# A taxonomic study of selected European taxa of the *Tortula muralis* (*Pottiaceae*, *Musci*) complex: variation in morphology and ploidy level

## Taxonomická studie vybraných evropských taxonů okruhu *Tortula muralis* (*Pottiaceae*, *Musci*): variabilita morfologických znaků a ploidní úrovně

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Four European taxa of the *Tortula muralis* complex (*T. lingulata, T. muralis* var. *aestiva, T. muralis* var. *muralis, T. obtusifolia*) were evaluated using multivariate analysis of morphological characters, a cultivation experiment and cytological screening (flow cytometry, chromosome counts). This study revealed that only *T. lingulata* is morphologically well defined within the complex and several new sporophytic characters that can be used to distinguish this taxon from the superficially most similar *T. obtusifolia*. The traditionally recognized taxa *T. muralis* var. *muralis, T. muralis* var. *aestiva* and *T. obtusifolia* showed continuous variation, with frequent intermediate plants. However, the main character of the gametophyte used for determination (costa excurrency) proved to be stable in cultivation, indicating that this character is under genetic control. Additionally, rather complex and only partly species-specific patterns of ploidy variation were found within the complex. *Tortula lingulata* and *T. obtusifolia* appear to be cytologically homogeneous; plants of *T. lingulata* were found to be diploid, whereas plants tentatively named as *T. obtusifolia* were haploid. In contrast, both haploid and diploid cytotypes were found in both varieties of *T. muralis*, with a marked predominance of diploids in var. *aestiva* and less marked predominance of diploids in var. *muralis*. Current varietal level of the evaluated infraspecific taxa of *T. muralis* was thus found to be warranted. It is suggested that plants previously recognized as *T. obtusifolia* should be treated as a subspecies of *T. muralis.*

Keywords: chromosome, cultivation experiment, determination key, flow cytometry, hybridization, morphometric analysis, polyploidy, taxonomy

## Introduction

The cosmopolitan genus *Tortula* Hedw. is a morphologically very diverse group within the family *Pottiaceae*. The delimitation of genera is difficult because of the wide range of morphological variation in these members of the family *Pottiaceae*. There is no worldwide revision of the genus. After the revision of the genus in South America (Cano & Gallego 2008), the genus *Tortula* includes approximately 100 species worldwide.

Although the infrageneric treatment of the genus *Tortula* is rather inconsistent, there are definitely groups of taxa within the genus, which are more closely related to each other. One such group, the taxa morphologically close to *Tortula muralis* Hedw. were the subject of this study. This complex in Europe includes predominantly epilithic taxa, with rather small and densely papillose leaf cells (ca 10 μm wide), broadly recurved leaf margin with ± isodiametric marginal cells, short to long excurrent nerve, rather small spores (ca 9–15 μm) and a variety of well developed, twisted to reduced peristome teeth. In Europe, the following generally recognized taxa might be included based on this definition: *T. israelis* Bizot & F. Bilewsky, *T. lingulata* Lindb., *T. muralis* Hedw. and *T. obtusifolia* (Schwägr.) Mathieu. Based on molecular data there would appear to be a close relationship between *T. muralis* and *T. vahliana* (Schultz) Mont (Werner & Guerra 2004). However, *T. vahliana* has several morphological characters, which differentiate it from the *T. muralis* complex (namely large leaf cells, only a slightly revolute leaf margin and less differentiated marginal leaf cells; Smith 2004, Cano 2006). Therefore, *T. vahliana* is not included in this study. For similar reasons, namely the unique type of leaf papillosity, *T. israelis* was also not evaluated.

The most common member of the complex, *T. muralis,* has a well developed peristome with spirally twisted teeth and leaves with costa excurrent into a hyaline hairpoint (Ignatov & Ignatova 2003, Frahm & Frey 2004, Smith 2004). Numerous infraspecific taxa are described within *T. muralis* and most authors recognize two varieties: var. *muralis* with leaves bearing a long hairpoint and var. *aestiva* with a rather short point. Most authors mention that var. *muralis* grows in more sun-exposed and dry habitats than var. *aestiva*. According to Fritsch (1982) there are numerous different chromosome counts reported for *T. muralis*, ranging from n = 13 to n = 66. However, most of these *T. muralis* chromosome counts are close to n = ca 24–30 and n = ca 48–52, respectively. This accords with the study of Newton (1968), who detected two cytotypes in British plants: a haploid with n = 26/27 and diploid with n = 50/52. Results of a recent molecular study based on chloroplast sequences (Werner & Guerra 2004) suggest that *T. muralis* is probably genetically diverse and might include several cryptic taxa, which are morphologically indistinguishable, but genetically distant.

*Tortula obtusifolia* is characterized by having leaves with only a short excurrent non-hyaline costa and a rather short or reduced peristome (Nyholm 1989, Cortini Pedrotti 2001, Ignatov & Ignatova 2003, Frahm & Frey 2004). *Tortula obtusifolia* occurs rather scattered thoroughout the whole of Europe, growing predominantly in mountain areas (Nyholm 1989, Cortini Pedrotti 2001, Frahm & Frey 2004). Chromosome count for *T. obtusifolia* is n = 26 (Lazarenko et al. 1968, Lazarenko et al. 1971).

*Tortula lingulata* is morphologically similar to *T. obtusifolia*, but differs by having a non-excurrent to very short excurrent coloured costa, and well developed leaf border formed by smooth and more thickened cells (Pilous 1957, Pilous & Duda 1960, Ignatov & Ignatova 2003). Plants of *T. lingulata* are described as dioicous (Lindberg 1880, Ignatov & Ignatova 2003), whereas other taxa of the complex are mostly referred to as being monoicous. Based on the literature, *T. lingulata* has a rather narrow distribution, growing in the Baltic area (Ignatov & Ignatova 2003, Ingerpuu et al. 2008), three nearby localities in the Czech Republic (Pilous 1957) and a single locality in Germany (Meinunger & Schröder 2007). Chromosome count of n = 24 is reported for *T. lingulata* (Mamatkulov 1976, Lazarenko et al. 1968, Vysotskaja 1975).

Although past treatments of the *Tortula muralis* complex are consistent, there are doubts about taxa delimitations. Some authors discuss the specific status of *T. obtusifolia*, which is morphologically similar to *T. muralis*, especially its var. *aestiva*. Juratzka (1882) and Loeske (1934) emphasize the difficulty of separating these taxa, Culmann (1921) treats *T. obtusifolia* as a subspecies of *T. muralis*, whereas Boros & Vajda (1957) designate it as a variety. Recently, Kučera & Váňa (2003) pointed out the clinal variability connecting *T. muralis* var. *aestiva, T. obtusifolia* and *T. lingulata*, which makes it impossible to distinguish among those taxa. Because the evaluated taxa also differ ecologically, it is not clear whether they represent distinct genotypes with different ecological requirements, or rather habitat modifications of the same genotype. The latter is suggested to be the case for *T. muralis* var. *aestiva* (Loeske 1934, Düll 1980, Smith 2004) and *T. obtusifolia* (Loeske 1934, Kučera & Váňa 2003).

In this study, the taxonomic status of the above mentioned taxa was evaluated using a complex approach, based on following methods: (i) a multivariate analysis of morphological characters; (ii) cultivation experiments, which focused on a particular leaf character, namely costa excurrency; (iii) evaluation of ploidy level. As at least some taxa of the complex proved to be cytologically variable, we decided also to evaluate cytological characters using flow cytometry (FCM, Doležel et al. 2007), together with classical chromosome counts.

## Material and methods

### *Morphometric study*

A total of 89 herbarium specimens were selected for the morphological measurements (Appendix 1). They originated from European collections, with the exception of plants close to *T. obtusifolia*. As this taxon is rather rare, the number of specimens was increased by including a few specimens from Asia. Twenty-six characters of the gametophore and 17 of the sporophyte were measured and scored (Table 1). These included all the characters reported to be of importance for taxa delimitation, together with others which were variable in the preliminary examination. From each specimen, three plants with undehisced mature capsules were selected for the measurements. Leaf characters of three different leaves from the upper part of the stem of three plants were measured. Leaf cell characters were measured on three different cells from three leaves, each from one of the three plants measured. Each specimen was treated as an operational taxonomic unit and characterized by average values for each morphological character.

This study revealed considerable variation in most diagnostic characters. Some specimens therefore could not with certainty be a priori classified to traditionally distinguished taxa. This was the case for plants morphologically intermediate between *T. obtusifolia* and *T. muralis*, *T. muralis* var. *muralis* and *T. muralis* var. *aestiva*, respectively. Therefore, herbarium specimens were classified into the following six groups using the following descriptions: (i) *T. lingulata* – according to Lindberg's description (Lindberg 1880) and lectotype specimen (deposited in TU): plants from sandstone areas in the region of the Baltic, peristome on average up to 400 µm long, costa never excurrent into a hairpoint, leaves with well developed leaf border; 14 specimens. (ii) *T. obtusifolia* – peristome strongly reduced, with or without well developed filiform teeth, on average up to 250 µm long, costa never excurrent into a hairpoint; 13 specimens. Unfortunately, Schwägrichen does not specify in detail the most important sporophytic character (peristome teeth) in the original description (Schwägrichen 1811). As the author's specimen from the type locality (deposited in BM) possess dehisced capsules with peristomes ca 250 µm long, we treated this character in the above mentioned way, which accords with recent treatments (Nyholm 1989, Ignatov & Ignatova 2003, Frahm & Frey 2004). (iii) Plants intermediate between *T. obtusifolia* and *T. muralis* var. *aestiva* – plants with reduced peristome without filiform

Table 1. – List of of the abbreviations and definitions of the morphological characters of the *Tortula muralis* complex that were measured. ‘Type’ refers to type of character: Q – quantitative, R – ratio computed from quantitative characters, S – semi-quantitative, B – binary.

| Character | Definition | Type |
|---|---|---|
| SetL | seta length | Q |
| CapL | capsule length | Q |
| CapW | capsule width | Q |
| CapL/W | capsule length / capsule width | R |
| SetL/CapL | seta length / capsule length | R |
| OpL | operculum length | Q |
| OpW | operculum width | Q |
| OpL/W | operculum length / operculum width | R |
| CapL/OpL | capsule length / operculum length | R |
| Turn | number of turns of peristome teeth [if < 1, than estimated with accuracy of ca 0.2 of a turn] | Q |
| PerL | peristome length | Q |
| Teeth | filiform teeth of the peristome; 0 – filiforme teeth lacking, 1 – filiform teeth well developed | B |
| Memb | basal membrane height [average value based on measurements at 3 different postions on the peristome] | Q |
| ExcW | exothecial cell width [3 different cells from middle part of the capsule] | Q |
| ExcL | exothecial cell length [3 different cells from middle part of the capsule] | Q |
| ExcL/W | exothecial cell length / exothecial cell width | R |
| SpoS | spore size [3 different spores from each capsule were measured] | Q |
| SteL | Stem length | Q |
| LfL | leaf lamina length | Q |
| CexL | length of excurrent part of the costa | Q |
| CexLr | relative length of excurrent part of the costa [expressed as % of leaf lamina length] | R |
| LfUW | leaf width across distal third of lamina | Q |
| LfUWr | relative leaf width across distal third of lamina [expressed as % of leaf lamina length] | R |
| LfUW/LfW | leaf width across distal third of lamina / leaf lamina width | R |
| LfW | leaf lamina width | Q |
| LfL/W | leaf lamina length / leaf lamina width | R |
| mLfWr | distance of the widest part of leaf lamina from the leaf base / leaf lamina length | R |
| LboLr | maximal length of the leaf border consisting of smooth cells [measured from leaf base; expressed as % of leaf lamina length] | R |
| rLboLr | relative length of the leaf border, defined as follows:<br>(length of the leaf border consisting of smooth cells [measured from leaf base] – length of the area of smooth basal cells [measured from leaf base] ) / leaf lamina length | R |
| LfBLr | length of the area of smooth basal cells [measured from leaf base] / leaf lamina length | R |
| CosW | costa width | Q |
| CosWr | relative costa width [expressed as % of leaf lamina width] | R |
| RevW | maximum width of the revolute part of the leaf lamina margin | Q |
| RevW/LfW | relative width of the maximaum width of the revolute part of the leaf lamina margin [expressed as % of leaf lamina width] | R |
| BLfcL | leaf basal cell length<br>[measured 3 different cells of one leaf of each of the 3 plants measured] | Q |
| BLfcW | leaf basal cell width<br>[measured 3 different cells of one leaf of each of the 3 plants measured] | Q |
| BLfcL/W | leaf basal cell length / leaf basal cell width | R |
| LfcW | upper leaf cell width [measured in a cross-section of the distal third of leaf lamina, 3 different cells, one on a leaf of each of the 3 plants, were measured] | Q |
| RevA | angle of leaf margin revolution on the distal part of leaf lamina<br>[measured on a cross-section, average of the values for both leaf margins] | Q |
| ThCos | width of costa on distal half of leaf lamina; 0 – costa widest on proximal half of leaf lamina, 1– costa widest on distal half of leaf lamina [average value for all 9 leaves was used in the analyses] | B |
| Hair | presence of costa excurrent into a hyaline hairpoint: 0 – none of the leaves had a hairpoint, 1 – at least some of the leaves had a hairpoint | B |
| Spiral | leaf arrangement when dry; 0 – spiral arrangement lacking, leaves irregularly twisted, 1 – spiral arrangement present, but rather indistinct, 2 – spiral arrangement present and distinct | S |
| GamAr | gametangia arrangement; autoicous or dioicous | B |

teeth, but costa excurrent into a hyaline hairpoint; or peristome on average up to 400 µm long, with shorter filiform teeth, costa with various type of excurrency; 17 specimens. (iv) *T. muralis* var. *aestiva* – peristome on average more than 400 µm long, with well developed filiform teeth, average costa excurrency up to 15% of leaf length, leaves when dry irregularly twisted; 18 specimens. Character of costa excurrency is not specified in Hedwig's description (Hedwig 1801). As far as we know, all later authors treat plants with costa shortly excurrent as var. *aestiva*. However, none of them specify the ranges of values of this character distinguishing var. *aestiva* from var. *muralis*. (v) Plants intermediate between *T. muralis* var. *muralis* and *T. muralis* var. *aestiva* – differ from those in the previous group by average costa excurrency ranging between 15–25% of leaf length; six specimens. (vi) *T. muralis* var. *muralis* – peristome on average more than 400 µm long, average costa excurrency more than 25% of leaf length; 21 specimens.

