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MOLECULAR IDENTIFICATION AND DIVERSITY ASSESSMENT OF ARMORED SCALED INSECTS FROM SOUTHEASTERN UNITED STATES

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Abstract

Diaspididae (the armored scale insects) are the largest family of scale insects, and consist of over 2600 species. These armored scale insects are economically importance due to the detrimental effect they have on their host. Scale insects found in Florida are a serious pest of palms and other ornamental plants. Severe damage to the host plant can cause wilting and death, which can lead to a decrease in agricultural production. This study aims to explore the genetic diversity of pest armored scale insects across the Southeastern United States. It also shows the need for molecular identification to determine any cryptic species using a common DNA marker 28S. Insect samples were collected in June 2010 in Florida, Alabama, Mississippi, Georgia and South Carolina. Polymerase Chain Reaction (PCR) was used to amplify DNA markers for molecular identification. We have identified over 25 species, many from multiple localities showing large infestations across the region. Molecular identification was done using BLAST and run through MRBAYES plug-in for phylogenetic analysis. These data will be helpful for growers in determining which species are attacking their plants and what the most effective measures will be to control outbreaks of armored scale insects.

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Introduction

The objective of this project was to collect and list different types of pests and invasive species found in family Diaspididae (the armored scales). The highest diversity of armored scale species are found in the Southeastern United States of America. The armored scaled insects make up more than one third of all scale insects and most species are classified within the two subfamilies Diaspidinae and Aspidiotinae (Miller & Davidson, 2005). They reside on a variety of plants that are mostly perennials. Such hosts include ornamental plants, forest trees, and shrubs. Even though the diversity among armored scales is vast the ability to distinguish between species is quite difficult due to most being morphologically cryptic.

The armored scales, the largest family of scale insects, consist of 2437 species according to ScaleNet (Ben-Dov et al., 2010). Their form of reproduction can be either asexual or sexual depending on the conditions and are variable in both shape and size. Armored scale size range can be from 1 to 2 mm (Miller & Davidson, 2005). Most of the armored scales lay eggs which are a result of mating. Males and females have different developmental stages with males containing five instars and females containing three instars. Instars are developmental stages that occur between each molt of the exoskeleton. Armored scales are sheltered by a separate covering made up of either waxy or non-waxy components (Ebstein & Gerson, 1971). The waxy covering is secreted by the pygidial wax glands found near the anal opening. Scale covering characteristics are unique to certain species and the shedding of their skin can be used as a flag to identify them in their habitat (Miller & Davidson, 2005).

Male and female covers differ in their composition and complexity. Female crawlers, first instar development, start secreting their waxy covering within one to two days after settling on a

host. The initial covering is cemented by secretions from the anal opening that helps maintain structure and protection (Miller & Davidson, 2005). As development continues the crawler grows and secretes another waxy material much denser than the initial wax covering. As the crawler stage of development ends the skin will be shed but will stay incorporated into the scale cover. The color of shed skin can differ among species and can be an important indicative quality for identification. Males can be covered by the same covering as females or can be covered by a white felted wax that is easily distinguished from other armored scale covers (Miller & Davidson, 2005). They usually start off diploid then change into a haploid organism for reproductive purposes (Morse & Normark, 2006). Their construction compared to females is more basic and the molting from second to fourth instar is not incorporated into the final scale cover (Miller & Davidson, 2005).

The development of the scale cover has altered the armored scales eating habits to feed on individual plant cells rather than targeting phloem sap. The latter eating habit is found in other scale insects closely related to the armored scales (Miller & Davidson, 2005). Previous research has shown a list consisting of over 200 species that are considered a pest to the host plant they inhabit (Morse & Normark, 2006). The development of the armored scales and generation time is dependent on temperature and humidity and can vary within species depending on the climate. Plants also depend on these factors making them accurate indicators in predicting the armored scales development. It is known that the armored scales have a high variability for host plant specificity in which a few species have been found on numerous host plants and conversely, numerous armored scale species have been found on a few host plants (Miller & Davidson, 2005).

The research on the feeding effects on plants due to armored scale insects is limited and the mechanism is not well understood. It is known that the armored scales feed on both the leaves and bark of its host plant, and large population infestation causes dieback and ultimately death of both leaves and branches. Most pesticides are used to regulate other pests that may compete with the armored scales for the host plant and can have an effect of also regulating armored scale populations (Miller & Davidson, 2005). Species-host specificity would be beneficial so that proper pesticides can be formulated and populations can be predicted for certain tendencies of armored scales and plant host.

