

Supplementary Info: Slippage of degenerate primers can cause variation in amplicon length

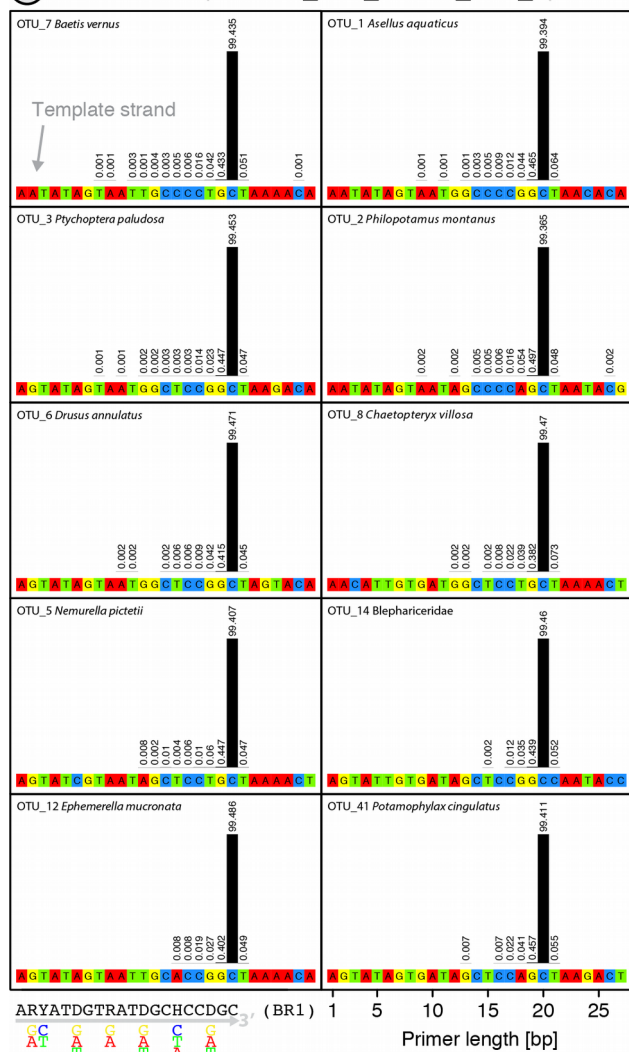
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Fig. S1: Plots of length variation for six additional primers.

Table S1: Raw length distribution data and number of sequences used for each taxon and primer. (Uploaded separately)

Scripts S1: R scripts used to analyze primer length distribution. (Uploaded separately)

A BR1 Primer (PCR: P5_BF1_0 + P7_BR1_4)



B BR1 Primer (PCR: P5_BF2_0 + P7_BR1_4)

