

Georgia Southern University Georgia Southern Commons

Electronic Theses and Dissertations

Jack N. Averitt College of Graduate Studies

Summer 2021

# Creating a Georgia Southern Spider Collection: Can DNA Barcoding Help?

Guy B. Hobbs

Follow this and additional works at: https://digitalcommons.georgiasouthern.edu/etd Part of the Biodiversity Commons, Biology Commons, Comparative and Evolutionary Physiology Commons, and the Genetics Commons

#### **Recommended** Citation

Hobbs, Guy B., "Creating a Georgia Southern Spider Collection: Can DNA Barcoding Help?" (2021). *Electronic Theses and Dissertations*. 2285. https://digitalcommons.georgiasouthern.edu/etd/2285

This thesis (open access) is brought to you for free and open access by the Jack N. Averitt College of Graduate Studies at Georgia Southern Commons. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Georgia Southern Commons. For more information, please contact digitalcommons@georgiasouthern.edu.

## CREATING A GEORGIA SOUTHERN SPIDER COLLECTION: CAN DNA BARCODING HELP?

by

#### **GUY HOBBS**

(Under the Direction of Lorenza Beati)

#### ABSTRACT

With over 280 spider (Araneae) species recorded within the State of Georgia, USA, the need for a well-documented natural history collection with a usable voucher system is critical to continually assess spider diversity and their future ecological impact in this region. Spider identification can be daunting for the inexperienced taxonomist; it is time consuming and sometimes requires destructive procedures. Previous works have successfully used an alternative method, DNA barcoding, to correctly identify spider species while preserving their morphology. This study set forth to create the core of a well-documented spider collection within Georgia Southern University's Institute for Coastal Plain Science and use DNA barcoding as a diagnostic tool. We collected 334 spiders from varying locations within the Coastal Plain and optimized a DNA extraction protocol from spider legs. Cytochrome C Oxidase Subunit I (COI), the gene commonly used in DNA barcoding, and an additional nuclear gene Histone 3 type A (H3A) were amplified and sequenced from a total of 132 and 150 spiders, respectively. The COI dataset identified 16 families, 33 genera, and 36 different species, while H3A identified 15 families, 23 genera, and 15 species. In addition, based on these gene sequences, we generated phylogenetic inferences of the COI and, when possible, of a concatenated dataset.

INDEX WORDS: Araneae, DNA barcoding, Cytochrome c oxidase subunit 1, Histone 3 type A

## CREATING A GEORGIA SOUTHERN SPIDER COLLECTION: CAN DNA BARCODING HELP?

by

## **GUY HOBBS**

B.A., Georgia Southern University, 2018

A Thesis Submitted to the Graduate Faculty of Georgia Southern University in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

## COLLEGE OF SCIENCE AND MATHMATICS

© 2021 GUY BRANDON HOBBS All Rights Reserved

## CREATING A GEORGIA SOUTHERN SPIDER COLLECTION: CAN DNA BARCODING HELP?

by

GUY HOBBS

Major Professor: Lorenza Beati Committee: Lance Durden William Irby

Electronic Version Approved: July 2021

### ACKNOWLEDGMENTS

Thank you to my Committee members for the continuous support throughout my academic career at Georgia Southern University. The scientific knowledge, scholarly advice, and general wisdom of my mentors has aided in my development as a scientist and as a better person.

## TABLE OF CONTENTS

3

Page
------

ACKNOWLEDGMENTS	2
LIST OF TABLES	4
LIST OF FIGURES	5
CHAPTER 1 INTRODUCTION	6
CHAPTER 2 MATERIALS & METHODS	8
Sampling	8
Preliminary identifications and imaging	8
DNA extraction	9
DNA amplification	9
Sequence assembly and preliminary identification through BLAST	10
DNA distances and phylogenetic analyses	10
CHAPTER 3 RESULTS & DISCUSSION	13
Sampling	13
Preliminary identifications and imaging	13
DNA extraction	13
DNA amplification	14
Preliminary identification through BLAST and K2P distance calculations	14
Phylogenetic tree comparisons	15
CHAPTER 4 CONCLUSIONS	24
REFERENCES	25
APPENDICES	30
APPENDIX 1: FIELD COLLECTIONS	30
APPENDIX 2: BLAST SEARCH RESULTS FOR COI AND H3A	52

## LIST OF TABLES

Table 2.1: PCR primers used in this study	11
Table 3.1: Optimized polymerase chain reaction programs for COI and H3A	16
Table 3.2: Comparison of four methods for DNA extraction from spider legs	17

## LIST OF FIGURES

Figure 2.1.: Collection sites	12
Figure 3.1.: Number of spiders collected in each type of environment	18
Figure 3.2: Counts of pairwise comparisons per K2P distance values (COI gene)	19
Figure 3.3.: Identifications by family (COI gene)	20
Figure 3.4.: Identifications by family (H3A gene)	21
Figure 3.5.: Phylogenetic analysis of Cytochrome C Oxidase subunit I (COI) sequences	22
Figure 3.6.: Phylogenetic analysis of concatenated COI and H3A sequences	23

#### CHAPTER 1

#### INTRODUCTION

The Coastal Plain of Georgia is an ecologically heterogeneous region. Many habitats from the marshes of the Altamaha River, to the sand hills and loblolly forests of the interior possess a plethora of spider species (Chamberlain and Ivey 1944). Anthropogenic influences, such as alterations of landscapes, introduced invasive species, and climate change, have already shown to have an impact on the biodiversity of Coastal Plain organisms (Guillebeau 2008; Hsiang et al. 2017; Mitchel et al. 2014; Pimentel 2005; Turner 1988), making this region a potentially important biomonitoring area.

Spiders (Arachnida: Araneae) play a vital role in terrestrial food webs by regulating population sizes and biodiversity of their prey and predators. Therefore, it is important to be able to monitor their diversity and density both spatially and temporally. However, with almost 40,000 species described worldwide and 3,000 in North America alone (World Spider... c2021), spiders are taxonomically challenging as the identification of many species requires extensive systematic expertise. Spiders of Georgia have been documented as early as the beginning of the 1800s with John Abbots' illustrations (unpublished illustrations, 1772-1804). Later, Chamberlain and Ivie (1944) published a seminal monograph on the spiders of Georgia that includes 282 species and 567 illustrations in part borrowed from Abbot's work. Recent publications have explored other regions of the Southern United States. In central and northern Florida, Corey and Taylor (1988) sampled and identified spiders in pine ponds, sand pine scrub and flatwood habitats recording a total of 57, 42 and 48 species respectively. Another study in southwest Georgia sampled three isolated pinewood wetlands describing 27 taxa from nine families of spiders (Tietjen et al. 2017). Within the wetlands, the families Tetragnathidae and Pisauridae were most abundant with Dolomedes triton and Tetragnatha laboriosa as the top two most common species. The most relevant publication in recent times is a study of ground-layer spiders of the Georgia Piedmont Floodplain (Draney 1997). This survey of natural floodplain forests, agricultural lands (tillage and non-tillage) and grasslands bordering these habitats in Clarke County, Georgia identified 145 species belonging to 26 families. All these studies involved endemic species exclusively occurring in Georgia and more widespread taxa found in other areas of the US.

While taxonomic keys (Bradley 2012; Gertsch 1979; Ubick et al. 2005), identification guides, and online resources (How to identify... c2021; Morphology and Physiology... c2015) are available for spider identification, the microscopic process of diagnosing spider species can be challenging and time consuming, particularly for less experienced taxonomists. Intraspecific polymorphism, sexual dimorphism, absence of keys for spiderlings, the need for dissections, all contribute to make spider identification complicated. With the advent of molecular methods, alternative procedures that, supposedly, do not require professional morphological taxonomic skills have been developed. Hebert et al. (2003a; 2003b) proposed a new genetic approach, called "barcoding of life." It involves the creation of a taxonomic depository of a highly conserved metazoan gene sequence (Cytochrome C Oxidase subunit I [COI]) from morphologically well identified specimens. The sequence depository is then used for sequence comparisons and taxonomic identification. This method has been used for many organisms, especially arthropods (Bucklin et al. 2011; Lavinia et al. 2017; Smith 2005) with spiders included (Barret and Hebert 2005; Blagoev et al. 2013; Blagoev et al. 2016). These three publications, which generated over 48,000 North American COI sequences deposited in GenBank and BOLD (Ratnasingham 2007), provided convincing evidence that the method can be used to correctly identify spiders molecularly.

