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Tortella mediterranea (Pottiaceae), a new species from southern Europe, its molecular affinities, and taxonomic notes on *T. nitida*

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ABSTRACT. *Tortella mediterranea* is described as a new species from the Mediterranean Basin of southern Europe. It is mainly characterized by (1) absence of a stem central strand, (2) lanceolate leaf shape, (3) rather gradual limb-sheath transition, (4) partial presence of elongate marginal cells in the upper half of leaf and (5) ventral costal stereid band usually stronger than the dorsal. Chloroplast *rps4* and nuclear ITS sequences were invariable within the studied specimens of the species, suggesting a low genetic variability, and the phylogenetic position is rather isolated with only obscure affinities to the group of closely related taxa around *T. tortuosa* and to *T. fasciculata*. The new species, seemingly a rather rare plant, was collected from dry and sunny to shaded limestone rocks. In addition, new molecular data for *T. nitida* s.str. and putative var. *irrigata* are provided and taxonomic problems within this variable species are discussed.

KEYWORDS. Balearic Islands, Croatia, DNA sequencing, Greece, nuclear ITS marker, *rps4* chloroplast marker, phylogeny, Spain, taxonomy.



For several years, the circumscription of the genus *Tortella* and the number of species included within it have been in a state of flux. The European checklist of Hill et al. (2006) cites 12 species and several varieties, one of which, *T. inclinata* var. *densa* (Lorentz & Molendo) Limpr., is better treated at species rank (Crundwell & Nyholm 1962; Hedenäs 2015; Nebel & Philippi 2000; Smith 2004). On the other hand, *T. limosella* (Stirt.) P.W.Richards & E.C.Wallace, known only from the type material, probably represents only an “aberrant form or short-lived genetic variant of another species” (Blockeel et al. 2014). Recent papers using molecular tools (Grundmann et al. 2006; Werner et al. 2005) demonstrated that *Pleurochaete* Lindb. is deeply nested within *Tortella*, suggesting its inclusion in that genus. Alternatively, however, it may be treated as a putatively young genus which evolved from the interior of an older one. *Tortella inflexa* (Bruch) Broth. was presumed to be excluded from the genus on the basis of a single

ITS sequence (Werner et al. 2005) but confirmation of its status from an additional marker seems desirable. Werner et al. (2014) showed that the former *Trichostomum arcticum* Kaal. molecularly represents an unambiguous member of *Tortella*, and therefore must be named *T. spitsbergensis* (Bizot & Thér.) O.Werner, Köckinger & Ros. In Europe, it is known only from Svalbard. The plant reported as *Trichostomum cuspidatissimum* Cardot & Thér. (in the sense of *T. arcticum*) from northern Sweden (Engelmark 1974) proved to be a hybrid between *T. spitsbergensis* and *T. arctica* (Arnell) Crundwell & Nyholm, and should be named *Tortella ×cuspidatissima* (Cardot & Thér.) O.Werner, Köckinger & Ros. Since both parents seemingly do not occur within its European range, an alternative approach is to treat it as an independent, hybridogenous species analogous to *T. rigens* Alberts., both reproducing only vegetatively. The report of one of its parents, *T. arctica*, from Sweden by L. Hedenäs and P. Eckel (in Ellis et al. 2011) has proved to be erroneous. Nuclear and chloroplast sequences of the Swedish material showed that it belonged to *T. tortuosa* (Hedw.) Limpr. s.l. (see

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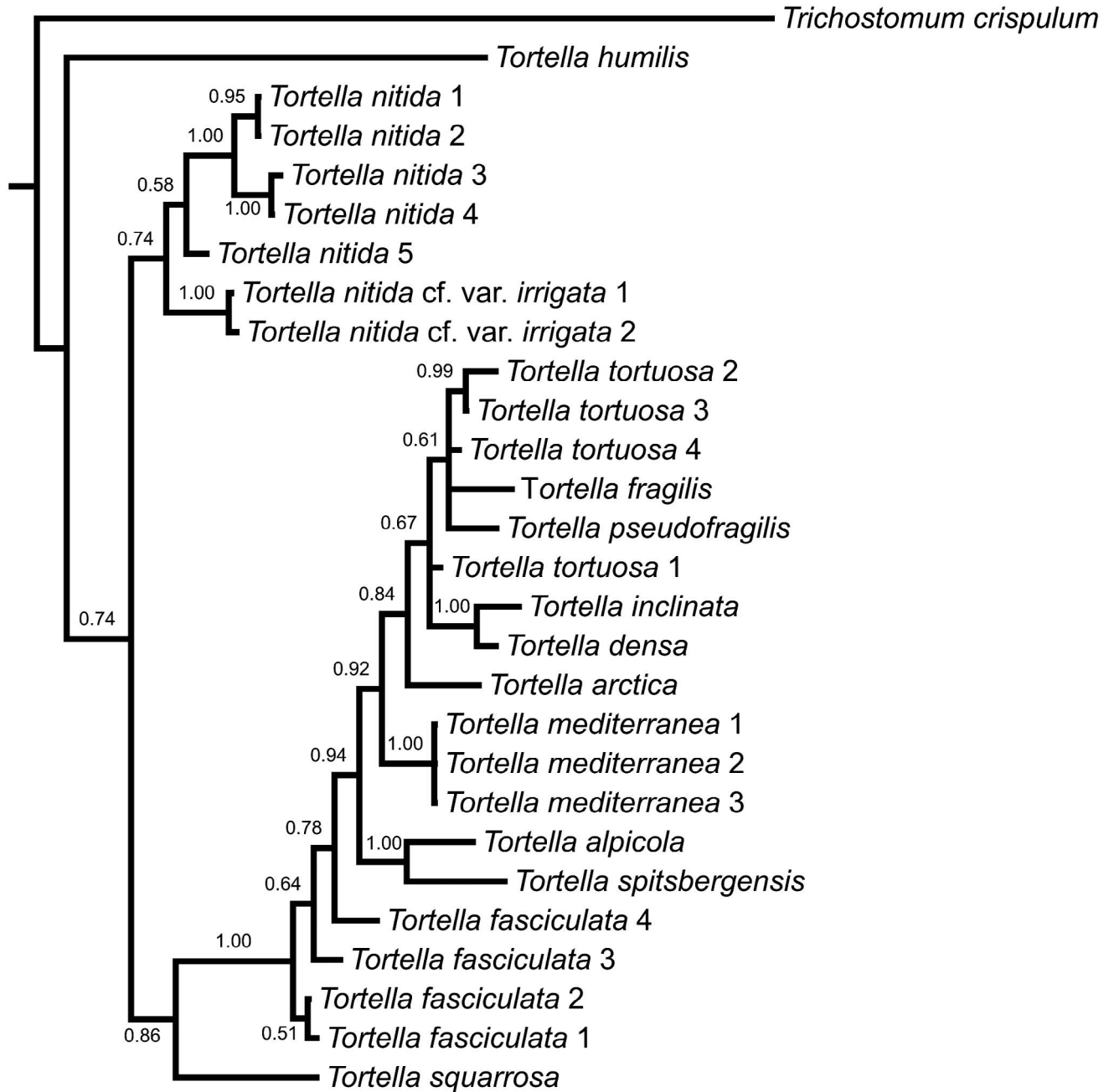