Morphometrical data were processed by multivariate analysis using Canoco for Windows 4.5 (ter Braak & Šmilauer 2002) and CanoDraw for Windows 4.0 (ter Braak & Šmilauer 2002). Principal components analysis (PCA) was used to visualize overall similarity among specimens. Linear discriminant analysis (LDA) was used to test differences between specimens of the most morphologically similar taxa, which was done by stepwise forward selection of the most reliable characters for delimiting the taxa (Monte-Carlo permutation test, 499 permutations). Additionally, for each morphological character considered, the marginal effect was tested using LDA of only the character evaluated. As LDA requires that the data analysed is normally distributed, distributions of average values of measured characters were tested using the Shapiro-Wilk test in Statistica for Windows 5.5 (StatSoft 1999). Values of characters significantly differing from a normal distribution were transformed using either a square root [$x' = \sqrt{(x+1)}$] or logarithmic transformation [$x' = \ln(x+1)$]. In case of five characters (*Turn, PerL, CexL, CexLr, LboLr*) transformation did not result in a normal distribution. However, LDA is generally considered to be a robust method for resolving such problems (Lachenbruch 1975). Finally, the gametangia arrangement character (*GamAr*) was excluded from all analyses, because all taxa examined proved to be autoicous, and all binary and semi-quantitative characters were omitted from LDA.

### *Cultivation experiment*

By growing plants under standard conditions it is possible to separate environmentally induced phenotypic variation from genetically determined variation. A total of 95 herbarium specimens were selected for cultivation (Appendix 1). From each specimen, 3–5 green shoot tips or several leaves were taken and rinsed in water to remove impurities. Shoot tips were transferred to Petri dishes containing a thin layer of crushed limestone, placed on window frame, at room temperature and occasionally watered to keep the substrate slightly wet. Approximately 3/4 of the cultivation attempts produced a protonematal mat. However, new gametophores developed from the protonematal mats or leaf axes of the plants only in approximately 30 out of 95 cultivation attempts. The oldest specimen of *T. muralis* var. *muralis,* which successful produced a gametophore, was 3 years old. Unfortunately, all attempts to cultivate *T. lingulata* failed because they only produced protonematal mats, but no gametophores.

The 23 successful cultivation attempts selected for statistical evaluation included two samples of *T. obtusifolia*, 11 of *T. muralis* var. *muralis*, six of *T. muralis* var. *aestiva* and four of plants intermediate between *T. muralis* var. *muralis* and *T. muralis* var. *aestiva* (Appendix 1; the above mentioned definition was used for taxa classification). Newly developed gametophores were harvested after 5–7 months of cultivation. From these, three well developed gametophores were selected and the following characters of three leaves from each gametophore were measured: leaf lamina length (*LfL*) and length of excurrent part of the costa (*CexL*). Additionally, the relative length of excurrent part of the costa (*CexLr*) was calculated and expressed as % of leaf lamina length. In the same way, three randomly selected gametophores from herbarium specimens were processed. Average values of costa excurrency were calculated for the original plants and cultivated plants, transformed using an arcsin transformation [$x' = \arcsin\sqrt{(x)}$] and compared using analysis of variance (ANOVA) for repeated measurements in Statistica for Windows 5.5 (StatSoft 1999). This analysis revealed the significance of the cultivation effect and interaction between cultivation effect and taxon, respectively. Finally, descriptive statistics were calculated using Statistica for Windows 5.5 (StatSoft 1999).

### *Evaluation of ploidy level*

DNA ploidy level was estimated using flow cytometry (FCM). Air-dried herbarium specimens up to 6 months old were routinely used for this analysis. However, in some cases even 3-year-old specimens were used. A total of 197 specimens from 82 localities were analysed (Appendix 1). Samples from places at least 500 m apart were treated as coming from different localities in the statistical evaluation.

From each specimen, 3–50 shoot tips were taken and rinsed in water to remove impurities. Diploid sporophytes, if present were removed together with gametangia and surrounding tissues in order to avoid including endopolyploid tissues (Lobachevska 1990). *Glycine max* (L.) Merr. cv. 'Polanka' (genome size 2.50 pg) was used as an internal standard. Both the sample and the standard were chopped simultaneously with a razor blade at room temperature in 1 ml of ice-cold buffer LB01 (Doležel et al. 1989). The buffer contained one of the following fluorochromes either: (i) 4,6-diamidino-2-phenylindol (DAPI) at a final concentration of 4 $\mu$L mL$^{-1}$ or (ii) propidium iodide (PI) and RNase IIA, both at final concentrations of 50 $\mu$L mL$^{-1}$. The suspension was filtered through a 42 µm nylon mesh and stained for ca 10 min at room temperature. Analyses were performed either on a Partec PA II flow cytometer equipped with a HBO mercury arc lamp (for DAPI analyses) or CyFlow cytometer equipped with a green (532 nm) solid-state laser (for PI analyses), respectively (both from Partec GmbH., Münster, Germany). Fluorescence intensity of 3000 particles was recorded. Ploidy level was estimated from the relative distances of the sample and standard peaks using Partec FloMax 2.4d software. Only analyses with a sample CV below 10% were considered.

Selected plants analyzed by FCM were also subjected to a standard karyological analysis (for list see Appendix 1). Chromosome counts were of meiotic chromosomes in spore mother cells (SMC) from living juvenile sporophytes (Steere 1954). Because counting the chromosomes proved difficult, because of the small size of the SMCs (ca 10–15 µm) and the chromosomes (ca 1 µm), chromosome numbers were measured to an accuracy of ±3 chromosomes.

## Results

### *Principal components analysis (PCA) of the morphological measurements*

This analysis revealed that 63.9% of the variation is explained by four factors. The results separate *T. lingulata* specimens from the other specimens (Fig. 1). The only exception is the *T. obtusifolia* specimen from France (RS03424), which is morphologically similar to *T. lingulata* and close to *T. lingulata* cluster. That the other taxa do not form discrete clusters implies there is no sharp morphological differentiation between them. Instead, along the first axis there is a conspicuous clinal variation in the groups of plants classified as *T. muralis* var. *muralis*, those intermediate between *T. muralis* var. *aestiva* and *T. muralis* var. *muralis*, *T. muralis* var. *aestiva* plants, those intermediate between *T. muralis* var. *aestiva* and *T.obtusifolia*, and *T. obtusifolia*.

### *Linear discriminant analysis (LDA)*

Analysis of *T. lingulata* and *T. obtusifolia* s.l. (i.e., including specimens intermediate between *T. obtusifolia* and *T. muralis* var. *aestiva*) confirmed the separation of both these groups (Fig. 2A). By using forward selection for searching for the most discriminating combination of characters, a model based on the following characters was identified: spore size (*SpoS*), maximum length of the leaf border (*LboLr*), exothecial cell length (*ExcL*) and exothecial cell width (*ExcW*); for details see Table 2. Among other characters with a significant marginal effect are the relative length of the leaf border (*rLboLr*) and upper leaf cell width (*LfcW*) (Table 3). Surprisingly, the marginal effect of the character mostly cited in the literature, costa excurrency (*CexL, CexLr*), was significant, but weakly so. As in the PCA, one specimen of *T. obtusifolia* (RS03424) is close to the *T. obtusifolia* cluster. However, this specimen can be correctly identified by using the most important discriminating character, i.e. spore size (*SpoS*).

LDA including *T. lingulata* and *T. muralis* var. *aestiva* revealed clear separation of both groups with no overlap (Fig. 2B). This analysis excluded the costa excurrency characters (*CexL, CexLr*), as they separate both taxa and were therefore used for defining the group.

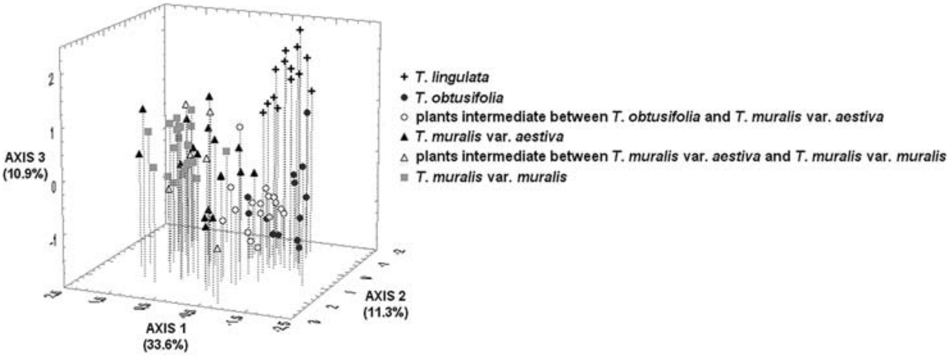
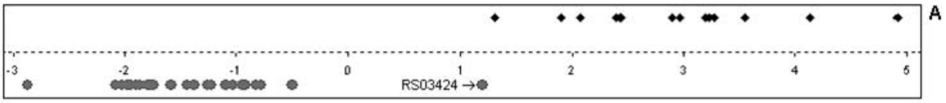


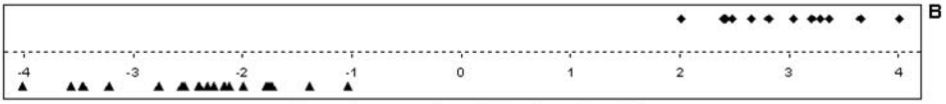
Fig. 1. – Principal components analysis (PCA) of the *Tortula muralis* complex, which included all the specimens. Percentages of variance explained by the axes are given in brackets.

Table 2. – Morphological characters of the *Tortula muralis* complex with significant conditional discriminant effect in particular linear discriminant analyses (LDA); forward selection, Monte-Carlo permutation test, 499 permutations. λ – discriminant effect of individual character after adding to the model (conditional effect), c % – cumulative percentage of explained variation after adding to the model, p – significance level after adding to the model, log – logarithmic transformation, sqr – square root transformation.

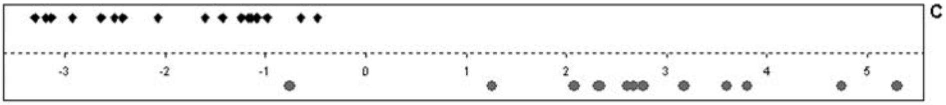
| Taxa analysed | Character | λ | c % | p |
|---|---|---|---|---|
| *T. lingulata* | SpoS | 0.636 log | 63.6 | 0.002 |
| *T. obtusifolia* s.l. | LboLr | 0.105 log | 74.1 | 0.002 |
| | ExcL | 0.030 log | 77.1 | 0.018 |
| | ExcW | 0.021 | 79.2 | 0.032 |
| *T. lingulata* | LboLr | 0.728 log | 72.8 | 0.002 |
| *T.muralis* var. *aestiva* | PerL | 0.087 log | 81.6 | 0.002 |
| | BLfcL/W | 0.017 | 83.3 | 0.030 |
| | LfUWr | 0.015 log | 84.8 | 0.048 |
| | Memb | 0.015 sqr | 86.4 | 0.044 |
| *T. obtusifolia* | LfL/W | 0.581 | 58.1 | 0.002 |
| *T.muralis* var. *aestiva* | RevW/LfW | 0.161 | 74.2 | 0.002 |
| | LfUWr | 0.039 | 78.1 | 0.048 |
| *T.muralis* var. *aestiva* | CosW | 0.535 | 53.5 | 0.002 |
| *T.muralis* var. *muralis* | LfcW | 0.079 | 61.4 | 0.008 |
| | ExcW | 0.076 | 69.0 | 0.010 |



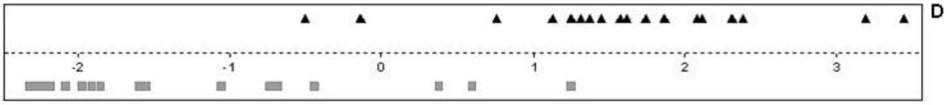
◆ *T. lingulata*, ● *T. obtusifolia* (including plants intermediate between *T. obtusifolia* and *T. muralis* var. *aestiva*); CC = 79.2



▲ *T. muralis* var. *aestiva*, ◆ *T. lingulata*; CC = 86.4



◆ *T. muralis* var. *aestiva*, ● *T. obtusifolia*; CC = 78.1



■ *T. muralis* var. *muralis*, ▲ *T. muralis* var. *aestiva*; CC = 69.0

Fig. 2. – Results of the linear discriminant analysis (LDA) of the *Tortula muralis* complex. CC – % of the total morphological variation explained by the first canonical component.

