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Using Spiderwebs to Capture Airborne Environmental DNA (eDNA) to Detect Animal Biodiversity in an Urban Park

Environmental DNA (eDNA) is the genetic material shed by organisms in the environment. It has emerged as a powerful resource for assessing biodiversity, particularly in aquatic settings. The application of eDNA methods in terrestrial environments is less developed, with researchers still exploring collection methods of eDNA from the air. One novel approach recently trialed is the use of spiderwebs, a passive, natural material whose sticky silk threads can capture tiny airborne particles, including eDNA. Here, we investigated the use of spiderwebs for airborne eDNA collection as part of a broader survey of biodiversity at the urban Ascot Hills Park in Los Angeles, California. Six spiderwebs were sampled from five native California plant species within a forest restoration site using sterile toothpicks, then taken for genomic DNA extraction, amplification, and sequencing of the Cytochrome Oxidate I gene. Processed sequence reads were searched against a curated species database for taxonomic assignment, resulting in the detection of 30 species, ~60% which were undetected in parallel visual surveys. The findings highlight the potential for spiderweb-based eDNA sampling as a complementary approach to traditional biodiversity monitoring techniques.

Keywords

Airborne eDNA, assessment, monitoring, terrestrial, species richness

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INTRODUCTION

Biodiversity is declining at an alarming rate due to anthropogenic global change (Schmeller et al. 2017; IPBES 2019). Effective monitoring through biodiversity surveys is vital for detecting changes in species composition and informing timely conservation actions (Moussy et al. 2022; Lindenmayer et al. 2012). It is therefore beneficial to have a range of survey techniques available to capture a more comprehensive picture of biodiversity. Traditional biodiversity sampling methods, such as roving surveys and pit traps, are well-vetted and relatively straightforward to implement, yet may also be intrusive, time-intensive, and limited in scope. On the other hand, direct observation surveys conducted during the day may miss crepuscular and nocturnal species (Levesque et al. 2023; Alvarez et al. 2025), and may lack species-level identification of small, quick-moving organisms, such as insects (Chua et al. 2023). Furthermore, identification of organisms to species level may require specialized taxonomic expertise that is not readily available (Schmeller et al. 2017), or not yet achievable through existing species identification tools such as iNaturalist (but see Mesaglio et al. 2025).

Environmental DNA (eDNA) metabarcoding is a complementary survey method to traditional tools. It is a powerful, non-invasive technique to detect species' presence from genetic material shed into the environment (Harrison et al. 2019). This technique has seen the broadest application in aquatic ecosystems, where water serves as a medium for capturing and transporting genetic material (Cowgill et al. 2025; Newton et al. 2025). Use for terrestrial environments is less developed, although sampling eDNA from soil has become increasingly common (Garrett et al. 2023). A promising, yet relatively unexplored application in terrestrial environments is the capture of airborne eDNA (Zhao & Andermann 2024).

One of the main difficulties in sampling airborne eDNA is identifying suitable substrates and techniques to obtain airborne genetic material (Newton et al. 2025). A potential method is using dust traps, as seen in Johnson et al. (2021), which showed that eDNA metabarcoding detected more plant species with less effort than traditional surveys. Several strategies have been explored on animal DNA, including leaf swabs, water vacuum, and fan vacuum sampling, with varying efficiency and usefulness (Lynggaard et al. 2022; Lynggaard et al. 2023). However, each method has certain limitations; for example, the water vacuum was loud and disturbed nearby animals (Lynggaard et al. 2022). Recently, spiderwebs have been identified as a potential passive filter to collect airborne eDNA. Spiderwebs are natural environmental traps, accumulating detritus, pollen, and microscopic organisms, which have led them to be previous targets for monitoring air pollutants and heavy metals (Rutkowski et al. 2018; Hose et al. 2002). The sticky silk threads of spiderwebs may also trap and retain DNA-bearing material, which may make them well suited for metabarcoding biodiversity research.

To our knowledge, Xu et al. (2015) was the first study to successfully recover DNA from spiderwebs, which was achieved in the laboratory under controlled conditions. Subsequent studies have expanded on this concept, demonstrating that spiderwebs can be a viable substrate to capture eDNA in various habitats, which can then be sequenced to detect a wide range of taxa, such as vertebrates and invertebrates. For example, Gregorič et al. (2022) examined the use of spiderwebs for biodiversity assessments, detecting bacteria, fungi, and animal species from forest habitats. Newton et al. (2024) also collected eDNA from spiderwebs in Australian forests and Perth Zoo

and isolated 32 vertebrate species from the wild and 61 species from the zoo environment, suggesting that the spatial proximity of animals significantly influences the ability of spiderwebs to capture eDNA. Kim & Kim (2024) recently explored spiderweb eDNA in agricultural contexts and concluded that it could be applied to identify both spider species and their prey, having possible implications for pest management practice.

In this study, we test the utility of eDNA from spiderwebs as a complementary biodiversity method in a recently established micro-forest at Ascot Hills Park, Los Angeles, California. eDNA was isolated from naturally occurring spiderwebs within the micro-forest, then sequenced in a molecular genetics lab to survey fauna biodiversity. Species detected and identified using the eDNA metabarcoding method were compared to concurrent traditional biodiversity survey data. We also described what taxonomic groups are detected most frequently in spiderweb eDNA and contrast the amount of eDNA recovered from spiderwebs collected across two different seasons.

METHODS

Sites

Ascot Hills Park (AHP; 34°04’14” N, 118°11’26” W) is a 93-acre urban open space located in northeast Los Angeles, California (Figure 1). For over a decade, local volunteer-driven habitat restoration efforts have been underway to improve the park’s ecological health (Siglar & Willette 2025). Over this time, AHP has also served as a canvas for scientific research on urban habitat restoration that includes field-based experiments (Mestetsky et al. 2024), valuation of ecosystem services (Wilson & Willette 2022), remote sensing (Siglar & Willette 2025), and survivorship of tree plantings by way of citizen science engagement (Willette et al. 2026). The latter study leveraged a new, densely planted Miyawaki microforest (Miyawaki 2004; Lewis 2022). Established in January 2024, this 10,000 sq ft (~930 sq m) plot included 850 1-gallon seedlings of 32 distinct native California plants ranging from canopy trees to perennial grasses. The microforest was manually watered five times between January and August 2024, and once in December 2024. The microforest is currently monitored on a bi-monthly basis for animal biodiversity using a combination of visual surveys, roving surveys, and insect pit traps, for which the 12-month inventory of observed species is used here for comparison to eDNA metabarcoding results (unpublished data). The Loyola Marymount University campus (LMU; 33°57’59” N, 118°25’27” W) is a 142-acre urban academic campus that served as a second field site to supplement sampling effort at AHP.

