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Spider webs as reservoirs of culturable fungal diversity: evidence from orb-weaving *Cyclosa mulmeinensis* spider in Thai rice agroecosystems

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Abstract

Spider webs are increasingly recognized as passive environmental collectors; however, fungi remain among the least explored biological components associated with spider silk, particularly when examined using culture-based and taxonomically resolved approaches. In this study, we present a proof-of-concept investigation of culturable fungal diversity associated with two-dimensional, debris-decorated orb webs constructed by the orb-weaving spider *Cyclosa mulmeinensis* in rice agroecosystems in Thailand. Using a standardized field-to-laboratory isolation workflow combined with genus-appropriate multilocus phylogenetic analyses, decorated orb webs were sampled from three provinces, and fungi were isolated via dilution plating on potato dextrose agar supplemented with chloramphenicol. A total of 112 fungal isolates were recovered, grouped into 45 colony morphotypes and resolved into 23 taxa across six genera: *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium* , *Penicillium*, and *Talaromyces*. Taxonomic placement was inferred primarily from multilocus phylogenetic analyses, with morphological characteristics used as supporting evidence. Notably, Several isolates formed well-supported lineages within *Cladosporium* and *Talaromyces* that could not be assigned to any described species, indicating the presence of potentially undescribed taxa. These findings demonstrate that spider webs can serve as a low-impact, non-destructive substrate for accessing viable fungal diversity in agricultural ecosystems. This approach enables repeatable culture-based recovery of taxonomically informative fungal lineages and highlights the potential of spider web sampling as a complementary tool for biodiversity assessment and environmental monitoring.

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Keywords

non-destructive sampling, multilocus phylogeny, debris-decorated webs, Ascomycota, spider-fungal interactions, DNA barcoding, checklist

Introduction

Spider silk is a protein-based biomaterial expressed in diverse structural forms and web architectures. At the molecular level, spider silk is composed primarily of spidroin proteins, and its fibre surface coated with glycoproteins and lipids that contribute to adhesion and hygroscopicity (Kelly et al. 2022, Opell and Stellwagen 2019, Römer and Scheibel 2014). These physicochemical properties allow webs to intercept, immobilize, and retain airborne particles and droplets, including dust, water-borne particulates, and biological materials. Consequently, spider webs have attracted increasing interest as low-cost passive sampling matrices for environmental monitoring. A synthesis of 33 experimental studies indicates that webs can capture a broad spectrum of environmental materials, but research effort has been unevenly distributed across target particle types and analytical approaches, with some biological components remaining comparatively understudied. As summarized in Fig. 1, heavy metals were the most frequently investigated materials, being assessed in all 33 studies, followed by vertebrate-derived traces (13 studies), organic micropollutants (12 studies), and particulate matter (nine studies). In contrast, biological components have been examined far less often. Only five studies assessed invertebrate traces, four reported bacteria, whereas fungi were reported in only three studies, a frequency comparable to that of microplastics (e.g., Del Fiol et al. 2007, Hubelova et al. 2021, Newton et al. 2024, Gregorič et al. 2022; Suppl. material 1) This comparative overview reveals a marked imbalance in research effort, with fungi among the least studied categories of materials associated with spider webs. Given the ecological importance of fungi and their ubiquity in aerial and surface-associated environments, their occurrence on spider webs remains poorly characterized—particularly using culture-based approaches coupled with taxonomically robust identification.

Fungi play fundamental roles in terrestrial ecosystems as decomposers and key drivers of nutrient cycling, thereby supporting plant productivity and shaping microbial communities (Bahram and Netherway 2021, Corbu et al. 2023, Lofgren and Stajich 2021). Although, more than 120,000 fungal species have been described, this represents only a small proportion of the global fungal diversity estimated to exist (Hawksworth and Lücking 2017, Hyde 2022, Hyde et al. 2020). Fungal propagules are abundant and widely dispersed across in air, soil, vegetation, and built environments, making them readily available for interception by exposed natural substrates, including spider webs (Wu et al. 2019, Vašutová et al. 2021). Despite this, direct evidence for microbial communities associated with spider-web matrices remains limited. Existing work in a small number of systems suggests that spider-produced structures can function as

microhabitats: for example, studies of the social spider *Stegodyphus dumicola* show that its three-dimensional nests—enriched with accumulated organic materials—harbor diverse bacterial and fungal assemblages (Nazipi et al. 2021, Lammers et al. 2022). In orb-weaving spiders, metabarcoding and spore-based investigations of *Araneus diadematus* webs have detected fungi predominantly from Ascomycota and Basidiomycota, with genera such as *Alternaria*, *Cladosporium*, and *Fusarium* frequently reported (Gregorič et al. 2022, Del Fiol et al. 2007). Beyond molecular detection, emerging experimental evidence indicates that webs can also function as biologically active microbial microhabitats. For example, bacteria isolated from orb webs were shown to interact with spider silk and increase its mechanical extensibility, indicating that at least some web-associated microorganisms may be active residents rather than solely passively trapped particles (Tsiareshyna et al. 2024). Nevertheless, most existing studies have relied on DNA-based detection or observational approaches, which limits recovery of viable fungal isolates and, consequently, constrains species-level taxonomic resolution as well as downstream functional interpretation.

