The *Didymodon tophaceus* complex (Pottiaceae, Bryophyta) revisited: new data support the subspecific rank of currently recognized species

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Abstract – Additional samples of *Didymodon erosus* and *D. sicculus*, barcoded using ITS sequences, necessitated the broadening of the morphological concepts and the reported ecology of these taxa, both of which were revealed to have considerable overlap. Additional molecular data from the chloroplast genome confirmed the earlier published phylogenetic inference based on the nuclear ITS, which groups *Didymodon tophaceus*, *Didymodon erosus* and *D. sicculus* into a highly supported lineage of closely related taxa. In view of the morphological overlap among taxa of the *D. tophaceus* complex, supported by the ITS non-monophyly of *D. tophaceus* in its current morphological concept, we advocate the reduction of *D. erosus* and *D. sicculus* to infraspecific rank within *D. tophaceus*. Sporophytes are described for the first time in *D. erosus*, and new country records for the latter two taxa are reported.

***Didymodon* section *Fallaces* / *Didymodon erosus* / *Didymodon sicculus* / nrITS / *rps4-trnS* / *trnM-trnV* / taxonomy / subspecies / Ecuador / Albania / Hungary / Czech Republic**

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INTRODUCTION

Didymodon tophaceus (Brid.) Lisa is a long-established member of the European bryoflora, also reported from several regions of the other continents (Asia, Africa, the Americas, Australia, Pacific Islands; Jiménez, 2005; Zander, 2007). Being a relatively common species in suitable habitats, which typically include wet calcareous soil and base-rich rock fissures in warmer regions, it has been acknowledged to be morphologically rather variable. About 25 infraspecific taxa have been formally described in the course of the 19th and 20th centuries (www.tropicos.org) but none of these infraspecific taxa have been commonly recognized in regional floras, and until the study of Werner *et al.* (2009) no additional species were identified as forming a complex of closely related taxa with *D. tophaceus*. However, the lineage of taxa closely related to *D. tophaceus*, which includes *D. sicculus* M.J.Cano, Ros, García-Zam. & J.Guerra and *D. erosus* J.A.Jiménez & J.Guerra, had already been discussed in the earlier phylogenetic analysis of *Didymodon* by Werner *et al.* (2005), based on molecular sequence data.

Cano *et al.* (1996) described *D. sicculus* from arid regions of Spain, a moss that has been subsequently reported from numerous other sites, particularly in the Mediterranean region (summarised in Papp *et al.*, 2012). The first Estonian record was reported by Vellak *et al.* (2015), the first Croatian one by Papp *et al.* (2016), and the first German record by Müller (2017). Outside Europe, *D. sicculus* has been reported from Cape Verde (Cano, 2016). Eight years after the publication of *D. sicculus*, Jiménez *et al.* (2004) described *Didymodon erosus*, another species of the complex, from two localities in northwestern Spain. While *D. sicculus*, which to a certain extent combines the characters of *D. luridus* Hornsch. and *D. tophaceus,* was originally thought to be related to *D. tophaceus*, *D. erosus* has been considered to be closely related to *D. sinuosus* (Mitt.) Delogne. Some of the morphological characteristics of *D. sicculus* and *D. erosus*, such as the mostly quadrate ventral costa cells in the upper part of the leaf, prevented Jiménez *et al.* (2005) from including the two species in their concept of section *Fallaces* (De Not.) R.H.Zander. Thus, the first formal recognition of the *D. tophaceus* complex in the sense of the lineage identified by Werner *et al.* (2005) did not occur before the treatment of Werner *et al.* (2009). These authors were able to amend the description of *D. sicculus* and particularly of *D. erosus* using the molecular barcoding of certain puzzling specimens, which mostly resembled stunted morphs of *Didymodon tophaceus*. The results of this study also extended the known distribution of *D. sicculus* north to the Netherlands, and additional localities of *D. erosus* were reported from the Iberian Peninsula, southern France, Austria and Germany.

The molecular sampling of Werner *et al.* (2009) included 12 specimens from Europe and the U.S.A., five specimens of *D. sicculus* from Spain and Netherlands, and seven specimens of *D. erosus* from Spain, Portugal, France, Germany and Austria. The specimens were sequenced only for nrITS. The results of the phylogenetic analysis suggested the monophyly of the complex, as well as the monophyletic nature of *D. sicculus* and *D. erosus*, but in *D. tophaceus* the situation was more complicated. One Austrian sample appeared as a separate lineage along with the lineages comprising most other *D. tophaceus* specimens, *D. sicculus* and *D. erosus*, while the American sample appeared in a sister position to all other taxa of the *D. tophaceus* complex.

Over the last couple of years, the first author (JK) was repeatedly consulted by multiple bryologists about specimens that did not match comfortably even the amended descriptions of the three species of the *D. tophaceus* complex, as provided by Werner *et al.* (2009). Although not a part of any other major project, JK was gradually able to barcode some of the puzzling specimens for ITS and later also for two chloroplast loci, *rps4-trnS* and *trnM-trnV*. This allowed him to verify the results obtained by Werner *et al.* (2009) using data from another genomic compartment, and to decide whether to broaden the morphological concept of the three taxa of the *D. tophaceus* complex, or describe new taxa, or rather merge the taxa of the complex into a single species.

MATERIAL AND METHODS

Molecular sampling of taxa

Twenty-one specimens of the *Didymodon tophaceus* complex (four identified as *D. tophaceus*, five as *D. erosus* and 12 as *D. sicculus*, of which only six were analysed for all loci and used in the analyses of concatenated data) were used in the molecular analyses. The nuclear genome was represented by the ribosomal ITS region (ITS1 + 5.8S rRNA + ITS2), and the chloroplast genome was represented by *rps4-trnS* and *trnM-trnV* loci, successfully applied in our earlier studies. Our accessions were supplemented with those used in Werner *et al.* (2009) in the separate analysis of ITS to make the results of the studies fully comparable. Outgroup selection was extended as compared with Werner *et al.* (2009), where only three other species of the section *Fallaces* were used. Based on the results of our other studies (particularly Kučera & Ignatov, 2015; Blockeel *et al.*, 2017), and on the preliminary analyses from JK's library of molecular data, we used all available species of *Didymodon* section *Fallaces* complemented with a limited selection of species from other sections and three representatives of the most closely related genera of Pottioideae (*Barbula* Hedw., *Pseudocrossidium* R.S.Williams and *Bryoerythrophyllum* P.C.Chen). A full list of analysed specimens is included in Appendix I.

