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# S1 Sequencing and sequence filtering

A total of 16,403,970 read pairs was produced by sequencing. The initial quality check showed quality scores only above 30 for forward and reverse sequence reads (Fig. S3.1). This means that one out of 1000 nucleotides is expected to be incorrect, which corresponds to 99.9 % accuracy. 14,602,042 read pairs could be assigned to their sample based on the tag combination. 1,709,787 read pairs (10.4 %) could not be assigned to their sample because primers did not match, a forward or reverse tag was missing or due to erroneous tags. 90,576 unique sequence types were retrieved of which 7,685 were at least 10 bp long and represented by at least 10 sequence counts. After de-noising (the removal of rare sequences) with *obiclean*, 2,043 unique sequence types were kept. After annotation, sequences corresponding to cultivated plants or plants exotic to the area (4.4 %) were regarded as inauthentic and excluded (S3). 72.2 % of the produced sequences (excluding contaminant sequences) showed 100 % sequence identity with an existing database entry.

# S2 Technical evaluation and authenticity of the data

Sedimentary ancient DNA (sedaDNA) datasets are prone to potential technical biases during DNA isolation, PCRs and sequencing. Hansen et al. (2006) highlighted that ancient DNA molecules from permafrost sediments are highly affected by interstrand-crosslinks, which may inhibit PCRs by preventing template denaturation. Besides, sequences are amplified with different efficiencies depending on the complementarity to the primer binding site (Pawluczyk et al., 2015) and the degree of DNA damage (Pääbo et al., 1989). The effect from lower PCR efficiencies cannot be distinguished from lower starting amounts of rare template molecules (Pawluczyk et al., 2015) and hence prevents true quantitative reconstructions. Overall constant high quality scores indicate a low probability for erroneous base-calls and a high sequencing data quality. Still, a high proportion of the unique sequences was not identical with an entry in the published sequence databases and can be attributed to PCR and sequencing errors. First, we found an inflation of sequence types derived by PCR-errors, e.g. 101 different sequences of *Carex* with best identities of more than 90%. However, they were rare sequences and in total accounted for 3.7 % of all read counts belonging to the genus *Carex*. As this pattern was noticed across the whole dataset, except for taxa assigned to food and cultivated plants, we assume that the analysed sequences are authentic and the high error rates triggered by DNA damage. In many cases it was impossible to objectively and adequately assign these sequences to their respective MOTUs, hence we kept only the sequences which were strictly identical to the references. Second, approximately 10% of the sequences could not be assigned to any sample, because primers did not match or one of the two barcode tags was missing or false. While non-matching primers could result from sequencing errors in the primer binding site, missing tags could result from incomplete sequencing and false tag combinations from tag switching. Tag switching can lead to the presence of rare sequences and are caused for example by mixed clusters during sequencing, cross-contamination with tagged primers or during sample preparation (Kircher et al. 2012, Schnell et al 2015). Schnell et al. (2015) found out that up to 2.6 % of the sequences in their metabarcoding studies had recombined tag combinations most likely due to the to the T4 polymerase activity during blunt-ending in the sequencing library preparation or the formation of chimeric sequences (merged artefacts of two or more highly similar molecules) during the sequencing library index PCR. We thus applied stringent filtering and denoising of rare sequences to keep as many true positives while excluding as many true negatives as possible.

# References

Hansen, A. J., Mitchell, D. L., Wiuf, C., Paniker, L., Brand, T. B., Binladen, J., Gilichinsky, D. A., Rønn, R. and Willerslev, E.: Crosslinks rather than strand breaks determine access to ancient DNA sequences from frozen sediments, Genetics, 173(2), 1175–1179, doi:10.1534/genetics.106.057349, 2006.

