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DNA extraction from spider webs

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Abstract Many spider species produce webs that represent a potential non-invasive source of DNA for conservation genetic analysis. Reported here is the successful isolation of target DNA from members of two families (Theraphosidae and Pholcidae) using a standard CTAB phenol–chloroform–isoamyl protocol. The isolated DNA was of sufficient quality to permit routine PCR amplification and sequencing of mtDNA COI fragments of various sizes (maximum 710 bp attempted). This adds to other studies in demonstrating that webbing offers an excellent resource for genetic studies of spiders across families. Applications of the technique include species identification and monitoring, faunistic surveys, population connectivity, subpopulation structuring, and ex situ breeding programs.

Keywords Spider webs · Ecological genetics · Non-destructive sampling · Non-invasive sampling · Conservation · Psalmopoeus · Pholcus

As DNA extraction techniques have improved, researchers in Arthropod conservation genetics have moved away from ‘non-lethal sampling’ (sampling of tissue which may impact the individual’s future life but does not kill (Vila et al. 2009)) and begun to explore ‘non-invasive sampling’ (sampling which confers minimal costs to the individual but that is targeted to a specific species (Feinstein 2004)) and environmental DNA (eDNA, genetic material from bulk environmental samples, not necessarily targeted toward a taxonomic group (Barnes and Turner 2016)). Spider webbing represents a potential source of DNA for such applications (Xu et al. 2015).

DNA isolation was tested on samples of webbing from two species (Psalmopoeus cambridgei Pocock 1895, Theraphosidae, and Pholcus phalangioides Fuesslin 1775, Pholcidae) that produce different web forms. Psalmopoeus construct vertical sheet webs in enclosed spaces in trees, which are then covered in loose material surrounding the web structure; primarily detritus and leaves (Bushell pers. obs.). Pholcus build ‘space webs’ which are used as prey-detection structures from which the spider hunts prey (Jackson and Brassington 1987). Both species produce different web forms to species investigated previously (Latrodectus spp., Theridiidae, Xu et al. 2015).

Samples of captive Psalmopoeus cambridgei webbing were cleaned of large particles of detritus, but the majority of the fine detritus (pieces of prey, faeces from the spider, and local substrates etc.) remained stuck to the web, a potential source of non-target DNA (Xu et al. 2015). Webbing was cut into pieces to give individual sample weights of 2.3–8.5 mg.

Webs known to belong to Pholcus phalangioides were collected from a house in Wales (52.4113, –3.9897). Four samples of webbing without visible exoskeletons from either the web holder or prey were used, weighing 2.4–7.4 mg.

DNA from web samples was extracted using a standard CTAB phenol:chloroform:isoamyl alcohol (PCIA) method (Winnepeppinckx et al. 1993), chosen for its relative low per-sample cost and applicability to low weight eDNA samples (Blake et al. 2015). Samples were placed in 350 μl of CTAB and 10 μl of Proteinase K and incubated overnight at 37 °C with occasional vortexing. 350 μl of PCIA was then added and the mixture shaken for 20 min.
Following centrifugation at 15,000 RPM for 20 min, the upper aqueous phase was removed and subjected to an ethanol precipitation with 1 ml of absolute ethanol. Following a second precipitation using 1 ml of 70% ethanol, the ethanol precipitation with 1 ml of absolute ethanol. Following centrifugation at 15,000 RPM for 20 min, the upper aqueous phase was removed and subjected to an ethanol precipitation with 1 ml of absolute ethanol. Agarose gel electrophoresis of neat DNA solution revealed high molecular weight DNA in all cases. The concentration of the extracted DNA, estimated using a Nanodrop 2000 (ThermoScientific), was 15.6–23.3 ng/µl for the Pholcus phalangioides samples of DNA from prey and detritus (Xu et al. 2015). Pholcus webbing, though these weights likely include material will also permit genotyping of a range of nuclear markers such as microsatellites, AFLP etc., as shown by other studies on low-quality environmental DNA (Nystro¨m et al. 2012; Calvignac-Spencer et al. 2013; Blake et al. 2015; Thomsen and Willerslev 2015). Studies on a variety of web producing species should greatly benefit from this technique, producing species should greatly benefit from this technique, including work on DNA barcoding, ecological genetic surveys, and fine-resolution population connectivity.

This work demonstrates that large fragments of COI (710 bp) can be amplified from a range of spider webs, joining Xu et al. (2015) and Sint et al. (2015) in the recent push toward advancing Araneae conservation genetics. However, caution should be used when using universal primers for species surveys due to the potential mixture of species contained within the DNA extraction, ideally taxa-specific primers should be developed and used where possible. The large fragment of mtDNA suggests that this material will also permit genotyping of a range of nuclear markers such as microsatellites, AFLP etc., as shown by other studies on low-quality environmental DNA (Nyström et al. 2012; Calvignac-Spencer et al. 2013; Blake et al. 2015; Thomsen and Willerslev 2015). Studies on a variety of web producing species should greatly benefit from this technique, including work on DNA barcoding, ecological genetic surveys, and fine-resolution population connectivity.

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**Table 1** Details of novel primers used in the study

| Taxon and targeted gene | Primer name | Primer sequence |
|------------------------|-------------|-----------------|
| Psalmopoeus cambridgei COI | Psal-333F | 5’-GGGGCCGCGGTGAACCTATTA-3’ |
| Psalmopoeus cambridgei COI | Psal-530R | 5’-TACAGACCCAAACGGCG-3’ |
| Pholcus spp. COI | Phol-415F | 5’-GGGTTTTCTATGATTTCGC-3’ |
| Pholcus spp. COI | Phol-459F | 5’-GGCTTCTCTATTATAGGGGC-3’ |
| Pholcus spp. COI | Phol-633R | 5’-GTCAGTCAAAATGTTAATGC-3’ |
| Pholcus spp. COI | Phol-694R | 5’-CAGCCGTAATTAAAACAGACC-3’ |

The number in the ‘Psal’ primer names denotes the position from the 5’ end of a complete mitochondrial genome of Psalmopoeus phalangioides from GenBank (accession number JQ407804.1). ‘F’ and ‘R’ at the end of the primer name refer to whether the primer is a forward or reverse respectively.

**Fig. 1** PCR success from Pholcus phalangioides webbing DNA extracts on a 3.5% agarose TBE gel with HyperLadder 50 bp (Bioline). Lane 1: Phol-415F+Phol633R. Lane 2: Phol-459F+Phol633R. Lanes 3, 4: Folmer primers on two different samples of P. phalangioides webbing. The 300 and 700 bp ladder markers are labelled.
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