Our study had several main goals: (1) assemble a core collection of spiders from the Georgia Coastal Plain, (2) optimize a method that allows the extraction of DNA while preserving diagnostic morphological features, (3) identify our samples by comparing COI sequences to GenBank accessions, and (4) add H3A nuclear gene sequences (Histone 3 type A) to the mitochondrial dataset for comparison of results and for phylogenetic analyses.

## CHAPTER 2 MATERIALS AND METHODS

#### Sampling

Spiders were collected from May 2017 to May 2020 from multiple habitats within the Coastal Plain of Georgia (Fig. 2.1 and Appendix 1) in an opportunistic way aiming at increasing diversity rather than monitoring densities. Older samples collected from March 2010 to June 2011 were added to our collection (Appendix 1). A variety of techniques were involved in our work: active hand catching both diurnal and nocturnal (visual detection in webs, on vegetation, under logs and stones), the usage of sweep nets, drag cloths, and pitfall traps with a 90% ethanol soapy solution.

The locations chosen for sampling were as follows: Georgia Southern University campus, two mixed forest habitats (Bo Ginn National Fish Hatchery near Magnolia Springs State Park and a private property south of Statesboro); one pond habitat (Bird Pond) south of Statesboro, where spiders were collected by the edge of the body of water and inside the surrounding mixed forest; one sandhill habitat, an east facing sandhill slope with cultivated slash pines and a small pecan grove transitioning into a riparian zone; and one saltwater marshland habitat located near St. Simons island. In addition, we received donated collected samples from multiple environments (homes, outbuildings, pools and other man-made public places) which we grouped under a "miscellaneous" section. Specimens are now housed within the Institute for Coastal Plain Science at Georgia Southern University. Each specimen is preserved in 90% ethanol, and each vial contains a label with a unique identifier (ex. ARA1, ARA2, etc.) and collection data (locality, geographical coordinates, date of collection, name of collector).

#### Preliminary identifications and imaging

Collected spiders were identified at least at the family level through traditional taxonomic methods. This included using identification manuals and keys available for spiders of this region (Bradley 2012; Gertsch 1979; Ubick et al. 2005). Identifications at the species or genus level were performed only for common and easy to recognize spiders that did not require dissection for a definitive diagnosis. By using a stacking BK Plus Lab System camera (Visionary Digital),

we generated voucher images of the frontal, dorsal and ventral sides of 173 of the collected spiders.

#### DNA extraction

In order to test our primer sets (Table 2.1) and optimize polymerase chain reaction (PCR) conditions, we first extracted DNA from whole bodies of spiders (after images were taken) by using the Qiagen DNAeasy Blood and Tissue Kit (Qiagen, Germantown, MD). Once PCR conditions were fully tested and optimized, and because one of our main goals was to preserve spiders and deposit them in a newly created collection, we developed an alternative method for extracting DNA that would preserve diagnostic voucher spider bodies. Three extraction kits, Qiagen DNAeasy Blood and Tissue kit, QIAamp DNAmicro (Qiagen, Germantown, MD), and ChargeSwitch (Invitrogen, Carlsbad, CA), were compared for their ability to extract DNA from 2 spider legs at a time. Twenty spiders from different families and of different body sizes were selected for the test. Six legs were removed from each specimen and, of those, 2 legs were removed to test the difference between DNA elution in H<sub>2</sub>O or in AE (Qiagen, Germantown, MD) buffer.

#### DNA amplification

Amplification of the mitochondrial COI (approx. 600 bp) (Hebert et al. 2003; Folmer et al. 1994) and the nuclear H3A (approx. 320 bp) gene sequences (Colgan et al. 1998) were performed by using primers described in the literature (Table 2.1). The amplification of COI from spider DNA often requires the combination of different primer sets (Astrin et al. 2016; Blagoev et al. 2013; Blagoev et al. 2016; Hebert et al. 2003b). Thermocycler conditions for amplifying spider DNA were optimized for each combination of COI primers and for the nuclear H3A gene primers by running annealing temperature gradients. Once PCR conditions were established, we used primer sets F1-R1 and F2-R2 (Table 2.1) for comparing amplicons from DNA extracted from legs with the four methods. Identical amounts of extracted DNA were amplified from each of the four extracts obtained from each spider by using the DreamTaq HotStart DNA Polymerase kit (Thermo Scientific, Waltham, MA). The PCR mixture included: 2.5µl of 10x DreamTaq Buffer, 0.5µl of a 10mM DNTPs mix, 1.25µl of each primer

(10pmole/µl), 2.5µl of DNA, 0.26 µl DreamTaq HotStart DNA Polymerase (1.25 U), and 16.74µl of molecular grade H<sub>2</sub>O. For each set of primers, four 1.5% agarose gels with 21 lanes each (20 for spiders and 1 for the molecular weight standard) were prepared. The four sets of amplicons were distributed in the same order in the gels and gel patterns were compared for specificity (one band versus, more than one band or an indistinct smear) and for intensity.

#### Sequence assembly and preliminary identification through BLAST

Successful DNA amplicons were purified by using ExoSAP-IT (Thermo Scientific, Waltham, MA) and shipped for sequencing of both complementary strands to Eurofins Genomics (Louisville, KY). The complementary strands were assembled by using the online platform, Benchling (Benchling... c2021). Consensus sequences were saved in the standard FASTA format. Each sequence was compared to homologous GenBank entries by using BLAST (Altschul et al. 1990). Query coverage and percent identity were recorded for the closest sequences retrieved with BLAST.

#### DNA distances and phylogenetic analyses

Sequences were aligned with Mesquite 3.61 (Maddison and Maddison 2019). Codon organization was considered when aligning the COI and H3A data sets. For the COI data matrix, Kimura-2 distance (K2P) (Kimura 1980) were calculated and used to generate a barcode histogram in order to verify whether our data provided a visible barcoding gap between intra and interspecific distances. When both sequences were available for the same spider, a concatenated COI and H3A aligned matrix was created. The COI and the concatenated data sets were analyzed by Bayesian analysis (BA) with a general time-reversible nucleotide substitution model and posterior probability values were calculated with MrBayes 3.2.4 (Huelsenbeck and Ronquist 2001; Ronquist et al. 2012b ). Two analyses, with four chains each, were run simultaneously for BA analyses (1,000,000 generations). Trees were sampled every 100 iterations with a 25% burning fraction. The 50% majority-rule consensus tree of the remaining trees was inferred, and posterior probabilities were recorded for each branch. Filistatidae were used as outgroups in our analyses because they were found to be a basal lineage in the Araneaomorpha (Garrison N et al. 2016). For the concatenated data set, we used Linyphiidae as outgroups because we did not obtain H3A sequences from Filistatidae samples.

Table 2.1. PCR primers used in this study.

Gene	Primer	Sequence (5'-3')	References
COI	chelicerate forward1 (F1)	TACTCTACTAATCATAAAGACATTGG	Hebert et al. (2003)
	chelicerate reverse1 (R1)	CCTCCTCCTGAAGGGTCAAAAAATGA	Hebert et al. (2003)
	chelicerate reverse2 (R2)	GGATGGCCAAAAAATCAAAATAAATG	Hebert et al. (2003)
	LCO1490 (F2)	GGTCAACAAATCATCATAAAGATATTGG	Folmer et al. (1994)
H3histone	H3AF	ATGGCTCGTACCAAGCAGACVGC	Colgan et al. (1998)
	H3AR	ATATCCTTRGGCATRATRGTGAC	Colgan et al. (1998)

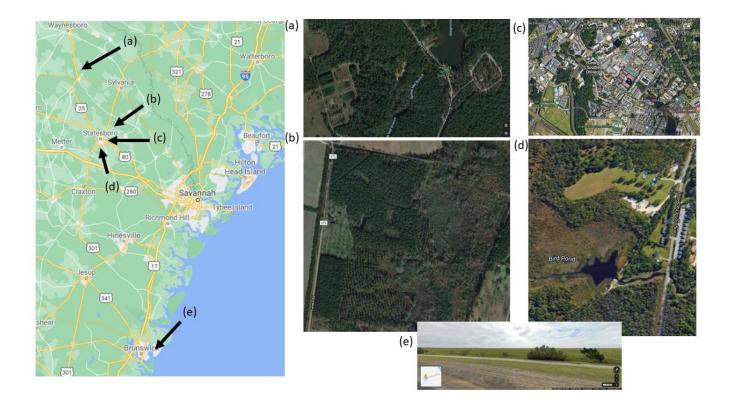


Figure 2.1. Collection sites. Site (a) is Magnolia Springs State Park, (b) a privately owned sandhill property, (c) Georgia Southern University campus, (d) Bird Pond, (e) Saltwater marsh near St. Simons Island.

