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Non-destructive DNA extraction methods that yield DNA barcodes in spiders

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NON-DESTRUCTIVE DNA EXTRACTION METHODS THAT YIELD DNA BARCODES IN SPIDERS

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Abstract

DNA barcodes are short sequences of nucleotides that differ from species to species (Hebert et al., 2003; Hebert & Gregory, 2005). DNA barcoding is very important in helping to reconstruct phylogenetic trees and to confirm the identity of threatened or endangered species in the wild. Additionally, it has become increasingly popular in the food industry to test the accuracy of the food being sold in fast food chains (Wong and Hanner, 2008). For scientific purposes, non-destructive DNA extraction techniques need to be explored because they allow for preservation of the voucher specimen, which is particularly important for rare or extinct museum specimens. Using a commercial DNA extraction kit could make the process of extracting DNA from small voucher specimens more regulated, and thus more universal. It is already known that DNA can be extracted from spiders in a non-destructive manner. This study addressed whether or not DNA can be extracted from small spiders (1-3 millimeters in size) in a non-destructive manner using a commercial DNA extraction kit, such as the Qiagen DNeasy Blood and Tissue Kit. From this research, it was determined that DNA can be extracted in a non-destructive manner using a commercial kit in fresh collections of spiders, thus allowing DNA barcoding while saving the specimen as a voucher. However, in preserved voucher specimens, four years or older, non-destructive DNA extraction methods using a commercial kit were not as effective, preventing DNA barcoding.

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Introduction

In a world where there are millions of species, DNA barcoding is one way that current species can be confirmed and new species can be discovered and logged (Hebert, Cywinska, & Ball, 2003; Barrett & Hebert, 2005; Hebert & Gregory, 2005; Rowley et al., 2007). DNA barcodes are short sequences of DNA nucleotides that are unique to a certain species (Hebert et al., 2003; Hebert & Gregory, 2005). These unique sequences, along with morphological descriptions, can then be entered into a database to help differentiate species and fill in gaps in phylogenetic trees (Hebert et al., 2003; Hebert & Gregory, 2005). DNA barcoding works by extracting mitochondrial DNA and amplifying the *cytochrome c oxidase I* (COI) gene via polymerase chain reaction (PCR; Hebert et al., 2003; Barrett & Hebert, 2005; Hajibabaei et al., 2005; Mitchell, 2008; Wong & Hanner, 2008; Moulton, Song, & Whiting, 2010; Zeale, Butlin, Baker, Less, & Jones, 2011; Newmaster, Grguric, Shanmughanandhan, Ramalingam, & Ragupathy, 2013). This gene is ideal to barcode because it is found in all animal species and has regions of conserved sequence that allow amplification with common primers (Hebert et al., 2003; Barrett & Hebert, 2005; Hebert & Gregory, 2005; Mitchell, 2008).

It was already known that DNA could be extracted from spiders in a non-destructive manner. The question to be answered from research was whether or not DNA could be extracted from small spiders (1-3 millimeters in size) in a non-destructive manner using a commercial DNA extraction kit, such as the Qiagen DNeasy Blood and Tissue Kit.

In the realm of spiders, DNA barcoding had the potential to be a more accurate way to identify what species a smaller organism belongs to (Rowley et al., 2007). Ideal candidates for this experiment were small in size (1-3 millimeters in size), and were not good candidates to use a leg for DNA extraction because of the small amount of DNA contained in their legs. Not

destroying these small organisms was important because many of them were voucher species that must be kept (Rowley et al., 2007).

The purpose of this quantitative research was to extract sequence-able DNA, known as a DNA barcode, from small spiders using non-destructive DNA extraction techniques and the Qiagen DNeasy kit (Qiagen, 2006). The DNA extracted from these spiders could be entered into a national database, such as GenBank or the Consortium for the Barcode of Life (CBOL), in order to identify existing species and confirm new species. Non-destructive DNA extraction techniques needed to be explored because they allow for preservation of the voucher specimen. In addition, rare or extinct museum specimens are ideal candidates for non-destructive DNA extraction techniques because these techniques do not harm the specimen. Additionally, using a commercial DNA extraction kit could make the process of extracting DNA from small voucher specimens more regulated, and thus more universal.

The significance of this study is that it would allow non-destructive DNA extraction techniques to become more universal. This would be especially important in logging DNA barcodes of museum specimens or voucher collections into GenBank or CBOL. With a more universal non-destructive DNA extraction method, DNA could easily be extracted from museum specimens that have gone extinct and voucher specimens that should not be harmed. Additionally, a more streamlined DNA extraction technique could allow small labs to extract DNA from several hundred-thousand samples a year, which would result in a more complete phylogenetic tree.

In looking at the research that has already been done on this topic, four distinct areas of research became apparent: what DNA barcodes are, efficacies of DNA extraction techniques, non-destructive DNA extraction techniques, and DNA extraction of spiders to gain DNA

barcodes. From looking broadly at the topic of non-destructive DNA extraction techniques, it is observed that little research has been done on whether or not DNA can be extracted using non-destructive methods and a commercial DNA extraction kit. When the topic is narrowed even further, it becomes evident that there is little existing research on the use of non-destructive DNA extraction techniques using a commercial kit in spiders.