For the scope of this study armored scale insects were collected in five states over a ten day period in late June 2010. Samples were collected from 14 localities across the southeastern United States which included Georgia, Florida, South Carolina, Alabama, and Mississippi (Figure 1). Samples were isolated, amplified, edited, and then identified using two different analysis methods. Both analysis methods were used to identify armored scale insects based off of molecular sequences.

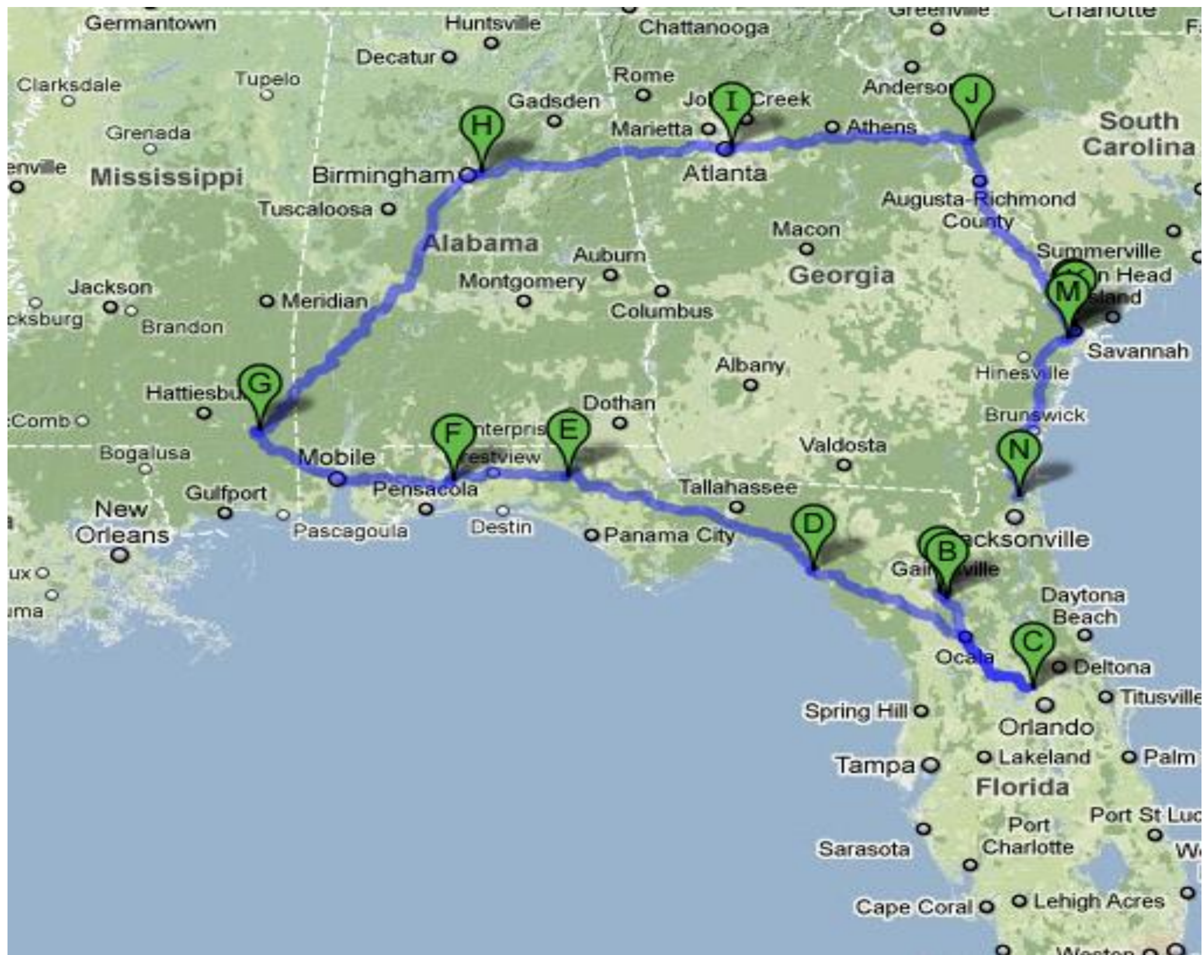

Figure 1: Shows the 14 different localities where samples of armored scale insects were collected. Florida, Alabama, Mississippi, Georgia, and South Carolina were among the states where collection occurred. Diversity and similarities were found throughout the five collection states (Table 2). The letters indicate different locations where collection of armored scales took place. Location A was in close proximity to location B. Location K and L is in close proximity to location M. Source: created using Google maps.