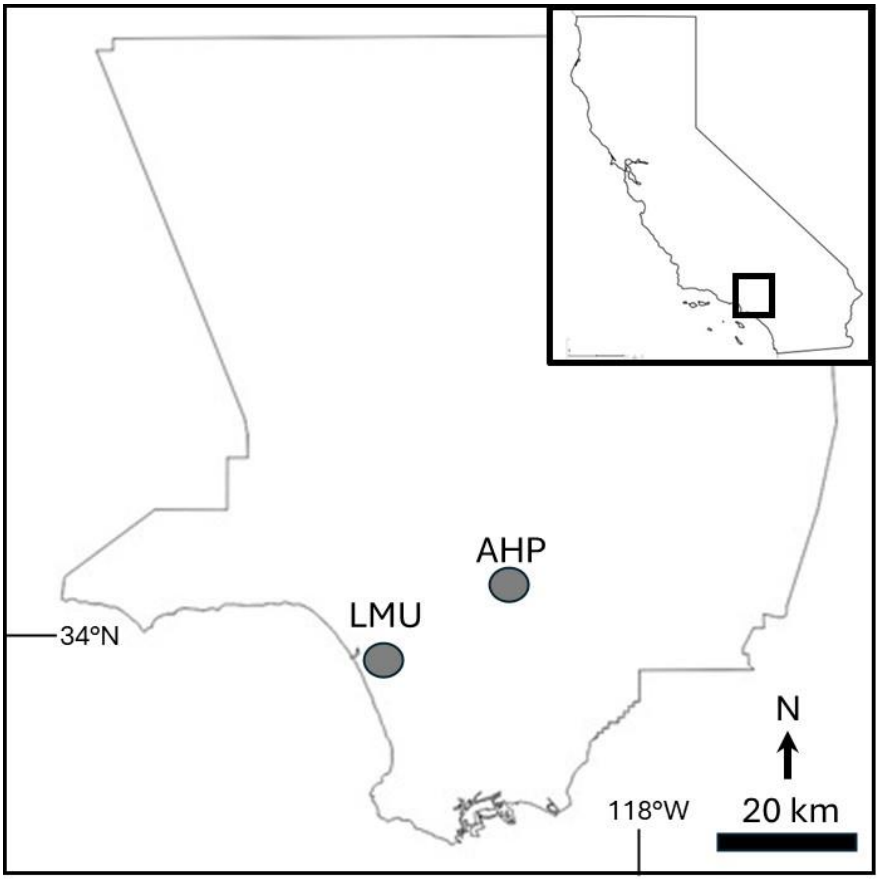

Figure 1 – Study site map showing the state of California (insert), with regional map of Los Angeles County, and two field sites of Ascot Hills Park (AHP) and Loyola Marymount University (LMU), both located within the city of Los Angeles, CA.

Field sampling

A total of four orb webs and two mesh webs were collected from the microforest in September 2024 from among the branches of five native California plants: coyote brush (*Baccharis pilularis*), elderberry (*Sambucus nigra caerulea*), coastal goldenbush (*Isocoma menziesii)*, California bush sunflower (*Encelia californica*), and coastal live oak (*Quercus agrifolia*). Six additional webs were collected in a similar fashion in February 2025, for a total of 12 for the AHP site. In the absence of a microforest, 12 spiderwebs were collected from an assortment of landscape shrubs from around the LMU campus. Each web was individually sampled using a sterile toothpick, moving the toothpick in a circular motion to concentrate the web at the tip of the toothpick. Effort was made to avoid sampling any debris, animals, or plant material caught in the web, and care was taken to coax any live spider onto the host plant. Each collected sample was transferred with the toothpick into a 2 mL tube containing 400 μL of biofluid & cell buffer and 40 μL of proteinase K (Zymo Research, San Diego, CA). Tubes were labeled and transferred to the lab for downstream processing.

DNA Extraction, Amplification, and Sequencing

Genomic DNA was extracted from each preserved sample using the Zymo Research Quick-DNA miniprep plus kit (San Diego, CA) following manufacturer's instructions for cell samples. DNA amplification and sequencing followed our lab's protocol as described in Willette and Joaquin (2026). Briefly, we amplified a 658-bp fragment of the mitochondrial COI gene using the HCO (Folmer et al. 1994) and IntF (Leray et al. 2013) primers with the addition of Oxford Nanopore Technology (Oxford, UK) (ONT)-specific FW (5'- TTT CTG TTG GTG CTG ATA TTG C -3') and RV (5'- ACT TGC CTG TCG CTC TAT CTT C -3') primer extensions. Each PCR cocktail included one Cytiva PuReTaq Ready-To-Go PCR bead (Marlborough, MA), 10 µl of water, 2.5 µl of each primer at 10 µM concentration, and 10 µl of gDNA. A first polymerase chain reaction (PCR) included a touchdown protocol of an initial denaturation step at 95°C for 3 minutes, then 17 cycles of denaturing at 95°C for 10 seconds, annealing at 62°C for 30 seconds, reduced by 1°C at each subsequent cycle, and an extension at 72°C for 1 minute. An additional 26 cycles followed with a denaturing step at 95°C for 10 seconds, annealing at 46°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C (see Willette & Joaquin 2026). Product was purified using AMPure XP beads (Agencourt, Beverly, MA), quantified using the Promega QuantFlor ONE dsDNA system (Madison, WI), both following manufacturer's instructions. At this point, only samples with at least 1.7 ng/µL of PCR product were carried through the rest of the library preparation step as follows. A second PCR added a unique ONT PCR barcode (ONT Expansion 1-12 kit) to each sample in a PCR cocktail of 2 µl of each barcode at 10 µM concentration, 100-200 *f* mol of the first PCR product in a total of 48 µl of water, and 50 µl of LongAmp Taq 2x master mix (New England BioLabs, Ipswich, MA). The second PCR protocol consisted of an initial denaturing step of 95°C for 3 minutes, followed by 12 cycles of denaturing at 95°C for 15 seconds, annealing at 62°C for 15 seconds, and extension at 65°C for 30 seconds, followed by a final extension of 65°C for 5 minutes. This second PCR was followed by purification, pooling all six sample products equimolarly to a total amount of 1 µg, and then taken through an enzymatic repair and end-prep step using the NEB Next FFPE DNA repair kit and Ultra II end-repair module (New England Biolabs, Ipswich, MA) following manufacturer's instructions. This resulting pooled DNA product was purified, taken through an adapter ligation using the ONT Ligation Sequence Kit v14 (Oxford Nanopore Technologies, Oxford, UK), sequenced on the ONT R10.4.1 flow cell with a final concentration between 5 and 50 *f* mol on the ONT MinION Mk1C device, and processed via the MinKNOW interface selecting a final quality score of Q7 for resulting sequence reads.