Table 3. – Contribution of individual characters to the discrimination of groups of the *Tortula muralis* complex (marginal effect) revealed by particular linear discriminant analyses (LDA), Monte-Carlo permutation test, 499 permutations. λ – marginal discriminant effect, P – significance level, log – logarithmic transformation, sqr – square-root transformation, predictor – character used for group definition (excluded from the analysis). Characters significant at the 0.05 level are printed in bold.

| Taxa analysed | *T. lingulata* *T. obtusifolia* s.l. | | *T. lingulata* *T.muralis* var. *aestiva* | | *T. obtusifolia* *T.muralis* var. *aestiva* | | *T. muralis* var. *aestiva* *T.muralis* var. *muralis* | |
|---|---|---|---|---|---|---|---|---|
| Character | λ | P | λ | P | λ | P | λ | P |
| BLfcL | **0.133** | 0.010 | 0.001 | 0.850 | 0.105 | 0.064 | 0.009 | 0.586 |
| BLfcL/W | 0.047 | 0.162 | 0.014 | 0.480 | 0.028 | 0.366 | 0.005 log | 0.662 |
| BLfcW | 0.059 log | 0.110 | 0.021 | 0.430 | 0.122 sqr | 0.060 | 0.068 | 0.126 |
| CapL | 0.006 | 0.600 | **0.295** | 0.002 | **0.286** | 0.006 | **0.383** | 0.002 |
| CapL/OpL | 0.030 | 0.262 | **0.224** | 0.006 | 0.083 | 0.118 | 0.046 | 0.166 |
| CapL/W | 0.034 log | 0.212 | **0.373** | 0.002 | **0.299** | 0.004 | **0.160 log** | 0.012 |
| CapW | 0.062 | 0.124 | 0.004 | 0.730 | 0.064 | 0.182 | **0.158** | 0.010 |
| in0CexL | **0.103** | 0.026 | predictor | – | predictor | – | predictor | – |
| CexLr | **0.171 sqr** | 0.004 | predictor | – | predictor | – | predictor | – |
| CosW | **0.186** | 0.004 | **0.456 log** | 0.002 | **0.145 log** | 0.042 | **0.535** | 0.002 |
| CosWr | **0.394** | 0.002 | **0.513** | 0.002 | **0.150** | 0.028 | 0.021 | 0.374 |
| ExcL | **0.401 log** | 0.002 | 0.004 | 0.728 | **0.491** | 0.002 | **0.195** | 0.010 |
| ExcL/W | 0.005 log | 0.638 | **0.282** | 0.008 | **0.397** | 0.002 | 0.057 | 0.158 |
| ExcW | **0.632** | 0.002 | **0.287** | 0.004 | **0.183 log** | 0.026 | **0.152** | 0.024 |
| LboLr | **0.574 log** | 0.002 | **0.728 log** | 0.002 | 0.088 sqr | 0.090 | 0.025 log | 0.388 |
| LfBLr | 0.006 | 0.596 | **0.272** | 0.002 | **0.342** | 0.002 | 0.039 | 0.244 |
| LfcW | **0.511** | 0.002 | **0.289** | 0.004 | **0.144 log** | 0.036 | **0.364** | 0.002 |
| LfL | 0.041 | 0.182 | **0.513** | 0.002 | **0.501** | 0.002 | **0.367** | 0.002 |
| LfL/W | **0.121** | 0.020 | **0.574** | 0.002 | **0.581** | 0.002 | 0.020 | 0.372 |
| LfUW | 0.014 | 0.400 | 0.002 | 0.784 | 0.028 | 0.356 | **0.458** | 0.002 |
| LfUW/LfW | 0.011 | 0.492 | **0.112** | 0.040 | 0.097 | 0.094 | **0.255 sqr** | 0.002 |
| LfUWr | **0.115** | 0.028 | **0.582 log** | 0.002 | **0.571** | 0.002 | **0.134** | 0.014 |
| LfW | 0.035 | 0.244 | 0.057 | 0.166 | 0.001 | 0.894 | **0.371** | 0.002 |
| Memb | **0.354 log** | 0.002 | **0.699 sqr** | 0.002 | **0.515** | 0.002 | 0.022 | 0.342 |
| mLfWr | 0.005 | 0.662 | 0.004 | 0.722 | 0.001 | 0.876 | **0.155** | 0.018 |
| OpL | 0.041 | 0.204 | **0.366** | 0.004 | **0.332** | 0.004 | **0.342** | 0.002 |
| OpL/W | 0.066 | 0.066 | **0.441** | 0.002 | **0.359** | 0.002 | **0.284 sqr** | 0.002 |
| OpW | 0.010 | 0.526 | 0.022 | 0.430 | 0.061 | 0.206 | 0.032 | 0.290 |
| PerL | 0.009 log | 0.488 | **0.721 log** | 0.002 | predictor | – | **0.450** | 0.002 |
| RevA | **0.120** | 0.024 | 0.087 | 0.088 | **0.353** | 0.002 | 0.067 | 0.110 |
| RevW | 0.023 | 0.354 | **0.161** | 0.022 | **0.243** | 0.004 | **0.447** | 0.002 |
| RevW/LfW | **0.141** | 0.006 | 0.023 | 0.422 | **0.321** | 0.002 | 0.032 | 0.264 |
| rLboLr | **0.547 log** | 0.002 | **0.613 log** | 0.002 | 0.041 log | 0.300 | 0.001 log | 0.826 |
| SetL | 0.011 | 0.492 | **0.267** | 0.002 | **0.423 log** | 0.002 | **0.208** | 0.002 |
| SetL/CapL | 0.063 | 0.098 | **0.115** | 0.042 | **0.292** | 0.008 | 0.012 | 0.518 |
| SpoS | **0.636 log** | 0.002 | **0.544** | 0.002 | 0.003 | 0.764 | 0.007 | 0.618 |
| SteL | **0.186 log** | 0.002 | **0.217** | 0.004 | 0.001 | 0.848 | **0.087** | 0.048 |
| Turn | 0.007 | 0.598 | **0.331 log** | 0.002 | predictor | – | **0.522 log** | 0.002 |

However, the LDA indicates that both taxa differ markedly in several other characters and seems to be well separated morphologically. Using forward selection, a model based on following characters was identified: maximum length of the leaf border (*LboLr*), peristome length (*PerL*), leaf basal cell length / leaf basal cell width (*BLfcL/W*), leaf width measured across distal third of lamina / leaf lamina length (*LfUWr*), basal membrane height (*Memb*); for details see Table 2. Among other characters with a significant marginal effect are relative length of the leaf border (*rLboLr*), leaf lamina length / leaf lamina width (*LfL/W*), spore size (*SpoS*), relative costa width (*CosWr*) and leaf lamina length (*LfL*) (Table 3).

LDA including *T. obtusifolia* and *T. muralis* var. *aestiva,* i.e. without plants intermediate between both taxa, revealed a considerable separation between these groups (Fig. 2C). This analysis did not include the following characters: costa excurrency (*CexL, CexLr*), peristome length (*PerL*) and number of turns of peristome teeth (*Turn*), as they clearly separated both taxa and were therefore used to define the group. Thus, this LDA indicates that plants with the typical characters of *T. obtusifolia* and/or *T. muralis* var. *aestiva* are distinct morphotypes and can be separated by using several other characters. Using forward selection, a model based on the following characters was identified: leaf lamina length / leaf lamina width (*LfL/W*), relative width of the maximum width of the revolute part of the leaf lamina margin (*RevW/LfW*) and leaf width measured across the distal third of the lamina / leaf lamina length (*LfUWr*); for details see Table 2. Among other characters with a significant marginal effect are leaf lamina length (*LfL*) and basal membrane height (*Memb*) (Table 3).

LDA including *T. muralis* var. *aestiva* and *T. muralis* var. *muralis,* but not plants intermediate between these taxa, revealed a considerable overlap between these groups (Fig. 2D). This analysis did not include the costa excurrency characters (*CexL, CexLr*) as they were used to define the group. Using forward selection, a model based on following characters was identified: costa width (*CosW*), upper leaf cell width (*LfcW*) and exothecial cell width (*ExcW*); for details see Table 2. As a result, the separation of these taxa based on costa excurrency alone seems to be rather arbitrary.

### *Cultivation experiment*

The cultivated plants differed from the original plants in having smaller leaves (data not shown). This may be caused by poor cultivation conditions or a consequence of the young age of the cultivated plants. However, the most important gametophyte character traditionally used for taxa determination, the relative length of the excurrent part of the costa (*CexLr;* significantly different among taxa, ANOVA: $P < 0.01$), was not affected by cultivation (no significant difference between original and cultivated plants; ANOVA: $P = 0.107$). Additionally, the effect of the interaction (cultivation effect x taxon) was not significant (ANOVA: $P = 0.236$), suggesting that this character was stable in all the taxa considered. Cultivation revealed that costa excurrency is genetically determined. All the plants determined as *T. muralis* var. *aestiva* had the typical phenotype of a relatively short costa excurrency. Similarly, *T. muralis* var. *muralis* plants retained their typical long hairpoint and in *T. obtusifolia* plants the relative length of the excurrent part of the costa was very short (Fig. 3).

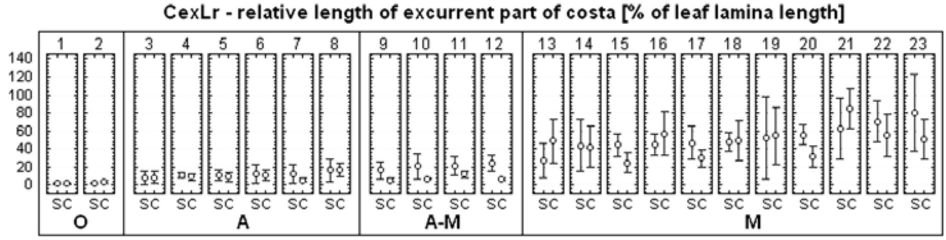


Fig. 3. – Comparison of leaf characters of plants of the *Tortula muralis* complex from herbarium specimens and plants that were propagated under standard conditions from material obtained from the herbarium specimens. Points – average values, bars – SD; O – *T. obtusifolia*, A – *T. muralis* var. *aestiva*, A-M – plants intermediate between *T. muralis* var. *aestiva* and *T. muralis* var. *muralis,* M – *T. muralis* var. *muralis;* S – original plants in herbarium collections, C – plants propagated by cultivation.

### *Evaluation of the variation in ploidy level*

The methods used in this study did not identify any aneuploid plants, which might account for several of the less frequent chromosome counts cited in the literature. This was due to the rather high CV of most of the analyses (usually 3–5%) and the low accuracy of the chromosome counts. However, both methods gave accurate estimates of the ploidy level.

### *Tortula muralis*

The ploidy level of 144 specimens from 82 localities, including 42 specimens of var. *aestiva*, 76 of var. *muralis* and 26 of intermediates between *T. muralis* var. *aestiva* and var. *muralis,* were examined. Two cytotypes were detected in both varieties of *T. muralis*: haploid with a ratio of 0.589±0.0264 for sample/standard and diploid with a ratio of 1.178±0.059 pg (average±SD, data obtained from PI analyses). Chromosome counts of four *T. muralis* accessions cytometrically determined as DNA-diploids also confirmed the diploid chromosome count (sensu Newton 1968): chromosome counts for *T. muralis* var. *aestiva* revealed n = 45–52 (KO1274: n = ca 47–52; KO1275: n = ca 45–49) and for *T. muralis* var. *muralis* n = 47–54 (KO1270: n = ca 50–54, KO1271: n = ca 47–51).

The relative frequency of both the *T. muralis* cytotypes is shown in Fig. 4. Generally, the diploid cytotype seems to be more frequent in *T. muralis.* When both *T. muralis* varieties are considered separately, the diploid cytotype predominates in var. *aestiva* and plants intermediate between both varieties, whereas in var. *muralis* the predominance of diploids is less distinct. In all taxa of *T. muralis* s. str. localities at which both the cytotypes are present are rare. The cytotypes appear to be rather randomly distributed in those areas cytometrically intensively screened (i. e. the Czech Republic, data not shown).