Literature Review

What is a DNA Barcode?

In recent years, the importance of DNA barcoding has increased because of the need to make as complete of a phylogenetic tree as possible in order to differentiate species and discover new species (Hebert et al., 2003; Hebert & Gregory, 2005). DNA barcodes are short sequences of nucleotides that differ from species to species (Hebert et al., 2003; Hebert & Gregory, 2005). A DNA barcode can be obtained by extracting DNA from the COI gene of most animals (Hebert et al., 2003; Hebert & Gregory, 2005). The COI gene is ideal for DNA barcoding because it provides a greater range of phylogenetic signal, and in order to obtain a DNA barcode, the DNA sequence must differ enough at genetic markers to show species resolution (Hebert et al., 2003; Mitchell, 2008; Casiraghi, Labra, Ferri, Galimberti, & De Mattia, 2010; Zeale et al., 2011). In addition, the COI gene has a high incidence of third-position base substitutions, so it has a higher rate of molecular evolution than 12s or 16s rDNA (Hebert et al., 2003). Lastly, the COI gene is ideal for DNA barcoding because there are many copies of the mitochondrial genome in each cell (Hajibabaei et al., 2005).

DNA barcoding is unique because it is one of the first tests that is accurate enough to differentiate between many different animals at the species level (Wong & Hanner, 2008). In fact, it has been estimated that DNA barcoding can provide species-level resolution in 95-97% of cases (Hebert & Gregory, 2005). DNA barcoding uses a standardized methodology to enter these "molecular identification tags" into a universal database (Hebert et al., 2003; Hebert & Gregory, 2005; Mitchell, 2008; Wong & Hanner, 2008; Dittrich-Schroder, Wingfield, Klein, & Slippers, 2012). Thus, reference sequences of the same species can be used to ensure that correct species identifications are made (Mitchell, 2008; Zeale et al., 2011; Dittrich-Schroder et al., 2012).

Furthermore, DNA barcoding and the use of DNA sequencing libraries, such as GenBank and CBOL, allow for quicker identification and differentiation of new species (Hebert & Gregory, 2005; Mitchell, 2008; Dittrich-Schroder et al., 2012). In contrast, DNA barcoding can also be used to prevent mislabeling an old species as a new species. Actually, DNA barcoding makes it easier to identify morphologically different sexes and life stages of the same species and can help clarify problems of synonymy (Hebert et al., 2003; Hebert & Gregory, 2005; Casiraghi et al., 2010; Dittrich-Schroder et al., 2012).

DNA barcoding has made taxonomic systems more accessible, which is beneficial to ecologists, conservationists, and food safety companies (Hebert & Gregory, 2005; Mitchell, 2008). The use of DNA barcoding is not just limited to science. Commercially, DNA barcoding can be used to detect contamination and substitutions in herbal products (Newmaster et al., 2013). In recent years, DNA barcoding has been used to detect market substitutions in North American seafood (Wong & Hanner, 2008). In fact, DNA barcoding was recently used to confirm that a toxic puffer fish had been smuggled into a Chicago market illegally and under a false name (Wong & Hanner, 2008). DNA barcoding is ideal for detecting product contamination and substitution because it is widely accessible as a commercial tool that is fast and cost effective (Hebert & Gregory, 2005; Wong & Hanner, 2008; Newmaster et al., 2013). These findings are important because contaminations and substitutions in herbal products and seafood markets can be a health risk to consumers and may not be approved by the Food and Drug Administration (FDA; Newmaster et al., 2013). Currently, there are no standards for the authentication of herbal products (Newmaster et al., 2013). However, the contaminants and substitutions that DNA barcoding has uncovered may urge the herbal industry to reconsider their

lack of standards. Clearly, DNA barcoding has made an impact in the realm of taxonomy and in market substitutions commercially.

Efficacies of DNA Extraction Techniques

Since DNA barcoding is fairly new in the field of science, the efficacy of DNA barcoding and DNA extraction is a big topic. Because DNA barcoding is so standardized, a generalization of the protocol for DNA barcoding would allow for more identifications to be made (Mitchell, 2008; Casiraghi et al., 2010). As DNA extraction is the critical first step in all DNA barcodes, it seems obvious that streamlining DNA extraction protocols could help standardize DNA barcoding (Ivanova, Dewaard, & Hebert, 2006; Lucentini, Caporali, Palomba, Lancioni, & Panara, 2006).

There are several facets that an ideal DNA extraction protocol should follow. For instance, an ideal DNA extraction protocol should yield the most DNA possible, limit amounts of DNA degraded, be cost-effective, and efficient in terms of time and labor (Chen, Rangasamy, Tan, Wang, & Siegfried, 2010). Additionally, DNA extraction protocols should not use toxic chemicals or produce hazardous wastes (Chen et al., 2010; Dittrich-Schroder et al., 2012).

