

Identification of New Crapemyrtle Bark Scale (*Acanthococcus lagerstroemiae*) Hosts (*Spiraea* and *Callicarpa*) through DNA Barcoding

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Summary

Global trade and international travel have led to the establishment of invasive pests in territories outside the pests' normal range. We have been following the distribution of crapemyrtle bark scale [(CMBS) *Acanthococcus lagerstroemiae*], an invasive insect first discovered in the United States in 2004. In addition to the rapid geographical expansion of the CMBS distribution, one crucial concern is its ability to infest a wide range of plant species, beyond its primary host - *Lagerstroemia*. Studying the molecular evidence helps determine the genetic relationships of CMBS specimens from different geographical locations and hosts. Naturally occurring CMBS infestations were confirmed on native plant species, American beautyberry (*Callicarpa americana* L.), and *Spiraea* (*Spiraea* L.) in the United States. The new infestation of CMBS found on *Spiraea* raises the alarm for

the green industry that other economically important crops in the Rosaceae family might potentially be susceptible to CMBS attacks.

INTRODUCTION

Crapemyrtle bark scale (CMBS; *Acanthococcus lagerstroemiae*) is exotic pest insect that has been raising concerns since its first discovery in the United States in 2004. To date, CMBS was found in at least 17 states, from south central to the east coast of the United States. The highest number of infestation reports are in Texas (115), followed by Oklahoma (34), Arkansas (30), Louisiana (29), Mississippi (25), North Carolina (23), and Virginia (23) (EDDMapS., 2021). With the rapid geographical expansion of its U.S. distribution, CMBS control and management is complicated by its polyphagous feeding habit - which allows it to infest a wide range of plant hosts.

A significant amount of host information for CMBS has been reported in the literature and online insect databases, such as Scalenet. Plant species from at least 22 genera (15 families) has been listed as CMBS hosts (García Morales et al., 2016). As the distribution of CMBS continues to expand beyond its native regions, specifically in the US, concerns have been raised regarding the expanded host range for CMBS beyond *Lagerstroemia*, and the potential threats that CMBS poses to the native and economically important plants in the United States.

For instance, in 2018, *Hypericum kalmianum* L. (St. Johnswort) was confirmed as a CMBS host (Schultz & Szalanski, 2019). We have conducted CMBS feeding preference studies, to confirm previous documented hosts and identify potential new hosts, especially native plants, in the US. From 2016 to 2019, we have confirmed several new species and cultivars as CMBS alternative hosts, including apple (*Malus domestica*),

Chaenomeles speciosa, *Disopyros rhombifolia*, *Heimia salicifolia*, *M. angustifolia*, twelve pomegranate cultivars (Xie et al., 2020) and nine *Callicarpa* species (Wu, 2021).

In 2020, a scale infestation (suspected to be a CMBS infestation) observed on *Spiraea japonica* (Fig. 1 A-B) at the University of Arkansas. In 2020, another incident of unknown scale infestation, later identified as CMBS, was reported by a homeowner in Concord, North Carolina. The infested plant was identified as *Spiraea thunbergia* (Fig.1 C-D), which was planted sometimes between 1953 and 2016 with no reported scale infestation. The scale infestation was believed to have been initiated after two crapemyrtle plants infested with CMBS were accidentally planted nearby the *S. thunbergia*.

Spiraea, or commonly known as spirea or meadowsweet, is a genus consisting of 100 to 120 species known worldwide; it is primarily found in the temperate region of the northern hemisphere (Wrońska-Pilarek, Wiatrowska, & Bocianowski, 2019). The phylogeny of Rosaceae has revealed a closer relationship between *Spiraeae* and *Maleae* (both under subfamily *Amygdaloideae*), while further from *Rubeae* (subfamily *Rosoideae*) (Xiang et al., 2017). Interestingly, this phylogenetic relationship is consistent with our previous findings, as CMBS infestation was found on apple and flowering quince (*Chaenomeles*) but not on blackberry and raspberry (*Rubus*) (Xie et al., 2020).

Although the insect morphology suggested the unknown scale on *Spiraea* was CMBS, observational evidence still has limitations in identifying species. For example, the azalea bark scale, a close relative of CMBS, is often mistaken as CMBS since the morphology closely resembles each other. Therefore, to confirm the identity, molecular

approaches were used to verify if CMBS caused infestations found on *S. japonica* and *S. thunbergii*.

MATERIALS AND METHODS

Insect Sample Collection and Handling. Naturally occurring scale infestations (suspected to be caused by CMBS) were found on *C. americana* (from Faulkner County, Arkansas), *S. japonica* (from Faulkner County, Arkansas), and *S. thunbergii* (from Concord, North Carolina). Live nymphs were then collected, using a fine pin or a fine brush, from infested twigs or branches of crapemyrtle (control) and alternative hosts (*Callicarpa* and *Spiraea*). Insects collected were used immediately or stored in -80°C freezer. DNA extractions were performed using DNeasy Blood and a Tissue Extraction Kit (Qiagen Inc., Valencia, CA).