### *Tortula obtusifolia*

The 35 specimens of *T. obtusifolia,* from 15 localities, analysed using FCM were all haploids. Their sample/standard ratio of 0.601±0.0234 pg (mean±SD, data obtained by PI staining) is close to that of the haploid cytotypes of *T. muralis*. Chromosome counts for *T. obtusifolia* revealed n = ca 23–25 (KO1544). All individuals intermediate between

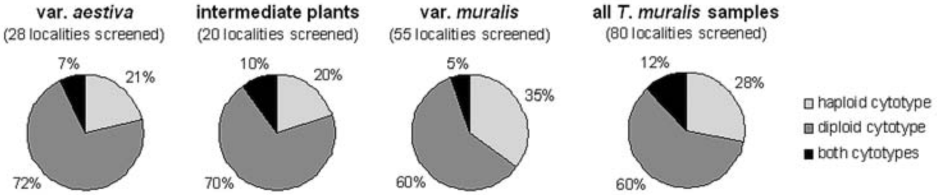


Fig. 4. – Relative frequencies of cytotypes in varieties of *Tortula muralis* (expressed as % of localities screened).

*T. obtusifolia* and *T. muralis* var. *aestiva* (10 specimens from seven localities) were haploid, with n = ca 25–31 (KO1277). These chromosome counts are close to the n = 26 reported for *T. obtusifolia* (Lazarenko et al. 1968, 1971).

*Tortula lingulata*

The seven specimens from the five Latvian localities, together with 1 sample from the Czech locality, tentatively determined as *T. lingulata*, were analysed using FCM. All these specimens were diploid. Their sample/standard ratio of 1.206±0.0357 pg (mean±SD, data obtained by PI staining) is close to that of the diploid cytotypes of *T. muralis*. Although the number of chromosomes was not determined for *T. lingulata* it is likely it is similar to that of the diploid cytotype of *T. muralis* (i.e. n = ca 45–54). This is different from the chromosome counts reported for *T. lingulata*. Vysotskaya (1975), Lazarenko et al. (1968) and Mamatkulov (1976) report n = 24 for this taxon. However, it should be noted that these counts are for plants from Ukraine and Tajikistan, and therefore there is doubt about the identity of these plants.

## Discussion

This study revealed that most of the morphological characters used for taxa delimitation are very variable and in some cases unable to distinguish traditionally recognized taxa. On the other hand, there are some morphological and cytological characters that can be used for identifying these taxa.

In *T. lingulata*, the putative dioicy of *T. lingulata* mentioned by Lindberg (1880), proved to be erroneous, as most specimens studied were heteroicous, with at least some plants bearing autoicous inflorescences. Such a gametangial arrangement was found in all the taxa of this complex and probably is not taxonomically important. The character costa excurrency was found to be reliable for distinguishing *T. lingulata* from both *T. muralis* varieties, but this character when used alone failed to distinguish *T. lingulata* from some morphotypes of *T. obtusifolia* (Fig. 1 or Fig. 5D, respectively). However, results of the LDA suggest that separation is always possible if other characters, especially the larger size of spores and exothecial cells, and the usually longer leaf border, are also taken into consideration (see Fig. 5). Interestingly, these characters were not previously used for identifying *T. lingulata*, although the larger spore size is mentioned in species descriptions (Malta 1919, Ignatov & Ignatova 2003). Apart from the above, *T. lingulata* was found to differ from *T. obtusifolia* in ploidy level. All specimens of *T. lingulata* proved to be diploid,

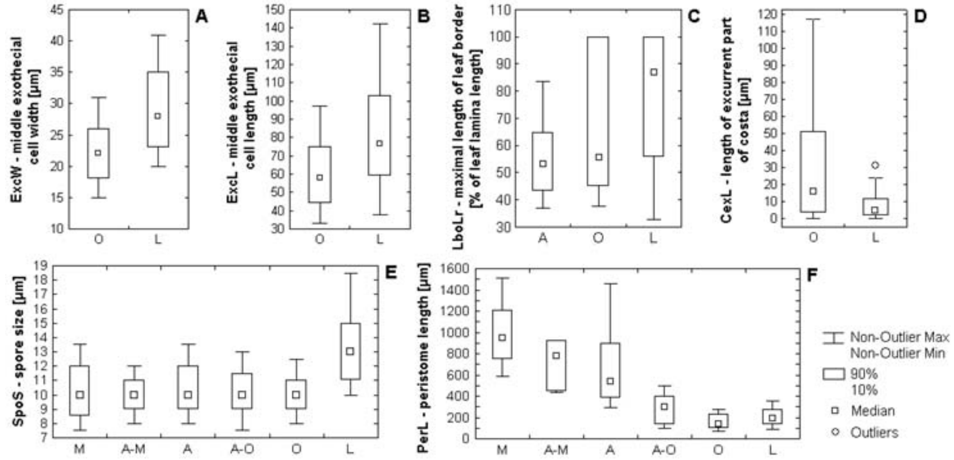


Fig. 5. – Descriptive statistics of selected morphological characters. A – *Tortula muralis* var. *aestiva*, M – *T. muralis* var. *muralis*, L – *T. lingulata*, O – *T. obtusifolia*, A–O – plants intermediate between *T. muralis* var. *aestiva* and *T. obtusifolia*, A–M – plants intermediate between *T. muralis* var. *aestiva* and *T. muralis* var. *muralis*.

whereas those of *T. obtusifolia* were haploid. This implies that both taxa exhibit important differences and *T. lingulata* therefore, should be recognized as a distinct species.

*Tortula lingulata* differs from other taxa of the complex in its ecology and restricted distribution, growing on ± base-rich sandstones in the Baltic area (namely Latvia and Estonia) and adjacent parts of Russia. Probably the specimens from the Czech localities (Pilous 1957) are identical with these plants. As the Czech plants lacked undehisced capsules they were not included in the statistical analysis. However, a recently collected specimen analysed using FCM (KO 577) was diploid and the spore size of the herbarium specimens ranges between 13–15 µm (Pilous 6.1943 – herb. J. Kučera CS 2758 – CBFS), which is typical for this species (Fig. 5E).

In *T. muralis*, two varieties are recognized, based mainly on costa excurrency. However, our study of a large number of specimens revealed that this character does not clearly distinguish these taxa. Moreover, the multivariate analysis of all the characters measured (PCA ordination, see Fig. 1) detected a great deal of variation in *T. muralis*, with frequent occurrence of intermediate plants. Similarly, when costa excurrency was not included in the LDA as the main taxa predictor, there was a distinct overlap between plants of var. *muralis* and var. *aestiva* (Fig. 2D), indicating that other characters do not completely separate these taxa. In addition, the results of the cultivation experiment revealed that costa excurrency is genetically determined (Fig. 3). Therefore, both varieties cannot represent mere ecological modifications, as suggested by Loeske (1934), Düll (1980) and Smith (2004). FCM screening revealed also that the predominance of diploids in var. *muralis* was less distinct in var. *aestiva*. Together with the somewhat different ecological requirements of var. *muralis* and var. *aestiva*, our results suggest that these varieties most likely have different genotypes but are not fully morphologically differentiated and therefore do not fulfil the criteria of morphologically defined species. Accordingly, the current practice of treating these taxa as varietes is appropriate.

Newton (1968) indicates there is a slight difference in size between haploid and diploid *T. muralis* plants, referring probably to var. *muralis*. A similar trend was detectable also in our specimens of *T. muralis* var. *muralis* (data not shown). The presence of two different ploidy levels in *T. muralis* raises questions about the evolutionary relationship between haploid and diploid cytotypes. The few localities in this study where both cytotypes co-occurred, might suggest multiple and recurrent autopolyploid origin of diploids. This is consistent with the considerable sequence divergence in the *rps4* chloroplast region, which suggest possible occurrence of cryptic taxa within *T. muralis* (Werner & Guerra 2004).

In most studies, *T. obtusifolia* is consistently regarded as a species. However, we found many plants that were intermediate between *T. obtusifolia* and *T. muralis* var. *aestiva*. Interestingly, these plants had one of two possible combinations of diagnostic characters, typical of neither *T. obtusifolia*, nor *T. muralis* var. *aestiva*: (i) peristome reduced and costa excurrent into a hairpoint, (ii) peristome well developed or somewhat reduced and costa only shortly excurrent, non-hyaline. In these plants the character of the peristome varied from the most reduced type without filiform teeth (Fig. 6A) to types with rather short teeth (Fig. 6B). This clinal variation between both types of peristome is mentioned by Loeske (1934).

The multivariate analysis (PCA, Fig. 1) revealed that plants of *T. obtusifolia* and *T. muralis* var. *aestiva* are distributed in one contiguous cluster. However, plants with the typical morphology of *T. obtusifolia* and *T. muralis* var. *aestiva* were distinctly separated when evaluated using LDA (Fig. 2C). Interestingly, this pattern is different from the above mentioned case of *T. muralis* var. *muralis* and *T. muralis* var. *aestiva*, where typical plants of both varieties were only weakly separated by characters other than those used for taxa definition. Another difference between *T. obtusifolia* and *T. muralis* var. *aestiva* is their ploidy level. Whereas all specimens tentatively named as *T. obtusifolia* were haploid, the diploid cytotype distinctly prevailed in *T. muralis* var. *aestiva*. This implies that *T. obtusifolia* deserves to be recognized as a distinct taxon, e.g. as a subspecies of *T. muralis*, as already proposed by Culmann (1921). This seems to be reasonable, as the overall morphological and cytological differences between *T. obtusifolia* and *T. muralis* are greater, than those between both varieties of *T. muralis*. It is unlikely that morphological variation in the characters used for distiquising between *T. obtusifolia* and *T. muralis* are environmentally determined, as costa excurrency is genetically determined, and characters of the peristome and sporophyte of mosses are considered to be generally little influenced by habitat conditions (Natcheva & Cronberg 2004). Additionally, plants with different combinations of diagnostic characters were observed several times growing at the same locality under similar conditions (J. Košnar, unpublished). Therefore, it is possible that hybridization may account for the cline in variation between *T. obtusifolia* and *T. muralis* var. *aestiva*. In general, hybridization has been rarely detected or even suggested for taxonomically difficult groups of bryophytes and its importance is probably underestimated (Natcheva & Cronberg 2004). Alternatively, the existence of two different types of plants, intermediate between *T. obtusifolia* and *T. muralis* var. *aestiva*, and the considerable variation in morphology and ploidy level in *T. muralis*, might suggest the existence of several cryptic or almost cryptic taxa. All those hypotheses need to be tested, preferably using molecular markers.

In conclusion, our study of the *T. muralis* complex demonstrates that a combination of morphological and cytological characters can be used to evaluate taxonomically difficult groups of bryophytes. Application of formalized statistical morphometrics, based on large

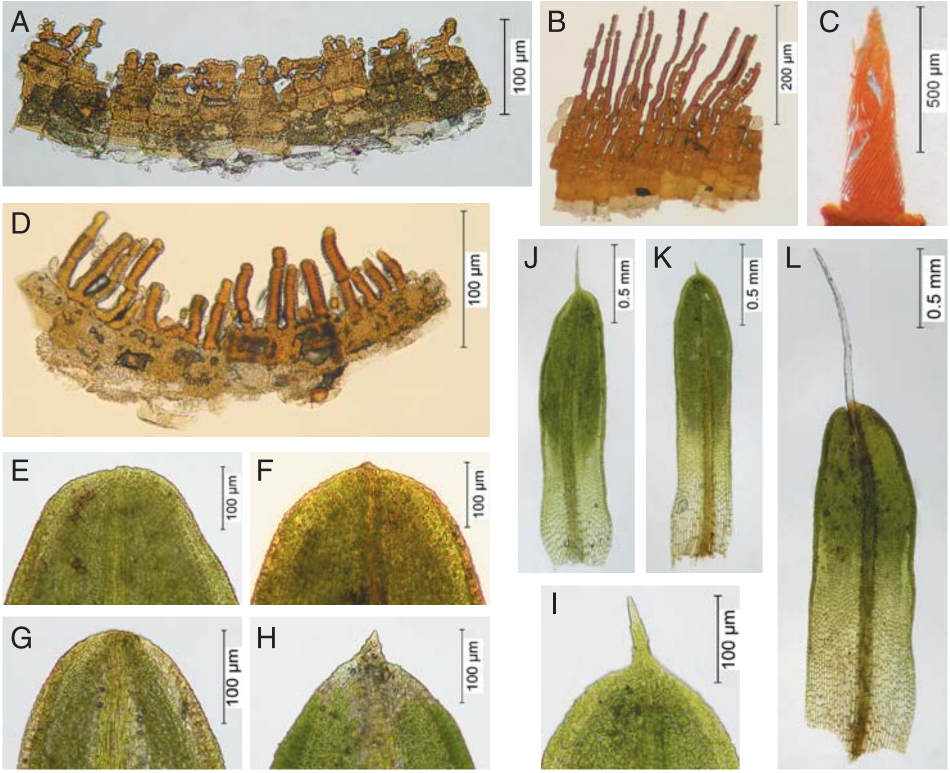


Fig. 6. – Variation in morphological characters of taxa of the *Tortula muralis* complex; peristome: (A) *T. muralis* subsp. *obtusifolia* (Demeter 1887 – BP), (B) plant intermediate between *T. muralis* subsp. *obtusifolia* and T. *muralis* subsp. *muralis* var. *aestiva* (Brotherus 1896 – S), (C) T. *muralis* subsp. *muralis* var. *muralis* (Košnar 2006 – KO771), (D) *T. lingulata* (Košnar 2006 – KO780); leaf apex and leaf shape: (E–F) *T. lingulata* (Košnar 2006 – KO795, Košnar 2005 – KO570), (G–H) *T. muralis subsp. obtusifolia* (Skrzypczak 2003 – RS03424, Košnar 2006 – KO631), (I–K) *T. muralis* subsp. *muralis* var. *aestiva* (Košnar 2005 – KO1013), (L) *T. muralis* subsp. *muralis* var. *muralis* (Košnar – KO771).