Figure 1. ITS phylogram obtained by Bayesian analysis. *Tortella mediterranea* is situated on a well-supported clade (pp=1) close to *T. arctica*, *T. densa*, *T. fragilis*, *T. inclinata*, *T. pseudofragilis* and *T. tortuosa*.

Köckinger & Hedenäs 2017, Fig. 1). The most recent change in the European *Tortella* flora was presented by Köckinger & Hedenäs (2017). They discovered that *T. bambergeri* (Schimp.) Broth. in its original sense belongs to *T. tortuosa* s.l., whereas the plants understood as *T. bambergeri* during recent decades belong to two distinct taxa, *T. fasciculata* (Culm.) Culm and *T. pseudofragilis* (Thér.) Köckinger & Hedenäs.

During morphological studies on the variability of European *Tortella tortuosa*, the first author's attention was attracted by photos with this name from Mallorca in Lüth's "Pictures of Bryophytes" (Lüth 2014), seemingly representing something outside the known variation of *T. tortuosa*. The loan of this plant and of additional Mediterranean collections of *T. tortuosa* from Lüth's private herbarium revealed a further specimen from Greece

almost identical to the Mallorcan plant. In addition, HK detected a third similar collection from Croatia in his own herbarium.

The main aim of the present study is to establish the correct rank and systematic position of this neglected plant. For this purpose, OW agreed to undertake a molecular investigation. We also checked the synonymy of similar species for any name that might be available for our plant. The overall appearance of this plant is \pm intermediate between *Tortella nitida* (Lindb.) Broth. and *T. tortuosa*. Since the first author, in collaboration with L. Hedenäs, is already engaged in a thorough investigation of the *T. tortuosa* complex, where this issue was taken into account, we concentrated our search on *T. nitida*. A detailed discussion of the general variation within this mainly Mediterranean species was provided by Eckel (1998). She also studied and illustrated the Lindberg type. We needed to pay particular attention to the younger synonym *T. cirrifolia* (Mitt.) Broth. and to *T. nitida* var. *irrigata* (H.Winter) R.H.Zander, since the descriptions of both these Macaronesian taxa come closer to our neglected plant than usual expressions of *T. nitida*.

METHODS

Morphological study. The description is based on all three known collections. To detect further material of the deviating plant many samples identified as *Tortella nitida* and *T. tortuosa* from MUB herbarium were revised by RMR and a series of doubtful plants also checked by HK. Tom Blockeel was searching for similar plants among his Greek collections and provided specimens. David Holyoak sent some *T. nitida* collections.

The type material of *Tortella cirrifolia* from NY and MO and *Tortella nitida* var. *irrigata* (H.Winter) R.H.Zander from JE was borrowed and studied by RMR. The types of *Trichostomum flavovirens* var. *medium* Boulay, var. *obtusum* Boulay, and var. *subtortuosum* Boulay were not available from LILLE nor PC.

Molecular study. Seven nrITS DNA sequences were newly created, corresponding to the three deviating *Tortella* specimens, two of *T. nitida* s.str. and two of *T. nitida* cf. var. *irrigata*. Other ITS sequences of 19 *Tortella* specimens belonging to 13 species available in public databases were added to represent the variability of the genus. Because of its

genetic variability, four samples of *T. fasciculata* were included. In the case of *T. tortuosa* we deliberately chose four genetically very different plants whose identity is known to us. *Trichostomum crispulum* Bruch was used to root the trees.

