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The four sample replicates of I-2015 (sampling day 8) were also analysed by paired-end (500 cycles) NGS. The zotus with 500 bp in length were reported as Zotu.n. 500 in parenthesis. Three Zotu.n. 500 were found identical to those observed by Sanger sequencing. However, a sequence identical to C5.3 at length ( 467 bp ) was found in one sample (D2.mar). This Zotun. 500 was denoted as Dn.500.0 in Table 1 and Supplementary Material S2. Of the additional seven Zotu.n. 500 for D2.mar, five matched to S6.5, and one matched to S9.5. The sequence of the other Zotu.n. 500 matched to S6.5. Thus, although the sequence of Dn.500.0 was identical to C5.3 and S6.5, the two Zotus were found in different sample replicates. Therefore, although a definitive conclusion cannot be made, it is suggested that Dn. 500.0 may have originated from an event involving C5.3 in I-2015. In this case, the number of C5.3 and Dn.500.0 is two and eight, respectively. The results of the NGS on the two sample replicates of another sampling trip (D7.mar and Dmax.mar) also produced two Zotus with a length of about 500 bp . In Figure3, these are designated Zotun.500.0 and n.500.1 in Supplementary Material S2. Their sequence matched to C5.3 and S6.5 at length. One Zotun. 500 was found in the reanalysed data of I -2015, and other Zotun. 500.0 and n .500 .1 were detected in the six re-analysed samples of D7.mar and Dmax.mar. Thus, it was concluded that one Zotun.500.0, which was identical to C5.3 at length, was the most common Zotu in the samples collected in 2014.

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