

Figure S2: Overview of the base composition of the COI Folmer region for the 15 most important freshwater groups. The plot of group base composition was generated with PrimerMiner and used to develop the BF / BR primers and manually evaluate other common metabarcoding primers.

The sequences for the Folmer binding regions (opaque colours) have been downloaded in February 2015 and had 26 bp clipping applied, as many clusters were affected by sequences which still contained the primer sequences. Sequences from the Folmer region were downloaded April 2015 not trimmed, as only the region amplified by the primers was used.