The chloroplast *rps4* region was chosen to represent the chloroplast DNA because together with the *atpB-rbcL* spacer it is the region with the highest number of available *Tortella* sequences at GenBank and the number of base differences between sequences are in the same range for these two regions. The fact that *rps4* is a protein coding gene reduces possible ambiguities in the alignment. For *rps4* region, the same three deviating *Tortella* specimens, two of *T. nitida* s.str. and two of *T. nitida* cf. var. *irrigata* were newly sequenced. The sequences of eleven *Tortella* species were added from public databases. Three of them belonged to *T. fasciculata*, four to *T. tortuosa*. *Trichostomum crispulum* was again used to root the trees. GenBank accession numbers of all sequences used in this study are given in **Table 1**.

DNA isolation and amplification of nrITS and *cprps4*. Total DNA was extracted from dry material using the NaOH extraction method as explained in Werner et al. (2002). The nrITS region was amplified with the primers AB 101F and AB 102R (Douzery et al. 1999). In the case of the *rps4* region, the primers used were *rps5* (Nadot et al. 1994) and *trnas* (Buck et al. 2000). Two μ L of stock DNA were used as template in 25 μ L reaction volume. To this were added 200 μ M of dNTP, 2 mM $MgCl_2$, 2 units Taq polymerase (DreamTaq, ThermoFisher), one μ L BLOTTO (10% skimmed milk powder and 0.2% NaN_3 in water) and the buffer (provided by the enzyme supplier). BLOTTO attenuates the PCR inhibition caused by plant compounds (De Boer et al. 1995). The amplification conditions were as follows: 3 min at 94°C, 35 cycles with 30 sec at 94°C, 30 sec at 50°C (*rps4*) or 55°C (ITS) and 1 min at 72°C, and a final 7-min extension step at 72°C. Amplification products were controlled on 1% agarose gels and successful reactions were cleaned using the a combination of 1 unit FastAP (Thermo Fischer) and 10 units ExoI (Thermo Fischer) for one hour at 37°C followed by a thermal inactivation of 15 min at 85°C. Cycle sequencing was performed with the BigDye Sequencing Kit (Perkin Elmer) using a standard protocol and the amplification primers. The annealing temperatures were set at 50°C. The reaction products were separated on an

Table 1. GenBank accession numbers for ITS and *rps4* sequences and voucher information for all sequences used in the study, including those from newly sequenced specimens.

Sample	Voucher information	Herbarium	ITS GenBank accession number	<i>rps4</i> GenBank accession number
<i>Tortella alpicola</i>	–	–	HM049809	–
<i>Tortella arctica</i>	–	–	AY854410	AY950375
<i>Tortella densa</i>	–	–	KM020638	KM020748
<i>Tortella fasciculata</i> 1	–	–	HM049810	–
<i>Tortella fasciculata</i> 2	–	–	KM020615	KM020724
<i>Tortella fasciculata</i> 3	–	–	KM020614	KM020723
<i>Tortella fasciculata</i> 4	–	–	KM020618	KM020727
<i>Tortella flavovirens</i>	–	–	–	AY950378
<i>Tortella fragilis</i>	–	–	KM020624	KM020733
<i>Tortella humilis</i>	–	–	AY854419	KM020751
<i>Tortella inclinata</i>	–	–	KM020636	KM020746
<i>Tortella mediterranea</i> 1	Greece, Lüth s.n.	MUB 56885, Lüth and Köckinger private herbaria	MH373392	MH365346
<i>Tortella mediterranea</i> 2	Croatia, Köckinger 15051	MUB 56886, GZU, Köckinger private herbarium	MH373393	MH365347
<i>Tortella mediterranea</i> 3	Spain, Lüth s.n.	MUB 56884; GZU, Lüth private herbarium	MH373394	MH365348
<i>Tortella nitida</i> 1	–	–	AY854421	AY950386
<i>Tortella nitida</i> 2	–	–	AY796264	–
<i>Tortella nitida</i> 3	–	–	KT380428	–
<i>Tortella nitida</i> 4	Portugal, Holyoak 07-505	MUB 56887, GZU, Holyoak private herbarium	MH373388	MH365342
<i>Tortella nitida</i> 5	Turkey, Stahl, s.n.	MUB 56889, Köckinger private herbarium	MH373389	MH365344
<i>Tortella nitida</i> cf. var. <i>irrigata</i> 1	Croatia, Köckinger 15050	MUB 56888, Köckinger private herbarium	MH373390	MH365345
<i>Tortella nitida</i> cf. var. <i>irrigata</i> 2	Croatia, Sule & Brodarovic, s.n.	MUB 56890, Köckinger private herbarium	MH373391	MH365343
<i>Tortella pseudofragilis</i>	–	–	KM020611	KM020720
<i>Tortella spitsbergensis</i>	–	–	KF356251	–
<i>Tortella squarrosa</i>	–	–	AY854406	AY950373
<i>Tortella tortuosa</i> 1	–	–	KM020625	KM020734
<i>Tortella tortuosa</i> 2	–	–	KM020627	KM020736
<i>Tortella tortuosa</i> 3	–	–	KM020628	KM020737
<i>Tortella tortuosa</i> 4	–	–	KM020631	KM020740
<i>Trichostomum crispulum</i>	–	–	KM020642	KM020752

ABI Prism 3700 automatic sequencer (Perkin Elmer).