The orb-weaving spiders, *Cyclosa mulmeinensis* (Thorell, 1887) (Araneae: Araneidae) represents a suitable model for investigating culturable fungi associated with spider webs. This species is widely distributed across South, East, and Southeast Asia, with confirmed records from India, Bangladesh, Myanmar, China, Taiwan, Japan, Singapore, and Thailand (Tanikawa 1992, World Spider Catalog 2026 2026). *Cyclosa mulmeinensis* is a small orb-weaver (adult female body length <6 mm) that commonly occurs in lowland and human-modified habitats, including agricultural landscapes, forest edges, mangrove forests, secondary vegetation, and exposed shorelines or riverbanks (Barrion and Litsinger 1995, Liao et al. 2009, Tseng and Tso 2009). Adult females construct two-dimensional orb webs typically less than 200 mm in diameter (Liao et al. 2009). A distinctive feature of *C. mulmeinensis* is the linear debris decoration (“trashline”) positioned along a single radial thread of the web (Fig. 2A). This debris line comprises discrete clumps of organic materials, including plant fragments, insect remains, exuviae, and other intercepted environmental particles (Blamires and Tso 2013, Chou et al. 2005; Fig. 2B). Such debris decorations may increase particle retention on the web and could therefore enhance the capture of microbial propagules, making decorated orb webs a promising matrix for culture-based recovery of viable fungi. The occurrence and composition of web decorations vary with developmental stage. Juvenile and subadult individuals may construct either decorated or undecorated webs, and when decorations are present, they are typically smaller and lack egg sacs (Fig. 2C). In contrast, adult females commonly incorporate egg sacs into the debris line, forming a conspicuous vertical structure extending from the hub toward the upper frame of the web, although some adults may also construct undecorated webs (Liao et al. 2009, Tseng and Tso 2009). Experimental and field studies further show that debris decorations contribute to camouflage, prey attraction, predator deterrence, and web persistence, thereby enhancing foraging efficiency and survival (Chou et al. 2005, Blamires and Tso 2013, Ma et al. 2020).

Despite accumulating evidence that spider webs can intercept a wide range of environmental particles, their role as substrates harboring viable fungi remains poorly characterized. Studies reporting fungi on spider webs have largely relied on DNA-based detection or spore-based observations, which provides limited access to living isolates and therefore constrains species-level taxonomic resolution and downstream functional interpretation. In addition, most investigations have focused on temperate systems, leaving tropical agroecosystems—where fungal diversity and airborne propagule loads are expected to be high—comparatively understudied. From a microbiological perspective, the orb webs of *C. mulmeinensis* represent a particularly promising yet understudied system: the combination of exposed silk surfaces and accumulated organic debris creates a heterogeneous microenvironment with the potential to intercept and retain fungal propagules present in the surrounding environment. Importantly, this study does not attempt to infer the environmental origin of web-associated fungi or to partition potential source pools (e.g. air, vegetation, or soil). Instead, we adopt an exploratory, culture-based approach to document the occurrence, observed richness, and taxonomic composition of culturable fungi associated with *C. mulmeinensis* orb webs. Using a standardized field-to-laboratory workflow, isolates were organized into colony morphotypes and identified using genus-appropriate multilocus phylogenetic analyses. This study provides a baseline characterization of viable fungal assemblages associated with *C. mulmeinensis* webs and establishes a reproducible framework for future ecological, taxonomic, and biomonitoring applications using spider silk. By expanding fungal sampling beyond conventional substrates (soil, plants, and air), spider-web-based isolation can complement biodiversity inventories in human-modified landscapes and support scalable monitoring of fungal diversity in agroecosystems.

Materials and Methods

Collection of *Cyclosa mulmeinensis* spider webs

Orb webs of adult female *Cyclosa mulmeinensis* were collected from trees and surrounding vegetation along rice-field embankments at three locations in Thailand. Because *C. mulmeinensis* constructs two-dimensional orb webs suspended between vegetation, intact webs were collected directly from the air without contact with surrounding surfaces using sterile 90 mm Petri dishes. Each dish was gently positioned to enclose a single web without direct hand contact with the silk, immediately sealed with parafilm, placed in a zip-lock plastic bag, and transported to the laboratory in an icebox. Upon arrival, samples were stored at 4 °C until further processing. Spider web sampling was conducted during two independent periods, each with distinct objectives and sampling designs.

First sampling: characterization of culturable fungal richness and composition

The first sampling was conducted in December 2022 to document the observed richness and taxonomic composition of culturable fungi associated with *C. mulmeinensis* orb webs across contrasting geographic and landscape contexts. Owing to logistical constraints associated with the collection, transport, and processing of intact spider webs, one debris-decorated orb web bearing egg sacs was collected per location, resulting in a total of three webs for this sampling. Sampling sites were located at: Ko Wai Sub-district, Pak Phli District, Nakhon Nayok Province (NN; 14°10'20.0"N, 101°17'48.0"E); Ban Pathum Sub-district, Sam Khok District, Pathum Thani Province (PT; 14°04'50.7"N, 100°34'58.6"E); and Ton Mamuang Sub-district, Mueang Phetchaburi District, Phetchaburi Province (PB; 13°04'15.7"N, 99°57'27.0"E) (Fig. 3). NN and PT are situated within the same broad geographic region but differ in surrounding habitat characteristics, whereas NN and PB differ geographically while exhibiting broadly comparable surrounding habitat characteristics. PT and PB differ in both geographic region and surrounding habitat characteristics. These contrasts were incorporated solely to provide ecological context and were not intended for formal statistical comparison. Because sampling was conducted on different dates, storage duration at 4 °C prior to processing differed among locations (NN: 22 days; PB: 13 days; PT: 12 days). To minimize handling-related and web-type variability, all webs collected during the first sampling were standardized to a single web category, namely debris-decorated orb webs bearing egg sacs, and were processed simultaneously for fungal isolation under identical laboratory conditions. As the first sampling was designed as an exploratory assessment of culturable fungal occurrence and taxonomic composition recovered from spider webs, a single web was analyzed per location. Accordingly, reported fungal richness and composition represent web-level observations rather than population-level estimates.

Second sampling: exploratory comparison of debris-decorated webs with egg sacs and debris-decorated webs without egg sacs

The second sampling was exploratory in nature and intended solely for qualitative assessment of fungal colony recovery between two different characteristics of orb webs: debris-decorated webs with egg sacs and debris-decorated webs without egg sacs. No quantitative analysis, species-richness estimation, taxonomic inference, or statistical comparison was undertaken based on this sampling. Sampling was conducted in January 2025 at Ko Wai Sub-district, Pak Phli District, Nakhon Nayok Province (NN). A total of three webs were collected: two samples of debris-decorated webs with egg sacs, which were processed independently, and one sample of debris-decorated web without egg sacs. Webs from the second sampling were stored at 4 °C for approximately 12 h prior to processing. Data derived from this sampling were used exclusively for qualitative observation of fungal colony abundance and density. While storage duration differed among sites, all webs were processed using identical media, dilution series, incubation

conditions, and morphotype-to-phylogeny workflows, and results are interpreted as web-level observations rather than population-level estimates.