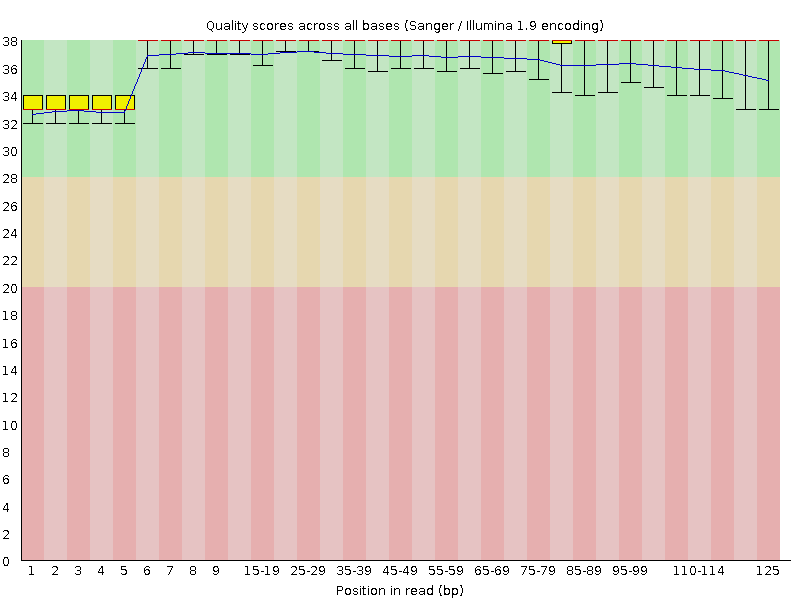
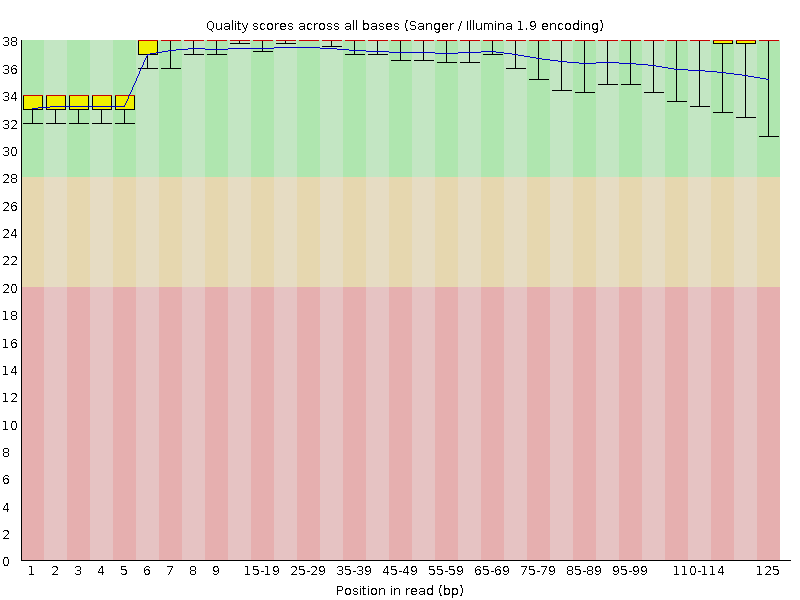
Kircher, M., Sawyer, S. and Meyer, M.: Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform, Nucleic Acids Res., 40(1), e3–e3, doi:10.1093/nar/gkr771, 2012.

Pääbo, S., Higuchi, R. S. and Wilson, A. C.: Ancient DNA and the Polymerase Chain Reaction: The emerging field of molecular archaeology, J. Biol. Chem., 264(17), 9709–9712, 1989.

Pawluczyk, M., Weiss, J., Links, M. G., Egaña Aranguren, M., Wilkinson, M. D. and Egea-Cortines, M.: Quantitative evaluation of bias in PCR amplification and next-generation sequencing derived from metabarcoding samples, Anal. Bioanal. Chem., 407(7), 1841–1848, doi:10.1007/s00216-014-8435-y, 2015.

Schnell, I. B., Bohmann, K. and Gilbert, M. T. P.: Tag jumps illuminated – reducing sequence-to-sample misidentifications in metabarcoding studies, Mol. Ecol. Resour., 15(6), 1289–1303, doi:10.1111/1755-0998.12402, 2015.

# Figures



B

A

Figure S3.1: FastQC quality scores across all bases of the raw sequencing data for (A) forward reads and (B) reverse reads. All read positions showed quality scores above 30 indicating high sequencing quality.

