities within the genus, is placed in the same clade as the three former species with good support in MP and ME analyses. The significant synapomorphic characters are: lanceolate to triangular leaf shape, red color reaction of the lamina with KOH, the presence of a hyaline area on the adaxial side of the costa in the upper part of the leaf, and the abundant bifurcate papillae of the upper laminal cells. Our data do not support the morphological cladograms of Zander (1998), which indicate a close relationship between *D. vinealis* and *D. rigidulus*. Based on our molecular data, the latter species is in a separate clade with other species of section *Didymodon*.

Didymodon luridus is sister to *D. maximus* and *D. spadiceus*, two species traditionally included in section *Fallaces*. Morphologically they are different, and their relationship is unclear. Nevertheless, some characters such as the red color reaction with KOH and the costa ending in or below the apex may be synapomorphic. *Didymodon luridus* is morphologically similar to *D. sicculus*, a species not yet included in any section. *Didymodon sicculus* can be distinguished from *D. luridus* by the papillosity of its laminal cells. Also the color reaction of the leaf lamina is red in *D. luridus* and green-yellowish in *D. sicculus* (Cano et al. 1996; Jiménez et al. 2004). Molecular data show that they are not as closely related as they appear morphologically. The sequence data suggest that *D. sicculus* is most closely related to *D. tophaceus* and *D. erosus*. Synapomorphies for this clade are the papillose upper laminal cells and the ovate to lanceolate leaves.

According to the molecular data, section *Vineales* could be maintained with *D. vinealis*, *D. insulanus*, *D. lamyanus*, and *D. nicholsonii*. Synapomorphies are the presence of a hyaline area in the adaxial upper part of the costa and the abundant bifurcate papillae in the upper laminal cells. The rest of the species previously included in section *Vineales* and species of section *Fallaces* with elongated cells on the adaxial side of the costa appear in several clades, and their precise infrageneric classification remains uncertain.

The species sequenced in section *Didymodon* are included in one clade supported by the molecular analysis. However, they do not form a monophyletic group, since members of other sections are also members of

this clade. One character that may define this clade is the presence of an excurrent costa in the leaf. This character may have been lost in *D. asperifolius* and *D. subandreaeoides*. *Didymodon cordatus* has been included in the section *Vineales* by Zander (1993), while Corley et al. (1981) place it in section *Didymodon*, which agrees with our molecular data.

Section *Didymodon* should be expanded to include the species of the *Didymodon* clade, including *D. asperifolius*, *D. cordatus*, *D. subandreaeoides*, *D. tectorum*, and *D. tomaculosus*. Besides the excurrent costa, other outstanding characters that may be synapomorphic are the presence of an adaxial stereid band and 1(2) layers of guide cells in the costa, and the leaves appressed when dry.

The concept of *Didymodon acutus* (Smith 1978; Kučera 2000, 2002; Casas et al. 2001; Cortini-Pedrotti 2001) is based on the presence of unistratose margins with non-papillose cells. Zander (1998) and Allen (2002) describe the species as having occasionally bistratose margins and usually papillose laminal cells. Our data indicate that the same name has been used for two different taxa. Because the exclusion of the gaps lowered the number of possible informative sides of the *D. acutus* clade, the species of this clade were reanalyzed. In the new analysis, the two samples of *D. acutus* are separated with good support under MP (84%) and ME (90%) and moderate support (88%) in the case of Bayesian inference (data not shown).

ACKNOWLEDGEMENTS. We thank the curators of BCB, DUKE, PAMP, and SALA for sending material, and to T. L. Blockeel, R. Düll, R. Skrzypczak, C. C. Townsend for loans from their personal herbaria. This research was supported by the Spanish Ministerio de Ciencia y Tecnología (Projects BOS2001-0276 and REN2003-00766) and Fundación Séneca of Murcia Region.

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APPENDIX 1

Voucher specimens and GenBank accession numbers for the taxa included in the molecular analysis.

Barbula unguiculata Hedw. Germany, Baden-Württemberg, *Ros & Werner s.n.*, [9 Aug 2000], (MUB). AY437129

Bryoerythrophyllum recurvirostrum (Hedw.) P. C. Chen (1). Italy, Trentino-Alto Adige, *Ros & Werner s.n.*, [30 Jul 2000], (MUB). AY437130; *Bryoerythrophyllum recurvirostrum* (Hedw.) P. C. Chen (2). Italy, Trentino-Alto Adige, *Ros & Werner s.n.*, [6 Aug 2000], (MUB). AY437131