## CHAPTER 3 RESULTS AND DISCUSSION

#### Sampling

A total of 334 spiders were collected: 93 from Bird Pond, 87 from the sandhill habitat, 15 from saltwater marshes, 24 from the GSU campus, 81 from mixed forest habitats, and 34 assigned to the "miscellaneous" section (Fig. 3.1).

#### Preliminary identifications and imaging

A total of 173 spiders were imaged. Identification attempts were performed on these samples and resulted in them being all identified at the family level. The most common spiders, such as *Leucauge* sp., *Micrathena* sp., *Neoscona* sp., *Peucetia viridans* and *Trichonephila clavipes* were identified to species level. The voucher images will be made available to the public through an ad-hoc web page (ongoing work). Once the non-destructive method for extracting DNA was fully optimized, because spiders could be preserved, the unnecessary imaging of the remaining 161 spiders was ended.

#### DNA extraction

Comparison of the four extraction methods showed that for both sets of primers, the QIAamp DNAmicro kit with elution in AE buffer gave the best results with 70-75% of the samples being amplified (one sharp band of the correct size and of high intensity), versus 55-60% for the DNAeasy Blood and Tissue kit, 45-65% for the Invitrogen kit, and 65-70% for the DNAmicro kit followed by elution in H<sub>2</sub>O (Table 3.2). After eliminating some of the most common spiders from our sample, in order to avoid too many redundant results, we extracted DNA from a total of 204 spiders (61%). One study (Blagoev et al. 2016) has managed to generate COI sequences from 27,269/30,679 (89%) spider DNA samples obtained from a single spider leg. Their method was more successful but based on fully automated procedures.

#### DNA amplification

By testing different annealing temperatures through temperature gradients, the best PCR conditions for our primer sets were determined to be as listed in Table 2.1. A total of 132 amplicons were obtained with the COI primers sets (64%), 150 for H3A (73%), and 70 for both (34%). H3A is a very short nuclear gene (~ 320 bp) that might be easier to amplify than a longer mitochondrial gene (~ 600 bp). Also, to reduce expenses, we used only the F1-R1 and F2-R2 combination of COI primers, while in some cases the F1-R2 or the F2-R1 combinations might have been more suitable. Once purified and sequenced, the amplicons yielded a total of 95 and 94 usable COI and H3A sequences, respectively.

#### Preliminary identification through BLAST and K2P distance calculations

We decided to use BLAST to compare our sequences to accessions in GenBank rather than use the BOLD platform for simplicity of use and because, while the BOLD sequences are also found in GenBank, not all GenBank accessions will be retrieved from BOLD. We are aware, however, that the algorithm used to identify species is slightly different in the two systems.

Of the 95 COI sequences, 79 were identified at the species level with BLAST similarity values > 98% (K2P > 97.93), 6 at the genus level only and 10 at the family level only. K2P distances between genera varied from 5.69 to 22.46% (in Theridiidae) indicating that distances between genera were sometimes as important as distances between families (12.00 to 29.39%). The barcoding gap histogram based on K2P distances showed that the intraspecific peak is clearly separated from the intraspecific peak (Fig. 3.2). The barcoding gap was not as definite in a previously published study (Barrett and Hebert 2005) which, however, included a larger variety of species. If the taxonomic breath of our sample had been more important, we might also have found taxa with higher intraspecific divergence values. Of the 94 H3A sequences, 30 were identified at the species level with BLAST similarity values > 98% (K2P > 98.20), 30 at the genus level only, and 34 at the family level only. COI was better at identifying specimens at the species level because there are more and more diverse COI sequences available for comparison in GenBank (over 48,000 vs. 1,075). Also, most available GenBank H3A sequences were generated from European or South American spider species (Arnedo et al. 2004; Piacentini and Ramirez 2019; Wheeler et al. 2017) that do not occur in North America, explaining why most identifications were at the supra-specific levels. Although H3A proved to contribute little to our

identification effort, at least for the overlapping samples, that were primarily identified though COI barcoding and microscopic confirmation, we can now provide the scientific community with a new set of H3A sequences.

The COI dataset include specimens from 16 families, 33 genera, and 36 different species. Considering the relatively small sample, this represents a diverse and heterogeneous group of taxa. The H3A dataset identified 15 families, 23 genera, and 15 species. Interestingly, COI and H3A primers did not equally amplify different spider families. For instance, COI primers appeared to be better at amplifying Lycosidae, Pisauridae, and Salticidae DNA (Fig. 3.3) than H3A primers which detected more easily Tetragnathidae (Fig. 3.4).

#### Phylogenetic tree comparisons

Phylogenetic trees were obtained after duplicate sequences had been removed from the aligned matrix. Bayesian analyses provide better distance evaluation based on more complex evolutionary models than K2P. The COI trees associated with barcoding in the literature are usually based on K2P distances of amino acid sequences and often show strong monophyly for each considered species, but little support for basal lineage topology (Barrett and Hebert 2005). By using DNA sequences, we achieved better overall resolution in some of the basal groups (families Desidae and Corinnidae) (Fig. 3.5). Nevertheless, the remaining families originated from a large polytomy. Within, the polytomy, all families were monophyletic (posterior probability values > 0.75) with the exception of Theridiidae. *Archaearanea tepidariorium* does not cluster with the other members of the family. The large divergence levels within a non-monophyletic Theridiidae has been observed in other more complex phylogenetic analyses (Liu et al 2016) where *Archaeaeanea* was found to belong to a different subfamily (Theridiinae) than *Latrodectus* and *Steatoda* (Latrodectinae), the other two genera represented in our sample.

While H3A has been used successfully, in conjunction with other genes, for phylogenetic analyses of spiders (Wheeler et al. 2017) these studies used morphologically identified specimens. Given the results we obtained, it does not appear that, until more H3A sequences from the United States are made available to the scientific community, this gene is a viable barcoding tool. Nevertheless, when the two genes were concatenated (Figure 3.6), the structure of the phylogeny was very well resolved even basally. With the exception of the Theridiidae, all families, genera, and species were monophyletic (with posterior probabilities > 0.75).

PCR step	Cycles	F1R1 & F2R2	Cycles	НЗА
Initial Denaturation		95°C for 5 min		95°C for 5 min
Denaturation		95°C for 20 sec		95°C for 20 sec
Annealing	10	$44^{\circ}C$ +0.4°/cycle for 45 sec	7	60°C -1°C/cycle for 30 sec
Elongation		65°C for 40 sec		72°C for 30 sec
Denaturation		94°C for 20 sec		95°C for 20 sec
Annealing	20	48°C for 45 sec	28	53°C for 30 sec
Elongation		65°C for 40 sec		72°C for 30 sec

Table 3.1. Optimized polymerase chain reaction programs for COI and H3A.