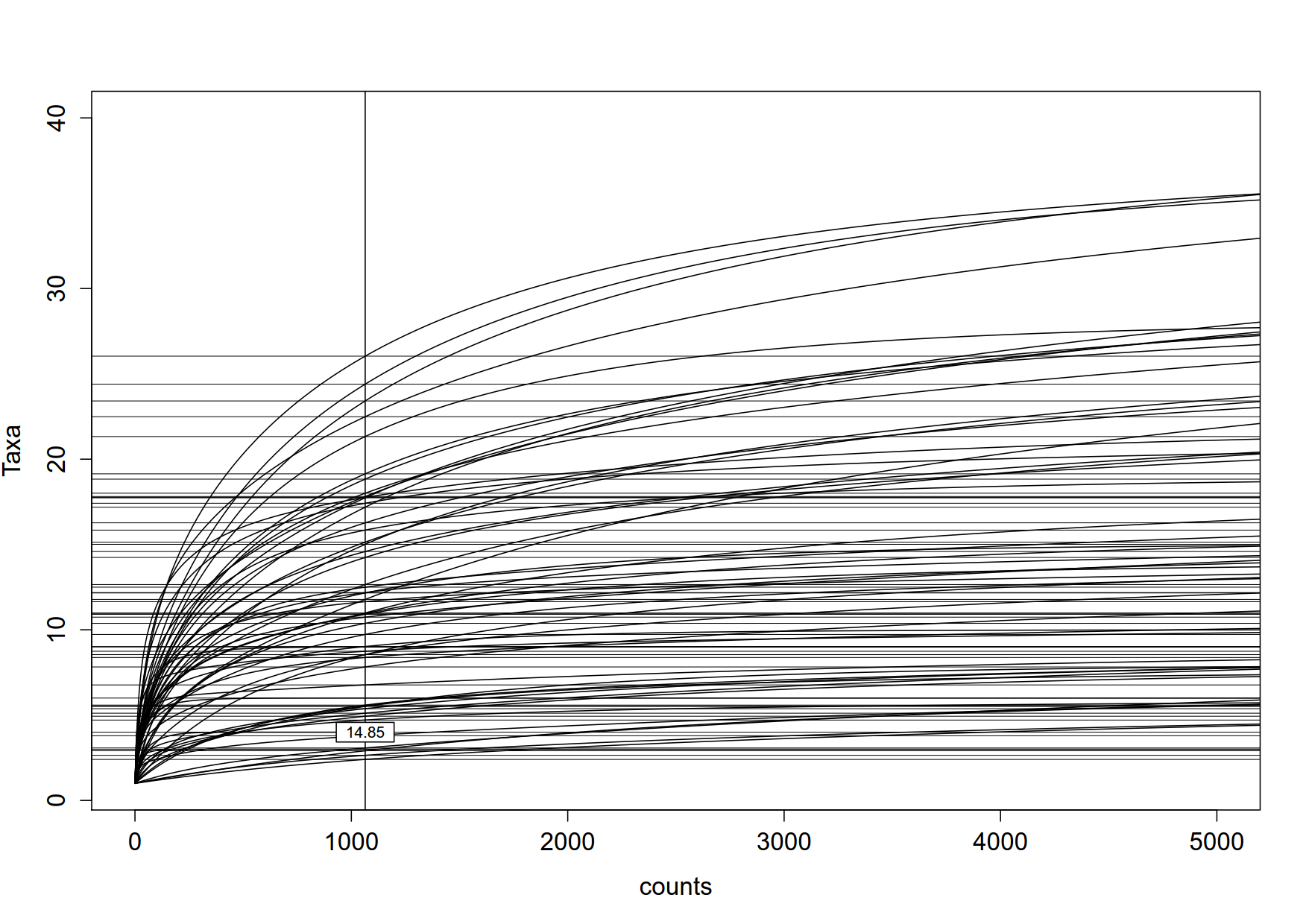


Figure S3.2: Rarefaction curves of the terrestrial sedaDNA dataset. The scale is limited to 5000 sequence count for better visibility. Flags show sample depth (m). Vertical lines indicate the minimum number of pollen counts (n = 1064) used for rarefaction. Horizontal lines indicate rarefied taxon richness values.