DNA Metabarcoding Analysis

Sequencing resulted in approximately 1 million reads per sample as FASTQ output files, which we processed using our lab's Python Flet-supported desktop application called the Genomic Analytics Tool (GNAT; available on GitHub, https://github.com/Intechgambit/giat). The GNAT application converts FASTQ files to FASTA files which were searched for closest species match using the National Center for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool (BLASTn) using the MegaBLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Within GNAT, we searched the FASTA files against a curated database of 140 species and genera (Supplemental Materials SM1) for taxonomic assignment. This database consisted of 58 species observed during complementary field surveys, with the other species of interest to the researchers

(*e.g.*, endangered species and other species reported from Los Angeles County). Sequences with a ≥98% percent identity match were summarized into descriptive statistics, and with taxa representing at least 1.0% of high-quality species-level matches reported. Post-bioinformatic output files are available upon request to the authors.

RESULTS AND DISCUSSION

Biodiversity Detection via Spiderweb eDNA

eDNA retrieved from the first six webs collected from AHP in September 2024 were the only samples with a sufficient concentration of PCR product (1.7 ng/µL or higher). None of the February 2025 AHP samples nor 12 January 2025 LMU samples had concentrations above 0.5 ng/µL and thus were not carried through the rest of the library prep. Notably, May 2024 to January 2025 was the second driest 7-month period in Los Angeles history, with no significant rainfall recorded over the duration of this study's field sampling. Spiderwebs sampled from all five types of native California plants and of the two web types did yield sufficient amounts of DNA to generate high quality sequences (Figure 2). The six September 2024 AHP samples yielded over 5.4 million COI gene region reads, with high-quality sequences detecting 30 unique species with an average 18.6 (SD±3.4) per sample (Table 1). Of these 30 species, one was of the Phylum Mollusca (Class Gastropoda; Order Vetigastropoda), four were Phylum Chordata (Class Mammalia; Order Chiroptera), and the remaining 25 were Phylum Arthropoda (Class Insecta; Orders Diptera, Lepidoptera, Hymenoptera, Coleoptera, Odonata, and Orthoptera). Although over 200 species were detected, including coyotes, hawks, lizards, and spiders, none of these detections exceeded the threshold of 1% of total reads per sample, suggesting traces of DNA from these taxa was very low on the sampled spiderwebs. The dominance of the insect taxa in the eDNA is consistent with the proximity and biological relevance of these organisms to spiderwebs, as insects are frequent prey of spiders (Nyffeler & Birkhofer 2017). It also suggests that web-based sampling may be particularly powerful for invertebrate detection. The identification of vertebrate species, although low in concentration, supports the notion that spiderwebs capture a diversity of eDNA, likely resulting from hair, skin cells, feather fragments, or fecal particles deposited into the air (Liu et al. 2025). The broad transport of airborne eDNA from distant sources and the variable persistence of eDNA in many environments are important considerations for using this metabarcoding approach for biodiversity surveys (Evans et al. 2017; Collins et al. 2018). Métris & Métris (2023) detected airborne eDNA more than 2,500 m from its source, suggesting that wind may carry eDNA far from its original location.

Table 1– List of detected species from spiderweb eDNA for each web sample, including common name, Phylum, and if species was observed in visual surveys. The percentage of sequence reads also reported per sample. Only species with at least 1% of detected high quality reads with 98% or higher match are reported; thus, sum percentage per sample may not equal 100%. The percentage of identified sequences (based on number of high-quality reads obtained after bioinformatic filtering) is influenced by amount of DNA in the sample, rate of DNA breakdown over time, and potential sampling bias. The higher the percentage, the more DNA was detected for a given species. In some situations, sequence count can be used as a crude proxy for species abundance or biomass.

Sample	Detected species	Common name	*Phylum*	HQ reads	% of sequences	In visual survey
1	*Bombus terrestris*	buff-tailed bumble bee or large earth bumble bee	*Arthropoda*	21,019	23.9	No
	Lasiurus blossevillii	southern red bat	*Chordate*	9,254	10.5	No
	Bombus californicus	California bumble bee	*Arthropoda*	7,451	8.5	Yes
	Sycophila novascotiae	black oak gall wasp	*Arthropoda*	5,909	6.7	No
	Tadarida brasiliensis	Mexican free-tailed bat	*Chordate*	2,357	2.7	No
	Bombus lucorum	white-tailed bumble bee	*Arthropoda*	1,894	2.2	No
	Allograpta obliqua	oblique streaktail	*Arthropoda*	1,822	2.1	No
	Myotis nigricans	black myotis	*Chordate*	1,545	1.8	No
	Bombus pensylvanicus	American bumble bee	*Arthropoda*	1,509	1.7	No
	Bombus pratorum	early bumble bee or early-nesting bumble bee	*Arthropoda*	1,397	1.6	No
	Aedes aegypti	yellow fever mosquito	*Arthropoda*	1,261	1.4	Yes
	Turbo castanea	chestnut turban	*Mollusca*	1,209	1.4	No
	Hyalophora cecropia	cecropia moth	*Arthropoda*	1,122	1.3	No
	Cecropterus toxeus	coyote cloudywing or coyote skipper	*Arthropoda*	1,031	1.2	No
	Sphaerophoria novaeangliae	black-striped globetail	*Arthropoda*	953	1.1	Yes
	Sphaerophoria cinctifacies	long hoverfly	*Arthropoda*	942	1.1	No
2	*Bombus terrestris*	buff-tailed bumble bee or large earth bumble bee	*Arthropoda*	61,548	26.2	No
	Bombus californicus	California bumble bee	*Arthropoda*	22,699	9.6	Yes
	Sycophila novascotiae	black oak gall wasp	*Arthropoda*	15,001	6.4	No
	Lasiurus blossevillii	southern red bat	*Chordate*	13,697	5.8	No