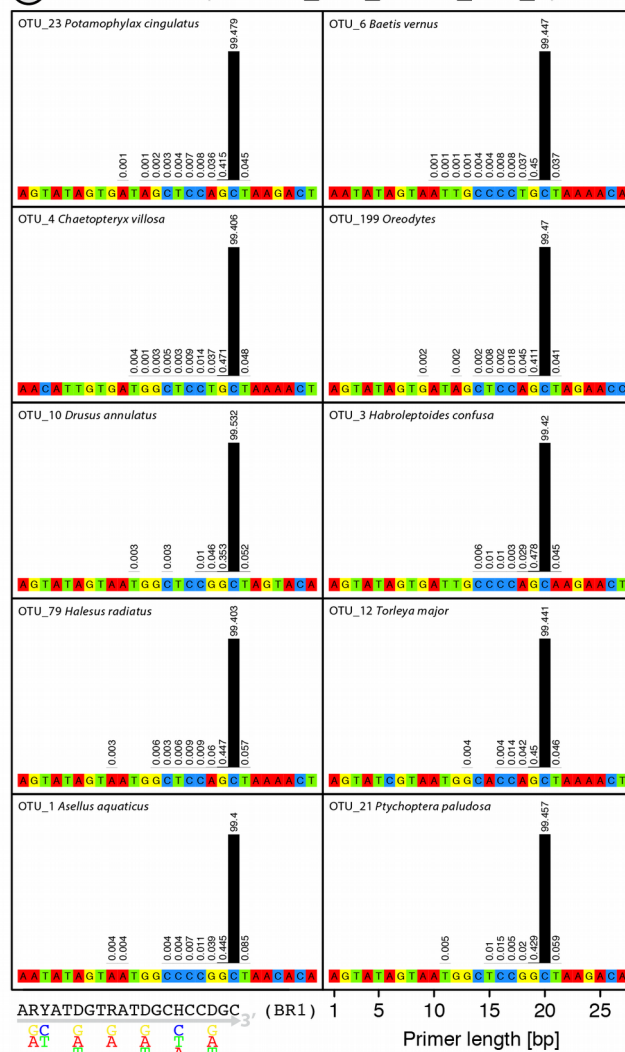
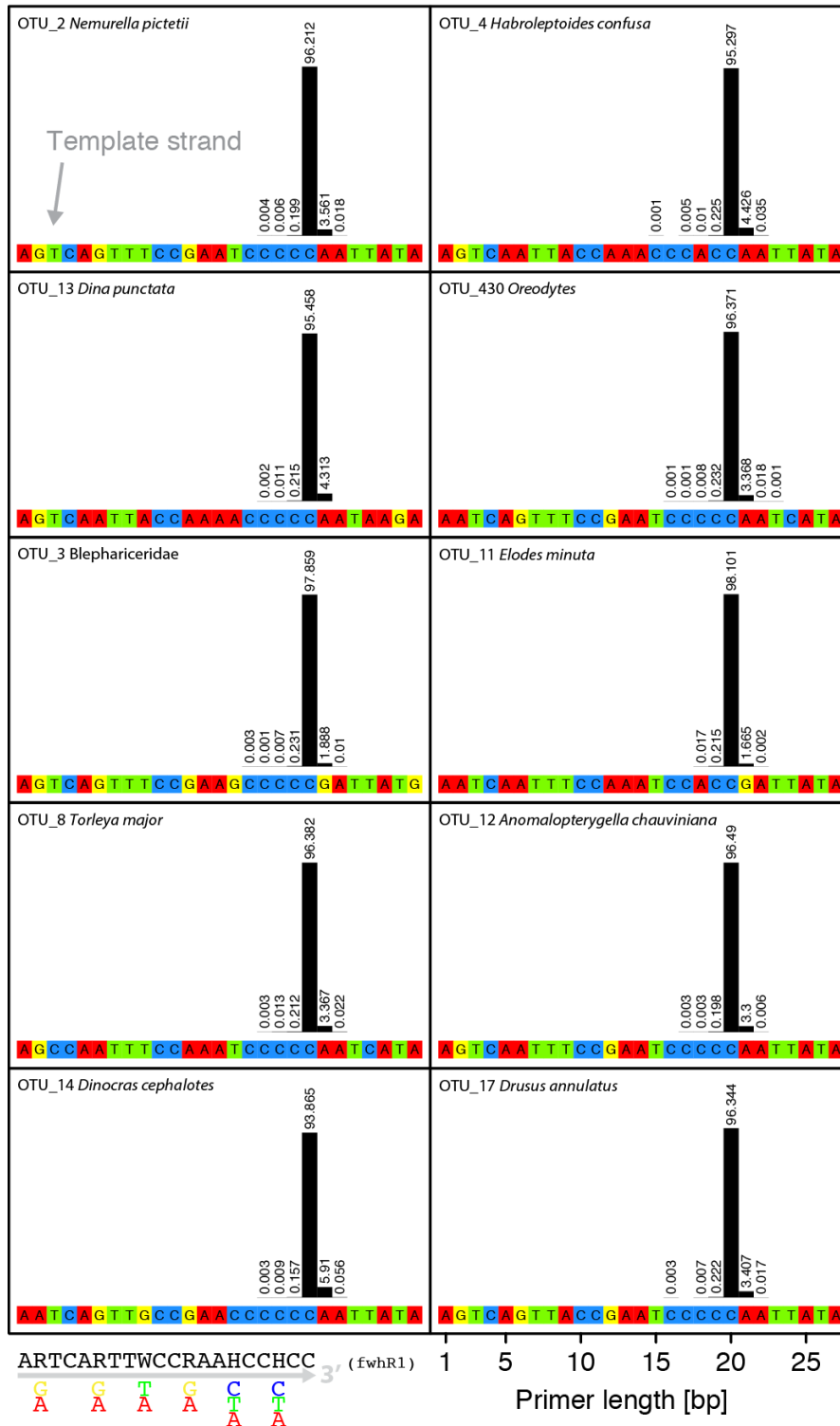


Figure S1: Plot of primer binding sites and bar plots depicting length of utilised primers for the 10 most abundant OTUs in mock sample B (sequence data from Elbrecht & Leese 2017). The presence and read abundance for different taxa can vary based on the primer set used, thus while amplifying the same mock samples, the order of and taxa the 10 most abundant specimens is not identical between plots as OTUs are sorted by read abundance. The sample was amplified with the P5_BF1_0 + P7_BR1_4 and P5_BF2_0 + P7_BR1_4 primer set, and the length distribution of the incorporated primers is shown for the BR1 primer (A and B). The percentage of amplicons that incorporated a particular length are shown above each bar.