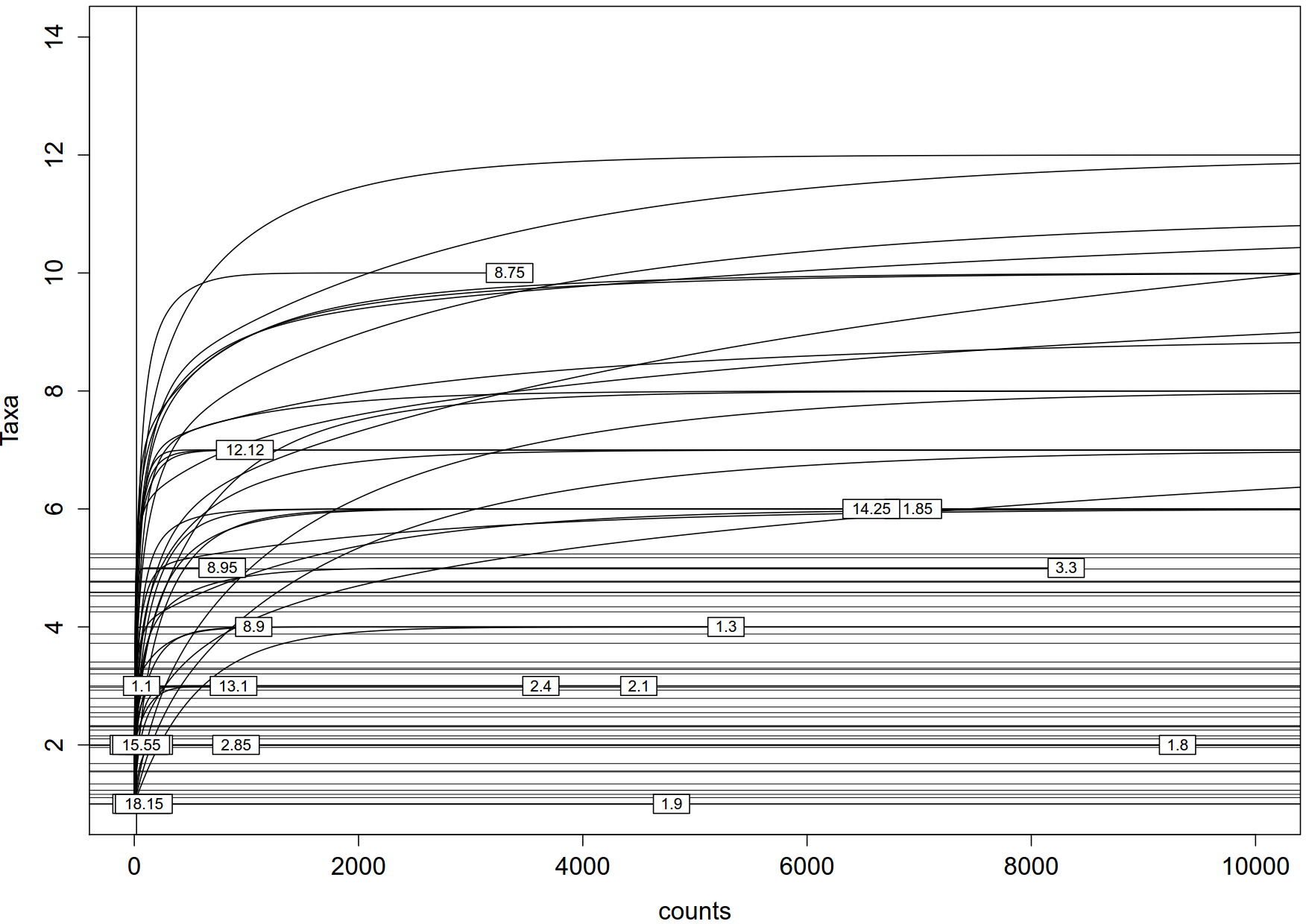


Figure S3.3: Rarefaction curves of the swamp/aquatic sedaDNA dataset. The scale is limited to 10000 sequence counts for better visibility. Flags show sample depth (m). Vertical lines indicate the minimum number of pollen counts (n = 20) used for rarefaction. Horizontal lines indicate rarefied taxon richness values.