Molecular protocols, sequence editing, alignment and data analysis

DNA extraction, amplification and sequencing of the target regions (ITS, *rps4-trnS*, *trnM-trnV*) was performed by JK, and followed the protocols described in Köckinger & Kučera (2011) and Kučera *et al.* (2013), respectively. The raw sequences obtained were edited and aligned using the procedures and software described in Kučera & Ignatov (2015). The final alignment is available on request from JK. Chloroplast and nrITS data were first evaluated both separately and together in a concatenated matrix, with partitions corresponding to the three analysed loci and indel data, which were scored using the simple indel coding method (Simmons & Ochoterena, 2000). Phylogenetic analyses were performed using the Bayesian inference (BI) in MrBayes v. 3.2.6 (Ronquist *et al.*, 2012) and maximum likelihood (ML), computed using the RAxML v. 8.2.4 (Stamatakis, 2014).

RESULTS

Molecular data

        The final ITS alignment of the matrix used for concatenation had 1141 site positions (of which 608 belonged to ITS1, 159 to 5.8S rRNA and 374 to ITS2), the chloroplast matrix had 1396 positions, of which 641 belonged to *rps4-trnS* and 755 belonged to the *trnM-trnV* region; 51 sites were coded as binary indel data. The extended ITS dataset which included the data of Werner *et al.* (2009) had 1132 site positions. Analysis of ITS data from the extended dataset (Fig. 1) resolved the *Didymodon tophaceus* complex in the sense of Werner *et al.* (2009) as a well-supported monophyletic group, sister to a clade that includes *Didymodon fallax* (Hedw.) R.H.Zander and *D. spadiceus* (Mitt.) Limpr. (hereafter the *D. fallax* clade), and *D. luridus* sister to the *D. fallax* clade with the *D. tophaceus* complex (virtually unsupported as a clade). The whole clade, consisting of *D. luridus*, the *D. fallax* clade and the *D. tophaceus* complex is highly supported and on a relatively long branch. The rest of sect. *Fallaces* (*D. ferrugineus* (Schimp. *ex* Besch.) M.O.Hill, *D. erosodenticulatus* (Müll.Hal.) K.Saito, *D. maximus* (Syed & Crundw.) M.O.Hill, *D. leskeoides* K.Saito, *D. giganteus* (Funck) Jur.) appears basal to that clade, and the accessions representing sect. *Vineales* (Steere) R.H.Zander (*D. vinealis* (Brid.) R.H.Zander, *D. occidentalis* R.H.Zander) appear basal to the well-supported sect. *Fallaces*. Within the *D. tophaceus* complex, the accessions cluster to three clades corresponding to *D. tophaceus*, *D. sicculus* and *D. erosus* but the American sample of *D. tophaceus* appears basal to the whole complex. The Austrian specimen Köckinger *12353*, interpreted as *D. tophaceus* in the treatment of Werner *et al.* (2009), clusters clearly with *D. erosus*. Another specimen from Ecuador, identified initially as *D. tophaceus*, belongs to the *D. erosus* clade.
        The chloroplast data (Fig. 2) show essentially the same picture of relationships except, e.g., for the clustering within sect. *Fallaces* and the missing clade of the two analysed samples of sect. *Vineales*. The analysis of concatenated ITS + cp data (not shown) displays results essentially identical to those retrieved by the analysis of chloroplast data alone, except for the weak support for the monophyly of *D. giganteus* and the clade consisting of members of sect. *Vineales*, and the generally higher support and longer branches of most clades, all driven by the signal from ITS data. Unfortunately, the above-mentioned American specimen was not available to us.

Morphological data

        As mentioned in the introduction, some of the newly investigated specimens did not fit within the variability of any of the three species of the *Didymodon tophaceus* complex, as described by Werner *et al.* (2009). For example, the specimen Papp *14/65/3* from Albania (Figs 5, 10, 14) shows rather large (to 2 mm long), lingulate leaves with a strong costa (to 100 μm wide with up to 3 ventral stereid layers) but the ventral costa cells in the upper part of the leaves are at most shortly rectangular. The specimen clustered molecularly clearly with *D. erosus* (Figs 1, 2). Ovate-lingulate leaves (among ovate-lanceolate ones) were present on plants of the sporulating specimen Blockeel *43/242* (Fig. 22), which clustered with *D. sicculus*; the costa was also relatively stout (to 80 μm wide with 1-2 ventral stereid layers), and the ventral costa cells in the upper part of the leaves were at most shortly

Barbula unguiculata Austria
Pseudocrossidium hornschuchianum Austria
Pseudocrossidium denticulatum Chile
Bryoerythrophyllum recurvirostrum Czechia
Didymodon sinuosus Czechia
Didymodon xanthocarpus South Africa
Didymodon torquatus New Zealand
Didymodon acutus Czechia
Didymodon rigidulus Czechia
Didymodon vinealis Spain
Didymodon occidentalis Canada
Didymodon giganteus Nepal
Didymodon giganteus China
Didymodon giganteus Austria
Didymodon giganteus Germany
Didymodon leskeoides Nepal
Didymodon leskeoides China
Didymodon maximus Ireland
Didymodon maximus Mongolia
Didymodon maximus Canada
Didymodon maximus China
Didymodon erosodenticulatus Russia
Didymodon ferrugineus Austria
Didymodon ferrugineus India
Didymodon luridus Czechia
Didymodon luridus Tenerife
Didymodon spadiceus Czechia
Didymodon spadiceus Russia
Didymodon fallax Czechia 2
Didymodon fallax Czechia 1
Didymodon fallax Ireland
Didymodon tophaceus USA EU835149
Didymodon sicculus Morocco AY437092
Didymodon sicculus Serbia 1
Didymodon sicculus Estonia
Didymodon sicculus Italy 2
Didymodon sicculus Croatia
Didymodon sicculus Spain EU835126
Didymodon sicculus Spain AY437091
Didymodon sicculus Greece
Didymodon sicculus Portugal EU835132
Didymodon sicculus Spain EU835123
Didymodon sicculus Serbia 2
Didymodon sicculus France
Didymodon sicculus Italy 1
Didymodon sicculus Hungary
Didymodon sicculus Netherlands EU835124
Didymodon erosus Austria
Didymodon erosus Ecuador
Didymodon erosus France
Didymodon erosus Hungary
Didymodon erosus Germany EU835145
Didymodon erosus Germany EU835146
Didymodon erosus Albania
Didymodon erosus Spain AY437094
Didymodon tophaceus Greece AY437093
Didymodon tophaceus var. *anatinus* France
Didymodon tophaceus Norway
Didymodon tophaceus Czechia EU835130
Didymodon tophaceus Greece EU835134
Didymodon tophaceus Malta EU835122
Didymodon tophaceus Spain EU835135
Didymodon tophaceus Spain EU835131
Didymodon tophaceus Spain EU835133
Didymodon tophaceus England MF536571
Didymodon tophaceus Hungary
Didymodon tophaceus Czechia EU835127
Didymodon tophaceus Czechia EU835128
Didymodon tophaceus England EU835129

