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Preparation of testate amoebae samples affects water table depth reconstructions in peatland palaeoecological studies

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Abstract. In peatland palaeoecological studies, the preparation of peat samples for testate amoebae (TA) analysis involves boiling of samples and microsieving them through a 15-µm sieve. We studied the effect of these preparation stages on the estimation of TA assemblages and on the reconstruction of water table depths (WTD). Our results indicate that the TA assemblages of boiled and unboiled samples are not significantly different, while microsieving reduces the concentration of small TA taxa and results in significantly different TA assemblages. The differences between microsieved and unsieved TA assemblages were reflected also in predicted values of WTD, which indicated drier conditions in case of unsieved samples than in microsieved samples. We conclude that the boiling of samples might be omitted if TA are extracted from the fresh peat samples. Microsieving may lead to erroneous palaeoecological WTD reconstructions and should be avoided if small TA taxa are present in samples.

Key words: testate amoebae, Protista, sample preparation, peatland, palaeohydrology, palaeoecology, water table depth.

INTRODUCTION

Testate amoebae (Protista) are unicellular organisms that live in various aquatic environments, being especially numerous in *Sphagnum* peats. These organisms produce tests that can persist in peat for thousands of years, making them useful in peatland palaeoecological studies. Because the distribution of testate amoebae (TA) in peatlands is controlled primarily by moisture (e.g., Charman & Warner 1992; Lamentowicz & Mitchell 2005), TA have been particularly valuable as indicators of past hydrological change (e.g., Charman et al. 2004, 2010).

Several methods have been used to extract TA from peat to examine and quantify their tests using light microscopy. Tests of TA can be counted on slides specially prepared for pollen analysis (i.e., after boiling samples in alkaline and acetulation) (E. Niinemets and S. Veski, unpublished data). Heal (1962) soaked *Sphagnum* samples in 5% formalin before filtrating and centrifuging. However, most techniques involve combination of boiling and sieving, but avoid any chemical treatments as they may destroy tests (Hendon & Charman 1997). For example, Grospietsch (1953) and Tolonen (1986) suggested boiling 3 cm^3 of peat in water and removing coarse material by sieving through a 600-µm sieve. Tolonen (1986) also recommended adding exotic markers (*Lycopodium* spores) during the boiling stage so that test concentrations could be calculated, although Warner (1987, 1988) suggested adding *Lycopodium* tablets after the boiling stage, as some tests might adhere to *Sphagnum* plants. Hendon & Charman (1997) found that the pollen preparation method is not suitable for TA analysis and suggested boiling and sieving samples similar to previous studies. They also found that the use of a 15-µm sieve to remove fine particulate matter from samples improved the clarity of samples and facilitated analysis (Hendon & Charman 1997). So, they complemented the preparation protocol by microsieving samples through a 15-µm sieve.

Most recent ecological and palaeoecological studies on TA have used the standard preparation methods described by Tolonen (1986) (e.g., Tolonen et al. 1992, 1994; Buttler et al. 1996; Woodland et al. 1998; Mitchell & Gilbert 2004) or Hendon & Charman (1997) (e.g., Charman et al. 2000, 2004, 2007; Hendon et al. 2001; Booth 2002, 2008; Booth & Jackson 2003; Wilmhurst et al. 2003; Hendon & Charman 2004; Sillasoo et al. 2007; Laggoun-Défarge et al. 2008; Lamentowicz et al. 2008; Sjögren & Lamentowicz 2008; Van der Linden et al. 2008a, 2008b; Payne & Pates 2009; Swindles et al. 2009), in some cases with minor modifications in storage liquid or mesh size of the coarse sieve. Only very few studies have departed from standard methods, for example Bobrov et al. (2002) did not add *Lycopodium* tablets and omitted the boiling stage.

While the issue of small taxa being lost through a microsieve has been raised in the literature (e.g., Hendon & Charman 1997; Charman et al. 2000; Payne 2009; Wall et al. 2010), the effect of such loss on water table depth (WTD) reconstructions has remained unclear. In

this study, we conducted a series of experiments to (1) assess the impact of the commonly used step of boiling peat samples prior to sieving and (2) further assess the influence of microsieving on the estimation of TA assemblage structure. We used alternatively processed TA samples for predicting WTDs to see whether the processing of samples affects results.