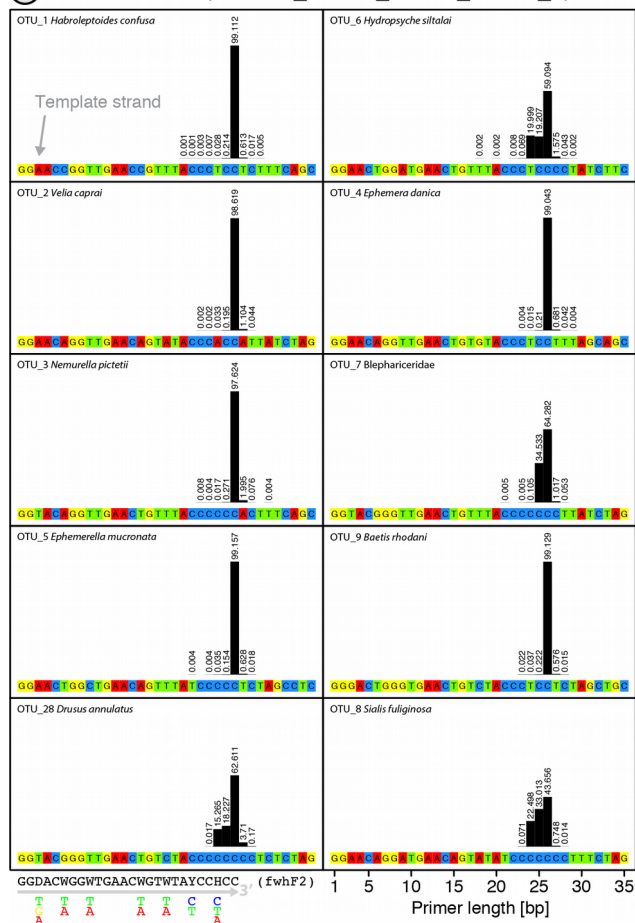
Additional plots for further primer combinations on the next 2 pages (C, D, E).

© fwhR1 Primer (PCR: P5_fw hF1_3 + P7_fw hR1_1)

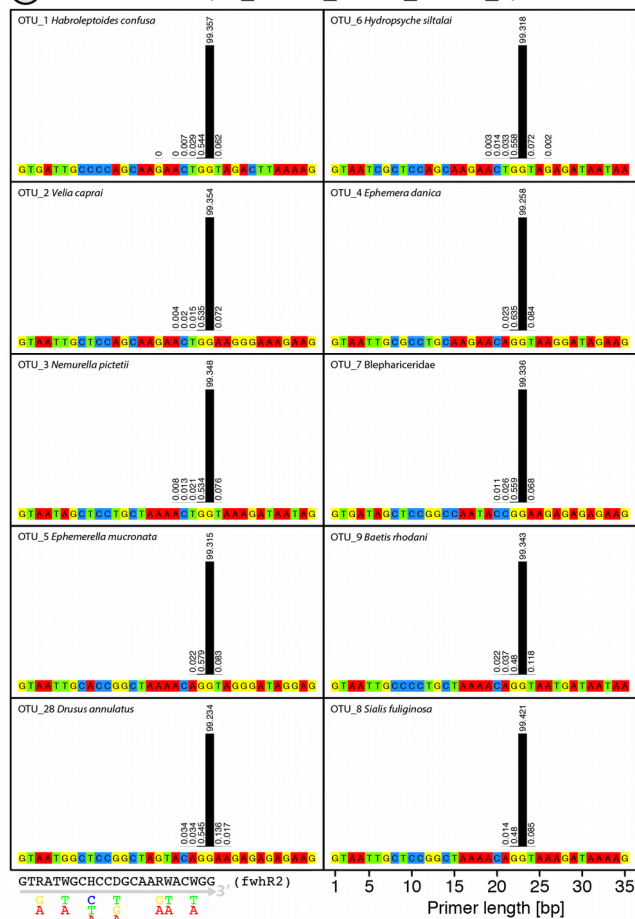


C: Length distribution of the fwhR1 primer (Sample B, P5_fw hF1_3 + P7_fw hR1_1, Vamos et al. 2017). The fwhF1 primer was not analyzed, as no data about the primer binding site was available (as it binds in the LCO1490 primer binding region which was not sequenced).