datasets, has been rather rarely used in studies on bryophytes. Interestingly, there are examples of this approach, the systematic studies of other closely related taxa of the genus *Tortula*, namely the *T. subulata* (Cano et al. 2005) and *T. laevipila* complexes (Gallego et al. 2005). Similarly, there are few cases of cultivation experiments being used in bryophyte studies (e.g., Shaw 1987 – *Weissia*; Briggs 1964 – *Dicranum*; Buryová & Shaw 2005 – *Philonotis*; Mishler 1985 – *Tortula*). This is striking when compared, e.g. with the incidence common garden experiments in studies on flowering plants. Some studies on mosses indicate that the characters used for taxa delimitation vary considerably when reared under experimental conditions (Mishler 1985). Therefore, it may often be necessary to cultivate bryophytes under standard condition in order to identify suitable taxonomic characters. Our study indicates that the cultivation of bryophytes is quite easy, as no sophisticated equipment is required, even to grow the almost strictly epilithic taxa of the *Tortula muralis* complex. When considering, e.g. tericolous mosses, one can assume that cultivation might be even easier. Finally, flow cytometry can serve as a fast and effective method for determining ploidy level, even when dealing with minute amounts of moss tissue obtained from air-dried specimens. In taxonomic studies of mosses this method has been used only in a study on *Sphagnum* (Melosik et al. 2005). Our study is the first to use FCM on taxa of the genus *Tortula*. However, probably there are taxa in all moss families that have variable chromosome counts (Fritsch 1982). Within the genus *Tortula*, the *T. subulata* complex (Cano et al. 2005) is another example of a taxonomically difficult polyploid complex. Therefore, it is likely that a wider application of FCM will greatly improve the systematic studies of bryophytes.

Key to taxa of the *Tortula muralis* complex

**1a** Spores (10–) 11–15 (–18.5) µm; leaves bordered by several rows of smooth cells, which at least in some leaves reach the leaf apex; peristome (100–) 140–280 (–350) µm long, filiform teeth slightly twisted, often irregularly developed; leaf apex with mucro or excurrent part of costa only 2–12 (–30) µm long, never forming a hyaline hairpoint; diploid plants, growing on ± base-rich sandstones .......................................... ***T. lingulata***

**1b** Spores (7.5–) 8.5–12 (–13.5) µm; leaf border of various length, usually not reaching leaf apex; peristome variable, ca 100–1500 µm long, well developed or sometimes reduced or without filiform teeth; leaf apex various, with short mucro, excurrent costa or hyaline hairpoint; haploid or diploid plants, growing on various epilithic substrates .......................................... 2

**2a** Peristome mostly reduced, sometimes without filiform teeth; leaf apex usually with costa excurrent into a short non-hyaline point; leaves often spirally arranged when dry; haploid plants, usually growing on base-rich rocks .......................................... ***T. muralis*** subsp. ***obtusifolia***

**2b** Peristome well developed, with spirally twisted filiform teeth; costa excurrency short to long, in upper part often hyaline; haploid or diploid plants, growing on base-rich rocks or artificial substrates .......................................... ***T. muralis*** subsp. ***muralis*** (3)

**3a** Costa excurrent into a rather short point .......................................... **var. *aestiva***

**3b** Costa excurrent into a rather long hyaline hairpoint .......................................... **var. *muralis***

Note: Sterile plants or plants with old dehisced capsules cannot be determined with certainty. *Tortula lingulata* is easily distinguished from *T. muralis* subsp. *muralis* even in the field, as the leaf apices have inconspicuous mucro or very shortly excurrent costa. However, for distinguishing between *T. lingulata* and *T. muralis* subsp. *obtusifolia*, spore size is the best character. Separation of *T. muralis* subsp. *obtusifolia* from *T. muralis* subsp. *muralis* var. *aestiva* is often impossible, as there are no reliable characters for distinguishing these taxa and intermediate plants are quite frequent. As peristome teeth are rather fragile, freshly dehisced capsules should be evaluated.

## Acknowledgements

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## Souhrn

Práce se zabývá problematikou taxonomie čtyř evropských taxonů komplexu *T. muralis* (*T. lingulata, T. muralis* var. *aestiva, T. muralis* var. *muralis, T. obtusifolia*). Vzorky studovaných taxonů byly zkoumány pomocí mnohorozměrné analýzy morfologických znaků, srovnávací kultivace a cytologických metod (průtoková cytometrie, počítání chromozomů).

Jako jediný morfologicky dobře definovaný taxon komplexu se jeví *T. lingulata.* Tento taxon lze odlišit od morfologicky nejpodobnější *T. obtusifolia*, pro praktickou determinaci se jako nejspolehlivější zdají být nově

zjištěné rozdíly ve znacích sporofytu (zejména velikost spor), naopak tradičně udávané znaky gametofytu (míra vybíhání listového žebra, délka listového lemu) byly shledány jako méně spolehlivé. Ostatní studované taxony komplexu se vyznačují klinální variabilitou, s poměrně častým výskytem rostlin přechodných mezi taxony *T. obtusifolia* – *T. muralis* var. *aestiva* – *T. muralis* var. *muralis*. V obou udávaných diagnostických znacích gametofytu i sporofytu (míra vybíhání listového žebra, resp. redukce obústí) byla u těchto taxonů zjištěna kontinuální variabilita. U vzorků přechodných mezi *T. obtusifolia* a *T. muralis* var. *aestiva* byly zaznamenány rostliny s oběma možnými kombinacemi diagnostických znaků gametofytu a sporofytu. Výsledky srovnávací kultivace gametofytů těchto tří taxonů přitom prokázaly, že tradičně udávaný determinační znak gametofytu (vybíhavost listového žebra) je geneticky podmíněný, a uvedené taxony proto nemohou být považovány za pouhé ekomorfózy. Tomuto závěru odpovídá i pozorovaný výskyt směsných populací odlišných morfotypů.

Analýza ploidní úrovně odhalila u taxonů komplexu výskyt haploidních a diploidních rostlin. U morfologicky podobných taxonů *T. obtusifolia* a *T. lingulata* byl objeven rozdíl v ploidii: pouze haploidní cytotyp byl detekován u vzorků *T. obtusifolia* (včetně rostlin přechodných k *T. muralis* var. *aestiva*), zatímco všechny vzorky *T. lingulata* byly diploidní. Haploidní i diploidní cytotyp byl detekován u obou variet *T. muralis*; u var. *aestiva* výrazněji převládaly diploidní rostliny, zatímco u var. *muralis* byla zjištěna pouze mírná převaha diploidního cytotypu.

Výsledky studie podporují stávající samostatný druhový status taxonu *T. lingulata*, a rozlišování obou variet *T. muralis*. Pro rostliny dříve rozlišované jako *T. obtusifolia* bylo přijato hodnocení jako subspecie *T. muralis*.

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Appendix 1. – List of herbarium specimens studied. Ploidy levels estimated by FCM are given in bold: n – haploid cytotype, 2n – diploid cytotype; CE – specimen used for cultivation experiment, CH – specimen used for chromosome counting, MA – specimen used for multivariate analysis; KO = private herbarium of J. Košnar, KU, CS, E = private herbarium of J. Kučera, RS = private herbarium of R. Skrzypczak. Specimens from KO, KU, CS & E herbaria are deposited in CBFS.