Fungal isolation from spider webs

Web samples consisting of spider silk, either with or without visible debris decorations, were processed using a modified dilution–spread plate method. Materials retained on the web surface, including macroscopic debris in decorated webs and microscopic particulate matter in undecorated webs, were gently rinsed with 200 µL of sterile 0.9% sodium chloride (NaCl) solution to generate a suspension, hereafter referred to as the *web-associated particle suspension*. Serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were prepared, and 100 µL aliquots from each dilution were aseptically spread onto potato dextrose agar (PDA; Difco, USA), with three replicate plates per dilution. Chloramphenicol was added to the medium at a final concentration of 250 mg L^{-1} to suppress bacterial growth. Plates were incubated at room temperature (25–28 °C) for three days and were monitored daily for colony emergence. All morphologically distinct fungal colonies were identified based on colony color, texture, and growth form, subsequently isolated, and subcultured onto fresh PDA plates and incubated for an additional seven days prior to downstream analyses.

DNA extraction and PCR amplification

Genomic DNA was extracted from all fungal isolates obtained from the first sampling. Single-colony isolates were cultivated on potato dextrose agar (PDA) at 25–28 °C for two weeks to obtain sufficient mycelial biomass. Mycelial biomass was harvested using a sterile spatula and transferred into 1.5 mL microcentrifuge tubes. DNA extraction was performed using the cetyltrimethylammonium bromide (CTAB) method of Doyle and Doyle (1987), with modifications described by Mongkolsamrit et al. (2020). PCR amplifications were performed in 25 µL reaction volumes. The internal transcribed spacer (ITS) region was amplified and sequenced for all isolates. Additional loci (β-tubulin (*BenA*), Calmodulin (*CaM*), Translation elongation factor 1-alpha (*TEF1-α*), Actin (*act*) and RNA polymerase II second largest subunit (*RPB2*)) were selectively amplified for isolates requiring higher taxonomic resolution, following established genus-specific phylogenetic frameworks. Primer sets and PCR cycling conditions are summarized in Suppl. material 2 , and full primer sequences and amplification profiles are provided in Suppl. material 3 . PCR products were examined by electrophoresis on 1.5% agarose gels, purified, and sequenced commercially by Macrogen Inc. (Seoul, Republic of Korea). Sequence chromatograms were assembled and trimmed using BioEdit v.7.1.3 (Hall 1999). All newly generated sequences were deposited in GenBank, and accession numbers together with isolate metadata are provided in Suppl. material 4.

Sequence alignment and phylogenetic analyses

Consensus nucleotide sequences were compared against the NCBI database using BLASTn to identify closely related taxa. For each genus, reference datasets were

assembled by combining sequences generated in this study with sequences from type or representative strains retrieved from published studies (e.g., Bensch et al. 2010, Sandoval-Denis et al. 2016, Yilmaz et al. 2016, Visagie et al. 2024, Houbraken et al. 2020, Nguyen et al. 2021). Multiple sequence alignments were generated using MAFFT v.7 with default parameters and manually inspected and edited in BioEdit v.7.1.3 (Hall 1999). Phylogenetic analyses were conducted using maximum likelihood (ML) inference implemented in IQ-TREE v.2.2.0 (Minh et al. 2020). Best-fit nucleotide substitution models were selected automatically by IQ-TREE for each dataset, and branch support was assessed using ultrafast bootstrap analysis with 1,000 replicates (Kalyaanamoorthy et al. 2017). Bayesian inference (BI) analyses were performed using MrBayes v.3.2.7 (Ronquist et al. 2012) with 10 million Markov chain Monte Carlo (MCMC) generations, sampling every 1,000 generations. Resulting phylogenetic trees were visualized using FigTree v.1.4.4 (Rambaut 2018) and finalized using graphic editing software. Taxonomic identification of isolates was inferred based on multilocus phylogenetic evidence. Final taxonomic assignments at the species level, species-complex level, or as unresolved lineages (sp.) are provided in Suppl. material 4.

Selection of representative isolates for morphological documentation

Following molecular identification, one representative isolate from each colony morphotype (n = 45) was selected for morphological documentation. Representative isolates correspond to the colony morphotypes and taxonomic assignments summarized in Suppl. material 4. Macroscopic colony features were examined after seven days of incubation on PDA using a stereomicroscope (Olympus SZ-PT, Tokyo, Japan). Microscopic structures were examined using a light microscope (Olympus CX31; Olympus Corporation, Japan). Diagnostic morphological traits, including conidiophores, conidia, phialides, and conidial arrangement, were documented following standard mycological references (Samson et al. 2010, Yilmaz et al. 2014, Bensch et al. 2010). Permanent microscopic slides were prepared for selected morphotypes. Morphological characters were used only to support descriptive interpretation and were not employed as primary evidence for species delimitation. All representative isolates were deposited in the culture collection of the Department of Biotechnology, Faculty of Science and Technology, Thammasat University, Khlong Luang, Pathum Thani, Thailand, and assigned accession codes prefixed with “BP”.

Literature survey and data synthesis

To contextualize the ecological relevance of spider webs as substrates for fungal isolation, a literature-based data synthesis was conducted. Published experimental studies investigating materials trapped by spider webs were retrieved using Google Scholar, employing combinations of keywords including *“spider web”, “environmental monitoring”, “indicator”, “eDNA”,* and *“metabarcoding”* in title and abstract searches. Only experimental studies reporting empirical detection of materials retained on spider webs were included; purely theoretical, review, or non-empirical studies were excluded. Materials reported in these studies were categorized into major groups, including heavy

metals, vertebrate-derived traces, organic micropollutants, particulate matter, invertebrate traces, bacteria, fungi, and microplastics. The compiled dataset was cleaned, organized, and summarized using RStudio (RStudio 2020). A figure was generated to illustrate comparative patterns among material categories and to highlight research gaps, particularly the limited number of studies reporting fungi associated with spider webs. This literature-based synthesis was conducted prior to data analysis and was intended to provide a structured contextual framework for interpreting the ecological relevance of fungal isolation from spider webs.

