Detecting incognito winter fungi: *Cladosporium* sp. in money spiders (Linyphiidae) and other arthropod-associated ascomycetes

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BBSRC Doctoral Training Partnerships

Research Questions

- What ascomycetes and other fungi reside in arthropods over the winter?
- What methods can be used to detect and identify symptomless fungal infections in arthropods?

SpiderLab Nottingham

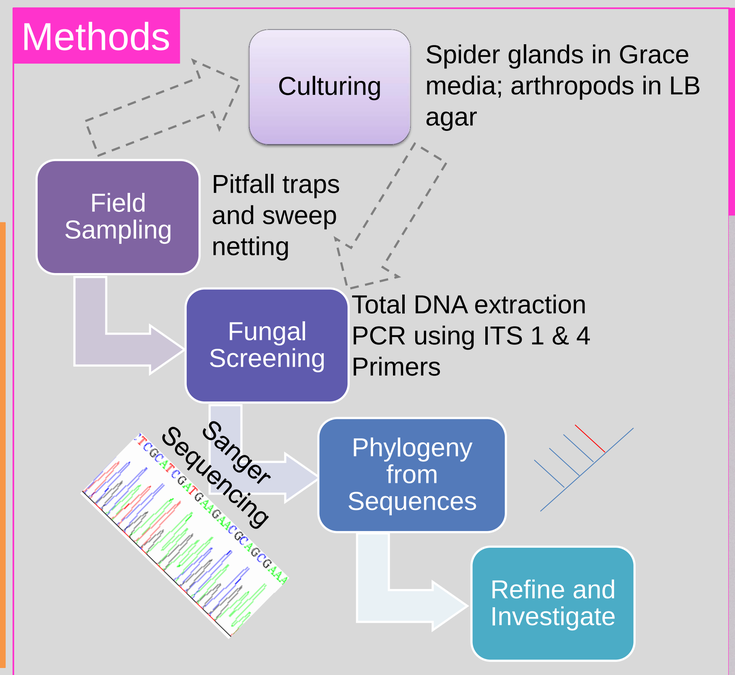
Rationale

Insect- and other arthropod-associated ascomycete fungi are well-studied in some respects, particularly the entomopathogens – which hold promising potential for biological control methods [1,2]. However, both the life cycles and taxonomic diversity of this guild of fungi remain poorly understood, particularly regarding the variation of their nutritional strategies [3]. This is an issue of great interest when one considers research in recent years relating to the effects of simultaneous endophytic and entomopathogenic fungi (EEPFs) on herbivore-crop systems [1,2]; evolutionarily nimble host-switching within taxa such as the Hypocreales (Sordariomycetes) [4]; and the enormous diversity of foliar endophytes found in plants [5]. Assessing the fungal diversity within arthropods such as herbivores and their predators can lead to agriculturally-important discoveries.

Spiders such as this Linyphiid (*Linyphia triangularis*) are important predators of herbivorous insects. Additionally, the ballooning behaviour of these spiders creates implications for the spread of hosted microbes such as fungi.

Selected References: 1. Posada & Vega (2005); 2. Vidal & Jaber (2015); 3. Meyling & Eilenberg (2007); 4. Vega *et al.* (2009); 5. Zhang *et al.* (2013); 6. Sunnocks & Hales (1996); 7. Aljanabi & Martinez (1997); 8. White *et al.* (1990); 9. Ownley *et al.* (2008); 10. Carbone & Kohn (1999); 11. Schubert *et al.* (2007); 12. Hibbett *et al.* (2007); 13. Cochrane *et al.* (2016); 14. Eken & Hayat (2009); 15. Abdel-Baky & Abdel Salam (2003); 16. Hoepfer *et al.* (2012)

Methods



Spider gland culturing: spider glands were dissected out of the garden cross spider *Araneus diadematus* (Araneidae) [below left] under sterile conditions and cultured in Grace media (containing 10% foetal bovine serum, penicillin, streptomycin, amphotericin B solution, and tetracycline) at 37ºC. Fungal hyphae grew out of the aciniform gland after 4 days [below right, before fungal growth].