Table 3.2 Comparison of four methods of DNA extraction from spider legs. QDBT= Qiagen DNAeasy Blood & Tissue Kit, ICS= Invitrogen ChargeSwitch Kit, QQDMW= Qiagen QIAamp DNAmicro Kit eluted in H<sub>2</sub>O and QQDMAE = Qiagen QIAamp DNAmicro Kit eluted in AE buffer

	QDBT	QDBT	ICS	ICS	QQDMW	QQDMW	QQDMAE	QQDMAE
Primers	F1-R1	F2-R2	F1-R1	F2-R2	F1-R1	F2-R2	F1-R1	F2-R2
Positive	11(55%)	12(60%)	13(65%)	9(45%)	13(65%)	14(70%)	14(70%)	15(75%)
Weak	4(20%)	0(0%)	4(20%)	5(25%)	3(15%)	3(15%)	5(25%)	0(0%)
Negative	5(25%)	7(35%)	3(15%)	6(30%)	4(20%)	3(15%)	1(5%)	5(25%)
Double	0(0%)	1(5%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Bands								

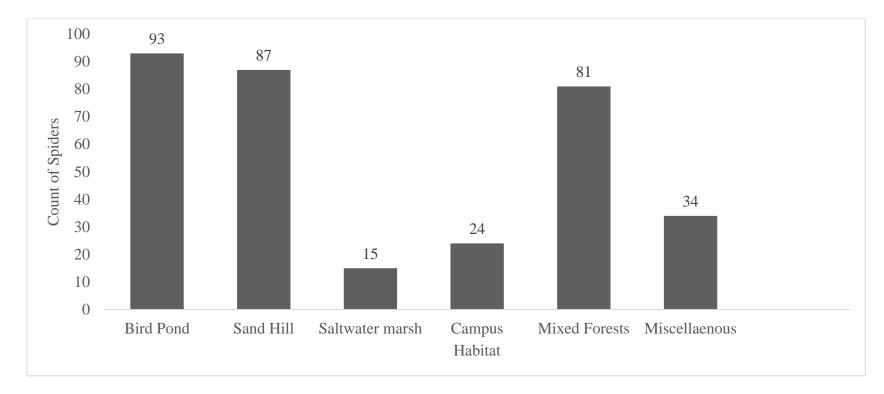


Figure 3.1. Number of spiders collected in each type of environment. Donated samples were assigned to the miscellaneous section.

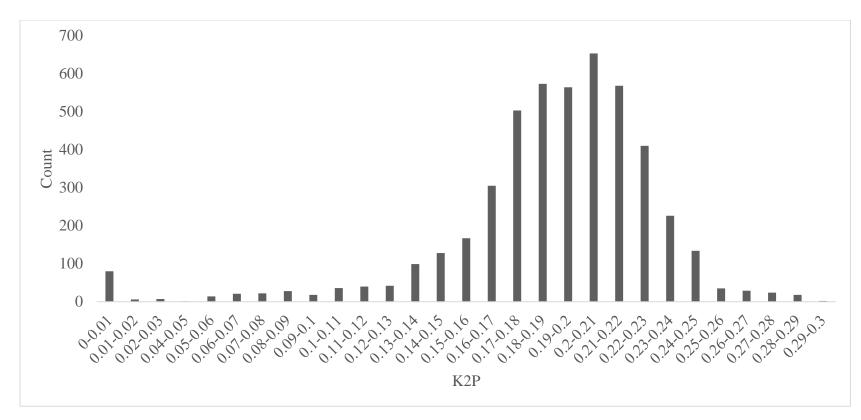


Figure 3.2. Counts of pairwise comparisons per KP2 divergence values.

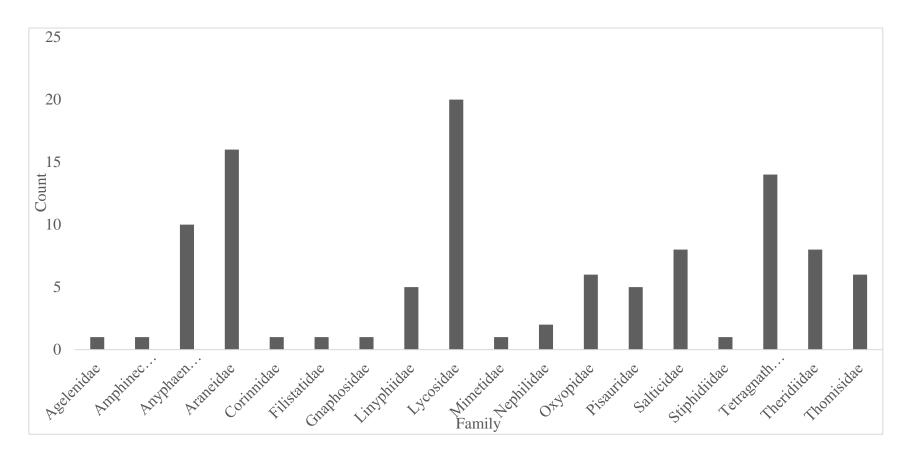


Figure 3.3. Number of identifications per spider family (COI gene)

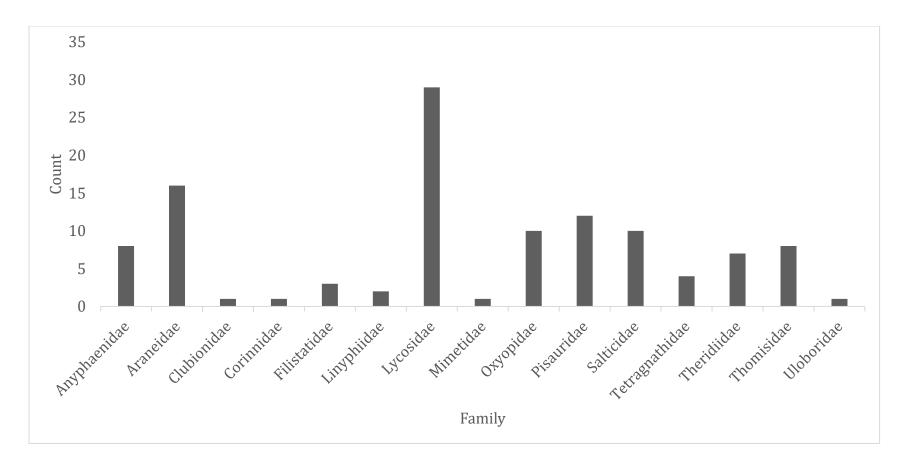


Figure 3.4. Number of identifications per spider family (H3A gene)

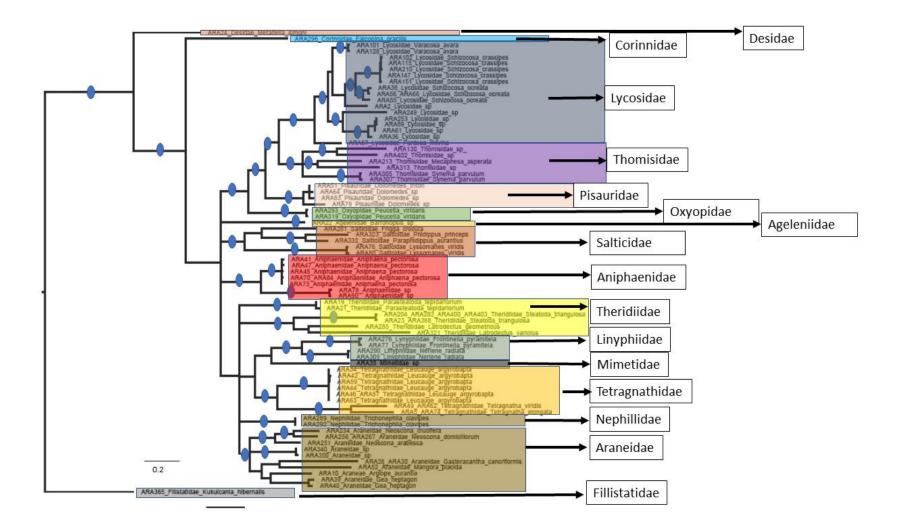


Figure 3.5. Phylogenetic analysis of Cytochrome C Oxidase subunit 1 (COI) sequences. Separate families are delimited by differently colored boxes. Supported lineages from Bayesian analysis (> 0.75 posterior probability) are marked on branches with a blue oval.