Climatic data sources and contextual analysis

Long-term climatic data were used to provide environmental context for interpreting spatial patterns in culturable fungal species richness and composition among sampling locations. Secondary climatic data, including mean annual temperature and relative humidity, were obtained from the Meteorological Department of Thailand for each province corresponding to the sampling sites. Data covering a 10-year period (2014–2023) prior to sample collection were compiled to represent prevailing regional climatic conditions (Suppl. material 12). These climatic data were not subjected to statistical analysis. Instead, they were incorporated descriptively to support ecological interpretation in the Discussion section, particularly with respect to potential influences of temperature and humidity on fungal occurrence and distribution.

Results

Fungal isolation and overview of taxonomic diversity

A total of 112 fungal isolates were recovered from the orb webs of *Cyclosa mulmeinensis* collected from three rice agroecosystems in Thailand. All isolates originated from debris-decorated webs bearing egg sacs collected during the first sampling. Isolates were preliminarily grouped into 45 colony morphotypes based on macroscopic colony characteristics to facilitate downstream analyses. All quantitative summaries presented below are based on isolates recovered from a single debris-decorated web per location during the first sampling and therefore represent web-level observations rather than population-level estimates. Details of isolate codes, collection sites, taxonomic identification, and GenBank accession numbers for sequenced loci are summarized in Suppl. material 4. Species identification was inferred primarily from multilocus phylogenetic analyses, with isolate codes explicitly linked to phylogenetic placement (Table 1; Suppl. materials 5, 6, 7, 8, 9, 10). In total, 23 fungal taxa were recovered, representing six genera and five families, including *Alternaria* (Pleosporaceae), *Aspergillus* and *Penicillium* (Aspergillaceae), *Cladosporium* (Cladosporiaceae), *Fusarium* (Nectriaceae), and *Talaromyces* (Trichocomaceae).

Molecular identification and phylogenetic placement of fungal taxa

Taxonomic assignments presented below are based exclusively on multilocus phylogenetic analyses.Corresponding isolate codes, sampling localities, and GenBank accession numbers are summarized in Suppl. material 4

Aspergillus, *Penicillium* and *Talaromyces*

Phylogenetic analyses based on concatenated ITS, *BenA*, and *CaM* datasets resolved five isolates of *Aspergillus* belonging to section *Nigri* (Suppl. material 5) including *A. aculeatinus* (three isolates: BP1-011, BP1-013, BP1-052), *A. brunneoviolaceus* (one isolate: BP1-004), and *A. niger* (one isolate: BP26-021). Within *Penicillium*, six isolates were resolved into four species based on multilocus phylogenetic analyses across sections *Citrina*, *Charlesia*, and *Lanata-divaricata* (Suppl. material 9). These included *Penicillium coffeae* (one isolate: BP1-003), *P. citrinum* (three isolates: BP29-001, BP26-029, BP26-064), *P. steckii* (one isolate: BP26-040), and *P. oxalicum* (one isolate: BP1-015). Five isolates of *Talaromyces* were resolved into two described species and one putative novel lineage within section *Talaromyces* (Suppl. material 10). The described taxa included *Talaromyces fusiformis* (one isolate: BP1-051) and *T. alveolaris* (one isolate: BP1-057). Three isolates (BP1-048, BP1-055, BP1-058) formed a distinct and well-supported clade and were treated here as *Talaromyces* sp. 1.

Cladosporium

Multilocus phylogenetic analyses of *Cladosporium* isolates based on concatenated ITS, *act*, and *TEF-1α* sequence datasets resolved 26 isolates into a total of 10 species, comprising seven described species and three undescribed lineages, distributed across the *Cladosporium cladosporioides* and *C. sphaerospermum* species complexes (Suppl. materials 6, 7). Within the *C. cladosporioides* species complex, four described species were recovered: *Cladosporium angulosum* (BP26-039), *C. lagenariiforme* (BP29-012), *C. perangustum* (BP2-010), and *C. xanthochromaticum* (BP2-012, BP29-005, BP29-011, BP29-015). In addition, two well-supported undescribed lineages were detected within this complex and are treated here as *Cladosporium* sp. 1 (BP1-002, BP1-012, BP1-054, BP2-001, BP26-025, BP26-037, BP26-047, BP29-013) and *Cladosporium* sp. 2 (BP26-032). Within the *C. sphaerospermum* species complex, three described species were identified: *Cladosporium dominicanum* (BP26-068, BP29-004), *C. fusiforme* (BP2-013), and *C. velox* (BP29-008). One additional isolate formed a distinct lineage within this complex and is treated here as *Cladosporium* sp. 3 (BP26-065).

Fusarium and *Alternaria*

A single isolate of *Fusarium pernambucanum* (BP26-033) was confidently resolved based on multilocus phylogenetic analysis of concatenated *CaM*, *TEF-1α*, and *RPB2* sequence datasets (Suppl. material 8).

One isolate of *Alternaria* (BP1-007) was identified to the genus level based on ITS sequence similarity, showing 100% identity in BLAST searches. Species-level identification was not pursued, as robust species delimitation within *Alternaria* requires multilocus phylogenetic analyses beyond ITS alone, typically including loci such as *GAPDH*, *TEF-1α*, and *RPB2*, together with additional diagnostic characters, in accordance with current taxonomic practice.

Descriptive comparison of fungal assemblages among sampling locations

The proportional composition of culturable fungal taxa differed among the three sampling locations (Fig. 4). The web collected from Nakhon Nayok Province exhibited the highest observed taxonomic richness, with 12 taxa representing five genera, followed by Phetchaburi Province with nine taxa from four genera, and Pathum Thani Province with six taxa from two genera. *Cladosporium* was the most species-rich genus at all locations.

Notably, the relative contribution of site-specific versus shared taxa varied among locations, with the Nakhon Nayok web being dominated by location-specific taxa, whereas the Pathum Thani web showed a comparatively greater representation of taxa shared among sites. Venn diagram visualization further illustrated both shared and location-specific taxa among the three webs (Fig. 5), indicating partial overlap in culturable fungal assemblages while also highlighting web-specific components of fungal diversity.