Data analysis. The sequences were edited using Bioedit 5.0.9 (Hall 1999) and aligned manually. The nuclear ITS and the chloroplast datasets (**Supplementary Tables S1 & S2**) were treated independently. Genetic distances were calculated with the help of MEGA7 (Kumar et al. 2016). Alignment gaps were recoded with the help of Seqstate (Müller 2005) according to the simple coding option of Simmons & Ochoterena (2000). The data were analysed by Bayesian inference implemented with MrBayes 3.2

(Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003; Ronquist et al. 2012). Gaps were treated as standard data. Trees were sampled across the substitution model space in Bayesian MCMC analysis itself (Huelsenbeck et al. 2004) using the option *nst=mixed*, removing the need for a priori model testing. Indels were treated as a separate unlinked partition, using the restriction site model (F81). Two runs with four chains were conducted with 10,000,000 generations. Trees were sampled every 10,000th generation and the first 2,000,000 trees were discarded (burn-in) in order to exclude the trees before the chain reached the stationary

phase. We checked for stationarity of the log likelihood values, that the Potential Scale Reduction Factor (PSRF) was close to "1" ($0.99 < \text{PSRF} < 1.01$), and that the estimated sample size was above 200. The final trees were edited with TreeGraph2 (Stöver & Müller 2010).

RESULTS

Molecular investigation. All accessions of the three deviating specimens (hereafter called *Tortella mediterranea*) had identical sequences for both of the investigated markers. In the case of the ITS region, the genetic distances (gaps excluded) from *T. fasciculata* were in the range of four to six and to *T. nitida* in the range of 11 to 14 changes. In the case of the *rps4* region, the distances were two to three changes between *T. mediterranea* and *T. fasciculata* and seven to eight between *T. mediterranea* and *T. nitida*.

The basal part of the ITS tree (Fig. 1) was not very well resolved, but *Tortella humilis* (Hedw.) Jenn., *T. nitida* and *T. squarrosa* (Brid.) Limpr. are the first diverging branches. Within the *T. nitida* clade, the two samples of "cf. var. *irrigata*" cluster together. The remaining *Tortella* species form a clade with a solid support ($\text{pp}=1.00$). But within this clade there are again some unresolved relationships. The *T. mediterranea* samples form a clade with solid support ($\text{pp}=1.00$), with *T. arctica*, *T. densa* (Lorentz & Molendo) Crundw. & Nyholm, *T. fragilis* (Hook. & Wilson) Limpr., *T. inclinata* (R.Hedw.) Limpr., *T. pseudofragilis*, and *T. tortuosa* situated on the sister clade.

The *rps4* tree shows similar patterns (Fig. 2). The basal part of the tree is poorly resolved. *Tortella humilis* is the first species to segregate, followed by *T. squarrosa* and *T. nitida*. *Tortella mediterranea* ($\text{pp}=1.00$) is situated on a polytomous clade together with unresolved *T. fasciculata* and a solidly supported clade with *T. arctica*, *T. densa*, *T. fragilis*, *T. inclinata*, *T. pseudofragilis* and *T. tortuosa*.

TAXONOMY

Because of the unambiguity of the morphological and molecular data and the lack of any available name, we describe this moss as a new species.

Tortella mediterranea Köckinger, Lüth, O.Werner & Ros, *sp. nov.* Fig. 3

Stem central strand absent, leaf shape narrowly lanceolate, leaf base ovate, marginal cells in upper half of leaf partially elongate, laminal cells small, limb-sheath transition rather gradual, ventral costal stereid band usually thicker than the dorsal.

TYPE: SPAIN. BALEARIC ISLANDS: Mallorca, Escoratorrent de Lluc (de Pareis), 200 m a.s.l., 16 March 2010, Lüth *s.n.* (holotype: MUB No 56884; isotypes: GZU, HB LÜTH).

Description. Plants medium-sized to large, in loose cushions or tufts, dull olive-green in shade, brown in sun, interior of cushions pale brown to brown. Shoots erect, up to 4 cm long, densely (rarely loosely) and evenly foliated, irregularly branched. Stems brown, in cross-section circular, up to 300 μm wide, hyalodermis present, cortex red-brown, small-celled, of 1–3 rows and moderately thick-walled, cylinder cells thin-walled and rather large, central strand absent. Rhizoids red to red-brown, tomentum present but usually hidden. Axillary hairs filiform, ca. 300 μm long, of about a dozen narrowly cylindrical, uncoloured cells, basal ones shorter. Leaves (2)3.0–6.0 \times (0.3)0.5–1.0 mm, lanceolate to linear-lanceolate, when dry straight in proximal half, incurved and \pm contorted in distal half, when moist erecto-patent to spreading, mostly straight, sometimes weakly flexuose or weakly secund, fragile, older ones usually transversely broken in upper third or in mid-leaf. Limb gradually and narrowly (in xeric extremes broadly) acuminate, canaliculate to tubulose or in shade \pm keeled distally. Sheath shortly ovate, in shade distinctly wider than limb and with indistinct shoulders at transition, non-coloured to distinctly yellowish, 0.5–1.0 mm long. Apex ending in a sharp, mostly edentate, sometimes flexuose, brownish or yellowish mucro, 150–450 μm long. Costa red-brown when mature, less coloured in young leaves, moderately glistening at back when dry, in shade-morphs in upper part rarely with indistinct spines (distally prorate), at leaf base (80)100–160 μm wide, gradually narrowed to apex, occupying about a quarter to a sixth of leaf width in mid-leaf, in cross-section at base elliptical and 2.5–3 times wider than long, in mid-leaf semi-circular to rounded triangular and about 2 times wider than long, isodiametric in apical part, adaxial epidermis usually continuously present, abaxial epidermis absent (rarely small patches of isodiametric cells in apical part), ventral stereid band 3–7 layers thick, dorsal band 2–5 layers thick, guide cells 8–10 in mid-

