Pembrokeshire Fungus Recording Network

DNA barcoding exercise 11th May 2019

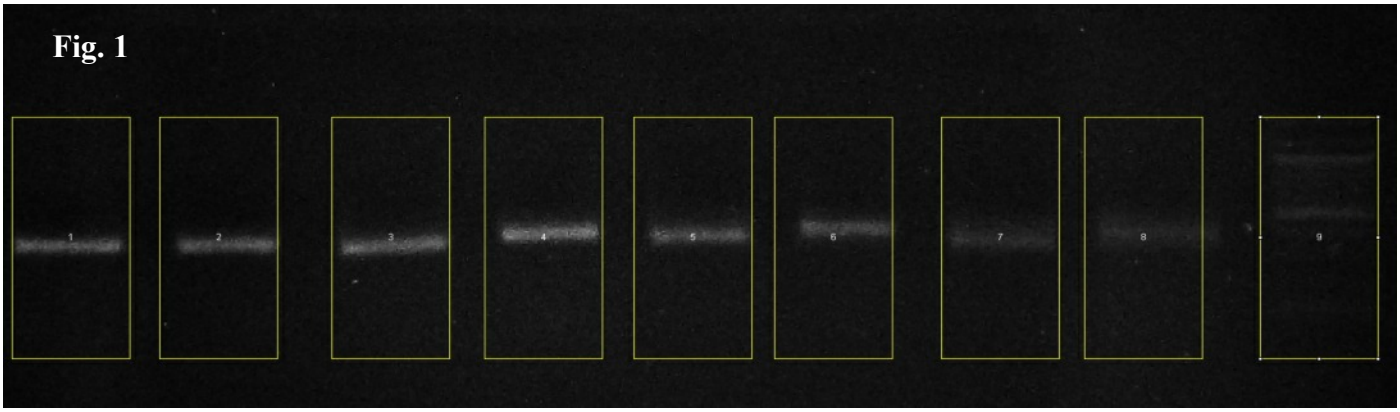
Method

Extractions were performed using a Quick Extraction technique based on Zou. Y, et al. (2017) with the DNA extract eluted into 30 µl TE buffer. Extraction buffer volume 100 µl, Wash buffer volume 1.0 ml.

Amplification was carried out using the Bento Lab thermal cycler with ITS3/ITS4 primers, Taq/dNTP reagent (RC1811) and TBT additive (Samarakoon, 2013), conditions:

$$95°C^{5\ min}\ [95°C^{30\ sec},\ 55°C^{30\ sec},\ 72°C^{1\ min}]_{35}\ 72°C^{10\ min}$$

The product was visualised on 1% agarose gel (fig 1). and the yield estimated by comparison with a standard DNA ladder using ImageJ software.



Results

All 8 extractions gave positive gel band consistent with those expected for a barcode amplified using the ITS3/ITS4 primer combination.

Extractions of the Spindle rust were less distinct than those obtained for the other specimens. All other bands were well defined (note that bands observed on the gel appear brighter and with greater contrast than those reproduced on the above photograph).

An estimate of the DNA yield is shown in in the table below.

Sample no.	Specimen	DNA yield (ng)
1	*Entoloma clypeatum* (dried). Shield pinkgill (basidiomycete)	380
2	*Entoloma clypeatum* (dried). Shield pinkgill (basidiomycete)	360
3	*Scutellinia scutellata* (fresh). Scarlet eyelash fungus (ascomycete)	430
4	*Entoloma clypeatum* (dried). Shield pinkgill (basidiomycete)	490
5	*Scutellinia scutellata* (fresh). Scarlet eyelash fungus (ascomycete)	370
6	*Puccinia smyrnii* (fresh). Rust on Alexanders	280
7	*Melampsora epitea* (fresh). Rust on Spindle	200
8	*Melampsora epitea* (fresh). Rust on Spindle	160

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