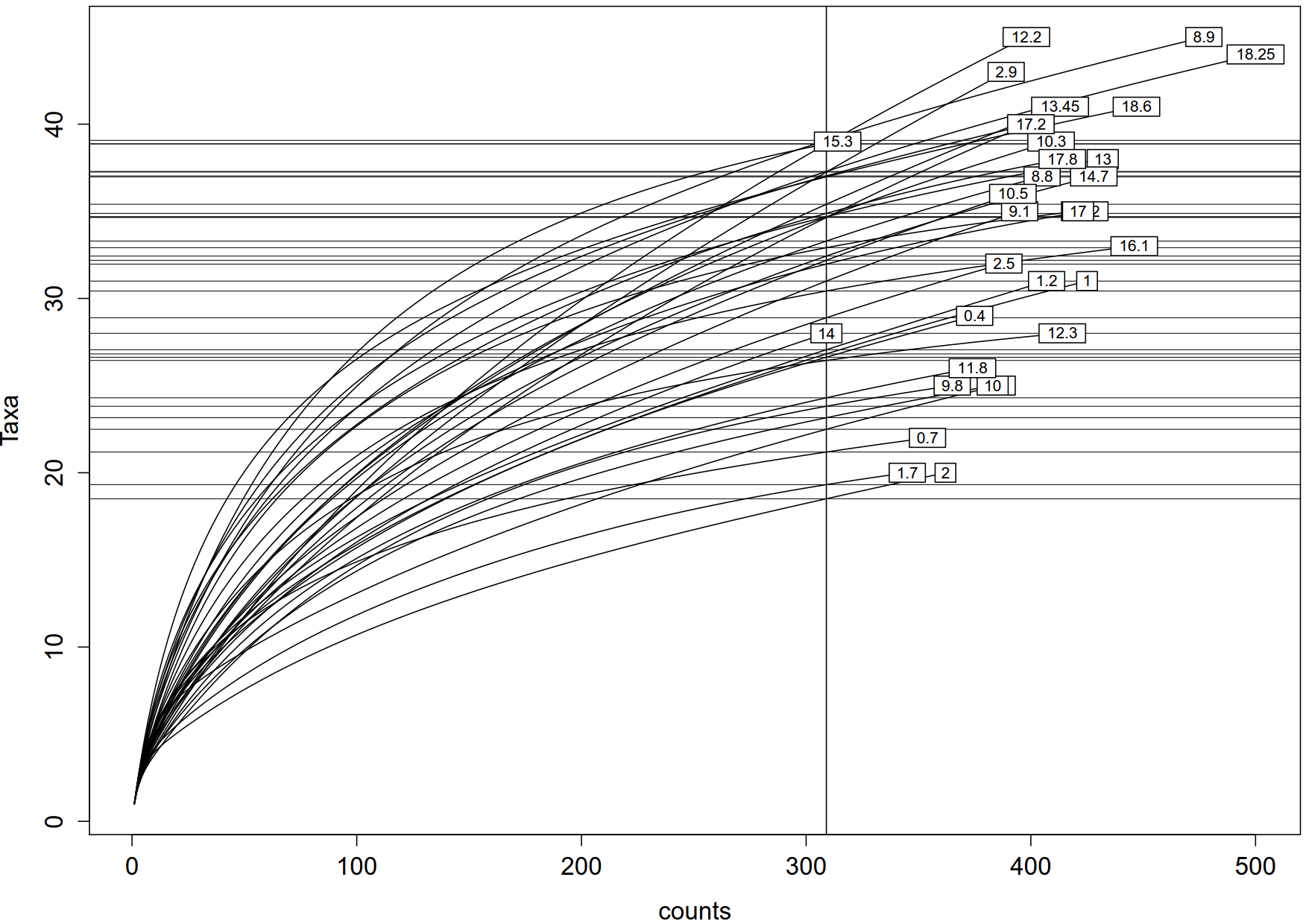


Figure S3.4: Rarefaction curves for pollen and non-pollen palynomorphs. Flags show sample depth (m). Vertical lines indicate the minimum number of pollen counts (n = 309) used for rarefaction. Horizontal lines indicate rarefied taxon richness values.