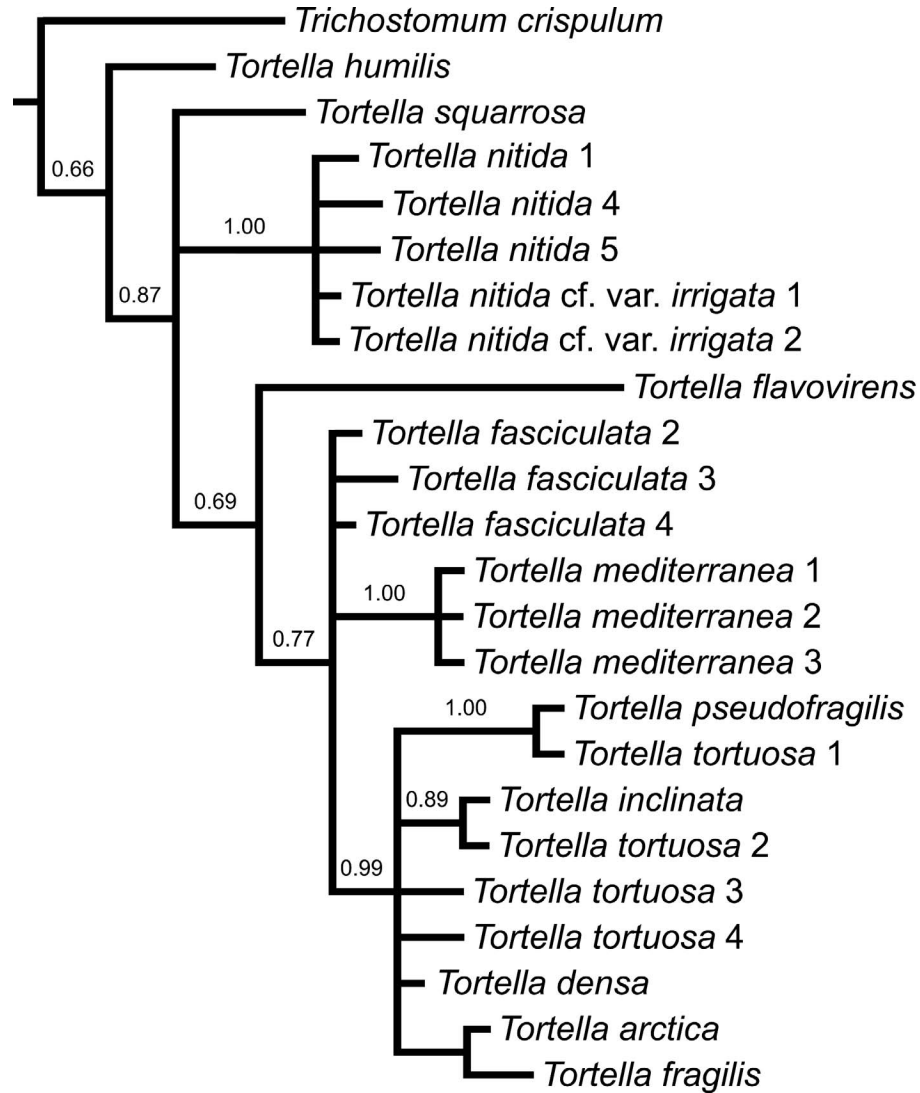


Figure 2. *rps4* phylogram obtained by Bayesian analysis. *Tortella mediterranea* is situated on a well-supported clade (pp=1) close to *T. arctica*, *T. densa*, *T. fasciculata*, *T. fragilis*, *T. inclinata*, *T. pseudofragilis* and *T. tortuosa*.

leaf, up to 12 at base, constantly in a single row. Margins somewhat incurved or flat (in xeric plants rarely partially recurved), entire up to the apex. Lamina mostly non-undulate, sometimes slightly so at limb base, unistratose, only at juncture with costa often narrowly bistratose, transition zone between limb and sheath V-shaped to broadly U-shaped, transition predominantly gradual (to very gradual) with a zone of short-rectangular, smooth and thick-walled cells, in shade-morphs frequently rather abrupt. Marginal cells in mid-limb and below apex longer than wide (elongate) in a single narrow row, $2\text{--}4 \times 6\text{--}16 \mu\text{m}$, in more xeromorphic plants in varying degrees partly or predominantly isodiametric, 5×5 to $8(10) \times 8(10) \mu\text{m}$, smooth to variably

papillose. Mid-limb cells mostly quadrate, partially rectangular or transversely rectangular, mostly in distinct longitudinal rows, $(4)5\text{--}8 \times (4)5\text{--}8 \mu\text{m}$, mostly thick-walled, with a dense and continuous ornamentation of fine papillae, in younger leaves of shade-morphs obscuring the areolation. Inner transition cells thick-walled, shortly rectangular and smooth. Basal cells non-coloured or yellow (to reddish), thin-walled, shortly rectangular, when inflated also elongate hexagonal, $20(30) \times 40\text{--}80 \mu\text{m}$, marginally reaching variably high into limb. Probably dioicous; gametangia not seen.