MATERIAL AND METHODS

For estimating the impact of boiling on the samples, 19 fresh surface samples were taken from different *Sphagnum* ecotopes from Õpetajasoo, Selisoo and Kalina bogs (NE Estonia). The samples were halved laterally for different processing. In the first step, the volume of the peat samples was measured using Archimedes' principle: the portion of peat was dropped into a measuring glass and 10 cm^3 water was added. The volume of a sample was a reading from the scale minus ten. Two *Lycopodium* tablets, dissolved in a drop of 30% acetic acid and diluted with about 50 cm^3 of water, were added and samples were transferred into boiling glasses. Samples A were soaked in water, by stirring well for about 10 min. Samples B were boiled for about 5 min in water. After that the processing of all samples was similar. All samples were washed through a coarse sieve (about 750 μm) into a boiling glass and the testate amoebae and peat debris were let to deposit for several hours. Clear water was decanted and the remaining sample was collected and stored in a 15-cm^3 glass tube, from which the clear water was decanted once more. Then some drops of glycerine were added to the concentrate and samples were stored in corked tubes.

For estimating the influence of microsieving through a 15-μm sieve, 20 pre-counted TA samples from Selisoo, Õpetajasoo and Kalina bogs were used, which were prepared without microsieving. Eleven samples contained concentrations of *Cryptodifflugia oviformis* as high as more than 10 000 tests in 1 cm^3 and ten samples contained concentrations of *Trinema lineare*-type tests as high as more than 1000 tests in 1 cm^3 of peat. The samples were washed through a sieve with a 15-μm mesh and counting of TA from the detritus remaining on the sieve was repeated.

All samples were counted under light microscope with magnification of 200–400×. The number of counted tests was 170–550 per sample, depending on the TA concentration and assemblage structure in the samples. If the proportion of *C. oviformis* was overrepresented, the number of counted tests was higher, but routinely at least 170 tests were counted. Both the percentages of TA in assemblages (relative abundances) and their concentrations in 1 cm^3 of peat were calculated. The effect of preparation treatments on the total concentration of TA tests in samples was tested using the two-sample Student's *t*-test. Prior to testing, the data were log-transformed in order to homogenize the variances. The comparison of TA assemblages (species composition based on concentrations and relative abundances of TA species) among treatments was carried out with nonparametric MANOVA (npMANOVA; Anderson 2001) and non-metric multidimensional scaling (NMDS).

Finally, the TA assemblages from different treatments were used to predict WTD. As a training set, ecological data on TA species abundances in different microhabitats in Estonian bogs were used (Avel-Niinemets et al. 2011). The weighted average model was used to develop the transfer function. The transfer function was applied to the treatment data (untransformed values) to estimate the sample-specific WTD. The mean values of predicted WTD of different treatments were compared with the Welch two-sample *t*-test. The packages *Vegan* (Oksanen et al. 2011) and *Rioja* (Juggins 2009) of the statistical program *R* (R Development Core Team 2011) were applied to multidimensional data analysis and WTD reconstructions, respectively. For all statistical tests $\alpha = 0.05$.

RESULTS

In the boiling treatments, the total concentrations of TA tests varied among samples from about 3000 to more than 80 000 tests in 1 cm^3 (Fig. 1A). This variation was not affected by the boiling of samples as the mean total concentration of TA tests was not different between unboiled (18 782 tests cm^{-3}) and boiled (17 372 tests cm^{-3}) samples (*t*-statistic = −0.3, df = 36, *p*-value = 0.8). In the sieving treatment, nearly all microsieved samples had a lower TA concentration than unsieved samples (Fig. 1B). The mean total concentration of tests was significantly different between microsieved and unsieved samples (18 474 tests cm^{-3} *versus* 116 996 tests cm^{-3}; *t*-statistic = 2.1, df = 38, *p*-value = 0.04).

Not surprisingly, microsieving samples through a 15-μm sieve affected primarily the concentration and abundance of small TA taxa (e.g., *C. oviformis*). As a result, the TA assemblages were significantly different among sieving treatments (npMANOVA, $F = 2.8$, df = 38, p-value < 0.001 for concentration data, and $F = 5.1$, df = 38, p-value < 0.001 for abundance data; estimation of both *p*-values based on 5000 randomizations). Contrary to sieving treatment, boiling had no effect on the estimated TA assembles ($F = 0.32$, df = 36, p-value = 0.98 for concentration data, and $F = 0.25$, df = 36, p-value = 0.99 for abundance data; estimation of both *p*-values based on 5000 randomi-