Since the aim of DNA extraction is to purify the DNA by binding it to a membrane or by fractionation (Hajibabaei et al., 2005), a streamlined DNA extraction protocol, such as one that uses a commercial kit, is more cost-effective and allows for hundreds of thousands of samples to be barcoded by the same facility each year (Hajibabaei et al., 2005; Wong & Hanner, 2008). There are several advantages to using a commercial kit. The Qiagen DNeasy Kit is convenient, efficient, and does not require the use of a fume hood (Chen et al., 2010). The Qiagen DNeasy kit purifies DNA by binding DNA to the membrane of silica (Hajibabaei et al., 2005, Qiagen, 2006). In general, silica-based approaches are ideal for high-throughput DNA barcoding of small

tissue samples (Hajibabaei et al., 2005). In addition, silica-based approaches, such as the Qiagen DNeasy Blood and Tissue Kit, have been found to be the best for extracting DNA from archived specimens (Hajibabaei et al., 2005). Thus, the Qiagen DNeasy kit is appropriate for this project.

Non-destructive DNA Extraction Techniques

There is one problem with the methods used in DNA barcoding; DNA barcoding often involves destroying the organism from which DNA is being extracted (Phillips & Simon, 1995; Rholand, Siedel, & Hofreiter, 2004; Rowley et al., 2007; Hunter, Goodall, Walsh, Owen, & Day, 2008; Miller, Beentjes, van Helsdingen, & IJland, 2013). This is problematic because specimens that are caught are often used as a voucher for a new species (Rowley et al., 2007; Hunter et al. 2008, Casquet, Thebaud, & Gillespie, 2012). Additionally, barcoding an endangered species or an ancient organism that resides in a museum should be avoided to protect the integrity of the museum collection (Wasko, Martins, Oliveira, & Foresti, 2003; Rohland et al., 2004; Hajibabaei et al., 2005). As a result, finding a non-destructive way to extract DNA from an organism for DNA barcoding is important (Phillips & Simon, 1995).

Several studies have shown that barcoding can be done with DNA that is extracted in a non-destructive manner (Phillips & Simon, 1995; Rholand et al., 2004; Pons, 2006; Gilbert, Moore, Melchior, & Worobey, 2007; Rowley et al., 2007; Hunter et al., 2008). However, as stated above, often times these experiments use harmful chemicals, such as phenol and chloroform, require overnight incubation periods, and are not universal (Hunter et al., 2008; Castalanelli et al., 2010). Additionally, in the majority of these experiments, DNA was not extracted with the use of commercial kits. In fact, little research has been done on whether or not DNA can be extracted in a non-destructive manner with a commercial kit (Lucentini et al., 2006; Castalanelli et al., 2010). Extracting DNA in a non-destructive manner with the use of a

commercial kit, such as the Qiagen DNeasy Blood and Tissue Kit, could make non-destructive DNA extraction quicker, more universal and more ideal for museum specimens, endangered species, and voucher specimens (Chen et al., 2010). Thus, by using this mechanism, species, old and new alike, could be barcoded efficiently.

DNA extraction of Spiders to Gain Barcodes

Since there are about 37,000 identified species of spiders and 40,000 suspected species of spiders, barcoding each and every one of these species is a huge undertaking (Barrett & Hebert, 2005). DNA barcoding is ideal in spiders because of the complexity of their morphology. Often, spider species cannot be differentiated unless the spider is an adult (Barrett & Hebert, 2005). This poses a problem because spiders at any other life stage cannot be differentiated. Additionally, adult spiders molt and grow as they age, which can make morphological differentiation approaches nearly impossible (Barrett & Hebert, 2005). Lastly, in many species of spiders, male and female spiders have differences in their morphologies that create challenges when trying to determine their species (Barrett & Hebert, 2005). Thus, DNA barcoding makes the most sense in helping differentiate spider species (Barrett & Hebert, 2005).

When trying to extract DNA from spiders, often time legs are removed to obtain DNA usable for DNA barcoding (Casquet et al., 2012; Miller et al., 2013). This is problematic because small spiders do not have enough DNA in their legs to produce a suitable DNA barcode. Thus, in order to extract enough DNA to barcode a small spider, the whole voucher specimen is crushed to obtain DNA (Castalanelli, et al., 2010). However, this technique is not ideal because it ruins the entire voucher specimen (Rholand et al., 2004; Castalanelli et al., 2010). Yet, research shows that DNA can be extracted from a small spider without removing its legs or crushing it (Castalanelli et al., 2010). In fact, studies show that non-destructive, whole body DNA

extractions of small spiders do not do any significant damage to the spider, which makes non-destructive DNA extraction techniques ideal for museum collections and voucher specimens (Miller et al., 2013). However, the amount of DNA that is extracted can be prohibitively small when derived from a non-destructive DNA extraction technique.