Differentiation. The new species is mainly characterized by (1) absence of a stem central strand, (2) narrowly lanceolate leaf shape with an ovate base

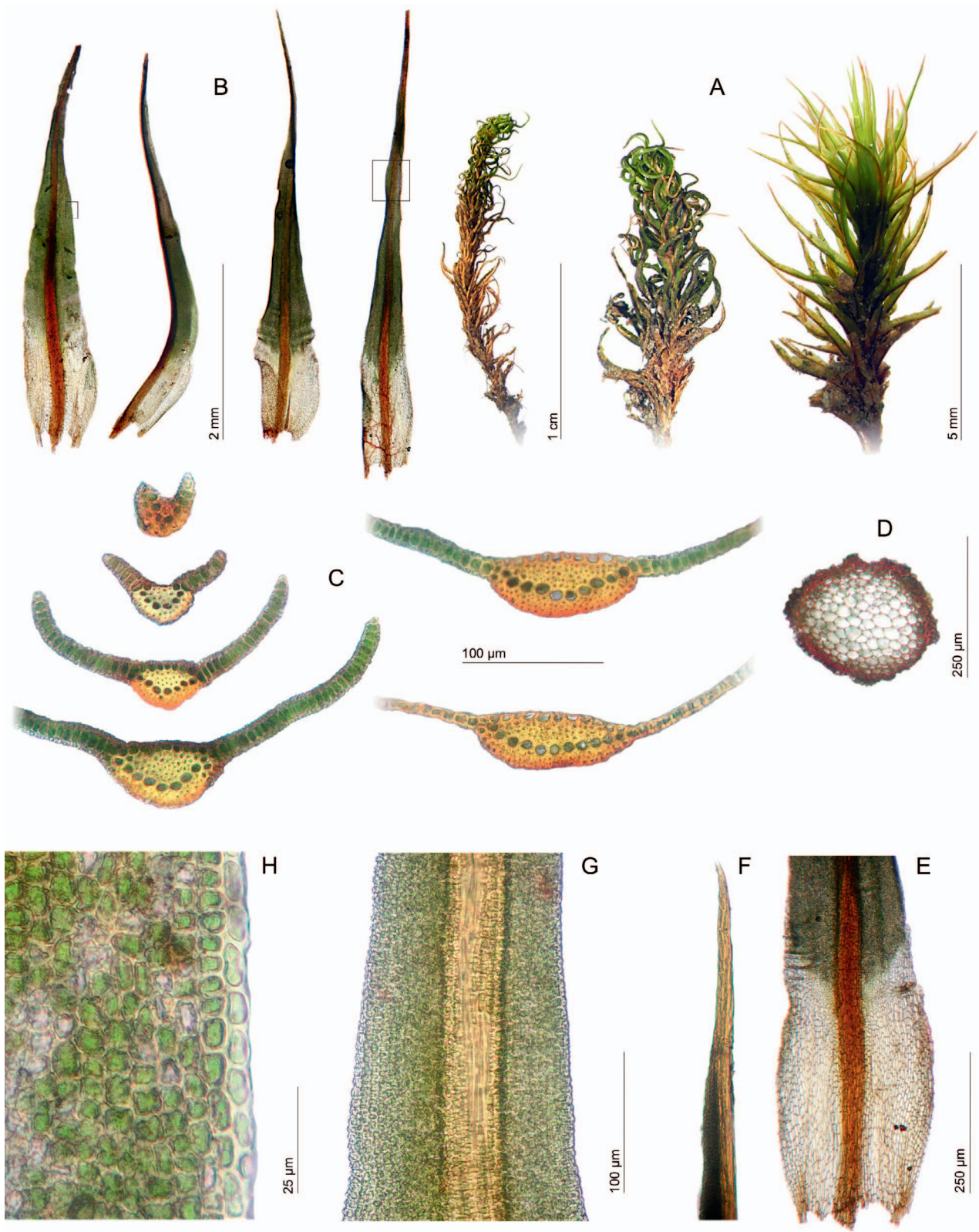


Figure 3. *Tortella mediterranea* Kückinger, Lüth, O.Werner & Ros. A. Shoot habit, moist and dry. B. Leaves. C. Leaf cross-sections. D. Stem cross-section. E. Leaf base. F. Leaf apex. G. Segment of upper limb. H. Segment of margin at mid-limb. Photographs from holotype by M. Lüth.

and a narrow mucro, (3) partial presence of elongate marginal cells in upper leaf, (4) small laminal cells, (5) rather gradual limb-sheath transition and (6) the ventral costal stereid band usually thicker than the dorsal.

Tortella nitida usually differs in having a lingulate-lanceolate leaf shape with a usually broadly acute and shortly mucronate leaf apex. When narrowly acuminate, the dorsal upper costa is covered with quadrate, papillose cells (var. *irrigata* and similar morphs). When dry the leaves are usually tightly incurved with the lamina inflexed, so that only the glossy dorsal surfaces of the costae remain visible. The laminal cells are usually larger and distinctly turgid, with coarser papillae covering only the lumina. Furthermore, the marginal cells are mostly transversely elongate (wider than long) in the upper part of the leaf. However, in shade morphs the laminal cells are sometimes as small as in *T. mediterranea*. Such plants may, furthermore, have fine papillae but not in a dense and rather thick layer. Xeromorphic extremes of *T. mediterranea* (collection from Croatia) come close to *T. nitida* in leaf shape with a short and not distinctly widened base and the apex more broadly pointed with a shorter mucro. In addition, the laminal cells are larger than in mesomorphic plants. Such expressions can be distinguished safely from *T. nitida* by the stem cross-sections showing no central strand. In *T. nitida* the central strand is constantly present and often strong (up to 50 µm in diameter), surrounded by rather thick-walled and often coloured cylinder cells which are larger, uncoloured and thin-walled in *T. mediterranea*.

