

Additional File 3. PCR conditions and primer sequences used in this research

	<i>wsp</i>	<i>16S</i>	<i>COI</i> and Sequencing	DIG-labelled <i>wsp</i> probe
PCR protocol				
Total volume	10µL / 20µL	10µL	20µL	50µL
GoTaq Reaction Buffer	1X	1X	1X	1X
MgCl ₂	4mM	2mM	2.5mM	5mM
dNTP	150µM each	125µM each	200µM each	2.5µL (DIG-dNTP labelling mixture)
Primer 1	0.8µM	0.63µM	0.4µM	0.6µM
Primer 2	0.8µM	0.63µM	0.4µM	0.6µM
GoTaq DNA polymerase	0.4u / 0.8u	0.5u	0.5u	1u
DNA Template	1µL / 2µL	1µL	2µL	2µL
Thermal Cycling				
Denaturation	94°C for 2min 94°C for 10s, 65°C for 30s, 68°C for 1min (x10 cycles) 94°C for 10s, 65°C for 30s, 68°C for 1min (x25 cycles, including an incremental increase of 20s at 68°C)	94°C for 5min 94°C for 30s, 54°C for 30s, 72°C for 1min (x35 cycles) 72°C for 10min	94°C for 5min 94°C for 30s, 55°C for 30s, 72°C for 1min (x35 cycles) 72°C for 10min	94°C for 2min 94°C for 10s, 65°C for 30s, 68°C for 1min (x10 cycles) 94°C for 10s, 65°C for 30s, 68°C for 1min (x25 cycles, including an incremental increase of 20s at 68°C)
Primers				
<i>wsp</i>				
Wsp-F	TGGTCCAATAAGTGATGAAGAACTAGCTA	Jeyaprakash and Hoy, 2000		
Wsp-R	AAAAATTAACGCTACTCCAGCTTCTGCAC	Jeyaprakash and Hoy, 2000		
<i>16S</i>				
wspecF	CATACCTATTCGAAGGGATAG	Werren and Windsor, 2000		
wspecR	AGCTTCGAGTGAAACCAATTC	Werren and Windsor, 2000		
<i>COI</i>				
Dick (C1-J-2441)	CCTACAGGAATTAATAATTTTAGATGATTA	Simon et al. 1994		
Pat (TL2-N-3014)	TCCATTGCACTAATCTGCCATATTA	Simon et al. 1994		
Sequencing				
T7 Promoter	TAATACGACTCACTATAGGG			
SP6	ATTTAGGTGACACTATAG			