D fwhF2 Primer (PCR: P5_fw hR2_2 + P7_fw hF2_3)

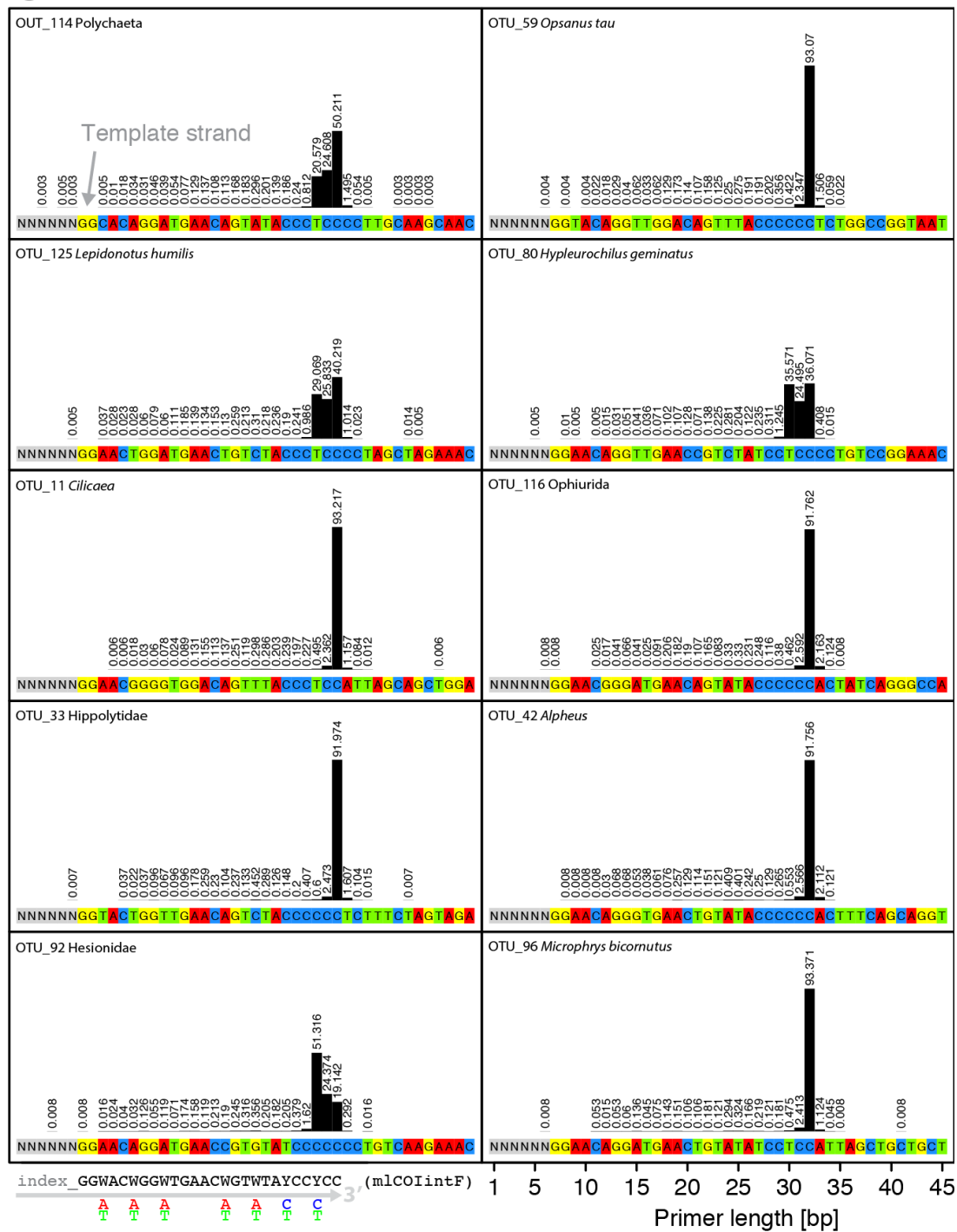


E fwhR2 Primer (PCR: P5_fw hR2_2 + P7_fw hF2_3)



D & E: Length distribution of the fwhF2 and fwhR2 primer (Sample B, P5_fw hR2_2 + P7_fw hF2_3, Vamos et al. 2017).

(F) mlCOLintF Primer (PCR: mlCOLintF + jgHCO2198, run 1)



F: Length distribution of the mlCOLintF primer (marine invertebrate sample from Leray & Knowlton 2017, mlCOLintF + jgHCO, run 1). The jgHCO primer was not analyzed, as no data about the primer binding site was available (as it binds in the HCO2198 primer binding region which was not sequenced).