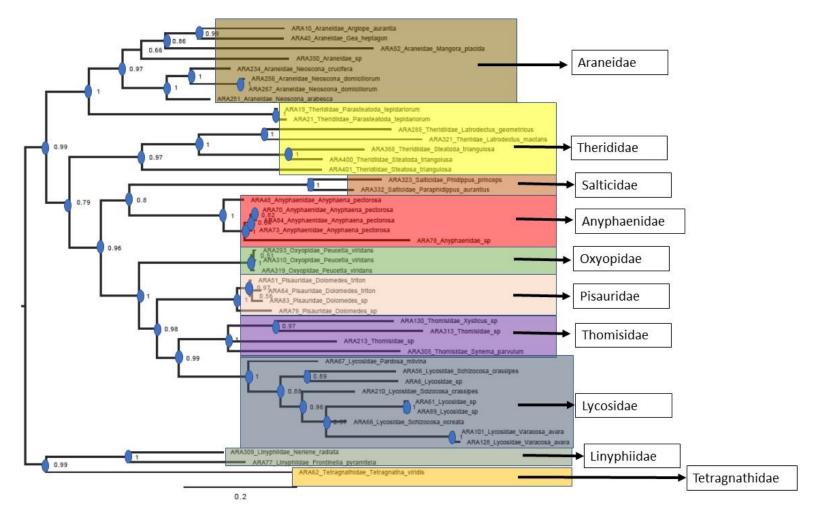


Figure 3.6. Phylogenetic analysis of concatenated COI and H3A gene sequences. Separate families are delimited in differently shaded boxes. Supported lineages from Bayesian analysis (> 0.75 posterior probability) are marked on nodes with a blue oval.

# CHAPTER 4

## CONCLUSIONS

In conclusion, this study provided us with a taxonomically diverse and well-documented spider sample that will constitute the initial core of the future GSU spider collection. DNA extraction methodologies preserving simultaneously the spider bodies, and more importantly, their diagnostic features were optimized as an essential part of a workflow that should allow this collection to rapidly grow.

COI barcoding proved to be useful because many of the species we collected had already been molecularly characterized and their sequences had already been deposited in GenBank. This was not the case for the H3A sequences. Nevertheless, one should not forget that morphological verifications are always advisable, because taxonomic identifications preceding GenBank sequence submissions are sometimes wrong. Of the samples that were only identified by COI barcoding at the genus and family level, morphological identification will also be necessary. They might correspond either to a known species for which a COI sequence has yet to be generated, or to a new species. Given the rate at which new spider species are validated (World Spider... c2021) it would not be surprising to discover unknown taxa for the U.S. and Georgia in particular.

Phylogenetically, the combination of COI and H3A sequences in a single analysis proved to be more informative than the use of COI only. When corroborated by COI identifications, our H3A dataset will constitute a small but interesting addition to the relatively limited set of sequences available in GenBank.

#### REFERENCES

Arnedo MA, Coddington J, Agnarsson I, Gillespie RG. 2004. From a comb to a tree: phylogenetic relationships of the comb-footed spiders (Araneae, Theridiidae) inferred from nuclear and mitochondrial genes. Molecular Phylogenetics and Evolution. 31:225-245.

Astrin JJ, Hofer H, Spelda J, Holstein J, Bayer S, Hendrich L, Huber BA, Kielhorn KH, Krammer HJ, Lemke M, et al. 2016. Towards a DNA Barcode Reference Database for Spiders and Harvestmen of Germany. y. PLoS ONE. 11(9): e0162624.

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ. 1990. "Basic local alignment search tool." Journal of Molecular Biology 215:403-410. Barrett RD, Hebert PD. 2005. Identifying spiders through DNA barcodes. Canadian Journal of Zoology. 83(3): 498-504.

Benchling: Biology Software. c2021. San Francisco (CA): Benchling. https://benchling.com.

Blagoev GA, Nikolova NI, Sobel CN, Hebert PD, Adamowicz SJ. 2013. Spiders (Araneae) of Churchill, Manitoba: DNA barcodes and morphology reveal high species diversity and new Canadian records. BMC Ecology. 13:44.

Blagoev GA, DeWaard JR, Ratnasingham S, DeWaard SL, Lu L, Robertson J, Telfer AC, HebertP.D. 2016. Untangling taxonomy: A DNA barcode reference library for Canadian spiders.Molecular Ecology Resources. 16:325-341.

Bradley RA, 2012. Common spiders of North America. 1<sup>st</sup> edition. University of California Press, Berkeley (CA), 288pp.

Brady AR. 2012. Neartic species of the new genus *Tigrosa* (Araneae: Lycosidae). The Journal of Arachnology. 40:182-208.

Bucklin A, Steinke D, Blanco-Bercial L. 2011. DNA barcoding of marine metazoan. Annual Review of Marine Science. 3:471-508.

Chamberlin RV, Ivey W. 1944. Spiders of the Georgia region of North America. Bulletin of the University of Utah. 35: 1-267.

Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR.1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*. **46**:419-437.

Corey DT, Taylor WK. 1988. Ground surface spiders in three central Florida plant communities. Journal of Arachnology. 16:213-221.

Curtis DJ. 1980. Pitfalls in spider community studies (Arachnida, Araneae). Journal of Arachnology. 8: 271-280.

Cushing PE, Graham MR, Prendini L, Brookhart JO. 2015. A molecular phylogeny of the endemic North American camel spider family Eremobatidae (Arachnida: Solifugae). Molecular Phylogenetics and Evolution. 92: 280-293.

Draney ML.1997. Ground-Layer spiders (Araneae) of a Georgia piedmont floodplain agroecosystem: species list, phenology and habitat selection. The Journal of Arachnology. 25:33-351.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology. 3:294-299.

Garrison NL, Rodriquez J, Agnarsson I, Coddington JA, Griswold CE. Hamilton CA, Kocot KM, Ledford JM, Bond JE. 2016. Spider phylogenomics: untangling the Spider Tree of Life. PeerJ. 4:e1719.

Gertsch WJ. 1979. American spiders. American Museum of Natural History, New York (NY). 274pp.

Graham AK, Buddle CM, Spence JR. 2003. Habitat affinities of spiders living near a freshwater pond. Journal of Arachnology. 31: 78-89.

Guillebeau LP, Roberts P, Hinkle NC. 2008. Summary of losses from insect damage and costs of control in Georgia, 2006. Georgia Agric. Exp. Stns. Spec. Publishers, available via: http://entomology.ent.uga.edu/IPM/sl00/sl00.htm

Hebert PD, Cywinska A, Ball SL, deWaard JR. 2003a. Biological identifications through DNA barcodes. Proceedings of the Royal Society. 270:313-321.

Hebert PD, Ratnasingham S, deWaard JR. 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society. 270(Suppl.1):S96–S99.

Hsiang S, Kopp R, Jina A, Rising J, Delgado M, Mohan S, Rasmussen DJ, Muir-Wood R, Wilson P, Oppenheimer M, et al. Estimating economic damage from climate change in the United States. Science. 356: 1362-1369.

Huelsenbeck J, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 17:754–755.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution. 16:111-120.

Lavinia PD, NuñezBustos EO, Kopuchian C, Lijtmaer DA, Natalia C, Garcia NC, Hebert PD, Tubaro PL. 2017.Barcoding the butterflies of southern South America: Species delimitation efficacy, cryptic diversity and geographic patterns of divergence. PLoS ONE. 12(10):e0186845. Maddison WP, Maddison DR. 2019. Version 3.61. Mesquite: a modular system for evolutionary analysis. <u>http://www.mesquiteproject.org</u>

Mitchell RJ, Liu Y, O'Brien JJ, Elliott KJ, Starr G, Miniat CG, Hiers JK. Future climate and fire interactions in the southeastern region of the United States. Forest Ecology and Management. 327:316-326.

Morphology and Physiology. c2015. Museum fur Naturkunde (Berline). International Society of Arachnology. <u>https://arachnology.org</u>.

Piacentini LN, Ramirez MJ. 2019. Hunting the wolf: a molecular phylogeny of the wolf spiders (Araneae, Lycosidae). Molecular Phylogenetics and Evolution. 136:227-240.