Materials and Methods

Scale insect collection

Samples were collected throughout the ten day trip in five southeastern states. Collection sites included seven localities in Florida, one locality in Mississippi, one locality in Alabama, four localities in Georgia, and one locality in South Carolina (Figure 1). Armored scale insects were identified on their plant host using a magnifying glass prior to extraction. The plant hosts with live samples were extracted using a variety of tree trimming tools depending on the size of the host plant and location of armored scale insect. Samples were stored with their plant host on ice for the duration of the trip to preserve insect DNA. At each collection site GPS coordinates where documented and each sample was given an identification number for future reference (Table 3).

DNA Isolation

Nucleic acids were isolated from 30 samples collected throughout five southeastern states. Isolation of insect DNA was performed using most of the steps found in the DNeasy Blood & Tissue Protocol (Qaigen, 2006). Fresh armored scale samples were first picked off of their host plants, poked with a size zero insect pin and soaked in 200 µl ATL buffer (DNeasy kit) in a clean 1.5 ml microcentrifuge tube. Samples were lysed using 20 µl of proteinase K, vortexed, and incubated at 56˚C overnight (Qiagen, 2006). The samples were vortexed for 15 seconds then mixed thoroughly while adding 200 µl of both Buffer AL (concentrate containing guanidine hydrochloride) and 100% ethanol. DNeasy Mini spin column was used to centrifuge the samples in two ml collection tubes three times. The first at 8000 rpm for one minute and second at 8000 rpm for one minute with the addition of 500 µl Buffer AW1 (concentrate

containing guanidine hydrochloride). The last was at 14,000 rpm with the addition of 500 µl AW2 Buffer (concentrate containing guanidine hydrochloride). Flow through was discarded after each centrifuge and new collection tubes were used. Column was then placed in a clean 1.5 ml collection tube with 100µl Buffer AE (10 mM Tris-Cl, 0.5 mM EDTA, pH 9.0) placed directly over the column center. Armored scale DNA was eluted with incubation at room temperature for one minute then centrifuged at 8000 rpm for one minute (Qiagen, 2006) and stored at -20°C.

Molecular Lab work

PCR, polymerase chain reaction, was used to amplify sequences found in successfully isolated armored scale DNA. Primers 28S3a and 28SB ordered from IDT (Integrated DNA Technologies) shown in Table 1 were used to amplify regions of 28S ribosomal sequences (Anderson et al., 2010). Their sequences were complementary to one another allowing for alignment to investigate molecular identification. PCR reaction tubes were made using Blue Nexus PCR Premix Taq Version tubes with the addition on ten µl water, one µl of each primer (28S3a and 28SB), and two µl of sample. PCR was done on the TC-3000X and TC-3000G machines run as 25 µl reactions and amplified using the following reaction conditions: 95˚C for two minutes, 30 cycles of 95˚C for one minute, 49˚C for one minute, 72˚C for one minute then held at 72˚C for ten minutes.

PCR reactions were visualized using 1.5% agarose gel electrophoresis with a buffer solution of 0.5X TBE. Each well contained one µl EZ Vision One, five µl DNA Dye Loading Buffer 6X, and five µl of PCR sample. Gel products were visualized using a Red Cell Bioscience UV transilluminator. Free nucleotides and primers were removed from the PCR reactions by

mixing ten µl of PCR product, one µl exonuclease, and one µl shrimp alkaline phosphatase and run on the same machines using the program Exo SAP-IT (Gruwell, Morse & Normark, 2007). After the Exo SAP-IT procedure five µl of PCR product and one µl of each primer were placed into a 96 well sequence plate. Cleaned samples were sent to Penn State Genomics Facility at University Park State College, PA where they performed cycle sequencing using a 3730 DNA analyzer (State College, PA). Direct sequencing was completed using primers 28S3a and 28SB. Sequences were edited and aligned preliminarily using Geneious Pro (5.3.4).