Table 1, *continued*

	Allograpta obliqua	oblique streaktail	*Arthropoda*	9,257	3.9	No
	Aedes aegypti	yellow fever mosquito	*Arthropoda*	7,683	3.3	Yes
	Tadarida brasiliensis	Mexican free-tailed bat	*Chordate*	5,810	2.5	No
	Sphaerophoria novaeangliae	black-striped globetail	*Arthropoda*	5,326	2.3	Yes
	Sphaerophoria cinctifacies	long hoverfly	*Arthropoda*	4,822	2.0	No
	Bombus pensylvanicus	American bumble bee	*Arthropoda*	4,750	2.0	No
	Bombus lucorum	white-tailed bumble bee	*Arthropoda*	4,058	1.7	No
	Bombus pratorum	early bumble bee or early-nesting bumble bee	*Arthropoda*	3,814	1.6	No
	Harmonia axyridis	multicoloured Asian lady beetle	*Arthropoda*	3,775	1.6	Yes
	Hyalophora cecropia	cecropia moth	*Arthropoda*	3,636	1.5	No
	Fazia fascifrons	N/A	*Arthropoda*	3,565	1.5	No
	Myotis nigricans	black myotis	*Chordate*	3,551	1.5	No
	Cecropterus toxeus	coyote cloudywing or coyote skipper	*Arthropoda*	3,428	1.5	No
	Pachydiplax longipennis	blue dasher	*Arthropoda*	3,317	1.4	Yes
	Terastia meticulosalis	erythrina twigborer or erythrina borer	*Arthropoda*	2,499	1.1	No
	Caenurgina erechtea	forage looper or common grass moth	*Arthropoda*	2,436	1.0	Yes
3	*Bombus terrestris*	buff-tailed bumble bee or large earth bumble bee	*Arthropoda*	26,904	24.4	No
	Bombus californicus	California bumble bee	*Arthropoda*	11,012	10.0	Yes
	Lasiurus blossevillii	southern red bat	*Chordate*	8,006	7.3	No
	Sycophila novascotiae	black oak gall wasp	*Arthropoda*	7,280	6.6	No
	Tadarida brasiliensis	Mexican free-tailed bat	*Chordate*	3,989	3.6	No
	Hyalophora cecropia	cecropia moth	*Arthropoda*	3,131	2.8	No
	Cecropterus toxeus	coyote cloudywing or coyote skipper	*Arthropoda*	2,618	2.4	No
	Bombus pensylvanicus	American bumble bee	*Arthropoda*	2,141	1.9	No
	Sphaerophoria cinctifacies	long hoverfly	*Arthropoda*	2,084	1.9	No
	Aedes aegypti	yellow fever mosquito	*Arthropoda*	2,005	1.8	Yes
	Ascalapha odorata	black witch moth	*Arthropoda*	1,786	1.6	Yes

Table 1, *continued*

	Sphaerophoria novaeangliae	black-striped globetail	*Arthropoda*	1,760	1.6	Yes
	Bombus lucorum	white-tailed bumble bee	*Arthropoda*	1,726	1.6	No
	Myotis nigricans	black myotis	*Chordate*	1,720	1.6	No
	Allograpta obliqua	oblique streaktail	*Arthropoda*	1,693	1.5	No
	Bombus pratorum	early bumble bee or early-nesting bumble bee	*Arthropoda*	1,676	1.5	No
	Caenurgina erechtea	forage looper or common grass moth	*Arthropoda*	1,624	1.5	Yes
	Fazia fascifrons	N/A	*Arthropoda*	1,573	1.4	No
	Terastia meticulosalis	erythrina twigborer or erythrina borer	*Arthropoda*	1,530	1.4	No
	Dione vanillae	Gulf fritillary or passion butterfly	*Arthropoda*	1,455	1.2	Yes
	Pachydiplax longipennis	blue dasher	*Arthropoda*	1,375	1.2	Yes
	Harmonia axyridis	multicoloured Asian lady beetle	*Arthropoda*	1,119	1.0	Yes
	Hyles lineata	white-lined sphinx or hummingbird moth	*Arthropoda*	1,080	1.0	No
4	*Bombus californicus*	California bumble bee	*Arthropoda*	11,907	15.0	Yes
	Sycophila novascotiae	N/A	*Arthropoda*	10,305	13.0	No
	Bombus terrestris	buff-tailed bumble bee or large earth bumble bee	*Arthropoda*	9,348	11.8	No
	Lasiurus blossevillii	southern red bat	*Chordate*	8,064	10.1	No
	Bombus hortorum	garden bumblebee	*Arthropoda*	8,006	10.1	No
	Aedes aegypti	yellow fever mosquito	*Arthropoda*	3,233	4.1	Yes
	Sphaerophoria novaeangliae	black-striped globetail	*Arthropoda*	2,427	3.1	Yes
	Pachydiplax longipennis	blue dasher	*Arthropoda*	1,586	2.0	Yes
	Myotis nigricans	black myotis	*Chordate*	1,566	2.0	No
	Ascalapha odorata	black witch moth	*Arthropoda*	1,200	1.5	Yes
	Hyles lineata	white-lined sphinx or hummingbird moth	*Arthropoda*	1,188	1.5	No
	Tadarida brasiliensis	Mexican free-tailed bat	*Chordate*	1,111	1.4	No
	Caenurgina erechtea	forage looper or common grass moth	*Arthropoda*	866	1.2	Yes
	Hedychridium roseum	N/A	*Arthropoda*	868	1.1	No