0.0   0.05   0.1

Fig. 1. Majority consensus tree from the Bayesian analysis of the extended version of ITS data. Branch lengths correspond to expected substitutions per site (scale). Branch widths correspond to obtained support (bold lines indicate very high support in both Bayesian analysis (BI) – posterior probability (PP) $\geq$ 0.99 and Maximum Likelihood (ML; bootstrap value (BS) $\geq$ 95 (650 replications)) analysis, semi-bold lines indicate moderate support – PP $\geq$ 0.95 and BS $\geq$ 75). Asterisk (above the branches for BI and below the branches for ML) denote maximum support (PP = 1.0, resp. BS = 100).
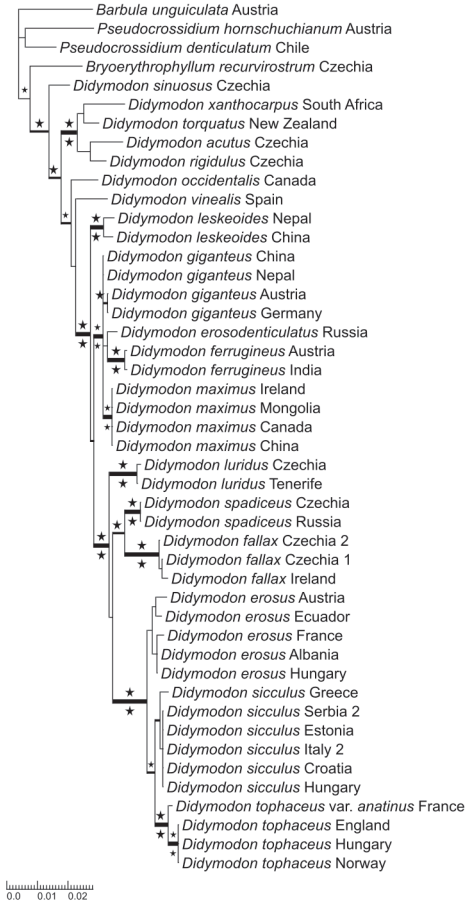
Barbula unguiculata Austria
Pseudocrossidium hornschuchianum Austria
Pseudocrossidium denticulatum Chile
Bryoerythrophyllum recurvirostrum Czechia
Didymodon sinuosus Czechia
Didymodon xanthocarpus South Africa
Didymodon torquatus New Zealand
Didymodon acutus Czechia
Didymodon rigidulus Czechia
Didymodon occidentalis Canada
Didymodon vinealis Spain
Didymodon leskeoides Nepal
Didymodon leskeoides China
Didymodon giganteus China
Didymodon giganteus Nepal
Didymodon giganteus Austria
Didymodon giganteus Germany
Didymodon erosodenticulatus Russia
Didymodon ferrugineus Austria
Didymodon ferrugineus India
Didymodon maximus Ireland
Didymodon maximus Mongolia
Didymodon maximus Canada
Didymodon maximus China
Didymodon luridus Czechia
Didymodon luridus Tenerife
Didymodon spadiceus Czechia
Didymodon spadiceus Russia
Didymodon fallax Czechia 2
Didymodon fallax Czechia 1
Didymodon fallax Ireland
Didymodon erosus Austria
Didymodon erosus Ecuador
Didymodon erosus France
Didymodon erosus Albania
Didymodon erosus Hungary
Didymodon sicculus Greece
Didymodon sicculus Serbia 2
Didymodon sicculus Estonia
Didymodon sicculus Italy 2
Didymodon sicculus Croatia
Didymodon sicculus Hungary
Didymodon tophaceus var. *anatinus* France
Didymodon tophaceus England
Didymodon tophaceus Hungary
Didymodon tophaceus Norway

0.0   0.01   0.02

Fig. 2. Majority consensus tree from the Bayesian analysis of the concatenated chloroplast data matrix, partitioned according to the loci (*rps4-trnS*, *trnM-trnV*, indels). For explanations of symbols, see caption to Fig. 1.

rectangular. An even stronger costa (to 95 μm wide) was present in the specimen Papp *14/5* (Figs 20, 28). Lanceolate, not shortly ovate leaves were also present in plants of Kučera *16279* (Fig. 23). Moreover, these plants showed clearly erose margins in the apex (Fig. 26) but again, the molecular accessions clustered clearly with *D. sicculus*. The specimen of *D. tophaceus* Blockeel *43/004* from England also has erose margins in the leaf apex and a percurrent costa (Figs 34, 40), typical of *D. erosus* in the sense of Werner *et al.* (2009) but the ventral costa cells are elongate as in *D. tophaceus* and also the molecular relationships are clearly with that clade. Overall, we were unable to define exclusive character sets that would unequivocally diagnose the three taxa, except mostly for the shape of the ventral costa cells (quadrate to short-rectangular in *D. sicculus* and *D. erosus*, always elongate in *D. tophaceus*), more apparently decurrent leaves in *D. tophaceus*, and the absence of a peristome in *D. erosus*. However, the absence of a peristome in *D. erosus* was seen in a single specimen from Ecuador and might be more variable (see Discussion).

Taxonomic evaluation

Given the existence of lineages that are molecularly (and morphologically) not clearly assignable, we believe that the best taxonomic solution at present is the recognition of the three taxa as subspecies of *Didymodon tophaceus*. This is formalised below with amended or corrected descriptions of morphology, ecology and distribution data.