Pimental D, Zungia R, Morrison D. 2004. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics. 52:273-288.

Ratnasingham S, Hebert PD. 2007. BOLD: the barcode of life data system. Molecular Ecology Notes. 7:355-364.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012b. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology. 61:539–542.

Smith MA, Fisher BL, Hebert PD. 2005. DNA barcoding for effective biodiversity assessment of a hyper diverse arthropod group: the ants of Madagascar. Philosophical Transactions of the Royal Society B. 360:1825–1834.

Tietjen WI, Becker S, Muenz T, Golladay S. 2017. Observations on the spider fauna of geographically isolated wetlands in southwestern Georgia. Georgia Journal of Science. 75: Article 14.

Turner MG, Ruscher CL. 1988. Changes in landscape patterns in Georgia, USA. Landscape Ecology. 1: 241-251.

Ubick D, Paquin P, Cushing PE, Roth V. 2005. Spiders of North America: an identification manual. American Arachnological Society, Keene (NH). 377pp.

Vetter, RS. How to identify brown widow spiders. c2021. UC Riverside (CA): Center for invasive species research. 2018. [accessed 2021 June 6]. <u>https://cisr.ucr.edu/</u>.

Wheeler WC, Coddington JA, Crowley LM, Dimitrov D, Goloboff PA, Griswold CE, Hormiga G, Prendini L, Ramirez MJ, Sierwald P, et al. 2017. The spider tree of life: phylogeny of Araneae based on target-gene analyses from an extensive taxon sampling. Cladistics. 33:574-616.

World Spider Catalog. c2021. Version 22.0. Natural History Museum Bern: World spider catalog; [accessed 2021 June 6]. <u>http://wsc.nmbe.ch.</u>

## APPENDICES

## APPENDIX 1: FIELD COLLECTIONS

Ascension #	Collection	Collection	Collection	Collector	Location	Location	Location	Location	Habitat	Lat	Long
	day	month	year		Country	State	County	town/village			
ARA1	27	VIII	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4299N	81.7838W
				Hobbs	States						
ARA2	27	VIII	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4299N	81.7838W
				Hobbs	States						
ARA3	27	VIII	2017	Guy	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
				Hobbs	States						
ARA4	1	IX	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4258N	81.7794W
				Hobbs	States						
ARA5	27	VIII	2017	Guy	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
				Hobbs	States						
ARA6	10	IX	2017	Holly	United	Georgia	Screven	Sylvania	Miscellaneous	NA	NA
				Kight	States						
ARA7	10	IX	2017	Holly	United	Georgia	Screven	Sylvania	Miscellaneous	NA	NA
				Kight	States						
ARA8	10	IX	2017	Holly	United	Georgia	Screven	Sylvania	Miscellaneous	NA	NA
				Kight	States						
ARA9	10	IX	2017	Holly	United	Georgia	Screven	Sylvania	Miscellaneous	NA	NA
				Kight	States						
ARA10	24	IX	2017	Guy	United	Georgia	Macon	Macon	Miscellaneous	NA	NA
				Hobbs	States						
ARA11	30	IX	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4258N	81.7794W
			Hobbs	States							
ARA12	30	IX	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4258N	81.7794W
				Hobbs	States						
ARA13	23	IX	2017	Guy	United	Georgia	Fayette	Tyrone	Miscellaneous	NA	NA
				Hobbs	States						

ARA14	23	IX	2017	Guy	United	Georgia	Fayette	Tyrone	Miscellaneous	NA	NA
				Hobbs	States						
ARA15	23	IX	2017	Guy	United	Georgia	Fayette	Tyrone	Miscellaneous	NA	NA
				Hobbs	States						
ARA16	23	IX	2017	Guy	United	Georgia	Fayette	Tyrone	Miscellaneous	NA	NA
				Hobbs	States						
ARA17	27	VIII	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4299N	81.7838W
				Hobbs	States						
ARA18	27	VIII	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4299N	81.7838W
				Hobbs	States						
ARA19	27	VIII	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4299N	81.7838W
				Hobbs	States						
ARA20	27	VIII	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4299N	81.7838W
				Hobbs	States						
ARA21	27	VIII	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4299N	81.7838W
				Hobbs	States						
ARA22	27	VIII	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4299N	81.7838W
				Hobbs	States						
ARA23	28	VIII	2017	Guy	United	Georgia	Bulloch	Statesboro	Miscellaneous	32.4539N	81.7395W
				Hobbs	States						
ARA24	25	VIII	2017	Trang	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4258N	81.7794W
				Nguyen	States						
ARA25	3	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4258N	81.7794W
				Hobbs	States						
ARA26	3	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4258N	81.7794W
				Hobbs	States						
ARA27	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA28	ARA28 5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA29	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						

ARA30	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA31	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA33	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA34	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA35	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA36	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA37	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA38	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA39	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA40	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA41	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA42	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA43	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA44	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA45	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA46	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						

ARA47	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA48	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA49	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA50	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA51	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA52	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA53	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA55	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA56	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA57	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA58	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA59	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA60	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA61	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA62	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA63	5	XI	XI 2017 Guy United Georgia Bulle	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W			
				Hobbs	States						

ARA64	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA66	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA67	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA68	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA69	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA70	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA71	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA72	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA73	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA74	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA75	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA76	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA77	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA78	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA79	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA80	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						

ARA81	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA82	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA83	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA84	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA85	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA86	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA87	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA88	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA89	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA90	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA91	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA92	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA93	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA94	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA95	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA96	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						

ARA97	5	XI	2017	Guy	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Hobbs	States						
ARA101	12	II	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA102	8	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA103	28	VI	2011	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA104	28	VI	2011	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA105	28	VI	2011	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA106	28	VI	2011	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA107	28	VI	2011	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA108	28	VI	2011	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA109	28	VI	2011	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA110	8	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA111	8	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA112	11	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA113	11	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA114	21	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA115	8	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						

ARA116	21	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA117	12	II	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA118	11	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA119	15	VI	2010	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA120	15	VI	2010	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA121	-	-	2019	Donation	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
					States						
ARA122	-	-	2019	Donation	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
					States						
ARA123	-	-	2019	Donation	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
					States						
ARA124	-	-	2019	Donation	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
					States						
ARA125	-	-	2019	Donation	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
					States						
ARA126	-	-	2019	Donation	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
					States						
ARA127	8	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA128	8	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA129	8	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA130	2	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA131	-	-	2019	Donation	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
					States						

ARA132	-	-	2019	Donation	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
					States						
ARA133	-	-	2019	Donation	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
					States						
ARA134	-	-	2019	Donation	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
					States						
ARA135	-	-	2019	Donation	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
					States						
ARA136	6	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA137	6	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA138	6	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207V
				Walker	States						
ARA139	6	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207V
				Walker	States						
ARA140	11	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207V
				Walker	States						
ARA141	6	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207V
				Walker	States						
ARA142	6	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207V
				Walker	States						
ARA143	6	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207V
				Walker	States						
ARA144	6	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA145	6	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207V
				Walker	States						
ARA146	25	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207V
				Walker	States						
ARA147	21	111	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207V
				Walker	States						

ARA148	21	111	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA149	21	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA150	21	111	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA151	21	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA152	29	VI	2011	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA153	2	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA154	15	VII	2010	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA155	21	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA156	5	VII	2011	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA157	12	II	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA158	21	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA159	21	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA160	21	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA161	11	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA162	11	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA163	11	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						

ARA164	11	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA165	21	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA166	15	Х	2009	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA167	8	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA168	8	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA169	8	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA170	8	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA171	21	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA172	8	Х	2019	Lorenza	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
				Beati	States						
ARA173	15	VII	2010	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA174	15	VII	2010	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA175	15	VII	2010	Swiss	United	Georgia	Bulloch	Statesboro	Bird Pond	32.3981N	81.7715W
				Class	States						
ARA177	25	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA178	21	III	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA179	11	IV	2011	Daniel	United	Georgia	Bulloch	Statesboro	Mixed Forests	32.3520N	81.8207W
				Walker	States						
ARA201	11	IX	2018 Guy United Georgia Bulloch Statesboro Sandhill 32.5113	32.5113N	81.7129W						
				Hobbs	States						