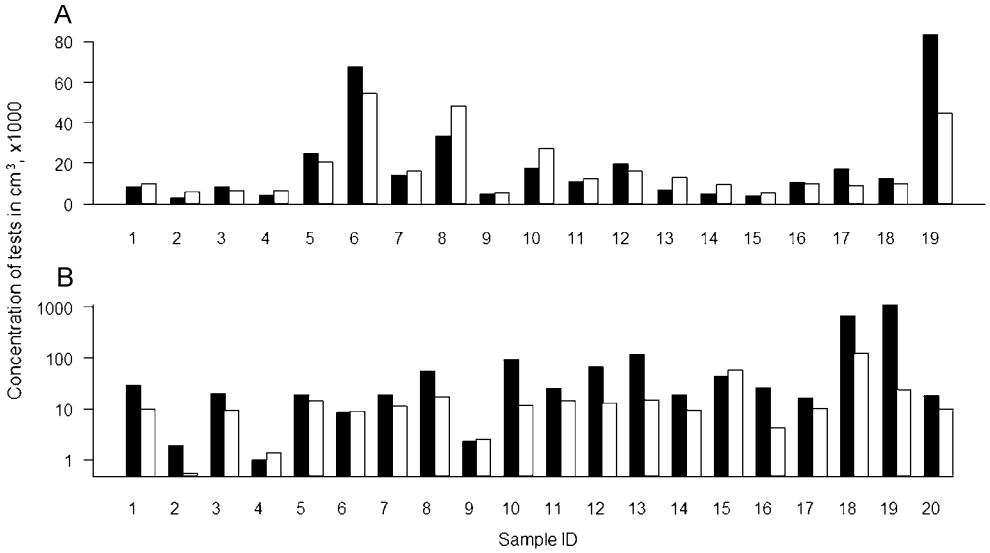

Fig. 1. Impact of boiling (**A**) and microsieving (**B**) on the total concentration of testate amoebae in peat samples. Black bars represent untreated samples and white bars denote treated samples. The impact of boiling was not significant (*t*-test, $p = 0.8$), while microsieving reduced significantly the total concentration of TA tests in peat samples ($p = 0.04$).

zations). An NMDS ordination also shows higher dissimilarity between paired samples of sieving treatment as compared with that of boiling treatment (Fig. 2).

The mean values of predicted WTD were not significantly different between the levels of both treatments (Welch's *t*-statistic = 0.42, df = 35.5, *p*-value = 0.68 for boiling treatment, and $t = 1.64$, df = 33.5, *p*-value = 0.11 for sieving treatment). However, in samples where microsieving affected TA assemblages the most (e.g., samples 18 and 19 from sieving treatment), differences in predicted WTD between unsieved and microsieved samples exceeded the error of prediction (Fig. 3). This indicates that microsieving of TA samples may alert the results of WTD reconstructions.

