Cantaurus, Vol. 29, 6-9, May 2021 © McPherson College Department of Natural Science

DNA barcode sequences of the spiders in the genus *Schizocosa*

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ABSTRACT

DNA barcoding is the process of using a unique short segment of DNA to identify a specimen and is commonly used by researchers to identify the different species they are studying. The genus *Schizocosa* of the wolf spider family, Lycosidae, have very similar morphologies making them hard to differentiate by species. For this study we extracted DNA from 45 samples of five different species of *Schizocosa.* The samples were amplified through PCR using LepF and LepR primers and then run through gel electrophoresis. The Samples were sent off for analysis and sequencing and came back with no results. The quality of DNA extraction can affect how well the PCR amplification works. The possibilities for impurities in the samples from extraction can also lead to less DNA being barcoded.

Keywords: DNA Barcoding*, LepF and LepR primers, Schizocosa.*

INTRODUCTION

DNA barcoding is the process of using a unique short segment of DNA to identify a given specimen. Starting in 2003, the idea of DNA barcoding related the uniqueness of DNA sequences to that of barcodes on supermarket items (Stoeckle 2011). DNA barcoding has been used to identify plants, animals, insects, and fungi (Madden 2019). DNA barcoding has also been very beneficial in both pure scientific research, aiding in species identification and species discovery (Goldstein 2019) as well as functional applications for society, including the identification of fungal pathogens on humans (Madden 2019), and the identification and authentication of plants for medical use (Vassou 2016). Also, government agencies such as Customs use DNA barcode sequencing as a way to identify potentially harmful insects and organisms coming into the country from foreign lands. With the access to the barcoding databases, Customs are able to identify the species accurately and efficiently to alleviate any sort of potential threat to our environments (Madden 2019).

Barcodes within specific species are expected to be very similar as they are specific to the species, but also expected to have slight variance due to mutations and replication errors. Based on research from Haiibabaei (2006), barcode sequences within the speices had a maximum variation of 0.46%. Species close together in the genus tree would be expected to have more similarities in their barcodes than species farther on the genus tree (Stratton, 2005). Once the barcodes are published, the publication would potentially create an identification process that is easier than the identification of species through morphology. Different genes are used based on the taxa when acquiring the barcode. For example, If the sample is a plant, rbcL and matK are recommended. If the sample is from an animal COI is recommended (CSH 2018).

The genus *Schizocosa* is just one genus of many in the wolf spider family Lycosidae. There are 27 different species in the *Schizocosa* genus. The genus is found all across North America. While some of the species are secluded to one or two states, some of the species span across multiple states and even into Canada (Eaton 2015). The *Schizocosa* genus has a wide range of habitats. An example is the species *Schizocosa ocreata* prefers upland forest areas and the species *Schizocosa rovneri* prefer bottom land forest and river flood plains. The species within the *Schizocosa* genus also have different breeding times and courtships, to potentially keep from crossbreeding (Uetz 1979). The different species within the genus can be challenging to differentiate morphologically. Identification of the species often requires the use of mature reproductive structures in males and females. Typically, juveniles of several species are found overlapping in nature. This makes it harder to identify them morphologically because they have not developed mature physical characteristics. With DNA barcoding, DNA could be used as a way to differentiate the species (Madden 2019).

I plan to take DNA extractions from different species in the *Schizocosa* genus to perform DNA sequencing. I will then use the DNA sequences to compare the separate species for differences among the DNA.

MATERIALS AND METHODS

Sample Collection

Specimens of five different species of *Schizocosa* were used in the study*; Schizocosa rovneri* (*N = 10), Schizocosa retrorsa (N = 9), Schizocosa uetzi (N = 5), Schizocosa ocreata (N = 8),* and *Schizocosa stridulans (N = 13).* Specimens were collected in the year range of 1985 to 2005 from Lafayette, Marshall, and Panola Counties in Mississippi, Henderson

County in Tennessee and Mason County in Illinois, and stored in alcohol for DNA isolation.

Isolation of DNA

Ten to 20mg of tissue were collected from the abdomens of the samples for DNA extraction. The samples were placed in separate 1.5mL tubes and labeled. 300uL of lysis solution was added to the samples, this is used to break down the cells for DNA to be exposed. The samples were grinded for 2 minutes with a plastic pestle and incubated in a water bath at 65°C for 10 minutes. The samples were then centrifuged for 1 minute at maximum speed. After centrifuging, the supernatant from the samples was transferred to a clean labeled tube. 3uL of silica resin was added to the supernatant and incubated for 5 minutes at 57°C. The silica resin is used to attract the DNA by the DNA molecules binding to the silica. The samples were then centrifuged for 30 seconds at maximum speed and the supernatant was removed. 500uL of the ice wash buffer was added to the pellet in the sample and then vortexed till the pellet was suspended. The sample then was centrifuged for 30 seconds at max speed. The supernatant was then removed and 500uL of ice wash buffer was added to the pellet. The pellet was then vortexed till suspended and centrifuged for 30 seconds at maximum speed. The supernatant was removed. This step is to eliminate unwanted material as the DNA should be bound to the silica in the pellet. 100uL of distilled water was added to the silica resin and mixed by vortex. The samples were then incubated in a water bath at 57°C for 5 minutes. The samples were centrifuged for 30 seconds at maximum speed. 90uL of the supernatant was transferred to a clean labeled tube and stored in -20°C till ready for amplification. This process was completed for all samples collected.