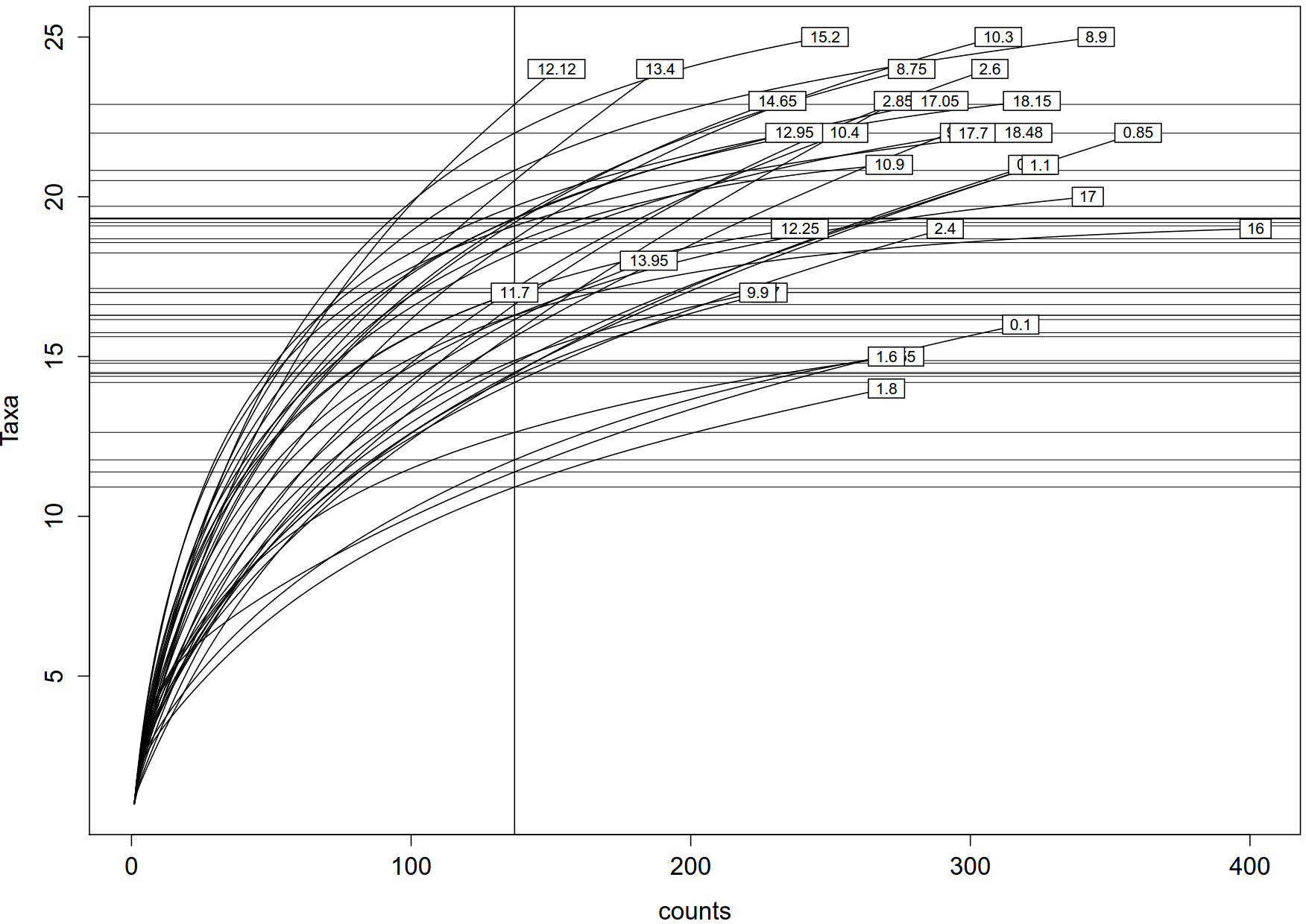


Figure S3.5: Rarefaction curves for terrestrial pollen. Flags show sample depth (m). Vertical lines indicate the minimum number of pollen counts (n = 137) used for rarefaction. Horizontal lines indicate rarefied taxon richness values.