Exploratory comparison of fungal recovery from debris-decorated webs with and without egg sacs

In a second, exploratory sampling conducted at Nakhon Nayok Province, fungal isolation outcomes were qualitatively assessed between debris-decorated webs with egg sacs and debris-decorated webs without egg sacs. Debris-decorated webs bearing egg sacs consistently yielded higher numbers and denser growth of culturable fungal colonies than debris-decorated webs without egg sacs (Suppl. material 11). This comparison was qualitative in nature and was not intended for species richness estimation, taxonomic inference, or statistical analysis, but rather to document observable differences in fungal recovery associated with contrasting web structures.

Discussion

Culturable fungi associated with *Cyclosa mulmeinensis* webs

This study provides a culture-based baseline of fungal biodiversity recovered from spider webs in tropical rice agroecosystems, demonstrating that spider silk is an underutilized substrate for isolating viable, taxonomically informative fungi. Using a standardized isolation workflow combined with multilocus phylogenetic analyses, we demonstrated

that spider webs can harbor a diverse assemblage of viable, culturable fungi. From three independently collected webs (one web per site), we recovered 112 isolates, which were grouped into 45 colony morphotypes and resolved into 23 fungal taxa spanning six genera: *Alternaria*, *Aspergillus*, *Penicillium*, *Talaromyces*, *Cladosporium*, and *Fusarium*.

Previous studies documenting fungi associated with spider webs have largely relied on spore observations or molecular detection approaches, such as metabarcoding or environmental DNA analyses (Del Fiol et al. 2007, Gregorič et al. 2022). While these methods are powerful for detecting taxonomic presence, they provide limited insight into fungal viability and restrict opportunities for downstream functional or taxonomic investigation. By contrast, the culture-based approach employed here demonstrates that a substantial fraction of fungi intercepted by spider webs remains viable and can be isolated, identified, and preserved for future study. This finding expands the conceptual role of spider webs from passive collectors of biological signals to substrates capable of retaining living fungal propagules. From a biodiversity and conservation perspective, non-destructive substrates such as spider webs offer a practical way to repeatedly sample fungal propagules without disturbing soil structure or plant tissues. This is particularly relevant in agroecosystems, where land-use intensity, pesticide regimes, and surrounding habitat mosaics can shape airborne and surface-associated fungal pools, making standardized, low-impact sampling valuable for long-term biodiversity monitoring.

Taxonomic composition and dominance of *Cladosporium*

Among the recovered taxa, *Cladosporium* was the most species-rich and frequently isolated genus across all sampling locations. Multilocus phylogenetic analyses resolved isolates into multiple species within the *C. cladosporioides* and *C. sphaerospermum* species complexes, including *C. angulosum*, *C. dominicanum*, *C. lagenariiforme*, *C. perangustum*, *C. velox*, and *C. xanthochromaticum*, as well as several unresolved lineages. The presence of multiple unresolved lineages within intensively studied genera underscores the capacity of spider-web-based sampling to access cryptic fungal diversity that may be underrepresented in conventional substrates such as soil or plant material.

The predominance of *Cladosporium* is ecologically consistent with its status as one of the most abundant and ubiquitous genera in outdoor air and on exposed substrates worldwide (Zalar et al. 2007, Bensch et al. 2015, Lee et al. 2023, Sandoval-Denis et al. 2016). Members of this genus are commonly recovered from plant surfaces, soil, insects, and aquatic habitats, and their frequent isolation from *C. mulmeinensis* webs supports the interpretation that spider silk functions as an efficient interceptor of airborne fungal propagules rather than as a selective growth substrate.

Recovery of Eurotialean fungi and implications for fungal discovery

In addition to *Cladosporium*, several taxa belonging to the Eurotiales, *Aspergillus*, *Penicillium*, and *Talaromyces*, were recovered and resolved using genus-appropriate multilocus phylogenetic frameworks (Houbraken et al. 2020, Visagie et al. 2024, Yilmaz

et al. 2014). These genera are well known for their ecological versatility and prevalence in the air, soil, and plant-associated environments, making their recovery from spider webs unsurprising. Notably, several isolates within *Talaromyces* and *Cladosporium* formed well-supported phylogenetic lineages that could not be confidently assigned to described species. This pattern suggests that spider webs may provide access to fungal diversity that remains underrepresented in conventional sampling efforts. Similar conclusions have been drawn from spider web–based metabarcoding studies, which revealed unexpectedly broad assemblages of organisms originating from surrounding habitats (Gregorič et al. 2022) The recovery of potentially undescribed taxa in the present study highlights the value of spider webs as complementary substrates for fungal biodiversity exploration.

Influence of web structure and organic debris

The qualitative comparison between debris-decorated webs with egg sacs and debris-decorated webs without egg sacs is presented only as a proof-of-concept observation and should not be interpreted as evidence of functional differences in microbial accumulation. A qualitative comparison between debris-decorated webs with egg sacs and debris-decorated webs without egg sacs suggested that decorated webs yield higher numbers and denser growth of culturable fungi. Although this comparison was exploratory and not intended for statistical inference, it is ecologically plausible that accumulated organic debris—such as plant fragments, prey remains, and soil particles—enhances the retention of fungal propagules and provides microhabitats conducive to fungal persistence. Debris decorations in *Cyclosa* webs are known to influence prey interception, camouflage, predator deterrence, and web persistence (Chou et al. 2005, Blamires et al. 2010, Ma et al. 2020). Our observations extend these functional interpretations by suggesting that debris accumulation may also increase the capacity of webs to intercept and retain microorganisms. Comparable patterns have been reported from silk-based structures of social spiders, where accumulated organic materials support diverse microbial communities, including fungi (Nazipi et al. 2021, Lammers et al. 2022).