Didymodon aaronis (Lorentz) J. Guerra. Spain, Almería, *Ros s.n.*, [19 Nov 1988], (MUB). AY437120; *Didymodon acutus* (Brid.) K. Saito. Canada, Quebec, *Hedderson 7631* (DUKE). AY437109; *Didymodon acutus* (Brid.) K. Saito. Greece, Peloponnisos, *Cano et al. s.n.*, [15 Mar 1999], (MUB). AY437111; *Didymodon asperifolius* (Mitt.) H. A. Crum, Steere & L. E. Anderson. Spain, Lérida, *Ballesteros s.n.*, [24 Aug 1986], (BCB). AY437089; *Didymodon australasiae* (Hook. & Grev.) R. H. Zander (1). Greece, Peloponnisos, *Cano et al. s.n.*, [15 Mar 1999], (MUB). AY437118; *Didymodon australasiae* (Hook. & Grev.) R. H. Zander (2). Morocco, Souss Massa-Draâ., *Draper et al. s.n.*, [19 Jun 2000], (MUB). AY437121; *Didymodon bistratosus* Hébr. & R. B. Pierrot. Spain, Málaga, *Hébrard s.n.*, [14 Apr 1988], (MUB). AY437124; *Didymodon cordatus* Jur. Spain, Lérida, *Cano et al. s.n.*, [17 Aug 1998], (MUB). AY437115; *Didymodon erosus* J. A. Jiménez & J. Guerra. Spain, Salamanca, *Elías s.n.*, [6 Feb 1985], (SALA). AY 437094; *Didymodon fallax* (Hedw.) R. H. Zander. Spain, Balear Islands, *Cano et al. s.n.*, [14 Apr 1999], (MUB). AY437099; *Didymodon ferrugineus* (Schimp. ex Besch.) M. O. Hill. Italy, Trentino-Alto Adige, *Ros & Werner s.n.*, [30 Jul 2002], (MUB). AY437100; *Didymodon giganteus* (Funck) Jur. Germany, Bavaria, *Cano s.n.*, [26 Jun 1999], (MUB). AY437101; *Didymodon glaucus* Ryan. Sweden, Väternorrland, *Hallingbäck 38777* (MUB). AY437110; *Didymodon icmadophilus* (Schimp. ex Müll. Hal.) K. Saito. France, Savoie, *Skrzypczak 2297* (Herb. R. Skrzypczak). AY437112; *Didymodon icmadophilus* (Schimp. ex Müll. Hal.) K. Saito. Canada, Northwest Territories, *Allen 19598* (DUKE). AY432213; *Didymodon insulanus* (De Not.) M. O. Hill. Morocco, Souss Massa-Draâ, *Draper et al. s.n.*, [19 Jun 2000], (MUB). AY437102; *Didymodon lamyanus* (Schimp.) Thér. France, Puy-de-Dome, *Pierrot 89054* (MUB). AY437105; *Didymodon luridus* Hornsch. (1). Greece, Central Greece and Euboea, *Cano s.n.*, [30 Aug 2003], (MUB). AY437098; *Didymodon luridus* Hornsch. (2). Morocco, Meknès-Tafilalt, *Cano et al. s.n.*, [22 Feb 1999], (MUB). AY437097; *Didymodon mamillosus* (Crundw.) M. O. Hill. Andorra, *Cano s.n.*, [17 Aug 2002], (MUB). AY437107; *Didymodon maximus* (Syed & Crundw.) M. O. Hill. Ireland, Sligo, *Jury s.n.* [24 Sep 1983], (BCB). AY437096; *Didymodon nicholsonii* Culm. Spain, Lugo, *Cano s.n.*, [31 Mar 2002], (MUB). AY437104; *Didymodon rigidulus* Hedw. England, Derbyshire, *Blockeel s.n.*, [9 Jun 2002], (Herb. Blockeel). AY437106; *Didymodon sicculus* M. J. Cano, Ros, García-Zamora & J. Guerra (1). Spain, Murcia, *Werner s.n.*, [27 Feb 2000], (MUB). AY437091; *Didymodon sicculus* M. J. Cano, Ros, García-Zamora & J. Guerra (2). Morocco, Guelmim, *Cano et al. s.n.*, [9 Mar 2001], (MUB). AY437092; *Didymodon sinuosus* (Mitt.) Delogne. Italy, Sicily, *Ros s.n.*, [16 Sep 2001], (MUB). AY437090; *Didymodon spadiceus* (Mitt.) Limpr. Spain, Navarra, *de Miguel s.n.*, [Aug 1982], (PAMP). AY437095; *Didymodon subandreaeoides* (Kindb.) R. H. Zander. France, Savoie, *Skrzypczak 2317*; (Herb. Skrzypczak). AY437108; *Didymodon tectorum* (Müll. Hal.) K. Saito. Rusia, Alania, *Townsend 85/813* (Herb. Townsend). AY437116; *Didymodon tomaculosus* (Blockeel) M. F. V. Corley. England, Wakefield, *Blockeel 31/417* (Herb. Blockeel). AY437114; *Didymodon tophaceus* (Brid.) Lisa. Greece, Central Greece and Euboea, *Blockeel 29/079* (Herb. Blockeel). AY437093; *Didymodon trivialis* (Müll. Hal.) J. Guerra. France, Alps-Maritimes, *Skrzypczak 1087* (Herb. Skrzypczak). AY437119; *Didymodon umbro-*

sus (Müll. Hal.) J. Guerra. France, Alps-Maritimes, *Skrzypczak 2460* (Herb. Skrzypczak). AY437123; *Didymodon validus* Limpr. Italy, Trentino-Alto Adige, *Düll s.n.*, [Aug 1995], (Herb. Düll). AY437117; *Didymodon vinealis* (Brid.) R. H. Zander. Spain, Balear Islands, *Cano et al. s.n.*, [14 Apr 1999], (MUB). AY437103

Leptophascum leptophyllum (Müll. Hal.) J. Guerra & M. J. Cano. Spain, Murcia, *Rams s.n.*, [Oct 1997], (MUB). AY437134

Pseudocrossidium hornschuchianum (Schultz) R. H. Zander. Spain, Almería, *García-Zamora et al. s.n.*, [13 Jun 1991], (MUB). AY437128

Tortula inermis (Brid.) Mont. Greece, Steréa Ellas, *Cano s.n.*, [28 Jul 1999], (MUB). AY437133; *Tortula muralis* Hedw. Yugoslavia, Serbia, *Sabovljevic s.n.*, [17 Sep 2002], (MUB). AY437132; *Trichostomum unguiculatum* (Mitt.) R. H. Zander. South Africa, Western Cape province, *Arts 107/18* (MUB). AY437127

Triquetrella arapilensis Luisier. Spain, Ciudad Real, *Fuertes s.n.*, [28 May 1996], (MUB). AY437127; *Triquetrella tristicha* (Müll. Hal.) Müll. Hall. South Africa, Western Cape province, *Arts 105/11* (MUB). AY437125