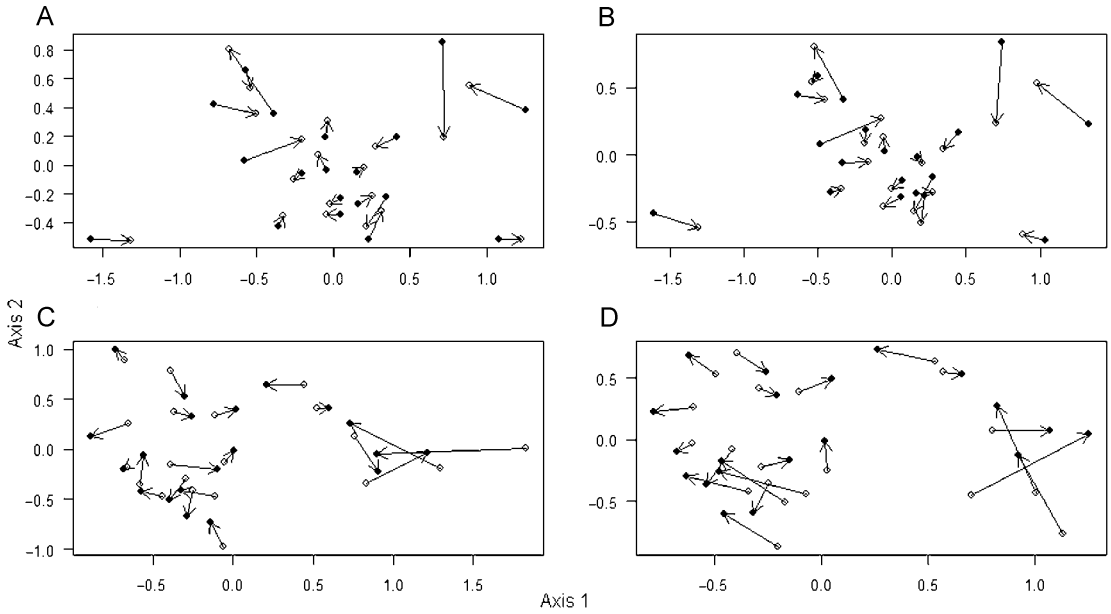

Fig. 2. Non-metric multidimensional scaling ordination plots showing the dissimilarity/similarity of the TA assemblages of differently treated samples (paired samples are connected with arrows; white circles are untreated samples and black circles are treated samples). (**A**) Boiling treatment, concentration data; (**B**) boiling treatment, relative abundance data; (**C**) sieving treatment, concentration data; (**D**) sieving treatment, relative abundance data. Note greater dissimilarity (longer arrows) between the paired samples of sieving treatment as compared with that of boiling treatment.

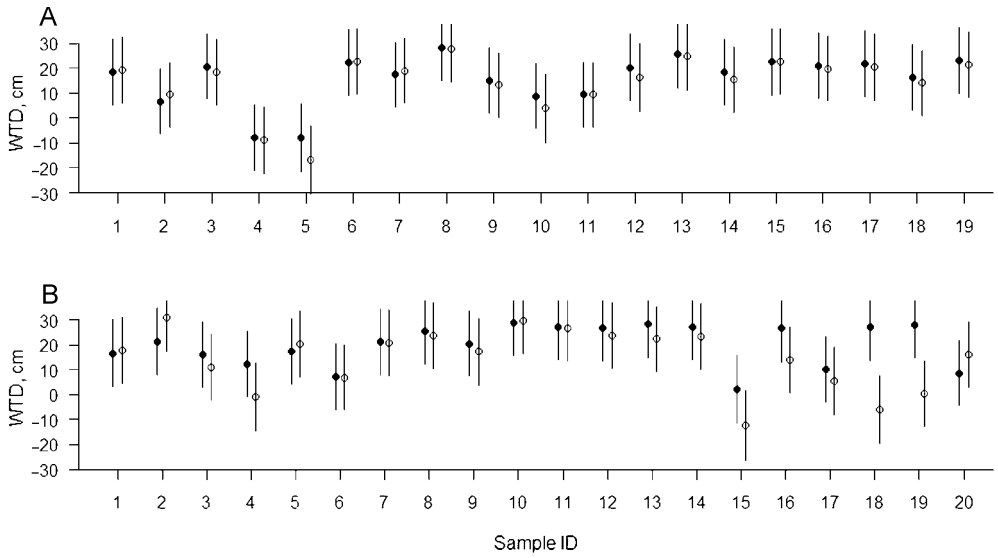

Fig. 3. Impact of boiling (**A**) and microsieving (**B**) on water table depth (WTD) values predicted from the TA assemblages of corresponding samples. Black circles represent untreated samples and white circles are treated samples. Bars show the error of prediction based on bootstrapping (n = 500). The concentration data of all TA species in unsieved and sieved samples are given in Appendix.

DISCUSSION

Our results show that the TA assemblage structure of fresh peat samples does not change markedly when the boiling of peat is substituted by soaking and stirring peat samples in water (usually a less time-consuming procedure than boiling, especially if the number of samples is high). Thus, at least for analysing the TA assemblage structure of fresh peat samples (i.e., taken from the living part of *Sphagnum* mosses), stirring could be used instead of boiling. In fact, many recent studies have prepared TA samples by just soaking and stirring them in water (e.g., Hendon et al. 2001; Booth 2002; Wilmshurst et al. 2003; Charman et al. 2004, 2007; Hendon & Charman 2004; Sillasoo et al. 2007; Lamentowicz et al. 2008; Sjögren & Lamentowicz 2008; Swindles et al. 2009). On the other hand, however, boiling might be necessary for preparation of fossil peat samples as it sterilizes samples and helps to disaggregate the particles from partly decomposed peat.