Table 1, *continued*

	Dione vanillae	Gulf fritillary or passion butterfly	*Arthropoda*	798	1.0	Yes
5	Sycophila novascotiae	N/A	*Arthropoda*	12,175	13.5	No
	Bombus californicus	California bumble bee	*Arthropoda*	9,393	10.4	Yes
	Bombus terrestris	buff-tailed bumble bee or large earth bumble bee	*Arthropoda*	9,348	10.4	No
	Bombus hortorum	garden bumblebee	*Arthropoda*	8,007	8.9	No
	Aedes aegypti	yellow fever mosquito	*Arthropoda*	6,886	7.6	Yes
	Lasiurus blossevillii	southern red bat	*Chordate*	3,580	4.0	No
	Sphaerophoria novaeangliae	black-striped globetail	*Arthropoda*	3,299	3.7	Yes
	Ascalapha odorata	black witch moth	*Arthropoda*	3,042	3.4	Yes
	Pachydiplax longipennis	blue dasher	*Arthropoda*	2,903	3.2	Yes
	Dione vanillae	Gulf fritillary or passion butterfly	*Arthropoda*	2,326	2.6	Yes
	Caenurgina erechtea	forage looper or common grass moth	*Arthropoda*	1,972	2.2	Yes
	Schistocerca nitens	vagrant grasshopper or gray bird grasshopper	*Arthropoda*	1,694	1.9	Yes
	Harmonia axyridis	multicoloured Asian lady beetle	*Arthropoda*	1,340	1.4	Yes
	Dasypterus xanthinus	western yellow bat	*Chordate*	1,061	1.2	No
	Allograpta obliqua	oblique streaktail	*Arthropoda*	1,056	1.2	No
	Bombus pensylvanicus	American bumble bee	*Arthropoda*	1,044	1.2	No
	Tadarida brasiliensis	Mexican free-tailed bat	*Chordate*	933	1.0	No
6	*Bombus terrestris*	buff-tailed bumble bee or large earth bumble bee	*Arthropoda*	23,164	17.9	No
	Bombus californicus	California bumble bee	*Arthropoda*	9,675	7.5	Yes
	Sycophila novascotiae	N/A	*Arthropoda*	8,293	6.4	No
	Lasiurus blossevillii	southern red bat	*Chordate*	8,141	6.3	No
	Bombus pascuorum	common carder bumble bee	*Arthropoda*	5,812	4.5	No
	Tadarida brasiliensis	Mexican free-tailed bat	*Chordate*	4,740	3.7	No
	Hyalophora cecropia	cecropia moth	*Arthropoda*	3,756	2.9	No
	Allograpta obliqua	oblique streaktail	*Arthropoda*	3,701	2.9	No

Table 1, *continued*

	Sphaerophoria cinctifacies	long hoverfly	*Arthropoda*	3,340	2.6	No
	Aedes aegypti	yellow fever mosquito	*Arthropoda*	2,831	2.2	Yes
	Cecropterus toxeus	coyote cloudywing or coyote skipper	*Arthropoda*	2,630	2.0	No
	Myotis nigricans	black myotis	*Chordate*	2,178	1.7	No
	Sphaerophoria novaeangliae	black-striped globetail	*Arthropoda*	2,161	1.7	Yes
	Bombus pensylvanicus	American bumble bee	*Arthropoda*	2,091	1.6	No
	Caenurgina erechtea	forage looper or common grass moth	*Arthropoda*	1,950	1.5	Yes
	Bombus lucorum	white-tailed bumble bee	*Arthropoda*	1,872	1.4	No
	Bombus muscorum	large carder bee or moss carder bee	*Arthropoda*	1,855	1.4	No
	Fazia fascifrons	N/A	*Arthropoda*	1,608	1.2	No
	Terastia meticulosalis	erythrina twigborer or erythrina borer	*Arthropoda*	1,584	1.2	No
	Ascalapha odorata	black witch moth	*Arthropoda*	1,536	1.2	Yes
	Harmonia axyridis	multicoloured Asian lady beetle	*Arthropoda*	1,422	1.1	Yes
	Pachydiplax longipennis	blue dasher	*Arthropoda*	1,300	1.0	Yes
	Bombus pratorum	early bumble bee or early-nesting bumble bee	*Arthropoda*	1,273	1.0	No