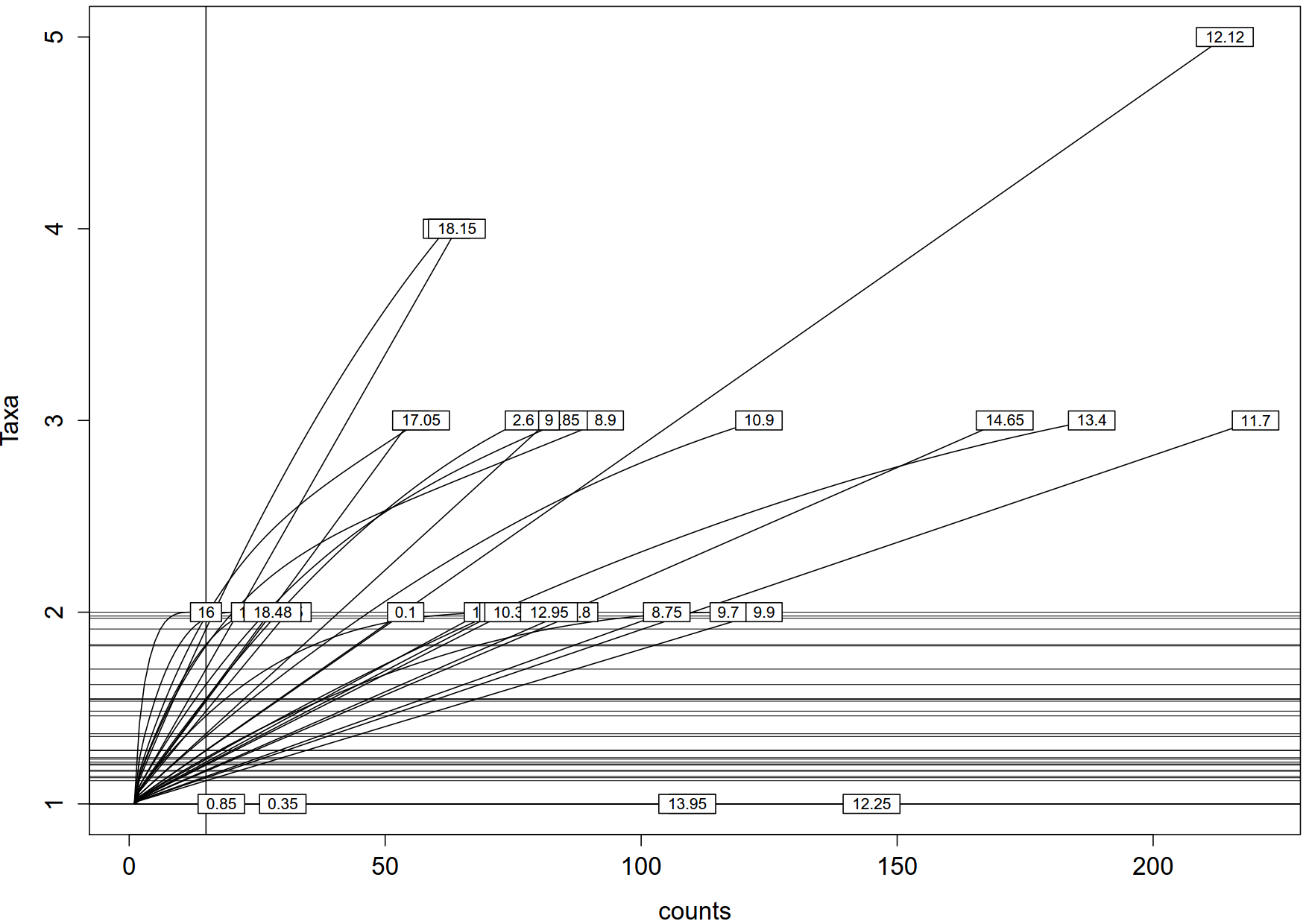


Figure S3.6: Rarefaction curves for swamp/aquatic pollen. Flags show sample depth (m). Vertical lines indicate the minimum number of pollen counts (n = 15) used for rarefaction. Horizontal lines indicate rarefied taxon richness values.