Molecular Identification and Bioinformatics

Complete edited sequences were all run through BLAST (Basic Local Alignment Search Tool) on the National Center for Biotechnology Information website (Alstrul et al., 1990). BLASTn was used to compare nucleotide similarities to the sample sequences found using 28S3a and 28SB primers. The BLAST search was run against the nucleotide collection (nr/nt) database and optimized for highly similar sequences. From BLAST results we imported three close BLAST hits to compare with original data in rigorous phylogenetic analysis. All the data was combined into a single data block in FASTA format and imported into a multiple sequence alignment program (MAFFT). The purpose of MAFFT is to look for sequence similarities and common domains. It was run using the E-INS-i algorithm, which specializes in a very slow less than 200 sequences with multiple conserved domains and long gaps which is made for ribosomal rDNA such as the data collected.

The MAFFT alignment block was imported back into Geneious to employ the MRBAYES plug-in for phylogenetic analysis. The analysis was run using gamma rate variation

under the HKY85 nucleotide substitution model for 1,100,000 generations with a burnin of 100,000 generations, set to err on the conservative side for a data set this size. Four heated chains were run simultaneously and they were resampled every 200 generations. From these analyses' the plug-in calculated Bayesian Posterior Probability scores which we used to support hypotheses of identification. Among other criteria, no relationship below a posterior probability score of 100 was accepted as an accurate identification.

	28S3a	**28SB**
Sequence	5'- AGT ACG TGA AAC CGT TCA GG -3'	5'- TCG GAA GGA ACC AGC TAC -3'
DNA bases	20	18
GC Content	50%	55.5%
Tm (50mM NaCl)	54.8˚C	54.0˚C

Table 1: Shows the two primers used for molecular identification of armored scales. These primers are used to amplify ribosomal rDNA. Primers were ordered from Integrated DNA Technologies.

Locality	GPS Coordinates	Sample Diversity	Sample # Collected	State
A	29º36'43.85" N 82º24'32.28" W	*Pseudaulacaspis cockerelli*	AS013, AS015	Florida
B	29º33'23.98" N 82º19'47.66" W	(*Thysanococcus pandani/ Acutaspis albopicta*)*	AS016	Florida
C	28º75'04.85" N 81º53'15.35" W	*Pseudaulacaspis cockerelli*	AS017	Florida
D	29º49'08.07" N 83º35'35.13" W	*Pseudaulacaspis cockerelli*	AS023	Florida
E	30º43'95.54" N 85º54'74.55" W	*Pseudaulacaspis cockerelli*	AS026	Florida
F	30º39'59.72" N 86º58'40.13" W	(*Thysanococcus pandani/ Acutaspis albopicta*)*	AS027	Florida
G	31º09'92.66" N 88º48'77.16" W	None	No sequences	Mississippi
H	33º54'43.21" N 86º76'08.55" W	*Pinnaspis Piperis*	AS030	Alabama
I	33º75'97.57" N 84º37'29.06" W	*Melanaspis obscura* *Carulaspis minima*	AS035, AS037	Georgia
J	33º50'16.58" N 82º05'76.96" W	*Melanaspis obscura* *Unaspis euonymi*	AS039, AS040, AS041 AS042	South Carolina
K	32º07'68.61" N 81º08'92.03" W	*Pseudaulacaspis cockerelli* *Carulaspis minima* *Parlatoria camelliae* *Aonidella aurantii* *Fiorinia theae* (*Thysanococcus pandani/ Acutaspis albopicta*)* (*Pinnaspis strachani/ Pinnaspis piperis*)*	AS043, AS044, AS045 AS046, AS047, AS048 AS049, AS050, AS051 AS053, AS054, AS055	Georgia
L	32º08'10.78" N 81º09'50.91" W	*Pseudaulacaspis cockerelli*	AS056	Georgia
M	32º00'26.50" N 81º11'64.70" W	*Pseudaulacaspis cockerelli*	AS057	Georgia

N	30º32'48.95" N 81º39'89.16" W	*Pseudaulacaspis cockerelli* (*Thysanococcus pandani*/ *Acutaspis albopicta*)*	AS058, AS059, AS060	Florida

Table 2: Shows the samples collected and armored scale insect diversity at each locality (Figure 1). Seven localities were visited in Florida which contained three different types of armored scales. Four localities in Georgia were visited which contained 12 different types of scales. Alabama and North Carolina had samples taken from one locality each. Collection was done in Mississippi but sequences were not able to be identified. *Identifies the differences in BLAST and phylogenetic ID results