Didymodon tophaceus (Brid.) Lisa **subsp.** *tophaceus*   **Figs 32-41**

For the list of synonyms (not revised in this treatment), description and illustration, see Jiménez *et al.* (2005).
New observations: stunted specimens, such as Lye *36934* (Figs 33, 36) show only a weakly developed stem central strand and sclerodermis, and leaf shape and size may completely match moderately developed plants of *D. erosus* and *D. sicculus*. Even in such specimens, however, the ventral costa cells seem to be generally elongated and rhizoidal tubers were not observed. Most descriptions emphasize the costa not reaching the apex; however, the specimen Hauguel *s.n.* (CBFS: 4465) has the costa excurrent in a stout mucro (Figs 35, 41), showing a habit similar to *D. validus* Jur. Such morphs are probably identical to var. *anatinus* Hamm. Interestingly, the unusual morphology is also matched by the somewhat isolated position in the phylogenetic tree (Figs 1, 2), although the North American sample is still much more distant. The possible absence of a peristome, reported by Zander (1993, 2007), might refer to *D. erosus* (see below) but more extra-European samples need to be studied to verify this fact. Jiménez *et al.* (2005) did not allow for the absence of a peristome in their description. The ecology reported in some collections, such as the above-mentioned Lye *36934*, matches that reported for *D. sicculus*; drier habitats are definitely able to support the presence of the species at some sites.

Didymodon tophaceus **subsp.** *erosus* (J.A.Jiménez & J.Guerra) Jan Kučera, ***comb. et stat. nov.***   **Figs 3-17**