ARA202	11	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA203	11	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA204	11	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA205	11	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA206	11	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA207	11	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA208	11	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA209	11	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA210	11	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA211	11	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA212	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA213	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA214	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA215	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA216	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA217	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						

ARA218	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA219	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA220	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA221	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA222	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA223	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA224	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA225	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA226	15	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA227	22	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Bird pond	32.3981N	81.7715W
				Hobbs	States						
ARA228	22	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Bird pond	32.3981N	81.7715W
				Hobbs	States						
ARA229	22	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Bird pond	32.3981N	81.7715W
				Hobbs	States						
ARA230	22	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Bird pond	32.3981N	81.7715W
				Hobbs	States						
ARA231	22	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Bird pond	32.3981N	81.7715W
				Hobbs	States						
ARA232	22	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Bird pond	32.3981N	81.7715W
				Hobbs	States						
ARA233	22	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Bird pond	32.3981N	81.7715W
				Hobbs	States						

ARA234	22	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Bird pond	32.3981N	81.7715W
				Hobbs	States						
ARA235	22	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Bird pond	32.3981N	81.7715W
				Hobbs	States						
ARA236	22	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Bird pond	32.3981N	81.7715W
				Hobbs	States						
ARA237	22	IX	2018	Guy	United	Georgia	Bulloch	Statesboro	Bird pond	32.3981N	81.7715W
				Hobbs	States						
ARA238	5	Х	2018	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA239	5	Х	2018	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA240	5	Х	2018	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA241	5	Х	2018	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA242	5	Х	2018	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA243	5	Х	2018	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA244	5	Х	2018	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	ts 32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA245	5	Х	2018	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			

ARA246	5	Х	2018	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA247	7	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA248	7	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA249	7	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA251	7	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA252	7	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA253	10	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA255	10	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA256	10	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA257	10	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA258	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA259'	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA259	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA260	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA261	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA262	19	Х	2018 Guy United Georgia Bulloch Statesboro Sandhill 32.511	32.5113N	81.7129W						
				Hobbs	States						

ARA263	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA264	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA265	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA266	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA267	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA268	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA269	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA270	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA271	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA272	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA273	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA274	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA275	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA276	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA277	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA278	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						

ARA279	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA280	19	Х	2018	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA281	3	IV	2019	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4258N	81.7794W
				Hobbs	States						
ARA282	3	IV	2019	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4258N	81.7794W
				Hobbs	States						
ARA283	3	IV	2019	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4211N	81.7898W
				Hobbs	States						
ARA285	3	IV	2019	Guy	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
				Hobbs	States						
ARA286	1	IV	2018	Lorenza	United	Georgia	Bulloch	Statesboro	Miscellaneous	NA	NA
				Beati	States						
ARA288	6	IV	2019	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4057N	81.7856W
				Hobbs	States						
ARA289	6	IV	2019	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4057N	81.7856W
				Hobbs	States						
ARA290	6	IV	2019	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4057N	81.7856W
				Hobbs	States						
ARA292	6	IV	2019	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4057N	81.7856W
				Hobbs	States						
ARA293	6	IV	2019	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4057N	81.7856W
				Hobbs	States						
ARA294	6	IV	2019	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4057N	81.7856W
				Hobbs	States						
ARA295	2	VIII	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA296	2	VIII	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA298	2	VIII	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						

ARA299	2	VIII	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA300	2	VIII	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA301	2	VIII	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA302	17	V	2019	Guy	United	Georgia	Bulloch	Statesboro	GSU Campus	32.4258N	81.7794W
				Hobbs	States						
ARA303	16	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA304	16	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA305	16	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA307	16	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA308	16	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA309	16	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA310	16	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA311	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA312	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA313	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA314	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA315	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						

ARA316	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA317	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA318	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA319	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA320	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA321	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA322	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA323	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA324	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA325	13	VI	2019	Guy	United	Georgia	Bulloch	Statesboro	Sandhill	32.5113N	81.7129W
				Hobbs	States						
ARA326	13	VI	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA327	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA328	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA329	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			

ARA330	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA331	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA332	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA333	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA334	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA335	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA336	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA337	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA338	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA339	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA340	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			

ARA341	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA342	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA343	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA344	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA345	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA346	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA347	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA348	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA349	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA350	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA351	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA352	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA353	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA354	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA355	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						
ARA356	23	V	2020	Guy	United	Georgia	Glynn	Brunswick	Saltwater Marsh	31.1674N	81.437W
				Hobbs	States						

ARA361	6	VI	2020	Guy	United	Georgia	Bulloch	Statesboro	Miscellaneous	32.4194N	81.775W
				Hobbs	States						
ARA362	6	VI	2020	Guy	United	Georgia	Bulloch	Statesboro	Miscellaneous	32.4194N	81.775W
				Hobbs	States						
ARA363	6	VI	2020	Guy	United	Georgia	Bulloch	Statesboro	Miscellaneous	32.4194N	81.775W
				Hobbs	States						
ARA364	6	VI	2020	Guy	United	Georgia	Bulloch	Statesboro	Miscellaneous	32.4194N	81.775W
				Hobbs	States						
ARA365	6	VI	2020	Guy	United	Georgia	Bulloch	Statesboro	Miscellaneous	32.4194N	81.775W
				Hobbs	States						
ARA366	6	VI	2020	Guy	United	Georgia	Bulloch	Statesboro	Miscellaneous	32.4194N	81.775W
				Hobbs	States						
ARA367	6	VI	2020	Guy	United	Georgia	Bulloch	Statesboro	Miscellaneous	32.4194N	81.775W
				Hobbs	States						
ARA368	6	VI	2020	Guy	United	Georgia	Bulloch	Statesboro	Miscellaneous	32.4194N	81.775W
				Hobbs	States						
ARA400	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA401	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA402	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			
ARA403	2	VIII	2019	Guy	United	Georgia	Jenkins	Magnolia	Mixed Forests	32.8828N	81.9601W
				Hobbs	States			Springs State			
								Park			

	H3A		COI	
ARA#	Species family	Species name	Species family	Species name
ARA3	Anyphaenidae	Anyphaenoides clavipes	Anyphaenidae	Hibana sp.
ARA5			Tetragnathidae	Tetragnatha elongata
ARA7	Lycosidae	Alopecosa sp.		
ARA8	Lycosidae	Alopecosa sp.		
ARA9	Lycosidae	Geolycosa sp.		
ARA10	Araneidae	Argiope aurantia	Araneidae	Argiope aurantia
ARA11	Filistatidae	Kukulcania hibernalis		
ARA13	Filistatidae	Kukulcania hibernalis		
ARA19	Theridiidae	Achaearanea tepidariorum	Theridiidae	Achaearanea tepidariorum
ARA21	Theridiidae	Achaearanea tepidariorum	Theridiidae	Achaearanea tepidariorum
ARA22			Agelenidae	Barronopsis sp.
ARA23			Theridiidae	Steatoda triangulosa
ARA24			Desidae	Metaltella simoni
ARA26			Araneidae	Gasteracantha cancriformis
ARA28			Tetragnathidae	Leucauge argyrobapta
ARA30			Araneidae	Gasteracantha cancriformis
ARA31	Salticidae	Lyssomanes viridis		
ARA33			Tetragnathidae	Leucauge argyrobapta
ARA34			Tetragnathidae	Leucauge argyrobapta
ARA35	Mimetidae	Mimetus sp.	Mimetidae	Mimetidae sp.
ARA36	Lycosidae	Tigrosa sp.	Lycosidae	Tigrosa sp.