Species Name	Accession Number
Thysanococcus pandani	DQ145391
Parlatoria theae	DQ145373
Parlatoria camelliae	GQ325519
Lindingaspis rossi	GQ325506
Nuculaspis californica	GQ325515
Aonidiella citrina	GQ325448
Aonidiella aurantii	DQ145289
Mycetaspis personata	DQ145366
Melanaspis bromiliae	DQ145360
Acutaspis albopicta	FJ040866
Melanaspis obscura	GQ325511
Unaspis euonymi	DQ145393
Diaspis coccois	DQ145317
Carulaspis juniperi	DQ145301
Carulaspis minima	DQ145302
Chionaspis wistariae	DQ145308
Aulacaspis difficilis	DQ145298
Megacanthaspis leucaspis	DQ145359
Pinnaspis strachani	FJ040871
Pinnaspis piperis	DQ145377
Poliaspis syringae	GQ325537
Fiorinia euryae	DQ145329
Fiorinia theae	DQ145332
Pseudaulacaspis pentagona	DG145386
Pseudaulacaspis cockerelli	GQ325545

Table 3: Shows species ID found through BLAST and used in Bayesian phylogenetic analysis (Figure 2 & Table 4). Accession numbers found from BLAST results are listed in this table.

Results

Results from both BLAST and phylogenetic tests are displayed in Table 4. The Bayesian phylogenetic analysis reporting posterior probabilities is presented in Figure 2. The purpose of running both BLAST and Bayesian phylogenetic analysis was to see both similarities and differences to correctly identify armored scale samples. Differences in identification were seen in samples AS016, AS027, AS059, AS060, and AS043 (Table 4). BLAST results showed these samples having an identity of *Mycetaspis personata* whereas phylogenetics identified them as closely related to *Thysanococcus pandani*. It also showed that exact matches were not found in BLAST which infer these samples have not been sequenced yet. Bayesian posterior probabilities were at 100% showing that this node branch is monophyletic and deserves consideration as a real entity (Figure 2). AS050 and AS051 were supported by both methods with an identity of *Parlatoria camelliae* and being monophyletic to *Parlatoria theae* (Table 4). Bayesian posterior probability was only 58% showing this node is very unlikely (Figure 2). AS054 was supported as being *Aonidella aurantii* by both methods an included a Bayesian posterior probability of 99% (Figure 2 & Table 4). Sample AS054 is closely related to *Aonidella citrina* (Figure 2). AS035, AS038, AS039, AS040, AS041, and AS042 were supported by both methods as *Melanaspis obscura*. Bayesian posterior probabilities were 99% for a monophyletic group of all six samples (Figure 2). AS042 supported by both as *Unaspis euonymi* which contained a 100% Bayesian posterior probability. AS037 and AS049 were supported by methods as being *Carulaspis minima*. Bayesian posterior probability was 68% showing less support for this node (Figure 2). AS030 and AS045 were supported by phylogenetics as being *Pinnaspis piperis* with a Bayesian posterior probability at 99% (Figure 2). AS030 was also supported as *Pinnaspis piperis* in BLAST, but AS040 BLAST results identified it as *Pinnaspis strachani*, a closely related species.

AS047 was supported by both phylogenetics and BLAST as being *Fiorinia theae*. Bayesian posterior probability was 99% for sample AS047 (Figure 2). Samples AS013, AS017, AS044, AS055, AS015, AS053, AS057, AS058, AS056, AS023, and AS026 were supported by both methods with an identification of *Pseudaulacaspis cocckerelli* (Figure 2 & Table 4). Bayesian posterior probability was 100% for all 11 samples (Figure 2).

Sample #	Top BLAST hit	Phylogenetic ID	Additional BLAST hits
AS013	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis pentagona* *Fiorinia theae*
AS015	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis pentagona* *Fiorinia theae*
AS016	*Mycetaspis personata*	*Thysanococcus pandani*	*Acutaspis albopicta* *Thysanococcus pandani*
AS017	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis pentagona* *Fiorinia theae*
AS023	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis pentagona* *Fiorinia theae*
AS026	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis pentagona* *Fiorinia theae*
AS027	*Mycetaspis personata*	*Thysanococcus pandani*	*Acutaspis albopicta* *Thysanococcus pandani*
AS030	*Pinnaspis piperis*	*Pinnaspis piperis*	*Pinnaspis strachani* *Megacanthaspis leucaspis*
AS035	*Melanaspis obscura*	*Melanaspis obscura*	*Mycetaspis personata* *Melanaspis bromiliae*
AS037	*Carulaspis minima*	*Carulaspis minima*	*Carulaspis juniperi* *Diaspis coccois*
AS038	*Melanaspis obscura*	*Melanaspis obscura*	*Mycetaspis personata* *Melanaspis bromiliae*
AS039	*Melanaspis obscura*	*Melanaspis obscura*	*Mycetaspis personata* *Melanaspis bromiliae*
AS040	*Melanaspis obscura*	*Melanaspis obscura*	*Mycetaspis personata* *Melanaspis bromiliae*