Tortella tortuosa differs in having a usually abrupt limb-sheath transition, mostly markedly secund (falcate) and undulate leaves, the absence of elongate marginal cells in the upper part of the leaf and the ventral costal stereid band usually thinner than the dorsal one.

Tortella fasciculata can be distinguished easily in having the dorsal surface of the upper costa covered with quadrate, papillose cells (also seen in *T. mediterranea* but only in small patches), the marginal cells in mid-limb transversely elongate (wider than long), a shorter mucro, and the presence of a stem central strand.

Tortella arctica is similar in the widened, ovate, often yellow leaf base and an acuminate, subula-like, canaliculate limb. Furthermore, it also has a very gradual limb-sheath transition and shares the

absence of a stem central strand. It differs in the non-fragility of the leaves, generally larger laminal cells with distinctly pitted cell walls in the basal part of the leaf, and the absence of a limb border of elongate cells.

Tortella fragilis shares the presence of elongate border cells in the upper part of the leaf, which are, however, much more distinct, non-papillose and with the outer wall usually very strongly thickened. It differs, among other features, in having a bistratose limb with a blunt apex, leaves only weakly curved when dry, and an abrupt limb-sheath transition.

Ecology. All three known collections originate from limestone rocks, one from a shaded crag (type material, **Fig. 4**), one from a sunny rock knoll and one from sunny roadside rocks. Although it grows predominantly on rather dry rocks, humid habitats also occur in close proximity, since two of the three records are from large gorges. Mosses collected at the same sites include *Grimmia dissimulata* E.Maier, *G. orbicularis* Bruch ex Wilson, *Schistidium crassipilum* H.H.Blom, and *Homalothecium lutescens* (Hedw.) H.Rob.

Distribution. The few single localities from Croatia, Greece and Spain suggest a Mediterranean distribution area, where it probably prefers mountainous regions with a rather high level of annual precipitation. However, it is probably restricted to altitudes below 1000 m; otherwise it could be expected to penetrate the warmer parts of Central and Western Europe. We are seemingly confronted with a rather rare plant, since no further material could be detected either in MUB or the private herbarium of T. L. Blockeel (pers. comm., 2017) with rich collections from Greece. Nevertheless, focused searches in suitable habitats will certainly reveal additional records. Because of the frequency of similar species like *Tortella nitida* and *T. tortuosa* in calcareous regions of the Mediterranean basin, the species has probably been widely overlooked.

Paratypes. CROATIA. LIKA-SENJ: Velebit Mts., Lopci SE of Senj, ca 500 m a.s.l., 18. 06. 1987, *Köckinger 15051* (MUB 56886, GZU, HB KÖCKINGER); GREECE. WESTERN MACEDONIA: Pindos, Vikos-Aoos National Park, top edge of gorge NW of Vikos, 620 m a.s.l., 18. 05. 2000, *Lüth s.n.* (MUB 56885; HB LÜTH, HB KÖCKINGER).

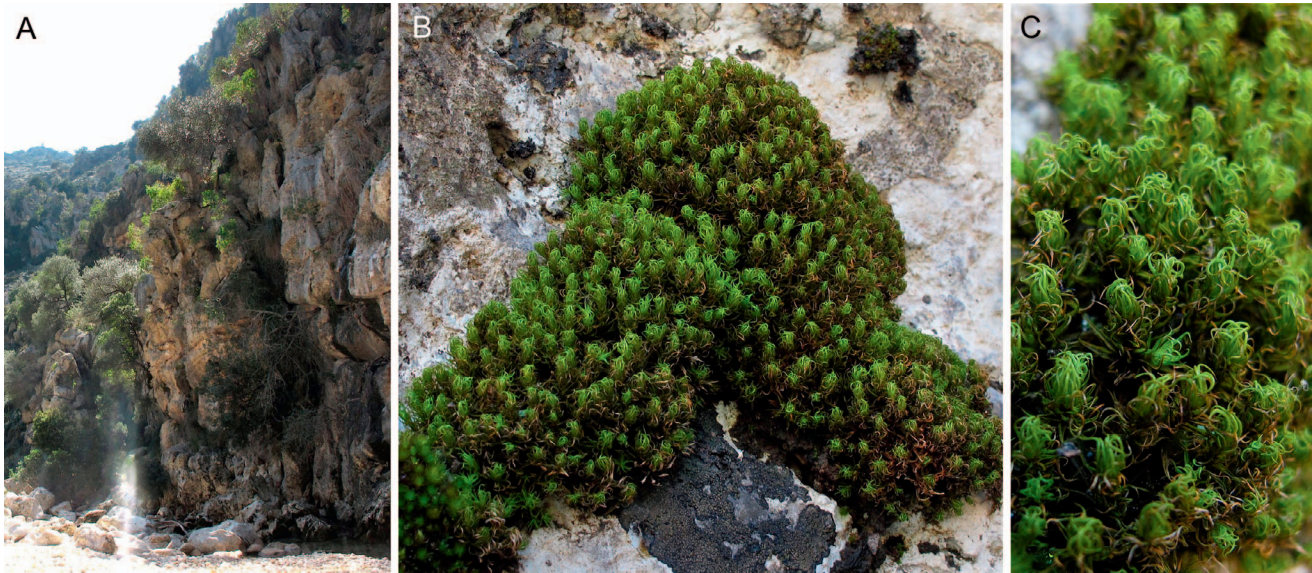


Figure 4. *Tortella mediterranea* in the field. **A.** Habitat at Mallorcan type-locality. **B.** Moderately dried cushion on shaded limestone crag at A. **C.** Detail of B. Photographs by M. Lüth.