Recent studies have shown that DNA has been extracted from museum collections of spiders, so fresh DNA is not necessary when attempting to barcode older spiders (Miller et al., 2013). This is important because these results show that voucher specimens or museum collections need not be harmed while extracting DNA. However, the tissue available for DNA extraction expires faster in smaller spiders than in larger spiders, so finding a non-destructive, commercial DNA extraction method that obtains enough DNA for barcoding is critical (Miller et al., 2013).

One way to ensure that small quantities of DNA will work for downstream analyses is to use a more specific primer. When extracting DNA that is usable for a DNA barcode sequence, primer design is critical to ensure amplification of the correct fragment during PCR (Hajibabaei et al., 2005; Casiraghi et al., 2010; Moulton et al., 2010). Using a primer that is more specific to spiders could allow for complete sequencing of mitochondrial genomes when there is less DNA available (Hajibabaei et al., 2005; Moulton et al., 2010). When barcoding spiders, several studies have been successful using a standard forward primer, such as LCO1490 (5′-GGTCAACAAATCATCATAAAGATATTGG-3′), and a spider-specific reverse primer, such as chelicerate reverse 2 (5′-GGATGGCCAAAAAATCAAAATAAATG-3′; Barrett & Hebert, 2005; Miller et al., 2013). Thus, spiders are an ideal candidate for non-destructive DNA extraction techniques using a commercial kit.

Methodology

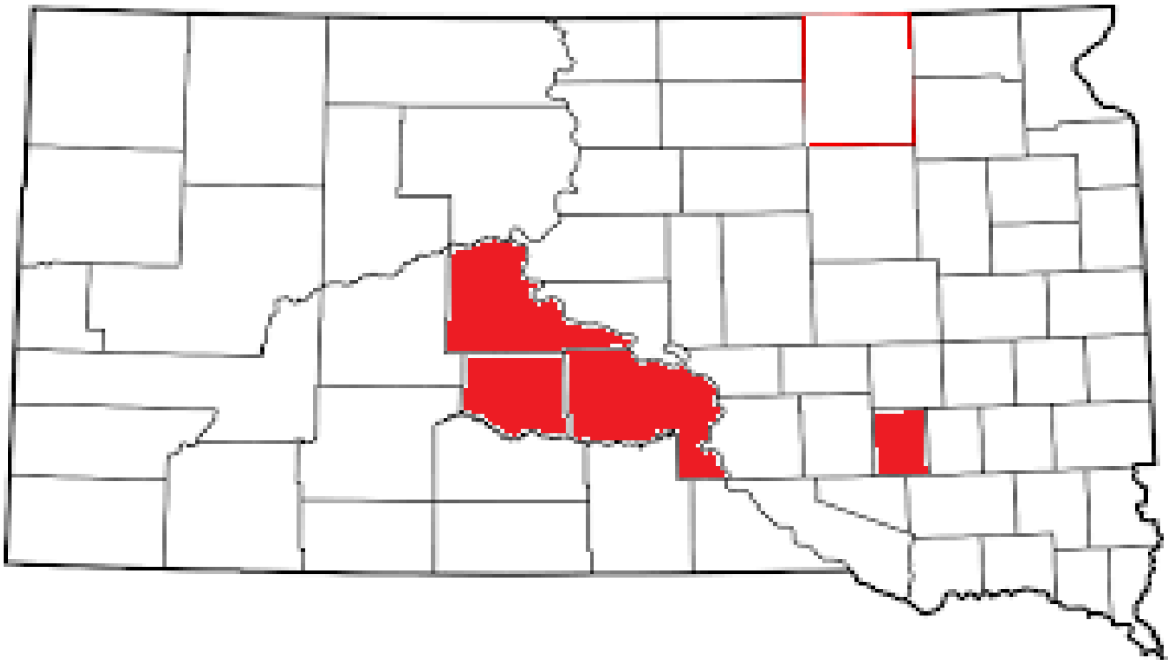
Fresh Collections

Fresh collections of spiders (1-3 mm in size) were caught in Mitchell, South Dakota, by hand, aspirator, or trap. After being caught, the samples were kept in 70% ethanol as a preservative until they were worked on. There were 17 fresh specimen tested.

Voucher Collections

Small spiders (1-3 mm in size) were selected from the voucher collection of Dr. L. Brian Patrick at Dakota Wesleyan University. These spiders were all four years old and were previously collected in Fort Pierre National Grasslands in Stanley County, Jones County, or Lyman County in South Dakota. These spiders were caught by a trap and identified by Dr. L. Brian Patrick. The specimens were stored in closed vials in 70% ethanol. These spiders were stored in a cabinet and were not exposed to light. There were 46 voucher specimen tested.

Figure 1. Stanley, Lyman, Jones and Davison County in South Dakota



Photographic Evidence

Before DNA extraction, each specimen was photographed under a Leica microscope. Specimens were removed from the vials using pipettes. Then, specimens were stuck in petroleum jelly and ethanol to prevent movement and to raise resolution. Next, the dorsal and ventral side of each specimen was photographed. After DNA extraction, the specimens were photographed on their dorsal and ventral sides again. If the legs of the spiders look more transparent in the after photographs, it was an indication that DNA was extracted.