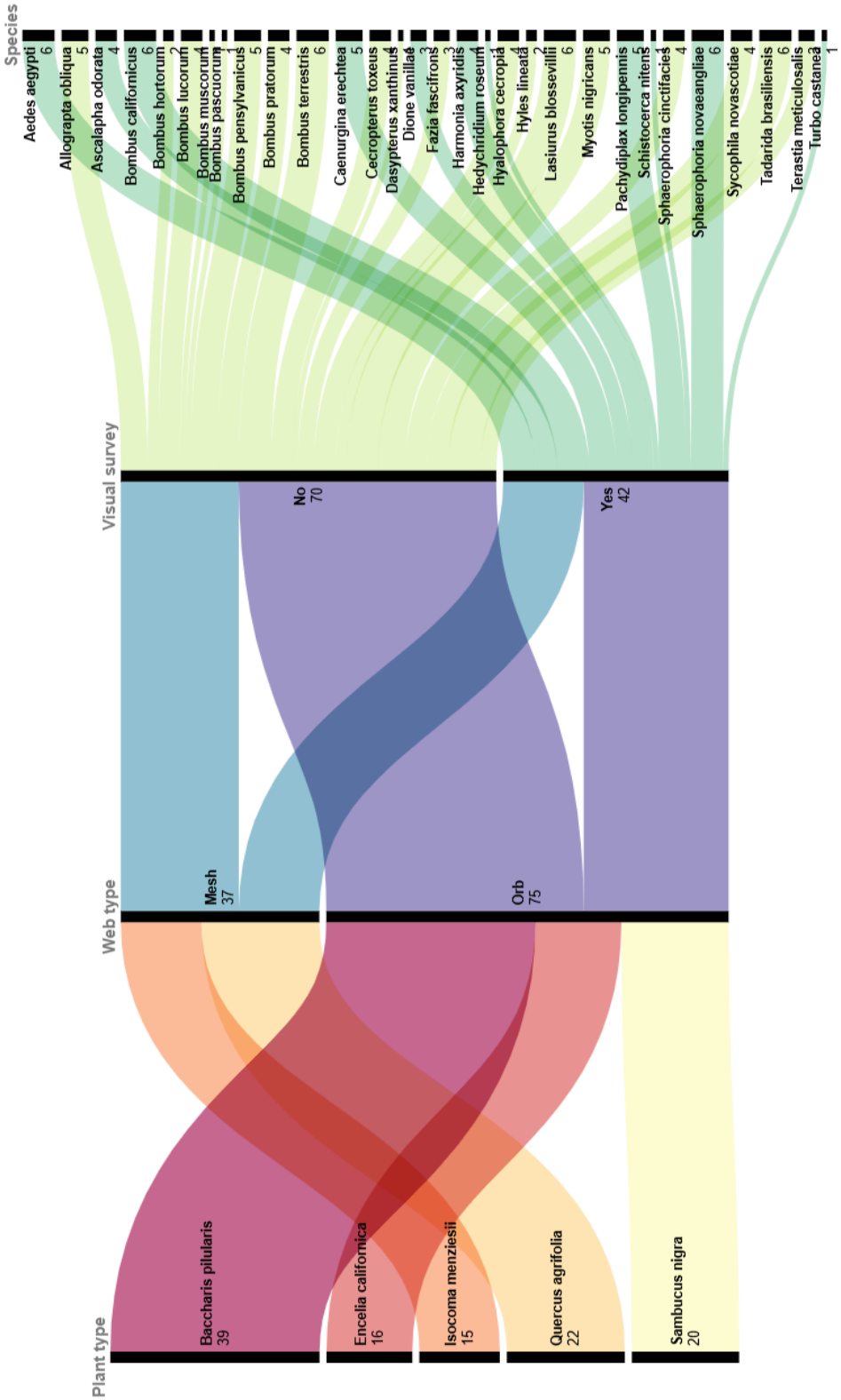

Figure 2 – Spiderweb eDNA detected species by plant species sampled from, type of spiderweb, if species was also detected in the visual surveys, and the molecular identification of detected species.

Visual vs. eDNA Survey Comparisons

Of the 30 species detected from the spiderweb eDNA, 11 (~36%) were also detected in the parallel field visual surveys, whereas 19 (63%) were uniquely detected using the eDNA method. Species detected by both eDNA metabarcoding and field visual surveys provide corroborated evidence of these species' presence in the micro-forest habitat. Notably, six species observed in the visual surveys lack sequence data in NCBI GenBank database, limiting their detection using our DNA metabarcoding approach. Conversely, the 12 months of field survey data documented 58 species (unpublished data), only 11 (19%) of which were shared with the eDNA results. Some of these species may have failed to be detected in visual surveys because of their small size, cryptic coloration, or nocturnal behavior. Several observed species are non-native to California, thus supporting the idea that traditional biodiversity methods may overlook segments of the community and emphasize the potential for eDNA, and spiderweb eDNA specifically, to complement visual surveys by expanding the temporal and spatial resolution of biodiversity assessments.

Seasonal Variation in DNA Capture

Spiderweb DNA concentrations differed between our two sampling periods and two locations. Webs collected at Ascot Hills Park in September (fall) showed higher DNA concentrations (~0.5–4.5 ng/μL), while those collected in January and February (winter) at both Ascot and Loyola Marymount University yielded consistently low values (<0.5 ng/μL). Note, although we did collect complete webs to the best of our ability (avoiding debris, etc.), we did not weigh or use other metrics to standardize the amount of web sample, a consideration for future work. The lower concentrations of DNA in winter could be because of reduced biological activity during winter months, for example, insects are far less active in the winter, with many species dormant (Nelms et al. 2013; Hickmann et al. 2025). This may mean that there are fewer species and less DNA for spiderwebs to pick up. Further investigation is needed to identify the optimal sampling period for spiderwebs in Los Angeles' Mediterranean-type climate.

Limitations and Next Steps

To our knowledge, this is the first study to apply airborne eDNA metabarcoding for biodiversity monitoring in Los Angeles, as well as one of the first reports of successfully using spiderwebs as a medium for capturing airborne eDNA. The scope of this study, however, was experimental and limited by a relatively small sample size, narrow temporal sampling window, and intentional comparison to species observed in the parallel field visual surveys. Given existing knowledge that the presence, composition, and concentration of eDNA varies in multiple environments, including soil (Zhang et al. 2025), aquatic (Collins et al. 2018; Altermatt et al. 2025), and air (Lyngaard et al. 2022; Métris & Métris 2023), this study is an early step to fully appreciate the potential of this complementary biodiversity monitoring tool. A consideration for advancing this research is to note web types and features. Here, we collected mesh and orb spiderwebs, both which yielded sufficient concentrations of eDNA for sequencing. Spiders weave webs and other silk-based constructions for various reasons, leading to variations of web types and web properties (Blamires et al. 2017). These differences are likely to influence the effectiveness with which eDNA is trapped and preserved. Gregorič et al. (2022) suggest that different web structures influence the type of eDNA capture. Web age and environmental direction (e.g., height, direction, exposure) may also influence DNA capture effectiveness and present additional variables to consider.

CONCLUSION

Our findings validate the application of spiderweb eDNA as a potential tool for terrestrial biodiversity monitoring. This is consistent with existing studies showing that spiderwebs can successfully collect airborne DNA from a range of taxa (Xu et al. 2015; Gregorič et al. 2022; Kim & Kim 2024; Newton et al. 2024). Spiderwebs' capacity to collect genetic material from various species indicates that they can act as passive, non-invasive samplers. When combined with our traditional survey data, spiderweb eDNA provides us with a comprehensive biodiversity analysis of our micro-forest field site, and it is suitable for repeated use in other field sites. Overall, this study contributes to the growing body of evidence for spiderweb eDNA as a valuable, non-invasive, and complementary biodiversity monitoring tool. This study builds upon previous work by applying the technique in a Mediterranean-climate urban micro-forest. Future studies can expand sample sizes, explore seasonal and spatial patterns, and integrate spiderweb eDNA with other biodiversity surveys to provide a general overview of local biodiversity and improve conservation and restoration planning.