DISCUSSION

Both molecular and morphological data support the recognition of this neglected moss at species rank. It does not seem to have very close affinities to other species within the genus. According to chloroplast sequences the closest relatives seem to be the recently resurrected *Tortella fasciculata* (Köckinger & Hedenäs 2017) and the large group of taxa around *T. tortuosa* (comprising *T. arctica*, *T. densa*, *T. fragilis*, *T. inclinata*, *T. pseudofragilis*, *T. rigens* and *T. tortuosa* s.l.), here informally called the *T. tortuosa* group. According to nuclear sequences it is closer to the cryophilous *T. arctica* than to the other taxa of the *T. tortuosa* group and rather remotely related to *T. fasciculata*.

The absence of a stem central strand is certainly an apomorphic trait in *Tortella*, otherwise only present in the majority of the species of the *T. tortuosa* group. From the morphological point of view, *T. mediterranea* therefore seems to have its strongest affinities to this large group, supported also by the lanceolate and acuminate leaf shape.

Because of its overall appearance, we first considered the possibility that *Tortella mediterranea* might be a hybrid (or a species of ancient hybrid-origin) between *T. nitida* and *T. tortuosa*, but this proved to be very unlikely based on molecular data. There is only a single joint mutation in the ITS sequence of *T. mediterranea* and two of the seven

samples of *T. nitida* and none in *rps4*, while there is one mutation each respectively with *T. tortuosa*.

Taxonomic notes on *Tortella nitida*. In the course of the search for available names for our new species, we had to check the types of *Tortella nitida* var. *irrigata* and *T. cirrifolia*, which also allowed an insight into the taxonomy of *T. nitida*. The var. *irrigata* is described as differing from *T. nitida* s.str. in having narrower, longly acuminate leaves and the dorsal surface of the upper costa covered with papillose, quadrate cells (May 1986). Two samples showing this combination of characters, both from the Dalmatian islands, proved identical to *T. nitida* s.str. in *rps4* sequences but moderately different in ITS, which would support the current infraspecific rank. The following observations, however, convinced us that much more work is necessary to come to a reliable conclusion and to settle the taxonomy. The type material of *T. cirrifolia* from Madeira (Funchal, leg. Johnson, NY, MO), a sporophyte-bearing plant with small and narrow leaves, curled when dry, looks only marginally different from the syntype material of *T. nitida* var. *irrigata* from Teneriffa (Icod de los Vinos, leg. H. Winter, JE), which has much longer and narrowly acuminate leaves. Both have very small, thin-walled and finely and densely papillose laminal cells, 4–6(8) μm wide. In the former plant, the dorsal surface of the upper costa normally shows exposed stereids reaching up to a quite cucullate apex, whereas it is variably

covered with quadrate, papillose cells in the latter. Careful search revealed some of these quadrate cells to be present, though rarely, shortly below the mucro in leaves of the type of *T. cirrifolia*, and single leaves of the type of var. *irrigata* possess exposed stereids almost throughout. Hence, the type of var. *irrigata* may well represent just a hygro-sciomorphic extreme of the same plant, already assumed by May (1986) and Crundwell et al. (1994). The two molecularly tested plants from Dalmatia, provisionally called “cf. var. *irrigata*,” show distinctly coarser leaves with some notches in the upper part and larger, rather thick-walled laminal cells with coarser, spaced papillae. They originate from shaded but dry sites. An extreme plant was collected by T. L. Blockeel (*Blockeel 45/143*, duplicate in GZU) from a “moist rock face by stream” on the Greek island of Andros showing mid-leaf cells up to $14 \times 20 \mu\text{m}$, a leaf length up to 7 mm and, despite a similar habitat, absolutely no similarity to the type of var. *irrigata*. Even in collections of quite “normal” *T. nitida* (e.g., in *Holyoak 07-505*, “*Tortella nitida 4*” in **Figs. 1 & 2**) short sections of quadrate, papillose superficial cells can be detected on the dorsal surface of costa just below the mucro. Seemingly, this character is a facultative one in *T. nitida* and largely determined by a shadier and moister environment, as with *T. fragilis*, rarely *T. tortuosa* (see Köckinger & Hedenäs 2017), and also *T. mediterranea* where small patches of these cells could be observed in shade morphs. On the other hand, this is a constant character, although of variable extent, in *T. alpicola*, *T. fasciculata* and *T. pseudofragilis*.

According to our present molecular data, *Tortella nitida* s.l., contrary to *T. mediterranea*, is a genetically variable species, which needs further study. In particular, it was not possible to sequence Macaronesian plants close to the types of *T. cirrifolia* and *T. nitida* var. *irrigata*, and the clarification of the taxonomy of *T. nitida* s.l. must therefore be postponed.

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Supplementary documents online:

Supplementary Table S1. Aligned sequences of nrITS used in the phylogenetic placement of *Tortella mediterranea*.

Supplementary Table S2. Aligned sequences of *rsp4* used in the phylogenetic placement of *Tortella mediterranea*.