DNA Extraction

DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, 2006). For destructive samples, all protocols were followed except: instead of 200 µL, only 100 µL of Buffer AE were added after the last extraction step to increase the final DNA concentration in the elute. For non-destructive samples, all protocols were followed except: the whole specimen was incubated rather than grinding up the specimen, the sample was allowed to incubate for 4 hours instead of 3 hours, the sample was mixed every hour using a pipette rather than a vortexer, and, after incubation, the sample was pulled out of the test tube before vortexing instead of vortexing the specimen with the sample. In order to avoid destroying small specimens, samples were placed into their microcentrifuge tubes via a pipette. This ensured that DNA was extracted in a non-destructive manner using a commercial kit. The amount of DNA extracted was measured using a Qubit Fluorometer, which can detect amounts of DNA equal to or greater than 25 ng/µL.

PCR

PCR was performed using the Takara *Taq* reagents. PCR was done on 25 µL reactions, and all Takara reagents and DNA template were calculated accordingly. As such, 421.875 µL of water,

62.5 µL of Taq buffer, 50 µL of deoxyribonucleotides, 12.5 µL of each primer, and 3.125 µL of Taq were mixed into a stock solution that performed 25 reactions each with a total volume of 25 µL. For each sample, 2.5 µL of DNA template were added to the PCR tube. The primers used were LCO-1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') (Folmer, BlacK, Hoeh, Lutz, & Vrijenhoek 1994) and Chelicerate Reverse 2 (5'-CCTCCTCCTGAAGGGTCAAAAAATGA-3') (Barrett & Hebert 2005), which have been shown to work well on spiders. PCR was performed with initial denaturing at 94°C for 60 seconds. PCR then went through 32 cycles of denaturing at 94°C for 45 seconds, annealing at 48°C for 45 seconds, and extension at 72°C for 30 seconds. The final extension was done at 72°C for 300 seconds. When PCR was complete, samples were refrigerated at -20°C. PCR sequencing was done by the CORE sequencing facility at Black Hills State University. Barcode sequences will be uploaded to GenBank. Samples C49-C60, C182-C183, C194-C204, C206, C208-C209, and C211-C212 were ran through another PCR machine after it was discovered that the first PCR machine broke.

Results

Fresh Collections

Of the 17 fresh samples, detectable DNA was extracted in a non-destructive manner using a commercial kit in all 17 samples (Table 1). This means that at least 25 ng/µL of DNA was present in each sample after DNA extraction. The average DNA concentration for the 17 samples was 217.59 ng/µL. After removing an outlier, the average DNA concentration of the 17 samples was 156.81 ng/µL. In addition, of these 17 samples, a PCR result was obtained from nine of them. This means that in about 53% of the samples, the DNA extracted in a non-destructive manner using a commercial kit allowed for DNA amplification and a DNA barcode could be obtained. The average DNA concentration of the samples that obtained a PCR result was 330.28 ng/µL. For sample C267, 290 ng/µL of DNA was extracted. A change can be seen between the before and after photographs in the legs of spider, especially at the joints (Figure 1 and Figure 2). For all samples, two bands on the PCR gel pictures indicates that a PCR result was obtained, and a single, small band represents a failed reaction (Picture 1). An arrow at the top of the gel picture indicates the placement of the target amplification product in non-destructive samples.

Table 1. DNA Concentration and PCR Result of Fresh Collection Samples

Sample Number	DNA [C] ng/ µL	PCR Result Obtained
C194	276	Yes
C195	325	Yes
C196	80.5	No
C197	288	Yes
C198	179	Yes
C199	134	Yes
C200	194	Yes
C201	37.5	No
C202	80	No
C203	44.5	No
C204	1190	Yes
C206	60.5	Yes
C208	27.5	No
C209	130	No
C211	36.5	No
C212	326	Yes
C267	290	No

Picture 1. PCR Results of Fresh Collections C194-C204, C206, C208-C209, and C211-C212