## APPENDIX 2: BLAST SEARCH RESULTS FOR COI AND H3A

ARA38			Lycosidae	Schizocosa ocreata
ARA39			Araneae	Gea heptagon
ARA40	Araneidae	Gea heptagon	Araneidae	Gea heptagon
ARA41			Anyphaenidae	Anyphaena pectorosa
ARA42			Tetragnathidae	Leucauge argyrobapta
ARA43	Pisauridae	Dolomedes sp.		
ARA44			Tetragnathidae	Leucauge argyrobapta
ARA46			Tetragnathidae	Leucauge argyrobapta
ARA47			Anyphaenidae	Anyphaena pectorosa
ARA48			Anyphaenidae	Anyphaena pectorosa
ARA49			Tetragnathidae	Tetragnatha viridis
ARA50	Pisauridae	Dolomedes sp.		
ARA51	Pisauridae	Dolomedes sp.	Pisauridae	Dolomedes triton
ARA52	Araneidae	Araneidae sp.	Araneidae	Mangora placida
ARA57	Lycosidae	Lycosidae sp.		
ARA58	Lycosidae	Lycosidae sp.	Lycosidae	Schizocosa ocreata
ARA59			Tetragnathidae	Leucauge argyrobapta
ARA61	Lycosidae	Lycosidae sp.	Lycosidae	Lycosidae sp.
ARA62	Tetragnathidae	Tetragnatha sp.	Tetragnathidae	Tetragnatha viridis
ARA63			Tetragnathidae	Leucauge argyrobapta
ARA64	Pisauridae	Dolomedes sp.	Pisauridae	Dolomedes sp.
ARA66	Lycosidae	Lycosidae sp.	Lycosidae	Schizocosa ocreata
ARA67	Lycosidae	Pardosa sp.	Lycosidae	Pardosa milvina
ARA69	Lycosidae	Pardosa sp.		
ARA70	Anyphaenidae	Anyphaena sp.	Anyphaenidae	Anyphaena pectorosa
ARA72	Anyphaenidae	Anyphaena sp.	Anyphaenidae	Anyphaena pectorosa
ARA73	Anyphaenidae	Anyphaena sp.	Anyphaenidae	Anyphaena pectorosa

ARA74			Tetragnathidae	Tetragnatha elongata
ARA76	Salticidae	Lyssomanes viridis	Salticidae	Lyssomanes viridis
ARA76	Salticidae	Lyssomanes viridis		
ARA77	Linyphiidae	Frontinella pyramitela	Linyphiidae	Frontinella communis
ARA78	Anyphaenidae	Anyphaena sp.	Anyphaenidae	Anyphaena sp
ARA79	Pisauridae	Dolomedes tenebrosus	Pisauridae	Dolomedes triton
ARA80			Salticidae	Lyssomanes viridis
ARA81	Araneidae	Araneidae sp.	Araneidae	Mangora placida
ARA82	Salticidae	Lyssomanes viridis	Salticidae	Lyssomanes viridis
ARA83	Pisauridae	Dolomedes sp.	Pisauridae	Dolomedes triton
ARA84	Anyphaenidae	Anyphaena sp.	Anyphaenidae	Anyphaena pectorosa
ARA87			Tetragnathidae	Leucauge argyrobapta
ARA88			Tetragnathidae	Leucauge argyrobapta
ARA89	Lycosidae	Lycosidae sp.	Lycosidae	Lycosidae sp.
ARA90			Anyphaenidae	Aniphaenidae sp.
ARA91	Uloboridae	Uloboridae sp.		
ARA92			Araneidae	Mangora placida
ARA96	Salticidae	Salticidae sp.		
ARA101	Lycosidae	Varacosa avara	Lycosidae	Varacosa avara
ARA107	Pisauridae	Dolomedes tenebrosus		
ARA113	Oxyopidae	Oxyopidae sp.		
ARA115			Lycosidae	Schizocosa crassipes
ARA118	Lycosidae	Tigrosa sp.		
ARA123	Pisauridae	Dolomedes sp.		
ARA126	Pisauridae	Dolomedes sp.		
ARA128	Lycosidae	Varacosa avara	Lycosidae	Varacosa avara
ARA129	Thomisidae	Thomisidae sp.		
ARA130	Thomisidae	Thomisidae sp.	Thomisidae	Thomisidae sp.

ARA131	Lycosidae	Lycosidae sp.		
ARA138	Araneidae	Araneidae sp.		
ARA140	Lycosidae	Tigrosa sp.		
ARA147			Lycosidae	Schizocosa crassipes
ARA151			Lycosidae	Schizocosa crassipes
ARA158	Pisauridae	Pisaurina mira		
ARA178	Thomisidae	Thomisidae sp.		
ARA204			Theridiidae	Steatoda triangulosa
ARA210	Lycosidae	Alopecosa farinosa	Lycosidae	Schizocosa crassipes
ARA213	Thomisidae	Thomisidae sp.	Thomisidae	Mecaphesa asperata
ARA214	Pisauridae	Dolomedes sp.		
ARA221	Lycosidae	Lycosidae sp.		
ARA223	Lycosidae	Lycosidae sp.		
ARA228	Lycosidae	Lycosidae sp.	Lycosidae	Schizocosa crassipes
ARA229	Lycosidae	Lycosidae sp.		
ARA231	Lycosidae	Lycosidae sp.		
ARA234	Araneidae	Neoscona sp.	Araneidae	Neoscona crucifera
ARA249			Lycosidae sp.	Lycosidae sp.
ARA251	Araneidae	Neoscona sp.	Araneidae	Neoscona arabesca
ARA253	Lycosidae	Lycosidae sp.	Lycosidae	Lycosidae sp.
ARA256	Araneidae	Neoscona sp.	Araneidae	Neoscona domiciliorum
ARA267	Araneidae	Neoscona sp.	Araneidae	Neoscona domiciliorum
ARA276			Linyphiidae	Frontinella pyramitela
ARA281			Salticidae	Frigga crocuta
ARA282			Theridiidae	Steatoda triangulosa
ARA285	Theridiidae	Latrodectus geometricus	Theridiidae	Latrodectus geometricus
ARA286	Corinnidae	Corinnidae sp.		
ARA288	Oxyopidae	Oxyopidae sp.		

ARA289			Nephilidae	Trichonephila clavipes
ARA290			Linyphiidae	Neriene radiata
ARA292			Nephilidae	Trichonephila clavipes
ARA293	Oxyopidae	Peucetia viridans	Oxyopidae	Peucetia viridans
ARA294	Thomisidae	Xisticus sp		
ARA295	Oxyopidae	Peucetia viridans		
ARA296			Corinnidae	Falconina gracilis
ARA299				
ARA300	Clubionidae	Clubionidae sp.		
ARA304	Thomisidae	Thomisidae sp.		
ARA305			Thomisidae	Synema parvulum
ARA307			Thomisidae	Synema parvulum
ARA308	Oxyopidae	Oxyopes sp	Oxyopidae	Oxyopes sp.
ARA309	Linyphiidae	Neriene radiata	Linyphiidae	Neriene radiata
ARA310	Oxyopidae	Peucetia viridans	Oxyopidae	Peucetia viridans
ARA313	Thomisidae	Thomisidae sp.	Thomisidae sp.	Thomisidae sp.
ARA319	Oxyopidae	Peucetia viridans	Oxyopidae	Peucetia viridans
ARA321	Theridiidae	Latrodectus sp.	Theridiidae	Latrodectus variolus
ARA323	Salticidae	Salticidae sp.	Salticidae	Phidippus princeps
ARA327	Oxyopidae	Peucetia viridans	Oxyopidae	Peucetia viridans
ARA332	Salticidae	Paraphidippus aurantius	Salticidae	Paraphidippus aurantius
ARA333	Lycosidae	Lycosidae sp.		
ARA340			Araneidae	Araneidae sp
ARA342	Araneidae	Araneidae sp.		
ARA343	Araneidae	Araneidae sp.		
ARA344	Araneidae	Araneidae sp.		
ARA350	Araneidae	Araneidae sp.	Araneidae sp	Araneidae sp.
ARA354	Salticidae	Salticidae sp.		

ARA363	Salticidae	Paraphidippus aurantius		
ARA365			Filistatidae	Kuculcania hibernalis
ARA368			Theridiidae	Steatoda triangulosa
ARA400	Theridiidae	Steatoda triangulosa	Theridiidae	Steatoda triangulosa
ARA401	Theridiidae	Steatoda triangulosa		
ARA403	Theridiidae	Steatoda triangulosa	Theridiidae	Steatoda triangulosa