Bas. *Didymodon erosus* J.A.Jiménez & J.Guerra, *Nova Hedwigia* 78: 502. f. 1-2. 2004.
New observations: despite a significant broadening of the morphological concept of the taxon by Werner *et al.* (2009), the description needs to be amended again. The leaves can be even larger than indicated earlier (up to 1.85 × 0.50 mm), with a lingulate apex and the margins broadly recurved to 4/5 of the leaf length (Figs 5, 10). The costa may reach *ca* 100 μm in section and show up to 3 layers of ventral and 4 layers of dorsal stereids. The stem cross section may have a well-developed sclerodermis. Such specimens nevertheless clearly show short (quadrate to shortly rectangular) ventral superficial cells over the costa in the upper part of the leaf (Fig. 10). On the other hand, stunted plants from Ecuador (Soldán *s.n.*, CBFS *19364*), showed elongate ventral costa cells throughout (Figs 7, 11), although better developed plants from the same site (CBFS *19365*, Figs 8, 12) also had short ventral costa cells. This variability calls for caution in evaluating this character, as already emphasized by Werner *et al.* (2009). The specimen Köckinger *12353* (Fig. 6) was evaluated as *D. tophaceus* by Werner *et al.* (2009), although the ventral costa cells are clearly short and the molecular data from ITS did not show a clear identification as *D. tophaceus*. The leaves of this plant are slightly larger and the costa stronger than in the stunted specimens of *D. erosus* which were available to Werner *et al.* (2009), but clearly fall within the variability of *D. erosus*, as indicated here. Nevertheless, both the ITS and chloroplast data suggest that the plant belongs to a
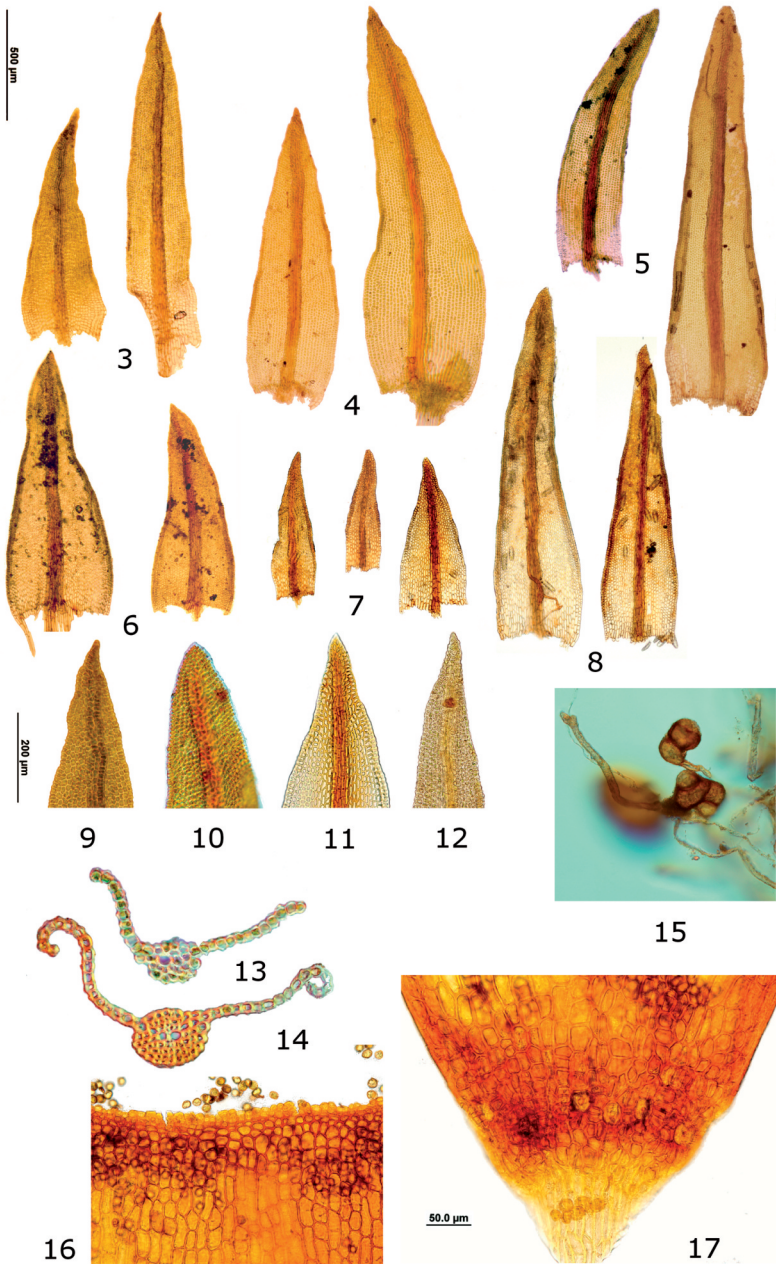
Figs 3-17. *Didymodon tophaceus* subsp. *erosus*. **3-8.** Leaves (**3.** Skrzypczak *s.n.* **4.** Papp *14/6.* **5.** Papp *14/65/3.* **6.** Köckinger *12353.* **7.** Soldán *s.n.*, CBFS *19364*. **8.** Soldán *s.n.*, CBFS *19365*.). **9-12.** Leaf apices (**9.** Skrzypczak *s.n.* **10.** Papp *14/65/3.* **11.** Soldán *s.n.*, CBFS *19364*. **12.** Soldán *s.n.*, CBFS *19365*.). **13-14.** Leaf sections in lower third (**13.** Skrzypczak *s.n.* **14.** Papp *14/65/3*). **15.** Rhizoidal gemmae, Papp *14/6.* **16.** Capsule mouth, Soldán *s.n.*, CBFS *19364*. **17.** Capsule base, Soldán *s.n.*, CBFS *19364*. Scales: 500 μm for 3-8, 200 μm for 9-12, 50 μm for 13-17.
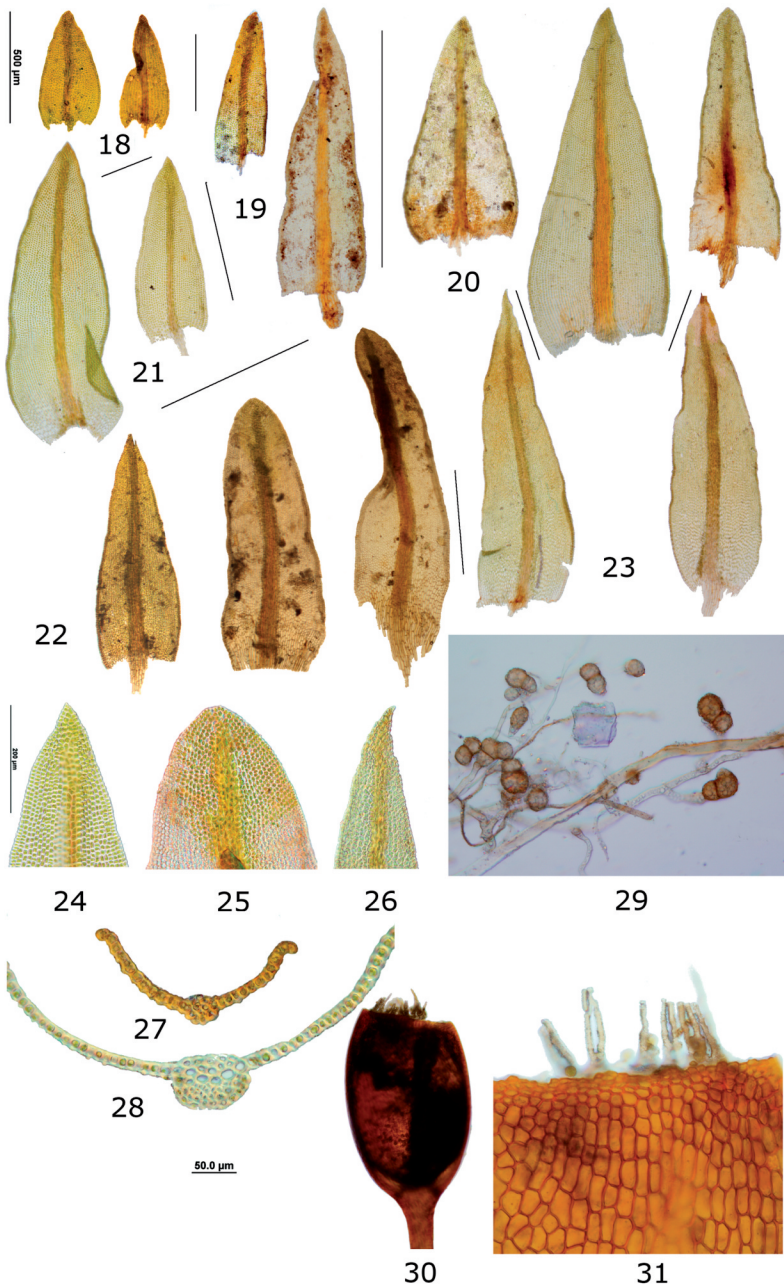
Figs 18-31. *Didymodon tophaceus* subsp. *sicculus*. **18-23.** Leaves (**18.** Oja *s.n.* **19.** Papp *s.n.* BP *185825.* **20.** Papp *14/5.* **21.** Kučera *17286.* **22.** Blockeel *43/242.* **23.** Kučera *16279*). **24-26.** Leaf apices (**24.** Kučera *17286.* **25.** Blockeel *43/242.* **26.** Kučera *16279*). **27-28.** Leaf sections in lower third (**27.** Oja *s. n.* **28.** Papp *14/5*). **29.** Rhizoidal gemmae, Kučera *17286.* **30.** Capsule, Blockeel *43/242.* **31.** Capsule mouth with peristome, Blockeel *43/242*. Scales: 500 µm for 18-23, 30, 200 µm for 24-26, 50 µm for 27-29, 31.
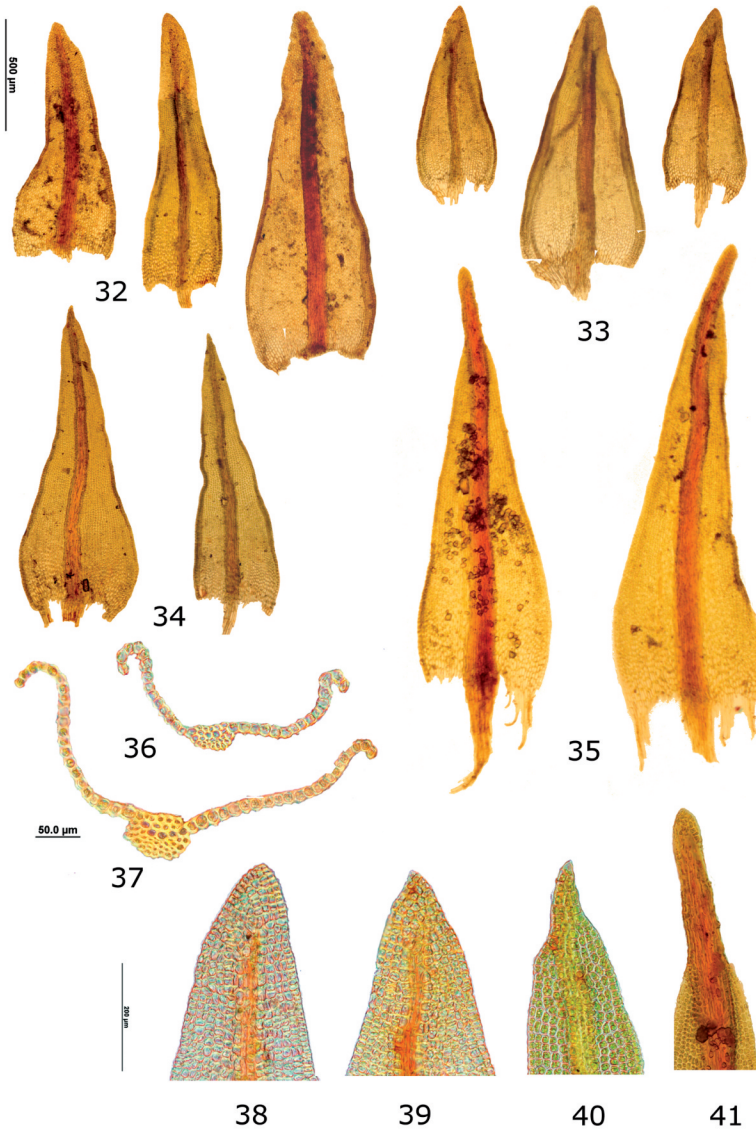
Figs 32-41. *Didymodon tophaceus* subsp. *tophaceus* var. *tophaceus*; **35, 41** – var. *anatinus*. **32-35.** Leaves (**32.** Erzberger *20917*. **33.** Lye *36934*. **34.** Blockeel *43/004*. **35.** Hauguel *s.n.*, CBFS *4465*). **36-37.** Leaf sections in lower third (**36.** Lye *36934*. **37.** Blockeel *43/004*). **38.-41.** Leaf apices (**38.** Erzberger *20917*. **39.** Lye *36934*. **40.** Blockeel *43/004*. **41.** Hauguel *s.n.*, CBFS *4465*). Scales: 500 μm for 32-35, 200 μm for 38-41, 50 μm for 36-37.