Arthropod samples: samples from pitfall traps were embedded in LB agar and incubated at 37ºC; subsequently DNA was extracted from growing fungi. Other pitfall arthropod samples and sweep net-caught samples were directly treated to total DNA extraction [6,7].

PCR primers: Interspersed Transcribed Repeat (ITS) sequence primers have been extensively used in mycology [8,9] to both identify taxa and build the fungal tree of life in the molecular age across both the Ascomycota and Basidiomycota.

ITS1(F) 5' CTTGGTCATTTAGAGGAAGTAA 3' and
ITS5(R) 5' TCCTCCGCTTATTGATATGC 3' primers were used to identify fungi where possible to genus or family level.

To clarify species identity in some cases, primers for actin, translation elongation factor 1, and calmodulin (after Carbone & Kohn 1999 [10,11]) were used.

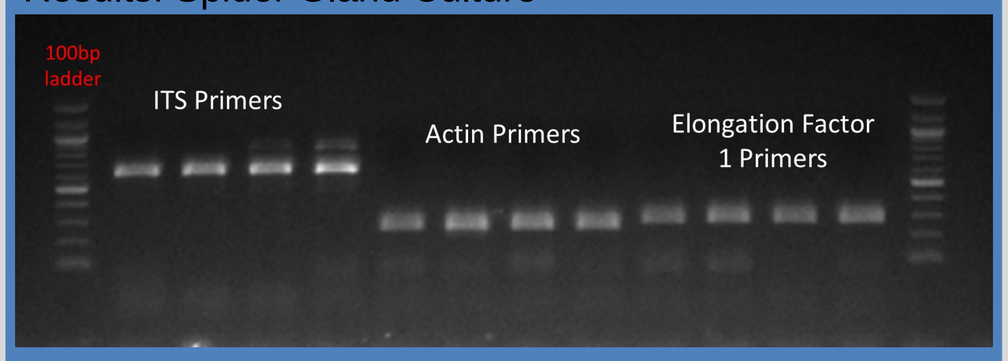
ACT-512(F)M 5' ATGTGCAAGGCCGGTTTC 3' **ACT-783(R)M** 5' TACGAGTCCTTCTGGCCCAT 3'
EF1-728(F)M 5' CATCGAGAAGTTCGAGAAG 3' **EF1-986(R)** 5' TACTTGAAGGAACCCTTACC 3'
CAL-228(F)M 5' GAGTTCAAGGAGGCCTTCTC 3' **CAL-737(R)** 5' CATCTTTCTGGCCATCATGG 3'

Discussion

There are clearly a range of methods for detecting symptomless fungal infections of arthropods; as well as many gaps in our understanding of the diversity of fungi living in these systems, the roles they play, and the evolutionary consequences of their presence. With regards to the cladosporin gene cluster's existence in the spider *Cladosporium*, PCR results indicate that some sequences are present, but there is a possibility that these are in a very different arrangement to those of *C. cladosporioides*, especially given the proximity of a hypothetical transposase-containing protein.