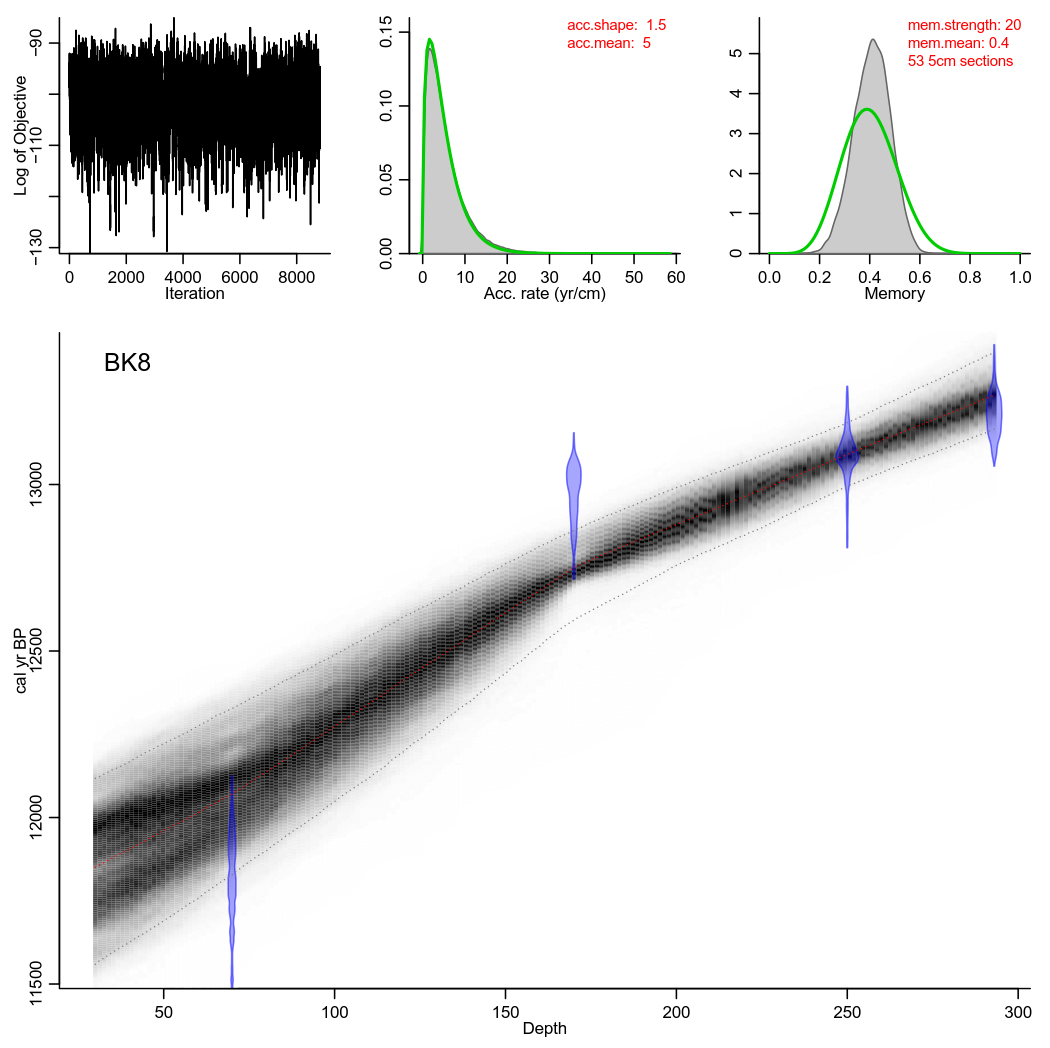


Figure S3.7: Age-depth model for permafrost sediments above the ice wedge. The calibrated ages are reported as calendar years before present (cal yr BP), the depths in cm.