Molecular Identification. To amplify DNA sequence more specifically from the CMBS COI gene, primers based on the CMBS COI sequence (GenBank accession number: AB439520.1) were developed. Primer pair: Forward: 5'-CCAGGATTTGGATTAATATCAC-3', and Reverse: 5'-TGAACCAATTGATGATAGAG-3', were designed and successfully amplified CMBS COI sequences with lengths around 640 bp in this study.

The PCR program used was as follows: the sample was held at 98 °C for 30s, then 30 cycles of 98 °C for 10s, 51 °C for 30 s, and 72 °C for 30s - followed by a final extension of 72 °C for 10 min. The successful PCR amplifications were checked on a 1% agarose gel following electrophoresis and visualized using a Gel Doc E.Z. imaging system (BIO-RAD, Inc., Hercules, CA). The PCR products were purified and sent to a sequencing lab (Eton Bioscience, Inc., San Diego, CA) for direct sequencing in both directions.

Data Analysis. A 475 bp section of COI sequence was used to analyze the relationship of CMBS from different hosts and two close related scale insects (*Acanthococcus azaleae*, and *A. abeliceae*). Crapemyrtle bark scale sequences used in this study have been deposited in GenBank (GenBank accession number: MZ312637:MZ312640). A phylogenetic tree was constructed using BLAST pairwise alignments (Johnson et al., 2008).

RESULTS AND DISCUSSION

Sequencing results reveal that amplicons from four insect samples (from crapemyrtle, *Callicarpa*, *Spiraea japonica*, and *S. thunbergia*) had sequence length above 600 bp (601~664). The BLAST results showed sequences from each sample had identity scores above 98% matched with CMBS COI sequences in the database (GenBank accession number AB439520.1).

To study the relationship of CMBS collected from different geographical locations and plant hosts, we constructed a molecular phylogenetic tree to reveal the genetic relationship of CMBS relative to its two close relatives from the US or Asia. The phylogenetic tree showed a clear separation between CMBS and azalea bark scale while sharing a common evolutionary origin compared to *Acanthococcus abeliceae* Kuwana (Fig. 2). All the CMBS collected from either crapemyrtle or alternative hosts (*Spiraea* and *Callicarpa*) in the U.S. location were grouped, versus CMBS from the Asia location, suggesting CMBS at different geographical areas might have been evolving differently.

Our results have confirmed that, in addition to the geographical expansion, the expanding host range of CMBS is not only a potential threat to the green industry but a reality. The latest findings have brought the total number of CMBS hosts in *Rosaceae* to

eight, making it one of the most prominent families hosting CMBS beside *Lythraceae*. According to the phylogeny within Rosaceae, confirmed CMBS host genera *Chaenomeles*, *Malus* and *Spiraea* had a closer genetic background with at least 55 genera under subfamily of *Amygdaloideae* (Potter et al., 2007), which includes many economically important crops such as apricot, almond, cherry, plum, and peach. Therefore, further investigation is needed for the potential threat of CMBS to other valuable crops in the Rosaceae.