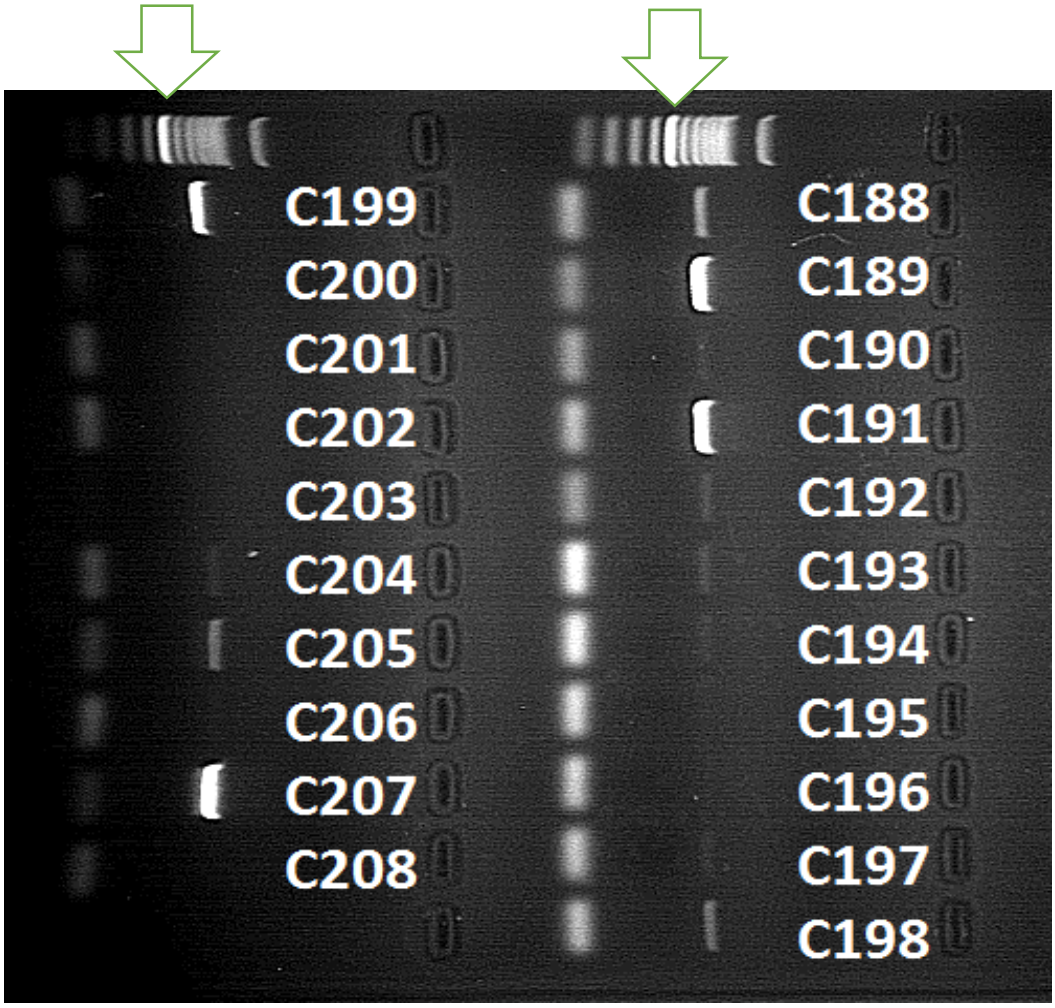
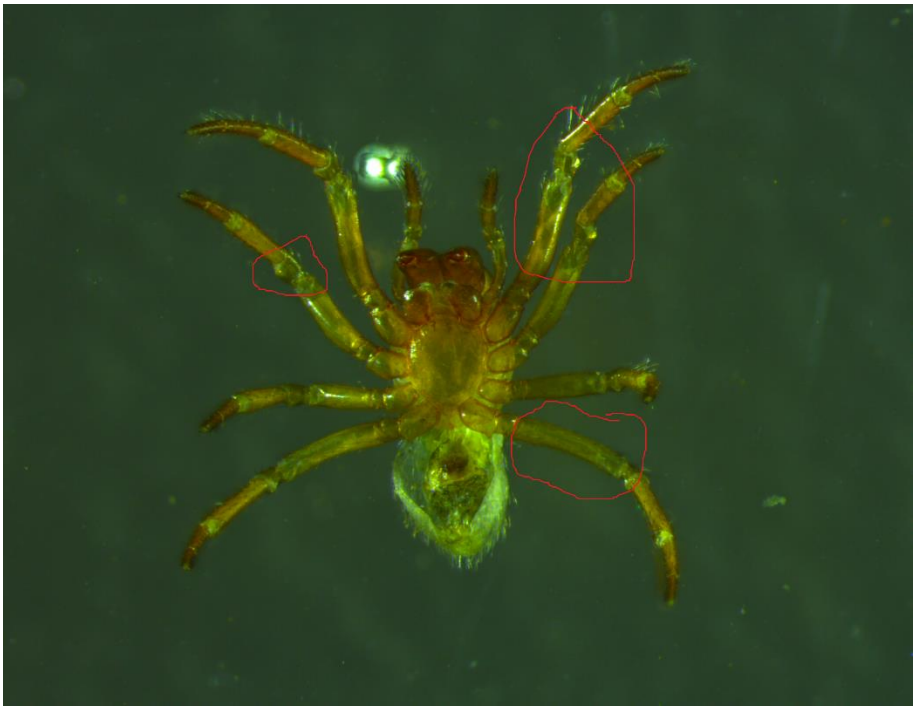


Figure 2. Sample C267 before DNA extraction

Figure 3. Sample C267 after DNA Extraction



Voucher Collections

Of the 46 samples voucher samples, detectable DNA (greater than 25 ng/µL of DNA per sample) was extracted in a non-destructive manner using a commercial kit in 16 samples (Table 2). The average DNA concentration of these 16 samples was 162.19 ng/µL. After removing an outlier, the average DNA concentration of the samples with detectable DNA was 114.33 ng/µL. Of these 16 samples, a PCR result was obtained in 4 samples. The average DNA concentration of those 4 samples was 375.13 ng/µL. Additionally, 4 samples that had no detectable DNA (less than 25 ng/ L of DNA per sample) obtained a PCR result (Table 3). This means that about in about 17% of the samples, the DNA extracted in a non-destructive manner using a commercial kit allowed for DNA amplification and a DNA barcode could be obtained.