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Supplemental Materials SM1 – Curated database of 140 target genera/species of interest used during the bioinformatics analysis of the spiderweb eDNA data analysis. The list was curated from species seen during traditional field surveys, endangered and special status taxa from the area, and species found in the Los Angeles area or of interest to the investigators.

Scientific name	**Common Name**	**Broad Taxonomic Group**	**Source**
Cheiracanthium punctorium	Yellow sac spider	Arachnid	Field survey
Zelotes subterraneus	Ground spider	Arachnid	Field survey
Habronattus sp.	Jumping spider	Arachnid	Field survey
Phidippus johnsoni	Red-backed jumping spider	Arachnid	Field survey
Menemerus bivittatus	Gray wall jumper	Arachnid	Field survey
Loxosceles deserta	Desert recluse	Arachnid	Field survey
Latrodectus geometricus	Brown widow spider	Arachnid	Field survey
Corvus brachyrhynchos	California crow	Bird	Field survey
Poecile rufescens	Chestnut-backed chickadee	Bird	Field survey
Melozone crissalis	California towhee	Bird	Field survey
Passer domesticus	House sparrow	Bird	Field survey
Calypte anna	Anna's hummingbird	Bird	Field survey
Selasphorus sasin	Allen's hummingbird	Bird	Field survey
Otala lactea	Milk snail	Mollusk	Field survey
Arphia sp.	Band-winged grasshopper	Insect	Field survey
Schistocerca nitens	Gray bird grasshopper	Insect	Field survey
Bemisia tabaci	White fly	Insect	Field survey
Andrena	Mining bee	Insect	Field survey
Euborellia annulipes	Ringlegged earwig	Insect	Field survey
Aphidoidea	Aphid	Insect	Field survey
Apis mellifera	Honey bee	Insect	Field survey
Bombus californicus	Bumble bee	Insect	Field survey
Xylocopa virginica	Carpenter bee	Insect	Field survey
Bombyliidae	Bee fly	Insect	Field survey
Hedychridium	Cuckoo wasp	Insect	Field survey
Paropsis atomaria	Leaf beetle	Insect	Field survey
Chrysopidae	Green lacewing	Insect	Field survey
Harmonia axyridis	Multicoloured Asian lady beetle	Insect	Field survey
Coccinella septempunctata	Red ladybug	Insect	Field survey
Aedes aegypti	Mosquito	Insect	Field survey
Hypera postica	Alfalfa weevil	Insect	Field survey
Drosophila melanogaster	Fruit fly	Insect	Field survey
Ascalapha odorata	Black witch moth	Insect	Field survey
Caenurgina erechtea	Forage looper moth	Insect	Field survey
Spilosoma vestalis	Vestal tiger moth	Insect	Field survey
Linepithema humile	Argentine ant	Insect	Field survey
Progomphus borealis	Gray sanddragon	Insect	Field survey
Halictidae	Sweat bee	Insect	Field survey
Pachydiplax longipennis	Blue dasher	Insect	Field survey
Muscina stabulans	False stable fly	Insect	Field survey
Musca domestica	House fly	Insect	Field survey

Junonia coenia	Buckeye butterfly	Insect	Field survey
Danaus plexippus	Monarch butterfly	Insect	Field survey
Dione vanillae	Gulf fritillary	Insect	Field survey
Papilio polyxenes	Black swallowtail	Insect	Field survey
Pieris rapae	White butterfly	Insect	Field survey
Pepsis	Tarantula hawk	Insect	Field survey
Chyliza	Rust fly	Insect	Field survey
Lycoriella sativae	Dark-winged gnat	Insect	Field survey
Sphex pensylvanicus	Black wasp	Insect	Field survey
Manduca quinquemaculata	Five-spotted hawkmoth caterpillar	Insect	Field survey
Sphinx libocedrus	Incense cedar sphinx moth	Insect	Field survey
Sphaerophoria novaeangliae	Black and white striped hoverfly	Insect	Field survey
Episyrphus balteatus	Marmalade hoverfly	Insect	Field survey
Eleodes	Desert stink beetle	Insect	Field survey
Ecnolagria	Darkling beetle	Insect	Field survey
Vespula vulgaris	Yellow jacket	Insect	Field survey
Sceloporus occidentalis	Western fence lizard	Reptile	Field survey
Taricha torosa	California newt	Amphibian	Endangered and/or special status
Spea hammondii	Western spadefoot	Amphibian	Endangered and/or special status
Ardea herodias	Great blue heron	Bird	Endangered and/or special status
Ardea alba	Great egret	Bird	Endangered and/or special status
Nycticorax nycticorax	Black-crowned night heron	Bird	Endangered and/or special status
Accipiter cooperii	Cooper's hawk	Bird	Endangered and/or special status
Falco peregrinus anatum	American peregrine falcon	Bird	Endangered and/or special status
Athene cunicularia	Burrowing owl	Bird	Endangered and/or special status
Calypte costae	Costa's hummingbird	Bird	Endangered and/or special status
Selasphorus rufus	Rufous hummingbird	Bird	Endangered and/or special status
Empidonax traillii extimus	Southwestern willow flycatcher	Bird	Endangered and/or special status
Riparia riparia	Bank swallow	Bird	Endangered and/or special status
Polioptila californica californica	California gnatcatcher	Bird	Endangered and/or special status
Vireo bellii pusillus	Least Bell's vireo	Bird	Endangered and/or special status
Setophaga petechia	Yellow warbler	Bird	Endangered and/or special status
Icteria virens	Yellow-breasted chat	Bird	Endangered and/or special status
Piranga rubra	Summer tanager	Bird	Endangered and/or special status
Spinus lawrencei	Lawrence's goldfinch	Bird	Endangered and/or special status
Myotis yumanensis	Yuma myotis	Mammal	Endangered and/or special status
Lasiurus cinereus	Hoary bat	Mammal	Endangered and/or special