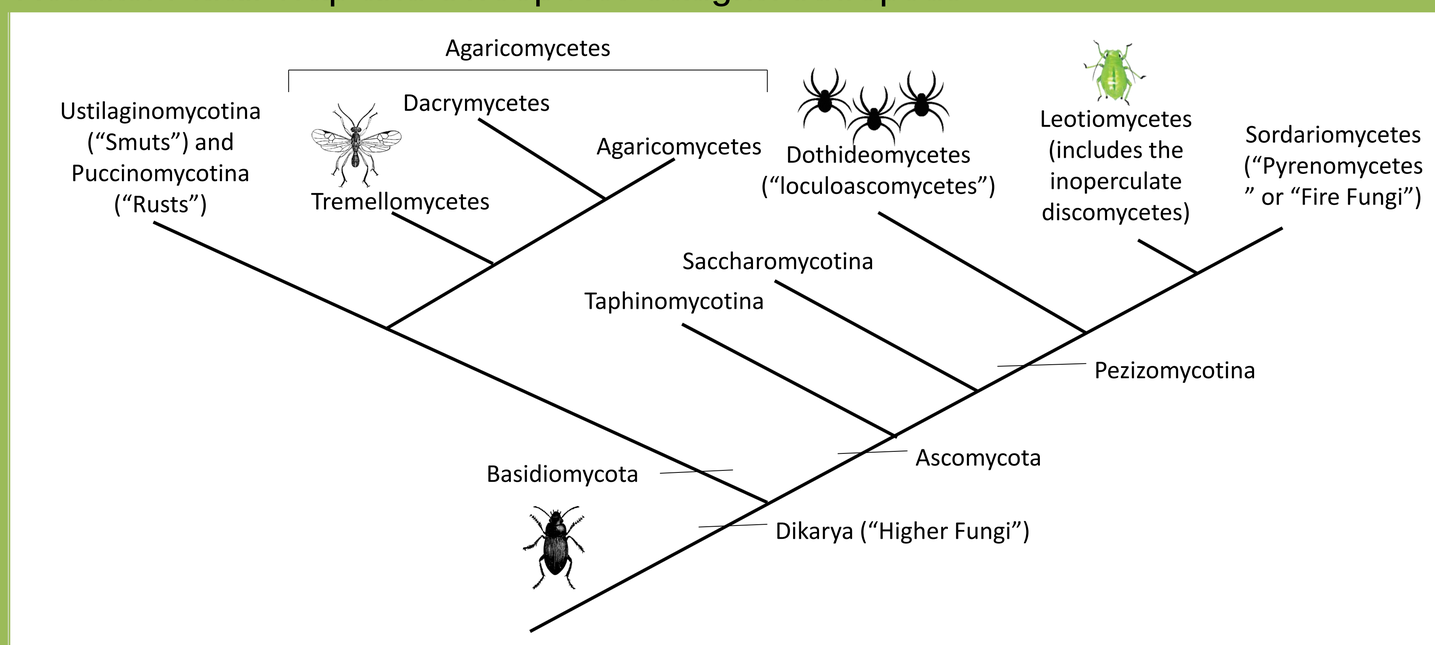
Further research: a thorough clarification of the species identity of the money spider *Cladosporium* sp., using more specific primers, and a survey of the distribution of this fungi spatially and with respect to spider taxa is required. Experiments to test the role of this fungus in spider ecology would require a practical method for curing and transfection.

Results: Spider Gland Culture



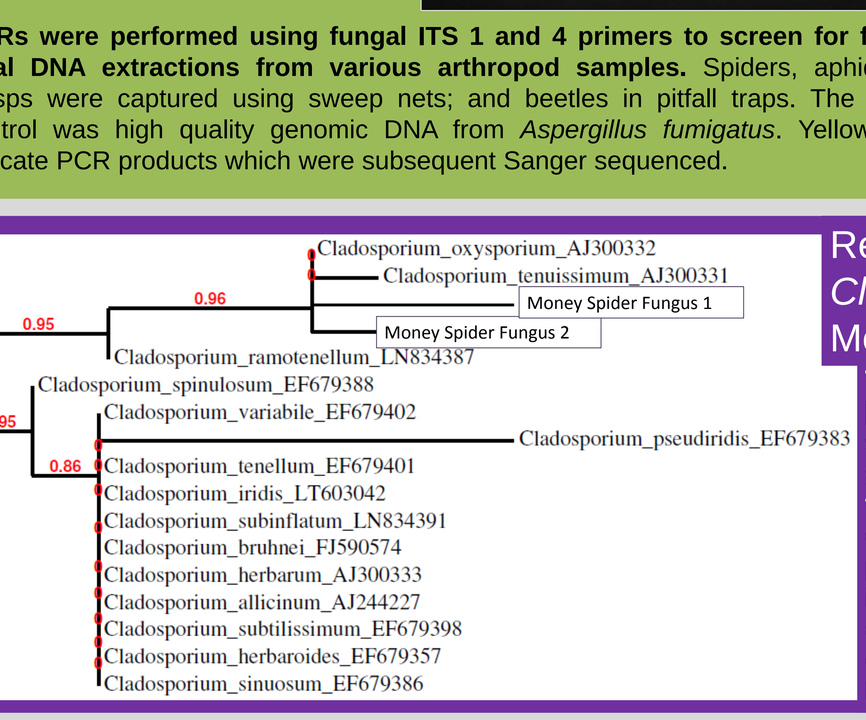
PCRs confirmed the presence of fungal growth from the aciniform gland in Grace media culture. The gel shows four separate DNA extractions each with sequences amplified using ITS, actin, and translation elongation factor 1 primers.