Table 2. Voucher Collection Samples that had Detectable DNA Concentrations

Sample Number	DNA [C] ng/ µL	PCR Result Obtained
C51	255	Yes
C52	234	No
C53	49.5	Yes
C151	91	No
C158	31	No
C160	35.5	No
C162	207	No
C163	32	No
C169	240	No
C171	50.5	No
C255	58	No
C261	30.5	No
C264	36	No
C268	49	No
C182	880	Yes
C183	316	Yes

Table 3. Voucher Collection Samples that had no Detectable DNA and Yielded a PCR Result

Sample Number	DNA [C] ng/ µL	PCR Result Obtained
C50	<25	Yes
C54	<25	Yes
C59	<25	Yes
C253	<25	Yes

Figure 3. Sample C261 before DNA extraction

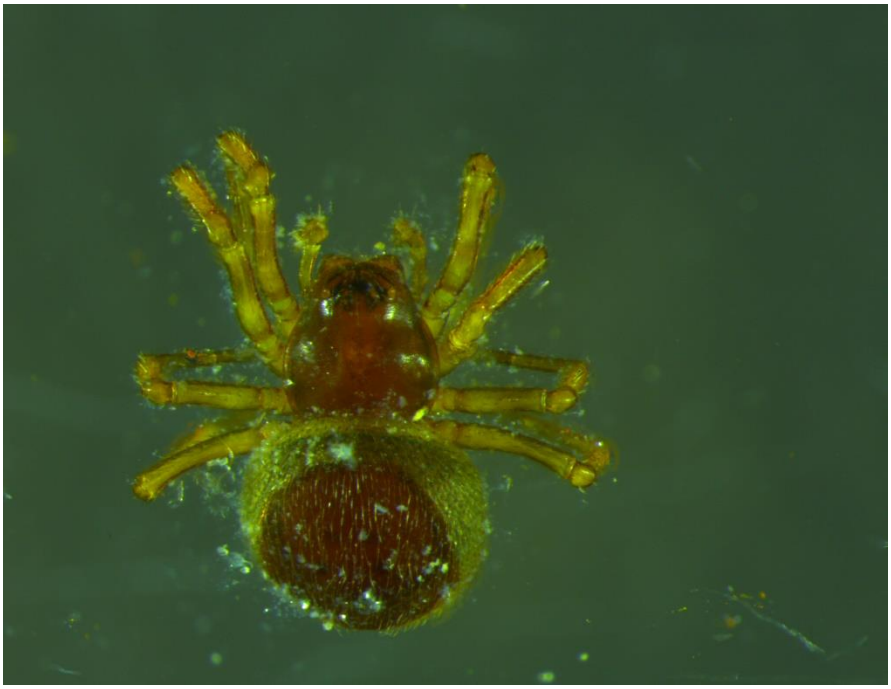
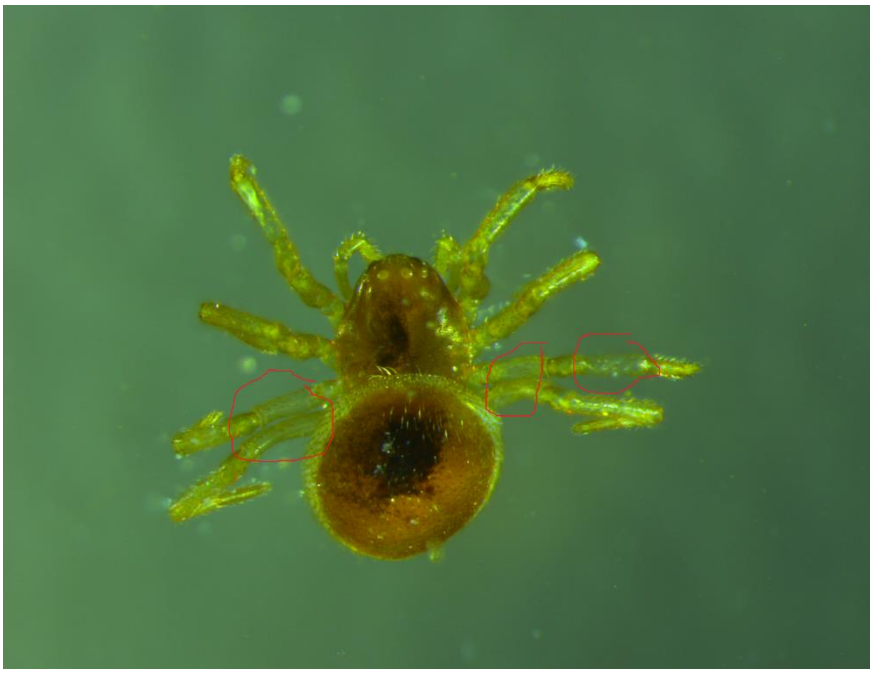