Amplification of DNA through PCR

The LepF and LepR primers were used for PCR amplification because of their success with spiders (Robinson, 2009). The forward primer sequence: LepF (5' ATT CAA CCA ATC ATA AAG ATA TTG G 3') and the reverse primer sequence; LepR (5' TGA TTT TTT GGA CAT CCA GAA GTT TA 3') were used in PCR amplification (Strutzenberger 2012). The PCR bead from the DNA kit was mixed with a LepF and LepR primer created using 3ul of working solution of LepF, 3ul of working solution of LepR, 5ul of the template, and 14ul of TE buffer. The mix was run through the PCR thermal cycling. The sample was run at 94°C for a minute, 5 replicates of 94°C for a minute, 45°C for 40 seconds, and 72°C for one minute. Then 35 cycles of a 94°C for a minute, 51°C for 40 seconds and 72°C for a minute. Then a final round of 72°C for five minutes for amplification of the DNA (Robinson, 2009). After amplification, the samples were stored at -20°C till ready to run electrophoresis. A second amplification was done on left over samples from each species. Two samples from each species were used for PCR amplification by mixing 22.5ul of insect-mammal COI, 2.5ul of the sample, and the PCR bead. The samples were then run through thermal cycling at 94°C for 30 seconds, 54°C for 45 seconds, and 72°C for 45 seconds for 35 cycles. After amplification the samples were stored at -20°C till read to run electrophoresis.

Analysis of PCR through Electrophoresis

2% agarose gel was created and added to gel-casting trays with well-forming combs to make five gels for electrophoresis testing. The gels were placed in electrophoresis chambers and 1xTBE buffer was added to the chamber to just covering the gel surface. 20ul of marker pBR322/BstNI was placed in the left most well of each gel and 5ul of the separate PCR samples were placed in the remaining wells. The electrophoresis was running at 130V till the marker reached three fourths of the way down the gel. The gels were then stained with Ethilium Bromide for 30 minutes each and stored till observed under UV light for DNA.

Sequencing PCR

The samples collected were labeled and sent to the DNA Learning Center for sequencing through the DNA subway.

RESULTS

DNA samples were extracted from *Schizocosa rovneri* (*N = 10), Schizocosa retrorsa (N = 9), Schizocosa uetzi (N = 5), Schizocosa ocreata (N = 8),* and *Schizocosa stridulans (N = 13).* The 45 samples were primed using the LepF anf LepR primers. After analysis with the electrophoresis gel, two of each species samples were primed with COI and analysed using electrophoresis. After PCR amplification there was only one sample that showed any recognizable bands (Figure 1). All the samples were sent off for sequencing, however, there were no results from the sequencing reactions due to no priming.

DISCUSSION

The samples extracted for amplification and sequencing came back with no results. There was no priming of the DNA extracted resulting in nothing able to be amplified and sequenced.

There are several factors that can contribute to the lack of DNA in the samples for sequencing such as the impurities in the DNA extraction and weak primers. In Burbach's research in 2015, they analyzed the results of 15 different manual DNA extraction kits. During the research they found that the quality of the DNA extracted can affect the effectiveness of the PCR. In

Figure 1. Stained Gel following PCR reaction.

extracted can affect the effectiveness of the PCR. In the research they found that different contaminations such at protein and reagents during extraction effected purity of the DNA extracted. In Bork's research in 2015 and Blagoev's research in 2009, they used a leg from the spider for DNA extraction while I used the abdomen for DNA extraction. This could have led to a higher rate of contamination in the samples. Burbach's research used absorbance to test the purity of the DNA extracted. When there were traces of protein contamination, the ratio of a 260/280, used for reading the purity of nucleic acids, was less than 1.0, meaning there are impurities in the samples (Burbach 2015). Burbach's research shows an example of how impurities can happen in DNA extraction kits. This could have led to the lack of DNA for sequencing and amplification. Research from Spooner in 2009 suggests that using DNA barcoding to identify and differentiate morphologically similar species can be complicated and lead to misreading of the species. DNA barcoding relies on well-defined species and when a new species is being sequenced there is a lack in DNA sequences that can be used for defining similar species (Spooner 2009).

According to research from Blagoev's research in 2009, very little has been done on DNA barcoding with spiders. As of 2009, there had only been one major study of spiders with barcoding for identification and there were several aspects that restricted the results. The study had a very low diversity and only five genera with more than four species (Blagoev 2009). The study also only took samples from a localized location, bring up the question of the effectiveness of identification through barcoding across vast areas.

Another complication could be with the Lep primers with the samples. In a study from Strutzenberger in 2012, none of the samples that used the LepF and LepR primers had amplification for sequencing. The study believes this to be due to the short fragments from the DNA collected. Another study showed that mixing different primers together created a better success rate for DNA amplification. The study used different combinations with the LCO 1490, HCO-700ME, Chelicerate Forward and Reverse, and Lepidoptera Forward and Reverse primers (Bork 2015). The study had 43 of the 43 samples amplified when using the LCO 1490 and HCO-700ME primer mixture. When using the LepF and LepR primer mixture, the study had zero of 43 samples amplified (Bork 2015). The other successful mixture was using the LCO 1490 and Chelicerate Reverse which had 29 of 43 samples amplified (Bork 2015).

During the Robinson study, there were four different primers used with the spiders; COI, M13, a universal metazoan primer, and Lep (Blagoev 2009). From the research of Boutin-Ganache in 2001, the use of M13 primers had better results microsatellite analysis than samples without the M13 primer. This could lead to better amplification and sequencing in DNA samples if there is the use of M13 primers.

The research with not result in DNA amplification and sequencing for barcoding, but it led to the discovery of different methods of DNA extraction, testing for the presence of DNA, and different primers to use for the DNA barcoding of spiders.

ACKNOWLEDGEMENTS

I would like to thank McPherson College and the McPherson College Natural Science Department with providing me with the resources for my research. I would also like to thank my advisor Dr. Wilgers for his support and feedback during this process.

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