lineage which is slightly divergent from the remaining *D. erosus* specimens, interestingly found to be most closely related to the analysed Ecuadorean specimen. Perigonia, calyptra and sporophytic characters could be observed for the first time on the specimens from Ecuador (Figs 16-17). Perigonia bud-like, in upper leaf axils, perigonial leaves with inflated base. Seta straight to slightly flexuose, 5.5-9.5 mm long, orange-brown. Urn ovate-cylindrical to cylindrical, 0.45-1.20 × 0.35-0.50 mm, lid long-conical to slightly obliquely rostrate, *ca* 0.60 mm long, calyptra cucullate. Exothecial cells irregularly rectangular, *ca* 15-25 × 30-80 µm, thin- to thick-walled (walls thickening with age), towards the mouth of capsule small and isodiametric in *ca* 3 rows but annulus of inflated cells absent; stomata at base of the urn present. Spores finely granulose, 13-17 µm.

New country records: **ALBANIA**. KUKËS COUNTY, Tropojë near Bajram Curri, 670 m a.s.l., on exposed serpentine rocks, 9.7.2014 coll. B. Papp *14/65/3*, herb. BP189898, dupl. CBFS. **HUNGARY**. CSONGRÁD COUNTY, Kiskundorozsma, Sziksós, 80 m a.s.l., saline grassland, 14.3.2014 coll. B. Papp *14/6*, herb. BP189928, dupl. CBFS. **ECUADOR**. Prov. Pichincha, NNE slope of Cotopaxi volcano, valley of Qebrada Tañiloma, 3850 m a.s.l., 26.-27.6.1999 coll. Soldán *s.n.*, herb. PRC, dupl. CBFS (19364, 19365).

Ecology: as acknowledged by Werner *et al.* (2009), the ecology of this taxon is not very well defined; reported habitats include both wet and dry calcareous sites in rock fissures and open places on soil. One specimen (Papp *14/6*) was collected in saline grassland, a habitat to-date considered to be typical for *D. sicculus*; the latter species was moreover found in close proximity, and with a similar ecology.

Didymodon tophaceus subsp. *sicculus* (M.J.Cano, Ros, García-Zam. & J.Guerra) Jan Kučera, *comb. et stat. nov.*   **Figs 18-31**

Bas. *Didymodon sicculus* M.J.Cano, Ros, García-Zam. & J.Guerra, *The bryologist* 99: 401. 1996.

New observations: parallel to the variability of *D. erosus*, larger morphs were observed in *D. sicculus*. In Kučera *16279*, ovate-lanceolate leaves up to 1.45 × 0.47 mm with erose apices were observed (Figs 23, 26), and similar sizes were observed in the upper leaves from below the perichaetium in the sporulating specimen Blockeel *43/242*, but here the apex was lingulate (Figs 22, 25). Generally, the plants from wetter sites had a more obtuse leaf apex and stronger costa, up to 95 µm wide, with one layer of ventral and up to 4 layers of dorsal stereids.

Setae were observed in the Hungarian specimen Papp *14/5*, and fully developed sporophytes were present in the Greek specimen Blockeel *43/242* (Figs 30, 31). Our description of fertile plants amends the recently published first description of sporophytes by Müller (2017), who observed somewhat larger capsules and longer peristome teeth, reflecting probably the site conditions. Perigonia bud-like, in upper leaf axils, perigonial leaves with inflated base. Seta 6-7 mm long, reddish in lower part, orange-brown upwards. Urn ovate-cylindrical, 0.7-1.0 × 0.4-0.5 mm, lid shortly and slightly obliquely rostrate, 0.4-0.6 mm long, calyptra cucullate. Peristome fugacious, composed of 16 irregularly anastomosing pairs of straight, filiform, low-papillose rami, *ca* 90-120 µm high, on very short basal membrane. Exothecial cells irregularly rectangular, *ca* 15-25 × 20-50 µm, moderately thick-walled, towards the mouth of capsule small and isodiametric in *ca* 3 rows but annulus of inflated cells absent; stomata at base of the urn present. Spores finely granulose, 14-16 µm.

New country records: **HUNGARY**. CSONGRÁD COUNTY, Nagy-Szék near Sziksósfürdő, ca. 80 m a.s.l., road ditch, 14.3.2014 coll. P. Erzberger *17010*, dupl. CBFS; Kiskundorozsma, Sziksós,

at an artificial lake, 80 m a.s.l., 14.3.2014 coll. B. Papp *14/5*, BP189927, dupl. CBFS; Bács-Kiskun County, Bába-szék saline area between Dunatetétlen and Akasztó along the road No. 53, 90 m a.s.l., saline area, 21.4.2012 coll. B. Papp *s.n.*, BP185822, dupl. CBFS; Pest County, Ócsa, along a road in a wetland, 100 m a.s.l., 2.3.2013 coll. B. Papp *s.n.*, BP185825, dupl. CBFS. Czech Republic. South Moravian Region, Sedlec: saline grasslands at the W edge of the Nesyt fishpond, 175 m a.s.l., 2.4.2017 coll. L. Janošík *s.n.*, herb. CBFS19420. Croatia. The first country record has been published by Papp et al. (2016) from Vukovar-Srijem County. New regional record: Primorje-Gorski Kotar County, Rab island, towards Ciganka bay at Lopar, 20 m a.s.l., 15.8.2014 coll. B. Papp *14/85*, BP190818, dupl. CBFS.

