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TECHNICAL NOTE

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. obvs*.). *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 (Winnepenninckx 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.

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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′-GGGGCCGGGTGAACTATTA-3′
Psalmopoeus cambridgei COI	Psal-530R	5′-TACAGACCACAAACGCG-3′
Pholcus spp. COI	Phol-415F	5′-GGGGTTTCTATGGATTTTGC-3′
Pholcus spp. COI	Phol-459F	5′-GGCTTCTTCTATTATAGGGGC-3′
Pholcus spp. COI	Phol-633R	5′-GTCAGTCAACAATATGGTAATAGC-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 *Psalmopoeus cambridgei* CO1 sequence from GenBank (accession number JQ412455.1), whilst in the 'Phol' primers this denotes the position from the 5′ end of a complete mitochondrial genome of *Pholcus 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

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, eluted pellets were air dried and resuspended in 100 µl of ddH_2O. 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 *Psalmopoeus* webbing, and 1.1–7.4 ng/µL for the *Pholcus* webbing, though these weights likely include DNA from prey and detritus (Xu et al. 2015).

Polymerase chain reaction (PCR) was used to amplify fragments of the cytochrome oxidase 1 (COI) gene in both species. Species-specific primers were designed for *Psalmopoeus cambridgei*, whilst within-genus primers were designed for *Pholcus phalangioides* (Table 1). The widely used universal invertebrate COI Folmer primers (Folmer et al. 1994) were also tested for both species. PCRs were performed in 20 µl volumes consisting of 10 µl of Biomix (Bioline), 1 µl of each primer (10 µM), 3 µl of DNA diluted from stock to 1/50, and 5 µl of ddH_2O. Thermocycler conditions for all reactions were: 95 °C/3 min, 45 × (95 °C/30 s, 50 °C (45 °C for the Folmer primers)/45 s72 °C/45 s), 72 °C/3 min.

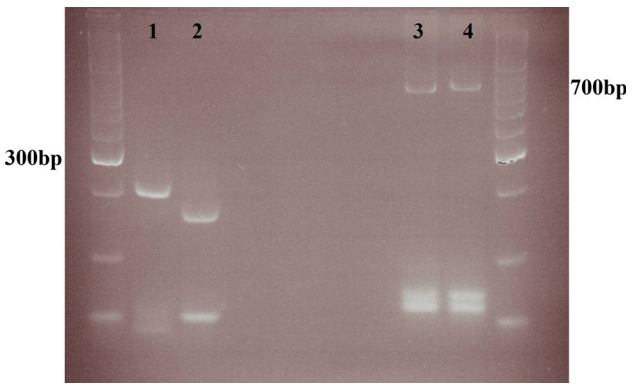

Fig. 1 PCR success from *Pholcus phalangioides* webbing DNA extracts on a 3.5 % agarose TBE gel with HyperLadder 50 bp (Bioline). *Lane 1*: Phol-415F+Phol633R. *Lane 2*: Phol-459F+Phol633R. *Lanes 3*, *4*: Folmer primers on two different samples of *P. phalangioides* webbing. The 300 and 700 bp ladder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

Most PCRs produced amplicons of the expected sizes, with the largest amplicon being generated by the Folmer primers (710 bp) (Fig. 1), though this reaction failed for the *Psalmopheus* material. Only the smallest *Pholcus phalangioides* sample failed consistently to produce amplicons. Sequencing of amplicons was performed using AB BigDye technology. BLASTn confirmed species identify for both *Psalmopoeus cambridgei* and *Pholcus phalangioides*, demonstrating amplification of the target region and species.

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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