***Tortula muralis* var. *aestiva***

AUSTRIA: Tirol, Innervillgraten. Ad saxa cal. 5200, VII 1885, Gander sub *T. obtusifolia* (BP) **[MA]**; Tirol, Innervillgraten. Am Kalksteinem in "Kalkstein", 5200, 3 VII 1882 Gander sub *T. obtusifolia* (BP) **[MA]**; CZECH REPUBLIC: Distr. Blansko, Arnoštovo údolí valley, ca 350 m a.s.l., N49°21.1851', E16°41.022', 21 IX 2005, J. Košnar – KO603 **[2n]**; Distr. Bruntál, Skály, ca 640 m a.s.l., N49°54.8452', E17°13.3103', 13 VIII 2006, J. Košnar – KO1049 **[2n]**; Distr. Český Krumlov, Loučovice, SSE-exposed slope of Mt. Svatý Prokop, ca 725 m a.s.l., N48°37.3652', E14°14.9418', 5 XI 2005, J. Košnar – KO1181 **[2n]**; Distr. Český Krumlov, NR Dívčí Kámen, 430 m a.s.l., N48°53.4297', E014°21.3684', 12 V 2005, J. Košnar – KO412 **[2n]**; Distr. Domažlice, Pila, ca 490 m a.s.l., N49°24.6679', E12°51.8888', 27 V 2005, J. Košnar – KO1888 **[n; MA]**; Distr. Jablonec n. Nisou, NNR Rašeliniště Jizerky, ca 860 m a.s.l., N49°50.8709', E16°7.9527', 6 VIII 2005, J. Košnar – KO554 **[MA]**; Distr. Jablonec n. Nisou, NNR Rašeliniště Jizerky, ca 860 m a.s.l., N50°49.3692', E15°20.2452', 6 VIII 2005, J. Košnar – KO553 **[2n]**; Distr. Jičín, Kost castle, ca 320 m a.s.l., N50°29.4475', E15°8.1374', 26 VI 2005, J. Košnar – KO561 **[2n; MA]**; Distr. Jičín, Kost castle, ca 320 m a.s.l., N50°29.4475', E15°8.1374', 26 VI 2005, J. Košnar – KO561 **[2n]**; Distr. Jindřichův Hradec, 2.2 km ENE of Velký Londýn, 485 m a.s.l., N48°53.8828', E14°59.2588', 3 IV 2005, J. Košnar – KO312 **[2n]**; Distr. Louny, ca 1,3 km W of the railway station Peruc, ca 340 m a.s.l., N50°19.958', E13°56.1352', 26 IX 2005, J. Košnar – KO575 **[2n]**, 16 IX 2006, J. Košnar – KO873 **[2n]**; Distr. Mělník, Kralupy n. Vltavou, Lobeč, 140–150 m a.s.l., N50°15.0608', E14°18.1343', 13 VII 2006, J. Košnar – KO816 **[2n]**; Distr. Mělník, Kralupy n. Vltavou, Lobeč, ca 140–150 m a.s.l., N50°15.4371', E14°18.0951', 13 VII 2006, J. Košnar – KO817 sub *T. muralis* var. *muralis* **[2n; MA]**; Distr. Mělník, Kralupy n. Vltavou, Lobeč, ca 150–160 m a.s.l., N50°15.1817', E14°18.0601', 13 VII 2006, J. Košnar – KO823 **[2n]**, 825 **[2n]**; Distr. Mělník, Kralupy n. Vltavou, Lobeč, ca 160–170 m a.s.l., N50°15.1991', E14°17.9808', 13 VII 2006, J. Košnar – KO826 **[2n]**; Distr. Mělník, Kralupy n. Vltavou, Lobeč, ca 170–190 m a.s.l., N50°15.0542', E14°18.1175', 13 VII 2006 leg. J. Košnar – KO827 **[2n]**, 828 **[2n]**; Distr. Náchod, Dolní Adršpach, ca 370 m WNW of the railway station, ca 510 m a.s.l., N50°36.9342', E16°7.1216', 28 IV 2006, J. Košnar – KO724 **[n; MA]**; Distr. Plzeň–jih, Kramolín, ca 510 m a.s.l., N49°26.8742', E13°34.8516', 2 VIII 2005, J. Košnar – KO563 **[2n]**; Distr. Svitavy, Borek, ca 520 m a.s.l., N49°48.3671', E16°9.6614', 3 VII 2005, J. Košnar – KO760 **[2n; CE]**, 761 **[2n]**; Distr. Svitavy, Budislav, ca 530 m a.s.l., N49°48.213', E16°9.9661', 20 VII 2006, J. Košnar – KO833 **[2n]**; Distr. Svitavy, ca 200 m WNW of Budislav, ca 520 m a.s.l., N49°48.3670', E16°9.6697', 21 V 2006, J. Košnar – KO764 **[MA]**; Distr. Svitavy, valley of the Novohradka river, ca 600 m N of Vranice settlement, ca 390–400 m a.s.l., N49°50.0878', E16°9.0796', 9 IV 2007, J. Košnar – KO1274 **[2n; CH]**, 1275 **[2n; CH]**; Distr. Tachov, ruin of Krasíkov castle, ca 610 m a.s.l., N49°52.7013', E12°55.9036', 11 VII 2006, J. Košnar – KO813 **[2n]**; Distr. Turnov, NM Údolí Žehrovky, ca 280 m a.s.l., N50°30.392', E15°11.206', 24 VI 2005, J. Košnar – KO560 **[2n; MA; CE]**; Distr. Vsetín, Vsetín, 360–370 m a.s.l., N49°20.3688', E17°59.9077', 20 VII 2005, J. Košnar – KO1036 **[2n]**; Distr. Vyškov, Kojátky, ca 250 m a.s.l., N49°9.9989', E17°0.998', 20 VIII 2005, T. Malinová – KO1035 **[2n; CE]**; Distr. Vyškov, Kojátky, ca 250 m a.s.l., N49°9.9989', E17°0.998', 20 VIII 2005, T. Malinová – KO1035 **[2n]**; ESTONIA: Lahemaa RP, Muuksi, Turje kelder, liivakivipaljand panga, 7 VII 1998, N. Ingerpuu sub *T. lingulata* – TAA **[MA]**; GEORGIA: Ossetia: Inter Kobi et Abano, VII 1881, A. H. & V. F. Brotherus sub *T. obtusifolia* – S **[MA]**; GERMANY: Distr. Rastatt, ca 0.3 km E of Hub, ca 240 m a.s.l., N48°39.8950', E8°8.4683', 20 IX 2007, J. Košnar – KO1604 **[2n]**; Distr. Rastatt, ca 0.55 km ENE of Neusatz, ca 340 m a.s.l., N48°39.7117', E8°9.605', 20 IX 2007, J. Košnar – KO 1601 **[n]**; HUNGARY: Com. Nográd. In rupibus umbrosis in sylvestribus montis Bugyíkó prope pag Diósjenö, montes Börzsöny, 16 VI 1957, L. Vajda sub *T. obtusifolia* (BP) **[MA]**; Com. Pest. In rupestribus andesit. silvaticis ripae rivi vallis Apátkuti–völgy prope Pilisszentlászló. ca 300 m.s.m., 22 VI 1952, A. Boros sub *T. muralis* var. *obtusifolia* (BP) **[MA]**; Comit. Háromszék. In rupibus andesit. silv. montis Bálványos supra Bálványosfüred prope pag. Torja, 1000 m, 12 VIII 1942, A. Boros sub *T. obtusifolia* (BP) **[MA]**; Comit. Hont. In rupibus andesit. sept. silvat. "Otlárkö" montis Csóványos prope Szkolya. 850 m, 30 VI 1957, A. Boros sub *T. muralis* (L.) Hedw. var. *obtusifolia* (Schleich.) Boros (BP) **[MA]**; Pest county, Prédikálószék hill, ca 2 km S of Dömös, 620–630 m a.s.l., N47°44.37', E18°55.25', 15 V 2006, J. Košnar – KO745 **[n]**, 746 **[n; MA]**, 748 **[n]**; Pest county, Visegrád, by the entrance to the ruin of the castle, ca 330 m a.s.l., N47°47.68', E18°58.92', 16 V 2006, J. Košnar – KO753 **[2n]**; LATVIA: Distr. Riga, Krimulda, ca 20–30 m a.s.l., N57°10.33', E24°50.23', 24 VI 2006, J. Košnar – KO775 **[n]**; Distr. Riga, Krimulda, SW near Mazā ala cave, ca 20–30 m a.s.l., N57°10.5396', E24°50.5030', 24 VI 2006, J. Košnar – KO778 **[2n; MA]**; SLOVAKIA: Distr. Krupina, NNR Čabraď, ca 250 m a.s.l., N48°14.71', E19°6.33', 16 IX 2005, J. Košnar – KO642 **[CE]**; Distr. Krupina, NNR Čabraď, ca 280 m a.s.l., N48°14.51', E19°6.42', 16 IX 2005, J. Košnar – KO635 **[n]**; Distr. Krupina, NNR Čabraď, ca 300 m a.s.l., N48°14.61', E19°6.34', 16 IX 2005, J. Košnar – KO648 **[2n; MA]**; Distr. Rožňava, N of Kečovo, ca 250–300 m a.s.l., N48°29.6725', E20°29.0659', 1 VI 2005, J. Košnar – KO1007 **[2n; CE]**; Distr. Zvolen, Zvolen castle, 280 m a.s.l., N48°34.33', E19°7.67', 16 IX 2005, J. Košnar – KO633 **[2n; CE]**.

### *Tortula muralis* var. *muralis*

ARMENIA: Goriskij rajon, Tatev monastery, ca 1515 m a.s.l., N39°22.7883', E46°15.0083', 15 VI 2007, E. Ekrtová & L. Ekrt – KO1646 **[2n]**; CZECH REP.: Distr. Beroun, Zlatý kůň National Nature Reserve, ca 470 m a.s.l., N49°54.9574', E14°4.0007', 13 V 2007, J. Košnar – KO1263 **[n; MA]**; Distr. Blansko, Arnoštovo údolí valley, ca 280 m a.s.l., N49°21.1607', E16°40.1045', 21 IX 2005, J. Košnar – KO765 **[n; CE]**; Distr. Blansko, Pustý žleb valley, 400–425 m a.s.l., N49°22.3624', E16°43.5078', 4 IX 2006, J. Košnar – KO858 **[2n]**; Distr. Blansko, Pustý žleb valley, ca 350 m a.s.l., N49°21.943', E16°42.6836', 4 IX 2006, J. Košnar – KO 862 **[n]**, 863**[n]**, 865 **[n]**, 866 **[n]**; Distr. Blansko, Suchý žleb valley, 400 m a.s.l., N49°21.8733', E16°42.6159', 4 IX 2006 leg. J. Košnar – KO841 **[n; CE]**; Distr. Blansko, Suchý žleb valley, ca 400 m a.s.l., N49°21.8733', E16°42.6159', 4 IX 2006, J. Košnar – KO843 **[2n]**; Distr. Bruntál, Skály, ca 640 m a.s.l., N49°54.8452', E17°13.3103', 13 VIII 2005, J. Košnar – KO1038 **[CE]**; Distr. Břeclav, Klentnice, 335 m a.s.l., N48°50.8659', E16°38,7235', 9 IV 2005, J. Košnar – KO350 **[n]**; Distr. České Budějovice, České Budějovice – Čtyři dvory, Institute of AS CR, ca 390 m a.s.l., N48°58.4679', E14°27.4749', 8 IX 2005, J. Košnar – KO1019 **[n; CE]**; Distr. Český Krumlov, Český Krumlov – Nádražní předměstí, ca 540 m a.s.l., N48°49.233', E14°18.7259', 15 XI 2005, J. Košnar – KO568 **[n]**; Distr. Český Krumlov, Český Krumlov – Nádražní předměstí, ca 550 m a.s.l., N48°49.2161', E14°18.6077', 15 XI 2005, J. Košnar – KO570 **[n]**; Distr. Český Krumlov, Český Krumlov castle, ca 530 m a.s.l., N48°48.7253', E14°18.643', 18 IX 2006, J. Košnar – KO885 **[2n; MA]**; Distr. Český Krumlov, Loučovice, ca 670 m a.s.l., N48°37.2692', E14°15.5382', 3 X 2005, J. Košnar – KO1037 **[n]**; Distr. Český Krumlov, NNR Vyšenské kopce, 555 m a.s.l., N48°49.1509', E14°17.7351', 19 IV 2005, J. Košnar – KO256 **[2n]**; Distr. Hodonín, Velká nad Veličkou, ca 290 m a.s. l., N48°52.8212', E17°30.8211', 23 IV 2006, J. Košnar – KO711 **[2n]**, 712 **[2n]**; Distr. Chrudim, ca 0.5 km SW of Vranice settlement, ca 425 m a.s.l., N49°49.3794', E16°8.7601', 24 IX 2005, J. Košnar – KO607 **[2n]**; Distr. Chrudim, NR Maštale, ca 0.5 km SW of Vranice settlement, ca 425 m a.s.l., N49°49.3794', E16°8.7601', 24 IX 2005, J. Košnar – KO607 **[2n; CE]**; Distr. Chrudim, Střemošice, 440–450 m a.s.l., N50°19.958', E13°56.1352', 22 V 2005, J. Košnar – KO1039 **[n; CE]**; Distr. Klatovy, Žichovice, ruin of Rábí castle, ca 510 m a.s.l., N49°16.7925', E13°36.9959', 1 V 2006, J. Košnar – KO663 **[2n]**; Distr. Louny, ca 1,3 km W of the railway station Peruc, ca 340 m a.s.l., N50°19.958', E13°56.1352', 16 IX 2006, J. Košnar – KO874 sub. *T. muralis var. aestiva* **[2n; MA]**; Distr. Louny, ca 1,3 km W of the railway station Peruc, ca 340 m a.s.l., N50°19.9580', E13°56.1352', 26 IX 2005, J. Košnar – KO578 **[CE]**; Distr. Louny, ca 1,3 km W of the railway station Peruc, ca 340 m a.s.l., N50°19.958', E13°56.1352', 16 IX 2006, J. Košnar – KO869 **[2n]**, 876 sub *Tortula muralis* s.l. **[2n]**; Distr. Mělník, Kralupy n. Vltavou, Lobeč, ca 160–180 m a.s. l., N50°15.0435', E14°18.1261', 13 VII 2006, J. Košnar – KO829 **[2n]**; Distr. Náchod, NE of Žďárky village, 440 m a.s.l., N50°28.3585', E16°14.0424', 30 IV 2006, J. Košnar – KO741 **[n; MA]**; Distr. Náchod, Police nad Metují, 450–460 m a.s.l., N50°32.1048', E16°14.3881', 27 IV 2006, J. Košnar – KO719 **[n]**; Distr. Plzeň–jih, Kramolín, ca 510 m a.s.l., N49°26.8742', E13°34.8516', 2 VIII 2005, J. Košnar – KO564 **[n]**, 565 **[n]**; Distr. Praha–west, Průhonice, Institute of Botany, ca 310 m a.s.l., N49°59.7285', E14°33.9962', 9 VIII 2006, J. Košnar – KO1179 **[2n]**; Distr. Svitavy, Budislav, ca 530 m a.s.l., N49°48.213', E16°9.9661', 20 VII 2006, J. Košnar – KO833 **[2n]**, 18 VIII 2006, J. Košnar – KO867 **[2n]**; Distr. Svitavy, Jarošov, ca 470–480 m a.s.l., N49°49.5120', E16°9.5304', J. Košnar – KO1271 **[2n; CE]**; Distr. Svitavy, Nové Hrady, ca 600 m N of Vranice settlement, ca 395 m a.s.l., N49°50.0878', E16°9.0796', 19 VI 2005, J. Košnar – KO464 **[2n]**; Distr. Svitavy, Polička, ca 550 m a.s.l., N49°42.8810', E16°16.3112', 19 II 2007, J. Košnar – KO1270 **[2n; CH]**; Distr. Tachov, along the touristic path to the ruin of Krasíkov castle, ca 580 m a.s.l., N49°52.8236', E12°56.1062', 11 VII 2006, J. Košnar – KO807 **[2n]**; Distr. Tachov, ruin of Krasíkov castle, ca 610 m a.s. l., N49°52.7015', E12°55.9119', 11 VII 2006, J. Košnar – KO811 **[2n]**; Distr. Tachov, Studánka, 600–610 m a.s.l., N49°46.6223', E12°36.3593', 11 VI 2006, J. Košnar – KO771 **[2n; MA]**; Distr. Třebíč, Senorady, ca 320 m a.s.l., N49°7.5272', E16°14.4705', 14 V 2005, J. Košnar – KO416 **[2n]**; Distr. Znojmo, ruin of Templštejn castle, ca 368 m a.s.l., N49°5.455', E16°14.9068', 14 V 2005, J. Košnar – KO418 **[n]**; Distr. Znojmo, Templštejn castle, ca 2 km NNW of Jamolice, ca 368 m a.s.l., N49°5.455', E16°14.9068', 14 V 2005, J. Košnar – KO418 **[n; MA]**; Languedoc-Roussillon, Montpellier, ca 30 m a.s.l., N43°36.6667', E3°52.1833', 13 XI 2005, T. Malinová – KO1033 **[2n; CE]**; GERMANY: Distr. Ortenaukreis, Hornisgrinde, ca 1150 m a.s.l., N48°36.1917', E8°12.2617', 19 IX 2007, J. Košnar – KO1597 **[n]**; Distr. Rastatt, ca 0.3 km ENE of Neusatz, ca 320 m a.s.l., N48°39.6633', E8°9.4233', 20 IX 2007, J. Košnar – KO1599 **[n]**; HUNGARY: Pest county, Visegrád, by the entrance to the ruin of the castle, ca 330 m a.s.l., N47°47.68', E18°58.92', 16 V 2006, J. Košnar – KO751 **[2n]**; LATVIA: Distr. Cesis, Metluri, railway station, ca 110–120 m a.s.l., N57°12.43', E25°14.073', 28 VI 2006, J. Košnar – KO800 **[2n]**; Distr. Riga, Krimulda, along the touristic path to Gūtmaņa ala cave, ca 20–30 m a.s.l., N57°10.330', E24°50.231', 24 VI 2006, J. Košnar – KO777 **[2n; MA]**; MONTENEGRO: Plužine Municipality, Mratinje, ca 760 m a.s.l., N43°15.8381', E18°48.5527', 28 VII 2007, J. Košnar – KO1367 **[2n; MA]**; Podgorica Municipality, ca 2.5 km E of Djurkovići, ca 90 m a.s.l., N42°29.1328', E19°18.4627', 3 VIII 2007, J. Košnar – KO1405 **[n; MA]**; Podgorica Municipality, ca 2.5 km E of Djurkovići, ca 90 m a.s.l., N42°29.1328', E19°18.4627', 3 VIII 2007, J. Košnar – KO1404 **[2n; MA]**; Podgorica Municipality, Žabljak, ruin of the fortress, ca 30 m a.s.l., N42°19.0481', E19°9.4468', 4 VIII 2007, J. Košnar – KO1409 **[n; MA]**; ROMANIA: Bihor county, Oradea, Parcul Petöfi Sándor park, ca 140 m a.s.l., N47°3', E21°56', 3 IX 2005, J. Košnar – KO1045 **[CE]**; Bihor county, Oradea, Parcul Petöfi Sándor park, ca 140 m a.s.l., N47°3', E21°56', 3 IX 2005, J. Košnar – KO1046 **[2n; CE]**; Mureş county, Calimani National Park, valley of Tihul brook, 1150–1200 m a.s.l., N47°5.9783', E25°5.4350', 4 VII 2007, J. Košnar – KO1347 **[2n; MA]**; Mureş county, Calimani National Park, valley of Tihul brook, ca 800 m a.s.l., N47°02.6950', E25°03.7183', 6 VII 2007, J. Košnar – KO1348 **[n; MA]**; Tulcea county, Culmea Pricopanului, ca 4 km ENE from Măcin, ca 200–250 m a.s.l., N45°14.0', E28°11.0', 31 VIII 2005, J. Košnar – KO1188 **[n]**; SLOVAKIA: Distr. Bratislava IV, Sandberg sandstone hill, ca 240 m a.s.l., N48°11.6657', E16°58.7592', 29 V 2005, J. Košnar – KO1043 **[2n]**; Distr. Bratislava IV, ca 1 km N of Devín, ca 280 m a.s.l., N48°11.2657', E16°58.8442', 29 V 2005, J. Košnar – KO1042 **[MA; CE]**; Distr. Košice, Turnianský hrad castle ruin, ca 370–380 m a.s.l., N48°36.6599', E20°52.4449', 2 VI 2005, J. Košnar – KO1009 **[2n]**; Distr. Košice, Turnianský hrad castle ruin, ca 370–380 m a.s.l., N48°36.6599', E20°52.4449', 2 VI 2005, J. Košnar – KO1010 **[n; MA]**; Distr. Levice, N along the road Šahy – Ipeľské Predmostie, N48°04.511', E18°57.97', 17 IX 2005, J. Košnar – KO996 **[2n]**; Distr. Lučenec, Belina, 200–230 m a.s.l., N48°14.5705', E19°51.2900', 31 V 2005, J. Košnar – KO1021 **[2n; MA]**; Distr. Nové Zámky, Čenkovská lesostep National Nature Reserve, ca 0.6 km WNW of Čenkov, ca 110 m a.s.l., N47°46.1081', E18°31.1531', 30 V 2005, J. Košnar – KO993 **[n; MA]**; Distr. Nové Zámky, Kováčovské kopce (Burda) hills, Velká dolina valley, ca 140 m a.s.l., N47°49.806', N47°49.806', 18 IX 2005, J. Košnar – KO998 **[2n]**; Distr. Nové Zámky, Kováčovské kopce (Burda) hills, ca 160 m a.s.l., N47°49.858', E18°48.847', 18 IX 2005, J. Košnar – KO1001 **[2n]**; Distr. Nové Zámky, Kováčovské kopce hills, near the touristic path to Skaly rocks, ca 160 m a.s.l., N47°49.9057', E18°44.1547', 30 V 2005, J. Košnar – KO994 **[2n; CE]**; Distr. Nové Zámky, Čenkov, ca 110 m a.s.l., N47°45.9428', E18°31.6069', 30 V 2005, J. Košnar – KO992 **[2n; MA]**; Distr. Piešťany, Záruby National Nature Reserve, ca 2.5 km SW of Buková, ca 590 m a.s.l., N48°31.3389', E17°22.4637', 4 VI 2005, J. Košnar – KO1016 **[n; MA]**; Distr. Rimavská Sobota, Hajnáčka, ca 310 m a.s.l., N48°13.0693', E19°57.3098', 31 V 2005, J. Košnar – KO1023 **[2n; MA]**; Distr. Snina, Stakčín, ca 260 m a.s.l., N49°00.05', E22°14.7333', 7 VII 2005, T. Malinová – KO1018 **[2n]**; Distr. Zvolen, Zvolen castle, 280 m a.s. l., N48°34.3', E19°7.67', 16 IX 2005, J. Košnar – KO 634 **[2n]**; SPAIN: Madrid–Barajas, Parque Juan Carlos I., N40°27.0', E3°36.0', 1 III 2007, E. Holá – KO1254 **[2n]**, 1255 **[2n]**; SWITZERLAND: Canton of Bern, SSW of Meiringen, Gschwantenmad, ca 1300 m a.s.l., N46°41.295', E8°9.705', 27 VIII 2006, J. Košnar – KO990 **[n]**; Canton of Bern, SSW of Meiringen, Schwarzwaldalp, ca 1450 m a.s.l., N46°40.575', E8°8.061', 27 VIII 2006, J. Košnar – KO988 **[n]**; Canton of Luzern, Luzern, St. Leodegar church, N47°3.0', E8°18.0', 27 VIII 2006, J. Košnar – KO991 **[n; MA]**.