The ecology of some of the collections is identical to previous reports, most often in periodically wet but seasonally dry saline grasslands (specimens from France, Croatia, Hungary, and the Czech Republic). Nevertheless, it seems that at some sites the species can also grow in permanently wet conditions (Kučera *16279* was found on the shaded wet calcareous stones of a water ditch in the Botanical Garden of Palermo). Periodically wet sites (such as that of Blockeel *43/242*, on seasonally wet soil on a roadside bank, or the Estonian site, which was on limestone in calcareous grassland) are not saline, and hence obviously intergrade with the ecology of both subsp. *tophaceus* and *erosus*.

Amended key to infraspecific taxa of *Didymodon tophaceus*

The key works for well-developed plants growing in non-extreme habitats in most, but certainly not all cases. In particular, large hygrophytic plants of subsp. *erosus* might key out to subsp. *tophaceus*, hygrophytic morphs of subsp. *sicculus* might key out to subsp. *erosus*, while weak morphs of subsp. *tophaceus* might key out to subsp. *sicculus*. Sporophyte characters have only been observed in single cases for subsp. *sicculus* and subsp. *erosus*, respectively. Hence, barcoding using one of the molecular markers described above is advisable in ambiguous cases.

1a. Ventral superficial costa cells on all leaves elongate (> 3:1), costa on leaves of well-developed plants often > 100 µm wide, leaf base markedly and broadly decurrent; rhizoidal gemmae not known .... subsp. *tophaceus* (2)

1b. Ventral superficial costa cells on most leaves short-rectangular to irregularly quadrate (mostly < 2:1), costa on leaves of well-developed shoots mostly < 70 µm wide, leaf base shortly, not conspicuously decurrent; rhizoidal gemmae often present (sufficient amount of substrate to be checked!) 3

    2a Costa ending below apex .... var. *tophaceus*

    2b Costa excurrent ....var. *anatinus*

3a Leaves ovate, ovate-lanceolate to ovate-lingulate (1.8-3.4:1), to 1.45 mm long; peristome present .... subsp. *sicculus*

3b Leaves lanceolate to lingulate (2.3-5.2:1), to 1.9 mm long; peristome absent (observation on plants from Ecuador)..subsp. *erosus*

DISCUSSION

The *Didymodon tophaceus* complex has been confirmed as a well-supported monophyletic lineage within *Didymodon* section *Fallaces* using both nrITS and chloroplast data. That section, recently also raised to generic rank (*Geheebia* Schimp.) by Zander (2013), appears to be molecularly well-supported after the

exclusion of *D. asperifolius* to sect. *Rufiduli* (Kučera & Ignatov, 2015), and *D. laevigatus*, *D. maschalogenus* and *D. tomaculosus* to sect. *Didymodon* (Werner *et al.*, 2005, and unpublished data of JK). Typical characters of the section include: (1) keeled leaves, (2) elongate cells on the ventral surface of the costa, (3) non-excurrent costa in the leaf apex, (4) decurrent leaf bases, (5) lamina cells with sharp simple conical papillae, and cell walls with a tendency towards strong corner thickenings, making the lumina angular, (6) stem with a weak central strand, thick-walled cylinder cells, a strong sclerodermis and no hyalodermis, (7) absence of axillary gemmae, and (8) yellow to orange KOH reaction of the cell walls. These characters are, however, well-developed only in the core members of the section (*D. giganteus*, *D. maximus*, *D. ferrugineus*, *D. erosodenticulatus*, all in a well-supported clade within the section), while the other members sometimes share individual characters with members of other sections, such as the well-developed ventral epidermis with isodiametric cells over the costa in *D. luridus*, to a certain extent also present in *D. erosus* and *D. sicculus*, the excurrent leaf costa in *D. tophaceus* var. *anatinus*, or the absence of papillae in *D. luridus*. On the other hand, elongate cells on the ventral surface of the costa are occasionally present in members of section *Didymodon* such as *D. maschalogenus*, *D. tomaculosus* or *D. laevigatus* but axillary gemmae typical of sect. *Didymodon* are present in the first species and the costa is regularly excurrent in the latter two species. Some members of sect. *Rufiduli* (*D. asperifolius*, *D. rivicola*, *D. rufidulus*) share their leaf shape with representatives of sect. *Fallaces* but have a rufous colour and a tendency towards bilaterally bulging lamina cells, not present in members of section *Fallaces*.

Although the taxonomy of the *Didymodon tophaceus* complex has recently been addressed by Werner *et al.* (2009), molecular barcoding and subsequent taxonomic evaluation have necessitated further broadening of and partly also changes in the morphological concepts applied to *D. erosus* and *D. sicculus*. Moreover, even the wider molecular analysis has left *D. tophaceus*, as presently defined morphologically, non-monophyletic. Several causes might underlie the observed paraphyletic pattern that applies to the current morphological circumscription of *D. tophaceus* in the strict sense. These include the existence of unsampled lineages within the complex, which might belong to additional cryptic species, incomplete lineage sorting, and gene flow among the lineages. The latter cause seems to be least probable in this particular case, as we found no incongruence between the pattern of chloroplast and nuclear data, and the ITS paralogs were observed very rarely and created monophyletic clusters of closely related accessions. On the other hand, it is impossible to decide whether congruence between gene trees and species trees has not yet been reached, or additional sibling species exist within the complex, given the insufficient molecular sampling over the entire geographic area of distribution. Moreover, the pattern of morphological variability described above does not allow the unequivocal identification of each of the species, and particularly the separation of *D. erosus* from *D. sicculus*, although tendencies for somewhat different leaf shapes and the generally larger stature of the former is obvious and has been used for developing the key above. Ecological preferences, emphasized by Werner *et al.* (2009) and confirmed recently also by Müller (2017), do not hold on a wider scale and the distribution pattern probably rather reflects inadequate knowledge, as both *D. erosus* and *D. sicculus* are here reported to have confirmed occurrences further north-west in Europe, and a South American locality is reported for the first time for *D. erosus*. Although sporophyte characters have been described for the first time here, and *D. erosus* seems to be clearly different from the other two taxa in the absence of a peristome, the discovery of additional fertile populations in the future

might reveal greater variability even in this character, as already reported for
D. tophaceus, in which eperistomate morphs (*Gymnostomum knightii* Schimp.) are
reported to occur (Zander 1993; we have however not investigated whether this
taxon belongs to *D. tophaceus* in its current definition).