AS041	*Melanaspis obscura*	*Melanaspis obscura*	*Mycetaspis personata* *Melanaspis bromiliae*
AS042	*Unaspis euonymi*	*Unaspis euonymi*	*Chionaspis wistariae* *Aulacaspis difficilis*
AS043	*Acutaspis albopicta*	*Thysanococcus pandani*	*Mycetaspis personata* *Thysanococcus pandani*
AS044	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis pentagona* *Fiorinia theae*
AS045	*Pinnaspis strachani*	*Pinnaspis piperis*	*Pinnaspis piperis* *Megacanthaspis leucaspis*
AS047	*Fiorinia theae*	*Fiorinia theae*	*Fiorinia euryae* *Poliaspis syringae*
AS049	*Carulaspis minima*	*Carulaspis minima*	*Carulaspis juniperi* *Diaspis coccois*
AS050	*Parlatoria camelliae*	*Parlatoria camelliae*	*Parlatoria theae* *Lindingaspis rossi*
AS051	*Parlatoria camelliae*	*Parlatoria camelliae*	*Parlatoria theae* *Lindingaspis rossi*
AS053	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis pentagona* *Fiorinia theae*
AS054	*Aonidiella aurantii*	*Aonidiella aurantii*	*Aonidiella citrina* *Nuculaspis californica*
AS055	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis pentagona* *Fiorinia theae*
AS056	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis pentagona* *Fiorinia theae*
AS057	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis pentagona* *Fiorinia theae*
AS058	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis cockerelli*	*Pseudaulacaspis pentagona* *Fiorinia theae*
AS059	*Acutaspis albopicta*	*Thysanococcus pandani*	*Mycetaspis personata* *Thysanococcus pandani*
AS060	*Acutaspis albopicta*	*Thysanococcus pandani*	*Mycetaspis personata* *Thysanococcus pandani*

Table 4: Shows the 30 samples isolated and identified throughout the five southeastern states. It includes the top three results when run through BLAST looking for nucleotide similarities. These results were then used for phylogenetic analysis which provided a phylogenetic ID (Figure 2). The collection of this table shows that most samples were identified as the same armored scale insect using both analysis methods. Six samples had conflicting identification which included AS060, AS059, AS045, AS043, AS027, and AS016.