### Plants intermediate between *Tortula muralis* var. *aestiva* and *T. muralis* var. *muralis*

CZECH REP.: Distr. Blansko, Suchý žleb valley, ca 400 m a.s.l., N49°21.8733', E16°42.6159', 4 IX 2006 leg. J. Košnar – KO837 sub *T. muralis* var. *aestiva* **[2n]**; Distr. Hodonín, S-exposed slope of Výzkum hill, ca 370 m a.s.l., N48°51.7828', E17°25.9029', 20 IV 2006, J. Košnar – KO709 sub *T. muralis* var. *aestiva* **[2n]**, 710 sub *T. muralis* var. *muralis* **[2n; MA]**; Distr. Chrudim, Luže, ca 370 m a.s.l., N49°53.0671', E16°2.1181', 18 VI 2005, J. Košnar – KO466 sub *T. muralis* var. *muralis* **[n; MA]**; Distr. Jičín, Kost castle, ca 320 m a.s.l., N50°29.4475', E15°08.1374', 26 VI 2005, J. Košnar – KO562 sub *T. muralis* var. *aestiva* **[2n]**; Distr. Louny, ca 1,3 km W of the railway station Peruc, ca 340 m a.s.l., N50°19.958', E13°56.1352', 16 IX 2006, J. Košnar – KO870 sub *T. muralis* var. *aestiva* **[2n]**, 871 sub *T. muralis* var. *aestiva* **[2n]**, 872 sub *T. muralis* var. *aestiva* **[2n]**; Distr. Mělník, Kralupy n. Vltavou, Lobeč, ca 140–150 m a.s.l., N50°15.0597', E14°18.1343', 13 VII 2006, J. Košnar – KO815 sub *T. muralis* var. *aestiva* **[2n]**; Distr. Mělník, Kralupy n. Vltavou, Lobeč, ca 200–210 m a.s.l., N50°14.8065', E14°18.18', 13 VII 2006, J. Košnar – KO832 sub *T. muralis* var. *aestiva* **[2n; MA]**; Distr. Náchod, Police nad Metují, 450–460 m a.s.l., N50°32.1037', E16°14.3855', 27 IV 2006, J. Košnar – KO721 sub *T. muralis* **[2n]**; Distr. Náchod, Teplice nad Metují, ca 480 m a.s.l., N50°35.717', E16°8.9307', 28 IV 2006, J. Košnar – KO734 sub *T.*

*muralis* var. *muralis* **[n]**; Distr. Náchod, Teplice nad Metují, ca 480 m a.s.l., N50°35.7197', E16°8.9299', 28 IV 2006, J. Košnar – KO733 sub *T. muralis* **[2n]**; Distr. Turnov, PLA Český ráj, NM Údolí Žehrovky, ca 270–280 m a.s.l., N50°30.3849', E15°11.2847', 24 VI 2005, J. Košnar – KO559 sub *T. muralis* var. *aestiva* **[2n]**; GERMANY: Distr. Ortenaukreis, Naturschutzzentrum Ruhestein, ca 910 m a.s.l., N48°33.6917', E8°13.32', 19 IX 2007 leg. J. Košnar – KO1598 sub *T. muralis* **[2n]**; Distr. Rastatt, ca 0.35 km ENE of Neusatz, ca 350 m a.s.l., N48°39.6850', E8°9.4767', 20 IX 2007, J. Košnar – KO1600 sub *T. muralis* **[n]**; GREECE: Santoriu?: Aufstieg zum Seargios? Krater. IV. 1911, V. Schiffner, det. J. Baumgarter sub. *Tortula aestiva* var. nov. *vulcanicola* Schffn. – Z **[MA]**; HUNGARY: Komárom-Esztergom county, Király-völgy valley, ca 2.5 km E of Dobogókö, 430–440 m a.s.l., N47°43.03', E18°55.95', 14 V 2006, J. Košnar – KO744 sub *T. muralis* var. *aestiva* **[2n]**; Pest county, Prédikálószék hill, ca 2 km S of Dömös, 620–630 m a.s.l., N47°44.37', E18°55.25', 15 V 2006, J. Košnar – KO747 sub *T. muralis* **[n; MA]**; Pest county, Visegrád, above the touristic path to the ruin of the castle, 280–290 m a.s.l., N47°47.55', E18°58.84', 16 V 2006, J. Košnar – KO757 sub *T. muralis* var. *aestiva* **[n]**; Pest county, Visegrád, by the entrance to the ruin of the castle, ca 330 m a.s.l., N47°47.68', E18°58.92', 16 V 2006, J. Košnar – KO752 sub *T. muralis* var. *aestiva* **[2n]**; ROMANIA: Bihor county, Oradea, Parcul Petöfi Sándor park, ca 140 m a.s.l., N47°3', E21°56', 3 IX 2005, J. Košnar – KO1047 **[2n; CE]**; Toplicza, 22 VII 1887, C. Demeter sub *T. obtusifolia* (BP) **[MA]**; SLOVAKIA: Distr. Detva, NNR Rohy, 410–420 m a.s.l., N48°32.54', E019°21.87', 15 IX 2005, J. Košnar – KO623 **[CE]**; Distr. Detva, NNR Rohy, ca 440 m a.s.l., N48°32.53', E19°21.91', 15 IX 2006, J. Košnar – KO630 sub *T. muralis* var. *aestiva* **[n]**; Distr. Lučenec, Belina, ca 200–230 m a.s.l., N48°14.5705', E19°51.29', 31 V 2005, J. Košnar – KO1022 sub *T. muralis* **[2n]**; Distr. Nové Zámky, Kováčovské kopce (Burda) hills, Velká dolina valley, ca 160 m a.s.l., N47°49.899', E18°48.833', 18 IX 2005, J. Košnar – KO1003 sub *T. muralis* var. *aestiva* **[2n]**; Distr. Piešťany, E of Buková, Horné mlyny settlement, 270–280 m a.s.l., N48°32.6536', E17°25.3361', 4 IV 2005, J. Košnar – KO1017 sub *T. muralis* var. *aestiva* **[2n]**; Distr. Rimavská Sobota, Hajnáčka, 260–270 m a.s.l., N48°13.0888', E19°57.4920', 31 V 2005, J. Košnar (KO1025) **[2n; CE]**; Distr. Rimavská Sobota, Hajnáčka, 260–270 m a.s.l., N48°13.0888', E19°57.4920', 31 V 2005, J. Košnar – KO1024 **[CE]**.