Given the incomplete morphological and molecular separation of the three
taxa but with clear tendencies, and in most cases also the clear molecular identification
of the lineages, we believe that the most appropriate taxonomic rank for the taxa of
the *D. tophaceus* complex is that of subspecies, with an additional variety
(*D. tophaceus* var. *anatinus*) for the conspicuous morph of the nominate subspecies
with an excurrent leaf costa. Subspecific rank has not been used very often in recent
taxonomic studies, and when such taxa have been recognized, the decision has
mostly been based on morphological evidence, such as the subspecies of *Schistidium
brunnescens* Limpr. and *S. elegantulum* H.H.Blom in the treatment of Blom (1996),
or the subspecies of *Pohlia nutans* (Hedw.) Lindb. in the treatment of Köckinger *et
al.* (2005). Molecular arguments supporting the earlier morphological evaluation of
subspecies in the *Tortula muralis* Hedw. complex (Košnar & Kolář, 2009) have been
presented by Košnar *et al.* (2012) in the partial gene flow between the taxa. Although
we could not find evidence of gene flow among the taxa of the *D. tophaceus* complex,
we believe that the arguments presented above constitute a further instance, based
on sound taxonomy, where the usage of subspecies might be a plausible solution for
a difficult complex of taxa.

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APPENDIX I

Specimens used for the molecular study

Genbank accession numbers refer to ITS, *rps4-trnS* and *trnM-trnV* loci, respectively. Sequences printed in bold were newly generated for this study. All specimens or their duplicates are filed in herbarium CBFS.

Barbula unguiculata Hedw. Austria, Kučera *12829*, HM147804, HM147777, JQ890366; ***Bryoerythrophyllum recurvirostrum*** (Hedw.) P.C.Chen. Czech Republic, Kučera *12925*, JQ890527, JQ890468, JQ890407; ***Didymodon acutus*** (Brid.) K. Saito. Czech Republic, Kučera *12925*, KP307477, KP307551, KP307667; ***D. erosodenticulatus*** (Müll.Hal.) K.Saito. Russia, Afonina *4405*, **MF536559**, **MF536597**, **MF536635**; ***D. erosus*** J.A.Jiménez & J.Guerra. France, Skrzypczak *s.n.*, CBFS 10080, **MF536573**, **MF536609**, **MF536646**; Albania, Papp *14/65/3*, BP 189898, CBFS 18581, **MF536577**, **MF536611**, **MF536648**; Hungary, Papp *14/6*, CBFS 18733, **MF536580**, **MF536618**, **MF536652**; Austria, Köckinger *12353*, CBFS 18742, **MF536584**, **MF536621**, **MF536655**; Ecuador, Soldán *s.n.*, CBFS 19364, **MF536585**, **MF536622**, **MF536656**; (from Werner *et al.,* 2009 additionally EU835145, EU835146, EU835144, AY437094); ***D. fallax*** (Hedw.) R.H.Zander. Czech Republic 1, Kučera *12448*, **MF536551**, **MF536590**, **MF536627**; Czech Republic 2, Kučera *13246*, **MF536556**, **MF536595**, **MF536633**; Ireland, Holyoak *04-112*, **MF536553**, **MF536592**, **MF536629**; ***D. ferrugineus*** (Schimp. *ex* Besch.) M.O.Hill. Austria, Kučera *12482*, **MF536549**, **MF536588**, **MF536625**; India, Lüth *6617*, **MF536560**, **MF536598**, **MF536636**; ***D. giganteus*** (Funck) Jur. Austria, Kučera *12897*, KP307468, KP307548, KP307669; Germany, Cano *s.n.*, MUB 6191, AY437101, AF480991, **MF536630**; China, Long *19107*, **MF536564**, **MF536601**, **MF536639**; Nepal, Long *20840*, **MF536565**, **MF536602**, **MF536640**; ***D. leskeoides*** K.Saito. China, Long *35856*, **MF536567**, **MF536604**, **MF536642**; Nepal, Townsend *92-51*, **MF536557**, **MF536596**, **MF536634**; ***D. luridus*** Hornsch. Czech Republic, Kučera *11262*, **MF536548**, **MF536587**, **MF536624**; Spain, Tenerife, Dirkse & Hochstenbach *28659*, **MF536561**, **MF536599**, **MF536637**; ***D. maximus*** (Syed & Crundw.) M.O.Hill. Ireland, Holyoak *00-725*, **MF536552**, **MF536591**, **MF536628**; Mongolia, Ignatov *01-792*, **MF536555**, **MF536594**, **MF536632**; Canada, Steere *76-779*, **MF536562**, **MF536600**, **MF536638**; China, Long *27238*, **MF536566**, **MF536603**, **MF536641**; ***D. occidentalis*** R.H.Zander. Canada, McIntosh *7521*, **MF536558**, KP307533, KP307599; ***D. rigidulus*** Hedw. Czech Republic, Kučera *1815*, KP307473, KP307589, KP307647; ***D. sicculus*** M.J.Cano, Ros, García-Zam. & J.Guerra. Hungary 1, Erzberger *17010*, CBFS:16809, **MF536568**, **MF536605**, –; Serbia 1, Erzberger *13773*, CBFS 14152, **MF536569**, –, – ; Serbia 2, Erzberger *13775*, CBFS 14151, **MF536570**, **MF536606**, **MF536643**; Estonia, Oja *s.n.*, TU 169462, CBFS 17183, **KX685409**, **KX685407**, **KX685408**; Greece, Blockeel *43/242*, CBFS 16881, **MF536572**, **MF536608**, **MF536645**; France, Kučera *17286*, **MF536574**, –, – **;** Italy 1, Kučera *16279*, **MF536575**, –, – ; Italy 2, Kučera *16338*, **MF536576**, **MF536610**, **MF536647**; Hungary 2, Papp *s.n.*, BP 185825, CBFS:18730, –, **MF536616**, – ; Croatia 1, Papp *14/85*, CBFS 18734, –, **MF536617**, – ; Croatia 2, Papp *13/23*, BP 190749, CBFS 18731, **MF536581/MF536582**, **MF536619**, **MF536653**; Hungary 3, Papp *14/5*, BP 189927, CBFS 18732, **MF536583**, **MF536620**, **MF536654**; (from Werner *et al.,* 2009 additionally AY437092, EU835132, EU835123, EU835126, AY437091, EU835124); ***D. sinuosus*** (Mitt.) Delogne. Czech Republic, Kučera *12059*, JQ890529, JQ890476, JQ890410;

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