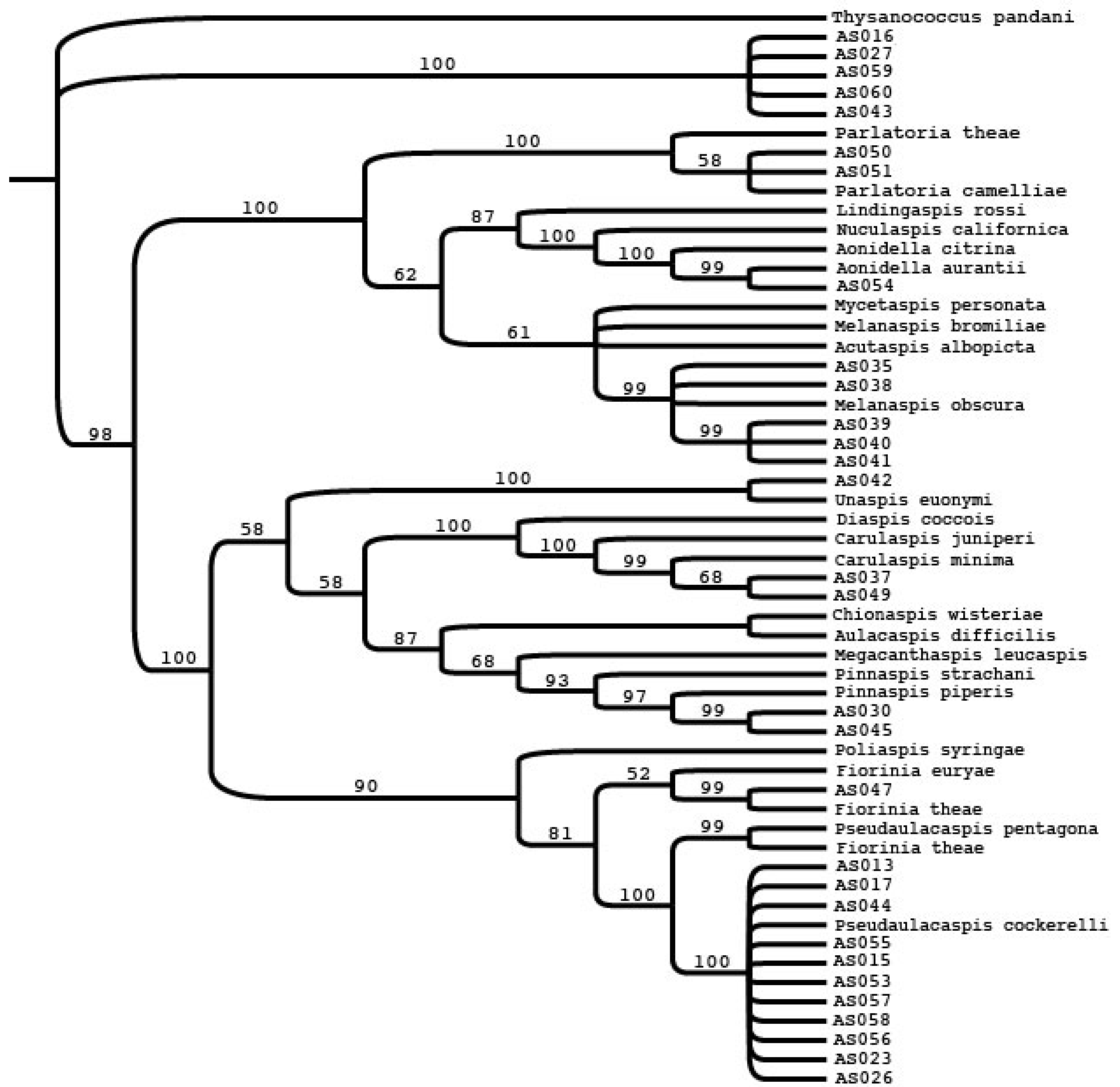

Figure 2: Bayesian topology used to identify new samples of armored scale collected in the Southern United States. AS numbers correlate with Table 2 and stand for new scale insect samples collected. Nodal support show above branches indicates Bayesian posterior probabilities.

Discussion

The infestation of armored scale insects can disrupt microecosystems by the damage they cause to their plant hosts. Over the five states where collection took place, we found 13 different armored scale insect species that are known to inhabit a specific spectrum of plant hosts and live in specific climates. It is also important to know generation time which will help in properly identifying the stage of development and best method for treatment. The most common species we found throughout the trip was *Pseuaulacaspis cockerelli*. We found these in 8 different localities across two states (Table 4). Also known as the white magnolia scale, this armored scale lives on over 30 genera of plants and can survive in climates found throughout most of the southeastern United States (Miller & Davidson, 2005). They are shown to undergo year round reproduction with generation times around 60 days (Tippins, 1968). Since their introduction into Florida in 1942, they have been identified as the most serious pest of ornamental plants (Dekle, 1970).

Melanaspis obscura, also known as the obscure scale, was collected in South Carolina and Georgia (Table 4). Commonly found in the eastern United States, these armored scales have one generation per year with eggs and crawlers emerging within the early summer months (Baker, 1933). They have over 20 different species plant hosts but are found to be most damaging to shade trees such as pin oaks (Miller & Davidson, 1990). Branch dieback is a result of increased infestation of these armored scales and pesticides have been unsuccessful in the past. Pesticides have been used to treat host plants during crawler stages but one explanation for their ineffectiveness has been the ability of new crawlers to settle underneath protective layers of dead scales. The knowledge of when generation times start is crucial for population control and

the best known time for control is using oil sprays during dormant stages of armored scaled insect development (Pinto, 1980).

Unaspis euonymi, the Euonymus scale, was collected at one locality in South Carolina (Table 3). A native to the Orient, these scales can have between two to three generations per year depending on their locality. Another determining factor of generation time is dependent on their host plant phenology which is known to consist of seven families in 14 genera (Mussey & Potter, 1997). It is known to be a serious pest of the Euonymus that has caused annual damages upward to a million dollars to this plant in the two northeastern states (Van Driesche et al., 1998). Euonymus scales are found throughout the entire United States so annual damages nationwide could potentially be in the multiple million dollar range. The major control method has been pesticides but research involving plant resistance and biological control has shown to be a safer and potentially more effective alternative (Miller & Davidson, 2005).