### *Tortula lingulata*

CZECH REP.: Distr. Louny, ca 1,3 km W of the railway station Peruc, ca 340 m a.s.l., N50°19.957', E13°56.1386', 26 IX 2005, J. Košnar – KO868 **[2n]**; ESTONIA: Ida–Virumaa county, Toila – Oru Park, 24 VI 2005, N. Ingerpuu – TU **[MA]**; Jõgeva Co, Kallaste town, on sandstone outcrop, 7 V 1990 leg. L. Kannukene – TAM **[MA]**; LATVIA: An Sandstein in Tale der Gauja (Livland Aa) bei Sigulda (Segewold), 12 X 1924, E. Kalis et J. Strautmanis (BP) **[MA]**; Distr. Cesis, Kārļu grava valley, ca 0.6 km SW of Kārļi, ca 100–110 m a.s.l., N57°14.358', E25°11.996', 27 VI 2006, J. Košnar – KO797 **[MA]**; Distr. Cesis, Kārļu grava valley, ca 0.6 km SW of Kārļi, ca 100–110 m a.s.l., N57°14.358', E25°11.996', 27 VI 2006, J. Košnar – KO799 **[2n; MA]**; Distr. Cesis, Cīrulīši, Cīrulīšu klintis rock formation, ca 30 m a.s.l., N57°18.128', E25°13.243', 28 VI 2006, J. Košnar – KO801 **[2n]**, 802 **[2n; MA]**; Distr. Cesis, Ieriķi, Sesîlu klintis rock formation, ca 70–80 m a.s.l., N57°13.1967', E25°9.6858', 26 VI 2006, J. Košnar – KO795 **[MA]**; Distr. Cesis, Ieriķi, valley of Kumada river Sesîlu klintis rock formation, ca 70–80 m a.s.l., N57°13.1967', E25°09.6858', 26 VI 2006, J. Košnar – KO796 **[2n]**; Distr. Riga, Krimulda, valley of Vikmeste river, ca 20–30 m a.s.l., N57°10.3123', E24°50.1642', 22 VI 2006, J. Košnar – KO773 **[2n]**, 774 **[2n]**; Distr. Riga, Sigulda, Krauklu aiza rock formation, ca 50–60 m a.s.l., N57°10.4147', E24°52.0223', 25 VI 2006, J. Košnar – KO786 **[MA]**; Distr. Riga, Sigulda, Pikenes krauja rock formation, ca 20–30 m a.s.l., N57°9.019', E24°49.025', 24 VI 2006, J. Košnar – KO780 **[MA]**, 783 **[2n]**; Lettland, an beschatteten Sandsteinfelsen der "Blauen Berge" bei Schlieterhof in Kurland, 28 VII 1920, N. Malta – B **[MA]**; Livland, Kreis Pernau, auf Sandstein bei Torgel, 17 X 1889, J. Treboux – TU **[MA]**; RUSSIA: European part. Leningrad Province, Tosna District, Sablino Settlement, Right bank of Sablinka River, on outcrop of red sandstone, 17 VI 1959, I.I. Abramov et A.L. Abramova – TAM **[MA]**; Gouv. Pleskau, Welikajamündung, auf Sandstein bei Njogotj, 28 VI 1916, N. Malta – B **[MA]**; Gouv. und Kr. Pleskau, auf Sandstein des Welikajaufers bei Ustje, Ende Juni 1917, N. Malta – B **[MA]**.

### *Tortula obtusifolia*

ARMENIA: Goriskij rajon, Tatev monastery, ca 1515 m a.s.l., N39°22.7883', E46°15.0083', 15 VI 2007, E. & L. Ekrt – KO1646 **[n]**; AUSTRIA: Kärnten, National Park Hohe Tauern, ca 950 m SE of the summit of Dritter Leiterkopf, ca 2500 m a.s.l., N47°2.9033', E12°44.77', 20 VII 2007, J. Košnar – KO 1541 **[n]**, 1542 **[n]**, 1544 **[n; CH]**, 1545 **[n]**; Kärnten, Hohe Tauern Mts., ca 1.6 km SSW of the summit of Mt. Gosinkopf, ca 1830 m a.s.l., N47°2.6883', E12°53.785', 21 VII 2007, J. Košnar – KO1556 **[n]**; Kärnten, Hohe Tauern Mts., ca 1.2 km S of the summit of Mt. Gosinkopf, 1910–1920 m a.s.l., N47°2.8217', E12°54.2767', 21 VII 2007, J. Košnar – KO1564 **[n]**, 1566 **[n]**, 1567 **[n]**, 1568 **[n]**, 1569 **[n]**; Salzburg, Radstädter Tauern, Grießnerkar, 1760–1770 m a.s.l., N47°8.3639', E13°23.9098', 14 IX 2006, J. Košnar – KO926 **[n; MA]**, 927 **[n]**, 928 **[n]**, 930 **[n]**, 931 **[n]**, 932 **[n]**, 933 **[n]**, 934 **[n]**; FRANCE: Du petit St. Bernhard entre la Savoie d'Italie (2100 mètres), 12 VII 1883, Philibert – Z **[MA]**; Savoie. Lanslebourg. Col du Mont – Cenis, 2084 m, 18 VI 2003, R. Skrzypczak – RS03424 **[MA]**; GEORGIA (RUSSIA?): Osetia, inter Lars et Kasbegi, 27 V 1881, V. F. Brotherus – S **[MA]**; HUNGARY: Pest county, Börzsöny Mts., ca 140 m SSW of the summit of Mt. Csóványos, ca 920 m a.s.l., N47°56.8717', E18°56.8867', 16 V 2008, J. Košnar – KO1845 **[n; MA]**; ICELAND: Rangárvallasýsla: Drangshliđ Vom Skógar. I taket i klippskreva, 5 VII 1983, Pär Johansson – S **[MA]**; ROMANIA: Ilva. A patak balparlján, 20 VII 1887, C. Demeter (BP) **[MA]**; Mesterháza, út m., egy szikláról, 21 VII 1887, C. Demeter (BP) **[MA]**; Ratosnya, 19 VII 1888, C. Demeter (BP) **[MA]**; Suceava county, Calimani Mts., Stâncile Lucaciu rocks, ca 1700 m a.s.l., N47°13.9483', E25°13.5150', 2 VII 2007, J. Košnar – KO1324 **[n]**, 1325 **[n]**; Suceava county, Calimani Mts., Stâncile Lucaciu rocks, ca 1700 m a.s.l., N47°13.9317', E25°13.59', 2 VII 2007, J. Košnar – KO1328 **[n]**, 1329 **[n]**; Suceava county, Calimani Mts., Doisprezece Apostoli rocks, ca 1700 m a.s.l., N47°13.4967', E25°12.7217', 2 VII 2007, J. Košnar – KO1332 **[n]**, 1337 **[n]**; Suceava county, ca 530 m WSW of the summit of Mt. Vf. Lucaciu, ca 1700 m a.s.l., N47°13.8967', E25°13.2933', 2 VII 2007, J. Košnar – KO1330 **[n; MA]**, 1331 **[n]**; Mureş county, Calimani Mts., ca 1.04 km N from the summit of Mt. Vf. Tihu, ca 1900 m a.s.l., N47°6.7717', E25°8.6717', 4 VII 2007, J. Košnar – KO1346 **[n]**; Mureş county, Calimani Mts., ca 2.5 km NW of Răstoliţa, ca 550–600 m a.s.l., N46°59.92', E25°1.965', 6 VII 2007, J. Košnar – KO1350 **[n]**, 1351 **[n]**; SLOVAKIA: Distr. Detva, NNR Rohy, 410–420 m a.s.l., N48°32.54', E19°21.87', 15 IX 2005, J. Košnar – KO623 **[CE]**; Distr. Detva, NNR Rohy, ca 420–430 m a.s.l., N48°32.54', E19°21.87', 15 IX 2005, J. Košnar – KO626 **[n]**, 628 **[n]**; Distr. Detva, NNR Rohy, ca 440 m a.s.l., N48°32.55', E19°21.94', 15 IX 2006, J. Košnar – KO631 **[n; MA]**, 632 **[n]**; Distr. Krupina, NNR Čabraď, ca 250 m a.s.l., N48°14.71', E19°6.33', 16 IX 2005, J. Košnar – KO639 **[CE]**; SWITZERLAND: Bern, Bachalp ob Grindelwald, 1980 m.s.m. auf Sandstein, 7 IX 1913, P. Culmann sub *T. obtusifolia* var. *brevifolia* (BP) **[MA]**; Jura. La Chaux puis Ste Croix, sur le calcaire, 1100", XI 1898, C. Meylan sub *T. obtusifolia* – Z **[MA]**.

**Plants intermediate between *Tortula muralis* var. *aestiva* and *T. obtusifolia***

ARMENIA: Caucasus, distr. Avakian, in pago Garni, apud ruinas templi vetusti. alt. 1600–1700 m s.m., 26 VI 1983, V. Vašák sub *T. obtusifolia* – B **[MA]**; Distr. Gegharkunik, Lchashen, 2020 m a.s.l., N40°29.6117', E44°54.625', 7 VII 2008, E. & L. Ekrt – KO1887 sub *T. obtusifolia* **[n]**; AUSTRIA: Carinthia, Hohe Tauern Mts., Glockner Gruppe: S slopes of Schwerteck and Leiterkopf, alt. ca. 2550 m, 15 VIII 1997, J. Kučera – E1858 sub *T. obtusifolia* **[MA]**; AUSTRIA: Kärnten, Hohe Tauern Mts., ca 1.2 km S of the summit of Mt. Gosinkopf, 1910–1920 m a.s.l., N47°2.8217', E12°54.2767', 21 VII 2007, J. Košnar – KO1565 sub *T. obtusifolia* **[n; MA]**; Kärnten, National Park Hohe Tauern, ca 950 m SE of the summit of Dritter Leiterkopf, ca 2500 m a.s.l., N47°2.9033', E12°44.77', 20 VII 2007, J. Košnar – KO1543 sub *T. obtusifolia* **[n; MA],** 1546 sub *T. obtusifolia* **[n]**; CZECH REP.: Distr. Blansko, Suchý žleb valley, ca 400 m a.s.l., N49°21.6941', E16°42.9847', 21 IX 2005, J. Košnar – KO601 sub *T. muralis* var. *aestiva* **[MA]**; Distr. Blansko, Suchý žleb valley, ca 400 m a.s.l., N49°21.6941', E16°42.9847', 10 IV 2007, J. Košnar – KO856 **[n]**, 1277 **[n; CH]**; Distr. Mělník, Kralupy n. Vltavou, Lobeč, ca 150–160 m a.s.l., N50°15.1833', E14°18.0567', 13 VII 2006, J. Košnar – KO824 sub *T. muralis* var. *aestiva* **[MA]**; U Kralup v skulinách kamen. pískovce, 1894, Velenovský sub *T. obtusifolia* – TU **[MA]**; FRANCE: Cantal: roches d'andèsite au dessus d'Enterroches près Murat, 950 m, 24 VIII 1921, P. Culmann sub *T. obtusifolia* var. *pilifera* – Z **[MA]**; Savoie. Lanslebourg. Mont – Cenis. 2000 m. Sur les rochers du sentier qui méne au fort de la Ronce, 30 VIII 2000, R. Skrzypczak – RS03455 sub *T. obtusifolia* **[MA]**; GEORGIA: Ossetia: Lehrinvall?, VII 1881, A. H. & V. F. Brotherus sub *T. obtusifolia* – S **[MA]**; HUNGARY: Komárom–Esztergom county, SSW–exposed slope of Árpád trónja, ca 500 m a.s.l., N47°44.29', E18°54.86', 15 V 2006, J. Košnar – KO750 sub *T. muralis* s.l. **[n; MA]**; Komárom–Esztergom county, SW–exposed slope of Árpád trónja, 500–550 m a.s.l., N47°44.29', E18°54.86', 15 V 2006, J. Košnar – KO749 sub *T. muralis* s.l. **[n; MA]**; Pest county, Visegrád, above the touristic path to the ruin of the castle, 280–290 m a.s.l., N47°47.55', E18°58.84', 16 V 2006, J. Košnar – KO756 sub *T. muralis* s.l. **[n; MA]**; KAZAKHSTAN (KYRGYZSTAN?): Alatau transiliensis: Djolbulak in valle fl. Kebin majoris, ad rupes praeruptas reg. silv. infer., 16 VI 1896, V. F. Brotherus sub *T. obtusifolia* – S **[MA]**; Inter diversoria Bjelaja vodi et Antonovka inter Tschimkent er Aulie Ata, ad rupes siccissimas conglomerat., 18 V 1896, V. F. Brotherus sub *T. obtusifolia* – S **[MA]**; ROMANIA: Tusnád–fürdö, 13 VIII 1883, C. Demeter sub *T. obtusifolia* (BP) **[MA]**; SWITZERLAND: Bachalp auf dem Faulhorn auf einem Steine, VI 1870, A. Metzler sub *T. obtusifolia* (BP) **[MA]**.