The results of sieving experiments confirm Payne's (2009) observations that sieving the sample through a 15-µm sieve should be avoided as it reduces both the concentration and relative abundances of small TA taxa in samples. Hendon & Charman (1997) have also shown the effect of sieving on the estimation of TA assemblages. They compared two processing methods to find the best solution for preparation of TA samples. Their first method (method A) involved boiling samples in water and sieving through 300- and 15-µm sieves. The second method (E) included boiling samples in 10% KOH and sieving through a 300-µm sieve. They found that the concentrations of TA species in samples prepared using method E were almost twice of those prepared with method A. The authors explained this difference with undisaggregation of sediment in water that caused the retaining of many TA tests on the 300-µm sieve, reducing the overall concentrations of TA species in method A. However, as the difference in concentrations between methods A and E was largely due to higher concentrations of *Cryptodifflugia sacculus* and *Cryptodifflugia paludosa* in method E, it is also possible that microsieving had affected the estimation of TA assemblages.

Many palaeoecological reconstructions of WTD are based on microsieved TA samples. Undoubtedly, microsieving allows faster analysis of TA samples by reducing debris in well-decomposed samples. The predicted WTD values have often wide error ranges, which may hide the differences in WTD caused by microsieving. In such cases using 15-µm sieves is justified. However, our results indicate that microsieving may occasionally lead to situations where differences in predicted WTD values are greater than the prediction error. Palaeoecological interpretation of such WTD values is impeded as microsieved samples may indicate wet conditions while unsieved samples indicate the prevalence of dry conditions (e.g., see WTD predictions based on samples 18 and 19 in Fig. 2B).

It has been stated that many small TA taxa with siliceous tests (small *Euglypha*, *Corythion–Trinema*-type) are rarely preserved in deeper layers of peat in *Sphagnum*-dominated peatlands (Swindles & Roe 2007).

Excluding small TA taxa from modern training sets is therefore not only a satisfactory practice, but it may in fact be advisable with respect to WTD reconstructions. However, large numbers of at least the species of genus *Cryptodifflugia* (particularly *C. oviformis*) are well preserved in deeper layers of peat (Niinemets et al. 2011). Including them into analysis (by not microsieving samples) might improve the precision of transfer functions and impact the accuracy of reconstructions of past water tables.

Based on the results of this study, we conclude that the boiling of fresh peat samples does not influence the concentration and relative abundance of TA species. Boiled and unboiled TA samples result in similar predicted WTD values. To spend less time on sample preparation, the boiling stage could be replaced by soaking and stirring the fresh peat samples in water. Microsieving decreases significantly the concentration of TA tests in samples be reducing selectively the concentration of smaller TA species. As a consequence, the reconstruction of WTD might be misleading. Thus, if TA samples are microsieved, the results must be interpreted with caution.

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APPENDIX

THE AVERAGE CONCENTRATION (TESTS IN CM^3) OF TA SPECIES AND THE DIFFERENCE BETWEEN SIEVED AND UNSIEVED SAMPLES

Species	Sieved	Unsieved	Difference
Amphitrema stenostoma	13.0	0.3	12.7
Arcella arenaria	15.4	5.5	9.9
Arcella artocrea	70.0	72.8	−2.8
Arcella catinus	4.2	2.8	1.4
Arcella discoides	202.2	75.5	126.7
Arcella megastoma	0.0	32.4	−32.4
Arcella muscicola	1 195.9	882.7	313.3
Arcella vulgaris	29.6	0.0	29.6
Archerella flavum	460.1	507.6	−47.6
Assulina seminulum	106.2	280.6	−174.4
Bullinularia indica	49.4	4.5	45.0
Capsellina aculeata	89.1	54.3	34.8
Centropyxis aerophila	504.8	408.5	96.3
Centropyxis arcelloides	57.0	104.9	−47.8
Centropyxis cassis	5.5	9.2	−3.7
Centropyxis compressa	0.0	16.4	−16.4
Centropyxis ecornis	23.7	13.4	10.3
Centropyxis gibba	1.1	0.0	1.1
Centropyxis platystoma	80.3	11.9	68.4
Corythion dubium	755.5	1 424.5	−669.0
Cryptodifflugia crenulata	0.0	18.1	−18.1
Cryptodifflugia horrida	7.4	0.0	7.4
Cryptodifflugia oviformis	2 341.8	100 811.7	−98 469.9
Cryptodifflugia pusilla	0.0	2.3	−2.3
Cryptodifflugia sacculus	0.0	4.5	−4.5
Cryptodifflugia voigti	15.9	22.4	−6.4
Difflugia bacillifera	0.0	8.0	−8.0
Difflugia glans	16.0	2.7	13.3
Difflugia globulosa	15.3	20.2	−4.9
Difflugia humilis	3.7	0.0	3.7
Difflugia lanceolata	0.0	4.0	−4.0
Difflugia leidyi	11.0	39.3	−28.4
Difflugia lucida	21.6	5.4	16.1
Difflugia oblonga	10.0	0.0	10.0
Difflugia penardi	21.3	0.0	21.3
Difflugia pristis	6 392.4	2 298.8	4 093.6
Difflugia pulex	170.1	496.9	−326.8
Difflugia rubescens	0.0	0.0	0.0
Euglypha ciliata	481.1	296.7	184.5
Euglypha compressa	27.5	15.4	12.1
Euglypha cristata	37.5	217.4	−179.9
Euglypha filifera	1.1	2.2	−1.1
Euglypha laevis	43.3	81.8	−38.6
Euglypha rotunda	278.4	836.9	−558.6
Euglypha simplex	52.1	173.1	−121.0
Euglypha strigosa	94.1	65.5	28.6
Euglypha tuberculata	368.9	303.6	65.3
Heleopera petricola	179.1	188.5	−9.4
Heleopera sphagni	20.9	0.0	20.9
Heleopera sylvatica	243.5	76.5	166.9
Hyalosphenia elegans	80.9	124.2	−43.3
Hyalosphenia minuta	0.0	0.0	0.0
Hyalosphenia ovalis	5.5	14.3	−8.9