Environmental context and spatial variation

Differences in culturable fungal richness and composition were observed among sampling locations. Although no formal statistical analyses were conducted, long-term climatic data and landscape context provide useful ecological background for interpreting these patterns (Suppl. material 12). Temperature, relative humidity, and wind are well-established drivers of fungal sporulation and aerial dispersal (Jones and Harrison 2004), and long-term variation in these factors may influence the pool of fungal propagules available for interception by spider webs. The Nakhon Nayok and Phetchaburi sites are situated within rural, vegetation-rich landscapes adjacent to mountainous terrain, environments known to support diverse plant-associated and soil-borne fungal communities (Bissett et al. 2011, Tedersoo et al. 2014). In contrast, the Pathum Thani site lies within a more urbanized and industrialized matrix, where land-use intensification has

been associated with altered or reduced fungal diversity in other systems (McGuire et al. 2015, Prober et al. 2015). Importantly, this study was not designed to identify environmental sources of fungi recovered from spider webs, and interpretations regarding environmental influence remain inferential rather than demonstrative.

Methodological implications and future directions

A central contribution of this study is the demonstration of a reproducible, culture-based workflow for isolating fungi from spider webs and resolving their taxonomy using multilocus phylogenetic analyses. This approach complements existing molecular detection methods by enabling the recovery of living isolates suitable for physiological, ecological, and taxonomic research. Future studies incorporating seasonal replication, multiple spider species with contrasting web architectures, and parallel sampling of surrounding air, vegetation, and soil will be essential to disentangle the relative contributions of web structure, habitat, and climate to fungal assemblages intercepted by spider silk. By establishing spider webs as viable substrates for fungal isolation, the present study provides a foundation for integrating arachnological and mycological perspectives in biodiversity assessment and environmental monitoring.

Conclusions

This study demonstrates that the orb webs of *Cyclosa mulmeinensis* can harbor diverse and viable assemblages of culturable fungi in tropical rice agroecosystems. By applying a standardized, culture-based isolation workflow combined with multilocus phylogenetic analyses, we recovered 112 fungal isolates from three independently collected webs, resolving them into 23 taxa across six genera, with *Cladosporium* representing the most species-rich group. Although the study was not designed to identify the environmental sources of these fungi, descriptive differences in taxonomic richness and composition among sampling locations indicate that spider webs can reflect locally available pools of culturable fungal propagules. Importantly, several isolates could not be confidently assigned to described species, highlighting spider webs as underexplored substrates with potential value for fungal discovery. The recovery of multiple unresolved lineages within well-studied genera such as *Cladosporium* and *Talaromyces* highlights the potential of spider-web-based sampling to reveal cryptic or under-sampled fungal diversity that may be overlooked by conventional substrates. By establishing a reproducible workflow for isolating and identifying living fungi from spider silk, this study advances the methodological integration of mycology and arachnology. Spider webs emerge as accessible, low-cost, and complementary substrates for fungal biodiversity assessment, with promising applications in ecological research, taxonomy, and environmental biomonitoring. More broadly, spider-web sampling can complement fungal biodiversity inventories and provide a scalable, low-cost approach for tracking changes in fungal assemblages across agricultural landscapes and management regimes.

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Integration of molecular and morphological data for species delimitation and biogeography in Argiope versicolor species complex 2nd year

Hosting institution

Thammasat Unversity

Ethics and security

The authors followed the ethical guidelines for the use of animals in scientific research in Thailand. However, official institutional ethical approval was not required for this study as the primary focus was on fungal isolation from non-living materials (spider webs). Adult spiders were only photographed and identified in their natural habitats without collection or invasive procedures. Furthermore, the egg sacs used for fungal sampling were at an early developmental stage prior to organogenesis. The authors also declare that no specific field permits were required because the sampling locations were not within

protected areas, and the study did not involve any endangered or protected species. All laboratory procedures were conducted following standard biosafety protocols at the National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand.

Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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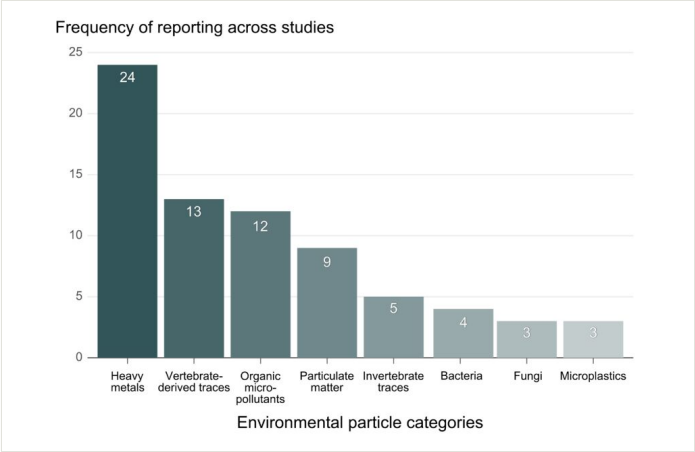

Figure 1.

Reported categories of environmental particles detected on spider webs across published studies. Data were compiled from 33 peer-reviewed articles retrieved via Google Scholar using predefined search queries. Bars represent the number of studies in which each particle category was reported at least once. A single study may contribute to multiple categories if more than one type of material was investigated or detected. The figure illustrates relative research attention across material types rather than absolute abundance or experimental frequency.

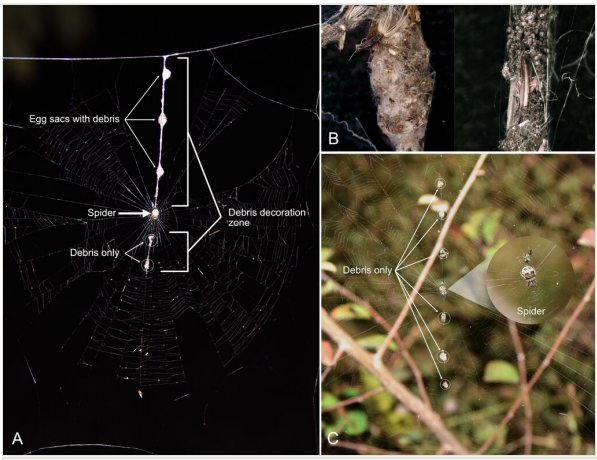

Figure 2.

Representative orb webs of *Cyclosa mulmeinensis* illustrating web architecture and debris decoration. **A** Debris-decorated orb web with egg sacs constructed by an adult female, showing a characteristic linear debris line with egg sacs extending along a single radial thread. **B** Close-up view of the debris line, demonstrating aggregated organic materials, including plant fragments and insect remains, covering an egg sac (left) and debris only (right) incorporated into the decoration. **C** Decorated orb web with a debris line lacking egg sacs, shown for structural comparison with (A). White circles denote debris fragments present along the debris line of the web.