			status
Eumops	Mastiff bat	Mammal	Endangered and/or special status
Nyctinomops macrotis	Big free-tailed bat	Mammal	Endangered and/or special status
Taxidea taxus	American badger	Mammal	Endangered and/or special status
Phrynosoma blainvillii	Blainville's horned lizard	Reptile	Endangered and/or special status
Diadophis punctatus modestus	San Bernardino ring-necked snake	Reptile	Endangered and/or special status
Thamnophis hammondii	Two-striped gartersnake	Reptile	Endangered and/or special status
Bombus pensylvanicus	American bumble bee	Insect	Endangered and/or special status
Bombus	Bee	Insect	Endangered and/or special status
Anodonta californiensis	California floater	Mollusk	Endangered and/or special status
Canis latrans	Coyote	Mammal	Found in Los Angeles/ investigator interest
Tadarida brasiliensis	Mexican free-tailed bat	Mammal	Found in Los Angeles/ investigator interest
Parastrellus hesperus	Canyon bat	Mammal	Found in Los Angeles/ investigator interest
Myotis californicus	California myotis	Mammal	Found in Los Angeles/ investigator interest
Aeorestes cinereus	Hoary bat	Mammal	Found in Los Angeles/ investigator interest
Eptesicus fuscus	Big brown bat	Mammal	Found in Los Angeles/ investigator interest
Myotis	Myotis bat	Mammal	Found in Los Angeles/ investigator interest
Lasiurus blossevillii	Western red bat	Mammal	Found in Los Angeles/ investigator interest
Lasiurus xanthinus	Western yellow bat	Mammal	Found in Los Angeles/ investigator interest
Nyctinomops femorosaccus	Pocketed free-tailed bat	Mammal	Found in Los Angeles/ investigator interest
Didelphis virginiana	Virginia opossum	Mammal	Found in Los Angeles/ investigator interest
Spilogale gracilis	Spotted skunk	Mammal	Found in Los Angeles/ investigator interest
Mephitis mephitis	Striped skunk	Mammal	Found in Los Angeles/ investigator interest
Procyon lotor	Common raccoon	Mammal	Found in Los Angeles/ investigator interest
Bubo virginianus	Great horned owl	Bird	Found in Los Angeles/ investigator interest
Tyto alba	Barn owl	Bird	Found in Los Angeles/ investigator interest
Sylvilagus audubonii	Audubon's cottontail	Mammal	Found in Los Angeles/ investigator interest
Thomomys bottae	Botta's pocket gopher	Mammal	Found in Los Angeles/ investigator interest
Turbo castanea	Chevron snail	Mollusk	Found in Los Angeles/ investigator interest
Simuliidae	Black fly	Insect	Found in Los Angeles/ investigator interest
Syrphidae sphaerophoria	Hoverfly	Insect	Found in Los Angeles/

			investigator interest
Syrphidae syrphus	Hoverfly	Insect	Found in Los Angeles/ investigator interest
Syrphidae allograpta	Hoverfly	Insect	Found in Los Angeles/ investigator interest
Neoscona crucifera	Hentz orb weaver	Arachnid	Found in Los Angeles/ investigator interest
Argiope argentata	Silver argiope	Arachnid	Found in Los Angeles/ investigator interest
Argiope aurantia	Yellow garden spider	Arachnid	Found in Los Angeles/ investigator interest
Lycosidae	Wolf spider	Arachnid	Found in Los Angeles/ investigator interest
Pholcidae	Cellar spider	Arachnid	Found in Los Angeles/ investigator interest
Opiliones	Daddy longlegs	Arachnid	Found in Los Angeles/ investigator interest
Latrodectus	Widow spider	Arachnid	Found in Los Angeles/ investigator interest
Loxosceles reclusa	Brown recluse	Arachnid	Found in Los Angeles/ investigator interest
Salticidae	Jumping spider	Arachnid	Found in Los Angeles/ investigator interest
Agelenidae	Grass spider	Arachnid	Found in Los Angeles/ investigator interest
Theraphosidae	Tarantula	Arachnid	Found in Los Angeles/ investigator interest
Synanthedon resplendens	Sycamore borer moth	Insect	Found in Los Angeles/ investigator interest
Lymantria dispar	Spongy moth	Insect	Found in Los Angeles/ investigator interest
Hyalophora cecropia	Cecropia moth	Insect	Found in Los Angeles/ investigator interest
Hofmannophila	Brown house moth	Insect	Found in Los Angeles/ investigator interest
Interpunctella	Indian-meal moth	Insect	Found in Los Angeles/ investigator interest
Terastia meticulosalis	Erythrina stem borer	Insect	Found in Los Angeles/ investigator interest
Antheraea polyphemus	Polyphemus moth	Insect	Found in Los Angeles/ investigator interest
Blabomma californicum	California meshweaver	Arachnid	Found in Los Angeles/ investigator interest
Mallos pallidus	N/A	Arachnid	Found in Los Angeles/ investigator interest
Yorima	N/A	Arachnid	Found in Los Angeles/ investigator interest
Dictyna	Mesh-web weaver	Arachnid	Found in Los Angeles/ investigator interest
Araneidae	Orb weaver	Arachnid	Found in Los Angeles/ investigator interest
Hyles lineata	White-lined sphinx	Insect	Found in Los Angeles/ investigator interest
Hemaris diffinis	Snowberry clearwing	Insect	Found in Los Angeles/ investigator interest
Eumorpha achemon	Achemon sphinx	Insect	Found in Los Angeles/ investigator interest
Cocytius antaeus	Giant sphinx	Insect	Found in Los Angeles/ investigator interest
Proserpinus	Hawk moth	Insect	Found in Los Angeles/

			investigator interest
Allograpta obliqua	Common oblique syrphid	Insect	Found in Los Angeles/ investigator interest
Sialia mexicana	Western bluebird	Bird	Found in Los Angeles/ investigator interest