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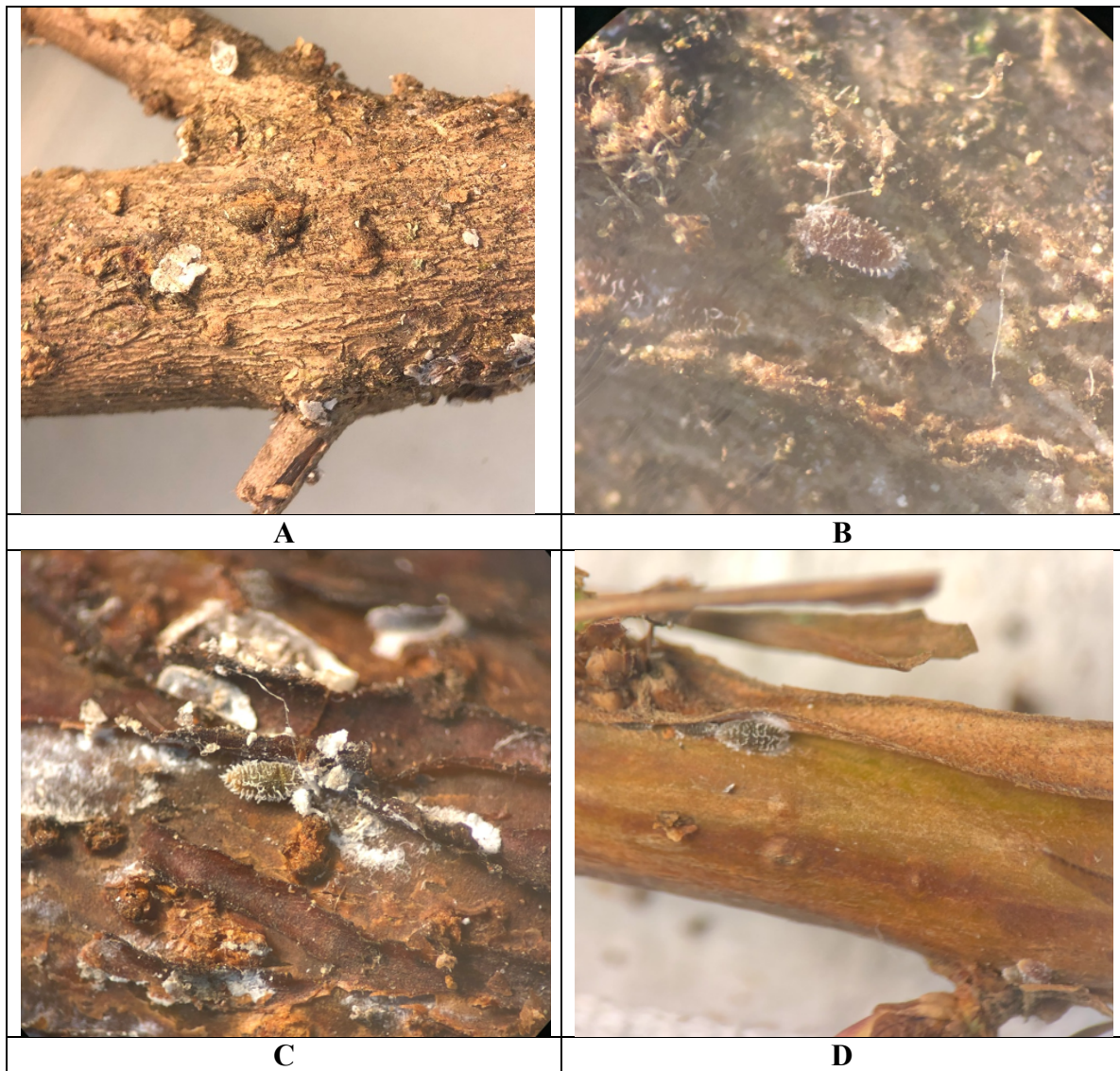


Figure 1. Microscopic images of crapemyrtle bark scale found on alternative hosts: (A-B) *Spiraea japonica*; (C-D) *S. thunbergia*.

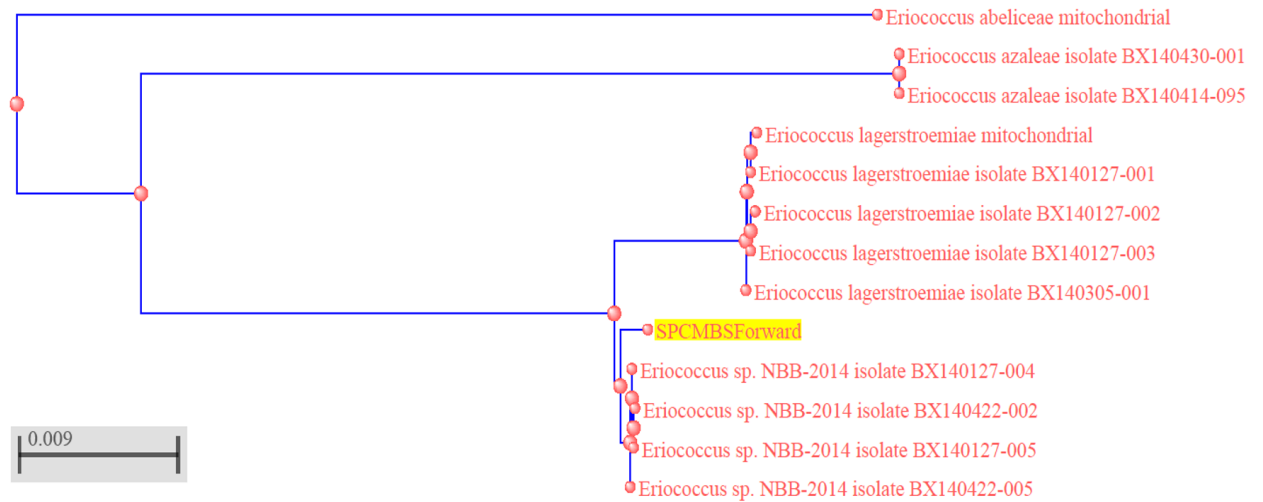


Figure 2. Phylogenetic tree shows the genetic relationship of *Acanthococcus* (= *Eriococcus*) *lagerstroemiae* (crapemyrtle bark scale from alternative hosts in this study were highlighted), *Acanthococcus azalea* (azalea bark scale), and *Acanthococcus abeliceae* from different geographical locations.