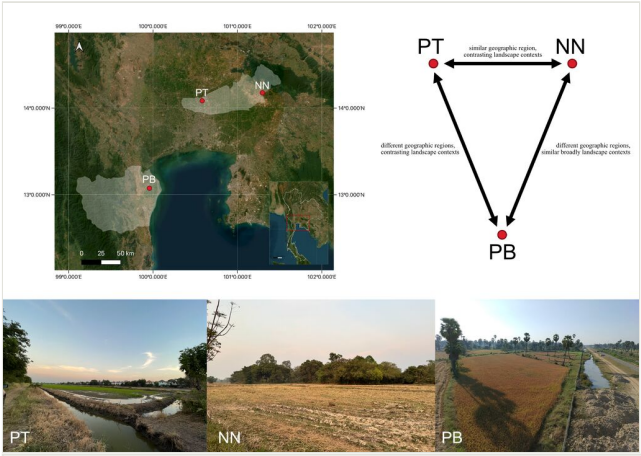

Figure 3.

Locations of paddy fields where *Cyclosa mulmeinensis* spider webs were collected in Thailand. Sampling sites represent different regional and landscape contexts: NN (Nakhon Nayok Province), PT (Pathum Thani Province), and PB (Phetchaburi Province). NN and PT are located within a similar geographic region but differ in surrounding landscape contexts. NN and PB differ geographically but are both embedded in predominantly rural landscapes with relatively close proximity to forested areas, whereas PT is situated in a more urbanized and industrialized landscape.

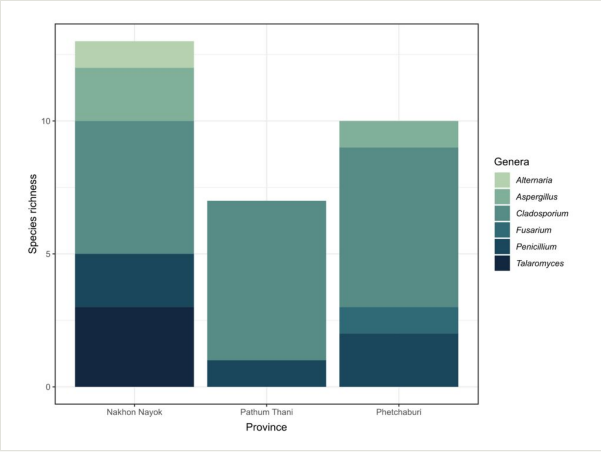

Figure 4.

Proportional composition of culturable fungal species richness among genera across sampling locations.

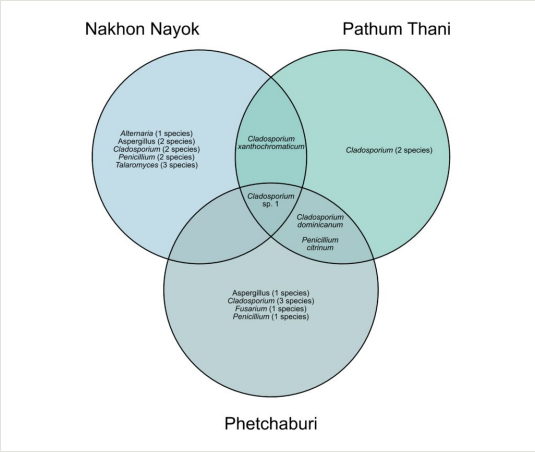

Figure 5.

Venn diagrams illustrating shared and site-specific culturable fungal taxa recovered from *Cyclosa mulmeinensis* spider webs collected from trees adjacent to rice fields in three provinces of Thailand (Nakhon Nayok, Pathum Thani, and Phetchaburi).

Table 1.

Details of fungal isolates obtained from *Cyclosa mulmeinensis* spider webs in Thailand including, taxonomic identification and isolated abundance. Taxonomic assignments are based on multilocus phylogenetic analyses and are reported at the species level, species complex level, or as unresolved lineages (sp.), depending on phylogenetic resolution

Family	Genus	Identified species	Series/Section/ Complex	Isolate abundance	Clade ID
Pleosporaceae	*Alternaria*	*Alternaria longissima*	-	1	-
Trichocomaceae	*Aspergillus*	*Aspergillus aculeatinus*	Japonici/Nigri	3	Suppl. material 5
	Aspergillus	*Aspergillus brunneoviolaceus*		1	
	Aspergillus	*Aspergillus niger*		1	
Cladosporiaceae	*Cladosporium*	Unidentified *Cladosporium* sp.	-	5	-
	Cladosporium	*Cladosporium angulosum*	Species complex cladosporioides	1	Suppl. material 6
	Cladosporium	*Cladosporium lagenariiforme*		1	
	Cladosporium	*Cladosporium perangustum*		1	
	Cladosporium	*Cladosporium* sp. 1		8	
	Cladosporium	*Cladosporium* sp. 2		1	
	Cladosporium	*Cladosporium xanthochromaticum*		4	
	Cladosporium	*Cladosporium dominicanum*	Species complex sphaerospermum	2	Suppl. material 7
	Cladosporium	*Cladosporium fusiforme*		1	
	Cladosporium	*Cladosporium* sp. 3		1	
	Cladosporium	*Cladosporium velox*		1	
Nectriaceae	*Fusarium*	*Fusarium pernambucanum*	FIESC	1	Suppl. material 8
Trichocomaceae	*Penicillium*	*Pencillium coffeae*	Phoenicea/Charlesia	1	Suppl. material 9
	Penicillium	*Penicillium citrinum*	Citrina/Citrina	4	
	Penicillium	*Penicillium steckii*		1	

	Penicillium	*Penicillium oxalicum*	Oxalica/Lanata-Divaricata	1	
Trichocomaceae	*Talaromyces*	*Talaromyces alveolaris*	Talaromyces	1	Suppl. material 10
	Talaromyces	*Talaromyces fusiformis*		1	
	Talaromyces	*Talaromyces* sp.1		3	