Fiorinia theae, the tea scale, was collected at one locality in Georgia (Table 4). The tea scale shows a 70 day life cycle with overlapping generations. They have been found living on 18 host genera in five families inhabiting ecosystems worldwide. It is known as the most important pest of the camellias but is also found on some species of holly (McComb, 1986). Even though this armored scale is a minor pest in some areas, it is considered to be major problem worldwide (Miller & Davidson, 1990). *Carulaspis minima*, known as the minute cypress scale, were collected in two localities in Georgia (Table 4). Commonly found in warm southern climates, these armored scales will show one generation per year in northern localities but will exhibit two generations in the south. Eggs hatch in early May with crawlers by the end of May (Miller & Davidson, 2005). They are found to inhabit seven genera of conifers and are known to be a

serious pest of juniper. It is also suggested that control by way of pesticide spray is necessary on ornamental hosts (Dekle, 1970).

Aonidiella aurantii, the California red scale, was collected at one locality in Georgia (Table 2). Generation cycle is hard to predict due to the excessive overlapping of each generation, but it is known that populations reached their largest size in autumn months (Hare, 1991). These armored scales inhabit 75 families and 202 genera of plant hosts and commonly settle in the midline of veins on the leaf. It is found to be one of the world's most important pests of citrus and has caused damaged to a variety of other fruits all across the world (Miller & Davidson, 2005). Effective monitoring of population size and pest control has been implemented through a program called SCALEMAN. SCALEMAN was developed as a way for cultivators to monitor and implement proper methods to eradicate this pest from their crops. It also provides insight about natural enemies which can be introduced to the population making this an effective method for pest control of the California red scale (Clift & Beattie, 1993).

Parlatoria camelliae was collected at one locality in Georgia (Table 2). They are known to inhabit 26 genera plant hosts, but the biology of generational time has not yet been investigated. This armored scale is widely distributed throughout the United States as well as internationally. It is known that they tend to congregate to the veins of the leaf they inhabit and have been identified as an occasional pest of camellia (Miller & Davidson, 2005). *Pinnaspis strachani*, the cotton white scale, was collected at the same locality in Georgia (Table 2). The cotton white scale has been found to inhabit 59 genera and 27 families nationwide as well as worldwide. This armored scale shows one generation per year and is found to be a serious pest to cotton, coconut palm, pineapple, citrus, and olives. Biological control programs for this species have been conducted in Peru but poor documentation has provided little information on

managing this pest (Miller & Davidson, 2005). Other species including *Mycetaspis personata*, *Pinnaspis piperis*, *Acutaspis albopicta*, and *Thysanococcus pandani* were suggested identities on samples found in Georgia, Alabama, and Florida (Table 2).

The wide host preference and variety in generational time of armored scale insects makes proper identification necessary for successful biological management programs. Identification of the 30 samples using BLAST provided a very fast way to find similar nucleotide sequences (Table 4). This provided us with names of various species that could be the identity of the sample. The utility of BLASTn as a tool for assigning species identification was dependent on the availability in the GenBak database on a reference sequence for a given species. BLASTn results could still come up for a particular sample but it might be a sequence from a closely related species. Further analysis would still need to be done to investigate the samples correct identity.

The second method used was Bayesian phylogenetic analysis. A compilation of all of the sample sequences as well as the sequences obtained through BLAST was run through Bayesian analysis. This was a more extensive analysis of our samples that took almost twelve hours to complete. This method provided a phylogenic tree with nodal support using Bayesian posterior probabilities (Figure 2). Most samples have the same suggested identities using both methods but a few showed differences. AS016, AS027, AS059, AS060, and AS043 were the samples that suggested an unidentified species of the armored scale insect. Bayesian analysis showed these samples being closely related to *Thysanococcus pandani* and 100% Bayesian posterior possibility show this branch (Figure 2). This example shows the failure of BLAST but the use of both methods has provided conclusive evidence of the identity of these samples.

Conclusion

The threat of armored scaled insects being a serious pest worldwide is contingent on proper identification so successful management programs can be implemented. Because armored scales are very small and have many cryptic species they are very hard to identify visually. Morphology can be studied by experts but can be impossible at times and very tedious. Molecular identification has given us the technology for a cheap, fast, and effective method to successfully identify the armored scales. This project demonstrates the importance of using BLAST and Bayesian phylogenetic analysis collectively in identifying samples of armored scale insects across the southeastern United States.

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