Figure 4. Sample C261 after DNA extraction



Discussion

The results of this study have many implications. First, since all the fresh collection samples had detectable DNA and over fifty percent of the fresh collections yielded a PCR result, it can be concluded that a non-destructive DNA extraction method using a commercial kit works reasonably well on fresh specimens. However, for the voucher collections, only four samples out of 46 had detectable DNA concentrations and only eight samples out of 46 yielded PCR results. Thus, it can be concluded that non-destructive DNA extraction techniques using a commercial kit are not as efficient when working with older or preserved samples.

On average, fresh collections that had detectable DNA concentrations had higher amounts of DNA than voucher collections that had detectable DNA concentrations. The fresh collections had an average of 55.4 ng/µL more DNA per sample than the voucher collections. Additionally, a higher average DNA concentration was needed to obtain a PCR result in the voucher collections. It took, on average, 44.85 ng/µL more DNA to obtain a PCR result in voucher collections. This may have something to do with the quality of DNA obtained in voucher collections.

Because this technique worked fairly well with fresh samples and did not work well with voucher specimens, it can be determined that this technique should only be used on fresh collections. This technique could be helpful in instances when a fresh sample has been caught and not yet identified. Knowing this information, researchers could make sure that they extract DNA in a non-destructive manner within a certain time period of catching a specimen. This could allow researchers to extract DNA in a non-destructive manner using a commercial kit, perform PCR, and enter sequences into a database, all while preserving the specimen as a voucher.

Conclusions

One limitation of this study was the age of the voucher collections. Since all of the voucher collection samples in this study were over three years old, an interesting extension of this project could be to see how long a voucher specimen can sit before efficacy of the technique drops significantly. One way to test this would be to test voucher specimens that had been collected six months previously, a year previously, a year and a half previously, two years previously, and 3 years previously to see if DNA extraction decreases significantly at any of these points.

Another limitation of this study was how long some of the spiders were in the traps after collection. Traps were set up in two week increments in Stanley, Jones and Lyman County in South Dakota. As a result, some spiders could have spent up to two weeks in the traps. The sooner a spider is drowned and preserved, the easier it is to extract DNA from it. If a spider dies and is not placed in 70% ethanol immediately, it can lose DNA as its proteins denature. Thus, the length of time that the spiders spent in the traps could have affected the amount of DNA extracted.

The way some of the voucher specimens were stored also could have led to less DNA extraction. On accident, three years previously, several samples had been stored in 70% ethanol that had been diluted with tap water rather than distilled water. These samples developed a residue on them when removed from their storage vials. This residue could have prevented DNA extraction or lead to degradation of DNA.

The last limitation of this study was that all of the fresh collections were caught in Davison County, South Dakota, while all of the voucher collections were caught in Stanley, Lyman and Jones Counties in South Dakota. To remedy this problem, fresh collections and

voucher collections from all four counties could be tested. If the same results were achieved, it would prove that there is a difference between fresh collections and voucher collections and not between geographical areas.

Additionally, some changes to the procedure could change the results of this experiment. For instance, before PCR, rather than adding 421.875 µL of water to the pre-made stock solution and then adding 22.5 µL of the stock solution with 2.5 µL of DNA, 16.88 µL of water and the 5.62 µL of the pre-made stock solution could be added to each individual DNA extraction sample. This would allow for less dilution of the stock solution and ensure that each sample of DNA was given an adequate amount of stock solution.

The purpose of this quantitative research was to extract sequence-able DNA, known as a DNA barcode, from small spiders using non-destructive DNA extraction techniques and the Qiagen DNeasy kit (Qiagen, 2006). The DNA extracted from these spiders could be entered into a national database, such as GenBank or CBOL, to identify existing species and confirm new species. It is important to explore Non-destructive DNA extraction techniques because they allow for preservation of the voucher specimen. Though this method worked better in fresh collections than voucher collections, it could still be used to preserve voucher specimens as long as DNA is extracted within a timely matter. Using a commercial DNA extraction kit could make the process of extracting DNA from small voucher specimens more regulated, and thus more universal. The methods described in this study were as simplified as possible to ensure that other labs could replicate the technique.

This study was significant in that it tested the efficacy of non-destructive DNA extraction methods using a commercial kit. Though the results were not as expected, they are still noteworthy. From this study, it was found that DNA can be extracted in a non-destructive

manner using a commercial kit if the specimen is fresh enough. Knowing this, researchers can be sure to extract DNA from voucher specimens as soon as possible. This guarantees that DNA can be extracted and the specimen can be saved as a voucher. Then, the DNA barcodes of those specimens can be logged into GenBank or CBOL. For this reason, it is important to understand and expand on the results from this experiment.

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