Supplementary materials

Suppl. material 1: Summary of published studies on spider webs as biological materials (n = 33), including spider species, web type, materials detected, and study approach (experimental or observational)

Authors: Thanakron Into
Data type: Table
Download file (179.52 kb)

Suppl. material 2: Molecular loci, primers, and PCR conditions used for multilocus phylogenetic identification of fungal isolates recovered from spider webs

Authors: Thanakron Into
Data type: Table
Download file (19.71 kb)

Suppl. material 3: Primer sequences and detailed PCR conditions used for amplification of fungal loci

Authors: Thanakron Into
Data type: Table
Download file (20.06 kb)

Suppl. material 4: Details of fungal isolates obtained from *Cyclosa mulmeinensis* spider webs in Thailand, including isolate codes, collection provinces, taxonomic identification, and GenBank accession numbers for sequenced loci (ITS, *BenA, CaM, act, TEF1-α and RPB2*)

Authors: Thanakron Into
Data type: Table
Brief description: Taxonomic assignments are based on multilocus phylogenetic analyses and are reported at the species level, species complex level, or as unresolved lineages (sp.), depending on phylogenetic resolution.
Download file (107.79 kb)

Suppl. material 5: Maximum Likelihood phylogenetic tree constructed using RAxML, representing *Aspergillus* section *Nigri* based on the combination of ITS, *BenA* and *CaM* sequence datasets

Authors: Thanakron Into
Data type: Image
Brief description: The RAxML Bootstrap support values (MLBS ≥ 70%) and the Bayesian inference posterior probabilities (BIPP ≥ 0.95) were shown as MLBS/BIPP with *Aspergillus candidus* CBS 566.65^{T} as the outgroup. Ex-type, ex-epitype, and ex-neotype strains were indicated by T, ET and NT, respectively. Strains isolated in this study were indicated in bold.
Download file (596.79 kb)

Suppl. material 6: Maximum Likelihood phylogenetic tree constructed using RAxML, representing *Cladosporium cladosporioides* species complex based on the combination of ITS, *Tef-1α* and *act* sequence datasets

Authors: Thanakron Into
Data type: Image
Brief description: The phylogenetic tree utilized *Cladosporium herbarum* CBS 121621^{T} as the outgroup for tree rooting. The RAxML Bootstrap support values (MLBS ≥ 70%) and the Bayesian inference posterior probabilities (BIPP ≥ 0.95) were shown as MLBS/BIPP. Ex-type, ex-epitype, and ex-neotype strains were indicated by T, ET and NT, respectively. Strains isolated in this study were indicated in bold.
Download file (12.32 MB)

Suppl. material 7: Maximum Likelihood phylogenetic tree constructed using RAxML, representing *Cladosporium sphaerospermum* species complex based on the combination of ITS, *Tef-1α* and *act* sequence datasets

Authors: Thanakron Into
Data type: Image
Brief description: The phylogenetic tree utilized *Cladosporium herbarum* CBS 121621^{T} as the outgroup for tree rooting. The RAxML Bootstrap support values (MLBS ≥ 70%) and the Bayesian inference posterior probabilities (BIPP ≥ 0.95) were shown as MLBS/BIPP. Ex-type, ex-epitype, and ex-neotype strains were indicated by T, ET and NT, respectively. Strains isolated in this study were indicated in bold.
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Suppl. material 8: Maximum Likelihood phylogenetic tree constructed using RAxML, representing *Fusarium incaarnatum-equiseti* species complex (FIESC) based on the combination of *CaM*, *RPB2* and *TEF-1*α sequence datasets

Authors: Thanakron Into
Data type: Image
Brief description: The RAxML Bootstrap support values (MLBS ≥ 70%) and the Bayesian inference posterior probabilities (BIPP ≥ 0.95) were shown as MLBS/BIPP with *Fusarium camtoceras* CBS 193.65 and *Fusarium neosemitectum* CBS189.60^{T} as the outgroup. Ex-type, ex-epitype, and ex-neotype strains were indicated by T, ET and NT, respectively. Strains isolated in this study were indicated in bold
Download file (891.09 kb)

Suppl. material 9: Maximum Likelihood phylogenetic tree constructed using RAxML, representing *Penicillium* section *Citrina*, *Charlesia* and *Lanata-divaricata* based on the combination of ITS, *BenA* and *CaM* sequence datasets

Authors: Thanakron Into
Data type: Image
Brief description: The phylogenetic tree utilized *Penicillium chrysogenum* CBS 306.48^{T} as the outgroup for tree rooting. The RAxML Bootstrap support values (MLBS ≥ 70%) and the Bayesian inference posterior probabilities (BIPP ≥ 0.95) were shown as MLBS/BIPP. Ex-type, ex-epitype,

and ex-neotype strains were indicated by T, ET and NT, respectively. Strains isolated in this study were indicated in bold.
Download file (3.16 MB)

Suppl. material 10: Maximum Likelihood phylogenetic tree constructed using RAxML, representing *Talaromyces* section *Talaromyces* based on the combination of ITS, *BenA* and *CaM* sequence datasets

Authors: Thanakron Into
Data type: Image
Brief description: The phylogenetic tree utilized *Talaromyces helicus* CBS 335.48 ^{T} as the outgroup for tree rooting. The RAxML Bootstrap support values (MLBS ≥ 70%) and the Bayesian inference posterior probabilities (BIPP ≥ 0.95) were shown as MLBS/BIPP. Ex-type, ex-epitype, and ex-neotype strains were indicated by T, ET and NT, respectively. Strains isolated in this study were indicated in bold.
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Suppl. material 11: PDA plate cultures illustrating variation in fungal colony enumeration associated with spider web structure

Authors: Thanakron Into
Data type: Image
Download file (890.65 kb)

Suppl. material 12: Weather data spanning ten years (2014–2023) were obtained for the three study locations, including (A) temperature, (B) relative humidity and (C) wind speed

Authors: Thanakron Into
Data type: Image
Brief description: The data were retrieved from the Information courtesy of the Meteorological Department of Thailand
Download file (635.64 kb)