Continued overleaf

APPENDIX. *Continued*

Species	Sieved	Unsieved	Difference
Hyalosphenia papilio	87.4	59.2	28.2
Hyalosphenia subflava	0.0	7.2	−7.2
Lesquereusia epistomium	0.0	1.3	−1.3
Nebela bohemica	108.3	65.1	43.2
Nebela carinata	1.8	4.0	−2.2
Nebela griseola	112.9	103.0	9.8
Nebela marginata	30.3	12.2	18.1
Nebela militaris	679.7	849.6	−169.9
Nebela parvula	56.7	78.0	−21.2
Nebela tenella	3.5	1.4	2.1
Nebela tincta	527.8	323.5	204.3
Nebela tubulata	9.0	16.3	−7.3
Phryganella acropodia	691.9	599.9	92.1
Placocista spinosa	116.4	112.2	4.2
Pseudodifflugia fascicularis	0.0	8.3	−8.3
Pseudodifflugia fulva	181.9	656.3	−474.4
Quadrulella symmetrica	0.5	0.3	0.2
Sphenoderia lenta	10.7	18.8	−8.0
Tracheleocorythion pulchellum	5.6	200.1	−194.5
Tracheleuglypha dentata	184.0	220.5	−36.5
Trigonopyxis arcula	620.1	751.1	−130.9
Trinema complanatum	265.3	419.5	−154.2
Trinema enchelys	71.0	58.5	12.5
Trinema lineare	106.3	2 362.3	−2 256.0

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Kodaamööbiproovide ettevalmistamine mõjutab soovee tasemete paleoökoloogilisi rekonstruktsioone

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Sooökosüsteemide paleoökoloogiliste uuringute raames kogutud kodaamööbiproovide ettevalmistamine näeb ette nii proovide keetmist kui ka nende sõelumist läbi 15 µm sõela. Käesolevas töös uuriti nende ettevalmistusetappide mõju kodaamööbikoosluste liigilisele koosseisule ja selle põhjal tehtud soovee tasemete rekonstrueerimisele. Töö tulemused näitavad, et keedetud ja keetmata kodaamööbiproovide liigiline koosseis ei ole erinev, sellal kui sõelumine vähendab oluliselt väiksemate kodaamööbiliikide esindatust sõelutud proovides. Erinevus sõelutud ja sõelumata proovide kodaamööbikoosluste liigilises koosseisus kajastus ka soovee tasemete rekonstruktsioonides. Sõelutud proovidega võrreldes näitasid sõelumata proovide põhjal tehtud rekonstruktsioonid oluliselt kuivemaid keskkonnatingimusi. Võime järeldada, et kodaamööbiproovide keetmise võib proovide ettevalmistamisprotsessis vahele jätta. Mikrosõelumise puhul tuleb arvestada, et kui proovides on esindatud ohtralt väiksemaid kodaamööbiliike, võib see viia valede paleoökoloogiliste järeldusteni.