Oligonucleotide probes applied in this study with horse radish peroxidase as the reporter on the 5'-end. For total eukaryotes a combination of three probes was used because of two known mismatches of the universal eukaryote probe with kinetoplastids and diplonemids. Stringencies are expressed as formamide concentrations.

Name	Stringency	Target	Probe Sequence (5'-3')	Reference
EKD	40%	All eukaryotes	combination of 3 probes:	
		Eukaryote (EUK516)	ACCAGACTTGCCCTCC	Amann et al. (1990)
		Kinetoplastids (KIN516)	ACCAGACTTGTCCTCC	Bochdansky & Huang (2010)
		Diplonemids (Diplo516)	ACCAGACTTGTCCACC	this study
KIN516	40%	Kinetoplastids (KIN516)	ACCAGACTTGTCCTCC	Bochdansky & Huang (2010)
LabY	50%	Labyrinthulomycetes	AACCCGAAATGTCCCTCTAAGAAG	Stokes et al. (2002)
PF2	40%	Fungi	CTCTGGCTTCACCCTATTC	Kempf et al. (2000)

Probe evaluation details

Stringencies were evaluated for individual probes as follows: EUK516 on *Cafeteria roenbergensis*, KIN516 on *Neobodo designis*, Diplo516 on *Diplonema papillatum***,** PF2 on *Rhizophydium littoreum*, and LabY on *Thraustochytrium gaertnerium*. Stringencies tested were 30, 40, 45, 50, 55, 60, 65, 70 % formamide concentrations. After the initial stringency test, the top 4 stringencies were tested again on cultures and then on samples from previous collections (Elizabeth River) and deep-sea samples (Atlantic Ocean). The PF2 probe is a reliable and broad fungal probe. In contrast, MY1574, considered a general probe for Eumycota (Baschien *et al*., 2008), did not hybridize with the chytrid *R. littoreum*. A BLAST search revealed that indeed neither MY1574 nor FUN1429 (Baschien *et al*., 2008) match any known chytrid sequences. Using these two probes would therefore underestimate the number of fungi in marine habitats as chytrids represent one of the most abundant groups of primarily parasitic fungi in the ocean (Kagami *et al*., 2014; Hassett and Gradinger, 2016). To date, there is still not a well-developed chytrid probe (Wurzbacher *et al*., 2010). However, because PF2 hybridizes well with a variety of fungi from *Saccharomyces cerevisiae* (Ascomycota) to *R. littoreum* (Chytridiomycota), we are reasonably confident that we captured the entirety of the true fungal branch (Eumycota) (Jobard *et al*., 2012). The EUK516 sequence is not as universal as previously thought as there are at least two groups of flagellates with mismatches: the kinetoplastids (1 mismatch) and the diplonemids (2 mismatches, Table S2). The reduced stringency and the mixture of three probes (EKD, Table S2) were necessary to be as inclusive as possible for eukaryotes.