Results: Pitfall Trap and Sweep Net-Caught Arthropods



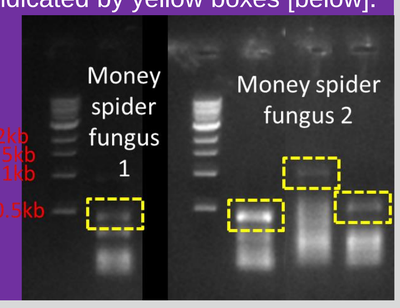
Cladogram depicting fungi detected in arthropod samples with respect to modern fungal phylogeny [12]. Fungi detected: *Cryptococcus* relative (Tremellales, Tremellomycetes); *Mortiella* sp. (Mortiellales, Mucoromycotina); *Cladosporium* spp. (Davidiellaceae, Capnodiales, Dothideomycetes); *Calloria* and *Hymenoscyphus* relative (Helotiales, Leotiomycetes) in wasp, ground beetle, money spider, and aphid samples respectively.

PCRs were performed using fungal ITS 1 and 4 primers to screen for fungi in total DNA extractions from various arthropod samples. Spiders, aphids, and wasps were captured using sweep nets; and beetles in pitfall traps. The positive control was high quality genomic DNA from *Aspergillus fumigatus*. Yellow boxes indicate PCR products which were subsequent Sanger sequenced.



Cladosporin synthesis gene cluster in *C. cladosporioides* described by Cochrane *et al.* (2016) [13]. *Cla1*: hypothetical transposase-containing protein; *Cla2*: HRPKS; *Cla3*: NRPKS; *Cla4*: lysyl-tRNA synthase; *Cla5*: hypothetical ANK-containing protein; *Cla6*: putative glycoside hydrolase.

[Above]: Primers designed for PCR attempts to amplify orthologues of the *C. cladosporioides* genes in money spider *Cladosporium*; [and below]: bands of expected size. primers involved are highlighted in red [above], and bands were cut out of the gel as indicated by yellow boxes [below].



Results and refinement: *Cladosporium* sp. in Money Spiders

The assembled Phylogeny [left], based solely on ITS sequence data, confirms the genus of the fungi detected in the money spiders to be *Cladosporium*, but does not provide species identity; and indeed it is broadly but not totally consistent with the accepted *Cladosporium* genus phylogeny based on multiple loci [11].

***Cladosporium* Importance:** the genus contains species of agricultural importance including *C. fulvum* (tomato leaf mould) and other plant pathogens. *C. cladosporioides* is one of the few species known to infect arthropods such as spider mites [14]; and *C. uredinicola* was shown to act as a natural biological control agent against aphids [15].

Cladosporin: *C. cladosporioides* produces the lysyl tRNA synthase-inhibitor cladosporin which has selective antimalarial properties [16], and potential for use as an antibiotic given a minimal effect on human protein synthesis [16].

The cladosporin synthesis gene cluster [above] contains highly-reducing and non-reducing polyketide synthase-encoding genes, as well as the lysyl tRNA-synthase gene for the fungus, on which resistance in conferred by the identity of two amino acid residues in the active site of the protein [13].

Photo credits. Linyphiid image: David Nicholls (naturespot.org.uk); garden cross spider image: wikipedia commons website; aciniform gland: Liaque Latif.